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Editorials

Monitoring HIV epidemiology using assays for recent infection: where are we?

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This issue of Eurosurveillance is dedicated to the widespread advances being made in Europe in the implementation of Serological Testing Algorithms for Recent HIV Seroconversion (STARHS). With the increasing interest in and the use of STARHS to estimate HIV incidence, we believe that the articles regarding the types of assays available [1], the implications for converting laboratory-based data into a real epidemiological measure of incidence [2] and the experiences from France [3], Germany [4] and Portugal [5] of incorporating STARHS methods into national HIV surveillance systems make this issue of Eurosurveillance opportune and of keen interest to a wide readership.

To date, the most important measure to monitor the HIV epidemic has been the reporting of newly diagnosed infections and national surveillance systems are now in place in nearly all European countries [6]. However, the major limitation of this measure is that it does not give an accurate picture of the evolving status of the epidemic as it comprises both people with recent infection and people with infection of several years' duration. In recent years this limitation has been brought into sharp relief in many European countries. Does the increase in many western European countries of diagnosed cases of HIV among men who have sex with men [6] represent a real increase in transmission or a reflection of a greater willingness to test for HIV? In countries such as Portugal, does the shift of newly diagnosed cases of HIV away from injecting drug users and towards those infected by sexual transmission [6] represent the true transmission dynamics of the epidemic? The anticipated benefit of STARHS is to provide answers to these questions by estimating HIV incidence, the number of new infections in a defined time period, and thus enable public health authorities better to target prevention campaigns and resources.

A decade ago, a new strategy based on a testing algorithm that combined two assays, one sensitive and one less sensitive, was proposed to identify a person in the period of early infection, when the antibody titre is increasing but before peak and persistently high antibody response [7]. This strategy requires the use of a commercially available enzyme immunoassay (the sensitive assay) and "detuning" it by increasing dilutions and decreasing incubation times (the less sensitive assay). A blood specimen from a person with early infection is reactive with the commercial assay, but non-reactive with the less sensitive detuned version. The detuned approach has been described using the Abbott HIVAB 3A11 and the BioMérieux Vironostika HIV-1 assay. Unfortunately, both assays were of the early generation immunoassays for HIV antibody screening and, as neither corresponds to the high sensitivity that is demanded, production of both assays has now ceased.

Another approach to identify recent HIV infections is to quantify the avidity of antibodies by modification of third generation anti-HIV assays that run on random access analysers [8]. A similar methodology has been successfully applied to diagnose primary infection by rubella virus, cytomegalovirus or toxoplasmosis during pregnancy, in order to provide individual counselling [9]. Although it is not common medical practice, improving the detection of recent infection by combining STARHS results with clinical and laboratory data may have benefit for the patient, by providing an opportunity to discuss enrolment in early intervention studies, and reduce the possibility of onward transmission, by enhancing partner notification procedures [10].

…assays can be developed that are designed specifically for the purpose of identifying recent infection.

There are a number of important obstacles and threats to the widespread use of STARHS in Europe and globally. The first is to assure the long-term supply of assays. The detuned and avidity STARHS assays require modifications of commercially available assays, and their long-term availability cannot be guaranteed. Alternatively, assays can be developed that are designed specifically for the purpose of identifying recent infection. Such assays can be developed commercially, such as BED-CEIA, or by collaboration between national reference laboratories and public health surveillance institutes, as has been done in France [3,11].

A second obstacle is that a window period must be defined for each assay, and then used for either determination of the frequency of recent infection in a given population or for incidence measurement. In a perfect world, one could imagine that every assay should identify a recent infection based on an identical window period. However, the few comparisons of the existing (past or present) assays clearly showed that there are many discrepancies between assays, particularly because the window period is not similar [12]. This is complicated by the fact that, even when using a single assay, the window period frequently differs when applied to a population different from that used initially for the development,

especially in areas where non-B subtypes predominate [13,14], There still remains important work to be done for the validations of the assays and algorithms for estimating incidence from cross-sectional blood specimens.

A third threat is the expertise required to implement the laboratory methods. As outlined in this issue in the article by Murphy and Parry [1], various quality control measures need to be implemented including external quality control procedures. This includes not only assuring and maintaining the operational characteristics of the assay, as outlined in the paragraph above, but also the logistics of rolling out the assay to a wider laboratory network beyond the currently small specialised group of laboratories.

A fourth limitation is the application and integration of STARHS data into routine public health practice. The proportion recently infected is often reported [10,16], but this measure is dependent on HIV testing patterns. The calculation of HIV incidence in the population is much more difficult, as highlighted in this issue by Le Vu et al [2], and will require significant enhancements and changes to current surveillance systems established to monitor the HIV epidemic. Not only will public health authorities need to obtain improved denominator data, but they will also need to enhance their knowledge of HIV testing patterns in different populations and develop current surveillance datasets to include more laboratory and clinical information with which to validate the results of any tests for recent HIV infection [10].

Although all the assays for recent infections have shown limitations, they have been already used in many circumstances to estimate either HIV incidence or, at least, the proportion of recent infection in various populations. Even if they cannot be recommended for routine use worldwide because of insufficient data on their performance to provide precise incidence in different populations, a few studies have already illustrated their usefulness [15,16,17].

The increasing momentum to incorporate STARHS methodologies within HIV national surveillance systems, particularly with the recent release of American estimates of the national HIV incidence [18], highlight the need for a European strategy to be formulated under the auspices and with the financial support of the European Centre for Disease Control and Prevention. Such a strategy should define the additional studies required not only to ascertain the operational characteristics of the assays but also the epidemiological needs for estimating incidence, thus providing best quality data to health policy makers for the implementation and evaluation of prevention campaigns. It is with the development of such a coordinated strategy that a European voice can provide a vital input into global STARHS initiatives.

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Review articles

Assays for the detection of recent infections with human immunodeficiency virus type 1

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The Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) is a generic term for several laboratory techniques that can be used to differentiate recent from long standing infections with human immunodeficiency virus-1 (HIV-1). There are several other approaches that identify acute seroconverters, but STARHS methods are distinguished by their ability to identify infections that occurred during an extended period of 4-6 months prior to sampling. While the STARHS techniques have been employed on an individual basis, their main usefulness lies in the potential of estimating the rate of acquisition of new HIV infection, or incidence, in a population by application to cross-sectional sero-surveys. This is substantially simpler and less expensive than cohort studies. As such, STARHS techniques facilitate the timely monitoring of the impact on HIV incidence of factors such as interventions, demographic factors and behavioural patterns.
The major STARHS techniques currently available are described. Furthermore, the principles behind the methods used are discussed and the limitations of the current assays and the confounding factors that may affect assay specificity are described. A model algorithm for the application of a STARHS assay is shown. Finally, we outline recommendations for laboratory quality systems that will improve the efficiency of STARHS testing, reproducibility of results and reliability of incidence estimates.

Introduction

The ability to segregate recently-acquired human immunodeficiency virus type 1 (HIV-1) infections (RHI), i.e. infections acquired in the previous few months, from long-standing ('prevalent') infections is a valuable tool for real-time measurement of the changing patterns of HIV transmission. Although the HIV infection process and the immune response to HIV afford opportunities to recognise recent HIV infection it is only in the last 10 years that these have been exploited to aid the determination of HIV incidence in populations. Differences between individuals present challenges to the application of serological tests of RHI on an individual patient basis.

Virological and serological events following infection

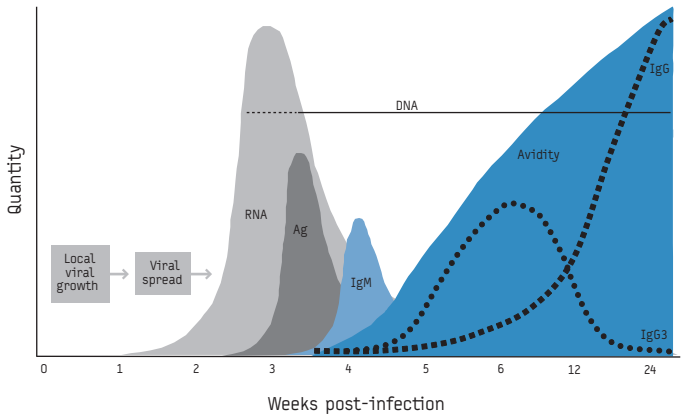
The typical evolution of viral and host markers of HIV infection are illustrated in Figure 1. Investigation of virological and serological events that occur during the very early phase of HIV infection indicate that, following local replication in proximity to the inoculation site a high titre viraemia occurs, generally during the second to third week after exposure [1,2]. This allows generalised seeding of the virus in susceptible tissues throughout the body. HIV genomic RNA is present before the patient has developed detectable anti-HIV antibodies and is therefore a powerful marker of recent infection. This phenomenon has been used to identify recent HIV-1 infection by some groups [3-6].

A protein component of the virus core, p24 antigen (p24Ag), is usually detectable [1] within a few days of the onset of viraemia [1]. As the host's immune system initiates a response, levels of both the virus and p24Ag fall. The p24Ag usually becomes undetectable until the degradation of the host immune system associated with progressive HIV-related disease, typically around 10 years later. In most cases HIV RNA remains detectable, albeit usually at levels much lower than in the acute phase. Detection of p24Ag in the absence of anti-HIV antibody may also be used as a marker of recent infection but its presence is unreliable and short-lived (1-2 weeks) and therefore has limited utility for measuring incidence.

The short duration of early p24Ag may in part be explained by it being masked due to complexing with the emerging anti-HIV antibodies. Heat or chemical treatment is able to disassociate antibody-antigen complexes, perhaps allowing extended detection of p24Ag further into anti-HIV seroconversion. However, many individuals with established HIV infection also have complexed

Figure 1

Typical evolution of key viral and serological markers during the first weeks following infection with HIV-1 (schematic diagram)



Viral markers: RNA, Ribonucleic acid; DNA, Desoxyribonucleic acid; Ag, Antigen. Immunological markers: IgM/IgG, Immunoglobulin M/G antibodies.

Tests that distinguish recently acquired HIV-1 infection from those that are long-standing take advantage of these events.

p24Ag [7]. Moreover, late in the infection, as the immune system fails, p24Ag is often produced in excess and can in many cases be detected even without the dissociation treatment [8,9]. The presence of p24Ag and/or a high level of HIV RNA after the seroconversion period are usually indicative of rapid disease progression and a poor prognosis [10].

The initial immune response is typically heralded by a virus-specific IgM response [11-13]. This IgM response is variable both in intensity and duration, generally peaking within 1-2 weeks, falling to background levels 1-2 weeks later [14]. Contemporaneously, the long-lived high-titre IgG response develops. A gradual increase in anti-HIV titre occurs over several months and this is the basis of both the 'detuned' and 'BED' assays, discussed later in the context of the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS).

Standard HIV screening and diagnostic assays

Standard commercial screening and confirmatory tests are mostly unable to distinguish between long-standing and recently-acquired infections. When specimens are taken during the short period, typically no more than 2-4 weeks, between onset of seroconversion and attainment of the maximum signal in the conventional diagnostic tests, it may be relatively straightforward to diagnose an incident infection on the basis of the rapidly evolving serological pattern. However, to be confident, a combination of supplemental tests needs to be done which may include some or all of the following: Immunoblot (Western blot/line immunoassay); and assays for the detection of: HIV RNA; p24Ag; and IgM anti-HIV. The Western blot assay involves the detection of antibodies against specific HIV-1 proteins separated by molecular weight. The presence and relative reactivity of each specific antibody can be identified, and a pattern typical of recent seroconversion may be recognised. A potential hazard of utilising limited Western blot patterns (i.e. reactivity with few HIV-1 proteins) as evidence of RHI, particularly during the earliest phase of anti-HIV seroconversion, is the significant risk of confusing non-specific reactions with HIV seroconversion. Furthermore, the interval during which this approach may be used, perhaps 3-4 weeks after infection, is too short to permit reliable measurement of HIV incidence on realistic population sizes.

The Serological Testing Algorithm for HIV Seroconversion (STARHS)

The typically rapid immunological response to HIV infection means that within less than a month of anti-HIV seroconversion commencing, standard HIV test kits are unable to distinguish recent from long-standing infections. However, a number of adapted or novel techniques have been developed that are able to identify recent infection over a longer time frame than that achievable with conventional assays. These methods are intended to be applied to individual specimens in which the presence of anti-HIV-1 antibody has already been confirmed, and the approach is known generically as the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS).

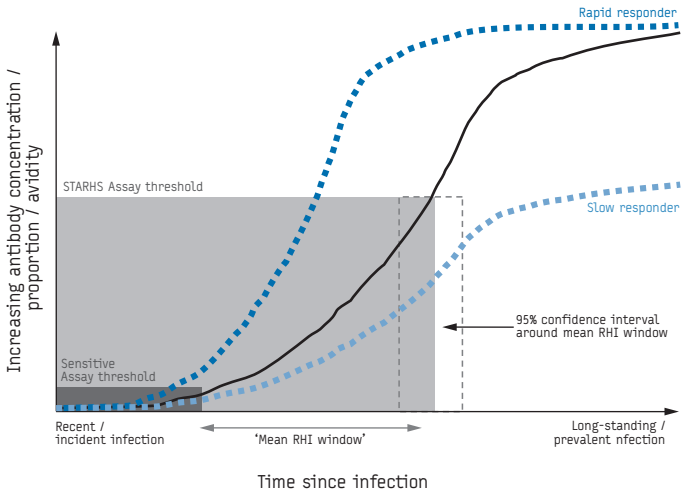
The STARHS approach offers a number of important advantages over other methods for determining HIV-1 incidence. Unlike cohort studies which require repeated testing of individuals, and where results may be biased by people leaving the study, STARHS testing can be carried out retrospectively on stored single specimens from cross-sectional sero-surveys. In comparison with cohort studies, applying the STARHS approach is cheaper, quicker and simpler to perform. Furthermore, STARHS testing can be performed on a real-time basis thus allowing a measure of recent infection at the time of a study as opposed to incidence derived from a cohort study which cannot be ascertained until after the follow-up sample has been collected and tested.

STARHS/RHI window period

The STARHS technique allows HIV-1 incidence to be determined from representative panels of stored anti-HIV-1-positive specimens gathered over a given period from a particular population whose size is known. The duration of the period between seroconversion in the original (sensitive) HIV-1 screening assay and conversion (from recent to long-standing) in the STARHS method must be well-defined and typically in the order of several months, and is critical to a STARHS assay being able to furnish a population incidence rate (Figure 2). The duration of this STARHS window needs to be determined carefully, and this requires panels of specimens from individuals whose date of seroconversion is known or closely approximated. Modelling these data allows the relationship between time since seroconversion and the expected average signal in the STARHS method to be described mathematically. From this, and additional data on known long-standing (>12 months) infections, the chosen cut-point, dividing recent from long-standing, may be set such that it provides an appropriate balance of sensitivity and specificity, and this is typically associated with a mean RHI window in the region of 3-6 months. The duration of the STARHS window is limited by the effects of individual variation on antibody titre and rate of antibody production and maturation. The longer the time after infection, the more pronounced these individual differences become, leading to increasing misclassification [15,16]. Although

FIGURE 2

Principles underpinning the serological testing algorithm for recent HIV infection (STARHS)



The STARHS approaches are applied to confirmed anti-HIV-1-positive specimens and, with the exception of the IgG3 and Inno-LIA approaches, rely on the marker employed, e.g. avidity, increasing over the first several months after seroconversion is detected by a sensitive screening method. If a test specimen gives a result below a pre-determined cut-point, it is deemed to have been a recently acquired HIV-1 infection (RHI). The cut-point is set such that it provides an appropriate balance of sensitivity and specificity, and this is typically associated with a RHI window in the region of 3-6 months. However, the uncertainties around this model should be considered, including the accuracy of the RHI window (95% confidence intervals) and the person-to-person variability shown in the diagram as outliers with either a 'rapid' or a 'slow' response. The former may appear to have a long-standing infection some time sooner than the average RHI window, and the latter may appear to be an RHI some for a considerably longer time.

of lesser magnitude, differences in seroconversion sensitivity between anti-HIV-1 screening tests employed in STARHS should also be taken into account, particularly when moving between generations of screening tests, for which the difference could be more than two weeks [17].

Definition of the STARHS window permits measurements of HIV-1 incidence to be made on achievable populations, but their robustness will depend on several factors, not least the accuracy of the mean STARHS window period employed. When applying STARHS on an individual (diagnostic) basis, the duration of the STARHS window period cannot be accurately defined, and arguably need not be. Importantly, it must be borne in mind that the STARHS windows described represent the mean interval between the earliest time at which an HIV-1 diagnosis may be made and conversion to long-standing status in the STARHS assay, and not the upper limit. This is derived by examining specimens from many seroconverting individuals in whom the immune response will mature differently. Accordingly, taking the 155 days' window advised for the BED-CEIA assay (described below), a substantial proportion (roughly one-half) of those infected will already have converted in that assay to a long-standing infection at under 155 days since seroconversion, and the remainder at over 155 days; very few will actually convert on day 155. The consequence of this, when applied to individuals, is that some are likely to be advised inaccurately that, in the former example, their infection is over 155 days-old and therefore long-standing, and in the latter, that it was under 155 days-old and therefore classified as recent.

Furthermore, STARHS results consistent with an RHI are known to arise and be persistent in a small proportion of those infected for years and in those presenting late in the course of infection [18], as discussed below. Because the rate of misclassification as an RHI is a key variable influencing the accuracy of population incidence estimates this is coming under closer scrutiny, and has led to proposed correction factors for incidence estimation [19,20]. However, these do not provide a means to ensure an error-free finding when applying STARHS individually. Nevertheless, an improved understanding of sensitivity, specificity and predictive values associated with STARHS testing should provide an appropriate platform for providing advice, care and public health action on an individual basis. It may be advisable when using STARHS as a diagnostic indicator to communicate the timing of infection less definitively, e.g. when the result is consistent with a RHI: 'The findings suggest HIV may have been acquired in the last 12 months'.

Assays for recent HIV-1 infection

A number of assays can be used within a STARHS programme (Table).

The 'detuned' assay

The 'detuned' assay was the first assay to be described as being able to identify specimens from individuals recently infected with HIV-1 for the purposes of incidence calculation. Employing the recommended assay cut-off, the technique recognises HIV-1 seroconversions that have occurred on average four to six months prior to collection of the positive specimen [15,16,21]. However, the period during which recent infection can be identified can be altered by changing the cut-off applied to the assay. The method relies on the generalisation that anti-HIV titres in the plasma rise gradually, and at a similar rate in each infected individual, over a period of several months following seroconversion.

The 'detuned' approach takes confirmed anti-HIV-1-positive specimens and re-tests them with an enzyme immunoassay (EIA) that has been made less sensitive ('detuned') by increasing the dilution at which each specimen is tested from 1/76 to 1/20,000 and by reducing the incubation times. Although assay variability is partially accommodated by the inclusion of a calibrator, obtaining accurate results by the detuned approach is technically demanding, requiring precise preparation of high serum dilutions and strict adherence to incubation conditions. Recent seroconversion is inferred if the confirmed anti-HIV-1-positive specimen is negative in the less sensitive EIA.

The 'detuned' approach has been described for two different immunoassays: the Abbott HIVAB 3A11 (Abbott laboratories, United Kingdom (UK)) and the bioMérieux Vironostika HIV-1 microelisa (bioMérieux, UK). Production of both assays has now ceased with the last lot of bioMérieux Vironostika assays expiring in summer 2008. Both these assays use a semi-purified viral lysate antigen adsorbed to the solid phase. In both cases the viral lysate derives from an isolate of the subtype B strain of HIV-1. The Abbott HIVAB 3A11 antigen is also 'spiked' with purified native gp41 antigen. The use of an antigen from a single HIV subtype means that

Table

Methods described which may be employed on serum/plasma specimens in serological testing algorithm for recent HIV seroconversion (STARHS) applications to distinguish recent from long-standing HIV-1 infection

STARHS method	Type	Principle	Reference
Abbott HAVAB (3A11)	Modified commercial (withdrawn 2003)	'Detuned' – standard assay, sensitivity reduced to extend seroconversion window	[15]
Abbott AxSYM HIV 1/2 gO	Modified commercial	Avidity of anti-HIV antibodies	[31]
Calypte BED EIA	Commercial	Proportion of total antibodies that are HIV-specific	[23]
bioMérieux Vironostika HIV-1 microELISA	Modified commercial (withdrawn 2008)	'Detuned' – standard assay, sensitivity reduced to extend seroconversion window	[16]
IgG3 anti-HIV	In-house	Transient presence of IgG3 isotype antibodies against HIV p24Ag	[36]
IDE-V3 EIA	In-house	Reactivity with two selected HIV antigens is used to predict likelihood of recent infection	[34]
Inno-LIA HIV	Modified commercial	Relationship of reactivity with various HIV antigens	[37]
Ortho Vitros *ECi* anti-HIV 1+2	Modified commercial	Avidity of anti-HIV antibodies	[33]
Particle agglutination (SeroDIA-HIV)	Modified commercial	'Detuned' – standard assay, sensitivity reduced to extend seroconversion window	[38]

heterologous antibodies (i.e. those formed against viral subtypes not utilised in the assay) may not bind the antigen as effectively as the homologous antibody. This generally causes an increase in the period during which the assay would determine a specimen to be from an RHI [22]. Reactivity in the 'detuned' assay is standardised against a calibrator specimen to give a standardised optical density (SOD), thus smoothing out run-to-run variability.

BED-CEIA assay

The BED-CEIA (capture enzyme immunoassay) is a commercial product (Calypte Biomedical, United States (US)) designed specifically for the purpose of identifying infections that were acquired recently [23]. Being a class-specific IgG antibody capture EIA, it differs in its mechanism from the 'detuned' assays as its reactivity is dependent, not on the absolute titre of HIV-1-specific antibody, but on what proportion of all the IgG captured from an HIV-infected person's serum is directed against the HIV antigens employed. In early infection, the proportion of HIV-specific antibody is lower than in a long-standing infection. As the BED-CEIA does not directly measure the amount of antibody present it is technically more robust than the 'detuned' techniques, the principle on which it is based being more forgiving about the accuracy of dilution of test specimens, incubation times and temperatures.

The BED-CEIA was designed to overcome some of the subtype differences associated with the 'detuned' assays, utilising a trimeric branched peptide. Each branch comprises a synthetic oligopeptide derived from the immunodominant region of the transmembrane gp41 glycoprotein of HIV-1 subtype B, CRF_01 AE and subtype D, hence the assay name 'BED'. These three peptides were selected to cover much of the breadth of antigenic diversity, in theory allowing a single window period to be used with the BED-CEIA test, whatever the infecting HIV-1 subtype. However, it has been shown that differences in window periods between subtypes do occur in the BED assay, though perhaps less pronounced than in the detuned assays (see below).

Avidity assays

A further approach to identifying recent infection is to investigate the maturity of the HIV antibody response by investigating its avidity. Antibodies of low avidity are usually indicative of recent infection and this approach has been shown to be valid for many viral infections [24-26]. Although avidity assays have previously been described for use with HIV-1 [27-30], it was not until recently that assays that could be used for HIV-1 incidence determination were described [31-33].

The method described by Suligoi et al. is a modification of the third generation anti-HIV-1/-2 assay that runs on the Abbott AxSYM random access analyzer, and is therefore easy to perform. It uses a method whereby the specimen is pre-incubated with the chaotropic agent guanidine (guanidine hydrochloride) [31,32]. Guanidine treatment of the specimen primarily disrupts the hydrogen bonds that help determine the secondary structure of the antibody, although it may also have a residual effect on the subsequent antibody-antigen interaction. The treatment has a greater effect on early antibodies, the active site of which has a less defined structure and can be degraded by mild denaturation so that they are less able to bind their homologous antigen, thus reducing the signal. As the antibody response matures, the active site becomes increasingly resistant to disruption.

When assessing the avidity of an antibody response, the level of signal obtained after chaotropic treatment is compared with the signal produced when pre-incubating the specimen in a neutral diluent such as phosphate buffered saline (PBS). When the antibody is highly avid and therefore largely resistant to the chaotrope, the two signals in the immunoassay will be very similar. The binding of early, less avid, antibodies on the other hand will be much reduced when treated with the chaotrope, and this will produce a reduced signal compared to the untreated aliquot. The RHI window for the AxSYM avidity assay has not yet been determined precisely, but it is thought to be close to six months.

Recently, an alternative antibody avidity assay has been described that also uses guanidine but runs on the Vitros analyzer (Ortho Diagnostics, UK). It has an RHI window of approximately 142 days when employing a threshold avidity index of 80% [33]. Currently no published data exist on the widescale application of these avidity assays, and work is continuing to refine their performance characteristics and the window period, particularly for HIV-1 non-B subtypes.

IDE-V3 assay

The IDE-V3 immunoassay is based on two conserved highly immunogenic epitopes found in the envelope glycoproteins of HIV-1 [34]. One is derived from the immunodominant epitope (hence 'IDE') of the transmembrane glycoprotein gp41; the second derives from the V3 loop of the outer glycoprotein gp120. The IDE antigen comprises two consensus oligopeptides of 30 amino acids, one from HIV-1 group M and one from subtype D. The V3 component comprises a blend of five oligopeptides derived from the HIV-1 subtypes A, B, C, D and CRF_01 AE. The IDE-V3 assay is not available as a commercial kit, but can be assembled by the user from basic ingredients that are available commercially.

Technically the assay is structured as a simple indirect enzyme-immunoassay, employing a 96-well microplate format, with the 8-well columns alternately coated with the IDE and V3 oligopeptides. A dilution of each specimen is tested against both the IDE and V3 antigens. In its current format this assay has to be assembled by the user from individual components and, although its principle is relatively straightforward, its wider availability as a robust STARHS approach awaits further standardisation of the reagents and controls.

To discriminate recent from long-standing infection this assay employs a mathematical formula which draws on reactivity of the specimen with the antigens from each region. The formula was derived from testing panels of specimens known to be from either recent (<6 months) or long-standing infections. Although the authors imply that the assay is able to identify recent infections that date back no more than six months, this appears to have been based on polarised specimen sets: specimens representing RHI, which had mostly been collected soon after seroconversion, and specimens representing long-standing infections, many of which may had been collected considerably later than six months following seroconversion. Consequently, the continuous relationship between the assay output and time since seroconversion has not been mathematically modeled, and the exact duration of the RHI window period has yet to be calibrated.

Sakarovitch et al., applying STARHS assays to seroconverting individuals in Cote d'Ivoire found that the IDE-V3 assay, while having good specificity (96.3%), had poor sensitivity (42.3%), and

this suggests strongly that its seroconversion window is likely to be considerably shorter than six months [35]. Currently this assay is being used as part of the French national screening programme to determine the proportion of newly diagnosed HIV infections that were recently acquired. Work is continuing to improve the estimation of the RHI window period for this assay.

Other STARHS approaches

A number of other approaches have been described that distinguish recent from long-standing HIV-1 infection. These include:

IgG3 anti-HIV: It is known that the IgG isotypes formed in response to an infection may vary during the course of an infection. Research investigating the IgG isotype response to a range of HIV-1 antigens using a Western blot approach identified that isotype IgG3 was usually present transiently during the first few months of HIV-1 infection [36]. The investigators found the antigen against which the IgG3 response was most reliable was p24. These findings were converted into a simple EIA based procedure whereby IgG3 to p24Ag is typically detectable for only the first 1-4 months of infection. Unfortunately, however, this method has not yet been translated into a commercial kit.

Inno-LIA HIV adaptation: The Inno-LIA™ HIV I/II Score is a line immunoassay, similar to a Western blot but employing only a limited selection of synthetic oligopeptides and recombinant antigens of HIV-1 and HIV-2. Its routine application is as a confirmatory test to investigate whether screen-reactive specimens are true or false. For the STARHS application the intensity of each band in the Inno-LIA test is read using a slightly modified scoring system. An algorithm is applied to the scores which allows the segregation of the results into recent or long-standing HIV infection [37]. The approach is expensive, but may have utility where it is already routinely employed as the confirmatory diagnostic test.

Several other approaches have been described, including one based on a particle agglutination test in a 'detuned' format [38] and an oral fluid assay [39], but neither of these assays has been applied on a large scale and the RHI window periods have not been established.

New STARHS approaches are under development and should be expected to become available over the next few years.

Limitations of STARHS assays

The accuracy of STARHS assays is affected by a number of factors that are likely to be encountered when testing populations of HIV-infected individuals, and these are outlined below:

Infecting HIV subtype

The detuned STARHS methods have been based on the use of HIV-1 clade B antigens. Because the immunodominant epitopes differ between HIV-1 clades it is likely that the heterologous antibody responses may show lower binding affinities and that this, in turn, could alter the RHI window period, in most cases extending it. Should this be the case, HIV incidence would be over-estimated unless the RHI window is adjusted. This presents serious difficulties when dealing with epidemics of mixed clades, such as are now established in at least some European Union countries.

Studies on populations infected with non-B viruses have indeed revealed that the period during which an infection is identified as recent is significantly different to that for clade B infections. For example, employing an SOD threshold of 1.0 in the Vironostika detuned assay the average RHI window is 170 days, whereas for the CRF_01 AE virus it is 356 days [22] and for clade C it is 360 days [40]. Comprehensive findings are not available on this issue, and few are actually published.

As discussed above, the BED-CEIA method was designed to overcome problems associated with the lower affinity of heterologous antibody responses by employing a multimeric antigen representing much of the antigenic diversity associated with the immunodominant region of gp41. The manufacturer's product insert for the BED-CEIA advises the use of a single mean RHI window period of 155 days [41]. However, studies have demonstrated that the mean RHI window period for clade C is substantially longer, at 181 days, and for CRF_01 AE it is much shorter, at 115 days [42]. The impact on more recently described methods like the avidity, IDE-V3 and IgG3 methods is as yet unknown.

Acquired immunodeficiency symdrome (AIDS)/low CD4 count

The failing immune system associated with advanced HIV disease has long been known to be associated with a decline in anti-HIV antibody levels [8], and this would be expected to impact the specificity of those STARHS methods that depend primarily on the quantification of antibody. Indeed, misclassification rates for the detuned methods have been published, and for Vironostika it has been estimated that approximately 5% of AIDS cases will be misclassified as a recent infection [16]. For the BED-CEIA approach, the AIDS misclassification rate has been estimated at 2-3% [41]. Misclassification of AIDS cases by the IDE-V3 assays is approximately 9% [34]. On the other hand, as the avidity of antibody binding is not related to the quantity of antibodies, it would be expected not to be similarly affected, and preliminary evidence suggests this may be so.

Antiretroviral therapy

It has been observed that combination anti-retroviral therapy (ART) leads to misclassification of long-standing infections as recent. The exact mechanism has not been elucidated, but simplistically, it is likely that the ART suppresses viral replication to such a degree that the chronic stimulus to the humoral immune response is removed, leading to a decline in anti-HIV antibody titre. The effect is most pronounced during the first few months after ART initiation (authors' unpublished findings). However, in comparison to the very high anti-HIV titres typically found in HIV-infected individuals this effect is modest and would not be sufficient to render state-of-the-art HIV screening tests negative.

Other confounders

In some cases there is no clear common factor associated with a misclassification by STARHS. In an extensive study among HIV-1-infected men who have sex with men in a UK city, several long-term infected individuals with naturally suppressed viraemia (<50 copies/ml) were flagged as a recent infection by the detuned assay [18]. There is some evidence that the BED-CEIA approach misclassifies a substantial minority of long-standing infections as recent and consequently leads to inflated incidence rates [19,20,43].

Quality control measures

As with any laboratory diagnostic method STARHS assays must be performed within an appropriate quality system. This includes the documentation of processes, use of standard operating procedures, appropriate training of staff and evidence of competency. In the authors' experience, the type and condition of equipment can significantly impact on the transferability of STARHS methods

between laboratories and lead to inconsistent results. Several of the key elements are discussed below:

Robust and reliable methods

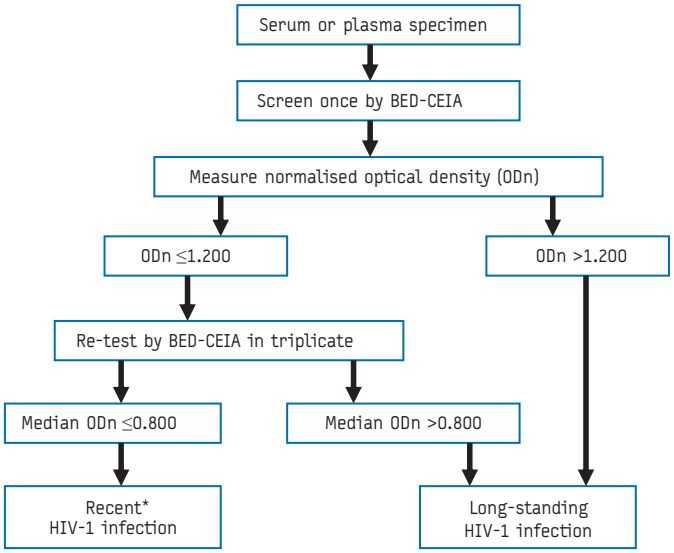
With the exception of the BED-CEIA the methods currently available are either modifications of commercial kits, or 'in-house' assays. Whichever sort of STARHS method is employed, it is important to select an assay that suits the laboratory's resources and skills and the population to which it is to be applied. The method should be capable of providing findings of acceptable accuracy and reproducibility. The use of modified or 'self-assembly' techniques is more vulnerable to inconsistency of performance and in those circumstances validated production and quality control processes must be in place to verify consistency of performance.

Confirmatory algorithms

In common with other diagnostic methods, the results of a STARHS assay will show some variability. To improve the reliability of the test result, the well-established methods ('detuned'; BED-CEIA) include an algorithm of triplicate retesting of specimens whose reactivity is in the range associated with recent acquisition, and a defined margin above, e.g. for the BED-CEIA a normalised optical density (ODn) of up to 1.200 (Figure 3). However, while this improves the accuracy of the STARHS measurement it does not identify the samples misclassified due to the factors discussed above such as advanced HIV disease. Similar approaches need to be developed for the other STARHS techniques.

Figure 3

Example of the STARHS testing process, employing the BED-CEIA procedure



* The duration of the recent HIV infection window that is advised in the BED product insert is 155 days. This is the mean duration, which is an important value when estimating population incidence rates. It is not the upper limit of the STARHS window. Consequently, when interpreting STARHS findings on an individual basis it must be borne in mind that a substantial proportion of those whose ODn is ≤0.800 will actually have been infected more than 155 days earlier. Similarly, some whose ODn is >0.800 will have been infected less than 155 days earlier. As one might expect, findings in close proximity to the cut-point of 0.800 are more likely to be a misclassification.

Assay calibrators and assay controls

A common approach to smoothing out lot-to-lot and run-to-run variation in performance is to employ one or more calibrator specimens which would show reactivity in the mid-range. They are employed to adjust the signal obtained with each test specimen against the reactivity of the calibrator, and thus control variations over time. At present, only the 'detuned' and BED assays incorporate a calibrator, generating respectively a 'standardised optical density' (SOD) and a 'normalised optical density' (ODn). In addition, other controls are normally included (e.g. non-reactive; long-standing). Even when all controls are supplied as part of a commercial STARHS kit it is best practice to include further controls of expected reactivity, either from a third party supplier or produced by the user laboratory, to provide the means to monitor assay performance independent of the kit manufacturer. Such controls provide a tool to ensure the assay is performing within expected parameters, and provide the basis for acceptance or rejection of each set of results.

External performance/quality assessment (EPA/EQA)

An important component of ensuring laboratories' performance is adequate is the blinded examination of small panels (typically 4-8 members) of specimens of unknown status. Such schemes require significant investment to establish and maintain. At present, EPA/EQA schemes exist only for the 'detuned' and BED assays. Furthermore, there is arguably a need for larger panels to qualify laboratories embarking on the application of STARHS methods.

Concluding remarks

A wide range of STARHS approaches have been described and new methods are under development. They clearly have a potentially important role both in public health monitoring and individual diagnosis. The evidence indicates that the current methods are generally able to distinguish recent from long-standing HIV-1 infections. However, the rigors of assigning an accurate duration to the interval between infection or seroconversion and the time at which the transition to a long-standing infection is assigned by STARHS remain challenging. This is due to the diversity both of the host immune response and of the antigenicity of HIV-1. When applying the method as an epidemiological tool to estimate incidence these variables may be controlled if there is a single prevalent HIV-1 subtype and its associated mean window is accurately known. In many parts of Europe, however, the HIV-1 epidemic is already heterogeneous. The BED-CEIA was designed to accommodate this, but despite this it has emerged that the mean RHI window, even for the small number of clades for which it has been derived, ranges from 115 to 181 days and this alone could lead to over- or under-estimates of incidence of approximately 50% [42].

It remains to be seen whether the assays currently being developed will provide improved accommodation of HIV-1 diversity. The complexities of the multiple variables involved in designing broadly applicable STARHS methods, optimising them, calibrating their performance and recognising their limitations present enormous challenges. A global initiative led by the WHO/UNAIDS has been created which is pooling the experience and resources of laboratory scientists, epidemiologists and statisticians working in the STARHS field. We should therefore expect improved STARHS methods and applications to emerge over the next few years.

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Review articles

Principles and uses of HIV incidence estimation from recent infection testing - a review

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Since the 1990s, the development of laboratory-based methods has allowed to estimate incidence of human immunodeficiency virus (HIV) infections on single samples. The tests aim to differentiate recent from established HIV infection. Incidence estimates are obtained by using the relationship between prevalence, incidence and duration of recent infection. We describe the principle of the methods and typical uses of these tests to characterise recent infection and derive incidence. We discuss the challenges in interpreting estimates and we consider the implications for surveillance systems.

Overall, these methods can add remarkable value to surveillance systems based on prevalence surveys as well as HIV case reporting. The assumptions that must be fulfilled to correctly interpret the estimates are mostly similar to those required in prevalence measurement. However, further research on the specific aspect of window period estimation is needed in order to generalise these methods in various population settings.

Introduction

Estimating HIV incidence, the number of new infections during a time period, is critically important for assessing the dynamics of human immunodeficiency virus (HIV) transmission and evaluating the impact of prevention policies. A conceptual improvement in surveillance methods has been made in the past ten years to make incidence estimation more feasible. By using a biomarker measurement to identify seropositive individuals who have recently been infected, incidence estimates can be obtained from a single specimen. This laboratory-based method can take advantage of the collection of specimen intended to assess prevalence (the proportion or number of persons cumulatively infected at a given time) and to obtain valid incidence data without the expensive and logistically complex requirement of following a cohort of uninfected individuals over time. However, as for other methods based on repeated prevalence data and mathematical modelling, the use of biomarkers to estimate incidence requires a substantial number of assumptions, some being difficult to assess, and an appropriate definition of the population the incidence is estimated for.

In this article based on the literature, we attempt to give an overview of the methods that allow estimating HIV incidence based on biomarker detection at the early stage of infection. After defining the principles, we review some typical uses of serological incidence assays and the challenges for each type of application.

Principles

Incidence based on detection of virological markers before seroconversion

In 1995, Brookmeyer and Quinn introduced a simple approach for estimating HIV incidence from a cross-sectional survey [1]. They used a two-step algorithm combining diagnostic tests for the p24 antigen and HIV-1 antibodies to determine the prevalence of p24 antigenaemia among antibody-negative individuals (Figure 1). The HIV incidence rate was then calculated by using the classical epidemiologic relation between prevalence, incidence, and duration of the period between the onset of detectability of p24 and the first HIV antibodies.

The disadvantage of this approach was that the time during which p24 antigen is detectable prior to seroconversion is short (the mean duration of this period was 22.5 days in 1995 and has become shorter since then due to the development of new diagnostic assays that allow to detect antibodies earlier [2]). The first consequence of this is that the estimation of this period comes with a considerable uncertainty which can have a large impact on the incidence estimate. The second consequence is that large samples and/or high HIV incidence are required to identify a sufficient number of individuals with detectable p24 antigen who have not seroconverted. Nevertheless, Brookmeyer and Quinn provided the conceptual framework for subsequent laboratory-based methods to estimate incidence from single cross-sectional surveys.

Within the range of methods to identify early infection through virological markers before seroconversion, testing of pooled HIV RNA now seems to be the most appropriate approach because RNA can be detected earlier than p24 antigen, which allows characterisation of a longer time period (Figure 1). Moreover, pooling of specimens improves the predictive value of the amplification assays and substantially lowers the costs. However, in order to obtain accurate incidence estimates, this method requires the inclusion of very large sample populations, such as those provided by blood donations [2] or by the large testing programme in the United States (US) described by Pilcher *et al*. [3].

Serologic incidence assays

Janssen *et al*. were the first to describe in 1998 an approach based on a test specifically developed for the purpose of estimating incidence [4]. This approach named "Serologic testing algorithm

for recent HIV seroconversion (STARHS)" aimed at detecting a transient state reached after the antibody conversion. It thus offered the advantage of testing only positive individuals and defining a period sufficiently short to fulfil the requirements of stationarity of the incidence over the study period, while sufficiently long to minimise the inaccuracy in its estimation. The work of Janssen *et al*. can be considered as a milestone for the concept of serological methods for the estimation of HIV incidence.

Following the same principle, various applications of laboratory-based incidence estimation from cross-sectional population surveys have been described and a growing number of assays have been developed (see the article of Parry *et al*. in this issue).These assays measure the immunological response against the virus, based on specific HIV antibody concentration [4-6], proportion [7], isotype [8] or avidity [9]. This measure should define a transient state from the onset of detectability by a standard HIV screening test to the cut-off value defining the "established" infection status of the test for recent infection (Figure 1). This period is called the window period. Because of the individual variability in antibody response, window periods may differ widely from person to person. Their mean duration is measured in advance by testing serial specimens from infected individuals with known dates of seroconversion [10]. The STARHS methods have been compared to classical incidence measurements obtained in cohorts to assess their validity [4,11,12]. Provided that the compared estimates are not affected by population sampling bias, the estimates are reported to be similar [10,12].

Incidence estimation

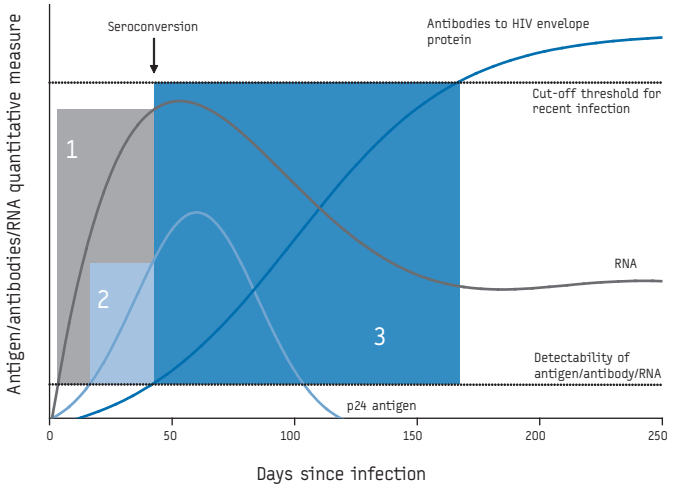
The incidence estimation is calculated as the frequency of the transient state (i.e. the prevalence of recent infection) divided by its duration (the mean window period). As stated above, this calculation is based on the relation "prevalence = incidence * mean duration". This relation assumes that the condition, in our context "recent HIV infection", is a rare event so that the prevalence odds can be approximated by the prevalence [13]. And the relation is valid for a stationary population with a constant level of incidence during the study period [1]. In Figure 2, we present an example of an incidence calculation using the formula developed by Janssen *et al*. with a window period of 180 days [4].

Various adjustments have been made to Janssen's formula in order to correctly express the number of people at risk and to account for misclassification of long-term infections. The first adjustment consisted in varying the assumed number of people at risk of having had a recent HIV infection during one year. As in the estimation of incidence in a cohort, HIV-negative individuals are considered at risk during the whole period, while infected individuals can be considered at risk during half a year on average [14].

In addition, concerns have been expressed that the mean window period for the BED capture enzyme immunoassay (BED-CEIA) does not properly take into account people who have a very long individual window period and can be falsely classified as

FIGURE 1

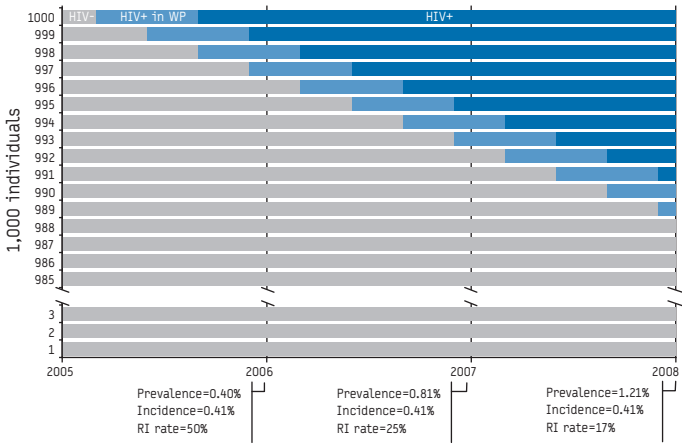
Kinetics of virological markers and host immune response used to define transient states in the early phase of HIV infection



1: RNA-to-seroconversion transient state as defined by Busch *et al*., 2005 [2]
2: p24-to-seroconversion transient state as defined by Brookmeyer *et al*., 1995 [1]
3: Antibody-based mean window period as defined by Janssen *et al*., 1998 [4]

FIGURE 2

Relation between HIV prevalence, recent infection rate, and the incidence estimation in a cross-sectional survey



A constant incidence rate of 0.4% persons/year is observed in a population of 1,000 individuals seronegative from the beginning of year 2005. Prevalence, incidence and rate of recent infection are estimated cross-sectionally at the end of 2005, 2006 and 2007. The number of HIV-positive inviduals includes those with recent infection, tested within window period (NR), and those with established infection, tested after the window period (NE), represented respectively in light blue and dark blue in the figure. HIV-negative individuals (Nneg) are represented in grey. While incidence estimates are nearly constant over the years, the recent infection rate, being influenced by the prevalence of established infection, is decreasing.

Estimates are calculated as follows [4]:

$$\text{Prevalence} = \frac{N_R + N_E}{N_{neg} + N_R + N_E}$$

$$\text{Incidence} = \frac{N_R}{N_{neg} + N_R} \times \frac{365}{\text{mean WP}}$$

$$\text{Recent infection rate} = \frac{N_R}{N_R + N_E}$$

This illustration was inspired by the presentation of Ruigang Song "Modeling HIV Testing Behavior and Its Impact on Incidence Estimation" at the 15th International AIDS Conference, July 15, 2004, Bangkok, Thailand.

WP = Window period; RI = Recent infection.

recent. This issue is probably a general one, affecting all the tests that have been calibrated using a disproportionate number of short term infections (for less than one year). It should have an impact on incidence estimation since the cross-sectional populations on which the method is to be applied are expected to contain a larger number of long-term infections. Two adjustments have been proposed to correct this issue about the specificity [15]. They share the principle of applying a corrective factor in the incidence formula to compensate for the false recent cases due to very long window period. Other algorithms have been proposed that, rather than correcting the formula, combine two incidence assays in order to avoid misclassification [12,16].

Applications

While a comprehensive review of applications for serological incidence assays is beyond the scope of this paper, the purpose of this chapter is to point out typical settings in which they may be used.

Typical applications

The most common context in which incidence assays are used are prevalence sero-surveys. Some were dedicated to incidence estimation, but the majority were set up to observe the recent infection status of stored HIV-positive serum specimens.

Numerous serial cross-sectional surveys have been applied in the setting of testing for HIV or other sexually transmitted diseases in countries such as the US [17-19], some European countries [20;21] or Brazil [22]. In these studies, temporal trends in incidence rate could be derived and helped to assess retrospectively epidemic phenomena among high-risk subgroups. But concerns about representativeness and selection bias can be raised about such voluntary testing sites (as reviewed below in the section 'Issues').

Similarly, already existing sentinel surveillance systems have provided insight into underlying trends in transmission in particular risk groups. Specimens gathered at enrolment in syringe exchange programmes or serial street surveys allowed the estimation of trends in HIV incidence among intravenous drug users in New York City, US [23] and San Francisco, US [24] over a long period.

For purposes of precision and as done for prevalence estimation, targeting a more general population than particular high-risk groups requires testing a very large number of people or setting the study in a country with a high incidence level.

At least one of these conditions was met in studies that estimated the HIV incidence by means of recent infection testing in antenatal screening programmes in Cambodia [25], South Africa [26], the US [27] and Brazil [28], in screening programmes for blood donation in the US [2;4], France [29] and the Ivory Coast [30], and a national household survey in South Africa [31].

In all these settings, specimens are collected routinely and can be tested for recent infection retrospectively or prospectively. Some demographic and behavioural data on the targeted population are usually collected along with the specimens, both for positive and negative individuals. Taking advantage of specimens from prevalence serosurveys allows to derive incidence data for these populations with only minor expenses in terms of cost and logistics.

In certain contexts, the most obvious added value of the incidence assays approach is that the incidence could not have been estimated by any other means. This is what happens when no accurate data on prior testing or exposure period can be obtained such as for the population of blood donors screened during their first donation [29].

Identifying recent infection

A particular use of incidence assays is identifying recent infection status per se, for individual patient management such as contact tracing or assessment of primary resistance. It is helpful to bear in mind that characterisation of recent infection was initially a by-product in the method described by Janssen *et al*. which considered incidence derivation as the main outcome. In particular, the use of the mean value of an incidence assay window period assumes that individual window periods are variable and that a certain number of individuals in a given population will have a window period shorter or longer than the mean. Consequently, some misclassifications of established infection (false positives) and of recent infection (false negatives) are to be expected. For the purpose of incidence estimation, the respective misclassifications are supposed to cancel each other out, so that the number of recent infection at a population level is correctly estimated. At the level of individual patients, however, this could lead to serious misinterpretation.

On the other hand, some assays have been developed for the specific purpose of classifying infections in individual patients as recent or established with given predictive criteria. This is the case for the enzyme immunoassay for recent HIV-1 infections (EIA-RI) developed by Barin *et al*. [6]. This assay uses a logistic regression classification algorithm in which the cut-off was chosen to detect individuals infected for less than 180 days with a enhanced focus on the level of specificity of detection It is to be noted that a lack of specificity, because it affects the population of established infections that is generally larger, should have a wider impact on misclassification than a lack of sensitivity, considering the low prevalence of recent infection status [30]. On-going development of the EIA-RI test aims to re-calibrate it for the purpose of incidence derivation.

Expressing the proportion of recent infection

Some applications define the proportion of recent infection in a population of positive individuals as an outcome. This is the way Puchhammer *et al*. analysed the results of the avidity assay among new diagnoses from case-reporting in Austria [32]. This is also the way that correlates of recent infection among new diagnoses are interpreted in France [33] (see also the article by Semaille *et al*. in this issue). However, this quantity that is somehow related to incidence depends also on the prevalence of non-recent infection and thus can not be considered as a good proxy for incidence. In fact, in the context of diagnostic testing, the proportion of recent infection has a lot to do with the testing framework capacity as well as the incidence rate in the population. Since the prevalence of undiagnosed infection affects the proportion of recent infection independently of any change in incidence (Figure 2), such results are difficult to interpret.

Incidence estimation from HIV case-reporting data

While it seems especially promising to take advantage of recent infection testing among reported HIV diagnoses at province or country level, there are several specific difficulties with regards to deriving a valid incidence measurement. Unlike cross-sectional surveys, a case-reporting system collects information only for individuals with positive test results and generally can not provide information on those who were negative. Therefore, the denominator of the formula, i. e. the number of people at risk, is not available. Another approach is needed to derive an incidence that can be generalised for the population targeted by the surveillance, and to take account of the fact that negative test results are not reported.

Such an approach has been described by Lee *et al*. for the estimation of the national HIV incidence in the US [34]. The statistical framework considers the reported cases identified as recently infected as a sample selected from all annual new cases, with a probability of inclusion related to their testing pattern. According to this probability, each case identified as recently infected is assigned a weight, and the sum of weights provides the incidence count. This approach represents a good opportunity to improve large scale surveillance of HIV dynamics, especially where a framework of HIV case reporting already exists and can provide data on testing patterns.

Finally, another approach has been described to bypass the issue that only positive individuals are reported to the surveillance system. In Ontario, Canada, an enhanced surveillance system has been established that requires diagnostic laboratories to collect information (number and risk factor) on a random subset of individuals with a negative test result in parallel to the information on those that were positive [35]. This system then allows the use of the Janssen's formula to derive the incidence in different risk groups.

Issues

There are issues that pertain to the estimation HIV incidence by characterising recent infections. We can distinguish issues that are related to the determination of recent HIV infection from those that affect the validity of incidence estimation.

Limitations in determining recent infection

The first issues are due to the limitations of the assays in detecting recent HIV infection. As the majority of assays are based on quantitative measurement of the antibody response, factors that affect the patient's immune response lead to some misclassification. Qualitative assays such as the avidity assay may be affected to a lesser extent [36].

Firstly, people with acquired immunodeficiency syndrome (AIDS) may falsely be identified as recently infected due to declining antibody levels. The same appears to be true in some individuals in the late stage of non-AIDS HIV infection. As for the AIDS stage, clinical data or CD4+ T-cell counts would need to be collected in order to exclude these patients from the calculation and avoid overestimation. A correction for misclassification due to late-stage non-responsive patients, has been proposed by Mc Dougal *et al*. and Hargrove *et al*. [15].

Secondly, antiretroviral drugs affect the antibody level by decreasing the viral load [37]. Again, to correctly assess recent infection, patients with ongoing treatment need to be identified and excluded by gathering declarative information (from clinician or patient) or alternatively by detecting drugs in serum specimens by, for example, mass-spectrometry.

Thirdly, test results are affected by the virus subtype and/or the patient's genetic background. It has been shown that all tests that have been developed mainly on specimens from patients infected with subtype B viruses give inconsistent results when used for infections with non-B subtypes. Therefore, an assessment of the test properties (cut-off and window period) in different population settings is needed before applying any method [30].

We have seen how the correct interpretation of test results relies on the availability of clinical data that characterise the population [38]. In order to further interpret incidence estimates, data on sex, mode of contamination, testing patterns, and possibly virus subtypes must be gathered along with tests results.

Representativeness and selection bias

A general issue of incidence estimation arises from the fact that the populations tested are not randomly selected and may not be representative of the populations at risk of infection. This is particularly the case in the context of HIV testing or sexually transmitted diseases clinics. The bias may go in either direction. People at high risk may seek testing more frequently with the consequence of raising the incidence estimation. On the other hand, people attending HIV testing settings as part of a prevention strategy might be at lower risk than people who do not do a test because they do not recognise the risk or are afraid of a positive result.

Schoenbach *et al*. raised this issue in 2001 and questioned the rationale of inferring HIV incidence in testing settings and in particular, whether it is possible to extrapolate these incidence estimates to a larger population [39]. With regard to generalising incidence, it may be preferable to collect specimens from surveillance settings such as blood donation facilities or antenatal clinics where people are not self-selected but tested in a systematic manner, and where large sample size can be obtained.

Nevertheless, it can be argued that every design of an incidence study suffers from some kind of selection bias, even longitudinal studies [11]. Moreover, studying the level of the infection among the attendees of testing sites can still provide insights over time, especially in conjunction with behavioural data.

Even more problematic seems to be the issue of a selection bias occurring if recently infected people tended to seek testing sooner than expected because of seroconversion illness or identified recent exposure. This leads to an increase in the number of detected recent infections and an overestimation of the incidence. Remis *et al*. refer to this bias as the "seroconversion effect" and proposed a way to measuring it by making different incidence estimates based on varying window periods [40]. Song *et al*. formulated the hypothesis of independence between testing and the occurrence of infection and proposed a procedure to test this hypothesis [41]. All these biases can be found when inferring HIV incidence from case-reporting of new diagnoses which also include individuals seeking testing or health care.

Finally, as it is not always possible to test the whole positive study population for recent infection, the proportion of recent infection obtained among those tested is classically assigned to those for whom a test result is not available. This extrapolation assumes that the availability of specimens for recent infection testing is randomly determined in the population.

Conclusion

Overall, the use of laboratory-based methods to estimate HIV incidence can add remarkable value to surveillance systems based on prevalence surveys or on HIV case reporting. The estimation of HIV incidence provides a clear public health benefit in that it allows better monitoring of HIV transmission and targeting of preventive initiatives. We have seen that the application of those methods in cross-sectional settings have been well described in terms of incidence estimation and limitations, one of the most important limitations being the lack of representativeness. The assumptions that must be fulfilled to correctly interpret the estimates are to a

large extent similar to those required in prevalence measurement. However, further research on the more specific aspect of window period estimation may be needed in order to generalise these methods. In particular, efforts are needed to correctly define the mean window periods for different virus subtypes and stages of infection so that the essential relation between prevalence and incidence holds true in various population settings.

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Surveillance and outbreak reports

Four years of surveillance of recent HIV infections at country level, France, mid 2003 – 2006: Experience and perspectives

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New systems of surveillance to better monitor the dynamics of HIV are needed. A national surveillance of new HIV diagnoses which included the collection of dried serum spots (DSS) to identify recent infections (<6 months) using an EIA-RI assay was implemented in 2003 in France. The collection of DSS is based on the voluntary participation by both patients and microbiologists. Multivariate analysis was used to identify factors associated with recent infection (RI). Between July 2003 and December 2006, 14,155 cases newly diagnosed for HIV were reported. A minority of patients refused the collection of DSS (3.3%) and the rate of participation of laboratories was 80%. The test was performed for 10,855 newly diagnosed HIV cases, the overall proportion of RI was 23.1% (95% CI, 22.3%-23.9%). The proportion of RI was higher among men who have sex with men (MSM) (42.8%) than among heterosexuals (16.3%). Among heterosexuals, it varied by current nationality: 27% among French versus 8.4% among Africans. The risk of RI was greater for MSM (aOR=1.8), those of French nationality (aOR=3.9), those with high-economic status (aOR=1.2), those tested after a risk exposure (aOR=1.4), those tested for HIV three or more times during their lifetime (aOR=2.5). The risk of RI decreased with age. A nation-wide implementation of RI monitoring is feasible. The information on RI is very useful for renewing prevention messages, particularly among population in which HIV transmission is on going, such as MSM.

Background

In most industrialized countries, HIV/AIDS routine surveillance is based on case reporting to monitor new diagnoses. Having the characteristics and trends of newly diagnosed HIV or AIDS cases is essential but not sufficient to monitor the dynamic of HIV transmission. Therefore, several countries implemented the surveillance of recent HIV infections at either regional or national level for a given period (e.g. Australia, Austria) or have started this monitoring recently (USA, Germany) [1,2]. To our knowledge, France is the only country where recent infection (i.e. infection acquired in the last six months) has been routinely monitored at the national level since 2003 among patients newly diagnosed with HIV [3].

This monitoring, defined as "virological surveillance" (VS), uses dried serum spots (DSS) taken at the same time as HIV diagnosis and notification. Here, we report the results of the first four years of this virological surveillance from July 2003 to December 2006. We also discuss the challenges in implementing such surveillance, the possibilities to introduce it in other countries, and the ways to use its results for public health action.

Methods

Case reporting of HIV

Mandatory anonymous HIV case reporting was implemented in France in 2003, and the procedures have been described previously [4]. The following patient characteristics are collected and entered into the national database: sex, age, country of birth, current nationality, region of residency, mode of transmission, socio-professional category, clinical stage at the time of HIV diagnosis (primary infection, asymptomatic stage, symptomatic not AIDS stage, AIDS stage), number of previous HIV tests and reasons for HIV screening. In this article, we analyze new HIV diagnoses dated from 1 July 2003 to 31 December 2006 which were reported to the Institut de Veille Sanitaire (InVS), the French Institute for Public Health Surveillance, up to March 2007.

The estimated proportion of under-reporting of new HIV diagnoses in France varies from 34 to 40%, depending on the year of diagnosis (40%, 37%, 34%, 36% in 2003, 2004, 2005, 2006 respectively). Every year, the estimated proportion of under-reporting and the reporting delay are based on the comparison between the number of HIV notifications and the number of positive serology results reported by all laboratories in France [5]. The case definition of new HIV diagnosis used in both systems is similar.

In this article the proportion of under-reporting and the reporting delay are taken into account when presenting the absolute numbers of recent infections in the results part. These absolute numbers were calculated separately for each year in order to take into account the different proportions of under-reporting which varied each year.

Virological surveillance

DSS was used to determine for each new HIV diagnosis whether or not the HIV infection was recent, i.e. occurred less than six months before diagnosis. For each case, the laboratory that made the original diagnosis was asked to take DSS from the stored serum sample and send it under the patient's anonymous code to the National Reference Center (NRC) by postal mail. Results from the NRC were then sent to InVS and linked to the epidemiological data

in the HIV national database using the patient's anonymous code. Although HIV notification is mandatory, VS is based on the voluntary participation by both microbiologists and patients. The patient's consent for VS is obtained by the reporting clinician through the HIV notification form.

Immunoassay to identify recent infections (EIA-RI)

The characteristics and properties of this assay have been described previously, and are also discussed in the article from J Parry *et al*. in this special issue of Eurosurveilllance [6]. Based on early evaluation of EIA-RI we estimated that this assay using DSS would be able to identify recent infections (RI) among all infected patients with HIV-1 (without AIDS) with a sensitivity of 87% and a specificity of 98%. The EIA-RI may misclassify patients at the AIDS stage as recently infected, and therefore patients known to have AIDS (information collected from the HIV reporting form) were classified as established infection whatever the result of the EIA-RI.

Statistical analysis

The chi test for trend was used to analyze the trend overtime of the proportion of recent infections among newly diagnosed HIV cases. The proportions were compared using standard chi-square tests. Variables that were significantly associated with recent infection status in the univariate analysis were entered in a multiple logistic regression model to identify factors independently associated to recent infections (using a global test). The goodness of fit was assessed by the Hosmer-Lemeshow test. All analyses were conducted with SAS® software version 08, and statistical significance was considered for p values < 0.05.

Results

Description of new HIV-1 diagnoses

Between July 2003 and December 2006, 14,155 newly diagnosed HIV cases were reported to the InVS. Males accounted for 61% of cases. More than half (53%) of the newly diagnosed HIV infections were attributed to heterosexual contact, whereas men who have sex with men (MSM) accounted for 25% of the cases (Table 1). Those infected through drug use constituted a low percentage of all cases (2%, n=306). Concerning the nationality of cases, one third (31%, n=4,383) came from sub-Saharan countries, and were mainly infected by heterosexual contact. The reasons for screening and the clinical stage at the time of HIV diagnoses varied by transmission categories. The proportion of cases that have undergone voluntary screening after an exposure is greater among MSM than among heterosexuals (33% vs 19%), and it is higher among French heterosexuals than African heterosexuals (22% vs 18%). The proportion of cases newly diagnosed at the time of primary infection ('primary infection' as filled in by clinicians whatever the results of the test of recent infection) was greater among MSM than among heterosexuals (19 vs 5%).

Recent infections among new HIV-1 diagnoses

From July 2003 to December 2006, the test for RI was performed for 10,855 new HIV diagnoses. Results were not obtained for 3,300 patients either because the laboratory did not submit DSS to the NRC (2,834 cases representing 20% of all new HIV diagnoses) or the patient did not consent to participation (466 cases, 3.3%). These cases were excluded from further analysis. Among the excluded cases, the proportion of MSM and of French nationals was lower than among the cases included in the investigation (19% vs 27% and 39% vs 46%, respectively), whereas the proportion of cases with unknown mode of transmission and unknown nationality was higher than among the included cases (24% vs 18% and 16% vs 13%, respectively).

The proportions of patients who refused to participate and of laboratories that did not send DSS for analysis were stable over time.

Among the newly diagnosed HIV-1 cases that were included in the analysis, 2,511 were identified as recent with the EIA-RI test (23.1%, 95% CI= 22.3 – 23.9). After adjustment for under-

Table 1

Newly diagnosed HIV cases by sex and transmission category, France, July 2003 – December 2006 (n=14,155)

Transmission category	Women N (%)	Men N (%)	Total N (%)
MSM		3,579 (41.6)	3,579 (25.3)
Heterosexuals Sub-Saharian Africa France Other/unknow	4,384 (79.1) - 2,359 - 1,150 - 875	3,168 (36.8) - 1,281 - 1,234 - 653	7,552 (53.4) - 3,640 - 2,384 - 1,528
Drug users	64 (1.2)	242 (2.8)	306 (2.2)
Other *	9 (0.1)	9 (0.1)	18 (0.1)
Unknown	1,084 (19.6)	1,616 (18.7)	2,700 (19.0)

MSM = men who have sex with men
*Hemophilia or transfusion recipient

Table 2

Proportion of recent infections among new HIV-1 diagnoses, France, July 2003 - December 2006 (n=10,855 newly diagnosed HIV-1 cases, of whom 2,511 were identified as recent)

	Number of recent infections	Proportion of recent infections	[IC 95 %]	p*
Sex				$p<10^{-4}$
Male	1863	27.8	[26,8 - 28,9]	
Female	648	15.6	[14,5 - 16,7]	
Age group (years)				$p<10^{-4}$
15 - 29	756	26.0	[24,4 - 27,6]	
30-39	969	24.3	[22,9 - 25,6]	
40-49	499	21.0	[19,4 - 22,7]	
> = 50	287	18.1	[16,2 - 20,0]	
Transmission category*				$p<10^{-4}$
Homosexual	1263	42.8	[41,0 - 44,6]	
Heterosexual	939	16.3	[15,4 - 17,3]	
Drug users	33	14.6	[10,0 - 19,2]	
Other/Unknown	276	14.3	[12,8 - 15,9]	
Current Nationality				$p<10^{-4}$
France	1707	34.4	[33,1 - 35,7]	
Europe (outsideFrance)	59	24.1	[18,7 - 29,4]	
Sub-Saharian Africa	285	8.4	[7,4 - 9,3]	
North Africa	40	18.6	[13,4 - 23,8]	
Other/Unknown	420	20.7	[18,9 - 22,5]	

* chi^2 test

Table 3

Factors independently associated with recent infections among new HIV-1 diagnoses. Results from the multivariate analysis France, July 2003 - December 2006 (n=10,855 newly diagnosed HIV-1 cases, of whom 2,511 were identified as recent)

		Univariate analysis				Multivariate analysis			
	Number of subjects	OR	95%	CI	p value[a]	aOR	95%	CI	p value[a]
Sex and transmission category									
Male heterosexual	2,414	1			<0.0001	1			<0.001
Male homosexual	2,949	4.07	3.57	4.65		1.85	1.59	2.15	
Other/unknown male	1,332	1.10	0.92	1.32		0.85	0.71	1.04	
Female heterosexual	3,340	1.10	0.96	1.27		1.12	0.96	1.32	
Other/unknown female	820	0.62	0.48	0.80		0.50	0.38	0.66	
Age group (years)									
≥ 50	1,587	1			<0.001	1			<0.0001
15 - 29	2,905	1.59	1.37	1.85		1.92	1.62	2.28	
30 - 39	3,991	1.45	1.25	1.68		1.43	1.22	1.67	
40 - 49	2,372	1.21	1.03	1.42		1.12	0.94	1.33	
Current nationality									
Sub-Saharan Africa	3,405				<0.0001	1			<0.001
France	4,962	5.74	5.02	6.57		3.95	3.36	4.64	
Other/unknown foreign country	2,488	2.89	2.47	3.37		2.59	2.18	3.08	
Reasons for HIV testing									
Pregnancy & systematic screening	1,934	1			<0.001	1			<0.001
Clinical symptoms or biological data	3,677	1.51	1.31	1.74		1.20	1.02	1.40	
Exposure	2,382	2.39	2.06	2.78		1.38	1.17	1.63	
Others	1,768	1.37	1.16	1.62		0.86	0.72	1.04	
Unknown	1,094	1.49	1.24	1.80		1.16	0.93	1.43	
Professional category									
Unknown and non-professional activity	4,816	1			<0.001	1			0.014
Employee	2,079	1.70	1.51	1.92		1.10	0.95	1.26	
Blue collar	1,454	1.03	0.89	1.20		0.91	0.77	1.09	
High level staff	2,506	2.16	1.94	2.42		1.17	1.02	1.35	
Testing frequency (during the whole life)									
One HIV test	3,804	1			<0.001	1			<0.001
Two HIV tests	2,731	1.65	1.45	1.87		1.47	1.28	1.68	
Three or more HIV tests	1,474	4.42	3.85	5.08		2.51	2.16	2.93	
Unknown	2,846	2.15	1.90	2.43		1.91	1.66	2.20	
Year of diagnosis									
Second semester 2003	1,628	1			0.65				
2004	3,160	0.97	0.84	1.11					
2005	3,397	1.04	0.90	1.19					
2006	2,670	0.98	0.85	1.14					
Region of residency									
Outside Paris area	5,661	1			0.0007	1			0.14
Paris area	5,194	1.17	1.07	1.28		0.93	0.84	1.03	

[a] global test, CI confidence interval
Note : Hosmer- Lemeshow statistic: chi^2 = 10,53; d.f. = 8; p = 0.23

reporting and reporting delays, the number of recent infections that occurred from mid 2003 to 2006 was estimated at around 4,000. Half of these cases (estimated at 2,010) were among men who have sex with men (MSM): representing 550 to 600 MSM per year. The number of drug users recently infected was very low (52 cases over the whole period). From 2003 to 2006, the adjusted number of cases newly diagnosed and identified as recent was greater among French heterosexually infected persons (805) than among sub-Saharan Africans living in France (454 cases).

The proportion of RI was higher in MSM (42.8%) than in heterosexuals (16.3%) (Table 2). Among heterosexuals, it varied by current nationality: 27.0% among French versus 8.4% among Africans ($p<0.001$). The year of diagnosis was not associated with recent infection in the univariate analysis. In the multivariate analysis the risk of recent infection was greater for MSM (aOR=1.8), those of French nationality (aOR=3.9), those of a high socio-economic status (aOR=1.2), those tested for HIV after a risk exposure (aOR=1.4) and those who had undergone three or more tests during their lifetime (aOR=2.5) (Table 3). However, the risk of RI decreased with age. Although the region of residency was not independently associated with recent infection ($p=0.14$), this variable was maintained in the model because it improved the goodness of fit ($p=0.23$).

Discussion

We found that a little less than one quarter of the newly diagnosed patients included in the study had been infected with HIV within the last six months. Among the newly diagnosed MSM, half had been infected recently This is consistent with results reported in several more restricted studies: the proportion of recent infections among new HIV diagnoses was 27% in Austria in 2002-2003, 26% in Switzerland in 2005-2006, 20% in ten cities in the United States in 1997-2001, and 45% among MSM in United Kingdom in 2005, and 36% in a study which mainly involved MSM in the Victoria region of Australia in 1999-2000 [1,2,7-9].

The proportion of recent infections should be interpreted with some caution because it depends on both testing patterns and HIV incidence. This is consistent with our analysis which found that the number of lifetime HIV tests performed is strongly associated with RI, and that the chance of detecting recent infections increased with the number of tests. Similarly, people screened for HIV after a risk-exposure are more likely to be diagnosed as a recent infection (aOR=1.4) than those screened for pregnancy.

Our results indicate that the largest population diagnosed as recently infected in France is the MSM population. This may result from both a relatively high HIV incidence and a more frequent testing among MSM. These findings are supported by other sources of epidemiological data which indicate that MSM have been engaging in high-risk sexual behaviors in recent years in France: (i) increase in the proportion of unprotected anal intercourse from 19% in 1997 to 33% in 2004 (Enquête Presse Gay 1997 and 2004) (ii) outbreak of syphilis ongoing since 2000, and (iii) emergence of rectal lymphogranuloma venereum in 2004 [10-12]. Behavioral surveys have also shown that MSM are more frequently tested for HIV: half of MSM were tested during the last 12 months before the study, whereas in the general population only 11% underwent testing during the last year [10,13,14]. However, the multivariate analysis, taking into account the variable "testing frequency" has identified MSMs as the subgroup with the highest risk of being recently infected.

Current nationality was also found to be strongly associated with RI. Persons of African origin were less likely to be diagnosed as a recent infection than French and other foreign nationalities. This may reflect the fact that HIV-positive Africans living in France are mostly immigrants who could have been infected with HIV many years before in their country of origin where HIV prevalence is high, and diagnosed only recently in France. A survey conducted in 2005 among the African community living in the Paris area showed that the testing frequency in this group was higher than expected: 65% of African respondents had been screened for HIV at least once in their life, compared to 51% in the general population (in 2004) [14,15]. However, the proportion of recent infections among newly diagnosed Africans living in France which we estimated to be 8% indicates that HIV transmission also occurred in this community while living in France.

The proportion of recent infections among drug users was found to be very low, and while surveys have shown that most drug users are aware of their HIV serostatus, these results reflect the positive impact of the harm-reduction strategy implemented in France since the beginning of the 1990s [16].

Socio-professional categories associated with high economic status were also independently related to the fact of being diagnosed as recent. This may reflect both a better access to HIV screening and a better assessment of the risk of HIV infection in this well-educated population with ongoing risk behaviors.

The proportion of cases with recent infection at the time of HIV diagnosis was also found to be higher among younger age groups, which can be explained by the fact that the probability of having a recent infection at the time of diagnosis increases with shorter exposure to the risk. Also, we need to take into account that in France young people are more frequently tested for HIV than older people (17% among 18-24 years old vs 4% among 45-54 years old) [14].

Our results have shown that the proportion of RI was stable between 2003 and 2006, and in the univariate analysis the year of diagnosis was not associated with the recent infection diagnosis. In parallel, HIV screening policies did not change during this period in France, and the rate of HIV screening per 100,000 population did not vary considerably (range from 79 to 81 per 100,000 depending on years) [17].

How feasible is the implementation of monitoring of recent infections among new diagnoses in other developed countries?

Our report summarizes the results of four years of long-term national monitoring of HIV infection by combining the surveillance of recent HIV infection with HIV case reporting. To our knowledge, France is the first country to have implemented such an integrated system at a national level. This was made feasible by using an assay which could be performed on samples collected on filter paper thus making the management and the cost of recent infections monitoring reasonable. The costs were estimated at around three euros per case (including filter paper, a hermetically sealable plastic bag for transportation, reagents, and the time spent by a technician to perform the test). This amount did not include the cost of validation and data entry performed at InVS. Furthermore, a DSS can also be used to determine the group, type and subtype of the virus by a serotyping method, and to genotype the virus in order to monitor the diversity of circulating viruses more closely

[3,18,19]. The EIA-RI assay used in France can also be used in other countries. Although the technique is transferable, other assays could also be chosen (see the article of J Parry *et al.*).

The nation-wide implementation of recent infections monitoring seems feasible in other countries. It has been possible in France with the collaboration of a very high number of laboratories (around 4,300) that perform HIV diagnosis and send the DSS to the NRC. However, the project requires a lot of continuous effort to inform and encourage thousands of laboratories to participate and sustain their collaboration overtime. The participation of laboratories in the voluntary virological surveillance is good (around 80%) and it is also well accepted by the patients (only 3% do not consent to participate). Knowing that other European countries do not have so many laboratories that perform HIV testing (their numbers range from a few dozens to a few hundreds), the implementation of a similar surveillance should, therefore, be more feasible than in France.

When starting the project we also had to resolve the ethical issue of informing or not the patients and their physicians about the patient's RI status. Considering that: a) the test for RI was designed for public health purposes and not for establishing an individual diagnosis as the positive predictive values are not high enough for diagnostic purposes; b) the information flow within the HIV notification channel is anonymous by law whereas giving back the results would mean maintaining correspondence between the anonymous code and the name of the patient; c) it is not clear whether the result (recent infection or not) would have an impact on the individual health since there is non consensus yet on the long term benefit of HAART during the early months of infection and contact tracing is not done in France; it was decided, after a collective discussion with patient associations and clinicians, not to inform either patients or physicians. Although this question was still being discussed at the time of implementation of the system, it ceased to be controversial when the first results of virological surveillance were reviewed with clinicians and patient associations and published in December 2003.

What is the impact of these results in terms of public health?

Nearly half of MSM newly diagnosed with HIV (43%) were shown to have been recently infected. Subsequently, these findings were actively communicated to the gay communities in France and had a major impact. The feedback of this group to associations for the fight against AIDS is critical. Moreover, the findings were used in several prevention campaigns and prompted the Ministry of Health to renew the prevention messages. Nevertheless, the extent of HIV transmission in MSM remains alarming, illustrating the difficulty to target and sustain prevention in this usually well-educated population. However, the high proportion of RI also indicates that screening strategies have been effectively adopted by the gay community: MSM more often than other groups undergo testing for HIV soon after a risk exposure. The data on reasons for screening show that the proportion of those who undergo voluntary testing after an exposure is higher among MSM than among heterosexual individuals (33% vs 19%). Therefore, the proportion of RI among MSM could also be an indicator for screening patterns among this population that would be interesting to monitor.

Although we showed that the proportion of recent infections among the newly diagnosed HIV cases of African origin is much lower than among the general population, our results indicate that HIV transmission in this group also occurs after arrival in France. In addition, we found out that one out of five newly diagnosed HIV cases of African origin were infected by subtype B, although this subtype is not common in Africa (data not shown in this article) [3,17]. The combination of these two results (proportions of RI and subtype B) has induced the Ministry of Health to adapt its policy regarding the African community living in France and encourage HIV screening and prevention within this population.

While the incidence of HIV infection has been previously estimated in defined risk groups such as prostitutes, IDUs, MSM attending STI clinics, this has not been done at the country level [9,20,21]. We are currently working on assessing the HIV incidence at the country level by combining, through mathematical modeling, the results of the test for recent infection with other factors such as screening patterns (see the article by S Le Vu *et al.* in this special issue of Eurosurveillance).

Conclusion

The information on recent infections is very useful for renewing prevention messages, particularly among populations in which HIV transmission is still ongoing, such as the gay community in France, and for promoting HIV testing among populations in which few recent infections have been identified. An overview of new testing strategies is ongoing in France in order to better define the use of the rapid HIV test on whole blood or serum samples, notably in a community context.

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Research articles

Settings for identifying recent HIV infections: the Portuguese experience

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Portugal has been the western European country with the highest rate of notified acquired* immunodeficiency syndrome (AIDS) cases since 1999 and human immunodeficiency virus (HIV) infection cases since 2000. Nonetheless, exact information on the magnitude and trends of recently acquired infections is missing. In a cross-sectional study we aimed to determine HIV prevalence, the proportion of recently acquired infections and the incidence among patients attending a Sexually Transmitted Infections (STI) clinic and among HIV positive cases tested at the AIDS Reference Laboratory (ARL), by using the Avidity Index (AI) of antibodies to identify recent HIV-1 seroconversions. Demographic and behavioural data were collected. At the STI clinic 253 patients were enrolled, 16 were found to be HIV infected (14 HIV-1, 2 HIV-2) and a prevalence of 6.3% was obtained. Four recent HIV-1 infections were identified and the HIV-1 incidence was 3.3% per year. At the ARL, 332 newly diagnosed cases of HIV-1 infection were studied, 59 (17.8%) were recent infections and an annual incidence of 4.1% was estimated. These findings support STI clinics as key sentinel sites for recently acquired HIV infections and illustrate the viability of testing for recent HIV infections in these settings and reinforce the value of this method in the surveillance for better monitoring current trends of the HIV/AIDS epidemic in Portugal.

Introduction

Portugal is the western European country with the highest rate of reported acquired immunodeficiency syndrome (AIDS) and newly diagnosed cases of human immunodeficiency virus (HIV) infection according to the EuroHIV end-year report for 2006 [1]. However, the proportion of recently acquired infections is unknown and information on trends is missing. After a decade (1990 to 2003) during which the epidemic was led by cases of HIV infections linked to intravenous drug use (IDU), the proportion of reported cases associated with sexual transmission (homosexual, bisexual and heterosexual) has progressively grown, accounting for over 60% since 2003, with heterosexual transmission currently being the driving force of the epidemic [2].

Similar to other countries, prevalence studies are used in Portugal for the epidemiological assessment of HIV infection as they provide relevant information necessary for the planning of health and social support services. Nevertheless, prevalence does not distinguish between infections acquired recently, i.e. in the past six months and established infections acquired more than six months ago, and the picture of recent trends of the epidemic is less clear compared to that obtained by incidence studies. The fact that longitudinal studies to calculate incidence are expensive, time-consuming and difficult to perform is well known [3-5]. Alternative approaches to estimate the incidence of HIV infections have been used worldwide [4, 5] and the interest in cross-sectional laboratory based studies has grown in the past decade. Different methods, based on various properties of maturing antibodies for HIV-1, that allow the identification of recent seroconversion have been described and were used for this purpose [5-10]. These laboratory methods, applied to a single serum sample for each HIV-1 infected case, allow the distinction between recently acquired and established HIV-1 infections. The avidity index (AI) of HIV-1 antibodies has been used for this purpose based on the principle that antibodies produced in the early phase of infection show a low avidity for the antigen [7]. Therefore, a low avidity is likely to indicate a recent infection.

In Portugal, the first cross-sectional study in which recent HIV-1 seroconversions were identified was performed in a group of IDUs entering a low threshold methadone programme for the period of one year [11]. Of those 24.5% were found to be HIV-1 infected and among them 18.4% were infected recently. HIV-1 incidence was estimated 7.2 % per year. These results encouraged us to explore other settings where we expected to identify recent HIV-1 infections and to collect useful epidemiological information.

Sexually transmitted infections (STI) are known to be associated with higher transmission or acquisition rates of HIV and patients referred to STI clinics are usually at increased risk for HIV infection [3]. STI clinics were identified as important settings for measuring the prevalence of HIV infection [12] and can also be important sites for estimating incidence. In Portugal STI clinics are rare. However, in Lisbon, a unique drop-in STI clinic exists at a primary healthcare facility, that provides free of charge same day appointments with specialists, laboratory diagnosis of STI (including HIV), treatment for diagnosed STI and counselling for risk reduction. Attending patients are mainly from the Lisbon area, self-presenting or referred by other primary healthcare services.

Laboratory based surveillance data on the number of new HIV diagnoses per year are available in some European countries such as France, allowing for studies to identify recent infections to be conducted [12,13]. However, such a system does not exist in Portugal where notification of HIV/AIDS cases has always been performed by clinicians, with limited laboratory information. In Lisbon, the AIDS Reference Laboratory (ARL) confirms over 300 new cases of HIV infection per year from various population groups, including IDUs, prison inmates and pregnant women.

The aim of our study was to identify population groups from specific settings where recent HIV infections are likely to be detected, to generate data on HIV prevalence and on the proportion of recent HIV-1 infections and to estimate the incidence of HIV-1 infection.

Methods

Study design and population

To achieve the aim of our study two settings, an STI clinic and the ARL, were selected as settings where recent HIV infections are likely to be detected.

STI clinic

A cross-sectional study was performed in a group of attendees at the Centro de Saúde da Lapa STI clinic, in Lisbon. Between February and August 2004, enrolment in the study was proposed by clinicians to all first time attendees and other patients eligible for HIV testing (i.e. patients reporting risk behaviour). Participation was voluntary and anonymous. Written informed consent was obtained from all participants and archived in the patient's clinical file. One blood sample was collected from each participant at the time of enrolment and data on demographics, behaviour and clinical condition were anonymously recorded by clinicians for each case.

AIDS Reference Laboratory

A cross-sectional study was also performed using anonymised serum samples from cases newly diagnosed with HIV-1 infection at the ARL during the year 2005. Demographical and behavioural data were collected of all cases selected for the study, Second time testers were excluded.

Laboratory methods

Prior testing for identification of HIV infection was necessary for samples from the STI clinic. Pools of five sera were prepared with an input of 100 µL for each sample and each pool was screened simultaneously for HIV-1 and HIV-2 antibodies using the third generation enzyme immunoassay AxSYM® HIV 1/2 gO (Abbott Diagnostics Division, Germany). Sera from pools with a positive result were individually tested using the same kit. Samples identified as reactive were further tested for confirmation using the HIV Blot 2.2 (Genelabs Diagnostics, Singapore), a Western blot assay for HIV-1 antibodies also harbouring a peptide for identifying HIV-2 antibodies. Samples reacting with this peptide were additionally tested with New LAV Blot II (Bio-Rad, France), a Western blot assay to confirm the presence of HIV-2 specific antibodies. All tests were performed according to the manufacturer's instructions.

Identification of recently infected HIV-1 cases

In HIV-1 positive sera the AI of the antibodies was determined following the method described by Suligoi et al [7] in order to identify recently acquired HIV-1 infections, i.e. seroconversions occurred six months or less prior to blood collection. For each sample a pre-test dilution of 1:10 was prepared on two aliquots of 50 µL: one with phosphate-buffer saline (PBS - PBS aliquot) and a second with 1M guanidine solution (G aliquot). After incubation at room temperature, both aliquots were tested with the AxSYM® HIV 1/2 gO (Abbott Diagnostics Division, Germany) assay following the manufacturer's instructions. The sample's AI was determined by the ratio between the results obtained for guanidine aliquot and buffer aliquot using the formula: AI = (S/CO of the G aliquot)/(S/CO of the PBS aliquot). S/CO standing for sample/cut-off value obtained in the test. The 0.8 cut-off for the AI was used to differentiate between recent (AI $<$ 0.8) and established (AI $\geq$ 0.8) cases of infection [14].

Epidemiological methods and concepts

Prevalence

For both settings, the STI clinic and the ARL, the proportion of HIV infections overall and by type of virus, as well as the proportion of recently acquired HIV-1 infections was determined.

Incidence

A cross-sectional approach was applied and six months was the window period assumed for the AI test used to identify recently acquired HIV-1 infections. The concept for the estimation of incidence [5, 6, 10] using data obtained from cross-sectional study at both sites is based on the following assumptions:

- cases identified as negative were also negative six months before blood sampling;
- cases identified as recently infected were negative six months before blood sampling;
- cases identified as established infection were positive six months before blood sampling;

The rate of seroconversion in the six months before blood sampling is obtained by the ratio between the number of recent infections and the number of susceptibles multiplied by two to obtain the annual incidence.

The following formula was used to estimate incidence in both groups:

$$\text{HIV 1 incidence (\%)} = \frac{N_R}{N_{neg} + N_R} \text{ x2 x100}$$

N_R Number of recently infected HIV cases

N_{neg} Number of cases with a negative HIV test result

Statistical methods and data analysis

Data were analysed using the Statistical Package for Social Sciences (SPSS) program for Windows, version 12.0. Descriptive analysis was performed for each variable, namely frequencies and proportions. Mean age values were compared using the t test for independent samples. Data from different subgroups were compared using two tailed Fisher's exact test or chi-square independence test. Results with a p value $<$ 0.05 were considered as being statistically significant. Odds Ratios (OR) and their 95% confidence interval (CI) were calculated in order to measure the strength of the associations found.

Results

STI clinic group

A total of 253 participants, 143 men (56.5%) and 110 women (43.5%), were enrolled in the study. The age ranged from 16 to

70 years, with a mean of 31.5 years (95% CI: 30.3-32.8) and a median of 28 years.

The majority of participants (90.9%) stated having only heterosexual contacts, 35.5% had more than one sex partner in the previous six months and only 15.9% declared to always use condoms in sexual contacts with unknown partners. Additional risk behaviours for HIV infection such as illicit drug use (not specified) and prostitution were acknowledged by 13 participants. Of all participants 45.1% had never been tested for HIV. Clinical data showed that, at time of enrolment, an STI other than HIV was diagnosed in 87 cases, with a high proportion of viral infections (43.5%). The majority of cases (81.3%) had no previous STI history.

Sixteen cases were found to be HIV infected 14 with HIV-1 and two with HIV-2. An overall HIV prevalence of 6.3% (95%CI: 3.3-9.3) was obtained. Type-specific prevalence was 5.5% for HIV-1 infection and 0.8% for HIV-2 infection. Prevalence by sex and sexual orientation showed a higher value in men than in women, 7.0% and 5.4% respectively and a high prevalence of 18,2% for homo/bisexual men. The comparative analysis between characteristics of HIV-positive and -negative cases is shown in Table 1.

Increased risk for HIV infection was found for homosexual/bisexual clients (OR = 3.33; 95%CI: 1.17-9.49), for those who had five or more sex partners in the previous six months (OR = 4.69; 95%CI: 1.70-12.82) and for those with an STI history (OR=2.91; 95%CI: 1.09-7.77). Although difference was not statistically significant, mean age in the HIV-positive subgroup (34.8 years) was higher than in the HIV-negative subgroup (31.3 years).

The AI of antibodies determined for the 14 HIV-1 cases ranged between 0.33 and 1.06 with a mean value of 0.85. Using 0.80 as cut-off value we were able to identify four recent HIV-1 infections. HIV-1 incidence in this group, as defined for the purpose of the study, was estimated to be 3.3% per year. No independent statistical associations were found between recent infections and the study variables.

Table 1

Comparative analysis of characteristics of HIV positive and HIV negative cases and characteristics of recent HIV-1 infection cases studied at the sexually transmitted infection (STI) clinic, Portugal, February to August 2004, (n=253)

Characteristics	HIV Antibodies			P[b]		HIV-1 Recent Infection[a]		P[c]
	Tested*	HIV Positive			Odds-Ratio (95%CI)	n'	%	
	N	n	%					
Sex								
Males	143	10	7.0	0.796	1.30 (0.46-3.70)	3	30.0	1.000
Females	110	6	5.4			1	16.7	
Age group								
<30 years old	144	6	4.2	0.122	0.43 (0.15-1.22)	0	--	0.221
>= 30 years old	109	10	9.2			4	40.0	
Sexual orientation								
Homo/bisexual	23	4	17.4	**0.045**	**3.33 (1.17- 9.49)**	2	50.0	0.520
Heterosexual	230	12	5.2			2	16.7	
Number of sexual partners (prior 6 months) †								
>= 5 partners	16	4	25.0	**0.015**	**4.69 (1.70-12.82)**	1	25.0	1.000
< 5 partners	225	12	5.3			3	25.0	
Condom use								
Always	40	5	12.5	0.147	2.61 (0.86-7.97)	2	40.0	0.580
Occasional / Never	212	11	5.2			2	18.2	
Additional risks for HIV infection								
No	239	14	5.9	0.196	0.34 (0.07-1.70)	3	21.4	0.505
Yes	13	2	15.4			1	50.0	
Prior HIV test								
Yes	139	8	5.8	0.797	1.22 (0.47-3.14)	3	37.5	0.559
No	114	8	7.0			1	12.5	
Prior STI history								
Yes	47	6	12.8	**0.040**	**2.91 (1.09-7.77)**	1	16.7	1.000
No	205	9	4.4			2	22.2	
STI other than HIV diagnosed at enrolment								
No	166	12	7.2	0.588	1.57 (0.52-4.74)	3	25.0	1.000
Yes	87	4	4.6			1	25.0	

* Information displayed for those where available
Note: CI - confidence interval
a – Among HIV-1 antibody positive cases
b - Fisher's exact test for associations between characteristics and HIV antibodies status
c - Fisher's exact test for associations between characteristics and HIV-1 Recent Infection status
† Only cases with one or more partners

AIDS Reference Laboratory group

During 2005, 372 (11.8%) of the 3,159 individuals tested for HIV at the ARL had a positive test result. Western blot testing of these positive samples revealed 360 HIV-1 (11.4%) and 12 HIV-2 (0.4%) infections. We studied 332 HIV-1 infections from the 336 cases found to be first time diagnoses. In this group, whose main characteristics are summarised in Table 2, females accounted for 83 (25.0%) cases and males for 245 (73.8%) cases, for four cases information on sex was missing. The age ranged from 17 to 85 years, with a mean of 35.5 years (95%CI: 34.2-36.69) and a median of 33 years. The majority (75.6 %) of cases lived in the Lisbon district.

Data on behavioural risk towards HIV infection were missing in 145 (45.7%) cases. Available information showed that, of 187 cases, sexual risk was present for 29.9% (n=56), in 12 cases associated with homo/bisexual contacts and in 44 (78.6%) with heterosexual contact. Drug use was mentioned in 70.1% (n=131).

After testing 332 samples to determine the AI, values obtained ranged from 0.24 to 1.09, with a mean value of 0.89. Cut-off value of 0.80 was applied and 59 (17.8%) cases were identified as recent HIV-1 seroconversions. No statistical association was found between recent HIV-1 infections and study variables. Even though the difference is not statistically significant, the proportion of recent infections was higher in females than in males (24.1% versus 15.5%). Estimated annualised incidence of HIV-1 infection among cases tested at the ARL in 2005 was 4.1%.

Table 2

Comparative analysis between characteristics of recent HIV (n=59) and established HIV-1 infection cases (n=273) identified at the AIDS Reference Laboratory, Portugal, 2005

Characteristics	HIV-1 Infections				p*
	Recent		Established		
	n	%	n	%	
Sex					
Males	38	15.5	207	84.5	0.195
Females	20	24.1	63	75.9	
unknown	1	25.0	3	75.0	
Age group (years old)					
≤25	7	19.4	29	80.6	0.506
26 - 30	17	27.9	44	72.1	
31 - 35	10	15.4	55	84.6	
36 - 40	7	15.2	39	84.8	
41 – 50	8	16.3	41	83.7	
51 – 60	1	7.7	12	92.3	
≥ 61	1	11.1	8	88.9	
unknown	8	15.1	45	84.9	
Origin of HIV test request					
Anonymous free test site	2	8.3	22	91.7	0.317
Prison clinical services	5	12.8	34	87.2	
External laboratories	7	13.2	46	86.8	
General practitioner	21	19.1	89	80.9	
Methadone programme	24	22.6	82	77.4	
Risk behavioural for HIV infection					
Sexual – Homo/bisexual	1	8.3	11	91.7	0.141
Sexual – Heterosexual	9	20.5	35	79.5	
Drug use	30	22.9	101	77.1	
unknown	19	13.1	126	86.9	

* χ^2 test

Discussion

Our study illustrates the first application of a cross-sectional approach to identify recent HIV-1 infections and estimate HIV-1 seroincidence in a group of attendees at a Portuguese STI clinic and in a group of newly diagnosed HIV infected cases detected at the AIDS Reference Laboratory in 2005. Recent HIV-1 seroconverters (less than six months) were identified based on the AI of antibodies.

The STI clinic group consisted predominantly of young, sexually active, heterosexual individuals. The fact that a very low proportion (15.9%) of participants stated to use condoms consistently in sexual intercourse with unknown partners and 45.1% had never been tested for HIV may derive from a lack of awareness to recognise the risk of contracting an HIV infection through unprotected sex. In the STI group authors did not only identify cases of HIV-1 infections (5.5%) but also HIV-2 (0.8%) cases, which mirrors the pattern of HIV infection in Portugal [2]. The overall prevalence rate of 6.3% and the 18.2% prevalence rate for homo/bisexual men are among the highest figures published [12]. A higher prevalence of HIV infection in homo/bisexual individuals than in heterosexuals without additional risk behaviour has been described in most European countries [12, 15]. The increased risk for HIV infection found in homo/bisexual participants is therefore consistent with the literature. Our data also show that a high number of sex partners increase the risk of HIV infection. The presence of STI indicates a risky sex behaviour that can lead to HIV acquisition or transmission. Accordingly, for the cases included in this study, a history of STI was also found to be an increased risk factor for HIV infection.

The determination of the AI enabled the identification of recent HIV-1 infections among HIV-1 infected participants. The proportion of recent HIV-1 infections (28.6%) identified in this study and the estimated incidence (3.3% per year), are similar to the highest values observed in published studies [3,16,17]. Nevertheless, caution is needed when comparing results obtained with different laboratory methods used to study other population groups as the window period varies from test to test and consequently the proportion of cases classified as recent.

The HIV-1 seropositive group assessed at the ARL consisted mainly of young individuals (median age 33.0 years) who were predominantly male (73.8%) and the majority of those with available information on risks were drug users (70.0%).

The high prevalence of HIV-1 (11.8%) infection in the ARL group may be associated with the fact that reference laboratories are likely to confirm more infected cases than other clinical laboratories. The proportion of drug users in this group and a 12 to 19% prevalence of HIV-1 infection described in Portuguese IDUs [18] may as well influence the result.

The proportion of recent HIV-1 infections (17.8%) found is lower than the one recently described in France [19] for newly diagnosed cases of HIV infection (24.9%). Possible explanations for this are different testing policies or rates, awareness of HIV risk, sample size and the use of different laboratory tests. The assessment of our group focused on cases of HIV-1 infection cases nonetheless incidence could be estimated since denominator was known, the result being a high value (4.1%).

We used the AI described by Suligoi et al. [7, 14] for identification of recent infections taking into account our previous experience, the availability of reagents and equipment, as well as being aware that the results would not be affected by disease, clinical stage or antiretroviral therapy [7]. Even if not adequate for individual and clinical use, this method has been found suitable for epidemiological studies, based on its sensitivity and specificity when the 0.8 cut-off value is used [14]. Also, performance with non-B subtypes of HIV was recently assessed and similar results have been obtained [20].This fact is of the utmost importance for using the method in Portugal were a high proportion of newly diagnosed patients carry non-B subtype viruses [21]. Although other methods for testing for recent HIV infections have been described, most of them are not available on the market and further constraints to their application have been clearly identified [10].

Pooling sera for HIV seroepidemiological surveys has been used before [22-24] and, due to economical reasons, this method was applied for the STI group. The amount of sera per pool is critical when looking for recent infections and our choice of using five was based on published data [22] where six samples per pool was the minimum format assessed. Even though the sensitivity of HIV tests has increased since 1993 we decided to pool five samples because STI patients are generally at higher risk towards contracting HIV infections and HIV-2 is also prevalent in our country.

There are several limitations and biases for this study: the voluntary participation in the STI clinic group and the fact that patients attending STI clinics are at high risk for HIV infection; the fact that reference laboratories are more likely to register a higher proportion of positive cases and detailed behavioural data are rarely collected in the laboratory setting and in our case were frequently missing in the ARL group. All these factors are likely to influence our results and need to be considered in the interpretation. However, it is the higher risk of the STI clinic patients that enables this population to serve as a sentinel for the wider community.

Conclusion

We were able to determine the prevalence of HIV infections and the proportion of recent HIV-1 infections and estimate an incidence for both groups. Determining the AI for identification of recent HIV-1 infections is possible and easy using a simple and automated method based on commercially available reagents. A high prevalence for HIV infection was found in both of our study groups at an STI clinic and the ARL. Detection of recent HIV-1 infections provides evidence of current transmission. The estimated incidences should represent a baseline for further assessments to enable temporal trends analyses in those settings. Due to the nature of our study which uses a convenience sample, the results can not be extrapolated to other similar health care settings or the general population in Portugal. Surveillance for recent HIV infections with serological methods is feasible and desirable for better monitoring current local trends of the HIV/AIDS epidemic.

* Erratum: The original phrase "Portugal has been the western European country with the highest rate of notified autoimmunodeficiency syndrome (AIDS) cases..." was corrected on 12 December 2008 to read "Portugal has been the western European country with the highest rate of notified acquired immunodeficiency syndrome (AIDS) cases...".

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Perspectives

Country-wide HIV incidence study complementing HIV surveillance in Germany

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Serological methods exist that allow differentiating between recent and long-standing infections in persons infected with HIV. During a pilot study in Berlin between 2005 and 2007 methodologies have been evaluated. In a cross-sectional study blood samples, demographic, laboratory, clinical and behavioural data based on a KABP survey were collected from patients with newly diagnosed HIV infections. The BED-CEIA was used to determine recency of infection. Recent HIV infections contributed 54% (CI [95%]: 45; 64) in MSM and 16% (CI [95%]: 0; 39) in patients with other transmission risks (p=0.041). Proportions of recent infections were significantly higher in MSM ≤30 years (p=0.019). The mean age was 33.9 (median 34 years) in recent compared with 38.6 years (median: 38 years) in long-standing infections (p=0.011). High-risk behaviour indicated through very low condom use in recently HIV infected MSM could be identified. The results of the pilot study support expectations that the modified application of the method may contribute to improving HIV prevention efforts in Germany. On this basis the Robert Koch Institute implemented a countrywide HIV incidence study to complement HIV surveillance in early 2008. The study is funded by the German Ministry of Health. Data on recent HIV infections and current HIV transmission risks are collected. Design, methods and impact are described in detail.

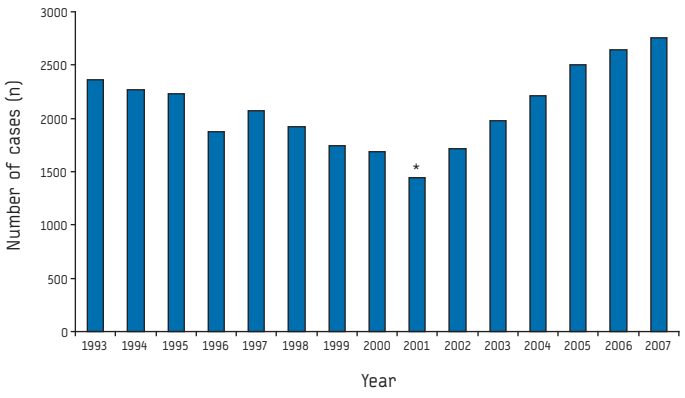
Background

In Germany newly diagnosed human immunodeficiency virus (HIV) infections reached a peak of 2,360 cases in 1993. The number of cases reported to the Robert Koch Institute (RKI), the institution responsible for the national surveillance of infectious diseases in Germany, dropped continuously in the second half of the 1990s, reaching the lowest level so far in 2001 with 1,443 cases. However, since 2001 this trend has been reversed and annual case reports increased to more than 2,750 cases in 2007 [1; Figure 1]. There are several possible explanations for these changes: an increase in HIV transmission ("true" incident infections); improved (earlier) case detection and reporting following the implementation of the "Protection against Infection Act" (Infektionsschutzgesetz - IfSG) in 2001; an increased number of HIV tests performed; changing attitudes towards HIV testing; and more widespread availability of testing facilities and better access to these facilities. The limited data available suggest that the increase in HIV cases is partly due to a rising willingness to test for HIV in groups with a high risk of transmission [2]. The higher number of HIV tests (ELISA and Western blot) performed in German laboratories when comparing the year 1999 to 2004 and the augmented use of HIV-NAT in primary HIV diagnosis additionally indicate changes regarding HIV testing [3].The rising number of cases reported between 1996 and 1997 may reflect increased testing for HIV following the implementation of highly active antiretroviral treatment (HAART). Recently the upwards trend in syphilis cases reported in Germany was discussed as a possible cofactor for increased HIV transmission in men having sex with men (MSM) [1]. However, the implications of these trends have not yet been analysed systematically.

The proportion of reported HIV cases without information on the underlying transmission risk decreased from 42% to 13% between 1993 and 2007, primarily reflecting amendments concerning case reporting [1]. In the same period the proportion of cases in MSM increased from 48% to 65%, whilst the proportion of cases with intravenous drug use decreased from 18% to 6%. Heterosexual transmission was constant at around 15-20%; persons originating from high prevalence countries (HPCs) as transmission risk for HIV contributed 11% of the total in 1993 and in 2007, with a peak of 25% in 2002 [1,2].

Figure 1

Number of newly diagnosed HIV cases reported in Germany, 1993 – 2007 (n=31,404)



* implementation of the "Protection against Infection Act" (Infektionsschutzgesetz - IfSG)

Standard reports of newly diagnosed HIV infections do not permit the differentiation between recently acquired (incident) and long-standing (prevalent) infections, since routinely applied serological HIV tests (screening and confirmatory tests) do not provide such information. The diagnosis of an HIV infection can be delayed by up to several years and the time between infection and diagnosis may be a number of years and vary considerably, thus estimating incidence rates accurately and effectively is difficult. However, incidence estimates are fundamental to understanding the current dynamics of the HIV epidemic.

Several other methods have proved suitable for the identification of recent (incident) HIV infections in patients with newly diagnosed HIV infections. The concept of recent infections in HIV usually covers a period up to six months prior to the diagnosis depending on the diagnostic assay used [4-9]. Testing for recent HIV infections was implemented as an additional component (anonymous and unlinked) of the national HIV surveillance systems in France [10,11], Switzerland [12] and in 22 federal states of the United States of America [13] and was used in selected population groups at risk for HIV infection in the United Kingdom and South Africa [14,15]. Collection of additional data on knowledge, attitudes, behaviour and practices (KABP survey) concerning HIV from patients identified as recently infected with HIV permits analysis of risks and protective factors effective in HIV transmission. Subpopulations at increased risk for acquiring HIV and with limited access to diagnostic services can be identified by comparing KABP data between risk groups.

After encouraging results from a pilot study in Berlin, a nationwide study including, testing for recent HIV infections and a KABP survey was started in Germany in March 2008. The study aims to provide a better picture of the current dynamics and drivers of the HIV epidemic based on incidence estimates. The results are expected to help amend the national prevention strategies.

Pilot Study in Berlin 2005-2007

A pilot study conducted in Berlin from 2005 to 2007 assessed the feasibility of the methodologies described above and the impact of the results for future HIV surveillance in Germany. The design was cross-sectional with voluntary sampling after obtaining patients' written informed consent. Sampling was anonymous and unlinked with no particular risk group being targeted. Exclusion criteria were clinical stage C HIV infection according to the US Centres for Diseases Control and Prevention (CDC) classification [16] and antiretroviral treatment. Clinicians in specialised private practices and clinic outpatient departments (OPD) collected venous blood and clinical data from adults aged 18 years or older with newly diagnosed HIV infections. Twenty of nearly 50 HIV-specialised facilities agreed to participate in the study. To determine a recent HIV infection the blood samples were tested using the BED-CEIA, one of the methods able to detect recent HIV infections serologically in patients with confirmed HIV diagnosis [17]. The BED-CEIA was established using a German HIV seroconverter sample panel with known time of seroconversion. Optimal cut-offs separating recent and long-standing samples in the reference panel were found with an optical density (ODn) of ≤0.8 for the BED-CEIA and duration of infection of 20 weeks [18]. KABP data with regards to HIV/AIDS were collected through patients' questionnaires. Test results were not delivered to the patients.

Results

Of 132 cases sampled, 114 were included in the study, 18 did not meet the eligibility criteria.

The 132 cases represent 27% of all newly diagnosed HIV cases reported to the RKI from the Federal State of Berlin during the study period between November 2005 and February 2007 (n=495). The total number of cases from Berlin accounted for 15% of all notifications from Germany. As far as data were available, all patients included had HIV-1 subtype B infections. Of the 114 cases meeting the eligibility criteria for the study, 102 were MSM (89%) and 12 had other HIV transmission risks.

Proportions of recent out of newly diagnosed HIV infections were found to be 54% in MSM (95% Confidence Interval (CI): 38-56) and 16% (95% CI: 32-0) in patients stating other risks. Proportions of recent infections were significantly higher in MSM ≤30 years (p=0.019), mean age was 33.9 (median 34 years) in patients with recent and 38.6 years (median: 38 years) in patients with long-standing infections (p=0.011). Symptoms of acute seroconversion correlated significantly with recent HIV infections (p=0.009). Mean viral load (VL) was significantly higher in recent HIV infections compared with long-standing infections (1,608,801 copies/µl and 141,951 copies/µl, respectively, p=0.009). A correlation was also found between recency of HIV infection and CD4 cell counts: counts >500/µml were indentified in recent HIV infections and counts ≤200/µml in long-standing infections; however, this correlation was not statistically significant (p=0.08).

Patients recruited for the pilot study showed a selection bias with samples from MSM being overrepresented (72% MSM in all cases reported from Berlin compared with 89% in the study sample). However, comparison of basic demographic variables in case reports of MSM from Berlin and MSM in the Berlin pilot study sample did not show statistically significant differences within the study period. High-risk behaviour indicated through very low condom use in recently HIV-infected MSM could be identified: >90% did not use condoms during sexual intercourse in the six months prior to HIV diagnosis and 19% stated that they did not use condoms despite being aware that their sexual partner had tested positive for HIV [19].

Conclusions

We were not able to produce incidence estimates since essential denominators are currently not available in Germany. Nevertheless, the results of the pilot study support expectations that the modified application of the method will contribute to amending and improving HIV prevention efforts in Germany.

National HIV Incidence Surveillance Programme 2008 - 2010

Since November 2007 the RKI initiated a nationwide study funded by the German Ministry of Health (BMG) to collect data on recent HIV infections and current HIV transmission risks. The results are expected to complement the available data on HIV from the general surveillance by identifying subpopulations presently at increased risk for acquiring HIV infections and the risks most recently having an impact on HIV transmission in Germany.

Design and methods

To obtain the desired information a cross-sectional unlinked anonymous study, with a case control component will be conducted from 1 March 2008 to 28 February 2010. Samples and data are collected over this period through either laboratories or specialised

clinical centres. Information on screening patterns for all cases is gathered in both the laboratory and clinical study arm. As data from the two study arms cannot be linked, overlapping of sampling from patients in both study arms cannot be excluded.

Laboratory study arm

Collaborating Institutions

Newly diagnosed HIV cases in Germany are reported to the RKI by more than 200 laboratories. Only 36 labs, however, contribute significant numbers to the reporting of newly diagnosed HIV infections (significant defined as providing each at least 1% of the total number of cases reported nationally). These 36 labs are responsible for almost 70% of all reported newly diagnosed HIV cases in Germany, with the remaining approximately 170 labs reporting another 30%. All 36 laboratories reporting high numbers of HIV infections agreed to participate in the national HIV incidence study (exhaustive sampling). Thirty-five of 51 randomly selected laboratories with HIV case reporting on a smaller scale also agreed to participate (random sample). Thus, a total of countrywide 71 laboratories will constitute the laboratory study arm.

Methods

Participating laboratories will collect plasma or serum samples from all newly diagnosed HIV cases during the study period. Samples are provided as "Dried Plasma Spots" (DPS) or "Dried Serum Spots" [20] and sent every month to the project group HIV Variability and Molecular Epidemiology at the RKI. All samples are tested for recency of HIV infection using the BED-CEIA. Clinical data are limited to information reported according to the national HIV surveillance regulations [21]. Data will allow to estimate recent HIV infections and incidence proportions by using basic demographic data and to analyse the risks to acquire an HIV infection. Data collected in this study arm are expected to be representative for Germany. The sample size is expected to include 1,600 cases annually representing around 60% of all new HIV diagnoses.

Clinical study arm

Collaborating Institutions

Over 80 clinical facilities specialised in HIV diagnosis and care from six regions in Germany will participate in the clinical study arm. The regions selected include those reporting the highest HIV case numbers nationally since 2001 (Figure 2) and they are characterised by a concentration of medical facilities specialised in HIV care compared with other regions. These facilities include private practitioners, clinic OPDs and counselling centres run by local health authorities or non-government organisations (NGO).

Methods

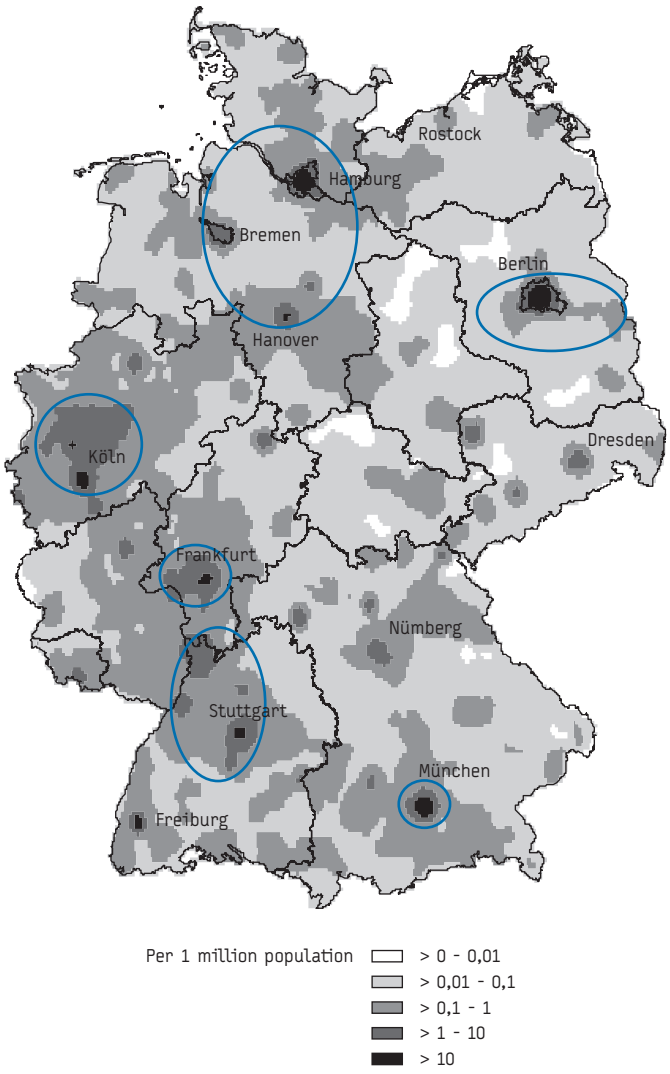
In this study arm clinicians specialised in HIV diagnosis and care will recruit patients with newly diagnosed HIV infections (cases) and patients undergoing an HIV test with negative result (controls). Cases and controls will be matched by basic demographic variables and their risk of HIV transmission. HIV testing for cases and their respective controls has to be performed within a three month period. After obtaining written informed consent, blood samples are collected from case patients as DBS [22]. The samples are analysed for recency of HIV infection by BED-CEIA at the HIV Variability and Molecular Epidemiology project group of the Robert Koch Institute. Clinical and medical history data from case and control patients are collected through a physician's questionnaire. KABP-data are collected from cases and controls by using a self-administered patient's questionnaire. The expected sample size is 600 cases and controls annually. Analyses of the data will allow comparison between patients with recently acquired HIV infection and persons undergoing HIV tests with a negative test result in the same clinical institutions and in an identical time frame. The analyses aim at obtaining information on the current status of general knowledge about HIV/AIDS, on the behaviour and attitudes towards prevention of HIV transmission, and on the risks taken with regards to HIV transmission.

Impact

The study offers an outstanding opportunity to identify recent HIV infections out of newly diagnosed cases and estimate HIV incidence. As a result of this a deeper insight into the transmission dynamics of the ongoing HIV epidemic in Germany will be available. To prevent further HIV infections, comparative analyses are aimed at identifying the risks for HIV transmission and the relevant behaviour and attitudes. However, the major limitations of our study are insufficient screening patterns that only reflect those patients requesting an HIV test. True incidence estimates will be

FIGURE 2

Cumulative incidence of newly diagnosed cases of HIV in Germany, 2001-2006 and six regions of the clinical study arm, Germany 2008



difficult to obtain as the denominators needed are not available in Germany. Despite these limitations the data are expected to have an impact on amending and improving national prevention efforts and strategies in Germany. Better knowledge of the factors driving the HIV epidemic and of the most recent dynamics of the epidemic revealing subgroups currently at increased risk of acquiring HIV will help to design targeted and prompt interventions.

Acknowledgements

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Meeting reports

Workshop on the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) and HIV Incidence Estimates, Stockholm, 11-12 March 2008

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The recent development of serological assays for human immunodeficiency virus (HIV) that are able to distinguish recent from long-standing infection has generated an important tool for HIV surveillance. In the European Union (EU), a number of different serological assays are being used, and there is the danger that that HIV incidence estimates in different countries, or even within a country, may not be comparable.

The former EU-funded project EURO HIV (http://ec.europa.eu/health/ph_projects/2004/action2/action2_2004_13_en.htm) included a work package on the investigation of several serological assays for recent HIV infection. It investigated the transferability of these tests, their comparative performance and their application in estimating HIV incidence in selected populations. Ten EU Member States contributed to this work.

The HIV experts (both epidemiologists and virologists) came together at a workshop held on 11 and 12 March 2008 at the European Centre for Disease Prevention and Control (ECDC) in Stockholm, Sweden, to discuss different approaches of the Serological Testing Algorithm for Recent HIV Seroconversion (STARHS) and their use for estimating HIV incidences.

EU-wide comparison of HIV serological methods

The EURO HIV *'work package 7'* was presented by John Parry (Health Protection Agency (HPA), London, United Kingdom (UK)). It aimed to increase networking and cooperation between reference laboratories with the goal of harmonising surveillance methods in the EU in order to obtain comparable data across countries. It investigated the use of the following serological assays:

- 'Detuned' enzyme immuno-assays (EIA) (modified commercial assays; bioMerieux Vironostika and Abbot HIV AB 3A11)
- BED-CEIA: an antibody capture EIA measuring the IgG proportion (commercial; Calypte Biomedical)
- AxSYM: an antibody avidity assay (modified commercial; Abbot)
- IDE-V3: EIA targeting two antigens at the same time (in–house assay; produced by Francis Barin)

Gary Murphy (HPA, London, UK) gave an overview of the existing serological assays that are able to distinguish between recent and long-standing HIV infection, pointing out the advantages and drawbacks of each method and identifying desirable criteria for an ideal assay. A more detailed description of the individual assays can be found in the article by Murphy and Parry in this issue.

The list of desirable characteristics for a STARHS assay includes a well defined, preferably long, window period, consistent discrimination between recent and long-standing infection, and accurate results for different cut-off values. The result should be independent of factors such as virus subtype, mode of transmission, opportunistic infections, pregnancy, and age, sex, race and therapy status of the patient. On the operational side, cost, availability, equipment requirements, ease of handling and storage, and the suitability for small volumes and different types of samples need to be taken into account, and the assay should ideally not depend on a single company. Moreover, a programme to standardise and control the performance of the assay needs to be in place.

It was concluded that no single assay at present fulfils all the desired characteristics.

All four STARHS methods were compared at the HPA Centre for Infections (CfI), London, though some were in use in other laboratories so that limited further comparisons using the same specimen panels were possible. The panel comprised 374 well characterised samples from England (CfI, London) and France (Université François Rabelais (UFR), Tours), as well as seven panels of around 200 samples from new HIV diagnoses that had been collected in England, Finland, Germany, Italy, the Netherlands, Portugal and Spain, a total of 1,736 eligible specimens. The results of the comparison are available in the final EURO HIV reports (available at: http://www.hpa.org.uk/webw/HPAweb&HPAwebStandard/HPAweb_C/1195733851609?p=1200660013708).

The intra-laboratory reproducibility was found to be satisfactory, with a reasonable correlation between original and repeat test results for the BED, Detuned, Avidity and IDE-V3 assays used at the CfI, and the IDE-V3 assay used at the UFR. However, certain issues were raised such as the need to define a window-period for the AxSYM and IDE-V3 tests, the need to set up a confirmatory test algorithm, particularly for specimens that give results in a critical range around the threshold value, and the evidence that a minority of patients may never develop an immune response sufficient to convert to a long-standing status in some assays. It was also seen as important to take into account the different factors that may bias the results, for instance anti-retroviral treatment, virus subtype, and disease stage.

There was some inter-laboratory variability that the participants thought was due to equipment calibration or maintenance issues and differences in the production lots purchased from the companies. They emphasised the importance of experience and training regarding the equipment and of suitable calibrators and controls, which are still to be developed. The lack of appropriate external assessment programmes to assure the quality of STARHS testing was seen as one of the greatest barriers to the transfer of any given method between different laboratories. It was suggested that it may be necessary to define a reference laboratory responsible for the development and standardised evaluation of new STARHS methods.

Andre Charlett (HPA, London, UK) presented an assessment of whether there was agreement between the four STARHS assays in the identification of recent versus long-standing HIV infection when using different window periods. The classification of the majority of specimens was consistent, but there were also intolerable inconsistencies, and none of the assays was found to be suitable for every specimen.

HIV incidence in the EU

With the laboratory methods still in need of improvement, more uncertainties arise when transferring laboratory data to incidence estimation. Part of *'work package 7'* was designed to test the applicability of the STARHS results for HIV incidence estimates in selected subpopulations in different EU Member States. Preliminary results from an HIV incidence estimation in three collaborating countries were presented by Daniela DeAngelis (HPA, London).

The estimates based on data from the four different STARHS assays differed substantially, and it was felt that more discussion will be needed on the interpretation of the results. Three main problems were put forward as possible reasons for the discrepant results: a) the data collection methodology may influence the interpretation of the test results; b) the difficulty of estimating the distribution of the window period, as the estimation procedure involves many assumptions and it might be based on a small panel of seroconverters; and c) misclassification of long-standing infections as recent. Other factors influencing the result include epidemiological data such as the testing pattern, the time since the last negative test and behavioural data.

Ongoing international activities in Europe

The second day of the meeting began with an overview on other ongoing European programmes focused on HIV incidence estimation. After a short presentation outlining the ECDC laboratory strategy, Valerie Delpech (HPA, London, UK) presented the EU-funded project 'Concerted Action on SeroConversion to AIDS and Death in Europe' (CASCADE), a network of epidemiologists, statisticians, virologists and clinicians from leading HIV institutions in 15 European countries, Australia and Canada that collects life-long data from local and national cohorts of seroconverters.

CASCADE's current activities include the ascertainment and follow-up of recently infected people in central and eastern Europe. Since most countries do not have the facilities to start a new large-scale surveillance project, CASCADE plans site visits to laboratories in order to create the infrastructure and train staff in suitable HIV tests. Whether STARHS methods are appropriate in this context, is being discussed.

The session was concluded with feedback from a recent meeting of the WHO working group on HIV incidence assays, a worldwide initiative to establish best practice in the calibration and evaluation of STARHS methodologies, to study the evidence on the use of these assays and to provide guidance on appropriate approaches to measuring HIV incidence. The next steps of the project foresee supporting the establishment of appropriate specimen panels, the calibration of existing and the development of new assays as well as their application, and the determination of a window period. A statistics working group will advise on how to interpret results and determine incidences. The use of incidence assays for purposes other than incidence estimates is being discussed.

Future objectives

In a third session the participants discussed, in two working groups, the laboratory and epidemiological aspects of using various STARHS assays, in order to define the next steps regarding the development and implementation of HIV serological assays and regarding incidence modelling in the EU Member States.

The workshop participants agreed that it is advisable to have at least two satisfactory standard STARHS methods established in all laboratories undertaking STARHS testing, in case one test should be temporarily unavailable. ECDC had hoped to conclude this workshop with a recommendation of one or two of these assays and to discuss the feasibility of their implementation in the EU. However, the experts felt that information for such a decision was lacking, and there was a general agreement that it is at present not possible to make such a recommendation. The laboratory experts were of the opinion that in the medium term it was more likely that five or six different assays would be in use across Europe and stressed that quality assessment programmes would be needed for all of them.

It was agreed that once an agreement has been reached on the test(s) to be used, ECDC should coordinate and fund the development of a framework or guideline for the implementation of STARHS for epidemiological use, detailing what epidemiological data are needed, from which populations, and which sampling strategy should be used. In the meantime, more work needs to be done with regards to the estimation of the window period, and a quality assurance and training programme needs to be developed. Further urgent issues for the near future include the development of an EU-specific panel of seroconverter samples for calibration of the assays, the realistic window period estimates, and a deeper analysis of the epidemiological information including validation of the results in different population groups according to the different factors that may bias the results.

It was decided that the WHO global initiative should be followed closely to avoid duplication of work. The overall conclusion was that, while HIV incidence testing may not become part of routine HIV surveillance in the very near future, all efforts regarding test development and epidemiological sampling frame should be targeted to reach this stage as soon as possible in order to improve the understanding of HIV epidemiology in the EU.

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News

United States Centers for Disease Control and Prevention release incidence estimates for HIV

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On 3 August 2008, the United States (US) Centers for Disease Control and Prevention (CDC) in Atlanta released for the first time estimates for HIV incidence based on a STARHS (serological testing algorithm for recent HIV seroconversion) [1]. In their communication, CDC report that the true HIV incidence for 2006 is around 40% higher than the previous estimate of 40,000 HIV infections. They also point out that this new figure of 56,300 does not indicate any increase in the annual number of new HIV infections, which is believed to be relatively stable since the late 1990s. Analysis by transmission category confirms that male-to-male sexual contacts accounted for 53% of the estimated new HIV infections in 2006, high-risk heterosexual contact for 31%, injection drug use (IDU) for 12% and male-to-male sexual contact and IDU for 4%. Further analyses by race/ethnicity revealed an uneven distribution with the highest percentage of new HIV infections occurring in African Americans (45%) followed white Americans (35%) and Hispanics (17%).

The results were obtained after using a STARHS assay, the BED HIV-1 capture enzyme immunoassay (BED-CEIA), to test 6,864 samples from new HIV diagnoses from 22 US federal states in 2006. Whereas standard HIV tests provide no insight into the time when infection was actually contracted, the BED-CEIA is able to identify HIV infections that occurred within around the previous five months. The test thus allows to distinguish between recent and long-standing infections and permits a more precise estimate of the true incidence. A total of 2,133 (31%) tests of the 6,864 were classified as recent infections and the estimated incidence rate for 2006 was 22.8 per 100,000 population. The detailed methods for the calculation of this incidence and an extended back-calculation model to estimate HIV incidence for the period 1977 to 2006 are reported in an article by Irene Hall et al. in JAMA [2].

The CDC state that the implementation of the STARHS-based surveillance system in the US will allow for reliable monitoring of incidence trends in the future, helping to pinpoint the populations at greatest risk and pave the way for more timely interventional measures.

Since it is estimated that one-quarter of HIV-infected individuals are unaware of their infection status and that they account for more than half of all new infections, CDC recommends testing everyone in the US aged 13 to 64 years for HIV. On a more positive note, the stability in the new HIV infections since 2000 is an indicator that prevention can, and does, work, especially if one takes into consideration that the number of people living with HIV increases over time – due to better survival of infected individuals - and subsequently the overall risk of HIV transmission increases.

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Editorials

Clostridium difficile: summary of actions in the European Union

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This week's issue of Eurosurveillance includes two papers on *Clostridium difficile* infection (CDI), also referred to as *C. difficile*-associated disease (CDAD). The term CDI is increasingly being preferred in recent international literature [1-4], mainly because CDAD is regularly used for *C. difficile*-associated "diarrhoea" as well [5-7], an entity that does not cover the entire clinical spectrum of the disease.

C. difficile is an anaerobic bacterium that was identified as part of the normal flora of neonates in 1935 and can be isolated from the stool of 3% of healthy adults and in at least 10% of asymptomatic hospitalised patients [7,8]. It was identified as the cause of antibiotic-associated pseudomembraneous colitis in 1974 and has since been recognised as the most common cause of healthcare-associated diarrhoea, often, but not always, in association with previous antibiotic use. The clinical spectrum of CDI ranges from mild diarrhoea to potentially life-threatening colitis that may result in toxic megacolon, colon perforation and multiorgan failure. The pathogenesis is mediated through the production of toxins, toxin-negative strains do not cause disease [8-10].

Since 2006, the European Centre for Disease Prevention and Control (ECDC) has been addressing the new CDI situation.

In recent years outbreaks of CDI and an increase in the incidence of healthcare-associated CDI have been described in the United States (US), Canada and several European countries, mostly associated with a new virulent strain characterised as toxinotype III, North American pulse-field type 1 (NAP1) and PCR ribotype 027 (Type 027) [9]. In the Euroroundup article published in this issue, E Kuijper et al. report that Type 027 has now been isolated in 16 European countries, and has been associated with outbreaks in nine of them. However, it has become clear that also other PCR ribotypes are associated with the increase of CDI, such as the new emerging Type 078 strain which has similar mechanisms for hyper-production of toxins as Type 027 and has been reported in Belgium, The Netherlands, Northern Ireland, Scotland, and possibly Spain.

The paper from Spain by A. Asensio et al. unfortunately lacks microbiological typing data, but it provides an interesting approach for a retrospective analysis of the increase of CDI at the national level using data from the EPINE study (Estudio de prevalencia de las infecciones nosocomiales en los hospitales españoles) a national prevalence survey of nosocomial infections performed repeatedly every year since 1990. Assuming a constant methodology over time, the study clearly shows an increase in the prevalence of nosocomial CDI from 0.039% in 1999 to 0.122% in 2007. This latter figure is still 10 times lower than the 1.21% hospital-associated CDI prevalence reported in 270 hospitals across the United Kingdom (UK) and the Republic of Ireland in 2006 [11]. However, differences in case-finding methods for CDI between the two surveys certainly account for a part of this difference.

Since 2006, the European Centre for Disease Prevention and Control (ECDC) has been addressing the new CDI situation. Considering the worrying evolution of CDI in Northern America [6,12,13], reports of Type 027 CDI outbreaks in Belgium [14], The Netherlands [15] and the UK [16] in 2005, and the preliminary results of an EU-wide study conducted in 2005 by the ESCMID (European Society of Clinical Microbiology and Infectious Diseases) Study Group for *C. difficile* (ESGCD) [17], ECDC convened a group of experts consisting of members of ESGCD, epidemiologists from healthcare-associated surveillance networks from the European Union (EU) and from the US Centers for Disease Control and Prevention (CDC).

This ECDC working group recognised the emergence of a new CDI problem in some EU Member States and the potential for spread to other countries and decided to act by:

- informing Member States and the scientific community;
- fostering the coordination of national surveillance activities and exploring the need for additional studies to assess the spread of Type 027 in Europe;
- exploring ways to improve microbiological standardisation, in particular typing methods, common typing nomenclature and sharing of reference strains; and
- developing best practice guidance to Member States.

Follow-up meetings were held at the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) in Nice (2006) and Munich (2007), at ECDC in January 2007 and at the Second International *C. difficile* Symposium in Maribor, Slovenia in June 2007. The ECDC working group produced the first background paper on the emergence of CDI in Europe that included interim case definitions for CDI as well as other recommendations for surveillance [9]. Similar, interim recommendations for surveillance were later published by a CDC working group [18], but their appropriateness in long-term care facilities (LCTF), in particular the attribution of

cases to either the hospital or the LTCF in delayed-onset cases was recently discussed in the context of public reporting of CDI rates in the US [5].

Members of the ECDC working group also communicated regular updates on the epidemiological situation in Europe at scientific conferences and in scientific journals [19]. Finally, the working group recently published a systematic review of infection control measures to limit the spread of *C. difficile* that can be used for the elaboration of evidence-based guidelines in Member States [20]. These should combine early diagnosis, surveillance, education of staff, appropriate isolation precautions, adapted hand hygiene and use of protective clothing before and after contact with symptomatic cases, environmental cleaning and cleaning of medical equipment, good antibiotic stewardship, and specific measures during outbreaks. The paper underlines the specific difficulties to prevent *C. difficile* transmission linked to the capacity of *C. difficile* to form spores that survive for months in the environment, may be excreted in large numbers by affected patients, cannot be destroyed by standard alcohol-based hand disinfection and persist despite usual environmental cleaning agents.

The ECDC is currently financing a European prospective CDI incidence survey coordinated by the Dutch National Institute for Public Health and the Environment (Rijksinstituut voor Volksgezondheid en Milieu, RIVM. The study aims at assessing the baseline incidence of hospital-acquired and community-acquired *C. difficile* infections in a selected number of hospitals from all EU Member States using the interim case definitions and will collect information on the severity of disease, the complication rate and the mortality of CDI as well. One of the major objectives of the survey is to build a network of laboratories with links to national surveillance institutes in all MS capable of isolating and characterising *C. difficile* isolates. This objective is pursued through training in typing techniques and distribution of reference strains of the most frequently occurring strains in Europe. It is expected that the project will result in a better standardisation of *C. difficile* typing. The resulting network of national *C. difficile* laboratories will be instrumental in setting up a future continuous surveillance of CDI in Europe: by performing typing of strains according to a EU-agreed laboratory and surveillance protocol; by improving the capacity of peripheral laboratories in the individual countries to diagnose CDI on a routine basis using standardised methods [21] allowing to follow-up the baseline incidence in healthcare institutions and to timely detect CDI outbreaks; and by assisting hospital infection control staff and public health authorities in implementing appropriate control measures.

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Editorials

Introducing human papillomavirus (HPV) vaccination - a challenge for European vaccine advisory committees and public health services

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The development of efficient human papillomavirus (HPV) vaccines has required long lasting and tremendous research and development efforts by both academia and industry. However, their availability now turns out to be a major challenge for the public health services of many member states within the European Union (EU) and beyond. Shortly after the two HPV vaccines, Gardasil from Sanofi Pasteur MSD and Cervarix from GSK, were found acceptable for the EU market by the Committee for Human Medicinal Products (CHMP) of the European Medicines Agency (EMEA) based in London, and were subsequently granted a community marketing authorization by the European Commission, the public perception was entirely focussed on the surprisingly high efficacy against cervical cancer caused by HPV high risk types 16 and 18 in a population that was not previously exposed to HPV types contained in these vaccines.

Both vaccines contain particulate recombinant L1 structural protein of HPV types 6, 11, 16 and 18 (Gardasil) or 16 and 18 only (Cervarix). The manufacturing processes as well as formulation and composition of both vaccines differ, however, markedly [1,2].

Protection from persistent HPV infection (virological endpoint) in parallel to prevention of CIN 2+ (histopathological endpoint) were chosen as surrogate parameters for efficacy. It is accepted within the scientific and regulatory community that compliance during clinical studies with predefined criteria regarding these two endpoints will correlate with prevention of and protection from cervical cancer caused by the vaccine specific high risk HPV types, thus supporting licensure of a given HPV vaccine.

…the integrated approach employed in the framework of the VENICE project aimed to facilitate the introduction of HPV vaccines in Europe…

This novel option, preventing cervical cancer by prophylactic vaccination has put enormous pressure on those institutions within the individual EU member states which are responsible for vaccination recommendations and also on those in charge of designing and financing vaccination strategies and campaigns. In some countries public demand was so explicitly expressed that neither vaccination advisory committees nor health insurance companies had options other than rapidly fulfilling these demands in a most generous manner. Other countries were following with their recommendations with some delay and a considerable number of member states has meanwhile chosen a more cautious position and continue to explore how HPV vaccines can be optimally used [3]

However, over the months following the initial licensure of HPV vaccines a number of issues have drastically modified the early enthusiastic views on HPV vaccination into apparently much more critical considerations. These issues evolved from the immediate widespread use of HPV vaccines in the countries that first introduced HPV vaccination and were faced with a phenomenon that is routinely observed upon introduction of new vaccines into a given population, i.e. side effects following vaccination not known from clinical studies. Adverse events ranging from mild to very severe conditions including autoimmune disease and some cases of death were reported in close temporal relationship to HPV vaccination [4]. Applying contemporary pharmacovigilance principles causality between vaccination and side effects can often be proven or disproven, nevertheless, public perception was distracted from the overwhelming efficacy of HPV vaccines and attention was focused on diffused and largely unfounded safety concerns. These public concerns were again and again taken up by virtually all media resulting in a massive confusion about the true value of HPV vaccines not only among those for whom these vaccines were officially recommended but also among health care providers and authorities. In many cases planned vaccinations were cancelled or missing remaining vaccinations rejected. Regulatory professionals have met such situations very frequently in the past and learned that explanatory efforts from agencies and official bodies aimed at adding science to what is communicated by headlines is hardly accepted or understood by the public either because the scientific or medical background is too complicated or the risk communication principles are not applied efficiently enough by the authorities.

For these reasons the integrated approach employed in the framework of the VENICE project aimed to facilitate the introduction of HPV vaccines in Europe (described in the article by King et al.) is of particular value and importance. Availability of a common platform for sharing scientific considerations which are based on hard data but also on modelling systems will become increasingly essential in the future when new vaccines and other novel medicinal products targeted at a significant proportion of the member states' population will be introduced in the EU. Strategies commonly acceptable to many if not all member states will put public health agencies in a much stronger position to justify and convincingly communicate the reasons why for a new prophylactic or therapeutic

approach the apparent benefits are considered to outweigh by far known and presumed risks. Closely linked to these questions is to an increasing extent the cost-effectiveness ratio of new therapeutic or prophylactic options. These economical aspects rapidly move into the foreground and are particularly applicable to HPV vaccination since HPV vaccines are the most expensive vaccines available on the common market and the size of target groups is especially large. The immediate and long term benefits of HPV vaccines for health care systems are, however, not instantly recognizable. For example, compared to the incidence of breast cancer, rates of cervical cancer are relatively low in the EU and mortality due to it is even lower [5] In addition there are reliable screening measures in place in all member states that are generally considered to be suitable and sufficient to prevent cervical cancer although not all eligible women will participate in screening programs and efficiency of screening programs might be overestimated when not sufficiently quality controlled [6]. Nevertheless their existence raises the question why widespread application of HPV vaccines should be financed in addition to screening programs by health care insurers or public health services without having clear evidence about the economical impact. Concerns have also been raised that HPV vaccination may induce false understanding of protection from disease prompting vaccinated women to deviate from or skip regular cervical screening. However, this kind of argumentation is always brought up following the development of new prophylactic or therapeutic antiviral solutions. Seriously following this line of argumentation would, however, ultimately block any progress in this field.

At the same time, public health services might ask themselves the question whether this money could be better invested in medical interventions where economic benefits are apparent or at least detectable easier and earlier. To answer this type of questions the VENICE platform might also be helpful since it may enable us to ask precise questions and get the most conclusive answers based on specific investigations or previous experience made in individual member states. Relevant questions to determine the cost-effectiveness of HPV vaccination may include:

- To what extent will HPV vaccination help to further reduce surgical intervention?
- Will this reduction outweigh the cost for HPV vaccination programs?
- Which population should be vaccinated to achieve optimal individual, population and economical effects?

It is very important to keep in mind in this context that we are at present just collecting first experience with the first generation of HPV vaccines. Extensions of indications of first generation HPV vaccines based on new data coming in from ongoing and additional studies are very likely and second generation HPV vaccines will follow providing options to also protect from other high risk HPV types. These vaccines will address concerns related to potential strain replacement probably triggered by the current HPV vaccines but may also allow to speculate about the elimination of cervical cancer if future HPV vaccines will contain all the high risk HPV types that are causative agents for virtually all cervical cancers diagnosed in the EU. This rather futuristic outlook should emphasize that integrated EU approaches to measure the value of new prophylactic and therapeutic options will follow a dynamic rather than a static principle meaning that what might not appear cost effective today might turn into an effective tool tomorrow reducing or abolishing significant health burdens to the EU population and financial burdens to national health systems in parallel.

Hopefully VENICE turns out to be an effective tool to reach that goal.

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Increasing rates in *Clostridium difficile* infection (CDI) among hospitalised patients, Spain 1999-2007

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Limited information is available on the burden and epidemiology of *Clostridium difficile* infection (CDI) in Spain. The present report communicates the secular trends in prevalence of CDI among hospitalised patients in Spain from 1999 through 2007. Data were obtained through the EPINE study (Estudio de prevalencia de las infecciones nosocomiales en los hospitales españoles), a point prevalence study series of nosocomial infections among patients admitted to hospital in Spain.

A total of 378 cases with CDI were identified. Median age was 74 years. Prevalence rates of CDI increased from 3.9 to 12.2 cases per 10,000 hospitalised patients and showed a significantly increasing secular trend from 1999 through 2007 (prevalence rate ratio per each year increment 1.09; 95% CI 1.05 – 1.14). Percentage of hospitalised patients receiving antimicrobials increased linearly from 36.0% in 1999 to 40.7% in 2007 (p <0.001) and was strongly correlated to CDI prevalence (R square = 0.73; regression coefficient =1.194, 95% CI= 1.192 – 1.196).

Introduction

Clostridium difficile is the most commonly diagnosed cause of infectious hospital-acquired diarrhoea [1]. Since 2003, outbreaks of severe nosocomial diarrhoea, caused by a new virulent strain of *C. difficile* Type 027, characterised as toxinotype III, North American pulsed-field type 1 (NAP1), restriction-endonuclease analysis group type BI and PCR-ribotype 027 have been recognised in Canada and the USA, and soon thereafter in several European countries, as well as in Japan, evoking great concern among public health authorities [2-5]. Limited information is available on the burden and epidemiology of *C. difficile* infection (CDI) in Spain. The present report communicates the secular trends in prevalence of CDI among hospitalised patients in Spain from 1999 through 2007 and factors associated with CDI prevalent cases.

Methods

Since 1990, a point prevalence study series of nosocomial infections among patients hospitalised in acute care facilities have been conducted in Spain (Estudio de prevalencia de las infecciones nosocomiales en los hospitales españoles – EPINE study).

Each year in May, acute care hospitals in Spain are requested to voluntarily join the EPINE prevalence study. Participating hospitals fill a standardised questionnaire on each hospitalised patient as well as overall data on the hospital and the hospital's wards.

CDI diagnosis relies on CDC case-definitions for nosocomial infections (note: the EU case definitions were not available at the time of the start of the study), and includes cases of either clinical diarrhoea or toxic megacolon with laboratory evidence of positive stool culture and/or toxin assay for *C. difficile*. Thus our analysis encompassed a symptomatic population with a positive microbiological confirmation of CDI by culture, toxin assay or both.

In addition to information on nosocomial infections, the patient forms collected from the hospitals included demographic data (age and gender); information on underlying clinical conditions such as diabetes mellitus, renal failure, inmunosuppression, chronic pressure ulcers and hypoalbuminemia; healthcare exposures such as previous surgery, enteral feeding, immunosuppressive therapy, use of antibiotics (as the proportion of patients receiving any antimicrobial on the day of the survey); type of ward (general medical as opposed to a surgical, intensive care, paediatric or obstetric ward); and size of the hospital as measured by number of beds (small: lesser than 200 beds; medium: 200-500 beds; large: greater than 500 beds). Hospital validated forms were sent to an independent central analysis unit for further validation and analysis. A hospital report was sent back to every participating hospital to avoid possible disagreements before final integration of the collected results in a centralized database. We focused our analysis on the period 1999-2007.

Prevalence rates were expressed as the number of patients with CDI per 10,000 hospitalised patients. Comparisons of facilities, clinical conditions, exposures and demographic features were made by chi-square test, likelihood ratio test, Student's t test or Mann-Whitney test if appropriate. Secular trends were evaluated by Poisson regression. For factors associated with CDI, prevalence rate ratios and 95% confidence intervals were computed. For correlation of the use of antimicrobial and the annual prevalence

rates Spearman correlation coefficient and regression coefficient along 95% confidence intervals were calculated. All calculations were performed with Stata/SE 9.0 statistical software.

Results

Between 1999 and 2007 on average 249 hospitals per year participated in the EPINE survey yielding a representative sample of almost 57,000 hospitalised patients per year. Most of the hospitals (82-85%) participating in the survey at any given year took part in the entire nine-year series. The mean age of patients increased from 56.2 years in 1999 to 58.7 years in 2007. A total of 378 CDI cases were identified. Prevalence rates of CDI ranged from 3.9 cases/10,000 patients in 1999 to 12.2 cases/10,000 patients in 2007, and showed a significantly increasing trend from 1999 through 2007 (prevalence rate ratio for one year increment 1.09; 95% CI 1.05 – 1.14) (Table 1). Prevalence rates were consistently

Table 1

Prevalence rates of *Clostridium difficile* infection (CDI) and use of antimicrobials in hospitals in Spain, by year of the survey

	1999 (N=233)	2000 (N=243)	2001 (N=243)	2002 (N=246)	2003 (N=241)	2004 (N=258)	2005 (N=257)	2006 (N=253)	2007 (N=266)	Prevalence ratio*	95% CI
Age group											
18-64 years											
Cases	6	9	11	11	12	11	14	8	26		
Patients	23,077	23,357	23,369	22,690	22,565	24,130	23,823	23,857	25,042		
Prevalence rate	2.6	3.9	4.7	4.8	5.3	4.6	5.9	3.4	10.4	1.12	(1.04-1.21)
65-79 years											
Cases	8	11	14	16	10	15	19	14	24		
Patients	18,569	19,164	19,718	18,752	18,542	19,488	19,256	18,513	19,466		
Prevalence rate	4.3	5.7	7.1	8.5	5.4	7.7	9.9	7.6	12.3	1.09	(1.03-1.18)
≥ 80 years											
Cases	7	17	10	13	9	17	13	17	23		
Patients	7,170	7,649	8,342	8,493	8,738	9,468	9,975	10,624	11,786		
Prevalence rate	9.8	22.2	12.0	15.3	10.3	18.0	13.0	16.0	19.5	1.03	(0.96-1.10)
All age groups											
Cases	21	39	35	40	33	45	50	40	75		
Patients	53,689	55,323	56,321	54,882	54,864	58,672	58,379	57,989	61,496	1.09	(1.05-1.14)
Prevalence rate	3.9	7.0	6.2	7.3	6.0	7.7	8.6	6.9	12.2		
Patients receiving antimicrobials (%)	36.0	36.7	36.4	37.0	36.9	38.6	39.4	39.4	40.7		

Prevalence rates are given per 10,000 hospitalised patients
N = number of participating hospitals
*Prevalence ratio for one year increment, estimated by Poisson regression

Table 2

Clinical and demographic characteristics of patients with *Clostridium difficile* infection (CDI) in comparison with non-CDI patients, hospitals in Spain 1999-2007

	CDI patients N = 378	non-CDI patients N= 511,237	Prevalence ratio (95% CI)	p value
Age in years, median (range)	74 (4-97)	64 (1-99)	10*	<0.001
Age > 65 years	207 (68.3%)	216,361 (48.1%)	2.3 (1.8-3.0)	<0.001
Male gender	203 (54.4%)	259,068 (51.6%)	1.1 (0.9-1.4)	0.273
Renal failure	80 (22.2%)	41,787 (8.5%)	3.1 (2.4-3.9)	<0.001
Diabetes mellitus	95 (26.1%)	97,475 (19.8%)	1.4 (1.1-1.8)	<0.003
Immunodeficiency	40 (11-1%)	18,305 (3.7%)	3.2 (2.3-4.5)	<0.001
Hypoalbuminemia	107 (30.3%)	29,093 (6.1%)	6.7 (5.3-8.4)	<0.001
Pressure ulcers	69 (19.1%)	24,051 (5.0%)	4.5 (3.5-5.9)	<0.001
Previous surgery	57 (15.5%)	151,639 (30.2%)	0.4 (0.3-0.6)	<0.001
Enteral feeding	60 (16.4%)	31,990 (6.5%)	2.8 (2.2-3.7)	<0.001
Immunosuppressive therapy	62 (17.2%)	41,468 (8.4%)	2.3 (1.7-3.0)	<0.001
Hospital size** < 200 beds 200-500 beds > 500 beds	 75 (19.8%) 145 (38.4%) 158 (41.8%)	 147,521 (28.9%) 213,673 (41.8%) 149,930 (29.3%)	 Reference 1.3 (1.0-1.8) 2.1 (1.6-2.7)	< 0.001
Medical wards***	266 (70.4%)	209,276 (40.9%)	3.4 (2.7-4.3)	<0.001

* Median difference
** Likelihood ratio test=30.7
*** In this study, we use the term "medical ward" to indicate internal medicine (and its subspecialties) wards as opposed to "non-medical wards" including surgical, intensive care, paediatric and obstetric wards.

higher in older age groups for every year. Furthermore, for adults, prevalence rates showed a statistically significant increasing time trend for every age group except for the group of patients aged 80 years and older (Table 1).

The prevalence of use of antimicrobials in the hospitalised population (given as the number of patients on antimicrobials per 100 hospitalised patients) increased linearly from 36.0% in 1999 through 40.7% in 2007 ($p < 0.001$) (Table 1) and showed a strong correlation with CDI prevalence rates ($R^2 = 0.73$; regression coefficient for percentage of use of antimicrobials 1.194, 95% confidence interval 1.192 – 1.196).

Comparison of CDI and non-CDI patients by main characteristics is displayed in Table 2. No differences were found for gender. However, CDI patients were older, presented more frequently underlying conditions such as renal failure, diabetes mellitus, immunodeficiency, pressure ulcers or hypoalbuminemia. CDI patients were also more frequently exposed to enteral feeding, and to immunosuppressive therapy, but significantly less often exposed to surgical procedures. Furthermore, being admitted to a general medical ward (such as internal medicine or its subspecialties: cardiology, pulmonology, etc.), as opposed to a surgical, intensive care, paediatric or obstetric ward was associated with a higher prevalence of CDI, and so was the size of the hospital (rate ratio 1.3 and 2.1 for medium and large size hospitals, respectively, compared with small size hospitals) (Table 2).

Discussion

One of the strengths of this prevalence series is that it represents more than half of the population hospitalised in acute care centres in Spain in a given day, and most data come from hospitals that have regularly participated in the survey every year. These data indicate that prevalence rates of CDI per 10,000 hospitalised patients over the period 1999-2007 increased significantly from 3.9 to 12.2, at an annual rate of 9%. Furthermore, this increase could also be demonstrated for patients pertaining to the age group of 18-79 years (the average annual increases for 18-64-year-olds and 65-79-year-olds were 12% and 9% respectively).

Several factors could explain this increase in CDI rates. When looking for potential outbreaks that could account for the differences between the various years, we were able to identify one hospital in 2002, two hospitals in 2004, another hospital in 2006 and three hospitals in 2007 showing point prevalence rates higher than 40 per 10,000 patients. Thus, even if prevalence surveys are not a powerful tool to detect outbreaks, the hypothesis of increasing trends related to more frequent hospital outbreaks in most recent years cannot be ruled out on the basis of our data.

Exposure to several classes of antimicrobials has been consistently found to be associated with CDI [6]. During the study period the proportion of patients receiving antimicrobials increased significantly and was found strongly correlated to the CDI prevalence rates. This increase in the use of antimicrobials suggests it could be one of the causes of the observed increase in CDI rates. Nevertheless, this hypothesis can not be proven from the ecological trend presented in our study since individual exposition should be taken into account for a causal association and we lacked data on individual patients' exposures to antimicrobials before their developing CDI.

It has been previously shown that the older and the sicker the patients the more prone they are to CDI. In fact, in our study, CDI patients were older than the patients not infected, and CDI rates were consistently higher for older age groups during the entire study period. Furthermore, the mean age of patients increased by almost 2.5 years from 1999 to 2007. The severity of the main underlying disease and/or the number of comorbidities also increased during the period [7] and could be another factor accounting for the increase in CDI.

The possibility that the new virulent strain of *C. difficile* Type 027 could account for the increasing trend observed is extremely remote. This strain has been identified in Spain in two cases only: an imported case of CDI in a patient transferred from a hospital in the United Kingdom, and another one in a laboratory technician who had worked with *C. difficile* isolates and subsequently developed CDI. However, no outbreaks associated with this strain have been communicated to date [8].

As previously reported, the underlying diseases and certain clinical characteristics were associated with a higher risk of CDI. We found diabetes mellitus, renal failure, immunodeficiency or hypoalbuminemia as well as being subjected to enteral feeding or immunosuppressive therapy to be associated with CDI. Furthermore, being admitted to a general medical ward and a large hospital, were both associated with a higher rate of CDI, whereas a history of previous surgery was associated with a lower rate of CDI. However, higher rates of CDI in larger hospitals could also be related to the more complex case-mix and to better awareness of CDI by clinicians in third care, including many referral, centres.

Our study has several limitations. Prevalence rates are not directly comparable to incidence rates that have been proposed for surveillance of CDI. Estimation of incidence rates from prevalence rates in the hospital framework is risky and has not been recommended [9]. A calculation from the formula proposed by Rhame and Sudderth [10] yielded an average incidence rate of 9.8 cases/10,000 patient-days for the whole period studied (1999-2007). This estimate would be within the range of other incidence estimates [11-14] before the emergence of the new virulent *C. difficile* Type 027.

It is also likely that the figures we obtained underestimate the actual prevalence, since testing for *C. difficile* is not a routine clinical practice in less severe cases, and is performed at the discretion of the attending physician. On the other hand, in recent years, clinicians have shown increased awareness of CDI in endemic situations and have more frequently tested for *C. difficile* toxins thus yielding a higher number of CDI diagnoses.

Further limitation of our study is that we lack information on strain identification therefore the importance of *C. difficile* Type 027 can not be definitely ruled out. Both cross-sectional and ecological studies are not a valid study design for risk factor research as they do not allow for establishing causal inferences, but they can point out potential risk factors for further evaluation. Another concern is seasonality. As the survey was performed every year during May, seasonal variations in time could not be assessed. Other studies have observed seasonality with rates peaking in winter months and lower rates in summer [15]. However, the fact that we performed the survey in the same month each year, although precluding a study of seasonality, allowed us to measure trends.

To conclude: over the 1999-2007 period prevalence rates of CDI increased significantly in Spanish hospitals. On-going surveillance systems are needed to closely monitor incidence, *C. difficile* strains characteristics, as well as the changing epidemiology of CDI in Spain.

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Introduction of human papillomavirus (HPV) vaccination into national immunisation schedules in Europe: Results of the VENICE 2007 survey

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The European Union Member States are simultaneously considering introducing HPV vaccination into their national immunisation schedules. The Vaccine European New Integrated Collaboration Effort (VENICE) project aims to develop a collaborative European vaccination network. A survey was undertaken to describe the decision status and the decision-making process regarding the potential introduction of human papillomavirus (HPV) vaccination into their national immunisation schedules. A web-based questionnaire was developed and completed online in 2007 by 28 countries participating in VENICE. As of 31 October 2007, five countries had decided to introduce HPV vaccination into the national immunisation schedule, while another seven had started the decision-making process with a recommendation favouring introduction. Varying target populations were selected by the five countries which had introduced the vaccination. Half of the surveyed countries had undertaken at least one ad hoc study to support the decision-making process. According to an update of the decision-status from January 2008, the number of countries which had made a decision or recommendation changed to 10 and 5 respectively. This survey demonstrates the rapidly evolving nature of HPV vaccine introduction in Europe and the existence of expertise and experience among EU Member States. The VENICE network is capable of following this process and supporting countries in making vaccine introduction decisions. A VENICE collaborative web-space is being developed as a European resource for the decision-making process for vaccine introduction.

Introduction

The availability of a new vaccine requires each country to decide whether to integrate the vaccine into the national immunisation schedule. The need for better knowledge about the decision-making process, and scientific contribution to decision-making regarding the introduction of a new vaccine across European Union (EU) Member States (MS) was one of the main justifications for setting up the VENICE project.

The VENICE project

The Vaccine European New Integrated Collaboration Effort (VENICE) project is a three-year European Commission (DG SANCO) sponsored project that was launched in January 2006. Twenty-eight European countries participate in the project, 26 EU MS (all except Malta) and two European Economic Area/European Free Trade Association countries (Iceland and Norway). The VENICE project aims to create an EU vaccination network capable of collecting and collating information on MS vaccination programmes. One of the ultimate goals of the network is to create a resource able to support MS and the European Commission by integrating available tools and knowledge on vaccine related issues.

In practical terms, the VENICE project is organized in technical work packages, which refer to different areas of activity and relate to the specific objectives of the program [1]. One of the VENICE technical work packages aims to encourage a rational approach to vaccination policy decision-making. This is achieved by promoting the exchange of experience and expertise, whenever a new vaccine is licensed in Europe, through sharing of information about recent and current studies performed, the methodologies used and the outcomes, and about vaccination strategies adopted.

In order to achieve the various objectives of the VENICE project, twenty-eight national gatekeepers were identified, one per participating country. Moreover, in each country, work package-specific contact points have been identified.

HPV vaccines in Europe

Two vaccines protecting against human papillomavirus (HPV) infections have been licensed in the EU based on the positive evaluation from the European Medicines Evaluation Agency (EMEA): a quadrivalent vaccine (Gardasil®) in September 2006 and a bivalent vaccine (Cervarix®) in September 2007 [2, 3, 4, 5]. Both vaccines have a prophylactic indication and aim to prevent pre-cancer lesions (CIN II +) and cancers due to persistent infection with HPVs 16 and 18 in women who have not been previously infected with these HPV types. HPV 16 and 18 have been estimated to cause 73% of cervical cancer cases in Europe [6]. The quadrivalent vaccine also prevents infection with HPV 6 and 11, viruses responsible for 80-90% of genital warts. [7 8].

Despite the high efficacy of these two vaccines, the decision to introduce HPV vaccination into a national immunisation schedule is complex and requires thorough epidemiological and economical analyses. Many factors must be considered, for example high vaccine cost and the added benefit of vaccination over an effective cervical screening programme. [9].

The European licensing of two HPV vaccines means that all MS are simultaneously considering the potential introduction of HPV vaccination into their national or, where applicable, regional immunisation schedules. These circumstances provide a unique opportunity to understand, in real-time, the decision-making process that precedes the introduction of a vaccine.

The objectives of this study were to identify the current decision-status of MS, describe the decision-making process and identify key information and methodologies used in the decision-making process for potential introduction of HPV vaccination into national immunisation schedules.

This report completes the preliminary analysis of this survey that was carried out early in 2007 and published in Eurosurveillance in April 2007 [10], and includes an update from January 2008.

Methods

Questionnaire

A web-based HPV vaccine questionnaire was developed in 2006 to explore the decision-making process for the introduction of HPV vaccination. The questionnaire was piloted in five countries (Italy, Ireland, France, Hungary and Greece) in August-September 2006 and posted on the VENICE website in January 2007 for completion by mid-February. The questionnaire was filled in by the project gatekeepers, or a designated contact point, in each country participating in VENICE using the dedicated web-based VENICE platform and stored on a secure domain of the website.

The questionnaire focused on several aspects of the HPV decision-making process, namely data sources available and ongoing or completed ad hoc studies to guide the decision-making process (or reasons not to conduct such studies), and the factors driving the decision to introduce HPV vaccination. Countries were also asked to describe their current status with regard to introduction of HPV vaccination.

After the European Commission (DG SANCO) requested additional information, a second version of the HPV vaccine questionnaire was posted on the VENICE website in September 2007. In addition to questions included in the first version, which could be updated if necessary, the second questionnaire asked for further information on the target population, infrastructure for vaccine administration and cost per dose of the vaccine (in countries where the vaccine had been introduced).

During the preparation of the European report of this survey in January 2008 [11] one of the countries participating in VENICE initiated an update of the results by sending an email to the other participants asking for information on their current HPV vaccine decision status. The received information was not standardised and varied in content and detail, nevertheless, we decided to take it into consideration when writing this article. The data that had been consistently supplied was therefore collated and added to the 2007 survey results.

Data analysis

Data from the completed second version of HPV questionnaires (posted in September 2007) were downloaded from the VENICE website on 31 October 2007 and analysed using Microsoft Excel® and Stata v8®.

Analyses were carried out to examine the factors associated with making a recommendation about the introduction of HPV vaccination. For each factor analysed, the proportion in countries where a national vaccine advisory body had made a recommendation (with or without a follow-on decision made by the national health authorities) was compared to the proportion in countries where a recommendation had not been made. In addition to the factors included in the HPV questionnaire, the analysis also took account of other available data potentially associated with making a recommendation, such as the country's Gross Domestic Product (GDP). Fisher's exact test (two-tailed) was used to generate p values, with $p \leq 0.05$ considered to be statistically significant. Quantitative variables were analysed by t-test comparison of means also using $p \leq 0.05$. It was not possible to conduct multivariable analysis, following univariable analysis, due to the limited number of observations.

Results

Completed second version HPV questionnaires were received from 27 of the 28 participating countries (all except Poland) in September/October 2007 (96% participation rate). The answers given by Poland in the initial questionnaire of January 2007 were used where possible, and so the study denominator value varies from 27 for the additional information requested by DG SANCO to 28 for unchanged questions. Following the email request to update the HPV vaccine decision-status initiated by one of the countries in January 2008, updated information was received from 27 of the 28 VENICE participating countries (all except Czech Republic).

Status of countries concerning the introduction of HPV vaccination

The process of introducing a new vaccine into the national immunisation schedule in European countries commonly occurs in two steps, firstly, a recommendation is made by a national vaccine advisory body, secondly, an official decision is taken by the national health authorities. As of 31 October 2007, the advisory bodies in 12 countries (44%) had made a recommendation (in all cases positive) regarding the introduction of HPV vaccination into the national immunisation schedule (Austria, Belgium, Denmark, France, Germany, Greece, Italy, Luxembourg, Norway, Slovakia, Spain and the United Kingdom (UK)). The national health authorities in five of these countries (Austria, Germany, France, Italy and the UK) had subsequently taken the decision to introduce HPV vaccination into the national immunisation schedule [12,13,14,15,16] whereas a decision was still pending in the remaining seven countries. No distinction was made in the questionnaire regarding the nature of the HPV vaccine (bivalent or quadrivalent) to be used in the national immunisation schedule.

Vaccination policy in countries where HPV vaccination was introduced

The HPV vaccination policies adopted in the five countries where HPV vaccination was included in the national immunisation programme are summarised in Table 1. The variation in the target populations by country is notable, with differences not only in the ages of targeted females, but in the targeting of boys/young males (recommended in Austria) and the catch-up campaigns to be

conducted (France and the UK). Only Italy anticipated differences in policies adopted between national and regional levels, as it is believed that some regions may decide to implement catch-up campaigns for females older than 11 years. The UK recommends administration principally via a school-based programme, but the final decision on delivery will be made at local level. The four remaining countries reported plans to use routine channels for vaccine administration.

The HPV vaccine is offered free of charge to the target population in Germany, Italy and the UK. In France, 65% of the cost is borne by the social security scheme and the remaining 35% is the responsibility of the individual or borne by a complementary voluntary insurance. A decision regarding reimbursement of HPV immunisation is still pending in Austria (as of October 2007).

Among the five countries that decided to introduce HPV vaccination, France, Italy and the UK reported that vaccine coverage data would be available for the primary target groups. All five countries reported the integration of HPV vaccination safety surveillance into the routine pharmaco-vigilance system. France and Italy also reported putting in place specific studies/systems to follow up the safety in adolescents/adults.

Basis for decision regarding introduction of HPV vaccination into immunisation schedules

Seven countries, including four that had taken the decision to introduce the vaccine (Austria, Germany, France, Italy) and three who in October 2007 anticipated taking such a decision in the future (Greece, Slovenia, Slovakia), reported the drivers for the decision. The principal drivers were favourable cost-effectiveness ratios and anticipated epidemiological impact on pre-cancerous and cancerous lesions (Table 2).

Epidemiological data available and ad hoc studies used to support a decision about vaccine introduction

Cervical cancer screening programmes were reported as operating in 24 countries (86%) (all except Belgium, Bulgaria, Cyprus and Luxemburg). The estimated proportion of the eligible age group reached by each country's screening programme varied from 9% to 100% (among the 16 countries submitting data), with 75% or more in Finland, Iceland, Norway, Poland, Spain, Slovenia, Sweden, UK and 25% or less in Ireland, Latvia and Slovakia.

Twenty-five countries (89%, all except Estonia, Greece and Romania) reported having data available on the incidence/prevalence of pre-cancer lesions (CIN2/3) or on the incidence of cervical cancer. Information on both conditions is available in 17 countries.

At least one ad hoc study was undertaken by 14 (50%) of the surveyed countries to support the decision-making process for HPV vaccine introduction. These included: disease burden studies, mathematical modelling studies and/or economical assessments. At the time of the survey, 11 countries (39%) had either completed or were currently undertaking HPV infection disease burden studies (Denmark, Finland, Germany, Iceland, Italy, The Netherlands, Poland, Portugal, Spain, Sweden, UK), including three of the five countries that have decided to introduce the vaccine.

Mathematical modelling projects to support the decision-making process for HPV vaccination introduction were reported as complete or ongoing by seven countries (Denmark, France, Germany, Italy, Norway, Portugal, UK), including four of the five countries that have decided to introduce the vaccine. Of these countries, two have used 'home-made models' (Denmark and UK) while the remaining five used models developed elsewhere. A state transition static model

Table 1

Details of HPV vaccination introduced into the national immunisation schedules of European countries as of 31 October 2007 (N=5); VENICE 2007 survey

Characteristic	France	Germany	Italy	Austria	United Kingdom
Target population	14-year-old females	12-17-year-old females	11-year-old females	Females/ boys/ young males before sexually active	12 -13-year-old females
Catch-up campaign	15-23-year-old female virgins or girls who started their sexual life <12 months ago (from July 2007)	No	No (maybe on a regional level)	No	Catch-up campaign to be conducted

Table 2

Principle drivers of decision to introduce HPV vaccination into the national immunisation schedules of European countries as of 31 October 2007 (N=7); VENICE* 2007 survey

Drivers of decision to integrate HPV vaccination	Average score from respondents*
Favourable cost-effectiveness ratios	4.0
Anticipated epidemiological impact on pre-cancer lesions	4.0
Anticipated epidemiological impact on cancer lesions	4.0
Social demand	3.6

* 1 = not considered in taking the decision, 5 = main driver of decision

Table 3

Principle reasons for undertaking neither mathematical modelling nor economical studies to support the HPV decision-making process (N=14*); VENICE* 2007 survey

Reasons for not undertaking studies	Countries	
	n*	%
Similar studies already performed by other countries sufficient	5	36
Lack of available financial resources	5	36
Usually not considered in decision process	3	21
Lack of expertise available	3	21

* Countries could select multiple answers. Numbers in table will therefore not add up to the denominator of 14 (and 100%)

was favoured by two countries (France and Portugal), a dynamic model by four and a combined model by one country (Denmark). All seven countries included the existence or absence of a current screening (pap smears) programme in their models. All, except one (Denmark), tested female-only immunisation strategies. The age range considered for the target population varied from 11-12 years to 10-26 years.

Economic assessments to support the decision-making process for HPV vaccination introduction were reported by 11 countries (39%) (Denmark, Finland, France, Iceland, Italy, Luxembourg, Netherlands, Norway, Portugal, Sweden and UK), including three of the five countries that have decided to introduce the vaccine. All of the countries submitting details of their analyses (N=10) had carried out cost-benefit or cost-effectiveness studies and eight countries (80%) had factored quality of life indicators into their assessment.

No studies to support the decision-making process, as defined by this survey, had been undertaken by 14 countries (50%) as of October 2007. In two of these (Greece and Slovakia) a recommendation favouring the introduction of HPV vaccine had been made and in one (Austria) a decision had been taken. The most commonly reported reasons for not embarking on such studies were the lack of available financial resources and the belief that similar investigations performed earlier by other countries were sufficient (Table 3).

Factors associated with making a recommendation about introducing HPV vaccination

The availability of epidemiological data to support analysis for the decision-making process (e.g. cancer registry data, cervical screening coverage figures, incidence of cervical cancer) does not appear to be a factor associated with having made a decision about HPV introduction (Table 4). A greater proportion of countries that made a recommendation had completed a mathematical modelling project or had undertaken an economic assessment although neither association attained statistical significance (Table 4).

In terms of factors not featured in the VENICE survey, larger country population (Eurostat 2006 data) and higher GDP (International Monetary Fund (IMF) 2005 data) were associated with making a recommendation (p values < 0.01) (Table 4). Countries having made a recommendation had a lower mean coverage rate of first dose of measles containing vaccine (MCV) according to World Health Organisation (WHO) 2005 data than countries not having made a recommendation (89.6% versus 94%, p=0.04). Geographic location was not statistically significant; however, 50% (6/12) of countries having made a recommendation about introduction are located in Western Europe (defined as Ireland, UK, France, Belgium, Germany, Austria, Luxembourg, The Netherlands) whereas countries in this region of Europe account for 30% (8/27) of surveyed countries (Table 4).

Table 4

Factors associated with making a recommendation about introducing HPV vaccination into the national immunisation schedule of a country (univariable analysis) (N=27); VENICE 2007 survey

Factor	Recommendation made (N=12)		Recommendation not made (N=15)		p value
	n	%/mean	n	%/mean	
Data to support analyses for decision-making process					
Availability of different types of epidemiological data to support analyses needed for the decision-making process (score[a] range per country 0-5)[b]	12	3.9[b]	15	3.9[b]	1.0
Ad hoc studies to support decision-making process					
1. HPV infection burden studies (completed project)	1	8	3	20	0.605
2. Mathematical modelling to evaluate the expected epidemiological impact of vaccination (completed project)	3	25	1	7	0.29
3. Economic assessment undertaken	6	50	5	33	0.45
Additional factors investigated					
1. Country population size (millions)[b] (Eurostat 2006 data)	12	30.7[b]	15	5.9[b]	0.004
2. Europe's geographic region:[c]					0.09
north (N=5)	2	17	3	20	
south (N=6)	3	25	3	20	
east (N=8)	1	8	7	47	
west (N=8)	6	50	2	13	
3. National GDP (millions $US)[b] (IMF 2005 data)	12	965,163[b]	15	115,633[b]	0.003
4. Coverage of first dose of MCV [b] (WHO 2005 data)	12	89.6[b]	15	94.0[b]	0.04

[a] Score based on a count of the five types of data surveyed in the questionnaire (five data sources: mandatory notification of cervical cancer, existence of cancer registries including cervical cancer, existence of a cervical cancer screening program, data on the incidence/prevalence of pre-cancer lesions (CIN2/3), data on the incidence of cervical cancer)
[b] Comparison of two means
[c] North: Norway, Sweden, Finland, Denmark, Iceland.
South: Portugal, Spain, Italy, Greece, Slovenia, Cyprus.
East: Estonia, Latvia, Lithuania, Poland, Slovakia, Romania, Bulgaria, Hungary.
West: Ireland, United Kingdom, France, Belgium, Germany, Austria, Luxembourg, the Netherlands.

Update of HPV vaccine decision status, January 2008

A change in the HPV vaccine status of seven countries participating in VENICE was noted as of 31 January 2008. Specifically, the vaccine advisory bodies of Bulgaria and Slovenia recommended the introduction of HPV vaccination, while the national health authorities in Belgium, Greece, Luxembourg, Portugal and Spain decided to introduce HPV vaccination into the national immunisation schedule. This takes the number of European countries that have made a recommendation, all favouring vaccine introduction, to 15 and the number of countries where an official decision has subsequently been taken to 10 (data as of 31 January 2008 for N=27, and 31 October 2007 for Czech Republic).

Discussion

This study is the first documentation of the status of European countries regarding HPV vaccination and it deconstructs the decision-making process which leads to the introduction of a new vaccine into the national immunisation schedule. Within the objectives of the VENICE project, the introduction of HPV vaccination in Europe has provided a unique opportunity for real-time examination of the decision-making process. The high participation rate in this study indicates the high level of interest in this issue among European countries and the effectiveness of the VENICE network as a means of collecting and sharing vaccination information at European level.

In the sixteen months (up to 31 January 2008) following the European licensing of the first HPV vaccine, Gardasil®, the national health authorities of ten MS decided to introduce HPV vaccination into the national immunisation schedule, while another five countries started the decision-making process with a recommendation favouring introduction. It is noteworthy that all advisory bodies that made a recommendation advised the introduction of the HPV vaccine and all national health authorities that made a decision opted for the integration of the HPV vaccination into the national immunisation programme. This suggests a high public health priority given to HPV vaccination which probably reflects the high expected gain from a vaccine that can prevent cancer.

The survey results show that the countries that decided to introduce HPV vaccination adopted varying vaccination policies. This is particularly evident in terms of target ages and catch-up campaigns. Such a result is not unexpected considering the variety in national immunisation programme delivery services and diversity of health services infrastructures in European countries. Regardless of the vaccination policy adopted, all four MS (as of October 2007) that made a decision about the reimbursement of the vaccine have chosen to reimburse vaccination either fully or partially.

Underlining the need for data to support the decision-making process, four of the five MS (as of October 2007) that decided to introduce HPV vaccination had undertaken at least two ad hoc studies (disease burden study, mathematical modelling study or economic assessment).

Countries where no such projects were undertaken reported the lack of financial resources and the belief that similar studies performed by other countries were sufficient as principle reasons for not carrying out ad hoc studies. This highlights the need for collecting information on such projects at European level and for collaboration between countries to share expertise and experience in order to minimise the number of redundant studies that can drain the limited health resources.

Germany, the UK, France and Italy, four of the five countries where HPV vaccination was introduced (as of October 2007) are the top four ranked European countries in terms of national GDP. This fact could explain the observed association between a higher national GDP and an introduction-decision being already made. A higher national GDP may also reflect a genuine greater capacity to fund routine HPV vaccination in these countries.

It is also worth noting that among the five northern European countries only two (Denmark and Norway) made a recommendation to introduce the HPV vaccination and none actually took the decision (as of January 2008) despite the fact that these countries generally have a well-developed public health infrastructure and also potentially have the resources needed to fund a routine HPV vaccination. Four of these countries (Sweden, Finland, Iceland and Norway) reported a target population coverage rate for the national cervical cancer screening programme above 75%, which raises a question about the possible impact of a successful screening programme on the decision not to introduce HPV vaccination.

The limited number of countries in the survey is likely to have affected the statistical power of the analysis of factors associated with making a recommendation about introducing HPV vaccination. We therefore cannot conclude from these data whether the availability of epidemiological data and the undertaking of ad hoc studies are associated with a more rapid decision making process.

The update initiated by one of the participating countries in January 2008 highlights the rapidly evolving situation once a new vaccine is licensed in Europe and the desire of the relevant authorities to have a European perspective on the introduction process. The VENICE project has developed a European network capable of answering this demand, not just for HPV vaccination but for other recently licensed vaccines such as rotavirus vaccines (for which a survey similar to that described here has been conducted) and combined MMR-varicella vaccines.

Conclusion

The deconstruction of the decision-making process concerning the introduction of HPV vaccine into national immunisation schedules has shown, in real time, that there is expertise and experience available among European countries that could be collated and shared. A collaborative space is being developed on the VENICE website that will serve as an inventory for information of this sort. This inventory will be available to participating countries and European institutions such as the European Centre for Disease Prevention and Control and DG SANCO. It is hoped that this web-based space will facilitate future collaborations between MS relating to vaccine-policy decisions and broader vaccination related activities.

The VENICE project is scheduled for completion in December 2008. It is planned that the European Centre for Disease Prevention and Control will take over responsibility for the project in 2009 with a view to maintaining and further developing an already well functioning network of European vaccination public health professionals.

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Survey of rotavirus surveillance, laboratory capacity and disease burden in the eastern part of the WHO European Region

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Following the licensure of two rotavirus vaccines in Europe, we aimed to assess factors, such as surveillance, disease burden and laboratory capacity, which will be relevant for making decisions about rotavirus vaccine introduction in the different countries.

We conducted an email-based survey of the national public health bodies in the World Health Organization (WHO) European Region in 2006 and report here the results from the 23 countries in the eastern part of the region. The survey included questions on rotavirus surveillance, laboratory capacity, burden (in children under the age of five years) and intention to introduce rotavirus vaccination. Countries were grouped into the four per-capita income categories defined by the World Bank.

Fourteen of the 23 countries responded to the survey. All except one country reported that less than a quarter of their laboratories had rotavirus diagnostic capacity. Four countries had some form of specific rotavirus surveillance, but half were of very limited coverage. Ten countries did not report data on the incidence of rotavirus hospital admissions, although nine were able to report some data on rotavirus burden. Six of the responding countries said they were likely to introduce universal rotavirus vaccination.

Rotavirus surveillance and laboratory capacity in the eastern part of the WHO European Region is limited but most countries had some estimate of rotavirus burden, often from special studies. The reported mortality rates were lower than those from a WHO mortality data source. Many countries in the eastern part of WHO European Region face a number of challenges before vaccine implementation, including strengthening surveillance, improving laboratory capacity and addressing financial barriers.

Introduction

The recent publication of the results of phase III trials of two oral rotavirus vaccines [1,2] showed vaccines that were effective in preventing serious clinical end points of rotavirus infection. The vaccines provide 85-95% protection against rotavirus infections severe enough to require hospitalisation, and 72-74% protection against all rotavirus infections [1,2]. However, vaccination appeared to protect only against disease, not to reduce the overall incidence of rotavirus gastrointestinal infection in the target group [2,3]. Following the licensure of both vaccines by the European Medicines Agency (EMEA) [4,5], there has been renewed interest in preventing rotavirus disease in Europe, with many countries considering the introduction of rotavirus vaccine into their routine immunisation schedule.

The introduction of these vaccines will depend upon a number of country-specific factors. These include local disease burden (mortality and morbidity), diagnostic and surveillance capacity, cost of the vaccine (which is relatively expensive [6]), vaccine effectiveness and adverse events profile, as well as competing healthcare priorities.

Rotavirus infections cause a considerable disease burden throughout the world. The burden of rotavirus disease tends to fall predominately on children under the age of five years [7,8], with an estimated half million deaths annually attributable to rotavirus in children under five years mainly in lower income settings [9].

A recent study estimated annual rotavirus disease burden in the (at that time) 25 countries of the European Union at 231 deaths and nearly 90,000 hospital admissions [10]. In the World Health Organization (WHO) European Region, which covers 53 countries, there is some evidence that the burden of acute gastroenteritis (AGE) is higher in some countries in the eastern part of the region [11,12]. However, there are fewer published studies of rotavirus disease burden in these areas. The countries without published burden studies may be able to supply burden estimates based on their own surveillance data, or special studies, helping to fill gaps in the burden profile.

Which countries might consider introducing universal childhood rotavirus vaccination? Each country may have different priorities in making such decisions. Higher-income countries may try to reduce primary care consultations, hospitalisations and nosocomial infections by vaccination. Countries with lower incomes and higher AGE mortality rates may find rotavirus vaccination to be life-saving in the under-fives. The GAVI Alliance (Global Alliance for Vaccines and Immunisation) supports rotavirus vaccination initiatives in low-income countries [13].

To provide an overview of the current situation in the eastern part of Europe, we conducted a survey of member states in that part of the WHO European Region. Our objectives were to identify and compare current laboratory capacity and surveillance for rotavirus infection, local disease burden, circulating rotavirus strains and priorities regarding possible vaccine introduction. This comparative information has been shared with the responding countries to assist national decision-making.

Methods

We sent, by email, questionnaires in English and Russian to 49 of the 53 countries in the WHO European Region. The surveys to the 23 countries in the eastern part of the region* were sent out from the WHO Regional Office for Europe (WHO/Europe) in Copenhagen, Denmark and the remainder from the Health Protection Agency Centre for Infections in London, United Kingdom (UK). The survey was addressed to the person in the national public health body in each country who was responsible for national rotavirus surveillance. Two email reminders were sent following the return deadline in January 2006.

We found initially that the response rate for surveys sent from the UK was very low (9 of 26; 35%), and the results suggested that laboratory capacity and data on rotavirus burden was better in the western part than in the eastern part of the WHO European Region. Therefore we present here only the results from the 23 surveys that were sent from the WHO office.

The questionnaire included sections on:

- country-specific details
- laboratory capacity for rotavirus diagnosis
- surveillance systems for gastroenteritis and rotavirus
- reported disease burden (deaths, hospitalisations and primary care consultations due to gastroenteritis and rotavirus)
- and country-specific literature.

It focused on the disease burden in children under the age of five years, as this is the age group most affected by rotavirus [7,8] and the age-range used in comparable literature [9,10]. In addition, countries were asked whether they would be likely to introduce rotavirus vaccination in the next five years, and which factors could influence this decision. Survey results were entered directly into a spreadsheet, and data validity was checked against the written surveys before the analysis.

We obtained per capita annual gross national income (World Bank, Atlas method, 2006 data [14]) for each country. For the burden calculations, countries were grouped according to the four income groups (low: below $905; lower-middle: $906 - $3,595; upper-middle: $3,596 - $11,115; high: over $11,116) defined by the World Bank [15].

Results

Response

Overall, 14 of 23 (Serbia and Montenegro sent separate returns) questionnaires were returned (61%). Countries that did not respond were: Armenia, Azerbaijan, Bosnia and Herzegovina, The Former Yugoslav Republic of Macedonia, Kazakhstan, Romania, Russia, Turkmenistan, and Ukraine.

Among the countries that participated in the survey were seven (of 13) low and lower-middle income countries and seven (of 10) upper-middle and high-income countries (see Table 1).

Laboratory capacity

The median proportion of laboratories with rotavirus testing facilities was 8% (range 0%-100%, Table 1). Among the low and lower-middle income countries, Belarus reported that 100% of the country's laboratories had such facilities, while the other six countries reported diagnostic facilities in fewer than a quarter of their laboratories.

The most common testing methods available were latex agglutination (7/14 countries), enzyme-linked immunosorbent assay (ELISA) (6/14 countries) and polymerase chain reaction (PCR) (4/14 countries). Serology was available in only one, and electron microscopy in only two countries.

Rotavirus surveillance

Regarding the type of surveillance in place the countries could choose rotavirus-specific surveillance systems, syndromic surveillance for AGE (with or without the quantification of rotavirus infections) or special studies.

Table 1

Laboratory capacity for rotavirus diagnostics and available methods

Country	GNI per capita category	Total laboratories for stool diagnostics	Percentage of laboratories with rotavirus diagnostics	Available methods
Kyrgyzstan	Low income	166	24%	ELISA, PCR
Tajikistan	Low income	70	0%	-
Uzbekistan	Low income	60	2%	ELISA
Albania	Lower-middle income	12	8%	ELISA, latex
Belarus	Lower-middle income	11	100%	EM, ELISA, PCR
Georgia	Lower-middle income	62	-	-
Republic of Moldova	Lower-middle income	50	2%	latex
Bulgaria	Upper-middle income	-	-	latex
Croatia	Upper-middle income	-	-	-
Montenegro	Upper-middle income	-	-	latex
Serbia	Upper-middle income	-	-	latex
Slovakia	Upper-middle income	60	17%	ELISA, PCR, serology, latex
Turkey	Upper-middle income	-	-	-
Slovenia	High income	8	-	EM, ELISA, PCR, latex

GNI: Gross national income; EM: Electron microscopy; ELISA: Enzyme-linked immunosorbent assay; PCR: Polymerase chain reaction

Four of the 14 countries, Kyrgyzstan, Uzbekistan, Belarus and Slovenia, reported having a specific rotavirus surveillance system. In Uzbekistan and Kyrgyzstan the system covered only hospitalised cases (with 0.02% coverage reported by Kyrgyzstan), whereas in Belarus and Slovenia community cases were also reported by the system.

Nine of the 10 remaining countries reported having syndromic surveillance for gastroenteritis. Two (Moldova and Slovakia) had systems that quantified the contribution of rotavirus to AGE and the remaining seven did not include quantification of rotavirus infections. Turkey did not answer this question.

Only two countries gave a percentage of the population covered by the system (40% in Albania, and 15% in Serbia).

AGE burden in children aged under the age of five years (Table 2)

Community burden (n=13 responses)

The median incidence of AGE for community cases was 21.8 per 1,000 children per year (range of 7 to 48 per 1,000 per year). The highest community burden was found in Tajikistan with 48 per 1,000 per year.

Hospitalisation burden (n=8 responses)

The median incidence of AGE hospital admissions was 18.9 per 1,000 (7.3 to 782 per 1,000) for hospital admissions, but only 9.9 per 1,000, if the extreme upper-outlier from Albania is excluded. The median community incidence was lower in high and upper-middle income countries (18.7, n=4 countries) than in low and lower-middle income countries (24.9, n=6 countries), and varied from three to 48 per 1,000 cases per year (overall median 21.8 per 1,000).

Reported rotavirus burden in children aged under five years (Table 3)

Nine of the 14 countries provided data on rotavirus burden. These data were based on special studies in five countries, routine data in three, and both routine and special study data in one country.

Community burden

The median incidence of community rotavirus infection was 2.3 per 1,000 per year in low and lower-middle income countries (n=2), and 0.17 per 1,000 per year (n=3) in upper-middle and high income countries (overall median 0.47 per 1,000). The highest estimate of community rotavirus incidence was reported from Belarus. The figures from Serbia and Slovakia were low-extreme outliers. The proportion of AGE due to rotavirus infection in a community setting were 0.7% (Slovakia) and 29.4% (Slovenia), both from routine data sources.

Hospitalisation burden

The incidences of rotavirus hospital admissions in children under the age of five years ranged from 0.13 to 3.2 per 1,000. The median incidence of hospitalised cases was 2.5 per 1,000 per year in low and lower-middle income countries (n=3) compared to 1.5 per 1,000 per year (n=2) in upper-middle and high income countries (overall median 2.5 per 1,000). The median proportion of AGE hospital admissions due to rotavirus was 20.0% (between 1.7% and 28%, n=7). This proportion was lower in high and

Table 2

Reported incidence of acute gastroenteritis in children aged under five years, WHO European Region (all data from routine sources except where specified)

Country (grouped by GNI per capita)	AGE in community (cases per 1,000 per year)	Year of data collection	AGE in hospital (cases per 1,000 per year)	Year of data collection (Special study)
Low income countries				
Kyrgyzstan			12.4	2004 **S**[a]
Tajikistan[b]	48	Not stated		
Uzbekistan				
Lower-middle income countries				
Albania			782	2005
Belarus	7	2005		
Georgia	19	2004		
Rep. of Moldova	29[c]	2005		
Upper-middle income countries				
Bulgaria	28[d]	2004		
Croatia[a]	11	1978 - 2005		
Montenegro[a]	34	2004		
Serbia	3	2004		
Slovakia	25	2003	7.3	1992-2005
Turkey				
High income countries				
Slovenia	42	2004	25.4	2004

GNI: Gross national income; AGE: Acute gastroenteritis.
[a] Internal report: Epidemiology and Rotavirus Disease Burden in Kyrgyzstan 2003-2006; results of hospital-based surveillance;
[b] Combined community and hospital figures;
[c] Age group 0-6 years, not 0-4;
[d] Figure unclear in returned questionnaire – presented as a percentage.

upper-middle income countries (median 6.5%, n=2) than in low and lower-middle income countries (median 25%, n=5).

Reported mortality and case-fatality ratios due to AGE and rotavirus in children under the age of five years

Six countries provided information on mortality due to AGE or rotavirus disease. Croatia, Serbia, Montenegro and Slovenia reported zero mortality due to diarrhoeal disease (including rotavirus) in children under five years. Belarus reported zero mortality due to rotavirus but did not provide any data on mortality due to diarrhoeal disease. Slovakia reported a case fatality rate of 0.5 per 1,000 cases of AGE in children under five years (data from 1954 to 2005, three deaths).

Serogroups

Two of the 14 responding countries supplied data on circulating rotavirus strains (Table 4). Between 56 and 81% of the strains were G1-G4.

Introduction of vaccine

Kyrgyzstan, Uzbekistan, Georgia, Albania and Slovenia stated that they would include the Rotavirus vaccine in routine immunisations given EMEA approval. Belarus gave a tentatively positive answer. Countries stating that they would not be likely to introduce the vaccine were: Republic of Moldova, Serbia, Slovakia, Croatia and Turkey. The countries were not asked about the timescale or degree of commitment to the introduction of the vaccination.

The six countries giving a "yes" or a tentatively positive answer to the question about vaccine introduction were relatively well-prepared, with four of the six having specific rotavirus surveillance systems and all six reporting the availability of one or more rotavirus diagnostic methods.

Disease burden was the most important factor influencing this decision (mean rank 2.2), followed by safety profile (mean rank 2.5), finances for new vaccines (3.9), and vaccine costs (4.0). Disease burden ranked first in all four upper-middle and high-income countries, but only in two of the eight low and lower-middle income countries. An additional influencing factor that was reported was the lack of laboratory capacity.

Table 4

Circulating rotavirus strains reported by responding countries (n=2)

Country	%G1	%G1-G4	%P8	Year(s) of data collection
Albania	12.5%	56.3%	No data	2001
Kyrgyzstan	56.5%	81.5%	63.0%	2004-2005

Table 3

Reported burden of community and hospital rotavirus disease, with contribution of rotavirus to acute gastroenteritis in each setting[a]

Countries (grouped by GNI per capita) Bold=EU 25	Community incidence of rotavirus in children under five years (per 1,000)	Hospital incidence of rotavirus in children under five years (per 1,000)	% AGE caused by RV in community	% AGE caused by RV in hospital
	(Year, Source: R=Routine data, S=Special study)			
Low income countries				
Kyrgyzstan[b]	0.47 (2005 S)[c]	3.2[d]		26% (2003-2006, RS)
Uzbekistan[b]				25% (2004-2005, S)
Lower-middle income countries				
Albania		2.5[e] (2001 S)		20[e] (2001, S)
Belarus	4.2[f] (2005 R)			
Georgia		1.4[g] (1984-1986 S)		28%[g] (1984-6, S)
Republic of Moldova				16.3%[b] (1992-2004, S)
Upper-middle income countries				
Serbia	0.11 (2004 R)			
Slovakia	0.17 (1992-ongoing, R)	0.13 (1992- ongoing,R)	0.66% (1992- ongoing,R)	1.7% (1992- ongoing,R)
High income countries				
Slovenia	12.3 (2004 R)	2.8 (2004 R)	29.4 (2004 R)	11.3 (2004 R)

GNI: Gross national income; AGE: Acute gastroenteritis; RV: rotavirus.
[a] No relevant data reported by Tajikistan, Bulgaria, Croatia, Montenegro and Turkey;
[b] One or more special studies listed in returned questionnaire but not traceable in PubMed;
[c] Possibly a hospital setting;
[d] Derived from AGE incidence (Table 2) and percentage of hospitalised AGE due to rotavirus;
[e] Role of rotaviruses in aetiology of AGE in children (university hospital of Tirana) – not traceable in Pubmed. In this study 20% of AGE admissions were due to rotavirus, so the total AGE admissions would be 12.5 per 1,000, suggesting the survey reply in Table 2 (782 per 1,000) is incorrect;
[f] Ages 0-6 not 0-4;
[g] Doctoral thesis: Epidemiology of rotavirus gastroenteritis in Georgian SSR of 1990.

Gaps in knowledge

Table 5 shows that less than one-third of the responding countries provided data on the incidence of rotavirus hospital admissions, and only half had information on the contribution of rotavirus to AGE hospital admissions. Only six countries returned data on mortality or case-fatality due to AGE or rotavirus, and of these only two reported an AGE or rotavirus mortality that was not zero.

Over a third (5 of 14) countries did not return any data on rotavirus burden. In four of the nine countries that sent some information about rotavirus burden, the data were derived from special studies only.

Discussion

This study was conducted in 2006, before WHO/Europe and its partners began to support the introduction of rotavirus surveillance in several countries of the WHO European Region (Azerbaijan, Georgia, Tajikistan and Ukraine). It describes the first review in the WHO European region of rotavirus surveillance, laboratory capacity, and willingness to introduce these newly developed and licensed vaccines. The results of the survey show, at least for the 14 countries that returned the questionnaire, that the current capacity for rotavirus surveillance and laboratory diagnosis is heterogeneous in the region. Gaps in the knowledge of rotavirus burden existed in a number of countries, although according to those countries that were able to provide data, rotavirus contributes considerably to hospital admissions due to diarrhoea.

Surveillance systems

Specific surveillance systems for rotavirus infections were present in less than one third of the surveyed countries, and in half of them the reported coverage was limited. Nevertheless, most responding countries had sufficient data from routine sources or special studies to give an estimate of the burden of rotavirus disease in the community or in hospitals, which would assist in making an informed decision regarding the potential introduction of the vaccine.

Laboratory capacity

In terms of laboratory capacity, most responding countries had access to either ELISA or latex tests for rotavirus detection. ELISA is currently the method of choice for most laboratories, being more sensitive than the latex assays [16] and more specific for clinically relevant infections than reverse transcription polymerase chain reaction (RT-PCR) which may also identify asymptomatic infections [17]. However, rotavirus diagnostic capacity was generally poor in the lower-income countries, in which – with one exception – less than one quarter of the laboratories had diagnostic facilities. Whilst diagnosis is not always clinically necessary in low-income settings, its lack limits the options for monitoring rotavirus burden. A regional laboratory network for rotavirus surveillance in the WHO European Region has recently been established [18], and this should improve laboratory capacity with development of standards, frequency of testing, and analysis of circulating strains. The initial members were Denmark, Finland, France, Germany, Hungary, Italy, Netherlands, Slovenia, Spain, Sweden and the United Kingdom. Further countries may be included as the network expands [18].

Table 5

Laboratory capacity and burden information, all countries responding to the survey (n=14)

Country (Bold for EU 25)	Percentage laboratories with rotavirus diagnostics	Any rotavirus burden data?	Incidence of rotavirus hospital admissions reported?	Rotavirus contribution to AGE admissions reported?	Rotavirus data sources
Low income countries					
Tajikistan	0%	No data	No data	No data	NA
Kyrgyzstan	10%	√	No data	√	Routine and special studies
Uzbekistan	2%	√	No data	√	Special studies
Lower-middle income countries					
Albania	No data	√	√	√	Special studies
Belarus	100%	√	No data	No data	Routine data
Georgia	5%	√	√	√	Special studies
Republic of Moldova	2%	√	No data	√	Special studies
Upper-middle income countries					
Bulgaria	No data	No data	No data	No data	NA
Croatia	No data	No data	No data	No data	NA
Montenegro	No data	No data	No data	No data	NA
Serbia	No data	√	No data	No data	Routine data
Slovakia	17%	√	√	√	Routine data
Turkey	No data	No data	No data	No data	NA
High-income countries					
Slovenia	100%	√	√	√	Routine data
Overall percentage of missing data	**43% (6/14)**	**36% (5/14)**	**71% (10/14)**	**50% (7/14)**	

NA: not applicable

Participation in the eastern part of the WHO European Region, however, remains limited.

Community and hospital burden

The community incidence estimates were not significantly greater than hospital incidences and showed greater variability. The true ratio of rotavirus community cases to hospital admissions has been estimated at eight [10], suggesting that the community incidences reported here are substantially underestimated. Differences in laboratory methods and testing policies may at least partially account for underestimation of community rotavirus incidences. For these reasons, and because the vaccines are more effective against severe disease [1,2], surveillance for rotavirus hospitalisations and deaths is likely to provide more useful indicators than surveillance for all infections.

The gaps in knowledge about the burden of severe rotavirus infections were especially marked for hospitalisation data with 71% of countries not able to provide data on the incidence of hospital admissions due to rotavirus infection. However, recent developments suggest that the situation is improving: Two low-income countries, Uzbekistan and Kyrgyzstan have started hospital surveillance for rotavirus, albeit with low overall coverage. Uzbekistan has recently undertaken a cost-effectiveness study for rotavirus vaccination [19]. Azerbaijan, Georgia, Tajikistan and Ukraine initiated sentinel hospital surveillance in late 2006. WHO/Europe has developed an accessible database of hospital admission statistics for a number of countries in the WHO European region [20]. This will be useful for future comparative studies of rotavirus burden.

Rotavirus mortality

Rotavirus infection is an important cause of death in low and lower-middle income countries worldwide [9]. None of the countries in these income categories were able to provide data on deaths due to rotavirus infections. The countries that did provide data on mortality, with one exception all reported that they had not had any deaths due to AGE or rotavirus.

The mortality data sent from those countries were at odds with WHO mortality data [21], which estimate that diarrhoeal disease contributes to between 0.3% (Croatia) and 6% (Serbia) of all deaths in children under the age of five years. It is likely that the death certification data supplied by countries to WHO is not collected in the same way or by the same departments as the data supplied by the people completing our survey. Public health authorities producing epidemiological data on the burden of gastrointestinal illness, which may be used by decision-makers for vaccine programmes, should use the death certification data to validate their mortality estimates.

Rotavirus strains

Only two countries returned data on circulating strains, and the available analyses were from different years. The strain categories correspond to those found in the licensed vaccines, G1P[8] in the monovalent Rotarix™ vaccine (GSK Biologicals, Belgium), and G1-G4 and P[8] in the pentavalent RotaTeq® vaccine (Merck&Co. Inc, USA)

The lack of up-to-date information suggests that strain analyses are not done routinely in this part of the European region. The literature on rotavirus strains circulating in the European Union has been reviewed, including considerably more information than was gathered in the survey [22]. The predominant strain can shift rapidly, as was recently observed in Spain where the usual G1 P[8] and G4 P[8] strains found between 1997 and 2004 were found to have been supplanted by G(9) P[8] in a 2005 study [23]. Therefore countries considering the introduction of rotavirus vaccination should, at least intermittently, monitor the circulating strains. Work needs to be undertaken to extend strain identification in the Eastern part of the European region.

Study limitations

The main limitations of our study are the low response rate, the challenge of responding to hypothetical questions on vaccine introduction, and the variability of responses relating to disease burden. Although only 14 of 23 countries responded, we did obtain responses from all the low income countries and nearly half of the low-middle income countries. The reported incidences of AGE and rotavirus infections varied widely and in some cases (Slovakia, Albania) were likely to be under- or over-estimated: The Albanian estimate of 782 admissions for AGE per 1,000 per year is at odds with their quoted incidence of rotavirus admissions (2.5 per 1,000). The low estimates of rotavirus incidence in Slovakia may be due to the reported low coverage of the surveillance system, with only 1% of stool samples being tested for rotavirus.

Conclusions

In summary, our study shows that rotavirus surveillance and diagnosis capacity was heterogeneous in the responding countries in the eastern part of the WHO European Region, with significant gaps in disease data and laboratory capacity. This lack of diagnostic and routine surveillance activity need not prevent countries from making a decision, based on their own measured disease burdens, on whether to introduce rotavirus vaccine. A time-limited epidemiological or surveillance study should be sufficient and indeed is necessary to provide an estimate of current rotavirus burden to make an informed decision regarding inclusion of any vaccine. Several countries have already undertaken such studies.

For countries that decide to adopt a universal rotavirus vaccination programme, it is critical to introduce and/or maintain surveillance for rotavirus infections or their contribution to the gastroenteritis burden in order to assess the programme's impact, effectiveness and safety. Focusing on hospitalised cases and deaths may be the most cost-effective method. Surveillance will require sufficient laboratory capacity, and should also include a facility or access to a facility for monitoring circulating strains (in case of strain replacement).

The financial implications of a possible introduction of universal vaccination will be a major issue due to the cost of the vaccine. This will be of particular significance in low-income countries where the burden of severe rotavirus disease is likely to be greater than in wealthier countries. Consideration will need to be given to financing schemes supporting the introduction of rotavirus vaccine at reduced cost in these settings, as recently proposed through second stage of GAVI investment in rotavirus vaccines, in which low income countries will be potentially supported [13].

* The 23 countries were: Albania, Armenia, Azerbaijan, Belarus, Bosnia and Herzegovina, Bulgaria, Croatia, The Former Yugoslav Republic of Macedonia, Georgia, Kazakhstan, Kyrgyzstan, Moldova, Romania, Russia, Montenegro, Serbia, Slovakia, Slovenia, Tajikistan, Turkey, Turkmenistan, Ukraine and Uzbekistan.

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Impact of infant pneumococcal vaccination on invasive pneumococcal diseases in France, 2001-2006

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Vaccination with the 7-valent pneumococcal conjugate vaccine (PCV) has been recommended in France since 2003 for children under the age of two years who are at risk due to medical or living conditions. From 2006, the recommendation has been extended to all children under two years. The impact of PCV introduction on the incidence of pneumococcal meningitis and bacteraemia and on the serotype distribution in French children and other age-groups was assessed using laboratory surveillance data. The coverage with three doses of PCV was 44% in children aged 6-12 months in 2006. From 2001/2002 to 2006, the incidence of pneumococcal meningitis decreased from 8.0 to 6.0 cases per 100,000, and the incidence of pneumococcal bacteraemia decreased from 21.8 to 17.5 cases per 100,000 in children under the age of two years. For the vaccine strains, the incidence of pneumococcal meningitis and bacteraemia decreased from 20,4 to 6.0 cases per 100,000, while the incidence of pneumococcal meningitis and bacteraemia due to non-vaccine strains increased from 9.4 to 17.5 cases per 100,000 in this time period. The incidence in older children and adults did not decrease.
Further expansion of PCV coverage is expected to increase the impact of the vaccination in both children and adults. However, the fact that cases caused by vaccine serotypes have been partially substituted by cases of non-vaccine serotypes is likely to reduce the overall benefit of PCV in France, should this early observation be confirmed in the future.

Introduction

Streptococcus pneumoniae causes a wide spectrum of diseases, ranging from upper respiratory tract infections to severe invasive diseases. *S. pneumoniae* is the main cause of bacterial meningitis in France [1,2]. Invasive pneumococcal diseases (IPD) are more frequent in young children and the elderly and are associated with high case fatality ratio. The fatality ratio for pneumococcal meningitis has been estimated at 11% in children under the age of two years in a recent French study [3].

Two pneumococcal vaccines are currently licensed in Europe. The 23-valent pneumococcal polysaccharide vaccine was licensed in the 1980s and, although recommended for high risk individuals and elderly in many European countries, is poorly immunogenic in children under two years of age [4]. The 7-valent pneumococcal conjugate vaccine (PCV) was licensed in Europe in 2001, is immunogenic in young children and covers serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. These serotypes account for between 43% and 75% of IPD in children under the age of 18 years in Western Europe [5].

Introduction of PCV in the United States in 2000 led to a dramatic decrease in those IPD that are due to vaccine serotypes, and an overall decrease of 80% in all IPD in children under the age of five years [6-8]. PCV vaccination of children had also a beneficial impact in older unvaccinated cohorts [6,7]. This herd effect is attributed to the reduction of pneumococcal carriage in the oropharynx of young children after PCV vaccination, reducing the transmission of vaccine-type pneumococcal strains to unvaccinated children and adults [9]. A slight increase in IPD due to non-vaccine serotypes was observed in American children after the introduction of PVC [7,8]. This did not significantly affect the overall reduction in pneumococcal disease incidence in American children [7,8], but has a been found to negatively affect the impact of PCV vaccination in high risk populations such as native Alaskan children [10].

In France, PCV has been recommended since 2003 for children under the age of two years who are at risk due to medical or living conditions (children in day care with at least two other children for more than four hours per week, children in families with more than two children, or children breast-fed for less than two months [11]). 79% to 89% of children under two years fall in this category in France [12]. Since June 2006, PCV vaccination has been extended to all children under the age of two years [13]. The French vaccination schedule for PCV contains three doses at the ages of two, three, and four months, administered together with the vaccines against diphtheria, tetanus, poliomyelitis, pertussis and *Haemophilus influenzae* type b, and then a booster at the age of 12-15 months.

The impact of PCV on IPD incidence at the national level has not been assessed in France. Moreover, as the serotype coverage of PCV appears to be lower in Europe than in North America, it is of particular interest to analyse the impact of this vaccine on pneumococcal incidence and serotype distribution in France and in other European countries.

We used surveillance data to evaluate the effect of PCV vaccine recommendations for children at risk, a definition that encompasses the majority of each birth cohort, on the incidence of pneumococcal invasive disease and serotype distribution in 2006, four years after the introduction of PCV.

Methods

Data collection

Pneumococcal surveillance in France relies on two hospital-based laboratory surveillance networks, Epibac and the National Reference Centre for Pneumococci (NRCP) network. Since 1987, Epibac, a national hospital-based laboratory network, has collected data on six severe invasive bacterial diseases including *S. pneumoniae*. Pneumococcal invasive cases are defined as the isolation of *S. pneumoniae* from blood (bacteraemia) or cerebrospinal fluid (meningitis). The participating hospital-laboratories collect information on pneumococcal invasive cases prospectively and report annually to the Institut de Veille Sanitaire. The collected data include age, sex, and site of isolation. In 2006, 307 laboratories participated, covering 79% of the French acute care hospital admissions.

Since 2001, all pneumococcal strains isolated from cerebrospinal fluid and from blood in children under 15 years-old have been collected from hospital-laboratories and sent to the NRCP by 22 regional laboratories organised into a pneumococcal surveillance regional scheme (Observatoires Régionaux des Pneumocoques). NRCP serotyped all collected strains using latex particles sensitized with a panel of antisera that was purchased from the Statens Serum Institut (Copenhagen, Denmark) and allowed to determine 90 serotypes. Pneumococcal strains with known serotypes from the Statens Serum Institut and from the NRCP collection were used as internal quality controls.

Data analysis

The annual incidence of pneumococcal bacteraemia and meningitis cases was calculated using the number of cases reported to the Epibac network as the numerator and the French population covered by Epibac participating hospitals as the denominator. The latter was estimated from the proportion of national public and private acute-care hospital admissions covered by the participating laboratories. This proportion was computed using the National Hospital Annual Activities Database which is an exhaustive source of information regarding inpatient hospital stays, managed by the Directorate for Research, Studies, Evaluation and Statistics (DREES) at the Ministry of Health. French population data is issued each year by the National Institute for Statistics and Economical Studies (INSEE).

Age-specific incidence rates were calculated in the same way using INSEE population data by age. Serotype/age-specific incidence rates for pneumococcal bacteraemia and meningitis were estimated by applying the age distribution of pneumococcal serotypes from the NRCP to age-specific incidence rates. For this analysis, serotypes 4, 6B, 9V, 14, 18C, 19F and 23F were grouped as vaccine serotypes (VT), and other serotypes as non-vaccine serotypes (NVT).

Data from 2001 and 2002, representing the pre-vaccination situation, were aggregated.

Confidence intervals (CI) for incidence rates were estimated using Poisson distribution. Differences in age-specific and serotype/age-specific pneumococcal bacteraemia and meningitis incidence rates between 2001/2002 and 2006 were tested using Fisher's test for binomial data. Statistical analysis was performed using Stata 9.0 (Stata Corporation, College Station, Texas).

Due to the very recent introduction of the PCV vaccine into the immunisation schedule in France, data are not yet available from the routine infant vaccination coverage monitoring tool, which is based on the health certificates filled in for each child at the age of 24th months. Instead we used data based on PCV sales for the trend analysis. In addition, three specific interview studies, one in 2004, one in 2006 and one in 2007, were conducted in representative samples of French mothers including 1,739, 1,008 and 1,005 mothers, respectively [12,14].

Results

Vaccine coverage

PCV sales increased from 0,6 to 1,6 doses per child under two years between 2003 and 2006. Coverage with three doses of PCV was estimated in three specific surveys at 27% in six month-old children in 2004 [12], at 44% in 6-12 month-old children in 2006 and at 56% in six to 12 month-old children in 2007 [14].

Pneumococcal meningitis and bacteraemia in 2001-2002

In 2001/2002, Epibac hospital-laboratories reported 7,469 cases of pneumococcal bacteraemia and 771 cases of pneumococcal meningitis. 181 (24%) cases of pneumococcal meningitis and 493 (7%) cases of pneumococcal bacteraemia occurred in children under two years; 194 (25%) cases of pneumococcal meningitis and 3,806 (25%) cases of pneumococcal bacteraemia occurred in adults over the age of 64 years. The reported number of cases and the estimated incidence by age-group are presented in Tables 1 and 2. The annual incidence of IPD in France was estimated at 9.4 cases per 100,000 population (95% CI [9.2, 9.6]) in 2001/2002.

Evolution of pneumococcal meningitis and bacteraemia incidence by age from 2001/2002 to 2006

From 2001/2002 to 2006, pneumococcal meningitis in children under two years decreased from 8.0 to 6.0 cases per 100,000 population, a decline of 25% (95% CI [2,43], p=0,04), and pneumococcal bacteraemia decreased from 21.8 to 17.5 cases per 100,000 population, a decline of 20% (95% CI [6,32], p=0,04). In the same period, pneumococcal meningitis incidence showed a not statistically significant increase from 0.69 to 0.73 cases per 100,000 population (+6% 95% CI [-7,21]) and pneumococcal bacteraemia incidence showed a statistically significant increase from 8.2 to 9.0 cases per 100,000 population (+11% 95% CI [6,15]) in older children and adults (Tables 1 and 2).

Incidence by serotype

In children under two years, the overall decrease in pneumococcal meningitis and bacteraemia incidences was associated with a shift in serotype distribution, NVT pneumococcal meningitis and bacteraemia cases partially replacing VT pneumococcal meningitis and bacteraemia cases (Figures 1 and 2). VT pneumococcal meningitis incidence decreased from 5.6 to 1.0 cases per 100,000 population, a 81% decline (95% CI [67,89], $p<10^{-3}$) and VT pneumococcal bacteraemia decreased from 14.8 to 5.3 cases per 100,000 population, a 64% decline (95% CI [53,72], $p<10^{-3}$) from 2001/2002 to 2006. In the same time period, NVT pneumococcal meningitis incidence increased from 2.4 to 4.9 cases per 100,000 population, a 102% rise (95% CI [41,191], $p<10^{-3}$) and pneumococcal bacteraemia increased from 7.0 to 12.2 cases per 100,000 population, a 74% rise (95% CI [39,114], $p<10^{-3}$).

Evolution of serotype distribution

We determined the serotype for 156 pneumococcal strains isolated from meningitis cases and 246 pneumococcal strains isolated from bacteraemia cases in children under two years,in 2001/2002, as well as 67 strains isolated from meningitis and 99 isolated from bacteraemia in 2006.

In children under the age of two years, VT pneumococcal strains accounted for 68% (274/402) of the serotyped strains isolated from pneumococcal meningitis and bacteraemia cases in 2001/2002 and 25% (42/166) in 2006.

Among NVT pneumococcal meningitis and bacteraemia cases that occurred in children under two years in 2006, serotypes 19A and 7F were the most frequent (Figure 3). Together they accounted for 37% of pneumococcal meningitis and bacteraemia. From 2001/2002 to 2006, the proportion of 19A strains increased from 8% to 19% in meningitis cases (p=0,03) and from 11% to

Table 1

Reported pneumococcal meningitis cases and estimated pneumococcal meningitis incidence by age in 2001/2002 and 2006, France (source: Epibac)

	No. of reported cases		Cases/100,000/year		Incidence rate ratio, CI 95%		
Age group	2001/2002	2006	2001/2002	2006	2006 vs. 2001/2002		p
< 2 years	181	74	8.0	6.0	0.75	[0.57,0.98]	0.036
2 - 15 years	74	41	0.5	0.5	1.02	[0.70,1.50]	0.922
16 - 64 years	322	199	0.6	0.6	1.11	[0.93,1.32]	0.254
> 64 years	194	106	1.4	1.3	0.97	[0.77,1.23]	0.857
Total	**771**	**420**	**0.9**	**0.9**	**0.98**	**[0.87,1.11]**	**0.785**

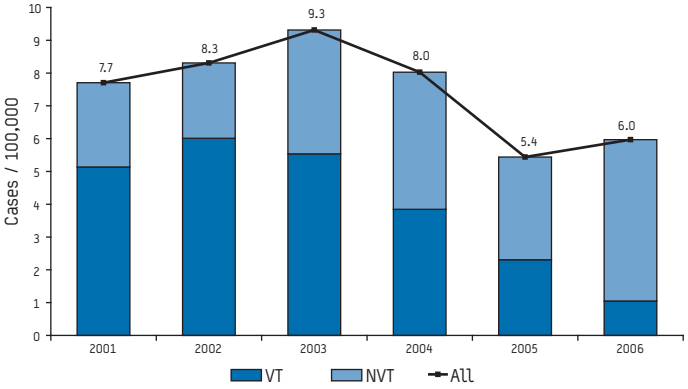
Table 2

Reported pneumococcal bacteraemia cases and estimated pneumococcal bacteraemia incidence by age in 2001/2002 and 2006, France (source: Epibac)

	No. of reported cases		Cases/100,000/year		Incidence rate ratio, CI 95%		
Age group	2001/2002	2006	2001/2002	2006	2006 vs. 2001/2002		p
< 2 years	493	217	21.8	17.5	0.80	[0.68,0.94]	0.007
2 - 15 years	416	274	2.7	3.3	1.22	[1.04,1.42]	0.013
16 - 64 years	2,754	1,681	4.,9	5.4	1.10	[1.03,1.16]	0.003
> 64 years	3,806	2,329	26.8	29.0	1.08	[1.03,1.14]	0.003
Total	7,469	4,501	8.5	9.2	**1.08**	**[1.05,1.13]**	**0.000**

Figure 1

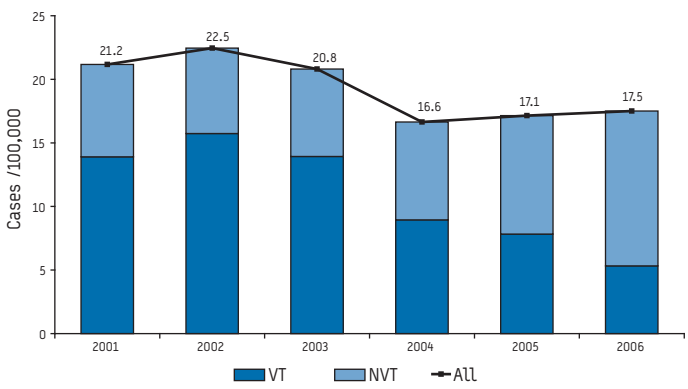
Estimated pneumococcal meningitis incidence by serotype in children under two years of age, France 2001-2006 (source: Epibac-NRCP)



VT: vaccine serotypes; NVT: non-vaccine serotypes

Figure 2

Estimated pneumococcal bacteraemia incidence by serotype in children under two years of age, France 2001-2006 (source: Epibac-NRCP)



VT: vaccine serotypes; NVT: non-vaccine serotypes

27% in bacteraemia cases ($p<10^{-3}$); the proportion of 7F strains increased from 3% to 18% in meningitis cases ($p<10^{-3}$) and from 1% to 10% in bacteraemia cases ($p<10^{-3}$). Other non-vaccine serotypes accounted for less than 8% of pneumococcal meningitis and bacteraemia cases in children under two years in 2006.

From 2001/2002 to 2006, the incidence of pneumococcal meningitis and bacteraemia caused by each of the seven vaccine serotypes decreased. The incidence of pneumococcal meningitis and bacteraemia due to serotypes which are not included in the vaccine but are part of the same serogroup as a vaccine serotype – with the exception of serotype 19A, i.e. serotypes 23B, 6A, 18B, 9N, and 23A – remained unchanged (Figure 3).

Discussion

French recommendations for PCV vaccination in 2003 included a large proportion of French children under the age of two years, while other European countries targeted only high risk children [4]. Between 2005 and 2006, vaccination with PCV has been extended to all children under two years in France as well as in other European countries such as Belgium, England, Germany, Luxembourg, the Netherlands, and Norway [15]. The early recommendations and the fact that the French surveillance for invasive pneumococcal diseases allows the analysis of trends in incidence and serotype distribution provided an opportunity to analyse the impact of PCV introduction in France. It is the second analysis of this kind in a European country at the national level following the analysis published this year from Norway [16].

Although PCV introduction in France was associated with a 71% decrease in vaccine-type IPD incidence between 2001/2002 and 2006 in children under two years, the overall decrease of IPD in this age group was only 21% (95% CI [10,31]). The fact that the decline was observed only in children under two years and only for cases due to vaccine serotype strains strongly argues for a role of PCV vaccination in this evolution.

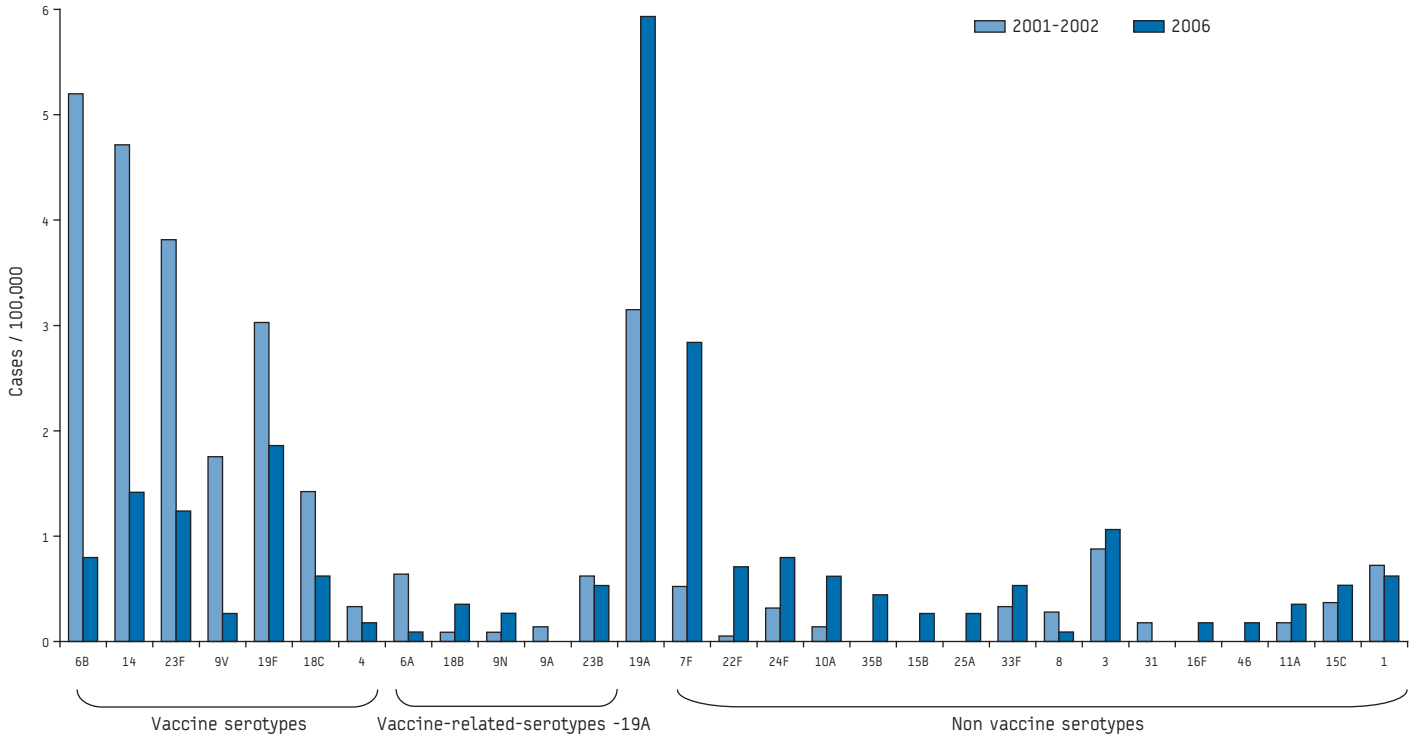
Impact of PCV vaccination on IPD incidence

The 21% decline of the disease in French children is far below the 77% reduction observed in children under the age of five years in the regions covered by the United States (US) Centers for Disease Control and Prevention's 'Active Bacterial Core surveillance' in 2005 and the 52% reduction observed in children under two years in Norway between 2004/2005 and 2007 [8,16]. Moreover, an indirect benefit of PCV vaccination in other age-groups has so far not been observed in France. The limited estimated vaccination coverage for PCV, below 60% in 2006, could explain in part this modest impact of PCV vaccination. Although PCV vaccine coverage has improved in the recent years, it remains well below the usual vaccine coverage for infants in France [14]. Expansion of the PCV vaccination coverage should lead to a further reduction in the IPD that are caused by vaccine serotypes in children and, through indirect effects, also in adults.

The overall reduction in IPD decrease that we found in young children is in agreement with the results reported by a French network of paediatricians which indicate a 28% decrease in the number of pneumococcal meningitides in 2-24 month-old children

FIGURE 3

Estimated incidence of pneumococcal meningitis and bacteraemia by serotype in children under the age of two years, evolution from 2001/2002 to 2006, France (source: Epibac-NRCP)



between 2001/2002 and 2005 [17]. Another survey conducted in 18 hospitals in northern France found a much greater reduction (82%) in pneumococcal meningitis incidence in children under two years between 2002 and 2005 than was found in the above and in our results [18]. A high PCV coverage in the northern region of France and the small number of cases involved in this retrospective survey (n<8 in 2005) are possible explanations for these differences.

Serotype replacement

The 71% reduction in IPD incidence due to VT strains was associated with a 85% rise in cases due to NVT strains in children under two years from 2001/2002 to 2006. The magnitude of this replacement impacted the overall effect of PCV vaccination: IPD incidence in French young children decreased between 2001/2002 and 2005 but did not decrease further from 2005 to 2006 despite a 20% rise in PCV sales. During that later period, the decrease in cases due to VT strains was balanced by an increase of the same magnitude in cases due to NVT strains. Replacement of VT by NVT strains has been observed to a smaller extent also in American children following PCV introduction. The increase of NVT pneumococcal disease in children under five years in the US estimated from 'Active Bacterial Core surveillance' data between 1998/1999 and 2005 was only 29% [8]. That this increase was higher in France may be due to the lower PCV serotype coverage in young children in France compared to the US. Before PCV introduction, 68% of IPD in children under two years were caused by PCV serotypes in 2001/2002 in France, compared to 83% in children under five years in 1998/1999 in the US [7].

Among NVT strains, two single serotypes – 19A and 7F – accounted for 37% of pneumococcal strains in 2006 in France. In the US, the 19A serotype has been found to be the predominant serotype in pneumococcal invasive cases in the years following PCV implementation, accounting for 40% of cases in children under the age of five 5 years in 2005, according to the results of the 'Active Bacterial Core surveillance' [8].

No decline in IPD was observed in older children and adults; on the contrary we identified a small but significant increase. However, as this trend had already been observed from 1998 to 2002 before the introduction of PCV in France, the possible contribution of vaccination to the increase cannot be conclusively assessed [19].

The evolution of pneumococcal invasive incidence in children in France can be compared with the situation observed in different areas of Spain after PCV introduction. Four regional Spanish studies were performed with the following results: no change in IPD incidence in a Barcelona district between 1999/2001 and 2002/2004 [20], a decrease in the Basque region between 2000/2001 and 2004/2005 [21] and in the Basque and Navarre regions between 1998/2001 and 2003 [22], and even an increase in pneumococcal invasive cases in Barcelona between 1997/2001 and 2002/2006 [23]. The reasons given by the investigators for this limited impact of PCV on IPD incidence refer to the conditions of PCV introduction in Spain: Vaccine coverage was low in Spain, where PCV is not subsidised, and the serotype coverage of PCV was significantly lower than the PCV coverage in the US (43% in the Navarre region) [20,22-24]. An increase in the frequency of pneumococcal invasive cases due to non-vaccine strains after PCV introduction was also found in three of theses studies [20,23,24].

Strengths and limitations of the study

We are confident about our incidence estimates because of the high and sustained coverage of the Epibac laboratory network combined with extrapolations made on a reliable source of information (the French national Hospital Annual Statistic database). Furthermore, we regularly monitored the reporting of cases by the participating laboratories through three sources capture-recapture analysis to ensure the exhaustiveness of the reports. The rate of underreporting were estimated at between 10% and 20% in these analyses [25,26].

We cannot completely exclude a change in the rate of pneumococcal case reporting in the last years. However, significant changes in reporting for pneumococcal cases alone are unlikely due to the following reasons: Firstly, the reporting rate of other bacterial diseases included in Epibac surveillance has not changed until 2005 as shown by a recent three sources capture-recapture analysis for invasive meningococcal diseases [26]; secondly, pneumococcal data show opposite trends for VT- and NVT-related cases; and thirdly, the cases are reported, by the vast majority of participating laboratories, through automatic extraction of microbiology isolates registration.

Incidence and serotype data are issued from two networks whose regional coverage is not identical. This may have introduced biases in the estimation of serotype/age-specific incidence evolution. However, each both networks covers more than 300 hospitals localised in all French regions, and the PCV serotype coverage did not vary with the geographical origin of pneumococcal strains (data not shown).

The evolution of individual serotypes should be interpreted with caution given the small number of strains involved in the 2006 analysis. Emergence of serotypes 19A and 7 F may not be due to PCV introduction alone, as changes in serotypes distribution can also occur for other reasons than vaccination pressure. The findings of this early analysis must be seen as a preliminary description of the PCV impact in France. IPD evolution and the extent of serotype replacement will be closely monitored in the next years through ongoing epidemiological and bacteriological surveillance.

Conclusion

In conclusion, PCV introduction was followed by a significant decrease in IPD in young children in France. Further improvement of PCV coverage should further increase the positive impact of PCV on vaccine-type pneumococcal invasive diseases in both children and adults in the next years, if a positive herd immunity effect is observed. If, on the other hand, the partial substitution of the cases that are caused by vaccine serotypes with cases caused by non-vaccine serotypes, that was observed in our early analysis in young children, is confirmed in the coming years, this would lead to a reduction of the positive impact of PCV vaccination in France.

Theses results emphasise the need for ongoing surveillance of the pneumococcal disease incidence and serotype in countries introducing PCV. The imminent availability of pneumococcal vaccines covering a broader range of the serotypes implicated in IPD in young children could limit the effect of serotype replacement and improve the impact of immunisation on IPD.

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Survey on legislation regarding wet cooling systems in European countries

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Wet cooling systems are often associated with large outbreaks of Legionnaires' disease. Several European countries have legislation for registering such systems. The authors aimed to obtain an overview of the situation in Europe. A questionnaire survey was sent to 35 of the countries that collaborate in the European Working Group for *Legionella* Infections. In two countries it was passed to a regional level (to three regions in both Belgium and the United Kingdom), so that 39 countries or regions were sent the survey; 37 responded. Nine countries stated having legislation for the registration of wet cooling systems. Separate legislation exists at a regional level for two regions in Belgium and all three regions in the UK, giving a total of twelve countries/regions with legislation. In nine of these countries/regions, the legislation has been introduced since 2001. All of these countries/regions require periodic microbiological monitoring between twice a year and weekly; in nine, the legislation requires periodic inspection of the systems. Regulations for the registration of wet cooling systems should be required by public health authorities. During an outbreak of legionellosis, a register of wet cooling systems can speed up the investigation process considerably. The authors believe that the European Centre for Disease Prevention and Control (ECDC) should take the initiative to propose European Community (EC) regulations for all Member States.

Introduction

Legionnaires' disease is an atypical pneumonic infection, acquired by inhaling aerosols containing *Legionella* spp. The *legionella* bacteria are commonly found in the natural and man-made aquatic environment, and enter the atmosphere through aerosol-generating outlets such as showers and cooling towers [1]. The first recognised outbreak of Legionnaires' disease occurred in 1976 at a hotel in Philadelphia [2] and was probably attributable to a cooling tower. Since then, wet cooling systems (including cooling towers, evaporative condensers and fluid coolers) have been established as some of the most common sources for outbreaks of legionellosis worldwide [1]. Wet cooling systems are heat rejection devices that utilise the evaporation of water to provide cooling. Common features are the recirculation of water which is sprayed or otherwise broken up into droplets in a counter current of air that is then ejected into the atmosphere. Some droplets may thus escape and form an aerosol outside of the cooling device. The recirculation of water can create good conditions for growth of *legionellae*.

Wet cooling systems can favour the growth of *legionella* by maintaining water temperatures of up to 35°C (temperatures in the range of 20°C to 45°C favour the growth of *Legionella* spp.) and by containing high levels of organic material and protozoa. About 2% of the water used in wet cooling systems escapes as aerosol and can drift more than 500 metres, in a few cases up to several kilometres, from its source [3,4]. When combined with poor maintenance and under-dosing of biocide, these systems can foster extensive growth of bacteria including *Legionella pneumophila*.

Every year the European Working Group for *Legionella* Infections (EWGLI) collects an aggregated dataset of all cases and outbreaks of Legionnaires' disease that have occurred in Europe during the previous year. Between 2002 and 2007, 44 outbreaks with cooling towers as the suspected source were reported in 11 countries, involving 1,175 cases (Table 1) [5-7].

For community-acquired outbreaks of Legionnaires' disease it is important to identify and treat the source as quickly as possible in order to prevent further infections. This can be a lengthy process if no register of wet cooling systems exists. Several European countries, especially those which have already experienced large cooling tower outbreaks, are known to have legislation for registering such devices. To obtain an overview of the situation in Europe, the authors conducted a questionnaire survey among the countries that participate in EWGLI.

Methods

A questionnaire was approved by the steering committee for the European Surveillance Scheme for Travel Associated Legionnaires' disease (EWGLINET) and sent to 35 EWGLI collaborating countries; it was passed to a regional level in Belgium (Brussels, Flanders and Wallonie) and the UK (England and Wales, Northern Ireland and Scotland). Therefore, 39 countries or regions were asked to participate.

The questionnaire included the following questions, and allowed space for further comments:

- Does your country have legislation for registering wet cooling systems?
- If yes, is the legislation national or regional?
- Which ministry issued the legislation?
- In what year was the legislation introduced?

- Is there an official requirement for periodical inspection of wet cooling systems?
- Who is responsible for the periodic inspection of wet cooling systems?
- Is there an official requirement for microbiological monitoring?
- Are there penalties imposed for unregistered wet cooling systems?
- Does a register of wet cooling systems exist?
- Who holds the register?
- How does the authority get the information?
- Who is responsible for maintaining the information?

The initial results were presented at the 22nd EWGLI conference in Stockholm [8], and comment and interpretation was sought from the collaborating countries.

Results

Representatives from 37 collaborating countries or regions (94.9%) returned the questionnaire. Of these, 12 (32.4%) reported having legislation requiring the registration of wet cooling systems at a national level (Andorra, France, Malta, The Netherlands, Norway and Spain) or a regional level (Belgium: Wallonie and Flanders; UK: England and Wales, Northern Ireland and Scotland; and the Russian Federation) (Table 2). The countries or regions that returned the questionnaire and do not have such legislation are: Austria, Belgium (Brussels), Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, Germany, Greece, Hungary, Ireland, Israel, Italy, Latvia, Lithuania, Luxembourg, Poland, Portugal, Romania, Slovak Republic, Slovenia, Sweden, Switzerland, Turkey.

In five countries or regions this legislation is issued by the Ministry of Public Health, in three by the Ministry or Department of the Environment, in two by the Ministry or Department of Trade and Industry, and in one by the Department of Industrial Construction. In the Netherlands, the registration is voluntary and is covered by environmental legislation; it is anticipated that legislation requiring the registration of new wet cooling systems will be introduced in 2009. In England and Wales, Scotland and Northern Ireland, legislation has existed since 1992 or 1994; in all other countries or regions the legislation has been introduced since 2001.

All countries or regions which have legislation require periodic microbiological monitoring between twice a year and weekly; 'microbiological monitoring' was not further specified in the questionnaire and the responses are likely to refer to dipstick tests rather than to full environmental sampling. In nine countries the legislation requires periodic inspection of the systems. In all twelve of the countries or regions which have legislation, a register of wet cooling systems exists. This register is held by national authorities (n=2), regional authorities (n=3) or local authorities (n=7), and in nine of these countries/regions, penalties are imposed for unregistered systems. In eight of the nine countries/regions where penalties can be imposed, the owner of the system is responsible for ensuring that the information on the register is correct.

Of the 25 (67.6%) countries or regions with no legislation for registering wet cooling systems (Table 3), five require microbiological monitoring and four stated that technical standards require periodic inspections; two will impose penalties for not

Table 3

Countries or regions without legislation on the registration of wet cooling systems, EWGLI survey, 2007

Number of countries or regions	Periodical inspections	Microbiological monitoring	Does register exist	Who holds register (authority)
25	21 no 4 yes	20 no 5 yes	23 no 2 yes	1 national 1 regional

Table 1

Outbreaks of Legionnaires' disease with wet cooling systems as the suspected source, as reported to the EWGLI annual dataset by collaborating countries, 2002-2007 (n=44 outbreaks)

Country (region) of outbreak	2002		2003		2004		2005		2006		2007	
	WCS outbreaks	Number of cases	WCS outbreaks	Number of cases	WCS outbreaks	Number of cases	WCS outbreaks	Number of cases	WCS outbreaks	Number of cases	WCS outbreaks	Number of cases
Austria											1	9
France	2	22;31	3	31;24;84			1	34	3	29;10;12	1	9
Italy			1	15								
Netherlands									3	31;9;10		
Norway	1	28					1	58				
Portgual									2	3;21		
Spain	2	108;9	4	11**;6;13;6	2	32;29	4	12;15;50;4	1	146	1	18
Sweden					1	32						
UK (England and Wales)	2	6;146	1	27	1	4	2	3;2				
UK (Northern Ireland)	1	3					1	3				
UK (Scotland)					1	7	1	3*				

WCS outbreaks = wet cooling system outbreaks
* Two Scottish cases and one English case
** Associated with an evaporative condenser
NB: These figures were provisional reports at the time of submission to EWGLI; subsequent reports may cite different case numbers. Some countries (Norway, Spain and Sweden) have provided updated data to reflect final case numbers for these outbreaks.

TABLE 2

Countries or regions in Europe with legislation on the registration of wet cooling systems, EWGLI survey, 2007

Country (region)	Legislation: national or regional	Which ministry issued legislation	Year of introduction	Content of legislation	Periodic inspections	Who is responsible?	Microbiological monitoring	Penalties for unregistered towers	Does register exist?	Who holds register (authority)	How does authority get information
Andorra	National	Ministry of public health	2002	Regulation for prevention and control of Legionellosis	Daily to annual	The owner; the local authority can verify at any time	Monthly	Yes	Yes	National authority	By periodic inspection
Belgium (Flanders)	Regional	Ministry of public health	2007	Regulation for prevention of Legionellosis in public places	No	[No response]	At least twice a year	No	Yes	Regional authority	Owner sends results
Belgium (Wallonie)	Regional	Ministry of the environment	2005	Regulation inbedded in the conditions for building permission	Yes, but not predefined	[No response]	Every two months; if negative, every three months	Yes	No	-	By the environmental permit
France	National	Ministry of the environment	2004	Concerns all cooling towers with evaporative cooling systems	Every two years by Ministry appointed company	Maintenance company ceritified by the Ministry of Health; the local authority can inspect	Monthly or bimonthly	Yes	Yes	Local authority	Owners sends results annualy
Malta	National	Ministry of public health	2006	Regulation for registration of cooling towers and evaporative condensers	Variable, according to checklist in regulation	The owner; the health authority can conduct their own monitoring if desired	Colony counts, monthly; Legionella every six months	Yes	Yes	National authority	Owner sends results, and audit inspections are conducted
The Netherlands	National	1. Ministry of Employment (if employee may be exposed to cooling tower aerosols) 2. Ministry of Environment (if the surrounding area [but not the employee] is exposed to cooling tower aerosols)	1.Ministery of Employment: 2004, amended 2007 2. Ministry of Environment: January 2009	1. Regulations for prevention are embedded in company risk analyses 2. Regulation for registration of new cooling towers. Also, local authorities may impose prevention legislation on cooling tower owners	Yes, but no period specified	1. The employer; inspection should ensure compliance. 2. The owner; local authorities should ensure compliance of the owner	Recommended; frequency depends on location of the tower (monthly, every three months or every six months)	No	Partly	Local authority	Owners are requested to register by the local authority
Norway	National	Ministry of public health	2005	Regulation to minimise the risk of spread of Legionella from aerosol generating equipment	Every six months	The owner	Colony counts, monthly;	Yes	Yes	Local authority	Owner sends results
Russia	Regional	Dept. of industrial construction	Not stated	Regulation for cooling towers and evaporative condensers of public objects	No	[No response]	Yes, planed ministry of public health	Not known	Yes	Local authority	Not stated
Spain	National	Ministry of public health	2001, amended 2003	Regulation for prevention and control of Legionellosis	No official inspections	The owner should have a maintenance programme in place.	Colony counts monthly ; *Legionella* every three months	Yes	Yes	Regional authority	Owners have to inform authority
UK (England and Wales)	National	Department of Employment	1992	Regulation for registration of cooling towers and evaporative condensers	No, but other legislation require inspection by the owners	The owner; enforcing authorities should ensure compliance of the owner	No, but other legislation require monitoring; colony counts, weekly; *Legionella* every three months	Yes	Yes	Local authority	Owners have to inform authority
UK (Northern Ireland)	National	Department of Enterprise, Trade and Investment	1994	Regulation for registration of cooling towers and evaporative condensers	Twice a year	The owner; enforcing authorities should ensure compliance of the owner	Colony counts, weekly; *Legionella* every three months	Yes	Yes	Local authority	Owner sends results
UK (Scotland)	National	Department of Trade and Industry	1992	Regulation for registration of cooling towers and evaporative condensers	Periodic inspection	The owner should have a management system in place	Depends on level of compliance with code of practice	Yes	Yes	Local authority	Business occupier is requested to register with local authority

following these standards. Of these 25 countries or regions, only one country (Luxembourg) and one region (Brussels) have a register of wet cooling systems, and because Brussels' register includes only new systems, it is not comprehensive.

Discussion

Minimising the number of cases of legionellosis caused by wet cooling systems should be an important target for public health authorities1. A preliminary risk assessment by Ambroise et al. [9] showed that exposure through cooling towers led to more cases of Legionnaires' disease (by a factor of 100-130) than exposure during showering, whilst Lock et al. detailed the high cost of an outbreak of Legionnaires' disease caused by a cooling tower [10]. The EWGLI annual dataset (Table 1) shows that between 2002 and 2007 there were an average of 7.3 outbreaks caused by wet cooling systems each year, involving 1,175 cases (an average of 195.8 cases per year and 26.7 per outbreak). In comparison, 215 outbreaks (35.8 per year) with 784 cases were associated with water systems (an average of 130.7 cases per year and 3.6 per outbreak) [6,7]. It should be noted that a large number of outbreaks are never properly attributed to sources [7], and that the larger ones (often associated with wet cooling systems) are more likely to be attributed to a source than smaller outbreaks [3,11,12].

In most of the countries or regions that have regulations for the registration of wet cooling systems, these were introduced following the recognition of outbreaks caused by such devices. Regulations were introduced in England, Wales and Scotland in 1992 [13] following Public Enquiries resulting from the Stafford hospital outbreak [14] and the BBC outbreak [15], both of which were caused by cooling towers. After a big outbreak in a town near Madrid in 1997 [16], the first regional law was issued in Spain. This was followed by laws in many other regions of Spain and by a national law in 2001 (later revised in 2003). In France a number of outbreaks, including the 2003 outbreak in Lens [3], led to specific regulations in 2004; in Norway regulations to minimise the risk of spread of *legionella* from aerosolizing equipment followed an outbreak caused by an air washer [4]. In the Netherlands a cooling tower related outbreak in Amsterdam in 2006 [17] was the impetus for the introduction of specific rules.

Of those eleven countries or regions that experienced wet cooling system outbreaks which were reported to EWGLI between 2002 and 2007 (Table 1), three reported having no legislation for registering wet cooling systems (Italy, Portugal and Sweden). However, the three countries or regions that have reported the most outbreaks over this period (Spain, France and England and Wales) all have legislation. These three countries or regions require frequent microbiological monitoring, keep a register of towers and impose penalties for unregistered systems. The only area where they may have less rigid legislation than countries or regions with fewer outbreaks is in regards to periodic monitoring. Spain suffers from the highest number of outbreaks and does not require periodic official inspection of systems, but there are different levels of response following positive *Legionella* spp. counts depending upon how infected the system is. France only requires inspections every two years, and England and Wales do not have a set frequency for inspections by local authorities (however the obligation to monitor rests with the wet cooling system owners and the enforcing authorities should ensure that they fulfil this obligation) [18].

It is difficult to draw solid conclusions from this data because there are many differences in ascertainment, data collection, and reporting systems between countries. Nevertheless, there is enough evidence to suggest that developing water safety plans for wet cooling systems, including system assessment, monitoring and management, is the preferred approach for managing the health risks associated with exposure to *Legionella* spp. [19,20]. Specific legislation is needed to ensure that authorities responsible for the safety of water systems or buildings develop and follow water safety plans. Most outbreaks associated with wet cooling systems are preventable, and such legislation could therefore lead to a substantial reduction in morbidity and mortality from Legionnaires' disease.

Regulations for the registration of wet cooling systems should also be required by health systems. During an outbreak of legionellosis, identifying and containing the source as quickly as possible should be one of the initial aims of an outbreak control team. In order to achieve this, improving surveillance to ensure the rapid detection of cases and clusters is important, but a register of wet cooling systems can also be an invaluable starting point and speed up the process considerably [21]. At present only 12 European countries or regions have specific legislation for this. Several EWGLI collaborating countries that do not currently have such legislation have suggested that European Community (EC) regulations for the registration of wet cooling systems and the prevention of legionellosis are required, and that the European Centre for Disease Prevention and Control (ECDC) should take the initiative to propose such regulations.

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List of responding EWGLINET collaborating centres: Andorra, Austria, Belgium (Wallonie, Brussels Capital and Flanders), Bulgaria, Croatia, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Israel, Italy, Latvia, Lithuania, Luxembourg, Malta, The Netherlands, Norway, Poland, Portugal, Romania, Russia, Slovak Republic, Slovenia, Spain, Sweden, Switzerland, Turkey, UK (England and Wales, Northern Ireland and Scotland)

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A cluster of hepatitis B infections associated with incorrect use of a capillary blood sampling device in a nursing home in the Netherlands, 2007

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In July 2007, two residents of a nursing home were diagnosed with acute Hepatitis B virus infection. To identify risk factors for HBV infection a retrospective cohort study among residents was performed. Case finding included discharged diabetes patients and those receiving home care. Among 32 residents one case of chronic hepatitis B was found that could be identified by genotyping as the source patient for the acute cases. Diabetes and finger sticks were risk factors for HBV infection. Most likely the cause of transmission was a multiclix finger stick device developed for use in individual patients but used in multiple patients. Education and training in the use of new equipment and hygiene audits remain the cornerstones in infection control practices.

Introduction

Hepatitis B virus (HBV) is transmitted by percutaneous and permucosal exposure to infected blood or body fluids, either directly or indirectly through contact with contaminated surfaces. Nosocomial transmission of HBV has previously been associated with unsafe injection practices, including contamination of multidose-multipatient vials and finger stick blood sampling devices with reusable components [1-7].

In mid-July 2007, the Municipal Public Health Service Rotterdam-Rijnmond (MPHS) received two notifications of acute hepatitis B in Dutch diabetic women in their late eighties and early nineties, hereafter called patient A and B. The onset of illness had been early July 2007. During the incubation period the two patients had lived in the same nursing home. An outbreak investigation was initiated in order to find the source of infection and to prevent further transmission. Our hypothesis was that HBV transmission had occurred through unhygienic capillary blood sampling. We considered this event a multiple needle stick injury with possible transmission of HBV, hepatitis C virus (HCV) and human immunodeficiency virus (HIV). To identify exposures associated with HBV infection, a retrospective cohort study was conducted among nursing home residents who lived in the home between 1 January and 31 July, 2007.

Methods

Inventory, environmental and other investigations

The nursing home, a separate unit of a larger institution, has 32 beds in four wards. The unit staff work exclusively in this unit, but some have additional tasks in a mobile team for home care. In August 2007, the nursing home had 32 residents. Since January 2007, 42 residents had been discharged and 14 residents had died. One of the deceased residents was known to be HBV-positive (patient C).

Infection control procedures were assessed through direct observation of activities of the pedicure and by interviews with nursing staff about protocols of nursing procedures. In a self-administered questionnaire the activities at work of health care workers applying finger sticks, as well as their HBV serostatus were assessed. Because finger sticks were suspected to be the cause of transmission, we additionally investigated six out of 42 discharged residents with diabetes mellitus and another eight patients on whom the mobile team had performed finger sticks at home, supposedly with devices from the nursing home.

Retrospective cohort study

The cohort consisted of 32 residents in August 2007 (including the two notified patients A and B) and the third patient C, for whom the medical history and serum were available for investigation. Informed consent was obtained from 31 residents and a relative of patient C. Risk factors were evaluated by reviewing the medical records for percutaneous and other possible exposures e.g. frequency and date of capillary blood sampling, insulin use, pedicure therapy and wound dressing.

Virological investigation

Serum specimens were tested for anti-hepatitis-B-core antibodies (anti-HBc; total and IgM) using standard assays (chemoluminescence assay; Siemens, Los Angeles, USA). In patients with a history of finger sticks, anti-HCV and anti-HIV testing was performed as well (both by enzyme-linked immunosorbent assay; Bio Rad, Paris, France). In anti-HBc-positive patients, hepatitis B surface antigen (HBsAg) and hepatitis B surface antibodies (anti-HBs) (chemoluminescence assay; Siemens, Los Angeles, USA) as well as hepatitis B envelope antigen (HBeAg) and hepatitis B envelope antibodies (anti-HBe) (enzyme-linked immunosorbent assay; Bio Merieux, Lyon, France) were measured. In anti-HBc-negative patients who were known to have undergone finger sticks, HBsAg was tested in order to detect a possible early infection. The HBV viral load was determined with a previously described *in house* developed real-time PCR assay that targets a 752 bp fragment of the HBV genome [8]. The PCR products obtained from the nursing

home patients were sequenced and compared with all HBV non-African genotype A fragments obtained from another contact tracing project of the MPHS [9]. The nucleotide sequences of the complete HBV genome obtained from a selected number of individuals were determined by methods described earlier [10,11].

Definitions

HBV infection was defined as infection in any resident who tested positive for HBsAg and total anti-HBc, and were either anti-HBc-IgM-negative (chronic) or -positive (acute). Individuals testing positive for total anti-HBc, negative for HBsAg and positive for anti-HBs were considered immune to HBV infection, and those testing negative for total anti-HBc and HBsAg were defined susceptible.

Statistical analysis

Univariate exact conditional logistic regression analysis was performed for various risk factors with dependent variable Hepatitis B infection, and the attack rates and percentage of cases exposed to the risk factor were calculated [12]. Proc logistic in SAS 9.1 was used (SAS Institute Inc., 2004, SAS/STAT 9.1 User's Guide, Cary, NC: SAS Institute Inc.)

Results

Inventory

Patient A had an acute hepatitis B infection in July 2007. In early 2007, she had had normal transaminase levels suggesting that she had not been infected at that time. Patient B also had an acute hepatitis B infection in July 2007 and normal transaminase levels in December 2006.

Patient C had been admitted to the nursing home on mid-January 2007 and died in early March 2007. This Dutch women in her mid-eighties had stayed in hospital after a hip fracture in November 2006, and was tested for hepatitis because of ascites – with a positive result. The diagnosis of hepatitis B had been reported to the MPHS and the serological pattern was interpreted as chronic infection with a flare-up including anti-HBc IgM. Patient C was not treated with antiviral therapy. All three hepatitis B patients had a viral load above $9x10^8$ genome equivalents/ml at the time of diagnosis (see Table 1 and Figure for details). All three patients had diabetes mellitus and underwent regular glucose monitoring. We found one blood sampling in the records for patients A and C that had been performed on the same day. One patient (B) had pedicure during the incubation period. No other risk factors were found in these patients. The three patients had no social contacts with each other during their stay in the nursing home.

The additional investigation showed that none of the discharged and home-based patients were recently infected with hepatitis B. None of the health workers was infected with HBV.

Environmental investigation

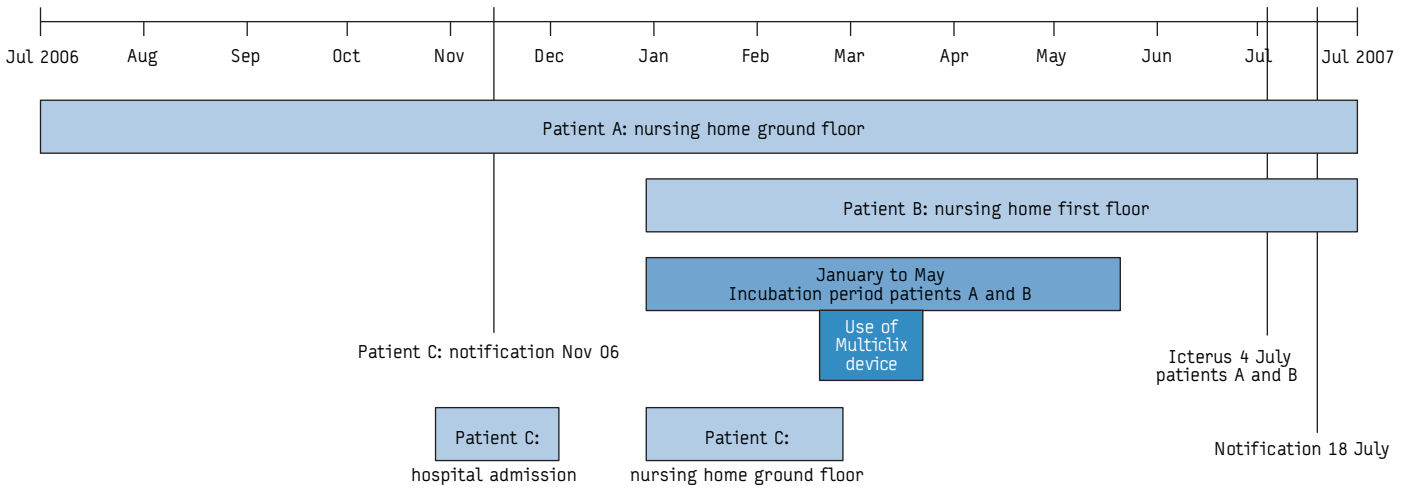
The hygiene audit informed us that HBV transmission was not likely to occur during pedicure. According to nursing procedures, gloves were used when disinfecting the skin and while taking capillary blood samples and discarded after use for one patient. However, some personnel admitted to wearing gloves irregularly during capillary blood sampling.

Until 12 February 2007, spring-loaded devices with a disposable platform had been used. After pressure on the device the lancet punctures the skin. It is technically impossible to use one lancet for more than one needle-stick. After use, both lancet and platform were disposed into a sharps-container. The devices themselves were re-used and occasionally shared between wards. They were not disinfected unless visibly contaminated with blood.

In the period from 13 February to 12 March 2007, a Multiclix device for capillary sampling was used in the nursing home (multiclix device "Accu-Chek® Multiclix"; F. Hoffmann-La Roche Ltd, Basel. Switzerland). This device has a drum with six lancets for rotating use. However, when rotating is forgotten, a lancet can be used twice. Even without re-using lancets, it cannot be excluded that one of the unused lancets comes into contact with blood remaining in the end cap of the drum. Staff at the nursing home applied this pen for multiple patients, but when they discovered that accidental re-use of lancets can occur, they stopped using it and re-introduced the spring-loaded device suitable for professional use in several patients [13].

Figure

Timeline for HBV patients A, B and C, July 2006-July 2007



It is obvious from the manufacturers guidelines that the "Accu-Chek® Multiclix" device is only meant for use in individual patients and not for use in institutions for several patients [14]. Sixteen of the 38 staff members performing capillary blood sampling had used the Multiclix device, eight of them worked on all four wards of the nursing home. None of the health workers was infected with HBV.

Cohort Study

The mean age of the cohort population was 80 years (range 53-96 years); 26 women and six men. The median admission time during the study period was 102 days (19-224 days). Except for the known patients (A, B and C) we found no other HBV-infected or immune people. Apart from one resident known to have a chronic hepatitis C infection (no finger sticks), no other HCV or HIV infections were found.

In the cohort, three of the eight diabetic patients were infected with hepatitis B compared to none of the 24 non-diabetics (Odds ratio 14.82 [95% confidence interval (CI) 1.448- infinity]; see Table 2). The attack rate for five residents receiving finger sticks during admission was 60% compared with none for the 27 residents not receiving finger sticks (Odds ratio 32.65 [95% CI 3.013 – infinity]). Undergoing blood sampling in the period of use of the multiclix device was associated with risk for HBV infection – although not statistically significant – compared to outside this period (Odds ratio 9.667 [95% CI 0.24-infinity]). Eleven of 32 residents were admitted from January to mid-March, i.e. they stayed in the home in the same period as patient C, as well as during the critical period of the use of the multiclix device. In this subgroup three of 11 residents were HBV-infected, while none of the patients admitted later got infected (Odds ratio 8.713 [95% CI 0.868-infinite]). Pedicure treatment was not a risk for Hepatitis B.

Table 1

Medical history of HBV patients A, B and C related to nursing home

	A	B	C
Sex	F	F	F
Age	89	91	85
Admission nursing home	Early July ground floor	Early January 1st floor	Mid-January 2007 ground floor
Onset of illness	Early July 2007	Early July 2007	NA
Date diagnosis HBV	Mid-July 2007	Mid-July 2007	Mid-November 2006
Anti-HBc	pos	pos	pos
Anti-HBc-IgM	pos	border line	pos
HBsAg	pos	pos	pos
HBeAg	pos	pos	pos
History transaminases	January/February 2007 normal	December 2006 normal	
Transaminases at diagnosis (N < 41 IU/L)	ASAT 151 IU/L, ALAT 126 IU/L	ALAT 1500 IU/L	ASAT 53 IU/L, ALAT 63 IU/L
Viral load at diagnosis (geq/l)	$4{,}18 \times 10^{9}$	$9{,}9 \times 10^{8}$	$2{,}1 * 10^{10\#}$
Geno-typing	Identical type A	Iidentical type A	Identical type A
Sero-conversion (HBsAG-neg)	Unknown (deceased October 2007)	Sep-07	Unknown
Diabetes mellitus	Insulin-dependent	Oral medication	Insulin-dependent

assessed August 2007; NA: not applicable; geq: genome equivalents

Table 2

Risk factors for Hepatitis B infection in the nursing home, 1 January – 31 July 2007

	Exposed			Non-exposed				Exact conditional logistic regression	
Risk factor	HBV infection	Total	Attack rate	HBV infection	Total	Attack rate	% cases exposed to risk factor	Odds ratio	95% CI
Diabetes mellitus	3	8	38%	0	24	0%	100%	14.82	1.448 - infinity
Finger sticks	3	5	60%	0	27	0%	100%	32.65	3.013 - infinity
Pedicure	1	14	7%	2	18	11%	33%	0.624	0.01 - 13.28
Capillary blood sampling in critical period*	1	1	100%	2	31	7%	33%	9.667	0.248 - infinity
Admission nursing home in critical period	3	11	27%	0	21	0%	100%	8.713	0.868 - infinity
Finger sticks in diabetes mellitus patients	3	5	60%	0	3	0%	100%	3.444	0.262 - infinity
Insulin use in diabetes mellitus patients	2	3	67%	1	5	20%	67%	5.784	0.158 - 587

HBV: hepatitis B virus; CI: confidence interval
* critical period is the period of use of the multiclix device

Relatedness of HBV isolates

Since the three nursing home patients were infected with genotype A, the 752 bp HBV-PCR fragment from these patients was compared with all HBV non-African genotype A fragments available in our MPHS contact tracing project [9]. In a total population size of 115 genotype A sequences and 298 non genotype A sequences, the HBV sequence of the three nursing home patients (A, B, and C) was part of a phylogenetic cluster of five completely identical sequences (A, B, C, G and H) and one completely identical sequence (F) with seven nucleotide ambiguity positions (not shown). The complete HBV genome (3,221 nucleotides) of the HBV strains from the five individuals in the cluster were determined and proved to be 100% identical over the complete length of the genome. We could not find an epidemiological link between the nursing home patients and patients F, G and H.

Discussion

Two concurrent acute hepatitis B infections in people that had lived in the same nursing for more than six months was suggestive of nosocomial transmission. Accounting for an incubation period of between six weeks and six months, the infection must have happened between early January and mid-May 2007. In our cohort study we did not find hepatitis B infections other than the acute cases (A, B) and case C. Patient C was highly infectious for hepatitis B when admitted to the nursing home in January 2007 for terminal care. Genotyping of the isolated Hepatitis B viruses of patients A, B and C showed that the viruses were completely identical, which confirmed that the three nursing home patients formed a transmission cluster. In view of the course of events, patient C was most likely the source patient for A and B. Since only patient B had pedicure treatment in mid-April it would be highly unlikely that this was the cause for transmission. Moreover, we did not observe any hygiene deficits in pedicure practice that could have led to a possible transmission of HBV.

Having diabetes and undergoing capillary blood sampling were clear risk factors for Hepatitis B infection; in fact, only diabetics were exposed to finger sticks. Outbreaks of hepatitis B through unhygienic use of finger stick devices have been reported before [1-6,15-17]. Most suspect in our case was the use of a multiclix device from mid-February to mid-March for multiple patients, for whom re-use of lancets could not be excluded. We could not establish a clear association between being sampled in the period of the use of the multiclix device and hepatitis B infection as according to the registration, only patient B had undergone finger sticks in this period. Since patient A had undergone a high number of finger sticks several times a week but not during this critical period this raises doubts about whether the registration of finger sticks was complete. The staff confirmed technical problems in their registration system and that missing registrations could not be excluded. We found staying in the nursing home during the critical period a risk for HBV infection, however, this coincides with the admission of the source case and is therefore not proof for a causal relation.

Could the HBV have been transmitted by the spring-loaded device? This device is developed for professional use in multiple patients and the lancet is disposed after use together with the platform which has been in contact with the skin of the patient [13]. The use of this spring-loaded device did not form a risk for transmission in our cluster. Despite our finding that gloves were not used every time when performing capillary sampling it seems unlikely that transmission via the hands of nursing staff can explain this cluster.

Patient A who frequently underwent capillary sampling stayed on the same ward as source patient C. Case B stayed on a different ward, but we have found a once-only registration of a glucose day curve carried out on the same day in cases B and C. Patient B could have been infected by rotating staff who used the multiclix device on several wards.

As patient C was highly infectious we would have expected even more HBV infections in the nursing home. By searching for early infections (HBsAg testing in exposed anti-HBc-negative residents) in mid-August, five months after the critical period, we excluded additional HBV infections. The death of patient C in early March 2007 and the discontinued use of the multiclix device may have contributed to the limited number of acute HBV infections. Had another procedure than the use of the multiclix device been the cause of transmission, new cases arising from the acute cases with high viral load should have occurred. Awareness of the HBV infection of patient C in nursing home staff may have led to increased vigilance regarding infection prevention. But even without that knowledge transmission of blood-borne pathogens in health care settings is entirely preventable by adherence to standards of care including infection control [1,18].

Recommendations and public health implications

As far as we know this is the first report of incorrect use for multiple patients of a device designed for individual use, which has most likely led to two acute HBV infections. It is striking that this device was used on multiple patients in the institution, although the instructions of the manufacturer clearly indicate "only individual use". When introducing new equipment, studying the instruction manuals, training the health care workers and evaluating the use of the new tools should be a routine. In yearly hygiene audits special attention should be paid to capillary blood sampling procedures. We consider it advisable to use personal finger stick devices in institutions for long term care as has been reported before [1].

These recommendations were discussed with the nursing home and reported to the health care inspectorate. The public health concern of our case is illustrated by the fact that a general practitioner group-practice in the Netherlands reported in December 2007 to have started an investigation among their exposed patients after having used the same multiclix device for multiple patients for several months. This was followed by another similar report from a clinic in the Netherlands. The inspectorate requested the manufacturer to issue a letter to all users of the multiclix device in the Netherlands in order to increase awareness of possible wrong use of the device [19].

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Travel-associated Legionnaires' disease in Europe: 2006

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Twenty countries reported 921 cases of travel-associated Legionnaires' disease to EWGLINET (the European Surveillance Scheme for Travel-Associated Legionnaires' Disease) with onset during 2006; 875 confirmed and 46 presumptive. Thirty three cases died, giving a case fatality rate of 3.6%.

Of the 124 new clusters detected in 2006, 43 would not have been identified without the EWGLINET scheme. A total of 146 investigations were conducted at cluster sites according to the standards of the EWGLINET investigation guidelines; 111 of these investigations were associated with the new clusters while 35 investigations were associated with re-offending sites (where additional cases had onset after a report was received to say that investigations and control measures had been satisfactorily conducted). The names of four accommodation sites were published on the EWGLI website.

Overall, there has been an upwards trend in case numbers since the scheme was founded, which has implications for the work load of public health authorities across Europe and for the tour industry. Despite this increasing pressure on public health authorities, environmental investigations are being conducted in a timely manner.

Introduction

In 1986, the European Working Group for Legionella Infections (EWGLI) was formed to facilitate the exchange of information and to collaborate in the management of Legionnaires' disease across Europe. A year later EWGLI members established the European Surveillance Scheme for Travel-Associated Legionnaires' Disease (EWGLINET), which aims to identify clusters of Legionnaires' disease cases in Europe that may not be detected by national surveillance systems alone, and to initiate investigation and control measures at the sites implicated. These measures are standardised in the European Guidelines for Control and Prevention of Travel Associated Legionnaires' Disease, which were endorsed by the European Commission in 2003 [1]. The history and current activities of EWGLI are described further on its website (www.ewgli.org).

This paper provides results and commentary on cases of travel-associated Legionnaires' disease reported to EWGLINET with onset in 2006.

Methods

EWGLINET uses standard case definitions to ensure that the data reported to the scheme are consistent regardless of the country of report. These definitions are available on the EWGLI website [2]. National surveillance schemes collect basic epidemiological, microbiological and exposure information on cases of travel-associated Legionnaires' disease that occur in residents of their country. These are reported to EWGLINET's co-ordinating centre at the Health Protection Agency Centre for Infections in London, which maintains a database of all reported cases. The database is searched each time a new case report is received in order to determine whether it is a single case or part of a cluster.

A single case is defined as a person who stayed, in the two to ten days before onset of illness, at a public accommodation site that has not been associated with any other previous case of Legionnaires' disease, or a person who stayed at an accommodation site linked to other cases of Legionnaires' disease but after an interval of at least two years. A cluster is defined as two or more cases who stayed at or visited the same accommodation site in the two to ten days before onset of illness and whose onset is within the same two year period [1].

The European Guidelines for Control and Prevention of Travel-Associated Legionnaires' Disease [1] were introduced in 2002 to standardise the investigations conducted across Europe in response to EWGLINET cluster alerts. The response required for single cases is minimal because the epidemiological evidence suggesting that the accommodation site is the source of infection is relatively low; as such, the responding collaborator is only required to send the accommodation site a checklist for minimising risk of legionella infections, so that the site can ensure that it is following the best practice.

However, if the site is associated with a cluster, the guidelines state that more detailed investigations must be conducted; these include a risk assessment, sampling and control measures. The collaborator in the country of infection must report the progress of these investigations to the co-ordinating centre after two weeks ('Form A') and six weeks ('Form B'). If these reports are incomplete or are not received on time, EWGLINET will publish details of the cluster site on its public website (www.ewgli.org), stating that the coordinating centre cannot be certain that risk of legionella infection is under control at the site. This notice is removed once the relevant form(s) have been received, confirming that measures to minimise risk are in place.

If a cluster is satisfactorily investigated under the guidelines and is subsequently associated with a further case, it is termed a 're-offending' site and a complete re-investigation is required.

Results

Cases and outcomes

Of the 35 collaborating countries in EWGLINET, 18 reported a total of 916 cases of travel-associated Legionnaires' disease with onset during 2006 (counting England and Wales, Scotland and Northern Ireland as one country). In addition, four cases were reported by the United States and one by Australia, two countries that do not form part of the official network. This brought the total number of cases reported to the EWGLINET scheme with onset in 2006 to 921, which is a major increase on 2005 when 755 cases were reported and continues the annual upward trend (Figure 1). The mean time between onset and report to EWGLINET was 36 days in 2006 in comparison with 29 days in 2005.

The countries that reported the most cases in 2006 were the United Kingdom (250 cases, from England and Wales, Scotland and Northern Ireland), France (174), the Netherlands (158) and Italy (130) (Table 1).

Cases in males outnumbered cases in females by a considerable margin and a ratio of 2.8:1 (677 males and 244 females), maintaining the gender profile seen in previous years (up from 2.5:1 in 2005). As in previous years, cases in 2006 were also skewed towards older age groups (with peaks in the 50-59-year age group for men and the 60-69-year group in women). The median age for male cases was 58 years (age range 18-91 years; two cases had unknown age) and for female cases 61 years (age range 18-93 years).

The peak month for onset of illness in 2006 was August compared with September in 2005, continuing the summer seasonal pattern of high incidence associated with this travel-associated scheme.

Among the 921 cases, 369 (40.1%) had an outcome provided and 33 deaths were notified (3.6%). This case fatality rate was similar to that in 2005 (29 deaths, 3.8%). The 33 deaths were reported for cases aged 34 to 84 years. Of these, 27 were male and six were female (4.5:1 compared with 4.8:1 in 2005), and the median age was 48. The majority of deaths (29) were associated with single cases (87.9%), and the remaining four (12.0%) with cluster cases. In 2005, 21 (72.4%) of the reported deaths were linked to single cases and eight (27.6%) to clusters.

Microbiology

The dataset of cases reported with onset in 2006 contains 875 confirmed and 46 presumptive cases; confirmed cases include those that are culture-positive, those diagnosed by urinary antigen and any *Legionella pneumophila* serogoup 1 cases diagnosed by serology four-fold rise, whilst presumptive cases include all other cases diagnosed by serology (fourfold rise non-*L. pneumophila* serogroup 1 cases and all single high titres) and those diagnosed by PCR. As in previous years the main method of diagnosis in 2006 was by urinary antigen detection at 89.2% (822 cases), the proportion increasing from 85.8% in 2005. The number of culture-proven cases rose from 37 in 2005 to 48 in 2006, but as a proportion of all cases remained similar in 2005 (4.9%) and 2006 (5.2%). Seven cases (0.8%) were diagnosed primarily by PCR (down from 2.3% in 2005). Serology as the main method of diagnosis has continued to decrease, falling to 44 cases (4.8%) in 2006 (compared with 7.0% in 2005); 11 cases (1.2%) were diagnosed by fourfold rise (2.5% in 2005) and 33 (3.6%) by single high titre (4.5% in 2005). Of the cases diagnosed by fourfold rise, five were *L. pneumophila* serogroup 1, whilst one was a *L. pneumophila* serogroup 6 and the others had unknown serogroup.

Travel

A total of 63 different countries were visited by the cases during their incubation periods in 2006 (Figure 2). Ninety four cases (10.2%) visited countries outside the EWGLINET scheme; 66 cases visited more than one European country, and ten visited more than one country outside Europe. Eleven cases were associated with cruise ships. The four countries associated with most cases of infection were Italy, France, Spain and United Kingdom. Together they accounted for 58.5% of the total data set in 2006 (538 cases); Italy was associated with 198 (21.7%) cases, France 159 (17.3%), Spain 126 (13.8%) and United Kingdom 55 (6.0%). In previous years, Turkey was the fourth country on the list but in 2006 it accounted for 45 cases (4.9%), less than United Kingdom.

Of the infections associated with travel in Italy, 58.1% occurred among Italian nationals travelling in their own country (115 cases). Likewise, 61.6% of cases visiting sites in France were French nationals (98 cases) travelling internally in their own country, as

FIGURE 1

Number of travel-associated Legionnaires' disease cases reported to EWGLINET since the scheme began in 1987 (n=6349)

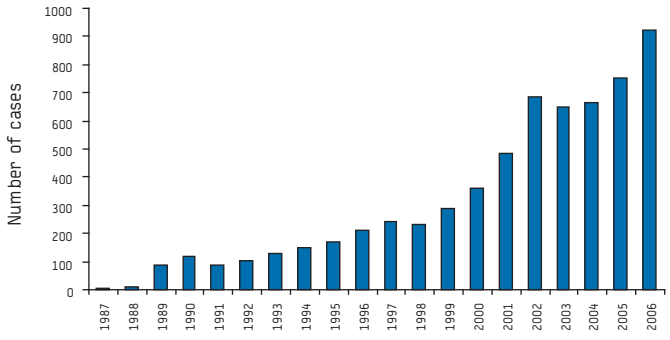

TABLE 1

Countries reporting more than 10 cases of travel-associated Legionnaires' disease to EWGLINET in 2005 and 2006

	Number of cases	
Country of report	2005	2006
United Kingdom	202	250
France	157	174
The Netherlands	134	158
Italy	96	130
Spain	30	73
Sweden	23	28
Denmark	40	26
Belgium	13	16
Austria	18	14
Norway	13	12

Note: In addition, ten other countries reported fewer than 10 cases, and are not listed here

were 47 of the cases linked to the United Kingdom (85.5%), and 35 (27.8%) of the cases linked to travel in Spain. Only one Turkish case was reported with travel within Turkey. The proportion of cases associated with clusters in Italy was 30.3% (60 cases). In France the proportion was 25.8% (41 cases), in Spain 42.1% (53 cases) and in the UK 5.5% (3 cases). In Turkey the proportion was 37.8% (17 cases) - a decrease from 53.2% in 2005 and 43.8% in 2004.

Clusters

One hundred and twenty four new clusters were identified in 2006, compared with 93 in 2005, 86 in 2004 and 89 in 2003. This does not include clusters which were identified in previous years and were associated with a subsequent case in 2006 ('cluster updates'); these clusters are included in the previous years' figures. The number of new clusters reported for 2006 represents a substantial increase of 33.3% compared with 2005. A total of 274 cases (29.8%) were part of clusters in 2006. Most of the clusters (107) comprised only two cases and 13 comprised three cases, so that 96.8% of all clusters fell into this group (Figure 3), compared with 93.5% in 2005. The largest cluster in 2006 involved five cases (down from eight cases in 2005). Forty three (34.7%) of the new clusters consisted of a single case that was reported by each of the two or more countries. These clusters would not have been detected without EWGLINET.

Clusters were detected in 27 countries, with Italy associated with the highest number (29), followed by Spain (24), France (23), Turkey (7), Greece (4) and Germany (4) (Table 2). Of the remaining clusters, 15 (12.1%) occurred in countries outside EWGLINET, a slight reduction on the 15.1% identified in 2005.

Ninety one of the clusters (73.4%) occurred during the summer period between May and October but clusters were detected during every month of 2006 (by date of onset of the second case in the cluster).

Figure 2

Countries visited by more than 10 cases of travel-associated Legionnaires' disease in 2006, by type of case, EWGLINET data

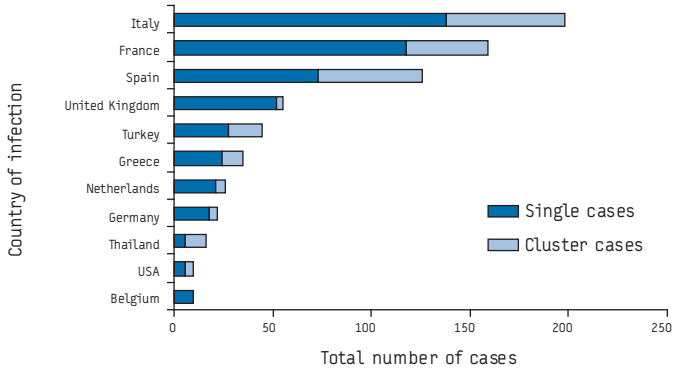

Figure 3

Number of cases of travel-associated Legionnaires' disease per cluster, by year, from 2003 to 2006, EWGLINET data

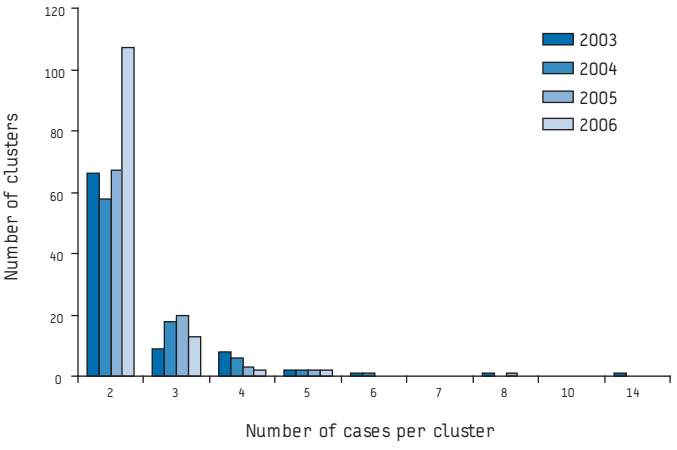

Table 2

Countries associated with clusters of travel-associated Legionnaires' disease in 2006, EWGLINET data

Country of infection	Number of clusters
Europe	
Italy	29
Spain	25
France	22
Greece	4
Germany	4
United Kingdom (England)	3
Sweden	2
The Netherlands	2
Poland Czech Republic	1
Poland	1
Malta	1
Luxembourg	1
Latvia	1
Germany/Italy	1
Jersey	1
Denmark	1
Croatia	1
Bulgaria	1
Austria	1
Non-Europe	
Turkey	7
Mexico	3
India	3
USA	2
Thailand	2
USA/Caribbean	1
USA/Mexico/Caribbean	1
Malaysia	1
Indonesia	1
Cuba	1

Investigations and publication

One hundred and thirty four accommodation sites were associated with the 124 new clusters in 2006. Twenty three of these sites were located in countries not signed up to follow the European guidelines, leaving 111 cluster sites that required EWGLINET investigations (an increase of 24.7% compared with the 89 sites that required investigations in 2005). Eighty two sites were associated with cluster updates issued in 2006, 33 of which included clusters where additional cases were detected after investigations had been completed and control measures were reported as satisfactory (re-offending sites); under the terms of the guidelines, these sites required further investigation. Two of the sites in 2006 fell into this 're-offending' category twice each. Thus, EWGLINET requested that a total of 146 investigations be conducted in 2006.

Ninety seven (66.4%) of the 146 Form B reports related to these investigations stated that *Legionella* spp. at concentrations equal to or greater than 1000 cfu/litre [1] were isolated from water samples taken at the accommodation sites. This compares with 57.4% of positive samples in 2005. Of the remaining 49 sites investigated, 46 (31.5%) reported that legionella was not detected in samples at the required levels, and three 'Form B' reports (2.1%) reported 'unknown' results due to site closures.

Whilst 35 investigations were conducted at re-offending sites, 33 distinct sites were involved with two sites re-offending twice (compared with 26 distinct sites in 2005, six of which re-offended twice). Fourteen of these sites were situated in Italy, six in France, five in Turkey, three in Greece and one each in Bulgaria, Malta, Poland, Spain and the United Kingdom. Twenty one of the 35 reinvestigations (57.1%) returned positive samples (compared with sixteen out of 32 reinvestigations in 2005 (50.0%)). One of the re-offending sites was part of a complex cluster (where the cases implicate more than one accommodation site as a potential source).

Only two accommodation sites (including one re-offending site) were published on the EWGLI website during 2006 for failure to return Form A or Form B reports on time, or for failure to implement appropriate control measures within the required period. Two further re-offending sites from 2006 were published in 2007. These sites were located in Bulgaria, France, Poland and Turkey. This represents a significant reduction from the nine site names published during 2005, four in 2004 and 27 published in 2003.

There is no requirement to investigate sites associated with a single case report within the EWGLINET guidelines. However, some countries do carry out these investigations and in 2006 reports were received for 82 such sites (114 sites in 2005), 48 (58.5%) of which were reported positive for *Legionella* spp. (at concentrations equal to or greater than 1000 cfu/litre [1].

Discussion

In 2006, the number of cases of travel-associated Legionnaires' disease reported to EWGLINET was higher than in any previous year, continuing the overall increasing trend in case numbers seen since the scheme began. Legionnaires' disease case numbers are increasing across Europe [3] (not only travel-associated cases), and several factors are driving this upward trend. Improved surveillance in national centres is an important factor contributing to the rise in cases; diagnosis, detection and reporting are being strengthened across Europe. However, some countries still only detect a handful of cases each year [3], and 17 of EWGLINET's 35 member countries reported no travel-associated cases to the scheme in 2006. Other factors that should also be considered as contributing to the increase in case numbers include climate change and generally warmer temperatures [4], and perhaps improved environmental conditions for growth of the legionella bacteria and therefore more opportunities for infection in travellers. In addition, it is known that leisure travel has increased markedly in recent years and that many active elderly (a more susceptible age group), are embarking on holidays further afield and outside Europe, increasing their risk of exposure to infection in countries where control and prevention programmes may be less well developed compared with European holiday destinations. The data set for 2006 showed that among the cases aged 70-79 years, 12.1% travelled outside Europe (19 out of 157 cases) compared with only 6.3% in this age group in 2005 (8 out of 127 cases*) (odds ratio=2.74, p=0.098). Among all cases associated with travel outside Europe, 24.0% were aged 70 or more in 2006 (25 out of 104 cases) compared with 11.8% in 2005 (10 out of 85 cases*) (odds ratio 4.64, p=0.031). (*Note that the figures for 2005 presented in this paragraph use data amended since last publication [3], and as such are not comparable with the other data for 2005 presented throughout this paper.)

The proportion of cases diagnosed by culture remained very low but relatively stable in comparison with 2005 although a rise in the absolute number of isolates was seen in 2006. Three quarters of the isolates came from single cases, a similar proportion to that observed in 2005 (73.7%) and were reported mainly by countries with a strong background in this methodology. Countries should be encouraged to increase the number of specimens taken for culture from cases associated with clusters in order to support the findings of epidemiological and environmental investigations, in addition to those collected prospectively and in advance of any case becoming part of a cluster. The number of isolates associated with cases that are known to have died is much higher than for other cases and was similar for 2005 and 2006 (13.2% in 2005 and 12.5% in 2006). This probably reflects the greater importance placed on thorough investigation of the illness when it has had a fatal outcome.

As case numbers have increased, so has the proportion of diagnoses conducted by urinary antigen detection. This has implications for investigators seeking to identify the source of an infection, since urinary antigen tests are mostly specific to *L. pneumophila* serogroup 1 infections and cannot distinguish between other serogroups or different strains within serogroup 1. Because *Legionella* spp. are ubiquitous in the environment, this is often insufficient evidence for legal purposes and compensation claims by cases. Also, if additional tests are not conducted on urine-negative cases, it is possible that non-serogroup 1 infections will be missed.

The case fatality rate decreased slightly in 2006, whilst the number of cases reported without a definitive outcome (i.e. reported as 'unknown outcome' or 'still ill') has increased. These two trends are probably linked, and it is likely that the 'unknown' or 'still ill' outcomes include some cases that died following the report to EWGLINET.

There has been a large increase in the number of clusters detected in 2006. This is due in no small part to Spain's retrospective reporting of 35 cases, most of which were associated with Spanish clusters; the majority of these case reports were submitted early

in 2007, which in turn accounts for the longer period between onset and report to EWGLI in 2006 compared with 2005. 2006 was the first year when Spain was able to report Spanish cases that had travelled internally within their country, following the relaxation of local reporting regulations. Spain should ordinarily have investigated these sites even without a EWGLINET cluster alert, since their public health authorities would have been notified of all of the cases associated with the particular accommodation site (whether through their national reporting scheme or through EWGLINET). Therefore we expect that the public health impact of Spain's change of reporting policy will be minimal for EWGLINET and the standards laid down in the European guidelines [1] since these in practice do not vary greatly from Spain's national investigation standards [5]. However, due to Spain's improved reporting, EWGLINET's case and cluster numbers are now more complete than in previous years.

The increase in cluster numbers has implications both for work load of EWGLINET's collaborators and the relevant national health authorities, and for the impact of Legionnaires' disease on the tourist industry. Tour operators are informed of clusters of three or more cases with onset of infection within three months of each other and about all clusters outside Europe. With the rise in travel to non-European countries, more clusters are expected to occur in countries where experience of legionella control and prevention is limited compared to Europe. When these happen it is costly for tour operators to relocate their guests, but the prevention of further, possibly fatal, cases of Legionnaires' disease is a public health priority and should be executed regardless of all costs.

Whilst the overall number of clusters has increased, those located in Turkey have decreased (seven in 2006 compared with 15 in 2005) and the number of reoffending sites has also decreased (five compared with 11 in 2005). This is very encouraging since Turkey has had difficulties with Legionnaires' disease in the past [6].

The proportion of positive environmental samples from cluster sites increased from 57.4% in 2005 to 67.8% in 2006. Since 2004, EWGLINET has been funded to hold annual training courses for collaborating countries in legionella outbreak management, risk assessment, sampling and control. Courses will also be held in 2008 and 2009. These training courses have led to an improvement in legionella detection and diagnosis in Europe and have positively contributed to higher quality surveillance programmes in many countries. However, as more cases are entered into the EWGLINET database, there is an increased likelihood of clusters occurring by chance, but with better microbiological expertise, we would expect these to return negative sampling results.

Despite the increase in the number of clusters and the related investigations, there was a reduction in the number of clusters published on the EWGLI website in 2006. This is encouraging, and indicates the timely investigation of these sites by EWGLINET collaborators and other public health professionals in the countries of infection.

Note: The data presented throughout this paper for 2005 (except where indicated by an asterisk) reflects case numbers as they appear in previous publications [3].

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*The list of EWGLINET collaborators is available at the following URL address: http://www.ewgli.org/collaborators.htm

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Effective control measures limited measles outbreak after extensive nosocomial exposures in January-February 2008 in Gothenburg, Sweden

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In January-February 2008, one imported case of measles initiated a series of exposures with around 380 nosocomial secondary contacts. Susceptible individuals were traced early and control measures were initiated that managed to limit the consequences considerably. Only four secondary cases were identified by the end of March. This minor outbreak illustrates the importance and efficiency of early control measures as well as the fact that the risk of measles outbreaks still exists in a country that has high measles, mumps, rubella vaccination coverage among children.

Introduction

Measles is one of the most contagious viral diseases and transmission in the community can only be prevented with efficient vaccination programmes. Such programmes have already reduced the incidence of measles in the European region. However, measles elimination in Europe is hindered by recurrent outbreaks in non-immune sub-populations. Non-immune subpopulations exist in all European countries due to:

- sub-optimal immunisation programmes with late implementation of measles, mumps, rubella (MMR) vaccination in a two-dose schedule,
- lack of catch-up campaigns for non-immune individuals of all ages,
- populations refusing MMR vaccination for a variety of reasons,
- and the window of susceptibility in infants between waning of maternal antibodies and the time when the first dose of MMR is provided.

Measles vaccination was introduced in Sweden in 1971. The vaccination coverage was initially only 40-60% [1] before the combined MMR vaccine was introduced in 1982. MMR has since then been offered in a two-dose schedule at 18 months and 12 years of age with a high vaccination coverage (>95%).

On 1 January 2007, the age for the second dose of MMR was lowered in Sweden from 12 years of age to 6-8 years since cases between the ages of six and 12 years had been observed in smaller outbreaks during the previous decade. At the same time it was decided to allow for the first dose to be given at any point between the age of 12 and 18 months. Under certain circumstances, such as international travelling or outbreak limitation, MMR may be provided between the ages of nine and 12 months.

Between mid-January and late March 2008, five cases of measles were notified to the medical officer in the Department of Communicable Disease Prevention and Control in Gothenburg, Sweden. All cases were unvaccinated. While four of the above patients were seeking medical attention for their measles infection, they exposed other patients, accompanying family members, and staff in the hospital or out-patient areas to measles on four separate occasions. Extensive nosocomial exposure of susceptible individuals to measles necessitated the implementation of control measures. These measures, described in the following, substantially limited the number of secondary measles cases.

Methods

Case definition

Measles is a notifiable disease in Sweden by the Swedish Communicable Diseases Act (2004:168). Case investigations include demographic characteristics, results from clinical and laboratory investigations, history of previous natural measles infection and vaccination. Contact-tracing of non-immune and exposed household-, school-, day care-, community- and nosocomial contacts should, if possible, be performed. A clinical case is defined as one having fever, a generalised maculopapular rash and one of the following: cough, coryza or conjunctivitis. A confirmed case is a clinical case with either laboratory confirmation (positive measles-specific IgM antibody test or positive PCR) or an epidemiological link to another case (two epidemiologically-linked cases are considered confirmed).

Prophylactic treatment

Prophylactic treatment should be offered to exposed non-immune children and adults. If less than 72 hours have passed since exposure, non-immune individuals should be either MMR vaccinated or offered immunoglobulin. If more than 72 hours but

less than seven days have passed since exposure, non-immune individuals should be offered immunoglobulin. The general immunoglobulin dose recommended for post measles exposure is 0.25 mL/kg up to a maximum of 15 mL intramuscularly, and for immunocompromised individuals, 0.5 mL/kg up to a maximum dose of 15 mL [2].

Laboratory investigations

Serological investigations (measles-specific IgM and IgG) are performed in the regional virus laboratories while virus isolation and molecular typing is performed by the national MMR laboratory at the Swedish Institute for Infectious Disease Control [3-4].

Results

Five cases of measles in unvaccinated individuals were notified to the Medical Officer in the Department of Communicable Disease Prevention and Control in the region Västra Götaland, Sweden between mid-January and late March 2008. Two cases were adults, aged 39 and 44 years, and three cases were children, aged 11 years, nine years and 18 months. Four of them were epidemiologically linked and the fifth case, for whom no epidemiological link has been established, had a link to the current outbreak through molecular typing of the isolated measles virus strains.

Index case

The index patient, an unvaccinated 11-year-old girl born in Sweden, developed fever and respiratory symptoms six days after returning from a visit to Paris, France, in mid-January 2008. On the third day of illness a rash was noted, and on the fourth day, she visited the paediatric emergency department at a local hospital. After 30 minutes in an open waiting room, she spent another five hours in an examination room without ante-room. During this period, a large number of young infants, children, accompanying parents and several hospital staff were present in the emergency department and the waiting room (Figure 1). Measles-specific IgM antibodies confirmed the measles diagnosis.

First generation of new cases

The first generation of cases included the index patient's younger sister, nine years old, and two visitors in the emergency department where the index patient sought medical attention, a 39-year-old pregnant woman and an 18-month-old boy.

Second generation of new cases

The second generation of cases included only one adult woman, 44 years old who had the same measles virus genotype and an onset of illness consistent with this outbreak. However, the source of her infection remains unknown and no epidemiological link has been established with the other cases.

Contact tracing and prophylactic treatment

Contact tracing and prophylactic treatment was initiated on the day the index patient was diagnosed with measles. All contacts to the index and subsequent cases were listed, traced and questioned about previous natural disease or immunisations against measles. On four separate occasions patients, accompanying family members and hospital staff in hospital and out-patient areas were exposed to measles, including a large number of susceptible and vulnerable individuals, i.e. pregnant women, infants and young children (Figure 1).

Immediate family of index case

A younger unvaccinated sister was exposed to the index case and developed measles nine days after the index case. During her incubation period, she was kept at home to avoid transmission of measles to non-immune class/schoolmates.

School of index case

The index case attended a school for children aged 12-16 years. The index case had attended school the day before developing fever, cough and coryza. Three unvaccinated children were identified in the school. Their parents were informed about the situation and the children were offered MMR vaccination. All children 11-12 years old had received only one dose of MMR at the age of 18 months and were due for the second dose the week after the index case fell ill. The second dose was given as planned. No further cases of measles evolved among the schoolmates.

Paediatric emergency department

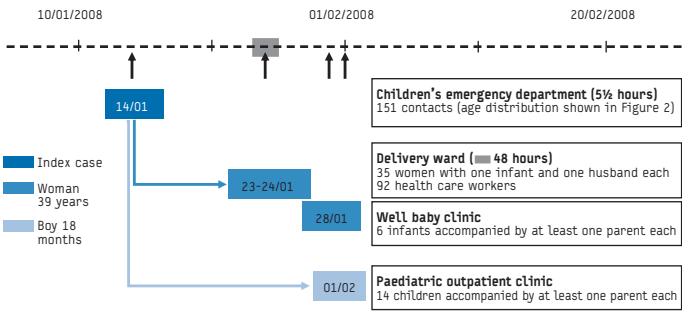
Altogether 151 visitors were exposed during the index patient's stay in the emergency department (see Table: nosocomial exposure I). All those that were uncertain of their immunity to measles, including those with no history of measles or incomplete MMR vaccination were offered post-exposure prophylactic treatment (see age distribution in Figure 2). By the time this could be arranged, 72 hours had passed and it was therefore too late for prophylactic MMR vaccine. Instead, polyvalent immunoglobulin (Beriglobin® CLS Behring 165 mg/mL) was administered to 61 contacts. Thirteen people who had only received the first dose of the MMR vaccine (MMR-I) were considered semi-immune and were therefore offered a second dose (MMR-II).

Delivery unit and postnatal ward

Among those exposed in the paediatric emergency department was a 39-year-old woman in late pregnancy. Due to natural immune suppression during pregnancy, she was considered immunocompromised and therefore received the maximum dose of 15 mL immunoglobulin. Nine days post exposure (six days post prophylaxis), she was admitted to the delivery unit for 48 hours and gave birth to a healthy full-term child. On the fourth day post partum the mother developed fever, cough and bilateral conjunctivitis. Since measles virus may be spread as early as several days before

Figure 1

Time line of nosocomial measles transmission and exposed contacts, Gothenburg, Sweden, January-February 2008 (n≥388)



Due to post exposure prophylactic treatment only two secondary cases were generated out of 74 susceptible contacts. These two, however, exposed approximately 237 persons (on three separate occasions).

onset of rash, the woman could have been contagious at delivery. Her child was given immunoglobulin prophylaxis of 0.25 mL/kg on the seventh day following birth. In retrospect, 35 pregnant women were identified as having been admitted to the delivery and postnatal ward during the same 48-hour-period in which the woman who developed measles had been a patient. In addition, their newborn infants, the accompanying family members that visited the delivery ward and postnatal ward, and the 92 hospital staff that had been on duty could theoretically have been exposed (see Table: nosocomial exposure II).

All 35 post-partum mothers were contacted and asked about their immunity to measles (i.e. previous natural disease or vaccination). Seventeen were uncertain of their status; therefore serology was performed on their antenatal sera. Laboratory results obtained for three of the women showed no measles-specific IgG antibodies. As a precaution only, since more than seven days had passed, the infants of these non-immune mothers were given immunoglobulin.

Seven hospital staff in the delivery and postnatal wards did not know their immune status and were temporarily suspended from further work (1-3 days) pending serology results. Serology result later revealed that all seven were immune.

Well baby clinic

Before measles was suspected, on the second day from onset of symptoms of fever, conjunctivitis and cough in the mother, the above 39-year-old measles case and her newborn child, visited the well baby clinic for a routine check-up of the baby at the same time as six other families (see Table: nosocomial exposure III).

Measles was initially confirmed in this woman by PCR performed on the nasopharyngeal aspirate and later by the development of measles-specific IgM (21 days post exposure and eight days after initial symptoms).

Paediatric outpatient clinic

Two weeks after visiting the emergency department, all immunoglobulin-treated individuals were contacted a second time (see the chapter on 'Follow-up' below). It was then noted that an 18-month-old boy was ill, with onset of fever and cough on day 14 after exposure (27 January). At this time, there were no signs of rash or conjunctivitis. On the scheduled follow-up in the department of infectious diseases on 2 February, he still had mild symptoms of fever and coryza. Viral PCR on a nasopharyngeal aspirate revealed respiratory syncytial virus and measles virus. It was interpreted as a mild case of measles, modified by the immunoglobulin but still contagious.

The family had visited a paediatric outpatient clinic on 1 February due to fever and coryza (see Table: nosocomial exposure IV). A further fourteen children (aged five months to 14 years), eight of whom were considered as non-immune to measles, were exposed in the paediatric outpatient clinic. However, at the time of diagnosis it was too late for immunoglobulin treatment of the exposed; therefore all these children were informed about the risk and symptoms of measles and followed clinically. No further cases of measles were identified.

Follow-up of immunoglobulin- treated individuals

Two weeks after visiting the emergency department, all 61 immunoglobulin-treated individuals were contacted a second time. It was then concluded that all were asymptomatic except for the 18-month-old boy mentioned above and the 39-year-old woman who had recently given birth. Her newborn child did not develop any symptoms. All immunoglobulin-treated individuals older than 12 months are still to be contacted again in three months for administration of MMR vaccine.

Table

Individuals, treatments and outcome at four nosocomial exposures to measles, Gothenburg, Sweden, January 2008 (n≥388)

	Exposed individuals	Susceptible and IgG-treated individuals	Number of measles cases among IgG-treated individuals
(I) Children's emergency department	151[a]	61[a]	2
(II) Delivery ward:			
mothers with infants	70[b]	3	0
accompanying spouses	35	0	0
hospital staff	92[c]	0	0
(III) Well baby clinic	≥ 12[d]	10	0
(IV) Paediatric outpatient clinic	≥ 28[d]	0	0

[a] Including 10 non-immune hospital staff.

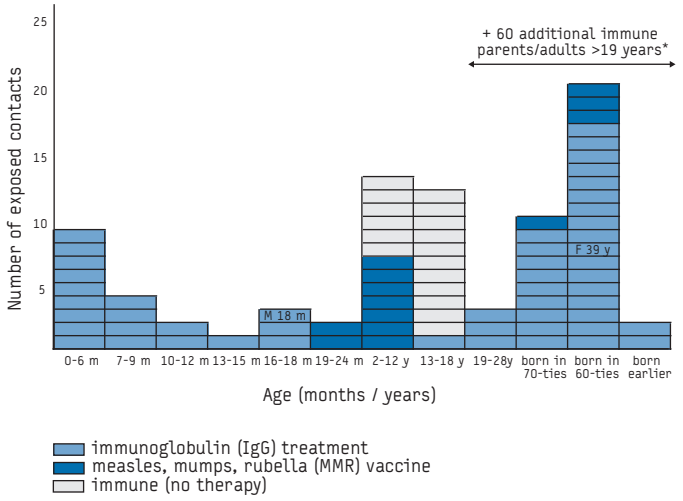
[b] Seventeen women with unknown immunity were tested, three were susceptible and their infants IgG-treated.

[c] Seven with unknown immunity were tested and temporarily suspended from work pending serology result.

[d] We estimated at least one parent accompanying each child to the clinic; the exact figures are not known.

Figure 2

Children, siblings, parents and hospital staff exposed to measles in the children's emergency department by age, Gothenburg, Sweden, January 2008 (n=151)



* The exact age of these 60 people was not known.

Of 151 exposed individuals, 74 were non-immune (10 hospital staff excluded from above chart) and were given post-exposure prophylaxis on the third day (>72 hours post exposure): Sixty-one were given immunoglobulin and 13 who had had one dose of MMR vaccine were given a second dose. Among those treated with immunoglobulin were an 18 month-old boy (M 18 m) and a 39 year-old pregnant woman (F 39 y) who later developed clinical illness.

Molecular typing of isolated measles virus strains

Serum and/or nasopharyngeal aspirate samples from four of the five patients with clinical symptoms of measles were available for laboratory investigations. In all four cases, measles-specific IgM or measles virus nucleic acid was identified. The patient without laboratory verification was the younger unvaccinated sister of the index case with an epidemiological link. Molecular typing of the isolated measles virus was performed on PCR products either from serum samples (in two cases) or from the nasopharyngeal aspirate (in one case). In all three individuals, identical sequences of the nucleoprotein gene were obtained and measles virus genotype D4 was identified. Molecular typing was instrumental in linking one of the cases to the current outbreak, since no epidemiological link could be established.

Organisation of control measures and use of media contacts

The implemented control measures involved a prompt and early response with regards to contacting susceptible, exposed individuals within hospital or out-patient settings. It required close multidisciplinary cooperation to identify and question all exposed individuals, initiate laboratory investigations and administer the recommended prophylaxis. Regular telephone conferences were held exchanging information and keeping all participants updated. Press releases were sent out and notices published on the website of the department of communicable disease prevention and control in Gothenburg. All general practitioners, emergency wards, infectious departments, paediatric departments, well baby clinics and paediatric outpatient clinics received continuous information via fax and mailing lists. Information about the nosocomial spread was disseminated through the media (television, local newspapers), alerting the general public to the symptoms of measles. In cases of a suspected measles infection, the public were advised to first contact the emergency medical services by telephone and if possible seek infectious disease departments where isolation routines are well established. The measles situation in Gothenburg was also continuously reported on the national level in the weekly newsletter EPI-aktuellt published by the Swedish Institute of Infectious Disease Control in Stockholm [5-7] to inform all health-care professionals in Sweden and increase their awareness of measles.

Discussion

In total, at least 388 people were exposed to measles in the context of the described outbreak. Seventy-four individuals were given immunoglobulin, another 13 individuals were offered a second dose of MMR vaccine, and three children in the index case's school were offered their first dose of MMR vaccine. Four of the exposed people developed measles. One of them was isolated at home, and one was not reached by the control measures, while two others were identified in time and received immunoglobulin treatment, but developed a milder form of the disease.

The number of measles cases reported in Sweden has varied from one to 77 cases per year during the last decade. The vaccination coverage in Sweden for one dose of MMR is over 99% and for two doses over 95%. No catch-up programme has ever been implemented targeting non-immune individuals, e.g. those that are too old to have been offered measles or MMR-vaccine or those that at one point in their life refused to be immunised but later may have been willing to receive the vaccine. In a recent study on measles-specific antibodies in antenatal sera from individuals born between 1965 and 1970, 7% of all women were susceptible to measles [8].

The five cases described here represent three different non-immune sub-populations in Sweden; the two adults had never contracted measles at the time it was circulating endemically and were too old to have been offered measles vaccination within the paediatric immunisation programme; the two older children belonged to a family that refused MMR vaccination; and the youngest child was still in the window of susceptibility as it had not yet received the first dose of MMR.

Nosocomial transmission generating clusters of secondary cases have recently been described [9, 10]. Physicians who seldom or never see measles cases in their practice, often have the misconception that measles is a mild disease. Reports from several recent outbreaks, however, describe a high (for European standards) mortality, and morbidity with frequent respiratory and central nervous system involvement [11-13]. Due to various complications, hospitalisation and additional supportive therapy is required in up to one third of the cases [14]. It is therefore very important to provide efficient protection at least for people at a high risk of developing serious disease, i.e. non-immune pregnant women, their newborn children and other immunocompromised individuals. A recent review of cases of measles in Sweden in 2005/2006 showed that more than half of all patients were hospitalised, often with pneumonia (unpublished data, Swedish Institute for Infectious Disease Control), suggesting that all measles-exposed individuals, irrespective of age, benefit from control measures.

Studies performed in the post-vaccination era indicate that young adults have lower antibody levels than the same age group at the time when wild type virus was still widely circulating [15,16]. Consequently, this decrease could also affect the amount of protective antibodies in the IgG fraction of pooled plasma obtained from vaccinated donors. In fact, none of the immunoglobulin preparations available on the Swedish pharmaceutical market today has measles prevention as an approved indication any longer. Nevertheless, we only observed two mild secondary cases, which did not require hospitalisation, among those treated with immunoglobulin in the course of the outbreak described here,

The lower antibody levels in young females, due to vaccine-induced immunity, also affect the time infants are protected by maternal antibodies [8]. The possible need for lowering the age for the first dose of MMR must be followed. It would be advantageous to have vaccines that are not affected by the amount of maternal antibodies and that could be given at any age. As the current live attenuated vaccines probably will continue to be used, the need for a third dose of MMR for young adults also ought to be assessed. Evaluating measles-specific antibodies in antenatal sera is an alternative strategy to identify susceptible women that could then be followed up by post-partum vaccination.

It is important to identify the non-immune sub-populations in a country. Different methods may be called for in different settings. Sero-epidemiological studies of the population and sub-populations may be helpful. A vaccination registry could in the long term be instrumental. Many countries are currently introducing a booster dose for diphtheria, tetanus and pertussis at the age of 14-15 years. This opportunity should be used to check whether all school children have received all doses of the recommended vaccines – including the MMR vaccine. Those who are behind in their schedules should be offered a final opportunity to receive the vaccines they have missed or refused earlier. However, it is vital

to exclude pregnancy before providing the live MMR vaccine to fertile young females.

Another important issue we observed in this outbreak was the lack of awareness among the healthcare workers of their own immune status, especially of those working in units where non-immune or immunocompromised patients are treated. It has previously been observed that employees working in medical facilities are at higher risk of being exposed to measles. Those that contract the disease may further transmit it and recommendations for preventive measures have therefore been given [2]. What preventive strategy that is most cost-effective, may be discussed in each institution and may differ between countries. Medical history should be obtained upon employment, and adequate immunisation recommended to those that are not immune, especially if they are likely to work with susceptible risk groups such as non-immune children, immunosuppressed transplant recipients or patients with malignant disorders. Alternative suggestions involve testing such people for their immune status upon employment or providing a booster dose of MMR, which would facilitate management, should any future exposure occur.

The genotype D4 identified in this outbreak has been reported from several European countries already in 2005/2006 [17]. Nine different measles virus genotypes were identified during this period throughout the World Health Organization (WHO) European Region, but all major epidemics were associated with the genotypes D4, D6 and B3. Highly mobile and unvaccinated communities have caused a massive spread of measles virus D4 throughout the whole region and this genotype is still causing outbreaks.

In conclusion, limiting outbreaks of measles with control measures is possible and should be done in order to avoid serious complications in the affected individuals, to prevent larger outbreaks, and to prevent the disease to become endemic again. In children with a recent history of travelling, both within and outside Europe, who develop a rash, a possible measles infection should be considered, and they should be kept in isolation until diagnosed. Finally, offering MMR-vaccination free of charge to susceptible individuals of all ages would significantly help to reach the goals set by WHO Regional Office for Europe to eradicate measles from the European region by 2010.

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First report of a *Salmonella enterica* serovar Weltevreden outbreak on Réunion Island, France, August 2007

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An outbreak of gastroenteritis involving 26 guests of a wedding dinner occurred in August 2007 in Réunion Island, a French Overseas Department. *Salmonella* was isolated in 61.5% of cases and the two isolates serotyped were of serovar Weltevreden. We believe this to be the first food-borne outbreak due to *S. enterica* serovar Weltevreden described in Réunion Island. The epidemiological and environmental investigations of this outbreak did not provide enough evidence to identify a single vehicle of infection. It is necessary to improve surveillance of salmonellosis by multidisciplinary cooperation between clinicians, epidemiologists, microbiologists and veterinarians on Réunion Island.

Introduction

Salmonellosis is estimated to affect three billion people and to cause 200,000 deaths every year [1]. *Salmonella enterica* is one of the most common causes of bacterial gastroenteritis worldwide and is often implicated in food-borne outbreaks. More than 2,500 serovars of *S. enterica* have been identified [2]. *S. enterica* serovar Weltevreden (hereafter referred to as *S.* Weltevreden) has been reported as a frequent and increasingly common cause of human infection in the restricted area of Southeast Asia [2,3]. The French National Reference Centre for *Salmonella* (Centre National de Référence des *Salmonella* – CNR-Salm) at the Institut Pasteur, Paris has identified sporadic cases of *S.* Weltevreden infection in Réunion Island and in other islands in the Indian Ocean (Weill FX, personal data) but no outbreak due to this serovar has previously been described on Réunion. In France, including French Overseas Departments, collective (at least two cases) food-borne poisoning is subject to mandatory disease notification and must be reported to the relevant Direction régionale or Direction départementale des affaires sanitaires et sociales (DRASS or DDASS). An outbreak investigation is then conducted by the DRASS environmental unit and by veterinarians from the Direction des services veterinaries (DSV), sometimes in collaboration with the epidemiologists from the Cellule interrégionale d'épidémiologie (CIRE) of the Institut de Veille Sanitaire (French Institute for Public Health Surveillance). The management of such outbreaks is the responsibility of the public health medical doctor of the relevant DRASS.

On 30 August 2007, 11 cases of acute gastroenteritis were reported to the DRASS of Réunion Island. All cases were guests of a wedding dinner which had taken place on the evening of 25 August. An outbreak investigation was conducted among the dinner participants to identify risk factors and the vehicle of infection. We report the results of this investigation.

Methods

An outbreak-associated case of gastroenteritis was defined as a person who had eaten at the wedding dinner on 25 August 2007 and developed diarrhoea (two or more liquid stools per 24 hours) or fever (≥38 ºC) in addition to at least one of the following three symptoms: nausea, vomiting, or abdominal pain within the 24 hours after the dinner. Eligible cases were defined as confirmed if *S.* Weltevreden was microbiologically isolated from stools, as probable if *Salmonella* was isolated from stools without serotyping, and as clinical cases when data on biological confirmation were unavailable.

An active case detection was conducted to assess the total number of cases. An unmatched case-control study was conducted to try to identify the vehicle for transmission. To do so, we proceeded to a telephone interview with a standardised questionnaire. These interviews were limited to voluntary guests who accepted to give their telephone numbers. Guests who accepted to answer the questionnaire and did not mention any symptoms after the dinner were considered as controls. Data were collected and analysed with WinTiac® version 1.6 software. Food-specific odds ratio (OR) and 95% confidence intervals (95% CI) were calculated for the consumption of food items. The Chi 2 test was used to compare proportions between groups (5% significance level). Serotyping of *Salmonella* isolates and antimicrobial drug susceptibility were performed at the CNR-Salm, as previously described [4]. Kitchen facilities were inspected but no food items could be sampled because of the long delay (five days) between the dinner and the notification of the outbreak.

Results

Descriptive findings

On 25 August 2007 at 8.30 PM, 285 guests were present at the wedding dinner. The meal was prepared by several guests at their homes and was brought to a communal building where the wedding took place. Food items were then warmed up in the communal kitchen and served by several guests to others. Most of those who had prepared and served the food refused to participate in the investigation. Active case detection found 26 persons who presented symptoms according to the case definition and were considered as cases. Among them, 10 cases were considered as

clinical, 14 were probable and two were confirmed. The mean age of cases was 30 years and the male to female ratio was 1:1. Diarrhoea was reported by all of the 26 cases, 16 experienced vomiting and 15 had fever. Other clinical symptoms were abdominal pain (n=1) and headache (n=1), the latter not included in the case-definition. None of the cases were hospitalised and all the patients recovered. The epidemic curve shows that the median time of illness onset was on Sunday 26 August 2007 at 8.00 AM [5.00 AM - 10.30 AM] (Figure). The median time of incubation was 11 hours and 50 minutes [8h50-14h00].

Microbiologic and environmental findings

Stool specimens from 18 persons were microbiologically tested, and in 16 of these (61.5% of the 26 cases) *Salmonella* was confirmed by culture. Two isolates were further analysed by serotyping, both were *S.* Weltevreden. These two isolates were susceptible to all 32 antimicrobial drugs tested.

No testing could be done on food items. However, an interruption of the hot and cold chain of food preparation was strongly suspected to have contributed to the outbreak.

Case control study

For the case control study, we included 26 cases and 26 controls. In univariate analysis, three exposures were statistically associated with risk of illness (Table). The most relevant food exposure was the chicken eaten by 88% of the cases and 58% of the controls (OR=5.62; CI 95% 1.34 to 23.56; p=0.01). The two other significant food items were: peas (OR=5.13; CI 95% 1.57 to 16.77; p=0.005) and rice (OR=4.03; CI 95% 1.08 to 15.09; p=0.03). However, none of these three food items could be considered as an independent vehicle of the food poisoning after adjustment with the Mantel–Haenszel method.

Discussion

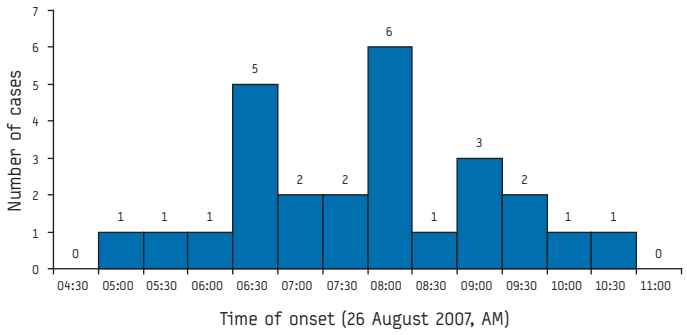
We believe this to be the first food-borne outbreak due to *S. enterica* serovar Weltevreden described in Réunion Island. The outbreak involved 26 guests of a wedding dinner. The serovar Weltevreden was isolated in two samples. These were the only two isolates serotyped because of the poor contribution of local laboratories in sending stool specimen to the CNR-Salm in Paris due to distance and cost of transport. However, the homogeneity of the clinical presentation of cases in the cluster, the shape of the epidemic curve, the isolation of *Salmonella* in 61.5% of cases (88.9% of tested stools) and the identification of the same serotype in the two tested specimens allowed us to strongly suspect this serotype as the cause of the outbreak.

The results of the case-control study suggested that none of the three food items statistically associated with the risk of illness (chicken, peas and rice) could be considered as an independent vehicle of infection after adjustment. There are several methodological limitations in the case-control study that should be noted. The small sample size available for the case-control study due to poor contribution of guests limited our ability to draw strong conclusions. Furthermore, environmental investigations such as testing of food items could have strengthened our findings, but were not conducted because samples were no longer available.

Before 1970, *S.* Weltevreden constituted less than 4% of the total number of cases of human salmonellosis in the world [3]. It was the most common serovar to cause human infections in India during the early 1970s [5], and the one most frequently

Figure

Distribution of cases of gastroenteritis among dinner guests by time of onset of symptoms, Réunion Island, 26 August 2007 (n=26)



Table

Frequency of selected exposures among cases and controls, outbreak of gastroenteritis, Réunion island, August 2007

Food item consumed	Cases (n=26) n (%)	Controls (n=26) n (%)	OR (IC 95 %)	p
Salmon petit four	24 (92 %)	21 (81 %)	2,86 (0,5-16,3)	0,42
Pork petit four	24 (92 %)	24 (92 %)	1 (0,13-7,69)	1
Pizza	24 (92 %)	24 (92 %)	1 (0,13-7,69)	1
Duck galantine	23 (88 %)	21 (81 %)	1,83 (0,39-8,59)	0,7
Pork roast	25 (96 %)	20 (77 %)	7,5 (0,83-67,5)	0,1
Minced cabbage	24 (92 %)	19 (73 %)	4,42 (0,82-23,79)	0,14
Raw vegetable	22 (85 %)	23 (88 %)	0,72 (0,14-3,58)	1
Chicken	23 (88 %)	15 (58 %)	5,62 (1,34-23,56)	0,01
Swordfish in combava sauce	13 (50 %)	14 (54 %)	0,86 (0,29-2,55)	0,78
Rice	22 (85 %)	15 (58 %)	4,03 (1,08-15,09)	0,03
Peas	19 (73 %)	9 (35 %)	5,13 (1,57-16,77)	0,005
Chili sauce	12 (46 %)	11 (42 %)	1,17 (0,39-3,5)	0,78
Fruit mousse cake	17 (65 %)	22 (85 %)	0,34 (0,09-1,31)	0,1

isolated from humans in Thailand during the years 1993-2002 [3]. Similar findings have been reported from Malaysia between 1983 and 1992 [6]. Thong et al. [7] found the same subtypes of *S.* Weltevreden among isolates infecting humans and those in raw vegetables, suggesting that this is a potential reservoir of this serovar in Malaysia. *S.* Weltevreden was the most common serovar in isolates from seafood, water, and duck in Thailand [3]. In a recent study in the United States, *S.* Weltevreden was the most common serovar found in seafood mainly imported from Thailand and Malaysia [8]. These observations could point to a water-related source for *S.* Weltevreden.

The results of the outbreak investigation described in this paper suggest that *S.* Weltevreden could be associated with a food-borne outbreak in Réunion Island in the Indian Ocean, as it was observed in other countries [9,10]. A better knowledge of the epidemiology of this serovar in humans and in animals is needed in this area to identify the source of transmission. Clusters of collective food-borne poisoning are subject to mandatory disease notifications in France and its Overseas Territories. Between 1996 and 2005, 72 food-borne outbreaks have been notified to the DRASS of Réunion. Among these outbreaks, 16 (22.2%) were due to *Salmonella* (Typhimurium=4; Enteritidis=1; unknown species=11) [11]. However, these data are certainly incomplete because of the recognized under-reporting of such events in Réunion. For a better knowledge of *Salmonella* epidemiology on the island and in the South-West Indian Ocean, it is necessary to raise awareness among physicians of the need of rapid notifications of food-borne outbreaks and to improve collaboration between epidemiologists, clinicians, microbiologists and veterinarians for future outbreak investigations.

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Epidemiological and virological assessment of influenza activity in Europe, during the 2006-2007 winter

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3. Members of EISS (2006-2007 winter), see article supplement

Influenza surveillance in Europe is based on influenza surveillance networks that cooperate and share information through the European Influenza Surveillance Scheme (EISS). EISS collected clinical and virological data on influenza in 33 countries during the 2006-2007 winter. Influenza activity started around 1 January and first occurred in Greece, Scotland and Spain. It then moved gradually across Europe from south to north and lasted until the end of March. In 29 out of 33 countries, the consultation rates for influenza-like-illness or acute respiratory infections in the winter of 2006-2007 were similar or somewhat higher than in the 2005-2006 winter. The highest consultation rates for influenza-like-illness were generally observed among children aged 0-4 years and 5-14 years. The predominant virus strain was influenza A (97% of total detections) of the H3 subtype (93% of H-subtyped A viruses; 7% were A(H1)). The influenza A(H3) and A(H1) viruses were similar to the vaccine reference strains for the 2006-2007 season, A/Wisconsin/67/2005 (H3N2) and A/New Caledonia/20/99 (H1N1) respectively. The majority of the influenza B viruses were similar to the reference strain B/Malaysia/2506/2004, included in the 2006-2007 vaccine.

In conclusion, the 2006-2007 influenza season in Europe was characterised by moderate clinical activity, a south to north spread pattern across Europe, and a dominance of influenza A(H3). Overall there was a good match between the vaccine virus strains and the reported virus strains.

Introduction

Influenza is an acute self-limiting viral disease of the upper respiratory tract. Influenza has a considerable public health impact in Europe each winter because of its ability to spread rapidly through populations by coughs and sneezes from infected people [1].

During seasonal influenza epidemics 5-15% of the population are affected with upper respiratory tract infections [2]. Seasonal epidemics are associated with substantial demands on healthcare resources and considerable costs due to increases in general practice consultation rates, clinical complications, hospitalisations, drug treatment and absence from work [3,4]. Although difficult to assess, it is estimated that worldwide between 250,000 and 500,000 people die from severe illness as a result of an influenza virus infection every year [2].

The European Influenza Surveillance Scheme (EISS) is a collaborative network of primary care physicians, epidemiologists and virologists that aims to contribute to a reduction in morbidity and mortality due to influenza in Europe by active clinical and virological surveillance of influenza. Effective influenza surveillance enables an early detection and characterisation of an epidemic as well as the isolation and antigenic characterisation of circulating viruses to assist in the formulation of the following season's vaccine and to provide new vaccine strains [5,6]. The participating national reference laboratories have functioned within EISS as the Community Network of Reference Laboratories for Human Influenza in Europe (CNRL) since 2003. They report virus detections and identification data to EISS and work on improving the virological surveillance [7,8].

EISS aims to cover all Member States of the European Union (EU), as required by EU Decision 2119/98/EC on the establishment of dedicated surveillance networks for communicable diseases [9]. During the 2006-2007 winter, EISS covered 26 of the 27 current EU countries (except Bulgaria), as well as Norway, Serbia, Switzerland and Ukraine. A total of 38 national influenza reference laboratories participated in EISS.

The identification of circulating viruses and the recognition of virological changes are major tasks for EISS in order to fulfil its early warning function [7]. There is a particular need to detect and monitor the emergence or re-emergence of viruses with pandemic potential, viruses that 'mismatch' with the vaccine strain, and to monitor the clinical impact of circulating viruses in the community.

During the winter period (week 40 to week 20 of the following year) a Weekly Electronic Bulletin is published each Friday on the EISS website (www.EISS.org), which allows members, public health authorities and the general public to view influenza activity in all participating countries.

This paper presents an analysis and interpretation of influenza surveillance data collected by European countries that were members of EISS during the 2006-2007 winter.

Methods

Population

All 30 countries that were members of EISS during the 2006-2007 winter actively monitored influenza activity from about week 40/2006 to about week 20/2007 (Table below). In this paper, England, Northern Ireland, Scotland and Wales are referred to as

countries as they have their own surveillance systems, and thus we considered EISS to include 33 countries. The characteristics of the sentinel networks are summarised in table 1 of the article supplement. The median weekly population under clinical surveillance by the sentinel networks during the 2006-2007 winter varied from 0.4% to 100% of the total population of a country, representing a median number of 30.8 million inhabitants of Europe. In total, about 25,500 general practitioners (GPs), paediatricians and other physicians participated in the sentinel surveillance during the 2006-2007 winter. However, the weekly number of physicians that actually reported was often lower. In general, the age distribution of the population under surveillance was representative for the age distribution of the total population in a country. However, in some countries the population under surveillance was skewed to the lower ages (partly due to a high proportion of paediatricians) and/or higher ages. Further information on the representativeness of the population under surveillance in EISS can be found for most countries in Aguilera *et al*. [10].

Table

Overview of influenza activity in European countries during the 2006-2007 winter[1]

Country (N=33)	Week of peak clinical activity	Most affected age groups[2]	Intensity (peak level)	Week(s) of peak virus detections[3]	Dominant virus type/subtype	Geographical spread (peak level)
Influenza-like illness:						
Austria	6	0-4	Medium	9	A(H3N2)	Local
Belgium	7	0-4, 5-14	Medium	6	A(H3N2)	Widespread
Cyprus	2	n.a.	n.a.	n.a.	n.a.	n.a.
Czech Republic	6	0-4, 5-14	Medium	6	A(H3)	Widespread
Denmark	10	0-4	High	9	A(H3N2)	Widespread
England	7	15-64, 0-4, 5-14	Medium	7	A(H3)	Regional
Estonia	10	5-14, 0-4	High	9	A(H3N2)	Widespread
Finland	n.a.	n.a.	n.a.	n.a.	A	n.a.
Greece	3	n.a.	Medium	3	A(H3N2)	Local
Hungary	6	n.a.	Medium	7	A(H3)	Local
Ireland	7	15-64, 0-4, 5-14	Medium	7	A(H3)	Local
Italy	8	15-64	Medium	5	A(H3N2)	Widespread
Latvia	9	0-4, 5-14	High	9	A(H3)	Widespread
Lithuania	9	n.a.	High	8	A	Regional
Luxembourg	7	n.a.	High	6	A(H3N2)	Widespread
Malta	5	n.a.	High	n.a.	n.a.	Sporadic
Netherlands	9	0-4	Medium	9	A(H3)	Widespread
Northern Ireland	5	n.a.	Medium	5	A(H3)	Sporadic
Norway	8	15-64, 0-4	High	6	A(H3)	Widespread
Poland	9	0-4, 5-14	Medium	9	A(H1)	Sporadic
Portugal	6	5-14	Medium	6	A(H3)	Widespread
Romania	5	0-4	Medium	4	A(H3N2)	Regional
Scotland	2	n.a.	Medium	6	A(H3)	Regional
Serbia	6	0-4, 5-14	Medium	6	A(H3)	Local
Slovakia	6	5-14, 0-4	Medium	4	A(H3N2)	Regional
Slovenia	8	0-4, 5-14	Medium	7	A(H3N2)	Local
Spain	6	5-14, 0-4	Medium	5	A(H3N2)	Widespread
Sweden	10	n.a.	High	9+10	A(H3N2)	Widespread
Switzerland	6	5-14, 0-4, 15-64	Medium	6	A(H3N2)	Widespread
Ukraine	n.a.	n.a.	n.a.	8	A(H1N1)+A(H3N2)	n.a.
Wales	7	15-64	Low	7	A	Sporadic
Acute respiratory infections:						
France	6	0-4, 5-14	Medium	5	A(H3N2)	Widespread
Germany	9	0-4	Medium	9	A(H3N2)	Widespread

[1] Sentinel data, except for dominant virus type/subtype for which sentinel and non-sentinel data were taken into account. For definitions of indicators see reference 13. n.a. = not applicable as no data was available or insufficient data was available. No peak = activity was not above baseline or was flat during the whole winter. Finland did not report clinical data. Cyprus did not report virological data and Sweden did not report sentinel virological data.
[2] Based on overall winter period consultation rates. If two or more age groups are shown the sequence is: most affected - less affected.
[3] Estimated where possible taking into account the percentage of influenza virus positive specimens and the absolute number of detections, if the percentage positive specimens was ambiguous only the absolute number of detections was used.

Clinical surveillance

In each of the countries except Finland and Ukraine, one or several networks of sentinel physicians reported consultation rates due to influenza-like-illness (ILI) and/or acute respiratory infection (ARI) on a weekly basis. Twenty-seven countries reported ILI consultations per 100,000 population; Malta and Cyprus reported ILI per 100 consultations and France and Germany reported ARI consultations per 100,000 population. In some countries doctors have patient lists, which mean that they have an exact population denominator. For other countries where patients have a free choice of doctors the population denominator has been estimated.

Virological surveillance

A proportion of the sentinel physicians, in most cases representative for the surveillance network in the country, also collect nose and/or throat swabs for virological surveillance using a swabbing protocol that guarantees representative swabbing during the winter period (table 1 article supplement) [10]. Combining clinical and virological data in the same population allows the evaluation of clinical reports made by the sentinel physicians and provides virological data in a clearly defined population, i.e. the general population that lives in the area served by the participating physician [11]. In addition to specimens obtained from physicians in the sentinel surveillance systems, the laboratories also collect and report results on specimens obtained from other sources (e.g. from hospitals and non-sentinel physicians). These data are called 'non-sentinel' and are collected in order to have a second measure of influenza activity (which contributes to early warning as the entire population is not covered by the sentinel system) and to validate the sentinel virological data [11]. Based on the collection of virological data, the total population under surveillance by EISS, during the winter 2006-2007, was about 497 million inhabitants living in the area covered by EISS [12].

The virological data included results mostly from cell cultures followed by virus type and subtype identification. Rapid diagnostic enzyme-immunological or immunofluorescence tests were also used to identify the virus type only. Many laboratories also use reverse transcription polymerase chain reaction (RT-PCR) routinely for detection, typing and subtyping. Almost 50% (16/33) of the countries reported antigenic characterisation data and about 30% (11/33) of the countries reported genetic characterisation data of the virus isolates during the 2006-2007 winter.

In addition to the circulation of the seasonal human influenza viruses, EISS laboratories monitored the occurrence of transmission of the highly pathogenic avian influenza virus A(H5N1) to humans in the countries covered by EISS.

Indicators

During the winter period, the weekly clinical and virological data were collected and analysed by the national centres and then entered into the EISS database the following week via the internet [13]. The clinical consultation rates, the indicators of influenza activity (the intensity of clinical activity and the geographical spread of influenza), as well as the dominant virus type/subtype circulating in the population were established on a weekly basis by the national coordinators based on agreed definitions that were published previously [8,14] (see Box). The dominant type/subtype for the whole winter period shown in the Table above was estimated per country using the algorithm published previously [14].

Spatial analysis

A spatial analysis of the timing of peak influenza activity across Europe was carried out using regression analysis of plots of the longitude and latitude of the centre of each country against the week of peak influenza activity of each country, as described previously [15].

Results

Epidemiological data

The seasonal influenza epidemic started around 1 January 2007 in Europe, with consultation rates for ILI or ARI above levels seen outside the winter period first reported in Scotland (week 52/2006) (graphs 1 and 2 article supplement). Eight countries reported a high intensity of clinical activity, Denmark in weeks 9-12/2007, Estonia in weeks 8-10/2007, Latvia in weeks 9 and 10/2007, Lithuania in weeks 8-10/2007, Luxembourg in weeks 4-7/2007, Malta in weeks 2-7/2007, Norway in weeks 7-10/2007 and Sweden in weeks 9 and 10/2007 (see Table above). Furthermore, Greece reported a local outbreak of influenza activity in week 40/2006 and Sweden reported an exceptional cluster of influenza A in northern Sweden (graph 2 article supplement). Most countries (21/33) reported a medium maximum intensity. Only one country (Wales) reported a low level of intensity throughout the season. Compared to the 2005-2006 winter, the consultation rates for ILI or ARI in the 2006-2007 season were similar in 17 countries that reported these indicators and higher in 12 countries. In particular, in Italy, the consultation rate for ILI in the 2006-2007 winter was much

Box

Definitions of indicators

Baseline

Level of clinical influenza activity calculated nationally representing the level of clinical activity in the period that the virus is not epidemic (summer and most of the winter) based on historical data (5-10 influenza seasons).

Intensity

The intensity of clinical activity compares the weekly clinical morbidity rate with historical data:

- Low ¬– no influenza activity or influenza activity at baseline level
- Medium – usual levels of influenza activity
- High – higher than usual levels of influenza activity
- Very high – particularly severe levels of influenza activity (less than once every 10 years)

Geographic spread

The geographical spread is a WHO indicator that has the following levels:

- No activity – no evidence of influenza virus activity (clinical activity remains at baseline levels)
- Sporadic – isolated cases of laboratory confirmed influenza infection
- Local outbreak – increased influenza activity in local areas (e.g. a city) within a region, or outbreaks in two or more institutions (e.g. schools) within a region; laboratory confirmed
- Regional activity – influenza activity above baseline levels in one or more regions with a population comprising less than 50% of the country's total population; laboratory confirmed,
- Widespread – influenza activity above baseline levels in one or more regions with a population comprising 50% or more of the country's population, laboratory confirmed

Dominant virus

The assessment of the dominant virus for the season is based on:

- Sentinel and non-sentinel data (primary assessment sentinel data)
- A minimum number of 10 isolates
- If more than 10% of total A isolates are H-subtyped the H subtype is taken into consideration
- If more than 10% of total A isolates are N-subtyped the N subtype is also taken into consideration
- The limits for co-dominant virus types/subtypes are: 45%:55%

higher than in the previous season (4,282 in 2006-2007 compared to 243 in 2005-2006) (graph 2 article supplement).

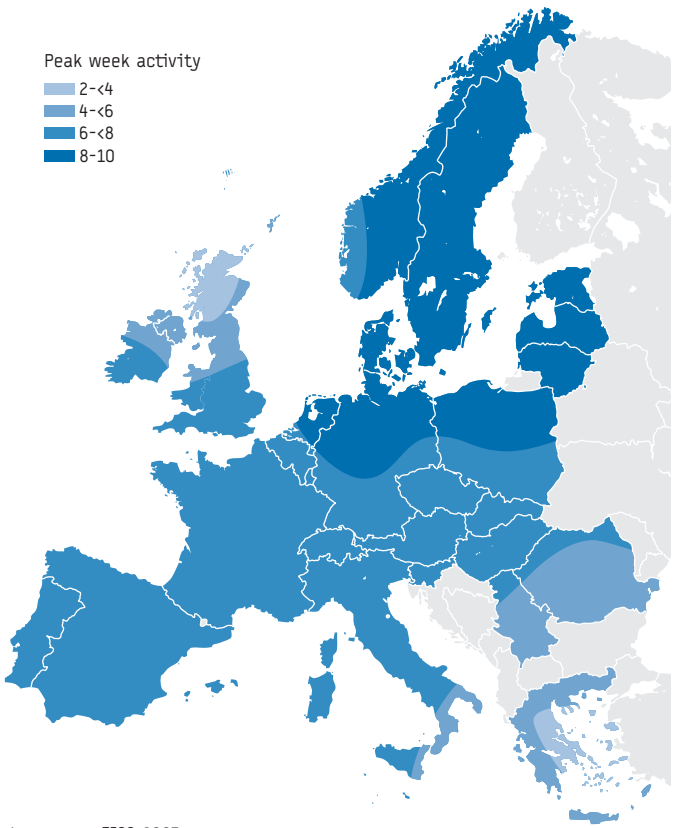
The ILI and ARI consultation rates in Europe reached their peak between week 02/2007 in Scotland and Cyprus and week 10/2007 in Denmark, Estonia and Sweden. ILI and ARI consultation rates started to increase first in the western and south-eastern parts of Europe, then in south-central Europe and finally in the North. Widespread influenza activity was reported across most of Europe by mid-February (week 07/2007). Although influenza activity was still increasing in some countries towards the end of February, in southern and western European countries it started to decline at that time. Clinical influenza activity gradually moved north across Europe and reached its peak around week nine in the Netherlands, Denmark, Germany and Poland, the Baltic states, Norway and Sweden (see Figure 1 below and graph 1 in the article supplement). A similar movement was seen when the timing of peak clinical influenza activity across Europe was analysed. A spatial analysis revealed a significant south-north pattern in the timing of peak influenza activity across Europe during the 2006-2007 winter (R^2=0.287; $p<0.05$ for south-north; R^2=0.060 for west-east) [16]. The timing of peak levels of clinical activity is visualised in Figure 1.

In individual countries, the week of peak ILI/ARI consultation rates coincided roughly with the week of peak sentinel influenza virus detections. In the 29 countries with paired data that could be evaluated the median week of peak ILI/ARI consultation rates was seven (range week 2 –10) and the median week of peak virus detections was seven (range week 3 – 10). In 14 (48%) of the 29 countries, the week of peak consultation rates coincided exactly with the week of peak virus detections. In 24 (83%) of the 29 countries the week of peak consultation rates either coincided exactly with the week of peak virus detections or the peaks coincided with a difference of one week.

In countries reporting age-specific data (N=22), the highest consultation rates during the influenza peak were observed among children in the 0-4 years and 5-14 years age groups, although consultation rates in England, Ireland, Italy, Norway and Wales were also high in the 15-64 years age group (see Table for an overview of the influenza activity).

Figure 1

Timing of peak clinical influenza activity across Europe during the 2006-2007 season



Data source: EISS 2007
Cartography and design: Institute for Hygiene and Public Health University Bonn, Bonn 2007

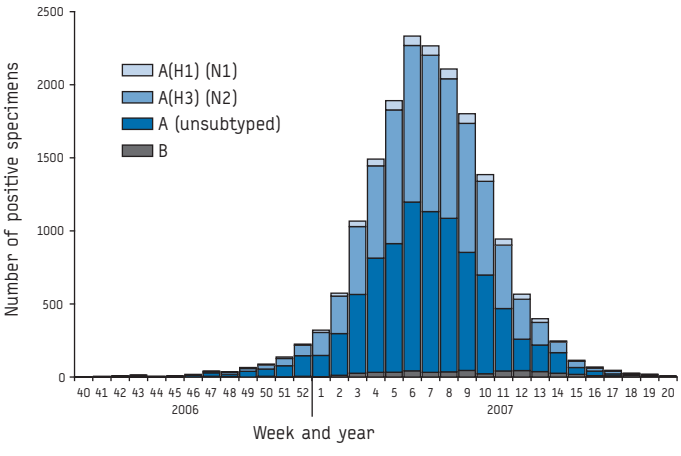
Note: The isobars on the contour maps represent interpolated time of peak activity distributed spatially at 2 week intervals. Countries included in this spatial analysis were: Austria, Belgium, Czech Republic, Denmark, England, Estonia, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, The Netherlands, Norway, Poland, Portugal, Romania, Serbia, Slovakia, Slovenia, Spain, Scotland, Sweden and Switzerland.

Virological data

For Europe as a whole, the largest number of influenza virus positive specimens was detected in week 6/2007 (N=2,254) (Figure 2). A total of 18,278 sentinel and non-sentinel specimens were positive for influenza virus: 17,759 (97%) were influenza A and 519 (3%) were influenza B. Of all haemaglutinin-subtyped viruses (N=8,934), 8,271 (93%) were H3 and 663 (7%) were H1. All 4,208 neuraminidase-subtyped A(H3) viruses were of the N2 subtype and all 504 neuraminidase-subtyped A(H1) viruses were of the N1 subtype. The predominant virus circulating in the individual countries was A(H3). In Poland A(H1) was the dominant subtype, in Romania A(H3N2) was co-dominant with B and in Ukraine A(H1N1) was co-dominant with A(H3N2) (Table). A relatively high proportion of influenza B viruses were detected in Romania (45%

Figure 2

Number of sentinel and non-sentinel specimens positive for influenza viruses, cumulated for all European countries by week, during the 2006-2007 winter (N = 18,278 as of 3 September 2007)



of all influenza viruses) and Ukraine (25% of all influenza viruses); in all other countries this was 14% at maximum (in Greece).

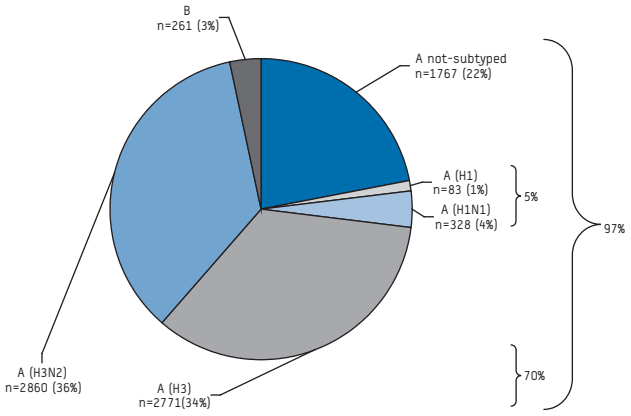
Five countries reported laboratory results for detection of the A(H5N1) virus but none of the 31 specimens from suspected and (possibly) exposed humans tested positive for the A(H5N1) virus. For a detailed breakdown of the virological data for Europe as a whole and by country by week and source (sentinel or non-sentinel) see Figure 3 below, as well as graph 2 and tables 2 and 3 in the article supplement.

Of all 18,278 influenza virus detections, 3,877 have been antigenically and/or genetically characterised: 326 (8%) were A/New Caledonia/20/99 (H1N1)-like, 55 (1%) were A/California/7/2004 (H3N2)-like, 3,318 (86%) were A/Wisconsin/67/2005 (H3N2)-like (a drift variant of A/California/7/2004 included in the vaccine for the 2006-2007 winter), 148 (4%) were B/Malaysia/2506/2004-like (B/Victoria/2/87-lineage) and 30 (1%) were B/Jiangsu/10/2003-like (B/Jiangsu/10/2003 is a B/Shanghai/361/2002-like virus from the B/Yamagata/16/88-lineage that was included in the vaccine for the 2006-2007 winter).

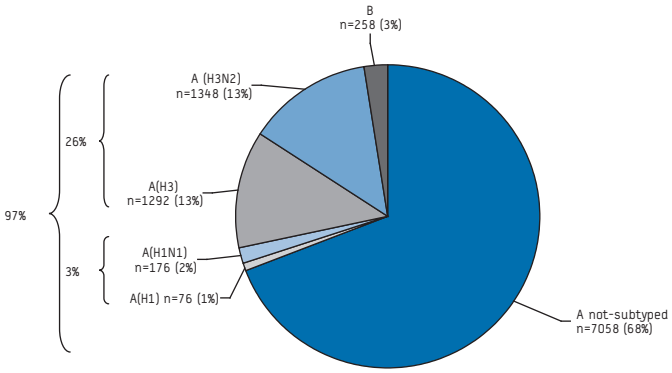
Figure 3

Breakdown of virus detections cumulated for all European countries by source (sentinel or non-sentinel) and by virus type and subtype, during the 2006-2007 winter

Sentinel virological data (N=8,070)



Non-sentinel virological data (N=10,208)



Discussion

The 2006-2007 influenza season was moderate in Europe in comparison to previous years and was predominantly due to influenza A infections, subtype H3, with a homogenous spread of viruses across Europe. Influenza activity in Europe started to increase around 1 January 2007 , which is earlier than in the previous winter, when influenza activity in Europe began late in January 2006. The peak clinical influenza activity by country was for the majority of countries (17/29 countries) similar to the 2005-2006 season, a season dominated by influenza B. For 12 out of 29 countries the peak clinical influenza activity was higher than in the 2005-2006 season. No country had a lower level of influenza activity. The higher peak levels of clinical activity in 2006-2007 compared to 2005-2006 can be attributed to the influenza A(H3) virus, which usually causes more severe disease symptoms, compared to the influenza B virus and the influenza A(H1N1) virus [4]. The total number of virus detections was 18,278, 61% more than the 11,303 detections during the 2005-2006 season when influenza B was dominant [14].

Compared to other seasons that were dominated by influenza A(H3) (e.g. the 2004-2005 season), the peak clinical influenza activity for 2006-2007 was similar or lower in the majority of countries (21/25 countries) [15]. The 2006-2007 season lasted from week 02/2007 to week 10/2007, which is relatively short compared to the previous seven seasons, when it lasted from 12 (1999-2000 season) to 19 (2003-2004 season) weeks [16]. Taking into account the relatively low clinical influenza activity and the relatively short duration, the 2006-2007 winter can be considered moderate compared to previous seasons dominated by influenza A.

For Europe as a whole, the 2006-2007 season showed a relatively homogeneous distribution of virus (sub)types across Europe with a dominance of influenza A(H3) virus. Only in some countries in eastern Europe, there was a relatively high proportion of influenza B virus (Romania) and A(H1) virus (Poland and Ukraine). However, in previous seasons, including the 2005-2006 winter, it has been shown that when investigated on a country level, virus type and even H-subtype dominance can be heterogeneous across Europe [14]. These observations stress the importance of analysing national or regional virus distribution data.

For the 2006-2007 winter there was a good correlation between clinical and virological data (an overall match of 83%, +/- 1 week) compared to the last eight seasons (an overall match of 72%, +/- 1 week) [16]. This result once again emphasizes the strength of the surveillance system in that it combines community-based clinical and virological data.

The direction of movement of increased influenza activity is unpredictable. In three of the eight preceding winters there was a south-north movement in the timing of peak influenza activity in countries across Europe [16]. The winter of 2006-2007 tended to fit into this pattern with a northward movement only becoming significant late in the season (Figure 1, graph 1 in the article supplement). Single clusters of influenza outbreak very early in the season, i.e. in Greece (week 51/2006) and Sweden (week 40/2006), did not succeed in country-wide spread of influenza, probably because conditions for further spread were not favourable. In Sweden a hypothesis about the contributions of local temperature and humidity to local epidemics in the north of the country is under investigation (personal communication Urban

Kumlin, Umeå University. It has been shown that type, subtype and antigenic characteristics of the founder virus, humidity, temperature, UV radiation and air traffic can drive the direction of the movement [17].

Influenza A(H3) viruses that circulated in the 2006-2007 season were antigenically closely related to the A/Wisconsin/67/2005-like vaccine viruses. Similarly, most of the influenza A(H1) viruses were antigenically closely related to the 2006-2007 vaccine virus A/New Caledonia/20/99-like vaccine virus. There were no detections of A(H1N2) in Europe and worldwide observations also suggest that A(H1N2) viruses have become extinct [18]. The majority of circulating influenza B viruses were of the B/Victoria/2/87 lineage and were antigenically and genetically closely related to the 2006-2007 vaccine strain B/Malaysia/2506/2004. To conclude, in the 2006-2007 winter in Europe there was in general a good match between the circulating influenza viruses and the vaccine strains.

In February 2007, The World Health Organization announced the composition of the influenza vaccine for the northern hemisphere to be used for the 2007-2008 influenza season [18]. Based on the available data on the recent influenza viruses provided from all over the world, the WHO modified the recommended composition of the 2007-2008 influenza vaccine compared to the 2006-2007 vaccine. The emergence of a different antigenic variant of A(H1N1) during the 2006-2007 season prompted the WHO to update the vaccine composition to include the A(H1N1)A/Solomon Islands/3/2006-like A(H1N1) strain. The recommendations for the vaccine reference strains for A(H3N2) and B virus remained the same. The European Agency for the Evaluation of Medicinal Products (EMEA) adopted the recommendations of the WHO [19].

In conclusion, the 2006-2007 influenza epidemic in Europe was characterised by moderate clinical activity and a south-north spread pattern across Europe. The dominant virus strain was influenza A(H3), and overall there was a good match between the vaccine virus strains and the circulating virus strains.

Contributors

The members of EISS contributed by weekly submission of influenza surveillance data to EISS during the 2006-2007 winter. JMS Arkema, TJ Meerhoff, A Meijer and WJ Paget carried out weekly analyses of the data and published the Weekly Electronic Bulletins during the 2006-2007 winter. JMS Arkema carried out the overall analysis of the data and prepared the body of the manuscript. A Meijer carried out the analysis for animations of the timeline of increased intensity of influenza activity and national geographic spread of influenza (Graph 1 article supplement). J van der Velden, as chair person of EISS, contributed by supporting the daily operation of EISS during the 2006-2007 winter.

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The authors thank F Ansaldi, U Buchholz, D Fleming and staff of the European Centre for Disease Prevention and Control for critically reviewing and commenting the Weekly Electronic Bulletins published during the 2006-2007 winter. EISS would not be able to monitor influenza activity throughout Europe without the commitment and participation of sentinel physicians across Europe. We would like to thank them for making this surveillance scheme possible. In addition we would like to thank all participating institutions during the 2006-2007 winter (for a full list, see article supplement). The authors thank I Wienand from the University of Bonn for carrying out the Kriging analysis of the timing of peak clinical activity across Europe and providing Figure 1.

Article supplement

The article supplement is available at: http://www.eiss.org/documents/eurosurveillance_supplement_2006-2007_winter.pdf

The article supplement contains:

i) Lists of persons and institutes participating in EISS during the 2006-2007 winter period,

ii) Characteristics of the influenza surveillance networks in EISS,

iii) Animations of the timing of the change of the clinical intensity and geographic spread indicators by country in Europe,

iv) Graphs of the weekly consultation rates and virus detections by country, and

v) Tables with a detailed breakdown by country of the virological data from sentinel and non-sentinel sources.

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Outbreak of *Salmonella* serovar Stanley infections in Switzerland linked to locally produced soft cheese, September 2006 – February 2007

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Salmonella serovar Stanley is rare in Europe. In Switzerland, the number of reported isolates has increased from 2 in 2000 to 25 in 2005. A nationwide outbreak of gastrointestinal illness due to *S.* Stanley occurred from September 2006 through February 2007. Eighty-two cases were documented. Males were 56%; mean age of the cases was 45.7 years (range 0-92). Forty-seven cases (57%) occurred in three western cantons: Vaud, Bern, and Geneva. Twenty-three cases (28%) were hospitalised. In the case-control study conducted to find the source of the outbreak, cases were more likely than controls to have eaten local soft cheese (OR 11.4, p=0.008). One clone of *S.* Stanley strain was isolated from soft cheese and from 77 cases (94%) who reported no history of having travelled abroad. The outbreak ended after the withdrawal of the cheese from the market. This is the first *S.* Stanley outbreak in Switzerland and the first in Europe unrelated to imported products, suggesting an increased local circulation of this previously rare serotype.

Introduction

Salmonella enterica subspecies *enterica* serovar Stanley (*S.* Stanley) is common in Asia, but rare in Europe, America and Australia. Most of the cases reported in Europe have a history of travelling in Asia or consumption of food products imported from Asia [1,2,3,4,5]. Contaminated peanut shells produced in China and alfalfa sprouts of unknown country of origin imported from Italy, Hungary and Pakistan were the source of two large international *S.* Stanley outbreaks in Europe and North America [6,7,8]. A high frequency of septicaemia during the sprout-borne outbreak in Finland in 1995 and cases of severe illness associated with *S.* Stanley have been reported in the literature [9,10]. Resistance to aminoglycosides, tetracycline and cotrimoxazol have been documented. In Europe, *S.* Stanley represents on average 27% of all multidrug-resistant salmonellae [2,5].

In Switzerland, the National Centre for Enteropathogenic Bacteria (NENT) is the reference laboratory for typing and molecular analysis of *Salmonella* sp. isolates nationwide. The annual number of *S.* Stanley isolates reported by the NENT to the Federal Office of Public Health (FOPH) increased from 2 in 2000 to 25 in 2005.

On 20 October 2006, the NENT reported 22 human isolates of *S.* Stanley detected since the beginning of the month and one isolate of this serotype from chicken meat tested during a routine quality control; the meat was imported from Hungary, processed as sliced fresh meat in Switzerland and distributed nationwide. Initially, this chicken meat was considered the most probable source of the human cases. However, although the incriminated meat was no longer on sale, cases continued to occur during the following two weeks. Therefore, the FOPH launched an investigation to identify the source of the outbreak in order to prevent the occurrence of further cases.

Methods

Epidemiological investigation

A case was defined as a resident in Switzerland, presenting with onset of gastrointestinal symptoms after 25 September 2006, and a stool or blood sample testing positive for *S.* Stanley.

Cases were identified by the NENT. In addition, the NENT sent an alert through Enter-net, the international surveillance network for enteric infections [11,12], in order to detect *S.* Stanley cases occurring in the countries participating in the network.

A retrospective case-control study was conducted between 3 and 17 November 2006, including the first 40 cases (onset of illness in weeks 39 - 44, 2006). A sample size of 120 with a ratio of cases/controls of 1/2 was estimated to provide a level of significance of 5%, and statistical power of 80% to detect an OR $\geq$3.

The controls were residents in Switzerland selected in two stages: households were randomly selected from the household database of the Swiss Federal Office of Statistics; in each household the person who celebrated his/her birthday most recently was selected to be interviewed.

Clinical data on cases were collected through interviews with treating physicians. For each case fulfilling the inclusion criteria, permission to contact the patient was obtained from the physician. Demographic data and information on food consumption, recent travel history and cooking hygiene were collected through telephone interviews with cases and controls. Cases were interviewed on food-borne exposures during the three days preceding the onset of illness whereas controls were asked about the food items they had consumed during the last week of October.

The association between investigated exposures and illness was estimated using crude odds ratios (OR) and ORs corrected for canton of residence and age (ORMH) and respective 95% confidence intervals (95% CI). Chi-square and Mantel-Haenszel tests were performed to assess whether observations differed from what would be expected by chance. A multivariate analysis through a logistic regression model was performed including variables with $p<0.1$ in bivariate analysis; the final model was build with STATA v9.1 using the backward method and looking at interactions.

Interviews with cases were continued after the end of the case-control study. Therefore, information on food consumption and other possible risk factors are available for more cases than included in the study (58 cases).

Analysis of food and environmental samples

The Food Safety Division of the FOPH coordinated the environmental investigations. The Federal Research Station responsible for testing food products of animal origin (ALP) conducted bacteriological testing of suspected food and environmental samples at the place of production.

Microbiological investigations

The NENT serotyped *Salmonella* sp. isolates collected nationwide from clinical, food and environmental specimens using commercial antisera according to standard protocols for slide agglutination. The NENT performed the molecular analysis of all isolates positive for *S.* Stanley using Pulsed Field Gel Electrophoresis (PFGE). PFGE profiles from extracted total DNA, restricted with XbaI, were generated using a harmonized protocol, and *S.* Braenderup (H9812) was used as the standard size marker [13].

Results

Description of the outbreak

Between 25 September 2006 (week 39) and 11 February 2007 (week 7), a total of 91 human isolates of *S.* Stanley were identified in Switzerland. Nine of these isolates were from patients not meeting the case definition: two were asymptomatic patients with stool samples (*S.* Stanley was an occasional finding) and seven had positive urine samples only. A total of 82 cases complied with the case definition. No other cases were notified by countries participating in Enter-net during this period.

Table 1

Characteristics and symptoms of *Salmonella* Stanley infection in outbreak-related cases (n=82) as reported by their treating physicians, Switzerland, September 2006 – February 2007

Characteristics of the disease	Value
Signs and symptoms (%)	
Diarrhoea	98
Fever	49
Abdominal cramps	35
Vomiting	18
Severe dehydration	9
Nausea	7
Muscle and joint pain	5
Asthenia	4
Other	16
Positive isolate from (%)	
Stools	96
Blood	4
Hospitalisation (%)	28
Mean duration of illness, in days (range)	9.4 (2-35)

Figure 1

Distribution of *Salmonella* Stanley cases (n=82) by week of onset of symptoms and by strain, Switzerland, September 2006 – February 2007

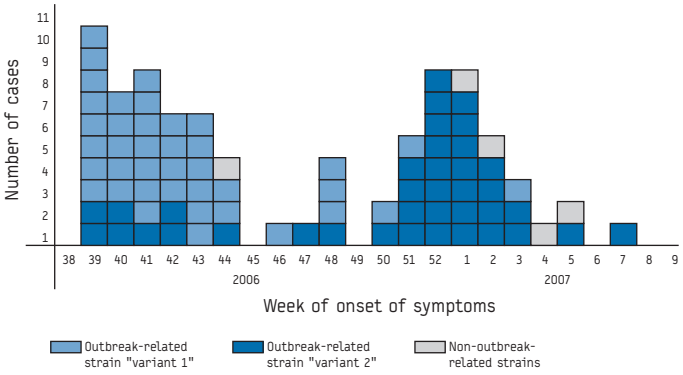


Table 2

Numbers of cases of *Salmonella* Stanley and incidences per 100,000 inhabitants in the cantons of residence of the patients, Switzerland, September 2006 – February 2007

Canton	number of cases	population	incidence
Vaud	21	662,145	3.2
Bern	19	958,897	2.0
Geneva	7	433,235	1.6
Zurich	6	1,284,052	0.5
Fribourg	5	258,252	1.9
Aargau	4	574,813	0.7
Basel-Stadt	3	187,920	1.6
Basel-Land	3	168,912	1.8
Grisons	3	267,166	1.1
Neuchatel	3	184,822	1.6
Valais	3	294,608	1.0
Jura	1	107,171	0.9
Lucerne	1	69,292	1.4
Nidwalden	1	359,110	0.3
St. Gallen	1	40,012	2.5
Zug	1	461,810	0.2
Total	82		

Of the 82 cases, 46 (56%) were male. The average age was 45.7 years (range 0-92 years). Ninety-eight percent of cases were of Swiss nationality. Twenty-three cases (28%) were hospitalised: 19 for acute severe gastroenteritis or resulting complications and four for underlying diseases worsening due to salmonellosis. One case died for reasons not directly related to the infection (invasive cancer). In seven cases (9%) the disease outcome was unknown, the remaining patients recovered. Forty-five cases (57%) were treated with antibiotics, most of them (36 cases) with ciprofloxacin. Reported symptoms are summarized in Table 1.

The distribution of cases by week of onset of symptoms shows a first peak in week 39/2006 and a second in weeks 52/2006 – 1/2007 (Figure 1). Cases were distributed in 16 of the 26 Swiss cantons; 47 cases (57%) were reported from three western cantons: Vaud, Bern, and Geneva. (Table 2).

Four cases occurred among two couples of siblings aged four months and three years, and two and five years, respectively. Four cases referred having a total of five relatives or contact persons who had developed similar symptoms in the same time period. None of those contacts was laboratory tested.

Case-control study

The study included 40 cases and 82 controls. The response rate among cases was 98% and among controls it was 62%. The proportion of people aged less than 35 years was higher among cases than among controls (43% versus 19% of controls; OR 3.5, p=0.005), as was the proportion of those living in French-speaking cantons (53% versus 24%; OR 3.4, p<0.0001) and reporting buying food in small dairies (28% versus 11%; OR 3.1, p=0.03) (Table 3).

As for food consumption, cases were more likely than controls to have eaten "raclette", a melted semi-hard cheese (13% of cases and 2% of controls; OR 9.8, p=0.03), sliced chicken (21% of cases and 4% of controls; OR 7.1, p=0.01), and a certain brand (henceforth referred to as "brand X") of soft cheese (35% of cases and 7% of controls; OR 7.4, p=0.0001) (Table 3).

The association between soft cheese of "brand X" and illness was higher among cases living in German-speaking cantons (OR 21.7, 95% CI 2.3–203.0) than in French-speaking ones and persisted when adjusting for cantons of residence (ORMH 5.4, 95% CI 1.7–17.2, p=0.02). For sliced chicken, the specific ORs for <35 and ≥35 years old were lower than the crude OR and the OR adjusted by age was not statistically significant (ORMH 4.7, CI95% 0.1 - 26.1).

Consumption of soft cheese "brand X" remained the only exposure associated with the infection after adjusting for the other factors in the multivariate model (adjusted OR 11.4, 95% CI 1.9 – 69.6) (Table 4).

Interviews with cases on food consumption and other risk factors were continued after the end of the case-control study. Of the total of 82 cases, 58 were interviewed about the food they had consumed prior to onset of symptoms, and of these 24 (41.4%) reported having eaten soft cheese "brand X".

Table 4

Multivariate analysis of risk exposure for *Salmonella* Stanley infection, Switzerland, September 2006 - November 2006

Risk factor/exposure	Adjusted OR*	95% CI	p value
Age <35 years	1.0	0.9-1.1	0.06
Resident in French-speaking canton	1.9	0.5-7.1	0.32
Buying food in small dairy	1.5	0.2-8.9	0.68
Sliced chicken	7.5	0.7-84.4	0.10
Raclette	4.8	0.3-71.6	0.25
Soft cheese "brand X"	11.4	1.9-69.6	0.008

Table 3

Demographic characteristics and food exposures of cases of *Salmonella* Stanley infection (n=40) and controls (n=82) included in the analytic study, Switzerland, September 2006 - November 2006

Risk factor/exposure	Cases exposed; number/total (%)	Controls exposed; number/total (%)	Crude OR	95% CI	p value
Age <35 years	17/40 (43)	15/81 (19)	3.5	1.4-7.5	0.005
Resident in French-speaking canton	21/40 (53)	20/82 (24)	3.4	1.5-7.6	0.002
Sex (male)	20/40 (50)	37/81 (46)	1.2	0.6-2.5	0.65
Buying food in small dairy	9/32 (28)	9/80(11)	3.1	1.1-8.7	0.03
Peanuts	7/35 (20)	11/79 (14)	1.6	0.5-4.4	0.41
Raw vegetables	21/35 (60)	47/74 (64)	0.9	0.4-2.0	0.72
Beef meat	22/32 (69)	46/76 (61)	1.4	0.6-3.5	0.42
Chicken meat Sliced chicken	18/34 (53) 7/34 (21)	44/77 (57) 2/57(4)	0.8 7.1	0.4-1.9 1.4-36.7	0.68 0.01
Pork meat	13/31 (42)	44/77 (57)	0.5	0.2-1.3	0.15
Eggs	11/33 (33)	64/76 (84)	0.1	0.04-0.2	<0.001
Mayonnaise	4/34 (12)	41/79 (52)	0.1	0.04-0.4	<0.001
Hard cheese (any) Raclette	21/35 (60) 4/31 (13)	72/80 (90) 1/67 (2)	0.2 9.8	0.1-0.5 1.0-91.5	<0.001 0.03
Soft cheese (any) Soft cheese "brand X"	20/35 (57) 12/34 (35)	43/79 (54) 5/73 (7)	1.1 7.4	0.5-2.5 2.4-23.4	0.79 0.0002

Microbiological analysis

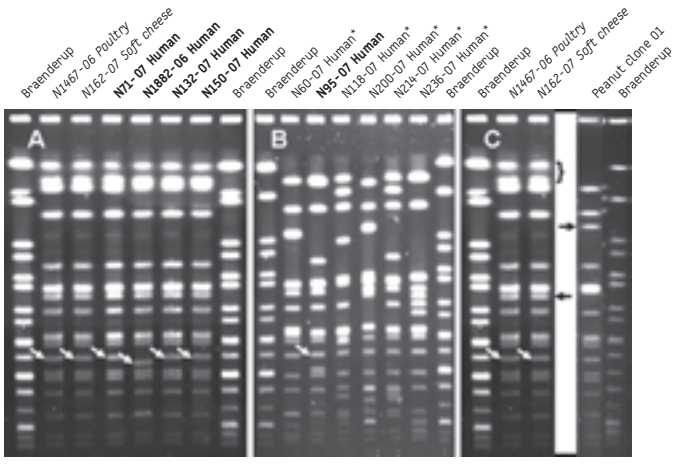
Within the outbreak period, NENT identified 91 isolates of *S.* Stanley from human samples, one from chicken imported from Hungary and two from soft cheese "brand X". Two variants of an outbreak related clone were identified by molecular analysis. Comparing the PFGE patterns, these variants differed in one single deviating band (Figure 3A). Both variants were distinctly different from *S.* Stanley strains isolated from human and environmental isolates collected during the weeks before the beginning of the outbreak (data not shown).

Of the 82 cases included in the outbreak, 77 (94% of all) carried either one of the two outbreak-related variants. "Variant 1" was identified in chicken meat, in soft cheese "brand X" and in 38 cases (46% of all cases), 28 of whom experienced onset of symptoms after week 49. No food isolates were available for "variant 2".

Of five cases carrying non-outbreak related strains, four reported having travelled in Thailand and Malaysia during the incubation period (Figure 3B). The PFGE pattern of the "variant 1" of the outbreak related strains was compared with the PFGE pattern of the peanut-related outbreak strain from United Kingdom [6]. They were closely related and differed by only two bands: one additional band of 550 Kb in the pattern of the peanut strain and one additional band of 260 Kb in the pattern of "variant 1" (Figure 3C).

Figure 2

Pulsed Field Gel Electrophoresis (PFGE) profiles of DNA from *Salmonella* Stanley isolates: A) selected isolates from patients related to the outbreak that occurred in Switzerland from 25 September 2006 – 11 February 2007, from samples of imported chicken meat and soft cheese "brand X" representing both variants of the outbreak clone; B) comparison of outbreak-related and non outbreak-related S. Stanley strains isolated from cases occurring during the outbreak period; C) comparison of the outbreak clone "variant 1" to the "peanut outbreak clone".



Legend: In bold: some outbreak-related cases; in italics: chicken and soft cheese strains; white arrows indicate single up-shifted band in "variant 1", and white arrowheads indicate single down-shifted band in "variant 2" of the outbreak clone; black arrows indicate differing bands in "variant 1" and peanut-related outbreak strain; parenthesis indicates technically artefactual bands (partial restriction digests); *: non-outbreak-related clinical isolates (mostly from cases imported from Thailand).

Analysis of food and environmental samples

Two series of cheese samples covering the entire production were collected in week 51/2006 in all 15 factories producing the soft cheese "brand X" in Switzerland. In total, 55 pools of scratch-samples were taken from the smeared surfaces of cheeses.

In week 1/2007 the analysis of the first series revealed *Salmonella Agona* in two specimens from one single producer. No other contamination was detected in any of the other production sites. The concerned producer blocked the release of new lots of cheese until they were completely checked for contamination with salmonellae and withdrew cheeses belonging to five different lots on sale. To trace the origin of *Salmonella* contamination in the concerned factory, 14 environmental samples from the production site, 10 environmental samples from ripening cellars and 14 samples of pooled milk from the suppliers of the dairy were collected. None tested positive.

At the end of January 2007, *S.* Stanley "variant 1" was isolated from several cheese samples of the second series taken in week 51/2006 in the same factory and of one of the five lots recalled in January.

Stool samples from workers of the incriminated dairy factory were collected by the concerned producer in the context of self control measures. All samples were negative and no employee declared having had diarrhoea or other gastrointestinal symptoms during the previous three months.

Discussion and conclusion

We described a nationwide outbreak involving 82 cases of *S.* Stanley infection in Switzerland. The overall number of cases was probably underestimated because only laboratory-confirmed cases were reported. The distribution of cases by date of onset of symptoms suggested a continuing common source disseminated in Switzerland in two successive periods.

Although chicken meat imported from Hungary was initially suspected on the basis of microbiological findings, our results suggested that this was not the source of the outbreak. Few cases were exposed to sliced chicken. The statistical association between chicken consumption and infection identified in the bivariate analysis was most likely confounded by age. Chicken meat was distributed all over the country whereas cases occurred mainly in the south-western part of Switzerland. Cases continued to occur when the chicken was no longer on sale.

The results of the case-control study indicated that soft cheese "brand X" was the most likely source of the outbreak. Having eaten soft cheese "brand X" was reported by at least 41% of cases. This relatively low percentage might be at least in part due to recall bias. No more cases were identified after the recall of suspected cheese and the strengthening of microbiological controls on new lots. This hypothesis was strongly supported by the microbiological confirmation of the contamination of cheese specimens from one cheese factory. The PFGE analysis of the *S.* Stanley isolates from cases and from cheese samples further confirms the link between the outbreak and soft cheese. The two outbreak-related variants were very closely related, differing only by one slightly deviating band, and were most likely two variants of the same clone [14]. Therefore, it is possible to exclude two parallel unrelated outbreaks; in total, more than 90% of cases carried the same clone as the contaminated cheese.

The "brand X" soft cheese is produced in the western (French-speaking) cantons of Switzerland. Even though distributed nationwide, it is more often consumed in the French-speaking cantons. It might appear contradictory that in these cantons, the association between "brand X" and illness was lower than in the German-speaking cantons. A possible explanation may be that in the French-speaking cantons, the population is generally more often exposed to this cheese whereby the probability to find controls who did not eat the cheese is lower than in the German-speaking cantons. "Brand X" is an artisanal cheese, made from thermized milk, produced from the end of September to March and ripened for a few weeks. The release of lots of contaminated cheeses ripened in two subsequent periods might explain the distribution of cases in two waves.

The origin of the contamination of the cheese factory remains unexplained. We hypothesise that the contamination occurred at the local level as two different lots produced by the same factory, distributed by different channels, were tested positive for *S.* Stanley "variant 1". The contamination of individual cheeses was probably not massive as only two family clusters were identified and there were only five symptomatic persons among contacts who shared a meal with cases during the critical days.

We could not explain why the outbreak-related strain was found in imported chicken meat. No human cases related to this source were reported in other European countries, including Hungary where the product came from. One hypothesis might be that the meat was contaminated by an asymptomatic carrier handling the chicken or that a laboratory contamination occurred during food quality control.

Food safety recommendations

Several types of soft cheese are known to be products at risk for outbreaks due to listeria and various *salmonella* serovars [15,16,17,18]. In Switzerland, cheese production is subject to the Hazard Analysis Critical Control Point (HACCP) conditions [19]. For the specific dairy product involved in this outbreak, routine investigations for bacterial contamination are performed in white cheese (early stage of production) whereas in ripened cheeses, at the latest stage of production, only controls for listeria are routinely done. Since bacterial contamination may occur at any stage of the production, in order to prevent further outbreaks linked to soft cheese "brand X" and similar dairy product we concluded that testing for *salmonella* should be systematically performed also in fully ripened cheeses, at the latest stage of production. Therefore, in Switzerland, the HACCP monitoring programme and the clearing procedures for the release of products on the market have been revised to intensify the measures aimed at preventing the risk of *salmonella* infections during production and ripening of cheese.

Conclusion

This is the first *S.* Stanley outbreak in Europe not linked to imported food items. However, the PFGE profiles indicated that the Swiss outbreak-related strain might have been derived through minor genetic changes from the peanut outbreak strain imported into Europe [6].

In Switzerland, during the years preceding this outbreak, an increasing number of *S.* Stanley isolates had been reported from human and environmental specimens. Routine testing of river water in February 2007 (cantonal laboratory of Aargau) yielded the isolation of *S.* Stanley in a canton only marginally affected by the outbreak. All these findings suggest an increased local circulation of this rare serotype.

S. Stanley is not known to be a particularly virulent serotype, although there are reports of severe cases [9,10]. However, during this outbreak the proportion of cases hospitalised was higher than in other salmonellosis outbreaks in Switzerland. In addition, this serotype has already been found to be resistant to some antibiotics [5]. The emergence of this serotype in Switzerland suggests the need to strengthen surveillance of salmonellosis, investigate outbreaks and implement preventive and control measures in order to avoid future outbreaks and prevent new serotypes from establishing in the country.

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Surveillance of giardiasis in Northwest England 1996-2006: impact of an enzyme immunoassay test

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The incidence of giardiasis in Central Lancashire increased following the introduction of a sensitive enzyme immunoassay diagnostic test in November 2002. We compared the epidemiological trends for 1996-2006 in Central Lancashire with a control area which used a standard wet preparation diagnostic method throughout. Poisson regression modelling was used to investigate trends in giardiasis before and after the introduction of the test. In the control area, incidence of giardiasis was four per 100,000 in 2005. In contrast, in Central Lancashire, the rates increased in temporal association with the introduction of the enzyme immunoassay test from 10.1 per 100,000 population in 2002 to 33.6 per 100,000 in 2006. The increase in giardiasis was unexplained by local factors including travel, outbreaks or sampling trends. The increase in giardiasis occurred in all age groups except for males aged 0-14 years and was most marked in males aged 25-44 years. The relative risk for trend post-test introduction in Central Lancashire was 1.11 (95% CI, 1.01-1.23). This suggests that the increase in giardiasis following the introduction of the sensitive enzyme immunoassay test was at least in part due to improved detection. There appears to be considerable under-diagnosis of giardiasis, particularly in adults. Additional research is required to evaluate the enzyme immunoassay test more widely. The test may assist in standardisation of diagnostic methods for giardiasis and enable more accurate estimation of disease burden and transmission routes.

Introduction

Giardia lamblia is a commonly diagnosed intestinal protozoan infection that causes a significant burden of disease worldwide. Although giardiasis is more prevalent than cryptosporidiosis in the population of England and Wales (33,431 cases of giardiasis were reported between 1995 and 2001 compared with 31,655 cases of cryptosporidiosis [1]), the true incidence and burden of disease attributable to giardiasis and the risk factors for its acquisition have not yet been fully characterised.

Historically the diagnosis of giardiasis has been made by the observation of Giardia cysts or trophozooites in a wet preparation of faeces by microscopy. However, since the early 1990s new antigen detection methods (e.g. enzyme-linked immunosorbant assays [EIA] and immunochromatographic assays) and molecular methods such as polymerase chain reactions (PCR) have been introduced for various infections. In England and Wales laboratory methods for diagnosis of giardiasis are currently not standardised. Most laboratories continue to use the conventional method of wet preparation and microscopy of stool samples. Between laboratories there is variable use of faecal concentration methods and application of selection criteria (e.g. age and travel) to determine which samples are assayed.

In 2002, a microbiology laboratory in the North West of England replaced their conventional wet preparation microscopy method with routine testing of all faecal specimens from patients with community-acquired diarrhoea using an EIA diagnostic method. Following the introduction of this new diagnostic method laboratory-based surveillance detected a temporal increase in the incidence of giardiasis in the population served by this laboratory. This report discusses the nature of these epidemiological changes and the possible implications of these findings on the surveillance and epidemiology of giardiasis in the wider setting.

Methods

In order to assess the impact of the introduction of the EIA test in 2002, surveillance data for the "intervention" area introducing the EIA test was compared to a neighbouring "control" area where the standard wet preparation/microscopy method based on selective "in-house" criteria for age and foreign travel had not changed. Statistical comparison of giardiasis trends for 1996-2006 was investigated using Poisson regression modelling.

The intervention area named "Central Lancashire" was served by a single laboratory and comprised a population of 337,600 people in the local government areas of Chorley, South Ribble and Preston. The control area named "North Lancashire and Cumbria" was served by two laboratories and comprised a population of 427,100 people in the local government areas of Blackpool, Wyre, Fylde and Carlisle.

Throughout 1996 - 2006 the microbiology laboratory serving the Central Lancashire screened for giardiasis all diarrhoeal samples submitted from the community by family doctors, hospital admission wards and paediatricians. Prior to November 2002 screening was done by light microscopy of a wet preparation. In November 2002 light microscopy was replaced by a monoclonal EIA antigen detection method (GIARDIA/CRYPTOSPORIDIUM CHEKTM, Techlab). Positive results indicating the presence of either *Giardia* or *Cryptosporidium* spp. were confirmed by light microscopy until April 2006, after which an immunochromatographic assay (RIDA®QUICK Giardia) was used.

All faecal samples were taken from clinical cases of diarrhoea. Laboratory-confirmed cases of giardiasis were identified through laboratory reports to the respective Health Authority (1996-2003) and to the Cumbria and Lancashire Health Protection Unit (2003-2006). Comparative national data was provided by the Health Protection Agency Environmental and Enteric Diseases Department surveillance database [1].

Statistical methods

Poisson regression modelling was performed to determine whether the observed increase in giardiasis following the introduction of the routine screening test was statistically significant and whether differences in age/sex specific incidence were significant. Giardia count was defined as the dependent variable, logarithm of the population at risk as the offset and age group (five-year age bands), sex, year, area (Central Lancashire versus North Lancashire and Cumbria) and test introduction phase (prior or post) as the independent variables. Baselines were arbitrarily chosen to be 0-4-year-olds, male, Central Lancashire and prior phase for the age, sex, area and phase variables respectively. As the introduction of the test occurred near the end of 2002, the statistical analysis took the years 1996 to 2002 inclusive to be the prior and 2003 to 2006 inclusive to be the post-test introduction phases respectively.

The modelling yielded relative risks either relative to a baseline or as a year-on-year increase in giardiasis.

The initial model consisted of all three-way interactions between the independent variables. Variables and interactions were considered significant if the associated p-value was less than 0.05. A backwards stepwise modelling procedure was adopted with the non-significant three-way interaction with the largest p-value being removed at each step until all three-way interactions were significant, at which point the non-significant two-way interaction with the largest p-value not involved in the remaining three-way interactions was removed at each step. The final model was reached when all interactions were significant. Independent variables were not removed from the model as they were all involved in one or more interactions. As the final model consisted of more than one interaction, a series of models were fitted, each with one interaction, thereby ignoring the other interactions. All statistical analysis was performed using STATA, version 9.2 [2].

Figure 1

Reported number of cases of giardiasis in England and Wales, 1996-2006

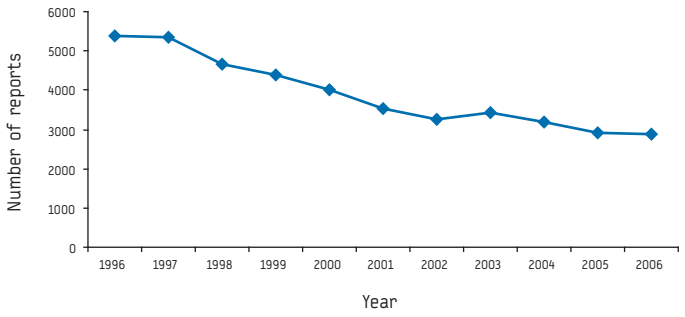


Figure 2

Reported number of cases of giardiasis in Central Lancashire versus North Lancashire and Cumbria, 1996-2006

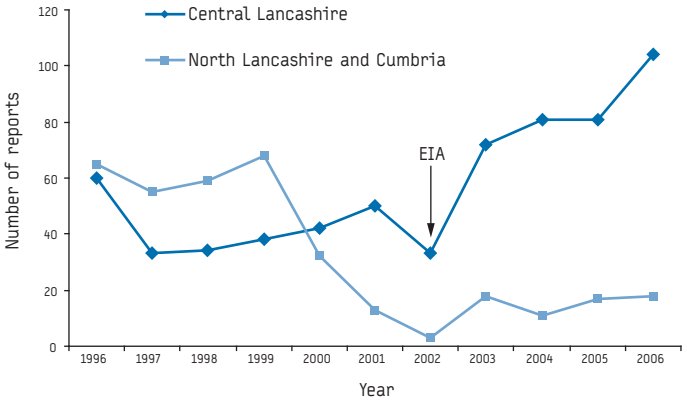
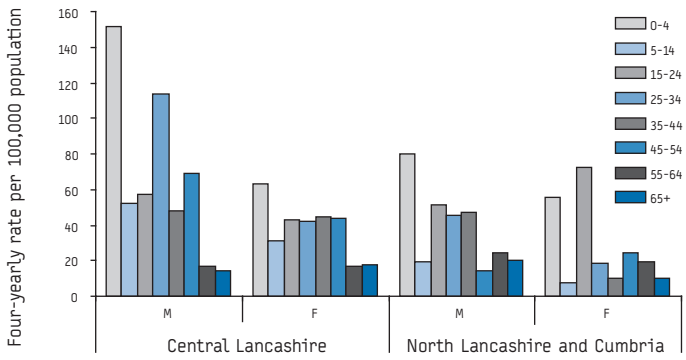


Figure 3

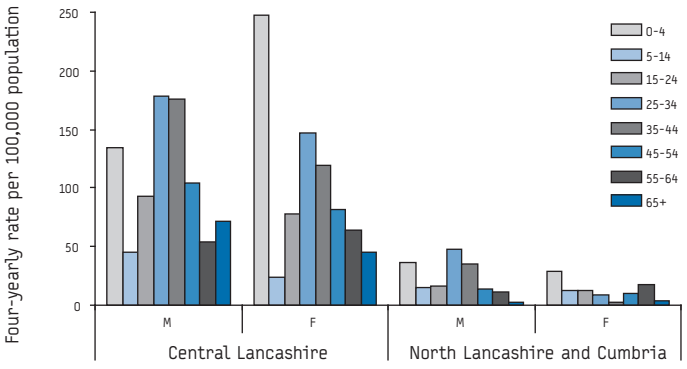
Age and sex-specific incidence of giardiasis, 1999-2002: Central Lancashire versus North Lancashire and Cumbria



Rates were calculated using 2000 population data available from: http://www.lancashireprofile.com

Figure 4

Age and sex-specific incidence of giardiasis, 2003-2006: Central Lancashire versus North Lancashire and Cumbria



Rates were calculated using 2004 population data available from: http://www.lancashireprofile.com

Results

Surveillance data for England and Wales demonstrate that the national number of reported cases of giardiasis has decreased steadily over the past decade falling from 5,379 cases in 1996 to 2,875 cases in 2006 [1] (Figure 1).

By contrast, in Central Lancashire there was a small increase in the number of reported cases of giardiasis between 1997 and 2001 and a marked increase from 2002 onwards. The start of the rise in 2002 corresponds in time with the introduction of the EIA diagnostic method. In North Lancashire and Cumbria, the reported cases of giardiasis decreased between 1999 and 2002, and have since remained at a low baseline (Figure 2)

In 2005 the incidence of giardiasis in England and Wales was 5.5 cases per 100,000 per year [1]. Similarly, the incidence of giardiasis in North Lancashire and Cumbria in 2005 was 4.0 cases per 100,000 per year. In Central Lancashire, however, the annual incidence of giardiasis increased from 10.1 cases per 100,000 in 2002 to 33.6 cases per 100,000 in 2006 – i.e. to more than six times the national rate. The increase in giardiasis in Central Lancashire was seen in all age groups except for males aged 0-14 years and was most marked in males aged 25-44 years and females aged 0-4 years (Figures 3 and 4).

The final Poisson regression model fitted was: AGE + SEX + INTRO + YEAR + AREA + AGE.SEX + AGE.AREA.YEAR + AREA.INTRO.YEAR, where INTRO referred to prior/post introduction of the screening test; and age, sex, area and intro were fitted as categorical covariates and year as a continuous covariate. The AGE.SEX, AGE.AREA.YEAR and AGE.INTRO.YEAR interactions had p-values of 0.007, <0.001 and <0.001, respectively. These interactions indicated that the incidence rates among the age groups were statistically significantly different between the sexes, the annual trends were statistically significantly different between each age group and area combination and age group and introduction phase, respectively.

The model clearly represented a complicated picture of the occurrence of Giardia. To try to understand the situation better, the following three models were fitted:

AGE + SEX + INTRO + YEAR + AREA + AGE.SEX,
AGE + SEX + INTRO + YEAR + AREA + AGE.YEAR.AREA,
AGE + SEX + INTRO + YEAR + AREA + INTRO.YEAR.AREA.

It appears that the rates are higher in males than in females with rates for males reaching their peak in the range 15-44 years of age, whereas for females the corresponding peak is in the range 15-34 years of age. The trend generally increases with age for Central Lancashire while for North Lancashire and Cumbria there is a decreasing trend in the rates which are consistent across all ages. There is an increasing trend following the introduction of the screening test in Central Lancashire, whereas for North Lancashire and Cumbria there is a decreasing trend prior to test introduction, but no statistically significant change in the post introduction phase (Table).

Table

Summary of the model of giardiasis incidence in Central Lancashire versus North Lancashire and Cumbria, 1996-2006

Model*	Variable	Age (years)	Relative risk	95% CI	
Averaging over AGE, YEAR and AREA, and INTRO, YEAR and AREA interactions	Males	0-4	1.00		
		5-14	0.31	0.21	0.45
		15-24	0.59	0.42	0.81
		25-34	0.79	0.58	1.06
		35-44	0.64	0.47	0.87
		45-54	0.43	0.30	0.60
		55-64	0.25	0.16	0.38
		65+	0.28	0.19	0.40
	Females	0-4	1.00	0.71	1.42
		5-14	0.21	0.14	0.32
		15-24	0.58	0.42	0.80
		25-34	0.51	0.36	0.71
		35-44	0.32	0.22	0.46
		45-54	0.32	0.22	0.46
		55-64	0.27	0.18	0.40
		65+	0.12	0.08	0.19
Averaging over AGE,SEX and INTRO,YEAR and AREA interactions	Trend (per year) by age group in **Central Lancashire**	0-4	0.99	0.92	1.07
		5-14	0.96	0.87	1.06
		15-24	0.94	0.87	1.01
		25-34	1.05	0.98	1.12
		35-44	1.10	1.02	1.18
		45-54	1.01	0.94	1.09
		55-64	1.06	0.95	1.18
		65+	1.04	0.94	1.14
	Trend (per year) by age group in **North Lancashire and Cumbria**	0-4	0.75	0.67	0.83
		5-14	0.70	0.70	0.89
		15-24	0.72	0.66	0.78
		25-34	0.80	0.73	0.87
		35-44	0.82	0.75	0.91
		45-54	0.76	0.67	0.85
		55-64	0.82	0.73	0.92
		65+	0.79	0.69	0.92
Averaging over AGE,YEAR and AGE,YEAR and AREA interactions	Trend (per year) by test introduction in **Central Lancashire**	Prior	0.97	0.91	1.03
		Post	1.11	1.01	1.23
	Trend (per year) by test introduction in **North Lancashire and Cumbria**	Prior	0.77	0.72	0.82
		Post	1.03	0.83	1.29

*AREA=Central Lancashire or North Lancashire and Cumbria, INTRO=pre or post EIA introduction
Note: The modelling yields relative risks for trend either relative to a baseline or as a year-on-year increase in giardiasis. Baselines are 0-4-year-olds, male, Central Lancashire and prior phase for the age, sex, area and test introduction variables respectively.

Discussion

This report describes a localised increase in the incidence of giardiasis after introduction of a sensitive diagnostic test. The results presented need to be treated cautiously for two reasons: firstly relative risks have been obtained for one interaction at a time, ignoring the others, and secondly there are various caveats with regard to the data, not least the low number of cases for North Lancashire in 2002 which was about the time when a new surveillance system came into operation. However it appears that the epidemiological change is in part due to increased detection following the introduction of the EIA diagnostic method. This was suspected from an "in-house" comparison of the sensitivity of microscopy versus EIA prior to EIA introduction. Some 601 faecal samples were tested and positive stools by either method were further tested by giardia PCR. The 18 samples that tested positive by EIA were all corroborated by PCR while microscopy missed three of these. Thus the additional yield of EIA in this survey was 17%. It is likely this would be greater in routine practice as the EIA is less demanding in terms of technical expertise.

Although statistical analysis is not conclusive it supports increased detection as the most likely explanation for the increased incidence as indicated by the relative risk of 1.11 (1.01, 1.23) post-EIA introduction in Central Lancashire. This explanation is further

supported by the association in time (Figure 2), the absence of other satisfactory explanations (i.e. no identified outbreaks, no systematic changes in overseas travel, water supply or stool sampling policy between the two surveillance areas) and the scientific plausibility of this explanation. For example, EIA diagnostic methods have been shown to be both highly sensitive (95% [3] and 88.6-100% [4]) and specific (100% [3] and 99.3–100% [4]). The sensitivity of conventional microscopy of single stool samples is operator-dependent and has been shown to be around only 70% [3,5]. PCR detection of *Giardia* and *Cryptosporidium* spp. is 22 times higher than that of conventional microscopy methods [6] suggesting that the currently used diagnostic systems are likely to considerably underestimate the incidence of these parasites.

The findings of this report have been based on arbitrary choices of baselines. Since the relative risks have been well estimated with these choices, different conclusions would not have been reached by choosing a different set of baselines. Indeed, some other choices may have led to relative risks being less well estimated.

From a practical perspective the EIA test was simple to perform and was readily incorporated into laboratory practice. The additional reagent costs were more than offset by the increased efficiency of skilled laboratory staff who no longer needed to undertake relatively time-consuming microscopy. The EIA also had the advantage of simplifying the diagnosis of cryptosporidiosis as it was a combined test. However the extra cost of the test is probably the main obstacle preventing laboratories from introducing the EIA test.

We have been unable to find similar reports in the literature of an increase in the incidence of giardiasis following the introduction of an EIA or similar method. For example, following implementation of a similar enzyme immunoassay screening test for *Giardia* and *Cryptosporidium* in a Canadian laboratory, although the timeliness and efficiency of diagnosis of these parasites improved, the total percentage of cases with enteric parasite infection remained stable [7]. However changes in laboratory methods have been associated with changes in epidemiology of infections caused by other organisms, e.g. *Bordetella pertussis* [8].

The introduction of the EIA method in 2002 does not fully explain the continuing increase in the reported incidence of giardiasis in 2006. The most likely explanation for this is the replacement of light microscopy confirmation by a more sensitive immunochromatographic assay in April 2006. Giardiasis is known to have a bimodal age distribution with a large peak in children under five and a smaller peak in adults aged 25-39 [9,10]. The high incidence in males aged 25-44 years in our series is particularly interesting as this is not a group that frequently seeks medical attention [11] and therefore has fewer stool samples collected. Given they are not a traditional high risk group for giardiasis this raises the question as to whether as yet undetermined risk factors may be contributing to the increased incidence and to the change in age- and sex-related epidemiology.

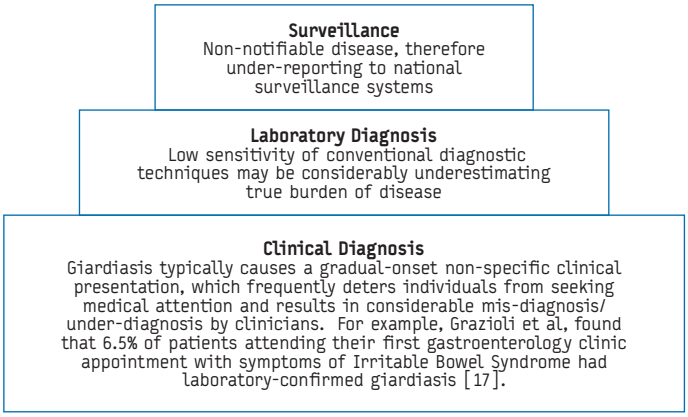
The majority of non-travel associated cases of giardiasis in the UK tend to be acquired sporadically rather than being associated with outbreaks. However, most information on risk factors for giardiasis has come from investigation of outbreaks abroad. A case-control study of sporadic giardiasis in Southwestern England identified swallowing water while swimming, recreational fresh water contact, drinking treated tap water and eating lettuce as independent risk factors for giardiasis [12]. Nevertheless, the relative importance of the various sources and transmission routes of giardiasis are poorly understood and a clear quantitative understanding is required [13].

This report highlights several general issues regarding the epidemiology and surveillance of giardiasis. Firstly, the true burden of clinical disease attributable to giardiasis may currently be considerably underestimated as a result of substantial under-diagnosis at all stages of reporting. Although this underestimation of community-acquired gastrointestinal diseases by national surveillance is a well recognised issue [14], this is likely to be particularly true for giardiasis [15,16] (Figure 5).

In one study *G. lamblia* was present in 9 out of 137 (6.5%) of patients with Irritable Bowel Syndrome, a finding which if replicated in further studies, would add to the public health importance of giardiasis [17]. Secondly, the non-standardisation of laboratory diagnostic methods makes interpretation of routine surveillance data and comparisons at regional, national and even international level difficult. Finally, the increasing incidence of giardiasis and the changes in age and sex-related epidemiology noted in this report emphasise the lack of knowledge regarding the relative importance of the various transmission routes for the acquisition of giardiasis in European countries such as England and Wales.

FIGURE 5

Underestimation of burden of disease due to giardiasis



Conclusion

The increase in giardiasis following introduction of the sensitive enzyme immunoassay test was at least in part due to increased detection. Additional research is required to evaluate the enzyme immunoassay test more widely. The test may assist in standardisation of diagnostic methods for giardiasis and enable more accurate estimation of disease burden and transmission routes, particularly in non-traditional high-risk groups.

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Changes in prevention and outbreak management of Legionnaires' disease in the Netherlands between two large outbreaks in 1999 and 2006

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We describe an outbreak of Legionnaires' disease in 2006 in Amsterdam, the Netherlands. Comparisons with the outbreak that took place in 1999 are made to evaluate changes in legionella prevention and outbreak management. The 2006 outbreak was caused by a wet cooling tower. Thirty-one patients were reported. The outbreak was detected two days after the first patient was admitted to hospital, and the source was eliminated five days later. The 1999 outbreak was caused by a whirlpool at a flower show, and 188 patients were reported. This outbreak was detected 14 days after the first patient was admitted to hospital, and two days later the source was traced. Since 1999, the awareness of legionellosis among physicians, the availability of a urinary antigen tests and more efficient early warning and communication systems improved the efficiency of legionellosis outbreak management. For prevention, extensive legislation with clear responsibilities has been put in place. For wet cooling towers, however, legislation regarding responsibility and supervision of maintenance needs to be improved.

Introduction

Legionnaires' disease (LD) is an acute pneumonia caused by infection with bacteria of the genus Legionella. Inhalation of aerosolized water containing the bacteria is the primary mode of acquisition. Although cases of LD are often sporadic, large outbreaks can be caused by communal sources, such as 'hot tubs' or 'spa pools' [1,2] and hospital or hotel showers [3,4]. Wet cooling towers can emit contaminated aerosols, with dispersal over long distances, sometimes causing major outbreaks [5-15].

In the Netherlands, the first large LD outbreak occurred in 1999; it affected 188 patients of whom 23 died. This epidemic was caused by aerosol transmission from a display whirlpool at a flower show, and was not recognized as an LD outbreak until 14 days after the first patient was diagnosed with pneumonia of unknown origin. The source was identified within a week after the epidemic was detected as an LD outbreak; 10 days after the show had ended, when already 71 patients had been admitted to various hospitals throughout the country. The 1999 outbreak was evaluated extensively [16] and this has led to changes in prevention policies, legislation and outbreak management strategies.

Here we describe the second large outbreak of LD in the Netherlands in 2006, and evaluate the effectiveness of changes in legislation, prevention management and outbreak management implemented after the first large outbreaks in 1999.

Methods

In the Netherlands, LD has been a reportable disease since 1987. Every diagnosed case has to be reported to the local Public Health Service (PHS), and is registered nationally by the Centre for Infectious Disease Control (CIb). Since 2002, the local PHSs report to CIb by the internet.

A confirmed LD case is a patient with pneumonia, confirmed by a positive laboratory test (urinary antigen test, positive culture, positive polymerase chain reaction (PCR), positive IgM antibody or a significant increase in IgG antibody ELISA test). After a case of LD has been reported to the PHS, patient information is gathered including demographics, diagnosis, underlying disease, domestic risk factors, risk factors at work, travel, and leisure activities in the 21 days before onset of disease, using a standardised questionnaire [17].

Any unusual number of reported cases in time or place will lead to an outbreak investigation as to a common source. In case an outbreak is suspected, depending on the suspected source, active case-finding is initiated by the PHS in order to detect and eliminate the source as soon as possible. Depending on the magnitude of the outbreak, active case-finding comprises alerting general practitioners and hospitals in the PHS area, other PHS branches and international early warning systems. Since 2002, in case an outbreak is suspected that is not confined to one PHS area, the CIb informs the other PHSs and other physicians by email service, which makes it possible to notify them instantly. The public can be warned by local or national press and television.

To strengthen local efforts to identify sources, a specialized team from the Regional Public Health Laboratory of Haarlem has offered sampling services to all public health services in the Netherlands since 2002, and serves as a reference laboratory where both human

and environmental strains are genotyped. The laboratory keeps a national register of sampled potential sources.

For the 2006 outbreak investigation, the following case definition was made: confirmed cases were patients with clinical signs of pneumonia, with fever > 380C, cough and shortness of breath, who had been to the eastern part of Amsterdam (with zip codes 1011 and 1018) between 8 June and 11 July and with a confirmed laboratory test (positive urinary antigen test; positive culture; fourfold increase in antibody titer or seroconversion in a paired sample).

All hospital laboratories were asked to send available cultures to the reference laboratory in Haarlem for genotyping, where Amplified Fragment Length Polymorphism (AFLP) was used for DNA fingerprinting.

Although wet cooling towers are a common source of LD outbreaks, in the Netherlands registration of these towers is not addressed in the law (Table 1). As soon as a cooling tower was suspected as the source of the outbreak, for tracing of this source wind directions were used as published by the Dutch National Meteorological Institute KNMI. [www.knmi.nl/klimatologie/daggegevens/index.cgi] All environmental samples were obtained by the department of Infectious Diseases of the PHS Amsterdam in cooperation with the Public Health Laboratory Haarlem

Results

Source tracing

On Thursday 6 July 2006, three cases of LD were reported to the PHS in Amsterdam, all diagnosed on the same day by a urine test indicating type I infection. On Friday 7 July, the second day when five cases were reported, the PHS Amsterdam continued the source tracing and started active case-finding by emailing all general practitioners who were on call that weekend (8 and 9 July). All six Amsterdam hospitals were called to alert and inform the microbiologists about the outbreak. Also, all other PHS branches in the Netherlands were notified by CIb email service and requested to report any unusual number of LD cases or cases that could be related to a recent visit to Amsterdam. During the weekend, nine additional cases were reported. Extensive interviewing did not suggest a common source for these infections. None of the patients had traveled recently. The majority of patients were living in the city centre, in an area about 500 meters east of the central railway station with zip codes 1011 or 1018, which is an area with a 2,5-3 km in diameter. Most of these patients reported onset of disease on the first of July (Figure 1).

On 8 July, the first sample was taken from a possible source, a newly installed display fountain, because most patients reported by then were living in the fountain area. This fountain was immediately closed.

Because it was possible that the outbreak was not confined to Amsterdam, on Monday 10 July, a national outbreak team was established, with participants from the PHS Amsterdam, the CIb and the Public Health Laboratory of Haarlem. The CIb started enhanced national active case-finding by contacting all infectious disease control physicians at PHS facilities in the regions surrounding Amsterdam. They were asked to telephone all hospitals in their region and ask if there had been any LD patients admitted. Also on 10 July, all general practitioners, microbiologists and infectiologists in Amsterdam were alerted by post. In order to alert as many people in the Netherlands as possible, a press release was issued on Monday announcing the unusual number of LD patients in Amsterdam.

Table 1

Legislation and supervision of preventive legionella source cleansing in the Netherlands, 2007

Laws		Supervisor	Location	Object/source
Law on drinking water	Chapter IIIC	Inspectorate of VROM	Hospitals, housing, camping sites, asylum seekers' centers, yacht-basins	Drinking water installations
	Chapter IIIC articles 17j, 17o, 17p, 17q	Inspectorate of VROM	Drinking water companies (waterworks)	Drinking water delivery
Law on occupational health and safety	Policy regulation* document 4.87-1	Labor Inspectorate SZW	Locations in companies with exposure risk for employees	***Cooling towers*** Humidifiers Industrial water installations**
		Food and Consumer product safety authority	Locations in companies with public exposure risk	
		Labor Inspectorate	Inland shipping	Drinking water installations Humidifiers Industrial water installations
		Inspectorate of Transport, Public Works and Water Management	Ocean shipping	
		Inspectorate of Transport, Public Works and Water Management	Airplanes	
Law on hygiene and safety public baths and swimming pools	Articles 2a-2d	Provinces	Public baths and swimming pools	Swimming and bathing water
Law on collective prevention in public health		Municipalities	Large-scale events	All atomizing installations

VROM: Ministry of Housing, Spatial planning and the Environment
SZW: Ministry of Social Affairs and Employment
* A policy regulation is not a law but a guideline; it describes best practice but does not have to be obeyed.
** Atomizing installations outside companies (such as fountains on squares or in shopping malls) are not part of this, or any other law.

In the ten days preceding the outbreak, the wind appeared to be mainly west and north-west (Figure 1). Therefore, the team started to look for fountains and wet cooling towers north-west of the affected area. Subsequently a second display fountain in this area was sampled and immediately closed. Since registration of wet cooling towers is not mandatory, a register of these cooling towers was not available. However, in 2003, a list of wet cooling towers was made in Amsterdam for a study on the prevalence of legionellae, but had not since been updated. With the help of Google Earth, we looked for new, not registered cooling towers, and also inspected the area. As a result, every cooling tower in the outbreak area was inspected and sampled. At the end of the day on 10 July, we detected one (previously not listed) wet cooling tower on ground floor level, a few meters east of a construction site just east of the central station. This cooling tower was installed on 10 June and was visibly not well maintained. Samples were taken from the tower and as a precautionary measure the tower was closed as soon as possible in the early morning of Tuesday 11 July. The next day, the laboratory results showed positive culture and revealed a concentration of 5 million colony-forming units per liter. In a follow-up press release issued on the same day, it was announced that most patients affected lived in or had recently visited the area east of Amsterdam Central Station, and that a cooling tower in this area was the probable source of the outbreak.

Active case-finding

On 10 July, all public health physicians in the country were updated about the outbreak by CIb email service and asked to query all LD patients about visits to Amsterdam, including specific locations visited. In total, active case-finding yielded seven LD patients who lived outside Amsterdam but all of them worked in or very near the construction site adjacent to the questionable cooling tower. These findings confirmed our suspicion that it was the source of the outbreak.

Active case finding within the Occupational Health Services of the construction companies working near the cooling tower revealed that one construction worker had died on 6 July from pneumonia. He fell ill on 4 July and refused admittance to hospital for further testing. A post-mortem lung specimen was tested and legionella bacteria could be detected by DNA isolation.

In July, many tourists visit Amsterdam. Because the LD source was so close to Amsterdam Central Station, the fear arose that international visitors could have been exposed, perhaps in large numbers. Therefore, on 12 July, the European Surveillance Scheme for Travel-Associated Legionnaires' Disease issued a community cluster alert to its participants [18] and a preliminary report was published in Eurosurveillance [19]. On 13 July, information on the outbreak appeared in ProMed [20]. No cases in tourists or visitors to Amsterdam were reported.

Characteristics of patients

In total, 31 patients with LD were reported in this outbreak: their characteristics are shown in Table 2. Seventy-four percent were men, and the case fatality rate was 10%. Sixty-five percent reported possible risk factors associated with developing LD.

Cultures and DNA fingerprinting

From seven patients epidemiologically linked to the contaminated cooling tower, cultures were available for DNA fingerprinting, enabling comparison with the bacteria obtained from the cooling tower. All seven matched. In Figure 3, three of these seven samples are shown (patient 2, 3 and 4) in comparison to another patient not related to this outbreak (patient 1) and samples from the cooling tower (samples 5,6,7 and 8). At the same time, at a routine control, legionellae were found in another wet cooling tower in Amsterdam, five kilometers south-west of Central Station. However, the strain found in this tower (samples 9 and 10) was evidently different from the strain found in the outbreak patients.

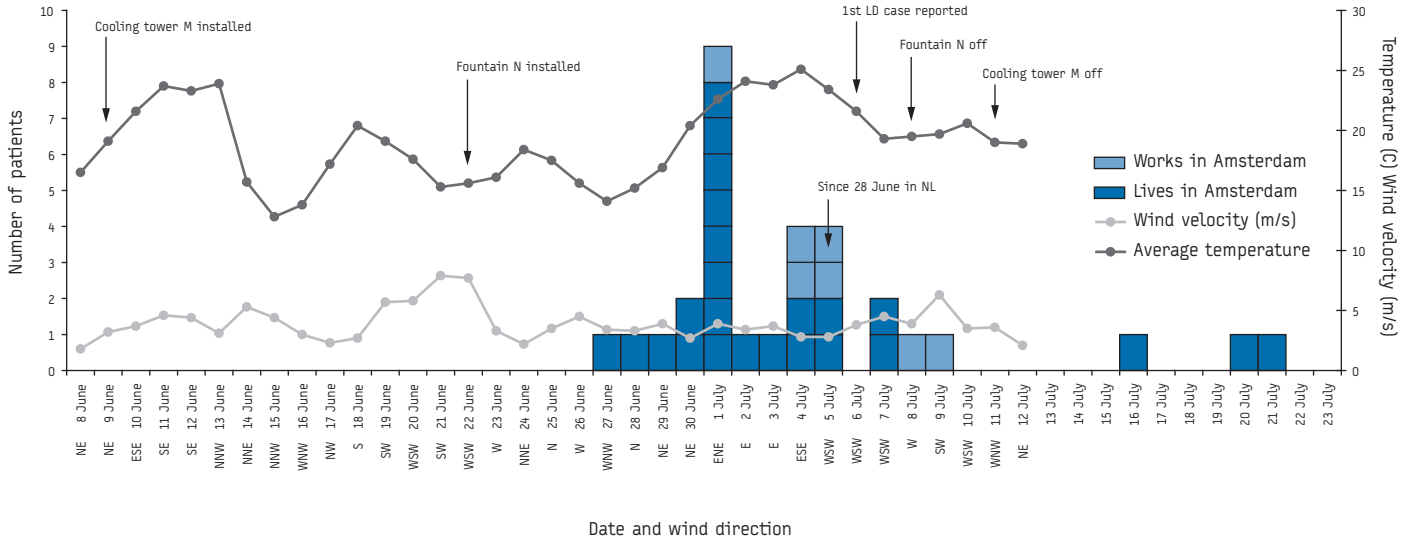
Discussion

Outbreak management

The most important development since the 1999 outbreak is that urinary antigen tests have become widely available and physicians more aware of LD. The 1999 outbreak was not recognized as an

Figure 1

Legionnaires' Disease (LD) patients in Amsterdam linked to a cooling tower, by date of onset of disease, June – July 2006 (n=31)



LD outbreak until 14 days after the first patient was hospitalized and diagnosed as a case of pneumonia of unknown origin. Hospital physicians were not aware that LD was a notifiable disease; they contacted the PHS because of the unusual number of pneumonia patients. In 2006, the first patient was diagnosed with LD within two days after hospital admission and reported to the PHS the same day the diagnosis was confirmed.

In the Amsterdam outbreak in which standardized questionnaires were used, the likelihood of a source outside a building (i.e. a cooling tower or a fountain) became clear after two days, by exclusion of communal sources. The actual source, a cooling tower, was located within four days after the first patient was diagnosed. In contrast, in 1999, a case control-study showed that it was likely that the source of the outbreak was situated at a flower show. Subsequent environmental risk assessment led to the most likely source, a whirlpool, and sampling revealed abundant legionella growth six weeks after the outbreak was recognized. [21]

Until 2002, national registration of reported LD cases was done by post from PHSs to CIb, where cases were subsequently entered in a database. This procedure resulted in delays in the 'early warning system'. Since 2002, national registration is done by internet reporting, which is much faster. Especially outbreaks in different PHS districts can now be detected faster than in 1999. Also, communication from the CIb to PHSs has improved by the installation of a CIb email service in 2002. The service makes it possible to notify public health and other physicians instantly. In 1999, this was done by telephone and facsimile, which was much slower. Also, internationally, early warning systems have been put in place. [28,20]

The work of the reference laboratory has also proven successful; in the first two years of the project, the lab discovered 17 LD clusters, 12 of which would not have been identified in a timely manner without this outbreak detection program. [22] Because the

Table 2

Characteristics of patients with Legionnaires' disease associated with cooling tower as most likely source of infection, Amsterdam, June – July 2006 (n=31)

Total number of patients	31	100%
Sex		
Male	23	74%
Female	8	26%
Age		
Average age (range) in years	56 (32-81)	
Age distribution in years		
30-39	3	10%
40-49	8	26%
50-59	7	23%
60-69	9	29%
70-79	3	10%
80-89	1	3%
Diagnosis		
Urinary test	31	100%
Urinary test + culture	7	23%
History taken in acute stage		
Patient	17	55%
Relative/proxy	14	45%
Deceased		
Number of deaths, case fatality rate	3	10%
Associated factors		
Diabetes type II	5	16%
Immune deficiency	2	6%
COPD	3	10%
Other lung disease	1	3%
Hypertension	2	6%
Smoker	11	35%
Alcoholism	2	6%
Any associated factor	20	65%

Figure 2

The annual number of reported cases of Legionnaires' disease in The Netherlands, 1987-2006

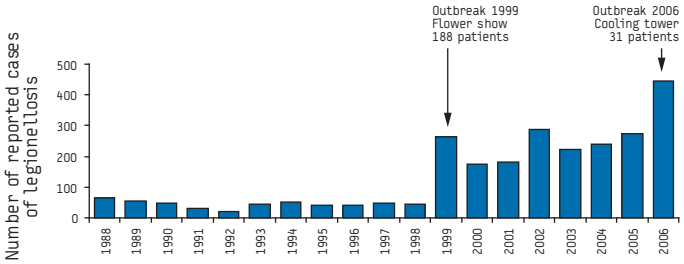
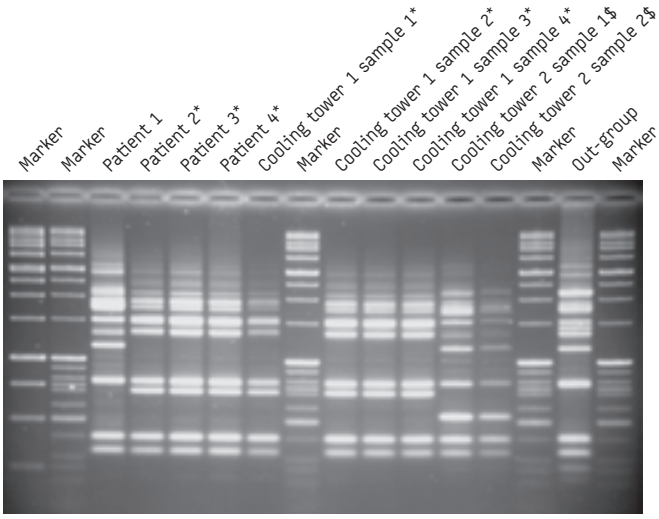


Figure 3

Results of DNA fingerprinting of four Legionnaires' disease patients and two cooling towers in Amsterdam, 2006



* Patients and cooling tower 1 match
$ cooling tower 2 was located 5 kilometers south-west of cooling tower 1

project was so successful, it was implemented in routine outbreak control and is coordinated by CIb since 2006. [23]

Because of their experience with sampling of possible legionella sources, in the 2006 outbreak the Regional Public Health Laboratory of Haarlem could take the first samples of suspected sources immediately after they were identified, starting on Saturday evening. Four days after the first patient was diagnosed, the actual source was sampled and one day later closed.

Increased awareness and availability of antigen tests are probably the reason why since the 1999 outbreak, the number of reported LD cases in the Netherlands has increased steadily (Figure 2). In 2006, the incidence of LD in the Netherlands was higher than in previous years. This increase cannot be explained only by the Amsterdam outbreak or increased awareness. The same trend was seen in the United Kingdom. [24] In both countries many sporadic cases spread all over the country were reported, which may be associated with certain weather conditions. In a recent study, warm and wet weather patterns, but not the hottest ones, were found to be associated with a higher incidence of LD in The Netherlands between 2003 and 2007 [25]

Legionella prevention and legislation

After the 1999 outbreak, the Dutch government launched a plan to combat Legionnaires' disease [26] which has resulted in the report 'Controlling Legionnaires' Disease', published by the Health Council in 2003 [27]. The report targets four areas in which the risk of infection could be reduced at acceptable cost: 1) European-wide agreement on guidelines (since about half of the patients are infected abroad); 2) rapid diagnosis and treatment; 3) modification of water fittings and implementation of management plans; and 4) stimulation of research to further rationalize prevention policies. The report states that some water atomizers (those used at large scale events, by residential properties, by small companies, and atomizers that are not connected to the main water system), and wet cooling towers used for comfort cooling need better maintenance.

New preventive legislation about control of legionella in water has been put in place, with clear responsibilities. In March 2005, the Ministry of Housing, Spatial Planning and the Environment (VROM) published a summary on the prevention and the legislation concerning the control of legionella in water. LD prevention is divided into pro-active and reactive source cleansing. For preventive pro-active cleansing, four laws are in place that apply to different water sources (see summary in Table 1). By law, samples to monitor the effectiveness of the preventive measures must be taken at regular intervals from all drinking water sources. Positive tests are reported to the VROM inspectorate. The local Public Health Service is notified in case of a positive culture with more than 1,000 colony-forming units per litre, so that it can give information to the users of the contaminated water installation and, if possible and applicable, communicate with reported patients.

Because the vast majority of cooling towers in the Netherlands are installed at company buildings, the Ministry of Social Affairs and Employment (SZW) is made responsible for the legionella control in cooling towers, as far as its risk for employees is concerned. It is assumed that this will also protect the general population. Registration of these towers in the Netherlands is not addressed by law.

As for preventive reactive legionella source cleansing, the infectious disease law is in place, stating that every physician must report LD patients to the local PHS within 24 hours of the diagnosis after which source tracing and elimination can take place as described above in the 'Methods' section [28].

Next steps

Although the Ministry of Social Affairs and Employment is responsible for legionella control in wet cooling towers, their actual supervision, so far, is limited. Registration of these towers is not addressed in the law but in a policy regulation, which is a guideline that describes 'best practice'. In response to the Amsterdam outbreak, the minister of Social Affairs and Employment stated that the responsibility for registration of cooling towers lay with the municipalities, and that voluntary registration was expected to be sufficient.

As for drinking water, it is urgently needed that wet cooling towers are sampled at regular intervals, and that these cooling towers, together with their test results, are registered nationally. Positive cultures should be fingerprinted and the results entered in the national database. This way, prevention will improve because maintenance will be monitored, and matches with patients' cultures can be made as soon as possible.

In 2007, a register of wet cooling towers was still not in place. In 2003, 30 wet cooling towers were registered in Amsterdam as part of a study. During the 2006 outbreak 14 new wet cooling towers were found. Although registration of cooling towers is not officially their task, in the beginning of the summer of 2007, the PHS Amsterdam decided to make a start with an updated list of wet cooling towers. At the end of the summer, 73 of such cooling towers were registered, more than twice as many as in 2003. Possibly, with a larger database that also includes cooling tower test results, more sources of such outbreaks as described in this paper can be found and prevented or eliminated faster in the future.

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Human immunodeficiency virus (HIV) and acquired immunodeficiency syndrome (AIDS) case reporting in the World Health Organization European Region in 2006

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This article presents information on HIV and AIDS case reporting systems as part of a survey on HIV/AIDS surveillance practices in the World Health Organization (WHO) European Region. A standardised questionnaire was sent to the 53 national correspondents of the European Centre for the Epidemiological Monitoring of AIDS (EuroHIV). The HIV and AIDS case reporting section of the questionnaire comprised four parts: data collection system, HIV/AIDS case definition for surveillance, variables collected, and evaluation of surveillance systems). Individual-based data collection systems for HIV case reports have been implemented in 43 of 44 countries in the WHO European Region and for AIDS case reports in all the countries. For HIV case reports, a coded identifier is used in 28 countries, and full names are used in 11 countries. The European AIDS case definition has been adopted in 35 countries (80%). Information on molecular epidemiology is available in 30 countries, and HIV drug resistance is monitored in 11 countries. HIV/AIDS case reporting systems have been evaluated for under-reporting in 17 countries and for completeness in 11 countries. This article outlines the future needs for HIV/AIDS surveillance and presents recommendations on how to improve data comparability across European countries in the WHO region.

Introduction

Originally, the focus of surveillance rested on reporting of AIDS cases, which was the main tool to monitor the epidemic trends but, with the introduction and widespread use of highly active anti-retroviral treatment (HAART), the number of AIDS diagnoses no longer reflects the underlying trends in the HIV epidemic satisfactorily. Hence, reporting of HIV diagnoses has progressively replaced AIDS case reporting as a surveillance instrument for monitoring the HIV epidemic in Europe.

Recommendations for HIV surveillance in Europe were published in 1998 based on the results of a survey that was conducted by EuroHIV among the group of experts and national coordinators from the countries of the World Health Organization (WHO) European Region [1]. The recommendations underlined the need for information regarding national reporting systems in order to facilitate international comparisons of HIV and AIDS data.

Since 1998, new treatment regimens have been introduced and the laboratory technologies have improved considerably. Therefore the detection of new patterns of resistance to antiretroviral treatments presents a number of challenges and opportunities in the context of monitoring HIV resistance in Europe.

A new survey on HIV and AIDS surveillance practices was conducted by EuroHIV in 2006 [2], which had the same aim as the original one conducted in 1998. This article presents the collected data regarding HIV and AIDS case reporting in the 53 member states of the WHO European Region

Aim and objectives of the survey

The survey on HIV and AIDS surveillance aimed to assess national surveillance systems for HIV/AIDS in order to make recommendations on HIV/AIDS surveillance across Europe.

The specific objectives of the survey as presented in this paper were:

- to determine HIV/AIDS surveillance practices across Europe, with special emphasis on HIV/AIDS case reporting and HIV/AIDS mortality surveillance,
- to develop technical recommendations and guidelines in order to improve data comparability across Europe,
- to provide baseline data needed to ascertain the feasibility of HIV/AIDS surveillance in Europe and coordinate its development in the future.

Methods

The questionnaire

The survey was conducted using a standardised questionnaire that was first tested in a pilot round among EuroHIV steering group members. The questionnaire was divided into the following four sections:

- HIV and AIDS case reporting,
- HIV testing practices,
- other surveillance practices (HIV incidence and prevalence estimates),
- mortality data.

The results of the first section of the questionnaire, on HIV and AIDS case reporting, are presented in this article. This section was made up of five sub-sections further described in the EuroHIV report [2].

Data collection and analysis

The questionnaire was sent out at the end of April 2006 to the EuroHIV national correspondents in all 53 countries in the WHO European Region. A Russian translation of the questionnaire was also available. Reminders were sent after one month and three months, and further contacts (email, fax and telephone) were made to improve the response. In December 2006, the questionnaire was also sent to WHO contact points from five countries. Data collection for the survey was completed in February 2007.

In this article, results will be presented with a particular focus on the following areas of HIV and AIDS surveillance:

- data collection system,
- HIV/AIDS case definition for surveillance,
- variables collected,
- evaluation of surveillance systems.

Results

The questionnaire was returned by 44 of the 53 countries (overall response rate of 83%): 26 of the 27 European Union (EU) countries (96%; non-respondent: Cyprus) and 18 non-EU countries (Andorra, Armenia, Azerbaijan, Belarus, Bosnia and Herzegovina, Croatia, Georgia, Iceland, Israel, Kazakhstan, Kyrgyzstan, Norway, Republic of Moldova, Russian Federation, Serbia, Switzerland, Turkey and Ukraine)

Case reporting systems

In 2006, there was an HIV case reporting system in place in 43 of the 44 responding countries (98%), the exception being Austria where HIV surveillance was operated through a cohort study (Table 1). In 37 countries (86%), data were collected directly at the national level (no regional intermediate for data collection). Individual data were collected by 40 countries (93%). Reporting was done by both laboratories and physicians in almost two-thirds of the countries (27/43), only by laboratories in nine countries and only by physicians (either hospital-based or community-based physicians or both) in six countries.

In 2006, there was an AIDS case reporting system in all the countries (Table 1). Data were collected directly at the national level in 41 of 44 countries (93%). Data collection was case-based at national level in 42 countries. AIDS cases were reported solely by physicians in 32 (73%) countries (in 11 of which reporting was done solely by hospital physicians), solely by laboratories in one country, and by both laboratories and physicians in eight countries.

HIV and AIDS case reports were compiled in one combined database in 30 of 43 countries (70%) and, for seven additional countries where HIV and AIDS case reporting were in different databases, there was a possibility of linking between the HIV and the AIDS databases. Thus, of the 43 countries, the minority (six) were unable to link HIV and AIDS databases (Denmark, Iceland, Italy, Malta, Norway and Spain).

HIV case reporting

HIV testing algorithms

Figure 1 shows the various HIV testing algorithms for surveillance purposes that are required for the diagnosis and reporting of an HIV case in an adult, an adolescent or a child aged 18 months or older. The most commonly used confirmatory tests were immunoblot (including Western Blot; used in 34 countries), or a second enzyme immunoassay (EIA; used in 17 countries). Four countries (Armenia, Kazakhstan, Portugal and Romania) required three positive tests for the diagnosis/reporting of HIV cases, including two EIA. A single positive test, i.e. detection of nucleic acid by PCR, p24 antigen testing or viral culture, was accepted in 10 countries although the number of HIV cases detected with one of these tests represented less than 10% of the cases reported in these countries in 2005.

Case identification

Forty of the 43 countries provided information on the case identifier in order to detect duplicate reports (information not reported for Austria, Belarus, Kazakhstan and Spain): Twenty-eight countries (70%) used a coded identifier based on the patient's name or part of the name (17 countries) or did not include the patient's name (11 countries). Twelve countries (30%) used full names (Figure 2).

Description of the cases and transmission categories

Information on sex and age was collected in all countries (see Table 2); data on ethnicity or place of birth (or both) were collected

Table 1

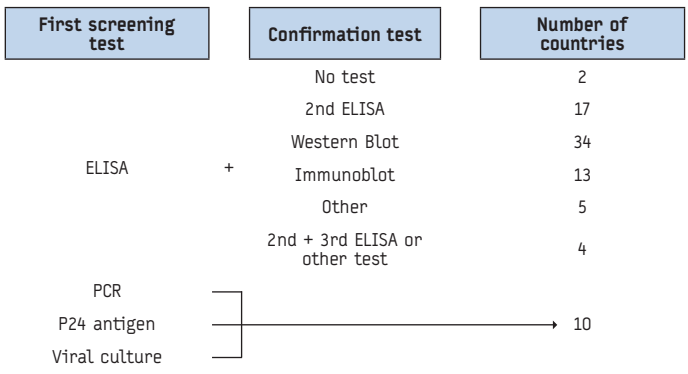
Information on data collection system, WHO European Region, 2006

	HIV		AIDS	
	%	(n/N)	%	(n/N)
Case reporting	98%	(43/44)	100%	(44/44)
National level	86%	(37/43)	93%	(41/44)
Individual data	93%	(40/43)	95%	(42/44)
Reporting by:				
Laboratories only	21%	(9/43)	2%	(1/44)
Physicians only	14%	(6/43)	73%	(32/44)
Both	63%	(27/43)	18%	(8/44)

n: number of countries with positive answer; N: number of participating countries

Figure 1

HIV testing algorithms used in the countries in the WHO European Region, 2006



in 34 countries (79%) and are planned to be collected in Bulgaria (not collected in Belarus, Estonia, Finland, Hungary, Poland, Republic of Moldova, Switzerland and Ukraine).

Information on the transmission category was collected by 40 countries, and on current drug injection status by 24 countries.

Clinical and virological characteristics

32 countries recorded the clinical stage at HIV diagnosis and four countries planned to do so in the near future (Bulgaria, Luxembourg, Republic of Moldova, Russian Federation). The definition used for clinical stage was the 2005 revised WHO clinical staging of HIV and AIDS for adults and adolescents [3] in 10 countries, the 1990 WHO clinical staging of HIV and AIDS for adults and adolescents in five countries, and the 2005 clinical staging system by the United States (US) Centers for disease control and prevention (CDC) in seven countries.

The CD4+ lymphocyte (CD4) count was documented in 21 countries and is planned to be collected in six countries.

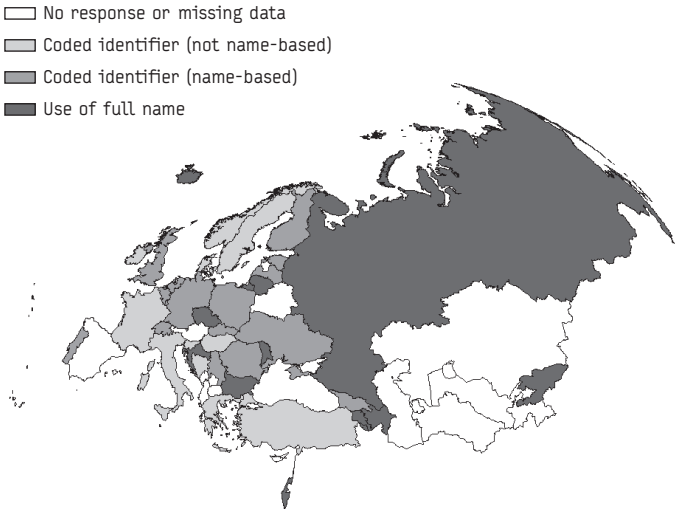
Some countries also collected data on molecular biology parameters: 10 countries collected data on HIV type, group and sub-type, four on type and sub-type, three countries collected data on sub-type only and 17 countries on types only. The laboratory methods used to characterise the virus were serological assays (16 countries), PCR (21 countries) and hybridisation (Belarus). Both PCR and serological assays were used in nine countries (Azerbaijan, Bulgaria, Croatia, France, Georgia, Hungary, Kyrgyzstan, Portugal, Sweden).

Monitoring death among HIV-infected persons

The HIV database could be linked to vital statistics or death certificate information in 18 countries (seven EU countries). Mortality data for HIV cases were reported in the routine HIV surveillance in 29 countries (66%). Date of death was recorded in all these countries, and in 23 of them also the cause of death. In 27 countries, death was reported by physicians, and in six countries by another source of information. The information collected was "death from any cause" in 13 countries and "death due to HIV infection (HIV infection is the only diagnosis at the time of death)" in 13 other countries. Both types of information (HIV-related and non HIV-related deaths) are collected in Azerbaijan and Portugal.

FIGURE 2

Case identifiers used for detecting duplicates, WHO European Region, 2006



AIDS case reporting

AIDS case definition

Different AIDS case definitions were used for AIDS case reporting [4]. Most of the countries in the WHO European Region (35, 80%) used the 1993 European AIDS Surveillance Case Definition [5]. Seven countries (Armenia, Belarus, Georgia, Latvia, Romania, Russian Federation and Ukraine) used the US CDC AIDS case definition [6]. Andorra and Belarus reported using the WHO 1994 case definition for AIDS surveillance in adults and adolescents.

The age cut-off for adolescent and adult AIDS surveillance case definitions varied between countries (Figure 3). In the 1993 European AIDS case definition, the age cut-off for adults and adolescents was 13 years and over. However, 17 of the 35 countries using that definition, set the age cut-off for adults and adolescents at 15 years, eight countries at 13 years (which is in accordance with the case definition proposed by the European centre for disease prevention and control (ECDC) [7]), and the 10 remaining countries used another or unknown age cut-off. In countries using the CDC or WHO case definition for AIDS, the age cut-off for adults and adolescents varied between 12 and 15 years.

Description of cases, clinical stage and transmission categories

Information on sex and age was collected in all the countries. Ethnicity or place of birth (or both) were documented in 35 countries (80%) and planned to be recorded in Bulgaria (not collected in Belarus, Estonia, Finland, Hungary, Moldova, Poland, Switzerland and Ukraine).

TABLE 2

Variables collected in the national HIV and AIDS case reporting systems, WHO European Region, 2006

Variables	HIV case reporting (N=43)		AIDS case reporting (N=44)	
	No. of countries	%	No. of countries	%
Sex	43	100%	44	100%
Age	43	100%	44	100%
Ethnicity and/or place of birth	34	79%	35	80%
Date of:				
HIV diagnosis	43	100%	41	93%
HIV report	40	93%	33	75%
AIDS diagnosis			42	95%
AIDS report			42	95%
Clinical stage	32	74%	32	73%
CD4 count	21	49%	26	59%
Transmission group	40	93%	42	95%
IDU status	24	56%	26	59%
ART			27	61%
ARV drug resistance	7	16%	9	20%
Mortality:				
Date of death	29	67%	42	95%
Cause of death	23	53%	33	75%

IDU: injecting drug users; ART: anti-retroviral treatment;

The CD4 count at the time of AIDS diagnosis was obtained in 26 countries (59%) and planned to be recorded in Moldova, Russian Federation and Slovakia.

The transmission category was recorded in 42 countries. Information on current drug injection status was collected by 26 countries.

Antiretroviral therapy (ART) and HIV drug resistance

The AIDS reports in 27 countries noted whether a patient was on ART at the time of AIDS diagnosis, and a further five countries (Belgium, Bulgaria, Estonia, Finland, Russian Federation) plan to start collecting this information in the near future.

Monitoring of resistance to ART was performed in nine countries among reported AIDS cases (and in seven countries among reported HIV-infected cases). Eleven additional countries plan to begin collecting this information within the next two years. The definition used for resistance was the "Stanford algorithm" in four countries, key resistance mutations defined by the International AIDS Society in four other countries, and another definition (not specified) in two countries.

Monitoring of death among AIDS cases

The AIDS database could be linked to vital statistics or death certificate information in 20 countries (nine EU countries). Mortality data on AIDS cases were reported in the routine AIDS cases surveillance system in 42 (95%) countries (all responding countries except Azerbaijan and Croatia). Date of death was recorded in all these countries and cause of death in 33 countries. AIDS death was reported by physicians in 39 countries and by another source of information in six countries. The information collected was "all causes of deaths among people living with AIDS" in 19 countries, "only deaths due to AIDS or AIDS-related illnesses" in 18 countries and "deaths from AIDS-defining illness" in two countries.

National evaluations of HIV and AIDS case surveillance systems

Over half of the countries (25 of 44, 57%) had not evaluated either their HIV or AIDS surveillance systems for under-reporting. Of the 17 countries that had done so, seven had assessed under-reporting of HIV reports only (i.e. HIV cases that are diagnosed but not reported), three reporting of AIDS only and eight reporting of both surveillance systems. The proportion of under-reporting in a country can be linked to the number of sources of information and can therefore vary widely between countries. For example, the proportion of under-reporting is low in the United Kingdom (UK) and Germany where only a few laboratories report HIV diagnosis. In France, the proportion of under-reporting is higher, but 5,000 laboratories report HIV diagnosis.

Nineteen of 44 countries (43%) had not evaluated the timeliness of either their HIV or AIDS surveillance systems (i.e. time from diagnosis to report). Of the 18 countries that had done so, three had assessed timeliness of HIV reports only, two of AIDS reports only and 13 of both surveillance systems.

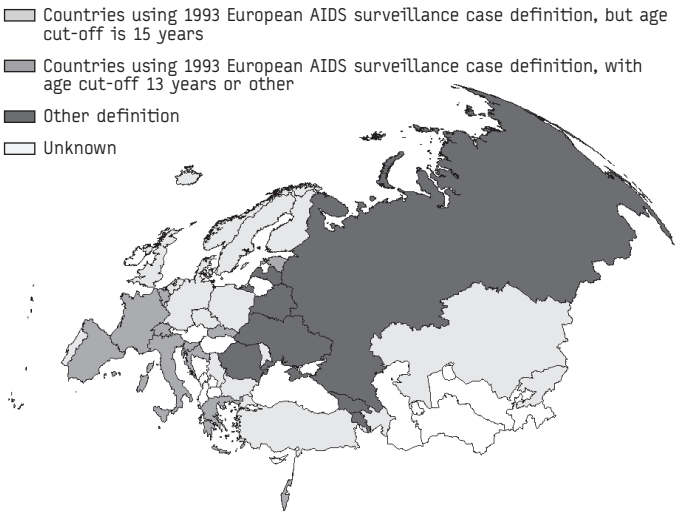
Of the 16 countries which reported the timeliness of their HIV reporting systems, all but three stated that 90% or more of HIV reports were received within six months (in Belarus, the UK and France, over 75% were received within six months). In contrast, of the 15 countries which reported the timeliness of their AIDS reporting systems, only eight stated that 90% or more of AIDS reports were received within six months, and six countries stated that 10% or more of AIDS reports were received with a delay of more than 12 months.

The validity of the HIV reporting system (e.g. comparison of the information provided on the original case report and the medical record) has been assessed in seven countries (100% in Andorra, Croatia and Czech Republic, 98% in Belarus). The validity of AIDS reporting system has also been assessed in seven countries (100% in Andorra, Croatia, Czech Republic and Republic of Moldova).

The completeness of HIV and AIDS reporting (i.e. percentage of cases with complete records on all variables) has been determined in 11 countries and varied from 23% to 100% for HIV cases and from 40% to 100% for AIDS cases. Separate percentages of completeness for the individual variables were not available.

FIGURE 3

Age cut-off for adolescent and adult AIDS case definition, WHO European Region, 2006



Discussion

In 2006, HIV and AIDS case reporting systems were in place in almost all the 53 countries in the WHO European Region. Overall, data collection is computerised and case-based in most of the countries. National coverage for HIV case reporting has not yet been achieved in two countries (Italy and Spain). In Austria, HIV case reporting was based on a national cohort of HIV-positive patients. In comparison with a previous survey on HIV reporting in Western Europe, conducted in 1999 [8], HIV case reporting systems have since been implemented in two additional countries (France and Ireland) and in the Netherlands the reporting system has become a national one. HIV reporting in Europe is based on newly diagnosed cases, except at the start of a new HIV case reporting system (a few years need to pass before the system has stabilised and data can be interpreted). Another exception is imported cases, which have been previously diagnosed in the country of origin.

AIDS surveillance data no longer reflects the underlying trends in current HIV infection satisfactorily. However, it still provides some objective indication of the number of people in the advanced stages of HIV infection. According to a survey that was conducted in 2005 [9], AIDS case reporting was considered "somewhat useful but not as much as before" in almost half (17/43) of the countries in the WHO European Region. For example, AIDS case reporting is useful to assess the number of late HIV diagnoses [10].

Linkage between HIV and AIDS individual reports, which allows for better case follow-up, is possible in most European countries (either within the same database or by linkage of databases). In a few countries with a high case load it is still not possible, mainly because different HIV and AIDS case identifiers are used for reasons of confidentiality. Linking HIV and AIDS databases could allow assessment of HIV disease progression and evaluation of modalities for HIV testing and care practices.

Fear of breach of confidentiality remains an important issue for HIV reporting. Although most of the European countries used a coded identifier to detect duplicate reports, the patient's full name is still used in 11 countries. While the use of full names needs strict and enforceable regimes of confidentiality to secure the registries, the use of unique coded identifiers depends on the reliability of the encoding system to be replicated and to identify duplicate reports [11]. Among the nine countries that had been using full names to identify HIV cases in 1998 [12], five were still using names in 2005 (Czech Republic, Israel, Lithuania, Republic of Moldova and Russian Federation) and two countries (Poland and Serbia) were using a code based on the name in 2006 (information unavailable for the two remaining countries). In contrast, HIV surveillance in the United States was name-based in 2006 in almost all the states, but not at federal level [13].

Although most countries used the 1993 European AIDS Surveillance Case Definition, some criteria need to be standardised across the European countries (e.g. the age cut-off for adults and adolescents, which was 13 years in some countries and 15 years in others). The AIDS case definition has been recently revised by the European Centre for Disease Prevention and Control (ECDC) and the age cut-off for adults has been defined as 15 years. This new case definition will be published in the near future. In parallel, in order to better monitor HIV treatment needs, the case definition for HIV surveillance has been recently revised by the WHO to include a clinical and immunological classification of HIV-related disease [3].

Of the variables included in HIV and AIDS reports at the European level, some are currently collected by more than 90% of the countries (e.g. sex, age, dates of diagnosis and report of HIV and AIDS, transmission categories) and others are not systematically collected by all the countries (e.g. ethnicity, date of death, ART at AIDS diagnosis or CD4 count at HIV diagnosis). Standardisation of variables is needed at European level, not only to understand better the epidemic but also to ensure that the countries have a minimum of data available to help design or improve interventions (e.g. HIV testing policies, monitoring of ART). Collecting information on CD4 count as well as clinical stage at HIV diagnosis is useful to monitor the proportion of cases diagnosed with advanced HIV infection, information that can be used to target efforts aimed at reducing late diagnosis. CD4 counts will be collected at European level for the first time in 2007. Several countries also monitor the molecular biology of HIV. This information is used to identify HIV strains that share the same genetic pattern, improving the characterisation of risk factors of genetic and environmental origin. This approach can also serve to understand better resistance to HIV treatment.

Information on HIV resistance was collected in only a quarter of the European countries. However, surveillance of HIV resistance is often not reported systematically; it can be based on cohort studies or networks of laboratories participating on a voluntary basis. Monitoring HIV drug resistance is useful for public health interventions or treatment monitoring [14]. While some guidelines recommend that HIV drug resistance surveillance should focus on individuals newly diagnosed with HIV in order to track transmitted resistance over time [14], other projects support genotypic resistance testing for all individuals who have not received antiretroviral drugs (recently and chronically infected) [15]. Different definitions are used to monitor HIV drug resistance, and the need to reach a consensus on the definition of drug resistance, especially for surveillance purposes, has been underlined [16].

In two-thirds of the countries, HIV and/or AIDS surveillance systems have been evaluated using one of four criteria: under-reporting, validity, completeness, timeliness. In countries where specific evaluations have been conducted, the percentage of under-reporting was higher and reporting delays longer for reporting of AIDS cases than of HIV diagnoses. In a survey conducted in 1996 [17], 32 European countries (71%) were able to provide quantitative estimates of under-reporting for AIDS cases. These estimates ranged from 0 to 25%. Completeness of HIV and AIDS reporting varied widely from one country to another (completeness of AIDS reporting has decreased in several countries, probably because clinicians no longer consider it equally important as before), and few countries have evaluated the validity of their reporting systems.

Although these four evaluation criteria were the ones most commonly used to evaluate HIV/AIDS surveillance systems, other assessment indicators (simplicity, flexibility, acceptability and representativeness) should also be used [18-20].

Conclusion and recommendations

HIV/AIDS case reporting data are crucial to support and guide public health policies for prevention and control of the HIV epidemic in the EU and the WHO European Region. Standardisation of HIV/AIDS surveillance system needs to be improved at European level in order to allow better comparability of data. The implementation of the revised European case definition for HIV/AIDS is the first step toward harmonisation and standardisation.

To achieve this goal, countries are advised by ECDC to have a surveillance system that collects individual data at a national level. Such a system should also ensure data confidentiality and respect the patients' human rights. Ideally, this surveillance system should integrate information on the three key stages of disease progression from asymptomatic HIV infection to death. For HIV diagnosis, the CD4 count at diagnosis provides valuable information for cases that present at a late stage of infection. For AIDS, information on treatment (HAART) is important to monitor access to care. For HIV/AIDS mortality, all causes of death, related to HIV or not, should be documented. Where possible, linkage between HIV/AIDS reporting systems and the mortality database is an added value. If this is not possible, other methods (e.g. surveys) can be conducted among HIV-infected persons. In addition, standard coding systems are needed to improve HIV/AIDS mortality surveillance [21].

Countries are further advised by ECDC to ensure that monitoring of HIV drug resistance is included in their current HIV surveillance system. WHO guidelines on this are available and these guidelines should be applied in the European Region [14].

The HIV epidemic is complex and its surveillance requires a multi-facetted approach, such as the development of "second generation" HIV surveillance which includes biological and behavioural data. This, as well as monitoring of HIV prevalence data, should be continued in addition to HIV case reporting.

Finally, it is advisable that the EU Member States evaluate their surveillance systems at appropriate and regular intervals as part of the data quality assurance process. A protocol for evaluation of surveillance systems would be a useful tool to strengthen HIV/AIDS surveillance in the WHO European Region.

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Situation of hantavirus infections and haemorrhagic fever with renal syndrome in European countries as of December 2006

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Hantavirus infections are widely distributed in Europe with the exception of the far north and the Mediterranean regions. The underlying causes of varying epidemiological patterns differ among regions: in western and central Europe epidemics of haemorrhagic fever with renal syndrome (HFRS) caused by hantavirus infections follow mast years with increased seed production by oak and beech trees followed by increased rodent reproduction. In the northern regions, hantavirus infections and HFRS epidemics occur in three to four year cycles and are thought to be driven by prey - predator interactions. Hantavirus infections and HFRS seem to be on the increase in Europe, partly because of better diagnostics, partly perhaps due to environmental changes. Unfortunately, hantavirus infections are still heavily under-diagnosed in many European countries. Here we report the results of a survey conducted in 2007 amongst the member laboratories of the European Network for diagnostics of Imported Viral Diseases (ENIVD).

Introduction

Hantaviruses (family *Bunyaviridae*, genus *Hantavirus*) are enveloped RNA viruses, carried primarily by rodents or insectivores of specific host species [1]. Three hantaviruses, Puumala (PUUV), Dobrava (DOBV) and Saaremaa (SAAV), are known to cause haemorrhagic fever with renal syndrome (HFRS) in Europe. Puumala virus, carried by the bank vole (*Myodes glareolus*, previously known as *Clethrionomys glareolus*) and Saaremaa virus, carried by the striped field mouse (*Apodemus agrarius*), are associated with mild HFRS. Saaremaa virus (also known as Dobrava Aa., Aa. standing for *A. agrarius*) has been found in Denmark, Estonia, southeast Finland, Germany, Russia, Slovakia and Slovenia. There are no well-documented SAAV-HFRS cases [2]. Dobrava virus (also known as Dobrava Af., Af. standing for *Apodemus flavicollis*), carried by the yellow-necked mouse (*A. flavicollis*) has been found in Albania, Bosnia-Herzegovina, Czech Republic, Croatia, Greece, Hungary, Russia, Serbia and Slovenia and causes severe HFRS [2]. In addition, the European common vole (*Microtus arvalis*) carries Tula virus (TULV), and rats (*Rattus norvegicus*, *R. rattus*) are carriers of Seoul virus (SEOV) [3]. However, these two viruses have not been definitely associated with disease in Europe [3], although both can infect humans as indicated by reports from Asia (China, South Korea) [4] and the United States where the virus has been associated with chronic kidney diseases [5]. In several European Union (EU) countries hantavirus infections are notifiable and in some countries (e.g. Belgium, France, Germany, the Scandinavian countries, Slovenia) their epidemiology was relatively well studied. In large areas of Europe, however, hantavirus infections and HFRS have not been studied systematically. In many countries the number of infections has been on the increase in recent years (Table 2). The European Network for diagnostics of Imported Viral Diseases (ENIVD, http://www.enivd.de) has conducted a survey of hantavirus infections and HFRS in order to learn more about the epidemiological features and public health impact and discuss factors that influence the occurrence of the disease.

Material and Methods

To obtain a more detailed overview of hantavirus infections in the EU, Bosnia-Herzegovina, Norway, Russia and Switzerland, a questionnaire was sent to all 30 members of the ENIVD. The following data were collected in 2007:

- Year in which laboratories implemented the diagnostic for hantaviruses;
- Methods used for screening and confirmation e.g. Enzyme-Linked Immuno Sorbent Assay (ELISA), Indirect Fluorescence Assay (IFA), Focus Reduction Neutralisation Tests (FRNT), Western Blot (WB) and RT-PCR.
- Notification status of hantavirus disease;
- Annual number of human cases;
- Years with increased hantavirus infections in humans
- Local rodent species (whether the species is known as hantavirus carrier or not)
- Circulating hantavirus serotypes in rodents
- Hantavirus serotypes known to cause human disease locally;
- Geographic distribution of the human cases in the country.

For the analysis of trends and identification of epidemic years, Belgium and France were chosen as examples (Figure 1) for western Europe, Finland, Norway and Sweden (Fennoscandia) represented northern Europe (Figure 2) and Austria, Bosnia-Herzegovina, Hungary and Slovenia, (Figure 3) represent central Europe. Our study covered the period from the start of hantavirus diagnostics in a given country until the end of the year 2006. Laboratories from 23 countries (19 EU Member States and four collaborating countries, i.e. Bosnia-Herzegovina, Norway, Russia and Switzerland) completed the questionnaire. No data were obtained from seven of 30 countries, i.e. Bulgaria, Estonia, Ireland, Latvia, Malta, Poland, Slovakia and the United Kingdom.

Table 1

Hantavirus cases by country in the European Union, Bosnia-Herzegovina, Norway, Russia and Switzerland, ENIVD study 2007

Country*	Year when diagnostic was started	Number of cases reported per year by the reference laboratory	Percentage of total cases reported in the European Union	Notifiable disease**	Hantavirus Serotype
Austria	Not available	198	0.60	No	PUUV
Belgium	1981	1856	5.66	Yes	PUUV
Cyprus	2005	0	0.00	No	
Czech Republic	1998	23	0.07	Yes	PUUV
Denmark	1999	0	0.00	Yes	PUUV
Finland	1979	24,672	72.22	Yes	PUUV
France	1987	1,536	4.68	No	PUUV
Germany	2001	1,320	4.03	Yes	PUUV/DOBV/SAAV
Greece	1997	37	0.11	Yes	DOBV
Hungary	1992	302	0.92	Yes	PUUV/DOBV/SAAV
Italy	1991	0	0.00	Yes	None
Lithuania	2000	9	0.03	Yes	PUUV/SAAV
Luxembourg	2000	16	0.05	Yes	PUUV
Netherlands	1994	43	0.13	Yes	PUUV
Portugal	1990	31	0.09	No	?
Romania	2005	2	0.01	No	PUUV/DOBV
Slovenia	1985	221	0.67	Yes	PUUV/DOBV
Spain	2001	0	0.00	No	None
Sweden	1994	3,516	10.73	Yes	PUUV
Bosnia-Herzegovina	1990	555	***	Yes	PUUV/DOBV
Norway	1990	1,084	***	Yes	PUUV
Russia	1980	89,162 (1996-2006)	***	Yes	PUUV/DOBV/TULV/ HTNV/AMRV/SAAV
Switzerland	2000	1	***	Yes	TULV

* no information obtained for Bulgaria, Estonia, Ireland, Latvia, Malta, Poland, Slovakia, United Kingdom
** hantavirus infection is a -by law- notifiable disease, within 48 hrs after confirmation in the laboratory
*** non-EU Member State.

Table 2

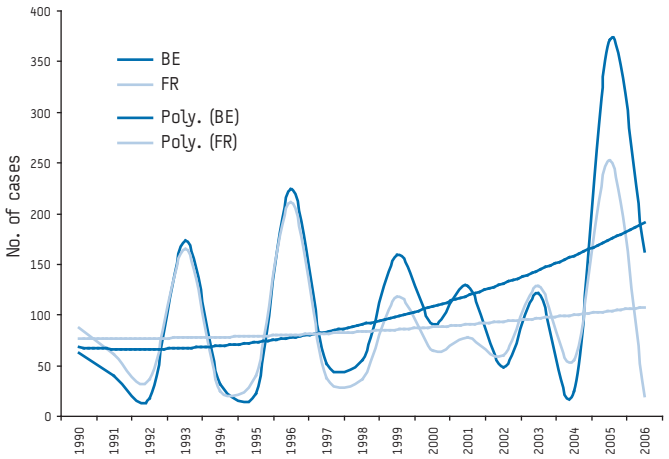
Hantavirus cases in 19 EU Member States and Bosnia-Herzegovina, Norway, Russia and Switzerland, ENIVD study 2007

	AT	BE	CY	CZ	DK	FI	FR	DE	EL	HU	IT	LT	LU	NL	PT	RO	SI	ES	SE	BIH	NO	RU	SW
1979		0				31																	
1980		0				9																	
1981		39				19																	
1982		4				64																	
1983		3				80																	
1984		3				108																	
1985		3				124											4						
1986		4				132											2						
1987		14				117	13										13						
1988		0				302	25										6						
1989		1				686	20										11						
1990		62				839	87								0		12			18	46		
1991		40				966	61								0		3				74		
1992		18				1,081	36			12					0		19				37		
1993	2	174				942	165			19					1		11				33		
1994	2	32				1,071	25			20				0	1		7		116		66		
1995	5	22				1,012	40			18				2	1		14		246	354	80		
1996	16	224				907	211			2				7	6		5		177		32		
1997	7	52				758	38		4	4				6	3		2		145		*81*		
1998	10	55		1		1,306	37		5	17				0	2		2		562		230		
1999	10	159		0		2,300	118		3	60				6	3		5		432		91	10,223	
2000	12	91		0		774	65		1	78			1	3	2		16		145		37	7,403	1
2001	13	129		0		1,057	78	185	3	19			2	5	2		6		360		61	8,356	0
2002	14	48		9		2,603	60	228	8	29			0	2	2		33		262	136	38	4,605	0
2003	7	122		4		1,566	129	144	2	11			1	3	2		6		179		39	6,161	0
2004	72	25		4		1,429	55	242	4	7		9	1	3	1		15		459		48	10,244	0
2005	16	372		3		2,526	253	448	5	6			8	3	1	1	24		330	21	65	7,348	0
2006	12	163		2		1,863	20	73	4				3	3	4	1	5		103	26	26	7,210	0
Total	198	1859	0	23	0	24,672	1,536	1,320	39	302	0	9	16	43	31	2	221	0	3,516	555	1,084	61,550	1

AT: Austria, BE: Belgium, CY: Cyprus, CZ: Czech Republic, DK: Denmark, F: Finland, FR: France, DE: Germany, EL: Greece, HU: Hungary, IT: Italy, LT: Lithuania, LU: Luxembourg, NL: Netherlands, PT: Portugal, RO: Romania, SI: Slovenia, ES: Spain, SE: Sweden, BIH: Bosnia and Herzegovina, NO: Norway, RU: Russia, SW: Switzerland.

Figure 1

Trends of hantavirus infections in Belgium and France, 1990-2006, ENIVD study 2007



Dark blue: yearly number of cases in Belgium
Light blue: yearly number of cases in France

Poly : Polynomial trendline: Calculates the least squares fit through points by using the following equation:
y = b + c1x + c2x2 + c3x3 + ... c6x6 , where b and c1... c6 are constants.

Trendlines in corresponding colour.

Results

Start of hantavirus diagnostics

Laboratory diagnostic of hantavirus infections had been initiated before 1990 in six of the responding 23 countries: Finland (1979), Russia (1980), Belgium (1981), Sweden (1984), Slovenia (1985) and France (1987). The remaining 17 countries started diagnostic testing after 1990.

Methods used for screening and confirmation

Commercial and/or in-house ELISA and IFA were used for screening. If a blood sample was available in the first four days after onset of symptoms, RT-PCR was an option in some cases. FRNT can be applied to recover the causal serotype from convalescent sera.

Notification status

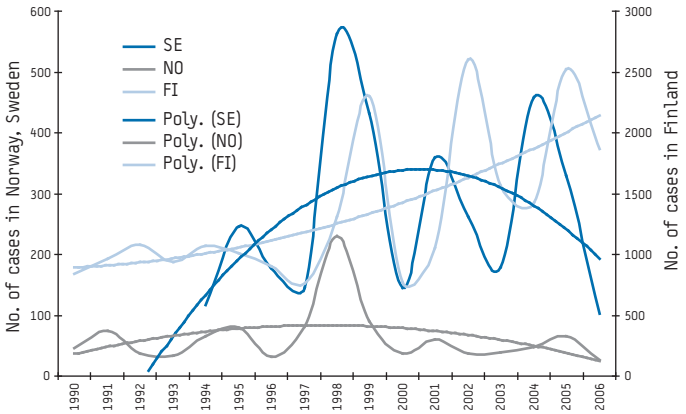
A notifiable disease is a disease which by law has to be reported to the appropriate authorities within a time frame defined by the national authorities, usually within 48 hours after laboratory diagnosis. Hantavirus infections are notifiable in 17 of the 23 reporting countries; they are not notifiable in Austria, Cyprus, France, Portugal, Romania and Spain (see Table 1).

Number of cases

Our survey accounted for a total of 35,424 laboratory confirmed cases, 33,587 (94.8%) of which were detected between 1990 and 2006. Finland reported 24,672 cases, accounting for 69.6% of all European cases. No hantavirus cases were reported from Cyprus, Denmark, Italy and Spain. In Russia, the European part accounted

Figure 2

Trends of hantavirus infections in Finland, Norway and Sweden, 1990-2006, ENIVD study 2007



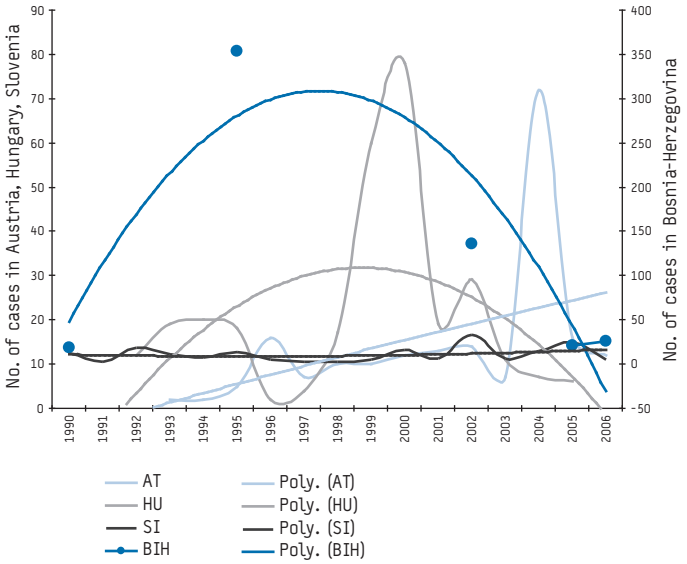
Dark blue: yearly number of cases in Sweden
Light blue: yearly number of cases in Finland
Grey: yearly number of cases in Norway

Poly : Polynomial trendline: Calculates the least squares fit through points by using the following equation:
y = b + c1x + c2x2 + c3x3 + ... c6x6 , where b and c1... c6 are constants.

Trendlines in corresponding colour.

Figure 3

Trends of hantavirus infections in Austria, Bosnia-Herzegovina, Hungary and Slovenia, 1990-2006, ENIVD study 2007



Dark blue: yearly number of cases in Bosnia-Herzegovina
Light blue: yearly number of cases in Austria
Dark grey: yearly number of cases in Slovenia
Light grey: yearly number of cases in Hungary

Poly : Polynomial trendline: Calculates the least squares fit through points by using the following equation:
y = b + c1x + c2x2 + c3x3 + ... c6x6 , where b and c1... c6 are constants.

Trendlines in corresponding colour.

for 95% of the nation's cases: Between 1996 and 2006, 89,162 cases were detected, the vast majority due to PUUV infection. In the Asian part of the Russian Federation, far fewer cases were noted, with DOBV, SAAV, SEOV, Hantaan (HTNV) and Amur virus (AMRV) as the causal agents (A. Platonov, personal communication).

The number of human cases is on the rise in almost all European countries and record numbers were noted in Finland over the last five years (2,603 cases in 2002, 2,526 cases in 2005), Sweden (459 cases in 2004) and Belgium (372 cases in 2005) (Table 1). The total number of reported cases by country is summarised in Table 1. Mild winters and more frequent and more productive mast events allow more rodents to survive the winter Particularly mild winters are responsible for an early start of the breeding season and, in consequence, for larger rodent populations [6-8].

Years with increased hantavirus infection activity

Increased hantavirus activity in epidemic years is not synchronised geographically and chronologically in the participating countries (Table 2). Based on the available data, we present the trends.

Belgium and France

In both countries the disease followed a three-year epidemic cycle prior to 1999 (1990, 1993, 1996, 1999) [9,10]. Between 1999 and 2005, a two-year cycle was observed (1999, 2001, 2003, 2005). The year 2006 (163 cases) was again an epidemic year, and 2007 with 262 cases detected emerged as the third consecutive epidemic year. As France and Belgium are geographically located in the temperate deciduous broad leaf-tree biome, rodent cycles are regulated by masting, i.e. the available food from - mainly - oak and beech trigger higher rodent population densities and increased virus circulation in the population, represented by considerable higher antibody seroprevalences (Figure 4 and 5). The 2006 epidemic was probably due to extensive (B. Van der Aa, Instituut voor Natuur- en Bosonderzoek, manuscript in preparation) masting of oak and beech in the autumn of 2004; this mast probably provided sufficient food for rodents even in autumn and winter 2005. In autumn 2006, an oak mast occurred again which was responsible for the increased hantavirus activity in 2007.

Adding a trend line to the dataset shows yet another remarkable feature; although both countries share a hyperendemic area, the trend for Belgium is increasing while the trend for France indicates a stabilisation of the situation. In 1985 the hantavirus activity in both countries was rather similar. The discrepancy between France and Belgium was marked in 2006: France (see: http://www.invs.sante.fr/surveillance/fhsr/points.htm) had very few cases, while Belgium had an increased number of cases (http://www.iph.fgov.be/epidemio/epinl/plabnl/plabannl/06_053n_v.pdf). The key factor, however, is the pattern change in 1999, which is so far unexplained. Abiotic factors like climatic conditions probably play a role [9,10].

Finland, Norway and Sweden

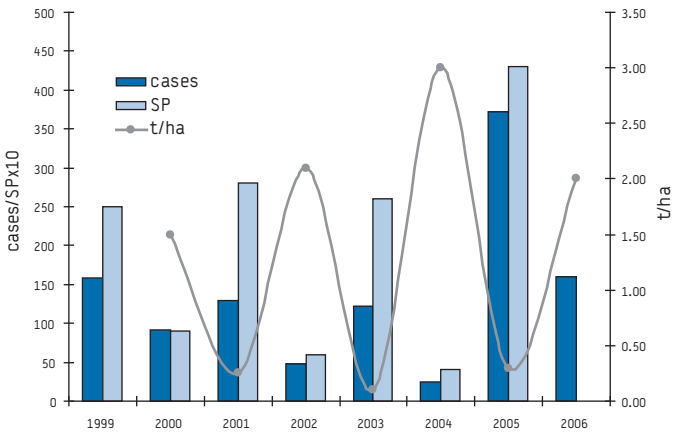
With 1,084, 3,516 and 24,672 detected cases, respectively, Norway, Sweden and Finland account for most of the hantavirus cases in Europe. Located in the boreal forest biome, rodent population density cycles depend mainly on predator-prey mechanisms. Incidences of HFRS almost as high as in Finland occur in parts of European Russia (e.g. Bashkiria and Udmurtia regions) and parts of northern Sweden. However, the epidemiological pattern on the national scale in Finland seems to be changing [11]. The 3-4 year cycles were less synchronised before the late 1990s, but more recently the whole southern part of Finland seems to be in a single cycle. The increasing trend, in the number of human cases also in 'low activity' years, may be due to better diagnostics.

Austria, Hungary, Slovenia, Bosnia-Herzegovina

With 198, 302, 221 and 555 detected cases, respectively, these countries experience the co-circulation of two or three hantavirus serotypes, PUUV, DOBV, and SAAV. Austria represents an interesting mixture of patterns: in low altitudes the mast-year pattern prevails while at higher altitudes the cyclic pattern is seen (S. Aberle, personal communication). The number of cases in

Figure 4

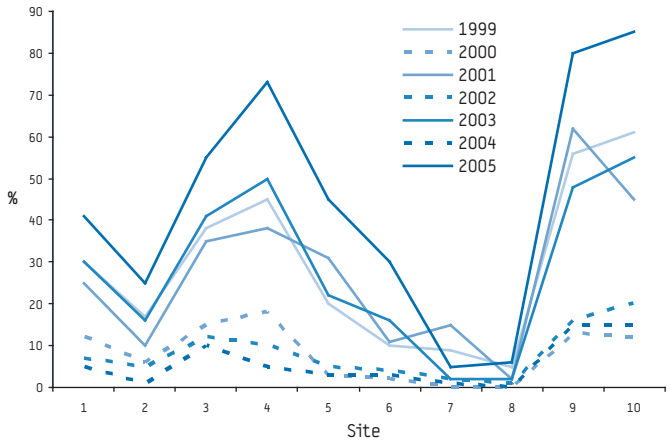
Relation between human cases, oak mast and Puumala virus seroprevalence in rodents



Cases: yearly numbers of cases 1999-2006 (dark blue bars)
SP: mean PUUV seroprevalence in rodents on ten sites in Belgium (light blue bars)
t/ha: tons of acorns per hectare (grey line).

Figure 5

Seroprevalence in *Myodes glareolus* at 10 trapping sites in Belgium (1999-2005)



Dotted lines: seroprevalence in *M. glareolus* at 10 trapping sites in Belgium in non-epidemic years
Lines: seroprevalence in *M. glareolus* at 10 trapping sites in Belgium in epidemic years

Bosnia-Herzegovina peaked significantly in 1995 (Table 2), during the conflict in that region.

Local rodent species and circulating hantaviruses

Although in most EU Member States and collaborating countries between 10 and 20 rodent species on average occur locally, only *M. glareolus*, *A. sylvaticus*, *A. flavicollis*, *A. agrarius*, *R. norvegicus*, *Microtus agrestis*, *M. arvalis*, *Mus musculus* and *Mus spretus* were reported as being present. The reported rodent species reflect those known or suspected of carrying a hantavirus serotype and other, non-suspect local rodent species were clearly not taken into account.

Puumala (PUUV), Tula (TULV), Dobrava, (DOBV), Saaremaa (SAAV) and Seoul (SEOV) hantaviruses were the serotypes reported as circulating both in humans and carrier rodents in the EU Member States and collaborating countries (Table 1).

Pathogenic hantaviruses in the EU

This survey confirmed that PUUV and DOBV have been causing the vast majority of human cases in the participating countries [11,12], with the exception of Switzerland, where the only case was associated with TULV [13]. Recently, SAAV was also found to be responsible for human cases in eastern Europe [14]. No confirmed cases of SEOV infection have so far been reported in the EU Member States, though an unpublished case, confirmed by focus reduction neutralization tests (FRNT), occurred in France (Å. Lundkvist, personal communication, see also [3]).

Imported hantavirus cases have been rare and were caused by DOBV in Sweden and Austria, HTNV in Austria, and Sin Nombre virus (SNV) in France.

In western and northern Europe (Fennoscandia), only PUUV infections were reported [11,12]. Increasing from the west and north to the east in the EU, the PUUV/ DOBV infection ratio varied from 3.6% in southern Germany to more than 50% in Slovenia and up to 100% in Greece. Notably, in Germany, SAAV infections have also been designated as DOBV-Aa. In south-eastern Europe, the DOBV-Af. variant is predominant.

Discussion

Before 1990, hantavirus infections were probably heavily underdiagnosed, due to lack of reliable diagnostic tools. We assume that a reasonable coverage of hantavirus surveillance is achieved if the level of surveillance and awareness in a country enables its national public health system to sufficiently determine, whether the disease is endemic and to what extent public health is affected by its presence. As of 1990, this has been achieved also for those countries with a passive hantavirus surveillance system (testing performed in regional and/or reference laboratories, regular reporting to the national public health authorities). It has been estimated that only 10% of PUUV infections lead to disease [1]. Furthermore, it should be kept in mind that, given the unclear clinical picture and the benign clinical symptoms in a number of patients, some cases escape the surveillance systems.

In all three described biomes of Europe, the human HFRS epidemiology follows the local rodent cycle, meaning that human cases occur in the same rhythm as the rodent cycles. However, the epidemiological pattern and the epidemic cycles in central Europe are less clear than in western or northern Europe.

Active surveillance of carrier rodents and circulation of pathogenic hantaviruses is seldom or not at all maintained on a regular basis in most participating countries. Possible reasons for this might be that active surveillance involving fieldwork (rodent snap- and /or live-trapping, sampling and subsequent testing) is an expensive and time-consuming exercise with often no immediate result for the funding public health authorities. This kind of surveillance is mostly performed by research groups targeting the pathogen(s) and their transmission ecology, or initiated as a response to an epidemic, the latter being too late to have a positive and immediate impact on public health.

Factors that determine the occurrence of hantavirus disease

Of the hosts of pathogenic hantaviruses in Europe, the bank vole, the carrier of PUUV, has a distribution range that includes most of Europe. Still, human disease incidence and epidemiological patterns vary greatly across the continent. The yellow-necked mouse has not quite as wide a range as the bank vole and DOBV has not been found in the western and northern parts of Europe. As far as we know, SAAV occurs in most areas where its host, the striped field mouse, is found in central and eastern Europe [15].

Multiple factors influence the occurrence of the disease in a region. The geographical location and the habitat composition is important and in Europe three major biomes occur (Figure 6):

- the boreal forest;
- the temperate deciduous broad-leafed forest;
- and the Mediterranean scrub zone [14].

The boreal forest in northern Europe can be defined as a large homogenous landscape with relatively low biodiversity, true cyclic rodent population dynamics with an extended peak phase of more than a year, and its rodent population is usually considered to

Figure 6

Terrestrial biomes in Europe

be regulated by predator-prey interactions [16]. In contrast, in the mid-European temperate deciduous broad-leafed forest zone, more stable and seasonal rodent population dynamics occur, with principally mast-driven peaks. Mast years are years in which trees (mainly oak and beech) produce more seeds than usually. The normal seasonal peak densities in autumn are of short duration, while after a mast event, increased winter survival results in extended high-density periods in the following year, coinciding with HFRS outbreaks (see Figure 4). Due to intensive agriculture and land use for building, the landscapes are highly fragmented and heterogeneous. The important trees for masting are oaks (*Quercus robur* and *Q. petracaea*) and beech (*Fagus sylvatica*). The more biomass these species produce, the more significant is the mast effect on rodent populations [17,18]. The less efficient spread of rodents and virus in the temperate deciduous biome is reflected in the ten-fold lower number of human cases when compared to Fennoscandia (the boreal forest biome) (see Table 2).

The Mediterranean scrub integrates well-developed and diversified herbaceous, shrub and arboreal strata that often are dense to almost impenetrable. Rodent population dynamics likely respond to food availability, which in turn is dependent on rainfall [19].

Human behaviour probably plays a crucial role in the likelihood of human hantavirus infections. In a long-term seroprevalence study in rodents at multiple locations in Belgium, both inside and outside the epidemic area for human cases, the presence of PUUV was detected in all examined bank vole populations [12]. Data on the incidence of human hantavirus disease, however, did not correlate with these findings; i.e. in some regions with a high seroprevalence in rodents, only a few or no human cases occurred at the same time and vice versa. Instead, the incidence correlated with the socioeconomic status of the inhabitants of a region [20]. The PUUV incidence rate was higher in areas with a high proportion of broad-leaved forests and a low level of urbanisation. A high level of urbanisation thus limits PUUV transmission, while income correlated negatively with the disease incidence.

Hantaviruses are unexpectedly stable over more than 10 days at room temperature and probably remain infectious for many months during winter in northern Europe [21]. The change of climatic conditions could have a significant impact on the magnitude and amplitude of the occurrence of hantavirus infections. Although it is still too early to draw firm conclusions, this effect has already been observed in Europe [9,16]. In France and Belgium a three-year epidemic cycle became a bi-annual cycle, and in Belgium, 2005, 2006 and 2007 can be considered three consecutive epidemic years (Table 2) [9]. In Finland, changes in the geographic synchronicity of rodent cycles have affected the incidence pattern at national level, although locally, human epidemiology follows the three-year vole cycles [16]. These spatial changes in the geography of rodent cycles have also occurred in Finland in the past. It may therefore be too early to draw conclusions regarding the effect of climate change.

Public health impact of hantavirus infections

DOBV infections, although relatively uncommon, cause severe HFRS with high case-fatality rate (CFR) around 10% [22]. Due to the varying severity of PUUV infections, only 5-10% of infected humans experience clinical problems severe enough to seek medical help [1]. Although the CFR due to PUUV infections is very low (~0.1% in Belgium and Finland) [1], about 5% of hospitalised PUUV-HFRS patients require dialysis treatment. A severe clinical course of PUUV-HFRS is strongly associated with HLA-B8 and mild with HLA-B27 haplotype. Fatal cases have been due to fluid imbalance after shock, haemorrhages and necrosis in the pituitary gland, and encephalitis. In a five year follow-up of hospitalized PUUV-HFRS patients, increased blood pressure, cardiac pulse and proteinuria were observed as long-term consequences in 20% of the cases [23]. After 10 years of follow-up the effect had largely, but not totally, disappeared.

Conclusions

We conclude that hantavirus infections are widespread in Europe and that they have clear effects on public health. Unfortunately, as documented by the present ENIVD survey hantavirus infections are currently underestimated or not recognised by the medical and public health authorities in many countries, largely because diagnostics are not available.

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Update of *Clostridium difficile* infection due to PCR ribotype 027 in Europe, 2008

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Outbreaks of *Clostridium difficile* infections (CDI) with increased severity, high relapse rate and significant mortality have been related to the emergence of a new, hypervirulent *C. difficile* strain in North America and Europe. This emerging strain is referred to as PCR ribotype 027 (Type 027). Since 2005, individual countries have developed surveillance studies about the spread of type 027. *C. difficile* Type 027 has been reported in 16 European countries. It has been responsible for outbreaks in Belgium, Germany, Finland, France, Ireland, Luxembourg, The Netherlands, Switzerland and the United Kingdom (England, Wales, Northern Ireland and Scotland). It has also been detected in Austria, Denmark, Sweden, Norway, Hungary, Poland and Spain. Three countries experienced imported patients with CDI due to Type 027 who acquired the infection abroad. The antimicrobial resistance pattern is changing, and outbreaks due to clindamycin-resistant *ermB* positive Type 027 strains have occurred in three European countries. Ongoing epidemiological surveillance of cases of CDI, with periodic characterisation of the strains involved, is required to detect clustering of cases in time and space and to monitor the emergence of new, highly virulent clones.

Introduction

Since the emergence of a new virulent strain of *Clostridium difficile* characterised as toxinotype III, North American pulsed-field type 1 (NAP1), restriction-endonuclease analysis group type BI and PCR-ribotype 027 (Type 027), multiple outbreaks have been reported in North America and Europe [1-9]. The increased virulence of *C. difficile* Type 027 is thought to be associated with a 1 base pair deletion at position 117 of the *tcdC* gene which leads to an increased or prolonged production of toxins A and B, and possibly the production of a binary toxin [1-3]. However, these virulence factors are not unique for Type 027 and are also present in other PCR ribotypes.

The first reports of outbreaks of *C. difficile* infections (CDI) due to Type 027 came from Canada, and the province of Quebec was the one affected first and most severely [4]. In the United States, cases of *C. difficile* Type 027 infection have been reported from at least 38 states (http://www.cdc.gov/ncidod/dhqp/id_Cdiff.html), and surveillance of community-acquired CDI has started [10]. By 2007, *C. difficile* Type 027 had been detected in 11 European countries [9]. The present report is an update on the situation in Europe in 2008.

Surveillance efforts

In 2005, the European Study Group for *Clostridium difficile* (ESGCD) performed a two-month surveillance study in 38 hospitals from 14 European countries [5]. Unfortunately, only hospital-acquired CDI were studied and no precise information on the severity and outcome was collected. The mean incidence of CDI was 2.45 +/-1.8 cases per 10,000 patient-days. The distribution of PCR ribotypes varied among hospitals and countries. Of 322 toxinogenic isolates, 20 (6.2%) belonged to Type 027 and were reported from Ireland, Belgium and The Netherlands. Patients infected with Type 027 were more likely to have a more severe disease, and to have been treated by metronidazole or vancomycin compared to patients infected by another PCR ribotype.

The European Centre for Disease Prevention and Control (ECDC) recognised this emerging new disease and undertook several actions to inform all European Union (EU) Member States. ECDC also offered support for surveillance studies at national and European level. Another pan-European surveillance study is presently being organised, which will collect epidemiological and microbiological data for one month in a selected number of hospitals from all EU Member States in order to estimate the incidence of hospital-acquired as well as community-acquired CDI. The results of this study are expected to be available in 2009.

Austria

In Austria, *C. difficile* Type 027 was reported once in 2006 in a British tourist suffering from pseudomembranous colitis. In May 2008, two cases of CDI due to Type 027 were found in patients who had no travel history in the year before their hospitalisation [11]. Typing patterns of isolates submitted voluntarily since 2006 demonstrate the occurrence of non-027 clusters of CDI cases in Austrian hospitals. The largest cluster affected a tertiary teaching hospital in 2006, where *C. difficile* PCR ribotype 053 represented 10 of 21 CDI cases. CDI is not a reportable disease in Austria. Hospital discharge data indicate a significant increase of CDI during the last years, from 777 cases (54 deaths) in 2003 to 997 cases (80 deaths) in 2004, 1,453 cases (88 deaths) in 2005 and 2,192 cases (150 deaths) in 2006.

Belgium

In Belgium, laboratory-based surveillance of CDI clusters performed by the national reference laboratory at the Université Catholique de Louvain as well as prospective surveillance of CDI incidence in acute care hospitals monitored by the Institute for Public Health were initiated in January 2006. Surveillance of CDI has become a legal obligation since July 2007. In 2007, 896 *C. difficile* isolates were analysed at the reference laboratory.

With 17.6% (158 isolates) Type 027 was the most frequently found type. Other frequently found types were PCR ribotypes 078 and 031, accounting for 6.3% and 5.6% of these isolates, respectively. Overall, the mean (median) incidence of CDI was 1.7 (1.6) cases per 1,000 admissions and 2.07 (1.86) cases per 10,000 hospital days. Sixty-eight percent of these cases occurred more than two days after hospital admission.

Denmark

In April 2006, Statens Serum Institut encouraged the Danish departments of clinical microbiology to report *C. difficile* cases on a continuous basis and to forward isolates for characterisation in cases of severe disease or in outbreak situations. In a retrospective survey covering a county in Region South Denmark, a cluster of eight patients with *C. difficile* Type 027 was detected. The isolates were recovered from 22 faecal samples that had been collected between November 2006 and March 2007. All eight cases were hospitalised in two hospitals in the region. Subsequently, active surveillance was initiated in the same region for the period June-August 2007, which resulted in five additional Type 027 cases among 22 *C. difficile* isolates tested. Interestingly, all 13 isolates were resistant to newer fluoroquinolones and cephalosporins, but susceptible to erythromycin and clindamycin.

Finland

The first case of *C. difficile* Type 027-associated disease was detected in Finland in October 2007 [12]. Since then the National Public Health Institute (Kansanterveyslaitos; KTL) has intensified surveillance and control of CDI. A few additional cases caused by Type 027 were detected retrospectively, indicating that this strain had previously been circulating in Finland. The Finnish Hospital Infection Programme (SIRO) prepared a protocol for CDI surveillance to detect severe cases and epidemics caused by *C. difficile*. Molecular methods for rapid detection of *C. difficile* Type 027 were set up at two clinical, university-affiliated laboratories in Helsinki and Turku, and genotyping methods for molecular epidemiology of *C. difficile* were set up at KTL.

During the five-month-period from mid-October 2007 to mid-March 2008, isolates of *C. difficile* Type 027 were reported from four of the nine health care districts that had sent the isolates to KTL, and originated from over 20 different health care facilities – most of them providing primary or long term care – located in southern and south-western Finland. Of the 268 isolates, 131 (49%) belonged to Type 027. The remaining isolates were distributed among more than 30 different PCR ribotypes.

France

In France, the CDI surveillance is based on the mandatory notification of severe cases or outbreaks of CDI to local health departments, regional infection control coordinating centres and the National Institute for Public Health (Institut de Veille Sanitaire;

InVS). Laboratories are encouraged to send the isolates from notified cases to a network of six French reference laboratories for *C. difficile*.

In April 2006, the first cluster of *C. difficile* Type 027 was reported in Northern France. From January 2006 to December 2007, 214 health care facilities reported at least one severe case or outbreak of CDI and a total of 1,247 cases. Sixty-four health care facilities (29 in 2006 and 35 in 2007, with no overlap between these 64) were affected by Type 027. Most cases originated from healthcare facilities in the Nord Pas-de-Calais region, but in 2007, small clusters of *C. difficile* Type 027 were reported from three other French regions, Picardie, Rhône-Alpes and Lorraine. Among the 1,227 isolates (511 in 2006 and 716 in 2007) sent for typing, 337 (27.5%) were identified as Type 027 (212 in 2006, i.e. 41.5% of the typed isolates, and 125 in 2007, i.e. 17.4% of the typed isolates). The large majority of strains were resistant to erythromycin and moxifloxacin, but susceptible to clindamycin. However, one hospital in Picardie reported an outbreak associated with a clindamycin-resistant strain that tested positive for the *ermB* gene encoding the macrolide-lincosamide-streptogramin B (MLSB) phenotype.

Unfortunately, no data are available on the occurrence of other PCR ribotypes.

Germany

Since October 2007, it is mandatory to report severe cases of CDI to the local authorities. Patient-based notifications are done by the physician treating the patient. Severe CDI cases are defined as cases which necessitate readmission to a healthcare facility due to the relapse of CDI, admission to an intensive care unit for treatment of CDI or its complications, surgery (colectomy) for toxic megacolon, perforation or refractory colitis, or lead to death within 30 days after diagnosis of CDI, if CDI is either the primary or a contributive cause to death. This mandatory surveillance was implemented shortly after the first outbreak of *C. difficile* Type 027 was detected in the region of Trier, Rhineland-Palatine in September 2007. To date, five of 16 Federal States (Länder), all of which are located in the south-west of Germany, have reported cases of CDI due to Type 027 [13,14].

Hungary (not included in the table)

A recently completed surveillance study in three different parts of Hungary revealed one isolate of Type 027 among 150 *C. difficile* isolates collected. The patient had systemic lupus erythematosus and developed severe CDI after antibiotic treatment for pneumonia in a hospital in Budapest.

Ireland

After the first report of *C. difficile* Type 027 in Ireland in 2007, this type was identified in six additional healthcare settings [15,16]. To date, more than 100 *C. difficile* Type 027 isolates from Ireland have been characterised by toxinotyping and 16-23S PCR ribotyping [15]. Isolates from two healthcare settings were susceptible to clindamycin (n=11: MIC90=4 mg/l). However, clindamycin-resistant Type 027 isolates (n=96, MIC90>256 mg/l) were identified in the five other healthcare institutions. All clindamycin-resistant Type 27 isolates tested positive for the *ermB* gene. Multiple locus variable number tandem repeat (MLVA) typing could clearly differentiate between clindamycin-resistant and -susceptible isolates from the same geographical region and sub-grouped them into two distinct clusters, with all isolates from the clindamycin-resistant cluster being were closely related [16].

CDI has become a notifiable disease in the Republic of Ireland since May 2008 under 'acute infectious gastroenteritis' using the case definition by ESGCD and ECDC. Only new cases will be reported, and this will enable data to be collected on the national level, but not on hospital-level. There are moves to make CDI notifiable in its own right to enable the collection of enhanced surveillance data (e.g. on origin and onset of CDI). National guidelines on surveillance, diagnosis and management of *C. difficile* have been published in May 2008 [17].

Luxembourg

During the period between October 2006 (start of CDI surveillance in Luxembourg) and February 2008, 96 (26%) of 368 submitted *C. difficile* strains were PCR ribotyped as Type 027. Type 027 was the type found most frequently, followed by types 001 and 106, but confirmation for the latter two is pending. The isolates came from all 10 hospitals in Luxembourg. The situation is ongoing and the total number of *C. difficile* isolates is now exceeding the number of salmonella and campylobacter isolates.

The median age significantly differed between patients with Type 027 (74 years) and patients with other ribotypes (59 years) (p=0.001). The mortality rate of CDI due to Type 027 within one month and within three months of isolate referral was 14.8% and 21.0%, respectively. In a logistic regression model, one-month mortality of CDI was significantly associated with age over 70 years (p<0.0001), but not with gender (p=0.66) or PCR ribotype (p=0.14).

Netherlands

Since October 2005, the Centre for Infectious Disease Control (CIb) at the National Institute for Public Health and the Environment (Rijks Instituut voor Volksgezondheid en Milieu; RIVM) and the reference laboratory for *C. difficile* at Leiden University Medical Center have encouraged microbiologists to send *C. difficile* isolates from patients with a severe course of CDI, or when an increased incidence of CDI was noticed. During the surveillance period from 2005 to 2007, Type 027 was reported from an increasing number of healthcare facilities in an endemic form or in outbreaks. At the end of 2007, 35 healthcare facilities have been affected, compared to 22 healthcare facilities until the end of 2006 [8]. During the surveillance period of 2006/2007, five outbreaks with Type 027 occurred, compared to 11 outbreaks in 2005/2006. One hospital was affected by an outbreak caused by both Type 027 and Type 017.

Comparison of clinical data of patients with CDI due to Type 027 (n=128) and other types (n=443) showed that CDI due to Type 027 was associated with older age, use of cephalosporins (mainly second generation) and fluoroquinolones (mainly ciprofloxacin). Patients with Type 027 CDI had more relapses and a more severe disease with a higher overall and attributable mortality [8]. *C. difficile* Type 027 was significantly more often acquired at a health care institution. Other frequently isolated PCR ribotypes in The Netherlands were types 014, 001 and 078.

Norway

In December 2007, the first two cases of CDI due to Type 027 in Norway were reported from a university hospital in Oslo [18].

Surveillance and infection control measures did not reveal other Type 027 isolates at this hospital. In February 2008, a third case of CDI due to Type 027 was detected at a nursing home in Oslo.

Since January 2008, the Department of Infection Prevention in cooperation with the Institute of Microbiology, both at Rikshospitalet University Hospital, Oslo, have performed genotypic characterisation of *C. difficile*. The most frequently found PCR ribotype is Type 014. Unfortunately, most medical microbiology laboratories in Norway do not cultivate *C. difficile*. As a consequence, the distribution of PCR ribotypes in Norway remains unknown.

Poland

No systematic CDI surveillance has yet been developed in Poland. Between 2005 and 2007, a surveillance study was performed in four hospitals in the Mazovia region. Of 400 *C. difficile* isolates, one isolate belonged to Type 027. As determined by E-tests, the isolate was highly resistant to fluoroquinolones (ciprofloxacin, gatifloxacin and moxifloxacin, MIC≥32 mg/l) and erythromycin (MIC≥256 mg/l), but susceptible to clindamycin (MIC=6 mg/l), metronidazole (MIC=0.38 mg/l) and vancomycin (MIC=0.75 mg/l). The most frequent PCR ribotype was Type 017, which accounted for approximately 40% of the *C. difficile* isolates studied.

Spain

Spain does not have a national surveillance programme to investigate cases of CDI or an official reference laboratory where hospitals could send *C. difficile* isolates for further characterisation. A surveillance study performed between January and June 2007 at a 1,750-bed, tertiary care hospital in Madrid revealed two cases of severe CDI due to Type 027. The index case was a Spanish patient admitted to the intensive care unit, who was transferred from a hospital in the United Kingdom. The other patient was a laboratory technician working with *C. difficile* isolates, who developed CDI shortly after antibiotic treatment. In this study, a non-specified PCR ribotype containing the genes for toxins A and B but not for the binary toxin, was detected in 103 of 388 typed *C. difficile* isolates (26.5%, 81 patients).

Since the *C. difficile* Type 027 has the binary toxin genes, testing for the presence of these genes is performed for all *C. difficile* isolates. Binary toxin-positive strains are subsequently ribotyped. In contrast to previous studies performed in this hospital, there was an increase of non-027 *C. difficile* containing the genes for toxins A and B and the genes for the binary toxin (13% of the total number of isolates studied). The PCR ribotype pattern of the binary toxin positive isolates probably corresponds to Type 078.

Sweden

Three sporadic 'historical' moxifloxacin-susceptible isolates of *C. difficile* Type 027 were found among 1,325 isolates collected between 1997 and 2001 in Sweden. In September 2006, the Swedish Institute for Infectious Disease Control (Smittskyddsinstitutet; SMI) alerted microbiologists and clinicians about *C. difficile* Type 027 and laboratories were encouraged to send *C. difficile* isolates to SMI for microbiological characterisation for patients with a severe course of CDI or when an increased CDI incidence was noticed.

Since epidemic Type 027 isolates have uniformly been moxifloxacin-resistant , a systematic screening of *C. difficile* isolates for moxifloxacin resistance was initiated during 2007 in four hospitals in Stockholm. In February 2008, this screening was extended to include all major hospitals in Sweden. Preliminary results indicate only one case of moxifloxacin-resistant Type 027 (found in May 2008), but there is currently no indication of outbreaks due to *C. difficile* Type 027. The most frequently PCR ribotypes isolated in Sweden are Types 012 and 014.

Switzerland

In Switzerland, the first outbreak of *C. difficile* Type 027 was observed in a geriatric hospital in Basel in 2006 [19]. The index case was an 82-year old female patient and the outbreak involved 15 other patients between October 2006 and May 2007. It is likely that the index patient acquired *C. difficile* Type 027 during a hospital stay in a foreign country. The median age of the 16 patients was 83.5 years (interquartile range: 79-92 years). A severe to moderate course of CDI was reported in seven (44%) of the patients and crude mortality was 19% (three deaths). All isolates were highly resistant to moxifloxacin (MIC>32 mg/l), erythromycin (MIC>256 mg/l) and clindamycin (MIC>256 mg/l). MLVA typing revealed one cluster of genetically highly related (STRD≤2) clindamycin-resistant Type 027 isolates which differed from the clindamycin-susceptible Type 027 control isolates and also from clindamycin-resistant isolates from Ireland.

United Kingdom (UK)

In England and Wales, mandatory surveillance of CDI in patients over 65 years has been included in the healthcare-associated infection surveillance system for acute trusts [20]. This mandatory surveillance programme is operated by the Health Protection Agency (HPA) on behalf of the Department of Health. Through its network of regional laboratories in collaboration with the *C. difficile* Ribotyping Network for England (CDRNE) and the Anaerobe Reference Laboratory (ARL) in Cardiff, the HPA further obtains *C. difficile* isolates from symptomatic patients in a structured, but random sampling scheme. In England, 110 out of 145 hospitals (76%) investigated between April 2007 and February 2008 showed the presence of *C. difficile* Type 027. Of 2,084 *C. difficile* isolates, 42% were typed as Type 027, 19% as Type 106 and 10% as Type 001. In Wales, 10 out of 16 investigated hospitals showed the presence of *C. difficile* Type 027.

In Scotland all diagnostic laboratories have been requested since September 2006 to submit *C. difficile* isolates to a UK reference laboratory in the case of severe CDI or outbreaks. The data are published quarterly [21]. Additionally, isolates from local research projects have also been submitted for ribotyping, which means that some hospitals/regions are over-represented in this collection of isolates. A total of 20 cases of *C. difficile* Type 027 were identified in Scotland in the period from September 2006 to April 2008. Among these were an outbreak with five cases in one hospital in the West of Scotland and an outbreak with three cases in a hospital in the North East of Scotland. In total, Type 027 has been detected in nine acute care hospitals in five different geographical regions of Scotland. One case was reported from a nursing home.

Until recently, *C. difficile* Type 027 was not a common PCR ribotype in Scotland. With the two recent outbreaks the frequency of 027 has reached 5.7 %. Since 2006, the most frequent PCR ribotypes in Scotland have consistently been type 106 (55% of *C. difficile* isolates) and type 001 (21%). Four isolates of the new emerging ribotype 078 have been identified in Scotland as well.

In Northern Ireland, a survey was undertaken between September and December 2006, and 60 samples (4.0% of the annual total of *C.*

TABLE

***Clostridium difficile* Type 027 in 15 European countries (due to differences in surveillance methodology the data cannot be directly compared)**

Country	Survey period	Population* (million inhabitants, 2006)	No. of hospitals* (2006, if not otherwise mentioned)	No. of hospital beds* (2006, if not otherwise mentioned)	No. of positive hospitals / No. of hospitals investigated for Type 027 (%)	No. of nursing homes positive for Type 027	No. of Type 027 isolates / Total no. of isolates tested (%)**	Attributable mortality of *C. difficile* infections	Updates available at
Austria	2006	8.3	total: 264; acute care: 183	total: 63,354; acute care: 52,894	1 / 43 (2%)	n.a.†	1 / 1,004 (<1%)	n.a.	http://webribo.ages.at
Belgium	2007	10.4	total: 215; acute care: 146	total: 55,158; acute care: 49,435	32 / 74 (43%)	n.a.	158 / 896 (18%)	see text	http://www.belgianinfectioncontrolsociety.be/
Denmark	Nov. 2006-Jan. 2008	5.4	total: 67 (2003); acute care: 62 (2000)	total: 20,646 (2004); acute care: 16,779 2004)	3 / 6	n.a.	13 / 44 (30%)	unknown	
Finland	Oct. 2007-Apr. 2008	5.3	total: 349; acute care: n.a. (20 health care districts)	total: 36,659; acute care: 12,730	4 / 9 health care districts	n.a.	131 / 268 (49%)	n.a.	
France	Jan. 2006-Dec. 2007	60.7	total: 2,856 (2005); acute care: 1,599 (2002)	total: 443,767(2005); acute care: 224,247 (2005)	64 / 214 (30%)	at least 6	337 / 1,227 (27%)	n.a.	http://www.invs.sante.fr/raisin
Germany	Oct. 2007-Apr. 2008	82.7	total: 3,359; acute care: 2,104	total: 683,484; acute care: 510,767	13 / n.a.	1	44 / n.a.	2.3%	http://www.rki.de > Infektionskrankheiten A-Z > Clostridium difficile
Ireland	2006	4.2	total: 179 (2004); acute care: 53 (2005)	total: 22,985 (2005); acute care: 12,094 (2005)	7 / n.a.	n.a.	n.a.	n.a.	http://www.ndsc.ie/hpsc/A-Z/Gastroenteric/CDifficile/
Luxembourg	2006-2008	0.5	total: n.a.; acute care: 9 (2004)	total: 2,871 (2004); acute care: 2,307 (2004)	10 / 10	n.a.	96 / 368 (26%)	see text	
Netherlands	2005-2007	16.3	total: 193; acute care: 104	total: 80,762 (2003); acute care: 49,715	35 / 70 (50%)	10	285 / 1,553 (18%)	4.1%	http://www.rivm.nl/cib/infectieziekten-A-Z/infectieziekten/Clostridium_difficile/Clostridium_difficile_draaiboek.jsp
Norway	Jan. 2008-Apr. 2008	4.6	total: 70; acute care: 60 (2000)	total: 19,193; acute care: 13,868	1 / 2	1	3 / 47 (6%)	n.a	
Poland	2005	38.1	total: 835 (2005); acute care: n.a.	total: 199,769 (2005); acute care: 178,074 (2002)	1 / 4	n.a.	1 / 400 (<1%)	unknown	
Spain	Jan 2007-July 2007	44.4	total: 788	total: 159,671	1/1	n.a	4 / 388	unknown	
Sweden	Nov. 2007-Febr. 2008	9.1	total: 81 (2003); acute care: 78 (2003)	total: n.a.; acute care: 25,492 (2005)	0 / 4	0	0 / 238‡		
Switzerland	2005-2008	7.3	total: 337 (2005); acute care: 192 (2005)	total: 41,196 (2005); acute care: 27,132 (2005)	3 / 10	n.a.	26 / 250 (10%)	unknown	
United Kingdom									
England	2006-2007	50.7	total: 170 trusts (acute care)	total: 126,976 (acute care, 2006/2007)	110 / 145 (76%)		879 / 2,084 (42%)		http://www.hpa.org.uk/infections/topics_az/clostridium_difficile/default.htm
Wales	2006-2007	3.0	total: 13 trusts (acute care)	total: 13,583 (2006/2007); acute care: 9,344 (2006/2007)	10 / 16 (63%)		unknown		
Northern Ireland	2006 (sept-dec)	1.7	total: 31 trusts (acute care)	total: 6220	n.a.	n.a.	0 / 60	unknown	
Scotland	Oct 2006-April 2008	5.1	total: 261 (2007); acute care: 45 (2007)	total: 26,463 (2007); acute care: 14,107 (2007)	9 / 18 (50 %)	1	20 / 253 (5.7%)	unknown	http://www.hps.scot.nhs.uk/haiic/sshaip/clostridiumdifficile.aspx

* Source: European health for all database (HFA-DB), World Health Organization Regional Office for Europe, Copenhagen, Denmark http://data.euro.who.int/hfadb/, except UK National Statistics, http://www.statistics.gov.uk/.
** Number of *C. difficile* isolates: may differ from number of patients mentioned in the text.
†n.a.: not available.
‡Total no. isolates screened for moxifloxacin resistance (n=238). PCR ribotyping was only performed on moxifloxacin-resistant isolates (n=60).

difficile reports) were ribotyped: the most common ribotypes were 001 (35%), 106 (11.6%) and 078 (8.3%). Ribotype 027 was not identified in this small sample. The first report of ribotype 027 in Northern Ireland related to a specimen in mid-June 2007. Since then there has been a large hospital outbreak associated with ribotype 027 (57 reports to date).

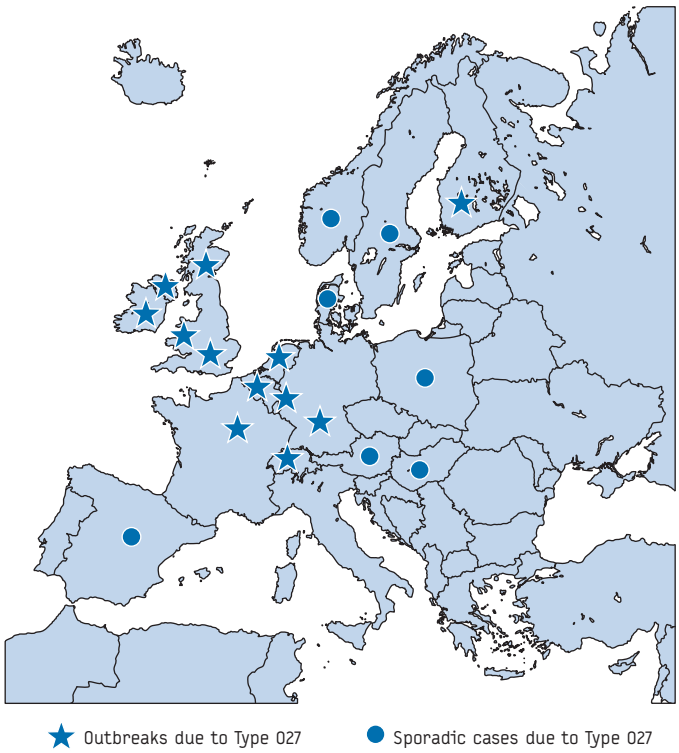
An enhanced ribotyping surveillance programme has recently been established: 59 specimens were ribotyped, of which 35% were Type 078, 25% were Type 001 and 8% were Type 014/20. The sample contained two reports of Type 027 (3%). Compared with the earlier survey in 2006 there has been a marked increase in ribotype 078 and a decrease in ribotype 001. Further investigations are underway to analyse this change in ribotype incidence.

Conclusion

As of June 2008, *C. difficile* Type 027 has been reported from healthcare facilities in 16 European countries (Figure, Table). Among those, nine countries have reported outbreaks and seven countries have reported only sporadic cases. Because of the lack of national surveillance programmes in many countries, it is at present impossible to estimate the incidence of *C. difficile* Type 027 in Europe. A new, emerging Type 078 strain, with similar mechanisms for the hyper-production of toxins as Type 027, is increasingly reported in Belgium, The Netherlands, Northern Ireland, Scotland, and possibly Spain.

FIGURE

Distribution of *Clostridium difficile* Type 027 by country in Europe* as of June 2008



* Not all countries have performed surveillance studies to *C. difficile* type 027 and this figure may underestimate the number of affected countries.

The occurrence of outbreaks due to clindamycin-resistant Type 027 isolates in three European countries is worrying. Clindamycin has been considered as a 'protective' antibiotic with regards to the development of CDI due to Type 027 [8]. However, resistance to clindamycin may increase the risk of CDI in patients receiving this agent and its use may be an important factor contributing to its persistence and spread. In addition, the report of erythromycin-susceptible and clindamycin-susceptible Type 027 isolates in Germany and Denmark indicates that antimicrobial resistance patterns are very dynamic and can no longer be used to identify *C. difficile* Type 027.

All European countries should now be aware about CDI in healthcare facilities, and specifically about *C. difficile* Type 027. Surveillance studies should be performed with uniform definitions, as proposed by ECDC [1]. These surveillance studies should not only focus on *C. difficile* Type 027, but include all major PCR ribotypes circulating in Europe since the distribution of these ribotypes varies greatly among European countries and over time.

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Laboratory investigation of the first suspected human cases of infection with avian influenza A(H5N1) virus in Bulgaria

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Reports of human cases of infection with avian influenza A(H5N1) virus have received increased public attention because of the potential for the emergence of a pandemic strain. In the end of 2005 and the beginning of 2006, avian influenza A(H5N1) virus caused outbreaks among domestic poultry and was isolated from wild swans in many European countries, including Bulgaria. Between January and March 2006, samples were collected from 26 patients who had been in close contact with ill or dead birds and developed a subsequent respiratory illness. The testing took place at the National Laboratory of Influenza in Sofia. Specific A(H5N1) assays were applied for screening (Sacace RT-PCR and real-time kit). Avian flu A(H5N1) virus was not detected in any of the patients tested. In three patients, human subtype A(H1N1) influenza virus, identifiable by RT-PCR was isolated and further characterized by hemagglutination inhibition test (HIT). The reliability of the molecular assays used in this investigation was demonstrated in an International Quality Control for Human and Avian A(H5N1) Influenza performed later in 2006 by INSTAND (Society for Promotion of Quality Assurance in the Medical Laboratories), Germany.

Introduction

Avian influenza (AI) viruses are divided into those of high and low pathogenicity (HPAI and LPAI). In domestic poultry, infection with LPAI may go undetected and usually causes only mild symptoms. However, the highly pathogenic form spreads more rapidly, and has a mortality rate that can reach 90-100% often within 48 hours. Influenza virus type A(H5N1) is highly pathogenic and very infectious for a number of bird species including most poultry species kept domestically. A(H5N1) virus does not usually infect people, but infections with these viruses have occurred in humans, with first cases detected in 1997, in Hong Kong. Most of the human cases have resulted from people having direct or close contact with A(H5N1)-infected poultry or A(H5N1)-contaminated surfaces. Although avian influenza viruses are currently poorly adapted to humans, the potential remains for the emergence of new pandemic strains either directly from avian viruses, or from their recombination with human or other animal viruses. [1,2,3,4].

In the end of 2005 and the beginning of 2006, avian influenza virus A(H5N1) caused a number of outbreaks among the poultry in countries close to Bulgaria, including Turkey, Romania and Ukraine [5,6,7]. In addition, Turkey registered its first two human cases of A(H5N1) infection in January 2006. During this period the virus has also been isolated from wild swans in many European countries [6]. In Bulgaria, the National Veterinary Services detected four cases of A(H5N1) infection in wild swans at the Black Sea coast and along the Danube river, in January 2006 [6,8].

In response to the increased circulation of A(H5N1) avian influenza viruses at the end of 2005 and the beginning of 2006, the Bulgarian Ministry of Health reinforced its activities related to influenza surveillance and control. The National Influenza Pandemic Preparedness Plan was approved. The National Influenza Pandemic Committee and Crisis Headquarters were established. The government provided additional financing for supplying antiviral drugs, protective equipment and diagnostic kits. Measures were also taken to inform the healthcare workers and the public about the risk of possible transmission of A(H5N1) virus among poultry and to humans.

In addition to these actions, the screening of potential human cases of infection with avian influenza virus A(H5N1) was undertaken. This required putting in place effective diagnosis not only of human influenza infections, but of avian influenza infections as well.

The laboratory identification of influenza virus infections is commonly accomplished by antigen detection, isolation in cell culture, or detection of influenza-specific RNA by highly sensitive and specific reverse transcriptase polymerase chain reaction (RT-PCR). This method, including genome detection tests, was subsequently adapted and tested during the investigation of the first suspected human cases of avian influenza A(H5N1) virus, as described below. [9]

Methods

For the purpose of the investigation, patients who experienced influenza-like symptoms and had been in close contact with ill or dead birds or had a history of travel to countries with registered human and and/or animal avian flu cases were considered suspected cases of A(H5N1) infection. Samples taken from these patients were sent to the National Laboratory of Influenza for screening.

Nasopharyngeal swabs from twenty six patients and post-mortem lung and tracheal tissue and bronchoalveolar lavage specimens from two patients were available for testing. The specimens were collected in the appropriate viral transport medium (Becton &Dickinson, USA) and were shipped immediately to the laboratory, in accordance with WHO guidelines' regulations for collection,

storage and transport of human and animal specimens for laboratory diagnosis of suspected influenza A infection. [10,11] The samples were tested immediately, or, in case it was not possible, they were frozen at –70°C. All the specimens were collected during the period of January to March 2006. Patients` data on sex, age and source of potential infection are shown in Table 1.

The initial specimens were screened for viral RNA by RT-PCR. RNA was extracted by using monophasic solution of phenol and guanidinium isothiocyanate-Trizol LS (Invitrogen life technologies, USA) an improvement of the single-step RNA isolation method as being developed by Chomczynski and Sacchi. [12]

For detection of avian influenza virus A(H5N1) we used the RT-PCR Avian Influenza A Virus (H5, H7) Screening and Typing kit (Sacace, Italy). For detection of human influenza viruses we applied conventional RT-PCR - One-Step Ready–to-Go RT-PCR Beads (Amersham Biosciences, U.K) kit. We used specific primer pairs for subtype A (H1N1), A (H3N2) and for type B(HA), directed against highly conserved regions of the hemagglutinin (HA) gene segment [13,14].

Table 1

Suspected human cases of A(H5N1) infection investigated in the National Laboratory of Influenza in Bulgaria during the period January – March 2006

Patient	Sex	Age	Potential risk factor
1	F	53	Exposure to wild bird
2	F	27	Exposure to ill bird
3	M	16	Exposure to dead bird
4	M	21	Chinese citizen
5	M	39	Exposure to dead swan
6	M	45	Exposure to dead swan
7	F	11	Exposure to dead swan
8[ab]	F	27	Fast food staff
9[b]	M	31	Exposure to dead bird
10	M	16	Exposure to dead swan
11	M	38	Exposure to dead swan
12	F	36	Exposure to dead swan
13	M	48	Turkish citizen
14	F	81	Exposure to dead swan
15	M	58	Exposure to dead swan
16	M	54	Exposure to dead swan
17	F	54	Exposure to dead swan
18	F	38	Veterinarian, Exposure to dead swan
19	M	60	Exposure to dead swan
20	F	22	Exposure to dead swan
21	M	45	Exposure to ill bird
22[a]	M	49	Exposure to ill bird
23[b]	M	9	Exposure to dead hen
24	M	36	Exposure to ill dove
25	M	47	Traveler to China
26	M	8	Exposure to ill bird

[a] Deceased after severe influenza-like-illness
[b] Isolated human subtype A(H1N1) viruses A/New Caledonia/20/99-like. Identified by HIT

Influenza virus A(H5) RNA from the commercial kits or standard laboratory human influenza strains A(H1), A(H3) and B(HA) were used as positive controls; ddH2O were used as negative controls. Viruses were isolated from those samples that were positive in the RT-PCR by one passage in the Madin-Darby canine kidney (MDCK) cell line and two subsequent passages in embryonated chicken eggs [13]. Viral isolation was carried out for the period of 7-10 days. The isolated strains were identified by hemagglutination inhibition test (HIT) using either WHO Influenza Reagent kit for identification of influenza isolates-2005-2006 or laboratory antisera to different human influenza A (H1N1), A(H3N2) and B standard strains.

In 2006, the National Laboratory of Influenza and Acute Respiratory Diseases also underwent an International Quality Control (IQC) facilitated by the Society for Promotion of Quality Assurance in the Medical Laboratories (INSTAND), Germany. We received 16 simulated samples for human and avian influenza viruses and respiratory syncytial viruses (RSV) with the aim to identify their antigenic and genomic composition. This IQC consisted of two stages: the first was performed in March while the investigation of the suspected human cases for A(H5N1) was still ongoing; and the second took place in October 2006.

The specimens sent from INSTAND were screened initially by the Directigen Flu A+B (Becton &Dickinson, USA) immunomembrane assay for detection of viral nucleoprotein (NP) and differentiation of type A and B influenza viruses. Specific H5 antigen detection kit (GeNet Bio, Korea) and RT-PCR Avian Influenza A Virus (H5, H7) Screening and Typing kit (Sacace, Italy) were used for detection of A(H5N1) avian influenza virus. For detection of human influenza viruses by RT-PCR, One-Step Ready–to-Go RT-PCR Beads (Amersham Biosciences, UK) were used as described before. The IQC specimens positive for A(H5N1) by conventional RT-PCR were confirmed once more by real-time PCR using Avian A Screening & H5N1 Typing FRT SC (Sacace, Italy), according to the two stage standard protocol of Sacace. We used the Chromo 4 (Bio-Rad, USA) real-time PCR system. Fluorescence was observed on the FAM channel for Avian A cDNA in the first stage (Real Time Amplification Kit), and on the FAM channel for Avian A cDNA H5, and on the Cy3 channel for Avian A cDNA N1. [14]

Results

On the basis of the case definition criteria, 26 patients were considered suspected cases of avian influenza (Table 1). All these patients exhibited influenza-like symptoms and either had contact with ill or dead birds or had travelled to areas affected by avian influenza. Four patients (nr 8, 9, 22, 23 in Table 1) with more severe symptoms were hospitalised and two of them subsequently died (8 and 22). From the investigated patients, 17 were men and 9 were women. Most of them were living in areas where A(H5N1) viruses were found in swans.

Clinical samples (nasopharyngeal swabs) of all 26 patients and post-mortem lung and tracheal tissue and bronchoalveolar lavage specimens from two deceased patients were collected between January and March 2006 and sent for testing at the National Laboratory of Influenza.

Avian flu A(H5N1) virus as a causative agent of respiratory disease was not detected in any one of the tested patients after the screening of the initial clinical specimens by RT-PCR.

Simultaneously performed, RT-PCR using HA specific primer pairs found three specimens positive for A (H1) human influenza viruses.

After PCR screening, influenza strains from the same patients' specimens were isolated and identified by HIT as human A/New Caledonia/20/99(H1N1) – like viruses. This investigation was performed because an epidemic of seasonal (human) influenza was taking place at the same time.

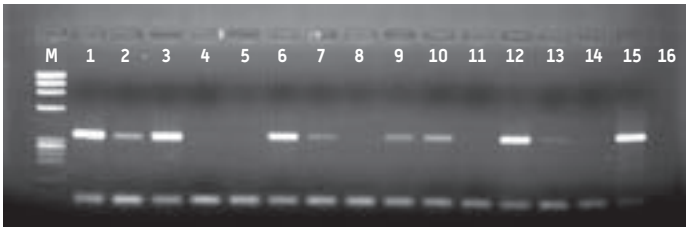
The accuracy of molecular testing used for the detection of suspected human cases of avian influenza A(H5N1) was demonstrated in the IQC. Nine of the 16 specimens received from INSTAND tested positive for A(H5N1) RNA by the use of conventional RT-PCR in March and October 2006 (Figure 1). The obtained sizes of the amplified products were 365 bp.

Positive results for A(H5) samples from IQC were confirmed also by real-time PCR. Figure 2 shows the positive results from three simulated samples in comparison with positive and negative control from the kit.

Since 2007 the National Laboratory of Influenza and Acute Respiratory Diseases as the Bulgarian National Reference Centre has participated also in External Quality Assessment (EQA) organized by the World Health Organization (WHO), and facilitated by Virology Division of the Department of Health in Hong Kong. The results of the investigation of the simulated samples we received for testing from Panel 3 of WHO EQA in February 2008 had 100% accuracy. [14]

FIGURE 1

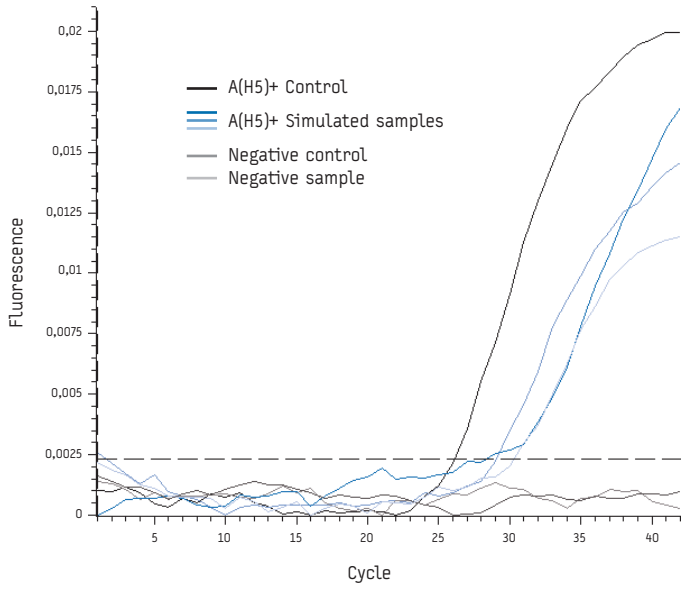
RT-PCR for detection of A(H5N1) avian influenza virus in simulated samples sent from INSTAND (Germany) for international quality control at the National Laboratory of Influenza, Bulgaria, 2006



Legend: Subtyping of influenza viruses and RSV by one-step RT-PCR. Lanes 1 to 15 are simulated samples. Lanes 1, 2, 3, 6, 7, 9, 10, 12, 13 are positive for A(H5); lane 15 is A(H5) positive control (Sacace kit); lanes 4, 5, 8, 11, 14, 16 are negative for A(H5); lane M (ΦX DNA) molecular size marker.
Each band is 365 bp.

FIGURE 2

Real-time PCR for detection of A(H5N1) avian influenza virus in simulated samples sent from INSTAND (Germany) for international quality control at the National Laboratory of Influenza, Bulgaria, 2006



Legend: Detection of influenza A(H5) viruses by real-time PCR.
The amplification curve in black represents A(H5) positive control (Sacace kit); in dark, medium and light blue – positive A(H5) samples; in dark grey – negative control (Sacace kit), in light grey – negative A(H5) sample.

Discussion and conclusion

As soon as the first cases of A(H5N1) in wild birds (swans) were detected in Bulgaria, the public health authorities considered it important to develop a more sensitive approach in defining and investigating suspected human cases of avian influenza in the National Laboratory of Influenza. In connection with the approval of the National Influenza Pandemic Preparedness Plan the National Laboratory of Influenza received the necessary equipment and tests for application of new diagnostic methods for detection of influenza viruses. The introduction of a complex of contemporary diagnostic methods was aimed to increase the preparedness of our laboratory in the conditions of a potential spread of avian influenza A(H5N1) virus.

Many countries conducted screening programmes for detection of A(H5N1) infection in humans. Thailand for example has large scale programme involving the testing of hundreds of people presenting with respiratory symptoms who have also had some exposure to poultry [15]. In Europe, Greece had a similar experience of examining 26 potential cases of A (H5N1) infection during the same period of 2006. The tests performed with molecular methods were all negative [16].

When examining the first suspected A(H5N1) patients in Bulgaria, we followed the routine diagnostic scheme stages developed by the National Laboratory of Influenza for human influenza viruses. [13,17]

By reason of the disease severity, the necessity of rapid diagnostic response, and the lack of appropriate Biosafety level-3 (BSL-3) in our laboratory for work with highly pathogenic strains, this scheme was carried out as follows:

- Screening of the initial clinical specimens by RT-PCR for avian and human influenza viruses (BSL-2);
- Isolation of influenza viruses after obtaining negative results for A (H5N1) by RT-PCR (BSL-2);
- HIT for identification of the isolated human strains.

The investigation for A(H5N1) infection in humans described here was the first of its kind for our laboratory practice in which we had to apply the scheme in a new approach. Although none of the samples tested positive for A(H5N1) we are confident that our performance of the tests by molecular techniques (RT-PCR and Real Time PCR) was correct because as shown in the quality control testing of the simulated samples in which we obtained

positive results for the A(H5N1) viruses. The RT-PCR and real-time PCR (Sacace, Italy) kits were adequately effective as regards the screening and genotyping of probable A(H5N1) specimens.

As described by other authors, real-time PCR finds more and more application in influenza diagnostics, due to its high sensitivity and specificity when making the diagnosis in a short period of time, and the possibility of simultaneous type and subtype differentiation of the viruses directly into the clinical specimens [18,19].

In the study we performed, in the identification of the IQC specimens, real-time PCR was applied only as a quality assurance method for the confirmation of the RT-PCR result in A(H5N1) diagnostics. This was the first time this technique was applied in our laboratory practice.

The negative results obtained by H5 GeNet Bio when testing the IQC samples which were positive by RT-PCR make us doubt the sensitivity of this rapid test for avian influenza virus antigen detection in clinical specimens. As a matter of principle rapid antigen testing is not currently recommended for the detection of avian influenza A(H5N1): a negative result does not exclude avian influenza, and a positive result of an antigen test (including immunofluorescence methods) does not differentiate between seasonal and avian influenza A viruses. Confirmatory testing and subtyping must be performed by molecular methods (e.g. reverse transcriptase polymerase chain reaction), virus culture or both [9].

In conclusion, the investigation of the first suspected human cases of A(H5N1) avian influenza virus allowed us to acquire skills needed when working with highly pathogenic infectious agents in our country and to gain the practical experience in applying the diagnostic methods necessary for the detection of influenza H5 antigens and genome in an extremely short period of time. The work described here has been of great importance for the public health system in Bulgaria in increasing the laboratory surveillance and preparedness. It also improved the collaboration between different institutions and persons responsible for public health in Bulgaria – epidemiologists, clinicians, human and veterinary diagnosticians working together under the direction of the Ministry of Health.

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EUREGIO MRSA-net Twente/Münsterland – a Dutch-German cross-border network for the prevention and control of infections caused by methicillin-resistant *Staphylococcus aureus*

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is associated with increased mortality and morbidity and a leading cause of hospital-acquired infections. Community-acquired (CA)-MRSA are a growing concern worldwide. In the last 10 years, an increase in the MRSA rate from 2% to approximately 23% has been observed in Germany, while a rate under 5% has been recorded for many years in the Netherlands and Scandinavia. In the Netherlands in particular, MRSA rates have become very low in stationary care due to a consistent 'search and destroy' policy. The main focus in Germany lies on hospital-acquired MRSA, whereas the Netherlands focus on the control of the importation of MRSA cases from abroad and on CA-MRSA. As MRSA in hospitals and in the community can be a problem in cross-border health care, the European Union-funded EUREGIO MRSA-net project was established in the bordering regions Twente/Achterhoek, the Netherlands and Münsterland, Germany. The main aim of the project is the creation of a network of the major health care providers in the EUREGIO and the surveillance and prevention of MRSA infections. A *spa*-typing network was established in order to understand the regional and cross-border dissemination of epidemic and potentially highly virulent MRSA genotypes. As the reduction of differences in health care quality is an important prerequisite for cross-border health care, a transborder quality group comprising hospitals, general practitioners, public health authorities, laboratories, and insurerance companies has been established since 2005 equalising the quality criteria for the control of MRSA on both sides of the border.

Introduction

Staphylococcus aureus is responsible for the majority of healthcare-associated infections worldwide. These infections include skin and mucosa infections, pneumonia and septicaemia. Infections caused by methicillin-resistant *S. aureus* (MRSA) are particularly critical because the therapeutic options are limited. Consequently, infections with MRSA are associated with a higher morbidity and lethality compared to other staphylococci. In the past ten years, an increase in the prevalence of MRSA infections has been observed in Germany. Although it has been assumed that the rate of MRSA isolations from blood cultures in Germany has stabilised at a level around 20-30%, this is still significantly higher than in neighbouring countries such as The Netherlands and Denmark, where the rates have been around 1% for many years [1]. This is a clear signal that the MRSA rates in hospitals can be minimised by adopting a consistent and co-ordinated "search and destroy" approach [2].

The EUREGIO MRSA-net project is a regional network designed to protect the population in the Dutch-German border region Twente/Münsterland (Figure 1) against MRSA infections [3,4]. It was launched to improve the implementation of MRSA prevention and control strategies within the EUREGIO by exchanging knowledge and technology. It represents a regional network for the control of

Figure 1

EUREGIO* MRSA-net project area

* The name EUREGIO stands for European region. It is used to refer geographically to a section of the Dutch-German border area covering parts of the Dutch provinces Gelderland, Overijssel, and Drenthe as well as parts of the German federal states Nordrhein-Westfalen and Niedersachsen. The MRSA-net project does not cover the whole of the EUREGIO.

MRSA involving local healthcare providers as recommended by the conference of the Germany's state health ministers in Dessau in June 2006. In Germany, the project is being co-ordinated by the Institute for Hygiene at the University Hospital Münster and the State Institute for Health and Work in North Rhine-Westphalia. In the Netherlands, it is co-ordinated by the laboratory Twente-Achterhoek and the University of Twente in Enschede.

Methods

The major objective of the EUREGIO MRSA-net project is to to improve patient safety and cross-border patient exchange in the EUREGIO. Its main activities are:

1. The creation of a euregional and cross-border network in the EUREGIO: 74 coordinator meetings, 21 round table discussions and four general meetings of all hospitals in the area have been organised to date;
2. Prevalence screening on admission of the patient to hospital and evaluation of regional risk factors: Over a four week period in November 2006, all patients in all participating hospitals in the region were screened at admission and asked for MRSA-associated risk factors;
3. Development of an MRSA prevention and control concept: Comparison and matching of recommended hygiene standards in the region [4,5];
4. Establishment of an international web-based communication portal for handling MRSA problems (24-hour help desks) for healthcare workers, patients and the public [5];
5. Training and professional development of healthcare personnel: 146 seminars and presentations for staff have been arranged to date;
6. Creating public awareness for MRSA and infections in general: The project was presented in 16 reports on national and regional television, seven radio reports and 45 contributions to local and national press;
7. Construction of an online *spa*-typing network for an early warning system.

Altogether, 40 hospitals in a region covering 8,000 km² and comprising 2.7 million inhabitants (950,000 inhabitants in the Dutch area) have participated in the project so far (Table 1). The healthcare structures in the EUREGIO vary strongly between the Dutch and the German side of the border: While on the German side there are six patient beds per 1,000 inhabitants, there are two patient beds per 1,000 inhabitants on the Dutch side. The same applies to doctors working outside hospitals. 163 doctors per 100,000 inhabitants (50% general practitioners (GPs) and 50% specialists) work in the Münsterland area compared to about 43 GPs per 100,000 inhabitants in the project area inTwente/Achterhoek.

The EUREGIO project involves 40 hospitals (four in the Dutch part), eight regional microbiological laboratories (one in the Dutch part), six public health offices (one in the Dutch part), and five professional institutions (e.g. Medical Order, Medical Association, health insurances such as the AOK Westphalia-Lippe). Patient interests regarding the quality of cross-border health were taken into account through collaboration with EPECS (European Patient Empowerment for Customised Solutions). In addition, nursing homes, ambulatory nursing services, and patient transportation services were included. The validation of special microbiological diagnostic procedures to detect MRSA was carried out by the Institute of Medical Microbiology at the University Hospital of Münster.

Results and discussion

Creating a crossborder network

The different actors involved in healthcare in the area were invited to round table discussions and informed about the project on several occasions. The motto for these round table discussions was "MRSA: One border, one problem, two results". The discussions showed that post-discharge case management of MRSA patients was not done regularly on the German side. Therefore, a 12-month-long case management system was established that required GPs to

Table 1

Comparison of healthcare structures between the Dutch and the German bordering regions in the EUREGIO MRSA-net Twente/Münsterland, 2006

	EUREGIO MRSA-net Dutch part (Twente)	EUREGIO MRSA-net German part (Münsterland)
No. of inhabitants	950,000	1,700,000
No. of acute care hospitals	4	33
No. of patient beds	2,200	10,139
Hospital patient beds/1,000 inhabitants	2	6
No. general practices (and specialists only in the German part)	358	3,128
General practices/1,000 inhabitants	0.4	1.8
No. of public health service offices	1	5

Figure 2

MRSA decolonisation planning tool for planning the 12-month-long case management period in hospitals and ambulatory care on the German side of the EUREGIO MRSA-net

recall a patient twice to control their colonisation status, first three to six months and again 12 months after discharge from hospital. Only after 12 months of negative screening results was a patient to be considered MRSA-negative, Patients admitted to a hospital during that period need to be screened before admission or isolated until they are excluded as persistent carriers of MRSA.

In order to improve the communication between hospitals and GPs, an MRSA patient management checklist was developed as well as a decolonisation planning tool (Figure 2) that facilitates the planning of the 12-month case management. The checklist informs the GP about the MRSA patient's condition at the time of hospital discharge and the treatment steps needed for his decolonisation. As consistent protocols for infection control outside the hospitals did not exist on the German side, such protocols were developed for patient transport services, nursing homes, ambulatory care.

Following the Dutch example of controlled decolonisation also after stationary care, an agreement was achieved between the Association Of Statutory Health Insurance for physicians (Kassenärztliche Vereinigung Westfalen-Lippe (KVWL)) and the primary health insurances (especially the AOK Westfalen-Lippe) regarding payment for GP services. According to this agreement, preventive decolonisation and control screening are now possible in ambulatory care after discharge from hospital and thus before next hospitalisation of the patient.

Finally, the public health offices were involved in the development of the network from the beginning. Acting within the scope of national legislation, the five German public health offices participated in the project as external quality controllers for all health institutions in the region. In order to make the regional MRSA epidemiology comparable, the hospitals were provided with the EpiMRSA software (Ridom GmbH, Würzburg) which enabled them to collect relevant data for standardised and cross-border analysis of MRSA-associated data (e.g. MRSA incidence, swabbing frequency and infection rates, *spa* types). These comparable data were regularly collected from all hospitals by the German public health offices, which allows for a better comparability and sustainability [5].

Prevalence screening and risk factor analysis

In November 2006, the MRSA-net project screened, during a four-week period, 86% of all inpatient admissions to German hospitals in the EUREGIO and interviewed all patients with regard to risk factors. This four-week prevalence screening was established in order to validate the already established screening recommendations. Preliminary analysis of the data indicates that the prevalence of MRSA varies between different districts and between different hospitals within the region.

Prevalence screening was also performed in one of the four Dutch hospitals in the EUREGIO. MRSA admission prevalence was shown to be about three-fold lower than on the German side of the border. Panton-Valentine leukocidin-producing community-acquired (CA)-MRSA infections only rarely contribute to the MRSA admission burden of regional hospitals (Netherlands: 8%, Germany: <1%). On the German side, the screening programme following the current national guidelines would have detected less than 50% of the MRSA carriers that were identified in our prevalence screening exercise. Screening of patients with a history of previous hospitalisation (not only in foreign "high-prevalence" countries, but also in German facilities) is therefore of great importance for successful MRSA detection for the hospitals in the EUREGIO.

Following this period of prevalence screening, MRSA prevention strategies and screening indications were adapted to a common euregional standard in all participating hospitals.

Prevention via the development of a web-based communication system for MRSA

The most important instruments needed to successfully implement prevention strategies and control measures are application plans and target group-specific infection control protocols.

We carried out 28 application tests with different target groups (doctors, nursing staff, and ward assistants) and examined infection control protocols of different hospitals on both sides of the border. The tests showed that information in the protocols was too difficult to understand or incomplete, or was not provided at all [6,7]. The tests also brought up over 160 practical questions about MRSA, to which the established infection control protocols and national guidelines did not provide answers.

We have developed a target group-oriented, user-friendly web-based portal. and practical questions and answers about MRSA. The national guidelines of the German Robert Koch Institute (RKI) and of the Dutch Working Group for Infection Prevention (WIP) provide the basis for the bi-lingual portal [8]. It can be accessed via www.mrsa-net.nl.

Further education and professional development

In order to create sustainable structures, more than 140 training courses have been organised to date for healthcare professionals in the EUREGIO. The medical association, the KVWL, the local doctors' association, and quality circles worked together to provide and carry out a series of professional educational courses for GPs and regional pharmacotherapy consultants. Regular analysis of the antibiotic prescriptions from all doctors in ambulatory care was established on the German side, following the Dutch experience.

Public Awareness

All information about the project is arranged according to target group and can be called up on the MRSA-net homepage. An around the clock "MRSA-net helpdesk" has been established in 2005. On average, more than 200 phone calls are registered per month by the helpdesk (80% of them on the German side). Two thirds of the phone calls come from health professionals seeking information about how to handle MRSA patients, and one third of the calls from patients or their relatives asking for general information about MRSA, its transmission in home settings, and the possible health risks for household contacts.

Leaflets and posters on the subject are available for patients and their relatives in printed or electronic form. The project has also been presented in a number of media reports (for details visit www.mrsa-net.eu). High priority is given to a systematic publicity campaign, because, especially in Germany, people feel they are not adequately informed about the MRSA problem.

EUREGIO MRSA-net *spa*-typing network

The Institute of Hygiene in Münster has developed a sequence-based typing strategy [9-11] that enables the online and real-time comparison of laboratory typing data on a region-wide and cross-border level for the first time. This method, which is based on *spa* typing, is used as a 'common laboratory language', elucidates epidemiologic correlations and helps to construct a molecular surveillance system [12].

Thirty-two hospitals in the EUREGIO (including four Dutch hospitals) agreed to *spa*-type the first MRSA-isolate from every patient. Five others *spa*-type MRSA from blood culture and in case of clusters. Furthermore, *spa*-typing data is collected and exchanged via the common server. On the German side, 20 sentinel GPs for CA- MRSA were encouraged to collect swabs from patients with soft tissue infections and send them to the euregional laboratories to be *spa*-typed. The typing data (i.e. regional distribution of *spa* types and occurrence of new types) have been analysed regionally and on both sides of the border since the project began. Table 2 shows the most prevalent *spa* types found in the EUREGIO.

On the one hand, it has been shown that certain *spa* types occur on both sides of the border. A comparison with data from the international typing initiative SeqNet.org (http://www.seqnet.org) has demonstrated that these types can also be found in other European countries and that they belong to 'epidemic' clonal lineages (e.g. t032, t003, t001). These types are also found all over the EUREGIO.

On the other hand, however, significant differences in the molecular epidemiology of MRSA have been found on both sides of the border (e.g. t026). These differences are illustrated in an online geographic analysis tool (Figure 3).

An early warning system based on a Z-Score analysis of current and historical *spa*-typing data [13] was designed to identify an unusual accumulation of specific MRSA *spa* types, which are considered to be particularly epidemic or virulent (Figure 4).

Conclusion

Because of the success of the EUREGIO MRSA-net in establishing regional and local measures against MRSA (see Box), the Robert Koch Institute considers this project as a prototype for regional networks dealing with infectious disease issues [4,5]. The differences in the prevalence of MRSA between Germany and

Table 2

MRSA prevalence and *spa* types in the EUREGIO Twente/Münsterland* between July 2005 and June 2006

	EUREGIO MRSA-net Dutch part	EUREGIO MRSA-net German part
Number of MRSA	54	1,034
Predominant *spa* types	t002, t012, t019, t026, t044, t065 (accounting for 87% of all MRSA)	t003, t032, t004, t011, t008 (accounting for 78% of all MRSA)
No. of PVL-positive MRSA (% of all MRSA), (associated *spa* types)	5 (8.3%), (t044, t016)	5 (0.6%), (t044, t019, t437)

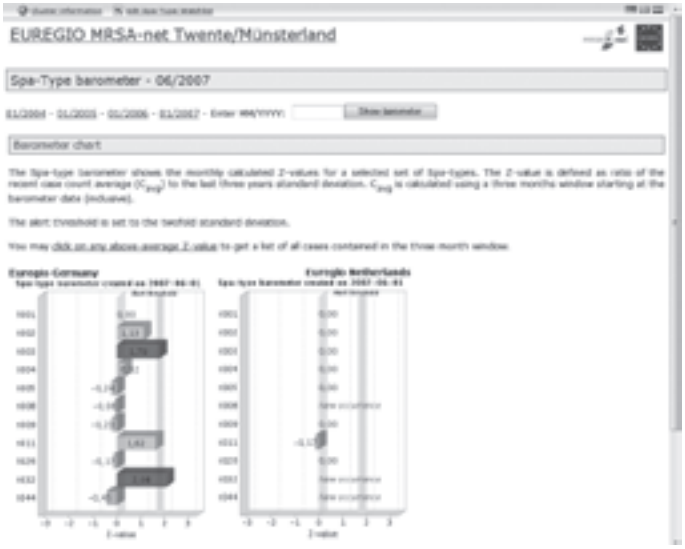
MRSA: methicillin-resistant *Staphylococcus aureus*; PVL: Panton-Valentine leukocidin.
* isolates obtained in 33 regional German acute care hospitals and four regional Dutch acute care hospitals

Figure 3

Online geographic illustration database showing the incidence rates (per 100,000 inhabitants) of MRSA *spa* types (here t032) isolated from patients in the hospitals of the EUREGIO MRSA-net Twente/Münsterland

Figure 4

***spa* type-based barometer for the identification of newly imported or emerging *spa* types as surrogate markers for highly epidemic or virulent clones in the EUREGIO**



Box

Milestones achieved of the EUREGIO MRSA-net Twente/Münsterland

1. Creation a cross-border MRSA network of all institutions involved in healthcare in the Münsterland/Twente region;
2. Comparison of national guidelines and creation of workable and user-friendly MRSA infection control protocols;
3. Further education and professional development of healthcare staff;
4. Enhancement of public awareness towards MRSA and prevention of infectious diseases in general by (regional) media reports;
5. Establishment of a *spa*-typing network for comparable molecular surveillance of MRSA and CA-MRSA in the EUREGIO;
6. Close co-operation with public health offices (Öffentlicher Gesundheitsdienst in Germany and Geneeskundige en Gezondheidsdienst in The Netherlands);
7. Creation of a quality euregional health net (with quality seal) and the creation of structures necessary to achieve a long-term decrease in the MRSA rate in the EUREGIO.

the Netherlands are considerable and have led to problems in cross-border healthcare activities and treatment of patients in the German-Dutch border region. However, the exchange of know-how and experience in MRSA management will improve the quality of patient treatment on both sides of the border and can provide an advantage for the people living in the border regions.

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Two severe cases of botulism associated with industrially produced chicken enchiladas, France, August 2008

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Two severe familial cases of botulism were reported to the health authorities in Brittany, north-west France, on 11 August 2008. An investigation was undertaken to identify additional cases, the vehicle of transmission, and to put in place adapted control measures.

Methods

Following notification of the cases, health authorities issued a communication to French hospitals, anti-poison and toxin centres and general practitioners to alert health professionals. No specific case definition was used for the investigation; the health professionals were requested to immediately report all clinical suspicions of botulism to the local health authorities using the routine mandatory notification system for the disease.

Serum samples from the cases and samples recovered from the food investigation were analysed by the National reference laboratory (NRL) for anaerobic bacteria and botulism at the Pasteur Institute, Paris. The presence of botulinum toxin was confirmed by intraperiteonal administration of patient serum to mice, and the toxin type was ascertained by the specific neutralisation technique.

The food history of the cases in the three to four days before onset of symptoms was documented.

Results

The two cases, a mother (in her 60s) and daughter (in her 20s), presented with gastrointestinal symptoms accompanied by dysphagia, blurred vision and facial paralysis on 9 August 2008. Both patients were hospitalised the day of symptom onset with a rapid evolution towards generalised and complete paralysis. The two women required intubation and mechanical ventilation. They remain in this condition in intensive care as of 3 September, with minor early signs of improvement. A trivalent antitoxin (toxin types A, B, E) was administered to the patients on 13 August. This antitoxin was imported from a commercial laboratory in Germany as botulism antitoxins are not commercially available in France. An authorisation for temporary usage of the product was issued by the French Health Products Safety Agency (Afssapsf).

The diagnosis of botulism (toxin type A) was confirmed for both cases by the NRL, by detection of botulinum toxin in blood samples of the patients. No other botulism cases associated with this episode were identified

The investigation of the food history for both women revealed that they had consumed an industrially produced pre-cooked Mexican-style "Tex-Mex" dish, chicken enchiladas, the day before onset of symptoms. These chicken enchiladas are sold as a pre-prepared kit consisting of several sachets containing a cheddar cheese sauce, a pre-cooked chicken and vegetable mix and two wheat tortillas. The product is consumed after reheating in a microwave oven. Microbiologic testing of the remaining chicken and vegetable mix revealed the presence of *Clostridium botulinum* and a high level botulinum toxin type A contamination ($2.8x10^5$ mouse lethal doses/g). The remaining cheese sauce was negative for botulinum toxin.

The epidemiological investigation of the two cases suggested that the contaminated enchiladas had been mistakenly stored at room temperature for two weeks between purchase and consumption, contrary to the producer's recommendation of refrigerated storage. They were consumed one day after the use-by date. However, the recommended storage conditions on the packaging are not easily visible to the consumer.

Risk analysis

The chicken enchiladas had been produced in France. The incriminated batch of enchiladas had a 'use-by' date of 7 August 2008. This batch was distributed only in France. Other batches of the enchiladas as well as pre-cooked chicken fajitas are also distributed in Belgium, Switzerland and Spain.

Stored production samples from the contaminated batch of enchiladas as well as other batches of enchiladas and fajitas and other products produced by the company around the same time were analysed and tested negative for botulinum toxin and *C. botulinum*.

A risk-analysis carried out on 14 August at the production plant concluded that the plant conforms to hygiene and safety regulations. An investigation of the fabrication protocols showed that the fabrication process includes a pasteurisation step of heating the product to 85°C for two hours.

Public health measures

The company issued a recall of the implicated batch of enchiladas on August 12. As a precautionary measure, this recall was then widened to include all enchiladas and fajitas produced by the firm. The population was informed of this outbreak through national

inter-ministerial press releases and posters placed in supermarket chains. European countries were informed via the 'Early Warning and Response System' and an alert in the 'Rapid Alert System for Food and Feed'.

In light of the potential role of incorrect product conservation in facilitating the multiplication of *C. botulinum* and toxin production in the contaminated enchiladas, a generalised reminder about respecting the storage conditions of such products was communicated by the French authorities. The producer of the enchiladas agreed to change the packaging of this and similar products to make the recommended storage conditions more visible for the consumer.

Discussion and conclusion

The two cases represent the clinically most severe cases of botulism reported in France in recent years. Botulism has been mandatorily notifiable in France since 1986, and 96 cases were reported between 2003 and 2006 [1]. Only two cases of botulism due to toxin type A, associated with the more severe form of the disease, were notified during this period, compared to 51 cases of toxin type B (53%) and four of toxin type E (4%) [1,2]. One-third of the cases notified during this period were not confirmed [1].

The epidemiological and environmental investigations support the hypothesis that the two cases ingested the toxin following incorrect storage of the chicken enchiladas which contained a strain of *C. botulinum* after production. Prolonged storage at room temperature could explain the unusually high level of toxin in the chicken and vegetable mix.

Intoxications with *C. botulinum* producing toxin type A are often associated with vegetable-based products that at some point contained soil with *C. botulinum* spores [2,3].

The thermo-resistance of *C. botulinum* spores varies by strain and according to factors such as the lipid and protein content of the food matrix [2]. Exposure to a temperature of 110-120°C for between 0.4 to 6 minutes is necessary to inactivate 90% of a population of *C. botulinum* A spores [2]. It is thus probable that the pasteurisation step during the enchiladas' fabrication process does not prevent the survival of spores present in primary ingredients or potentially introduced during the fabrication process. Thus, correct refrigerated storage of such processed food products is essential to avoid germination of the spores and toxin production.

Certain ingredients used in the production of "Tex-Mex" food products, including industrially produced cheddar-cheese sauce and home-canned jalapeno peppers, have previously been implicated in outbreaks of botulism in the United States [4,5].

This family cluster highlights the potential public health threat of *C. botulinum* spores in incorrectly stored processed food products and underlines the importance of clear labelling of storage conditions for products purchased in the refrigerated sections of supermarkets. In addition, the episode, widely reported in the national media, has served to remind the general population in France that compliance with food storage recommendations is a prerequisite for food safety.

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Nationwide outbreak of *Salmonella enterica* serotype Kedougou infection in infants linked to infant formula milk, Spain, 2008

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On 5 August 2008, the National Reference Laboratory of Salmonella (NRLS) noted an increase in the number of isolates of *Salmonella enterica* serotype Kedougou. As of 22 August, 29 isolates have been reported during 2008, which is ten times more than the average number of isolates identified by the NRLS during 2002-2007. All isolates have a typical, indistinguishable Pulse Field pattern (SALKEDXB-1, Spanish code) and are fully sensitive to the standard suite of antimicrobials.

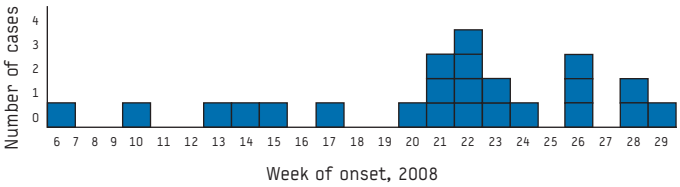
Of the 29 patients with *S.* Kedougou, 12 were male. Twenty three patients were younger than one year while the remaining six were aged between seven and 76 years. From the available information we know that one of the adult patients is the father of one infant infected with *S.* Kedougou.

In the context of this outbreak we defined a case as an infant younger than one year old with clinical symptoms compatible with a *salmonella* infection and an isolate of *S.* Kedougou from stools, blood or urine, since 1 January 2008. As of 22 August, 23 cases fulfilling the case-definition were identified with the onset of symptoms between 4 February and 28 July 2008 (Figure).

To date, 19 of these cases have been investigated. The children live in seven different regions throughout Spain. The parents of all 19 infants reported feeding them with powdered formula milk of the same brand in the week before onset of symptoms. The main symptoms were diarrhoea (100%), fever (32%), nausea (21%) and vomiting (21%). Six cases were hospitalised.

Figure

Cases of *Salmonella* Kedougou infection in infants by week of onset of symptoms, Spain, 2008 (n=23)



A matched case control study was carried out by the Surveillance National Network, and included 10 cases and 36 controls. The study showed that illness was significantly associated with the consumption of a particular brand of formula milk for infants (chi-square=26.03; df=1; P<0,0001).

These preliminary results strongly suggest that the infant formula milk was the source of the outbreak. On 26 August, based on the preliminary results of the epidemiological investigation, and as a precautionary measure, the Spanish food safety authorities recalled five batches of formula milk produced under the incriminated brand. These batches had only been distributed in Spain.

An urgent inquiry was posted trough the European Centre for Disease Prevention and Control (ECDC) to the European Network of Food and Waterborne Diseases (former ENTER-net) on 7 August. From the responses received until 22 August it seems that no country had detected an increase in *S.* Kedougou isolates. Although the infant formula milk has only been distributed in Spain, an alert to the Rapid Alert System for Food and Feed (RASFF) (number 2008.1034) was sent on 27 August by the Spanish Food Safety Agency.

S. Kedougou is one of approximately 2000 *Salmonella* serotypes that can cause illness in humans but it is rarely reported in Spain. On average, three isolates per year were identified by the NRLS between 2002 and 2007. We have found only two outbreaks of *S.* Kedougou described in literature, one associated with salami [1], the other with jam and turkey meat [2]. We are not aware of any outbreak of *S.* Kedougou caused by the consumption of infant formula milk. However, other serotypes of *Salmonella* have been associated with outbreaks linked to infant formula milk [3-12], one of them in our country [3,4].

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A multi-country outbreak of *Salmonella* Agona, February - August 2008

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An outbreak of gastroenteritis affecting residents in the United Kingdom, Ireland and Finland is currently being investigated. As of Wednesday 13 August, a total of 119 cases have been identified. An investigation that includes interviews of persons with *Salmonella* Agona infections, comparison of pulsed field gel electrophoresis (PFGE) profiles of *S.* Agona isolates from cases and also food samples from an Irish food production company and retail outlet chain supplied by the company, suggests that food products from that company may be related to some of these cases. A number of food products including beef steak strips, chicken in various forms, bacon in various forms, and pork have been withdrawn (see: http://www.fsai.ie/ for details). The investigation is ongoing.

Background

On 15 July, the Irish National Salmonella Reference Laboratory reported to the Health Protection Surveillance Centre six isolates of *S.* Agona received over the previous three weeks. This was an unusual finding as there were a total of three isolates in 2007, five in 2006 and 10 in 2005. The temporal association of six isolates of an uncommon serotype suggested a possible link between cases. Early descriptive data showed that the patients affected were mainly young adult males between 20 and 45 years of age. No link between cases was immediately apparent and a food-borne source was considered most likely. An outbreak was declared on 16 July. Colleagues in United Kingdom Surveillance Centres were notified on 16 July. Colleagues in England, Scotland and Wales informed about an increase in reports of *S.* Agona during the end of June and early July. Alerts were posted through the Food- and Waterborne Diseases (former ENTER-net) network and the European Union (EU) Early Warning and Response System (EWRS) on 23 July. Subsequently, single cases were reported in Northern Ireland and Finland.

The following case definition was used:

Table 1

Case definition for *Salmonella* Agona

Case	Definition
Confirmed	*S.* Agona with PFGE* profile designated as SAGOXB.0066
Probable	*S.* Agona phage type (PT) 39
Possible	*S.* Agona where PT unknown or PFGE profile unknown

* pulsed field gel electrophoresis

To date, 119 cases have met the case definition. Of these, 110 cases are confirmed, seven (six in England, one in Wales) are probable and two (in Scotland) are possible awaiting definitive analysis. The most recent date of onset reported is 29 July 2008 (Figure 1). Cases range in age from three months to 79 years with a median age of 27 years. The three-month-old infant is a secondary case. Most cases (56%) are in males, the ratio is 67 male versus 52 female cases (Figure 2).

To date, 14 cases are known to have been or are currently hospitalised.

There has been one death associated with the outbreak. An elderly female patient in the United Kingdom aged 77 years contracted *S.* Agona and subsequently died. The cause of death is reported as ischaemic colitis secondary to *salmonella* infection.

Investigations to date

The epidemiological descriptive study has demonstrated that at least 10 cases had eaten sandwiches containing one of the products from the company in question. A case control study is underway to test the hypotheses that cases are more likely to have eaten at outlets supplied by the company in question, and foods supplied by it. The study is complex due to the multitude of products and outlets involved in this investigation. Microbiological investigations

Table 2

Number of confirmed, probable, and possible *Salmonella* Agona cases, by country and month of onset*, 1 February - 13 August 2008 at 16:00 (n=119)

Country	Feb	Mar	Apr	May	Jun	Jul	Aug	Total
England	2	0	7	14	19	27	0	69
Finland	0	0	0	1	0	0	0	1
Ireland	0	0	0	0	2	8	0	10
N. Ireland	0	0	0	1	0	0	0	1
Scotland	0	0	0	4	13	13	0	30
Wales	0	0	0	3	3	2	0	8
Total	2	0	7	23	37	50	0	**119**

* Date of onset is unknown for n=29 cases; where the date of onset is unknown, the specimen date or a calculated date (lab receipt date - mean diff of lab receipt - onset) is used.

demonstrate *S.* Agona isolates with the identical PFGE profile SAGOXB.0066 in isolates from cases, and food samples in the factory and outlets supplied by the factory.

As it may take several weeks from onset of illness to the results of detailed molecular analysis it is expected that more cases fitting the case definition will be diagnosed.

Control measures to date

Working closely with the Food Safety Authority of Ireland, the Department of Agriculture Fisheries and Food, the company has ceased production from the implicated part of the plant that is a focus of concern. In addition, the company has instituted a product withdrawal of some product lines from an implicated production line/thermal zone in the plant. The withdrawal has focused on products intended primarily for consumption in the made-to-order sandwich trade.

The withdrawal also includes selected batches of cooked beef, cooked chicken and cooked bacon products processed on the same line for the made-to-order sandwich trade. The company has an extensive product distribution list with produce from the plant distributed through UK, Republic of Ireland and many European countries. A confirmed case in Finland has eaten beef strips in Finland from a branch of the retail outlet chain implicated in Ireland and the UK.

Certain other cooked meat products from this production line/thermal zone have not been withdrawn at this point on the basis that they are intended for further cooking before consumption. This position remains under review.

A Rapid Alert System for Food and Feed (RASFF) was released on 4 August and updated on 8 and 11 August by the Food Safety Authority of Ireland (see: http://www.fsai.ie).

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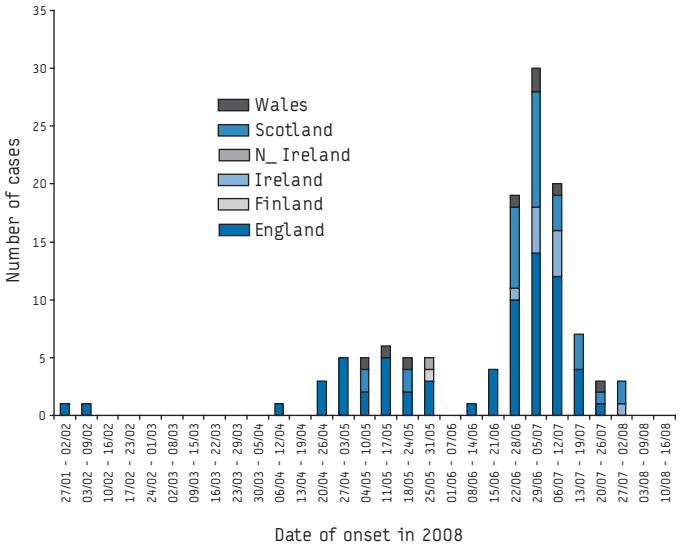
Figure 1

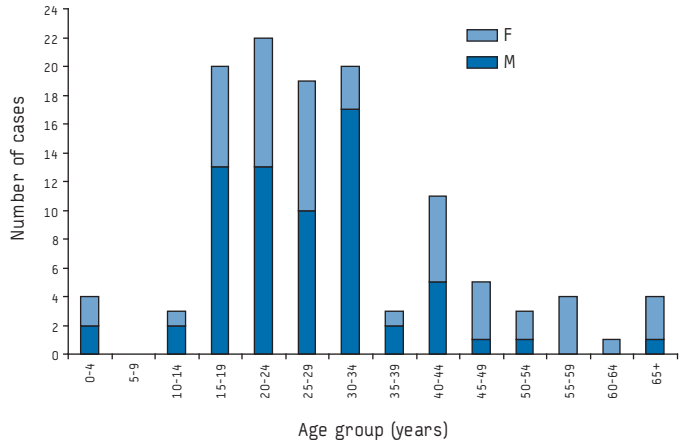
Reported number of confirmed, probable, and possible *Salmonella* Agona cases by date of onset* and country, 1 February - 13 August 2008 at 16:00 (n= 119)



* Date of onset unknown for n=29 cases
Where the date of onset is unknown, the specimen date or a calculated date (Lab receipt date - mean diff of lab receipt - onset) is used.

Figure 2

Reported number of confirmed, probable, and possible *Salmonella* Agona cases by age and gender, 1 February - 13 August 2008 at 16:00 (n=119)



Large ongoing outbreak of infection with *Salmonella* Typhimurium U292 in Denmark, February-July 2008

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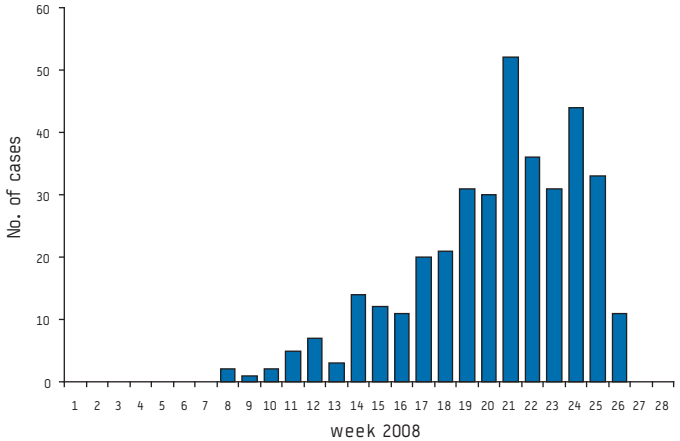
Denmark is currently experiencing an unusually large outbreak of gastrointestinal illness caused by *Salmonella* Typhimurium, phage type U292. The outbreak was discovered in early April by molecular typing (MLVA typing) of S. Typhimurium isolates at the Statens Serum Institut (SSI); the first patients reported onset on illness in February, but the number of reported cases has been particularly high in May and June (Figure 1). There are currently (as of 7 July) 366 confirmed cases, effectively making this the largest outbreak of *salmonella* infections in Denmark since 1993 [1].

Based on two urgent inquiries through the European Centre for Disease Prevention and Control's Food- and Waterborne Disease network (on 17 April and again on 18 June), the outbreak appears to be fully confined to Denmark; no cases have been reported from other countries including neighbouring Scandinavian countries or Germany. The outbreak affects all parts of Denmark, although the incidence varies in different parts of the country. The gender distribution is even (49.7% males), but the age distribution is skewed towards young age groups (Figure 2) with roughly 50% of cases being younger than 15 years compared to roughly 30% in the group of S. Typhimurium patients reported in previous years.

Figure 1

Number of registered *Salmonella* Typhimurium cases of the outbreak MLVA type by week of submission of stool sample to the laboratory, Denmark 2008, n=366

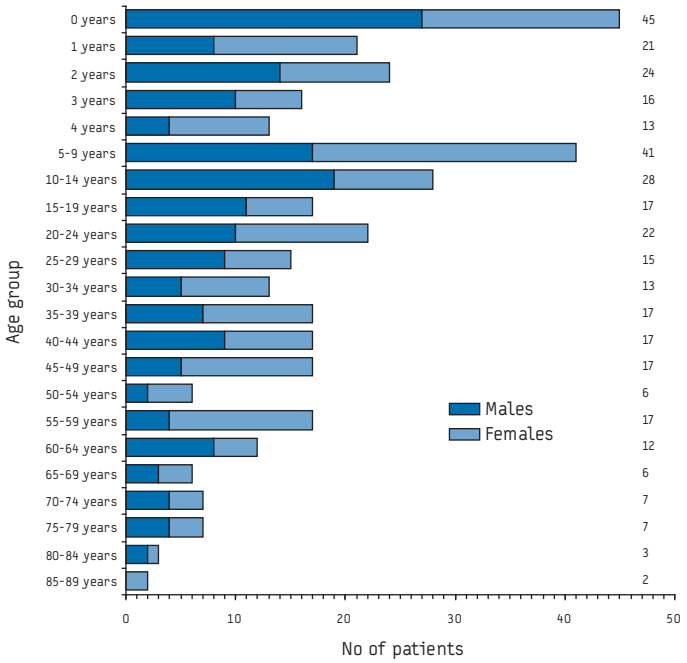


There are several instances in which two cases belong to the same family, but otherwise no embedded outbreaks.

The source of the outbreak has so far not been found and the outbreak appears to be ongoing. This outbreak has led to an extensive investigation using different methods among which are patient interviews (including focus group interviews and home visits), two case-control investigations, comparative analyses of patients' shopping lists obtained from supermarket computers, geographical and trace-back analyses, subtyping of isolates obtained in the surveillance programmes of food, animals and slaughterhouses in Denmark, microbiological analyses of food collected from patients' homes and of selected food production facilities. The results of these investigations indicate that the outbreak may be caused by several types of food vehicles. The main working hypothesis is that

Figure 2

Age and gender distribution of registered *Salmonella* Typhimurium cases of the outbreak MLVA type, Denmark 2008, n=366



the outbreak originates from pigs, but other ideas are also under investigation. Should anyone have information that may be of value to the investigation team please contact the authors.

The phage type U292 is very rarely detected in Denmark and other countries. The phage type pattern is: phage 11: +++, phage 14: +++/SOL, phage 26: +/++, phage 35: +/- and with negative reaction in all other routine phages. The MLVA pattern is (in base pairs): 162-246-341-369-524, in the order: STTR9-STTR5-STTR6-STTR10-STTR3. The outbreak strain is fully susceptible to all antibiotics in the Enter-net panel.

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Extensively drug-resistant tuberculosis: first report of a case in Ireland

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Extensively drug-resistant tuberculosis (XDR-TB) is an emerging global threat. Between 2002 and 2007 sixteen countries in the European Union (EU) reported at least one case of XDR-TB [1]. Infection is characterised by alarming mortality rates in both HIV and non-HIV populations.

We report the first case of XDR-TB in Ireland and describe a successful outcome after 20 months of treatment. We also discuss the implications for public health in our country as well as the international community.

Case report

In January 2005, a 25-year-old Lithuanian female was admitted from the emergency department with a three-month history of productive cough. There was no associated haemoptysis, weight loss or night sweats. The patient denied previous treatment with anti-tuberculous drugs or exposure to patients with active TB.

There was no significant medical history. The patient was an ex-smoker and drank occasionally. She had been living in Ireland for two and a half years.

The patient was afebrile with oxygen saturations of 99% on room air. Respiratory examination was normal. The chest radiograph on admission showed bilateral pulmonary infiltrates and a cavity in the right mid-zone (Figure 1).

Auramine staining of sputum demonstrated acid-fast bacilli. Mycobacterium tuberculosis was cultured from sputum. Screening for HIV and hepatitis B/C was negative. Isoniazid 250 mg od, rifampicin 600 mg od, pyrazinamide 1500 mg od and ethambutol 400 mg bd were commenced for presumed pan-sensitive tuberculosis.

In February 2005 preliminary drug susceptibility tests (DST) showed a profile of resistance consistent with MDR-TB. The

Figure 1

Chest X-ray of a patient diagnosed with XDR-TB in Ireland (on admission)

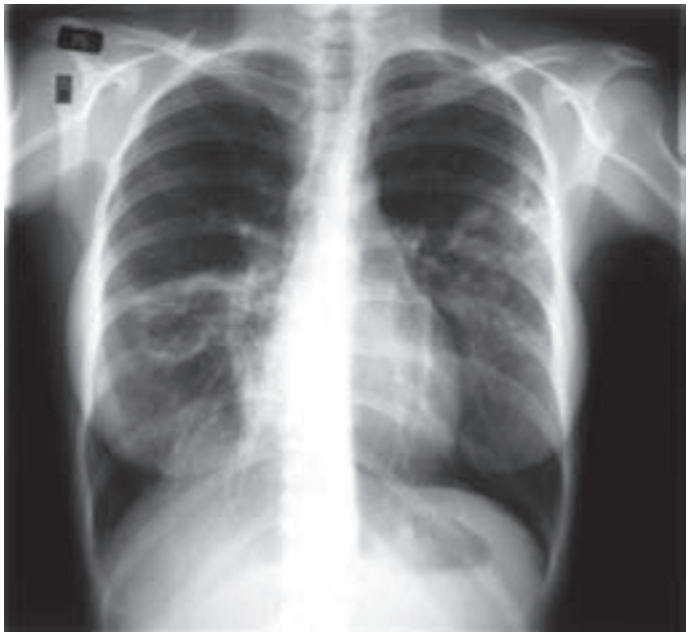
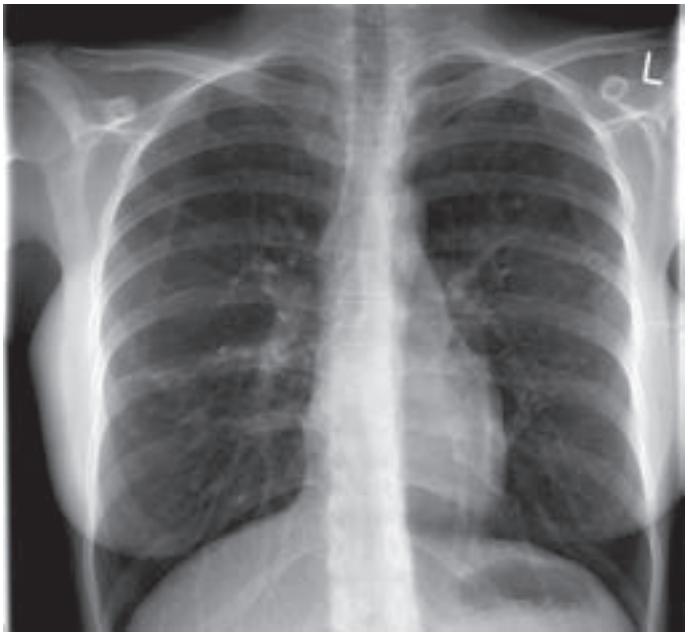


Figure 2

Chest X-ray of a patient diagnosed with XDR-TB in Ireland (after treatment)



drug regimen for drug-sensitive TB was stopped. Treatment was recommenced with capreomycin 1g im od, moxifloxacin 400mg od, prothionamide 750 mg bd, cycloserine 250 mg bd and P-aminosalicyclic acid (PAS) 4g tds. The patient was placed under directly observed therapy (DOT)

After four months PAS was stopped when the final report of DST demonstrated resistance. The final drug resistance profile is shown in Table 1; this profile is consistent with XDR-TB. No drug susceptibility test was available for Moxifloxacin. No further drugs were added to the regimen as the patient was responding clinically and radiologically.

Sputum culture was negative for TB after 96 days; capreomycin was consequently reduced to thrice weekly administration. Treatment for XDR-TB continued until October 2006 for a total of 20 months. During this time there was no clinical or radiological evidence of disease recrudescence. The final chest X-ray showed bilateral fibrocalcific changes only (Figure 2).

Adverse events were noted. A mild, transient transaminitis (AST 33 □ 82) occurred in the first week. Nausea persisted until the fourth month and required anti-emetics. Bilateral tinnitus developed after eight months; capreomycin was discontinued at this point and an audiogram showed high frequency hearing loss in the right ear (5 db below normal). The final 12 months of the total 20 months of treatment passed without complication.

Once treatment was discontinued the patient failed to attend for follow-up. She did not re-present to our hospital. She is still living in Ireland. Since her treatment was stopped 21 months ago, Ireland has not reported any further cases of XDR-TB.

Discussion

The treatment of patients with multi-drug resistant (MDR) tuberculosis (i.e. resistance to both isoniazid and rifampicin) is a daunting medical challenge. Isoniazid and rifampicin are the most potent anti-tuberculous agents but by definition are ineffective in MDR-TB. Second line agents replace them but these drugs are less efficacious, more toxic and more costly.

XDR-TB is defined as resistance to isoniazid, rifampicin, any flouroquinolone and any one of amikacin, capreomycin or kanamycin [2]. In these circumstances therapeutic options are further restricted because of resistance to both first and second line agents. Consequently the XDR-TB treatment regimen often consists of older drugs (i.e. serine analogues, thioamides) that are predominantly bacteriostatic rather than bactericidal.

The poor efficacy of XDR-TB chemotherapy is reflected in the alarming mortality rates. In Western European countries mortality among non-HIV patients has been reported as 36% [3]. Infection in immunocompromised patients is even more devastating; an outbreak of XDR-TB in a HIV positive population killed 52 out of 53 infected patients [4].

Although XDR-TB carries a high mortality rate, our patient had a successful outcome. In this case the patient was treated with an antimycobacterial cocktail of capreomycin, moxifloxacin, prothionamide and cycloserine. Current World Health Organisation (WHO) guidelines recommend treatment of MDR-TB with at least four drugs whose efficacy against the isolate is certain or almost certain [5]. Formulating an appropriate regimen is a crucial component of treating MDR-TB (and XDR-TB). If DST shows susceptibility, pyrazinamide and ethambutol should be included. An injectable agent should also be added i.e. amikacin, kanamycin or capreomycin; if tolerated these agents should be continued for a minimum of six months. Next a flouroquinolone should be considered e.g. moxifloxacin or levofloxacin. Finally oral second line agents (i.e. PAS, cycloserine or prothionamide) should be added until the drug cocktail consists of four to six drugs to which the isolate is susceptible. Once the regimen has been commenced, patients should be placed on DOT. Treatment should continue for at least 18 months [5].

Drug resistance in TB arises from ineffective TB control programmes. Patient non-compliance, poor quality drugs or incorrect prescribing engender resistant strains. Furthermore resistance cannot be detected if resources for TB culture and drug susceptibility tests are lacking. Thus in the absence of appropriate resources and infrastructure there is improper identification and treatment of resistant cases which ultimately leads to uncontrollable disease.

Although XDR-TB has been recognised since 2000 [6], epidemiological data describing its distribution worldwide only became available in February 2008 [1]. South Africa has so far reported the greatest absolute number of XDR-TB cases worldwide [1]. The vast majority of XDR-TB cases reported in the EU have occurred in Estonia, Latvia and Lithuania [1].

However assumptions that XDR-TB is limited to resource-limited countries are incorrect; all G8 countries have now reported at least one case [1]. The emergence of XDR-TB in industrialised nations may be linked to issues of immigration; 76% of United States cases reported from 2000-2006 occurred in foreign-born persons [7]; 63% of XDR-TB cases in Germany and Italy have occurred in non-nationals [3].

Table 1

Results of final drug susceptibility tests, XDR-TB case, Ireland 2005-2006

Drug	Resistant (R) / Sensitive (S)
Isoniazid	R
Rifampicin	R
Ethambutol	R
Pyrazinamide	R
Streptomycin	R
Amikacin	R (highly resistant)
Prothionamide	S
PAS	R (highly resistant)
Cycloserine	S
Capreomycin	S
Ciprofloxacin	R
Rifabutin	R
Clarithromycin	R

In October 2006 the WHO Global Task Force on XDR-TB announced its response to XDR-TB [2]; infection control measures and surveillance systems required strengthening; laboratory facilities must be augmented to improve access to drug susceptibility tests; low-priced, high-quality second-line drugs must be more readily available. A US$ 2.15 billion plan to implement these recommendations was launched by the WHO and Stop TB partnership in June 2007 [8].

This is the first report of a case of XDR-TB in Ireland. Further cases are likely as immigration from European countries with a high burden of MDR-TB continues. How can the threat be averted? Suspected cases of tuberculosis should be referred for chest X-ray as well as sputum staining and culture. Patients should be isolated until infectivity is excluded. If Mycobacterium tuberculosis is cultured, drug susceptibility testing should be performed to detect resistance; MDR-TB or XDR-TB is more likely in patients previously treated for TB or in immigrants from countries with a high burden of MDR-TB. If MDR-TB or XDR-TB is diagnosed, treatment in a specialist centre is advised. Public health authorities should be notified to identify contacts and offer chemoprophylaxis if appropriate.

These measures must be followed carefully; this will ensure that the devastation wrought by tuberculosis in 20th century Europe is not repeated in the modern day.

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Outbreak of measles in two private religious schools in Bourgogne and Nord-Pas-de-Calais regions of France, May-July 2008 (preliminary results)

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To date, 110 cases of measles have been identified by local health authorities in the Bourgogne and Nord-Pas-de-Calais regions of France, with onset of symptoms between 3 May and 19 July.

The first three cases were reported on 25 June by a general practitioner to the French national institute of health (Institut de Veille Sanitaire, InVS) regional office in Bourgogne, in the centre of France. The three unvaccinated cases were students at a private religious school for girls located in Bourgogne and had onset of rash between mid-May and 23 June. On the same day, another general practitioner in Nord-Pas-de-Calais reported a case of measles in an unvaccinated 14 year-old boy attending a private religious boarding school for boys. The boy had developed a rash on 19 June and happened to live in the same place in Bourgogne where the above-mentioned girls' school was located.

The two schools have elementary to secondary students. Both are private religious boarding schools. Most of the students come from the surrounding areas, some resident pupils are from other French regions, and some from abroad.

All students returned home on 26 June for summer holidays.

An epidemiological investigation was initiated in both regions by the local health authorities. In Bourgogne, the index patient was found to be a Swiss pupil vaccinated against measles with a single dose. She developed a fever on 28 April and a rash on 3 May. During mid-April she had spent a few days visiting Switzerland and Austria, and had been in contact with a cousin who had measles at the time of her visit. Her cousin is living in Feldkirch, Austria, where a measles outbreak was ongoing at the time.

Regarding the first case diagnosed in Nord-Pas-de-Calais, the investigation showed that three of the boy's sisters attended the school in Bourgogne and had been diagnosed with measles, with rash onset on 6, 7 and 15 June respectively*. The four siblings were not vaccinated against measles.

In order to identify possible further cases in the two places, the students' parents were asked by phone or through a questionnaire sent by mail to provide information regarding history of measles and vaccination status of their whole family. In addition, general practitioners and laboratories were asked to report possible cases of measles to the health authorities and to perform laboratory diagnostic tests to confirm the cases.

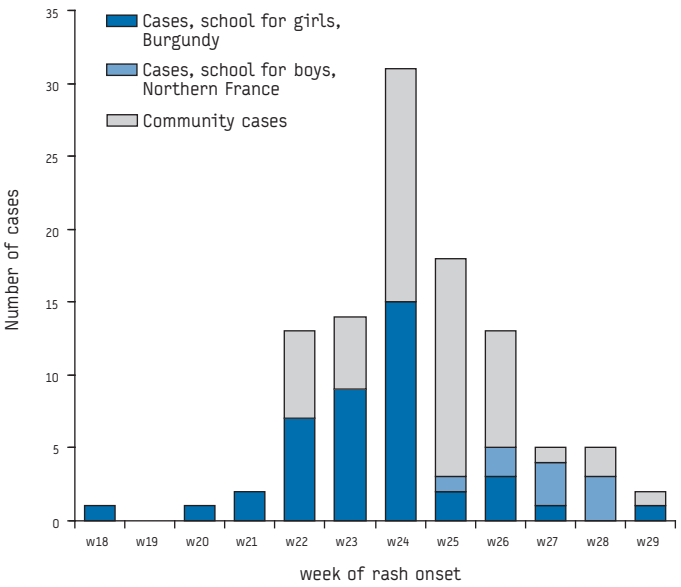
A clinical case of measles was characterised by fever and a generalised maculo-papular rash in association with cough, coryza, conjunctivitis or Koplik spots. Laboratory criteria for the diagnosis of measles were the detection of a significant rise in measles IgG antibody titre, the identification of measles IgM antibodies or the detection of measles virus nucleic acid by PCR.

Outbreak description

The figure shows the epidemiological curve for those 105 of the 110 cases for whom information on the date of onset was available.

Figure

Measles cases for whom the date of rash onset was available (n=105), by week of rash onset, outbreak in two schools, France, May-July 2008



Cases in the two schools (n=53)

Bourgogne

Between 3 May and 16 July, 43 cases were identified among the 147 girls attending the private school in Bourgogne (attack rate=29%). The mean age of the cases was 12 years (range six to18 years). Five cases (12%) were laboratory-confirmed (salivary or serological IgM or PCR).

Thirty-nine cases (91%) were not vaccinated against measles. Measles immunisation coverage among the pupils of the school estimated through the questionnaires returned by their families was 40% for the first dose and 26% for the second dose of vaccine.

All the girls in the school were French except two Swiss girls, including the index case.

Among the 20 adults working with the children, one unvaccinated teacher in her 30s developed a rash on 30 May.

Nord-Pas-de-Calais

Between 19 June and 11 July, nine cases were identified among the 154 students attending the above-mentioned boys' school in Nord-Pas-de-Calais (attack rate=6%). The mean age of the cases was 14 years (range: eight to 17 years) and none of the cases were vaccinated against measles. Four cases were laboratory-confirmed, including one Canadian student. There were no reports of cases with complications or hospitalisation. Measles immunisation coverage among the pupils of the school, estimated from the returned questionnaires, was 65% for the first dose and 44% for the second dose of vaccine.

Twenty students attending that school returned home abroad for the holidays: 10 to Belgium, seven to the United Kingdom (Kent), two to Canada (Québec) and one to Luxembourg. Cases occurred in three British students from the same family (on 2 and 11 July) and one Canadian (on 7 July), once they were back home.

Community cases (n=57)

Of the 57 secondary cases between 26 May and 19 July that were linked to school cases, 52 occurred in siblings of cases (mean age: nine years, range: nine months to 21 years) and one in a parent (in their 30s). Three cases aged two, six and 13 years occurred in two other families who were close friends with measles cases. One adult case in their late 20s was in the general practitioner's waiting room at the same time as one laboratory-confirmed case.

All these cases were French. Two secondary cases were laboratory-confirmed. Two cases, both in their early 20s developed respiratory complications, and one of them was hospitalised. Fifty-five of the 57 cases (96%) were not vaccinated against measles, including two infants aged eight and nine months.

Microbiological investigations

Among the clinical specimens (throat swab, serum) sent to the French national reference centre for measles, measles virus was detected by RT-PCR in the samples obtained from five French cases. The sequences of the N-terminal part of the viral nucleoprotein gene were identical in all cases and belonged to genotype D5.

Control measures

Although the first case report was much delayed and most of the cases were identified retrospectively, the parents and local general practitioners in both areas around the two schools were given information about eviction measures and immunisation of contacts. The health authorities in the United Kingdom and Canada were informed about measles cases on their territories.

Nevertheless, many parents declined immunising their other children due to personal beliefs and did not consult a general practitioner when additional cases occurred in their household. These factors explain why only eight of the 110 cases were notified to the French health authorities through the mandatory notification system, although physicians had been reminded explicitly of the importance of reporting.

Discussion

With more that one hundred cases, this is the first important outbreak of measles that has been investigated in France since the national plan for the elimination of measles and congenital rubella was launched in 2005 [1,2]. The target of the elimination plan is to achieve in all the French *Départements* a minimum vaccination coverage of 95% for the first dose and at least 80% for the second dose at 24 months of age, and at least 90% for the second dose at six years of age. Currently, the average national vaccination coverage at two years of age is estimated to be 87%. In 2006 and 2007, 44 and 40 cases, respectively, were reported through the mandatory notification system, while 108 cases were reported for the first six months of 2008.

This outbreak proved to be linked to outbreaks reported by the public health authorities in other European countries, namely the ones ongoing since November 2006 in Switzerland [3] and since March 2008 in Austria [4], which are also caused by the measles virus D5 genotype. It shows once more how easily and rapidly the virus can spread in susceptible communities. The outbreak has also led to the exportation of three cases to the United Kingdom and one case to Canada. The main difficulty encountered in this outbreak was that the schools were already closed when the investigation started on 26 June, and that most families had already left for the holidays, which explained the - still ongoing - delay in reporting the cases to the health authorities.

The religious community in which the outbreak occurred appeared to have a relatively low vaccination coverage which explains the high attack rate in the school in Bourgogne. This outbreak highlights the presence of population subgroups that are susceptible to measles and represent specific risk groups for measles outbreaks.

* All the dates mentioned in the following are dates of rash onset.

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Measles outbreak in an anthroposophic community in The Hague, The Netherlands, June-July 2008

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An ongoing outbreak of measles linked to anthroposophic communities in The Hague, The Netherlands has been identified since mid-June 2008. Thirty-four cases have been reported until 25 July. In addition, two cases have been reported in other cities (Leiderdorp and Utrecht). Both are epidemiologically linked to the cluster in The Hague.

Introduction

Measles is a statutorily notifiable disease in The Netherlands. The case definition for surveillance purposes includes patients with clinical symptoms in combination with an epidemiological link and /or laboratory confirmation.

The most recent large measles outbreak in The Netherlands took place in 1999-2000. Over 3,200 cases were reported, of whom three children died [1]. The outbreak was predominantly situated in the so called 'bible belt' where many people choose not to immunise their children based on religious conviction.

The Dutch national immunisation programme has included routine measles vaccination since 1976. A two-dose measles-mumps-rubella (MMR) vaccine schedule was introduced in 1987 for children aged 14 months and nine years. In 2007, the national vaccination coverage rates for the first and second dose of MMR were 96% and 93%, respectively (birth cohorts 2005 and 1997) [2]. Corresponding figures for The Hague were 98% and 84%, respectively.

Current outbreak description

On June 17, a general practitioner (GP) reported a suspected case of measles with strong clinical evidence in a previously unvaccinated eight-year-old boy. Urine, throat swab and blood specimens were subsequently submitted for PCR testing. All specimens were found positive for measles virus.

It is yet unknown where this case (the index case) acquired the infection from. There was no relevant travel history. The child attends a school of 210 pupils, of whom many come from the anthroposophic community in which parents opt not to vaccinate their children. From June 18 to July 3, nine further cases from the same school were reported. One was laboratory-confirmed and eight were epidemiologically linked.

On June 26, a seven-year-old child from another school (population: 450 pupils), also with many pupils from the anthroposophic community, was diagnosed with measles and confirmed by PCR. The child is a cousin of one of the cases from the first school. From July 3 to July 22, 15 other cases from the second school were reported. All were epidemiologically linked. The vaccination coverage amongst children at the two schools is unknown. However, we estimated the second dose coverage at the second school to be 65% in 2007 [3].

Initially, the measles outbreak (Figure) seemed confined to the two school clusters. Eight incidental cases outside the two schools were reported, but all were family members of the affected school children. Recently, however, two cases outside the school clusters have been reported in other cities (Leiderdorp and Utrecht). Both are epidemiologically linked to the outbreak in The Hague.

Age and vaccination status

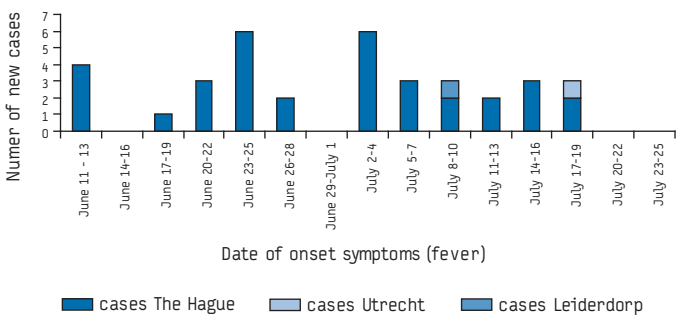
The median age of the affected children in The Hague (n=32) was eight years, (range 4-16 years). Two affected adults (a mother and a father of affected children from different families) were aged 35 and 48 years, respectively. Male to female ratio was 1:1.

Of the 34 cases, 31 were non-immunised children; one child received the vaccine (first dose) during the outbreak and developed measles three days later. This is therefore not considered a vaccination failure.

Regarding the adults, one was vaccinated with a single dose in 1978 and the other has never been vaccinated.

Figure

Number of cases of measles by day of onset of symptoms defined as first day of fever, The Netherlands, June 11 – July 25 (n=36, including n=34 in The Hague, n=2 in other towns)



Microbiological investigation

Clinical specimens (urine, throat swab, serum) were obtained from six cases. The presence of measles virus was detected in all cases by RT-PCR. The sequences of the N-terminal part of the nucleoprotein gene of the viruses were identical for five cases and belonged to genotype D8. In one case, genotyping is pending. In two cases, measles-specific antibodies (IgM) were detected in serum.

Control measures

The municipal health centre of The Hague (GGD) has implemented several outbreak control measures. Since the outbreak was initially limited to the specific anthroposophic population associated with the two schools, measures were aimed at this target group. All parents of children attending the two schools received an information letter. MMR vaccination was offered to all unvaccinated children and to the family members of cases.

However, the school authorities had rightfully predicted that very few would use this opportunity, as most parents in this community had deliberately chosen not to immunise their children. In total, only 10 vaccinations were administered (two to adults, eight to children).

For case-finding, the local GPs and microbiologists were asked to be alert and report possible cases of measles.

In the general population awareness of the importance of vaccination was raised with the help of media releases. An elaborate fact sheet with questions and answers for the public was published on the GGD website [4]. Until July 21 about 500 visits were registered.

Discussion

We report the largest cluster of measles that has occurred in The Netherlands since the large outbreak of measles in 1999/2000. It is yet unclear where the virus involved in the current outbreak originated from. Although genotype D8 has been detected in Europe before [5], recent outbreaks of measles in several European countries have, to our knowledge, not been associated with genotype D8 [6]. The present outbreak is linked to the anthroposophic community. The relatively low vaccination coverage in combination with social clustering, e.g. at schools, makes this community particularly prone to outbreaks of vaccine-preventable diseases. As the vaccination coverage of the general population in The Netherlands is relatively high, the risk of spread of measles outside these communities, whether antroposophical or on religious, is limited when compared to the risk of spread within these communities in outbreak situations [7].

In the last few years the infectious potential of measles seems to be increasing, with outbreaks currently being reported in the United States [8] and several European countries including Italy [9], Spain [6], Switzerland [10] and the United Kingdom [11], some of which were also linked to antroposophical communities [12]. Based on the high overall vaccination coverage and the low incidence of measles, The Netherlands appears to be near to the 2010 WHO Euro measles elimination goal [13,14]. However, there is strong social clustering of people who deliberately (on various principal grounds) choose not to vaccinate. As a result, a large measles outbreak associated with religious or anthroposophic communities can still occur.

Acknowledgements

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Measles resurges in Italy: preliminary data from September 2007 to May 2008

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Following an incidence rate of 1/100,000 inhabitants in 2006 [1], Italy has been facing an upsurge of measles cases since September 2007, with outbreaks being reported in various regions. In Italy, measles vaccination is currently offered free of charge as combined measles-mumps-rubella (MMR) vaccine. The current national vaccination schedule recommends two doses of MMR vaccine, given respectively at 11-12 months and 5-6 years of age. Although childhood vaccination coverage has increased in recent years, reaching the national average of 88% in 2006 (source: Ministry of Health), with some regional variability (Figure 1), it is still below the target of 95% set by the National Measles Elimination Plan (MEP) launched in 2003 [2], and outbreaks continue to occur.

Measles is a statutorily notifiable disease in Italy and in the last six-year period (2001-2006) an average of approximately 5,400 cases were reported annually, with a range from 215 cases (in 2005) to 18,020 cases (in 2002). According to the MEP, sensitivity, specificity, and timeliness of case reporting had to be improved and an enhanced surveillance system was therefore established in April 2007 [3]. According to the new system, physicians have to report suspected cases of measles within 12 hours and laboratory diagnostic testing (never requested previously) of blood, saliva and urine specimens is recommended for all sporadic cases. Outbreaks of suspected measles must be investigated with collection of specimens from at least 5-10 cases for confirmation and characterisation of the viral strain. Case report forms are collected centrally at the Ministry of Health and the National Health Institute (ISS). In order to support case ascertainment, a National Reference Laboratory was also established at the ISS.

This report is a preliminary description of the main epidemiological features of 2,079 cases reported to the enhanced measles surveillance system from September 2007 to May 2008. Cases with negative laboratory results have been excluded from the present analysis.

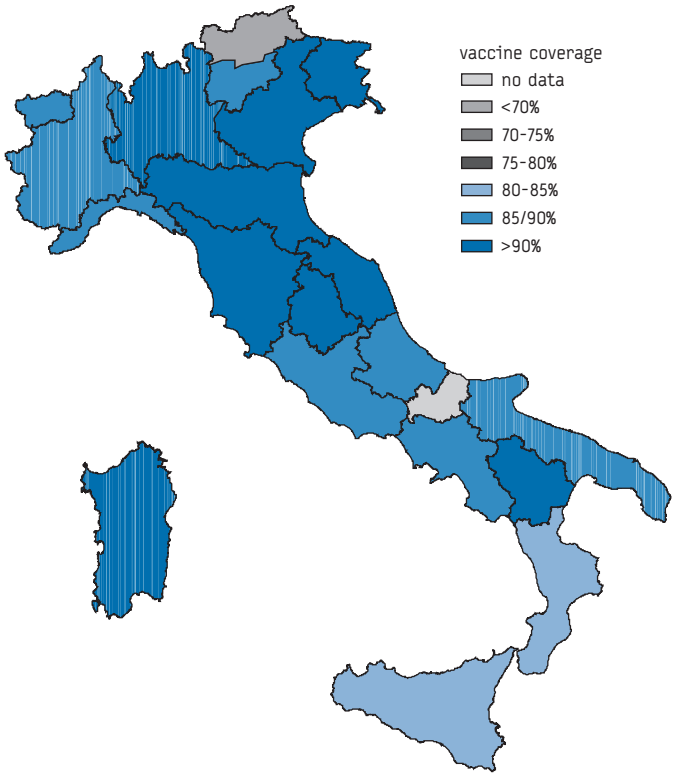
Place and time

Most reported cases occurred in the Piemonte region where a large outbreak began in September 2007, among a group of unvaccinated adolescents. [4].

The first case was a 17-year-old girl who developed symptoms two days after returning to Italy from the United Kingdom. In the following weeks and months the outbreak spread in Piemonte and increased measles activity was also reported in other regions. Since September, clusters of cases and larger outbreaks have been detected in 15 of the 21 Italian regions, with the monthly number of nationally reported cases reaching a peak of 434 cases in February 2008 (Figure 2).

Figure 1

Vaccine coverage for the first MMR dose in children at 24 months of age, by region, Italy, 2006 (source: Ministry of Health)



Over the nine-month period between 1 September 2007 and 30 May 2008, the estimated national cumulative incidence of measles was 3.4 cases per 100,000 inhabitants. Ninety-three percent of the cases were reported from six regions: Piemonte (966 cases – 47 % of the total), Lombardy (452 cases- 22%), Lazio (183 cases – 9% of total), Tuscany (128 cases – 6%), Emilia Romagna (113 cases – 5%) and Veneto (87 cases – 4%). The remaining cases were reported in Sardinia, P.A. Trento, Liguria, Valle D'Aosta, Marche, Abruzzo, Friuli-Venezia Giulia and Puglia [5].

Figure 3 shows the reported measles incidence per 100,000 inhabitants, by region, from September 2007 to May 2008. The highest incidence was reported from Piemonte followed by Lombardy, Tuscany and Lazio with 22.2, 4.7, 3.5 and 3.3 per 100,000 inhabitants respectively.

Transmission occurred in families, schools, hospitals, Roma/ Sinti communities, and groups opposed to vaccination. In several regions cases also occurred among healthcare workers.

Age and vaccination status of cases

The age was reported for 2,008 cases (97%). The median age of cases was 17 years (range: 0-77 years). Almost 60% of cases (1,247) were aged 15-44 years (Figure 4). More specifically, 23% cases were aged 15-19 years, 15% were aged 20–24 years and 21% were aged 25-44 years.

Using national age-specific population figures as denominators, adolescents aged 15-19 years had the highest incidence rate, followed by infants (<1 year of age): 15.8/100,000 and 11.3/100,000 respectively.

Of the 1,932 cases for whom vaccination status was known, 1,772 (91.7%) were unvaccinated against measles at the time of infection, 130 (6.7%) had received only one dose of measles-containing vaccine (MCV), 12 (0.6%) had received two doses, while 18 (1%) were vaccinated but the number of doses was unknown (Figure 4).

Microbiological investigation

Overall, 631 cases (30%) have been laboratory-confirmed. Preliminary molecular sequence analyses have identified genotype D4 in all positive samples tested up to early May 2008, with the exception of one sample from Emilia Romagna (genotype D8, 99% similar to viruses identified in 2007 in UK and in 2008 in Canada).

Hospitalisations and complications

Information on hospitalisations and complications was available for 1,227 cases. Of these, 371 (30%) were hospitalised. One case of encephalitis was reported as well as three cases of thrombocytopenia, 22 cases of pneumonia and 27 of otitis media.

Figure 3

Reported measles incidence per 100,000 inhabitants, by region, Italy, September 2007- May 2008 (n=2079)

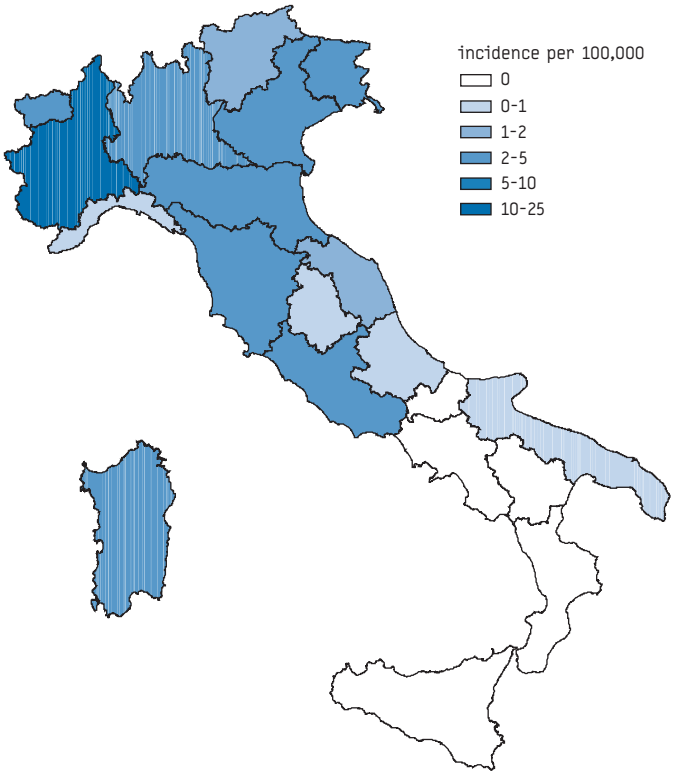


Figure 2

Number of reported measles cases*, by month, Italy, September 2007 - May 2008 (n=2079)

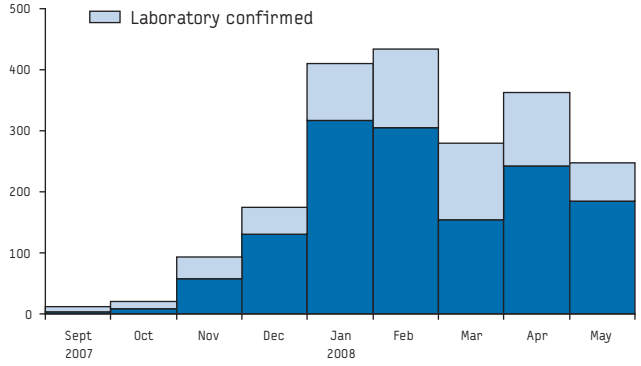
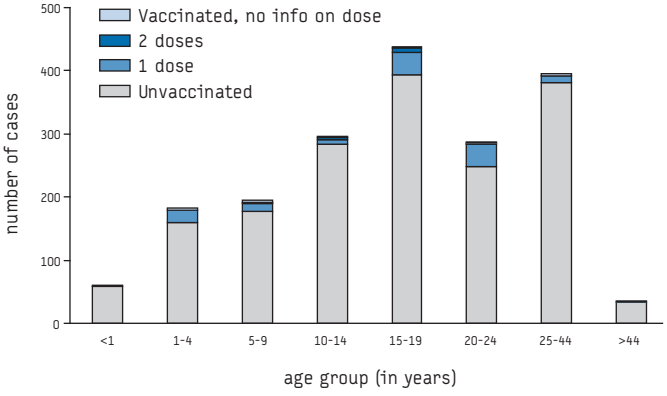


Figure 4

Vaccination status of measles cases, by age-group, Italy, September 2007 - May 2008 (n=2079)



One death due to measles pneumonia occurred in a laboratory-confirmed case in the Piemonte region, in an unvaccinated 10-year old child with a genetic immunodeficiency syndrome.

Public health measures

In each region, outbreak investigation and control measures were initiated by the local health authorities, according to the procedures indicated in the MEP and in the enhanced surveillance circular [2,3]. The type and extent of the public health response measures varied amongst local authorities but generally included basic epidemiologic investigation of suspected cases, identification and vaccination of susceptible contacts and laboratory confirmation of diagnosis. Primary care paediatricians and general practitioners were alerted for prompt reporting of measles cases and further investigation.

Discussion

Although some progress has been made in Italy since the implementation of the MEP, as shown by the increase in routine immunisation coverage (from 84% in 2003 to 88% in 2006 in two-year old children, source: Ministry of Health) and the introduction of a routine second dose, the ongoing outbreaks indicate that much still needs to be done.

Molecular characterisation studies indicate that the first case reported in the Piemonte region was imported from the United Kingdom (genotype D4) [6] showing the importance of international efforts in controlling the current upsurge of the disease in Europe [7-9].

In Italy, adolescents and young adults have been particularly affected and most reported cases were unvaccinated or incompletely vaccinated. Nosocomial transmission occurred in several regions and cases were also reported among healthcare workers. As in 2006, cases have once again been reported among the Roma/Sinti population [10].

In conclusion, there is an urgent need to improve vaccination coverage with two doses of MMR in Italy, not only among children, but also among adolescents and young adults. More efforts should also be made to prevent measles transmission in healthcare settings by implementing effective infection control practices and ensuring that all healthcare workers are immune to measles, and to raise immunisation coverage in hard-to-reach populations. Surveillance and laboratory confirmation have improved but outbreak control should be further strengthened as viral transmission was not effectively interrupted. Finally, vaccination coverage in adolescents and young adults and second dose coverage in children should also be closely monitored. A national vaccination coverage survey is currently being conducted and will provide updated information on coverage in children and adolescents as well as on reasons for non-vaccination.

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A case of Crimean-Congo haemorrhagic fever in Greece, June 2008

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Case description

A 46-year-old woman with disseminated intravascular coagulation (DIC) died in a hospital in Alexandroupoli, in north-eastern Greece, in the end of June 2008. The woman was admitted to the hospital four days earlier, with fever, malaise, myalgia, chills and abdominal pain. One day before death, her condition deteriorated rapidly and she developed heavy hemorrhage from the genital tract, DIC and multi-organ failure.

The patient reported a tick bite four days before admission, and that she had tried to remove the tick herself. No travel abroad was reported. She was engaged in agricultural activities in a rural area near the town of Komotini, in Rhodope prefecture, south of the Greek-Bulgarian border (see Figure).

Figure

Map of Greece showing the area where a case of Crimean-Congo haemorrhagic fever was reported in June 2008

Laboratory investigations

After the patient's death, stored serum sample taken upon admission was sent to the World Health Organization (WHO) Collaborating Centre for Reference and Research on Arboviruses and Haemorrhagic Fever viruses in the First Department of Microbiology, Aristotle University of Thessaloniki, Greece. An RT-nested PCR which amplifies a 240-bp fragment of the S RNA genome segment of Crimean-Congo haemorrhagic fever virus (CCHFV) [1] was applied and resulted positive. A quantitative real time PCR revealed high CCHF viral load, as is usually seen in fatal cases [2]. Sequence analysis of the PCR product showed that the causative CCHFV strain was similar to other strains detected or isolated in the Balkan peninsula (Albania, Bulgaria and Kosovo), Russia and Turkey, which are associated with severe, and sometimes fatal, disease in humans [2].

Control measures

Laboratory diagnosis and confirmation by sequencing was achieved in 24 hours from the time of sample receipt. The case was immediately notified to the European Centre for Disease Prevention and Control (ECDC) and WHO and information on it circulated through the Early Warning and Response System (EWRS) and ProMed. Immediately, the Hellenic Center for Disease Control and Prevention (HCDCP) sent guidelines to all hospitals in northern Greece for management of suspected hemorrhagic fever cases, infection-control measures and handling of clinical specimens. The case definition for suspected cases included patients with a clinical picture compatible with CCHF and a history of tick bite; or contact with tissues or blood from a possibly infected animal; or a health-care worker with a history of contact with a CCHF case occurring within the previous 14 days and within the prefecture of Rhodope.

In addition, residents of Rhodope and the neighbouring prefectures of Drama, Kavala, Xanthi and Evros were informed about measures for tick bite prevention and about the importance to refer as soon as possible to the closest hospital or general practitioner for tick removal. At the same time guidelines were disseminated to health-care workers for proper removal of attached ticks.

To date, no secondary or other cases have been observed in Greece. Extensive surveys have been launched recently by HCDCP to test seroprevalence in humans and to interview residents of the Thrace region (Xanthi, Rhodope and Evros prefectures) about

the history of tick bites and any associated symptoms. Surveys in animals have also started through the Hellenic Ministry of Rural Development and Food, and studies aiming at establishing what species of ticks are circulating in the Thrace region per season and estimating the rate of CCHF infection per species.

Discussion

During March and April 2008, six probable CCHF cases have been reported in a known endemic area in Bulgaria, close to the border with Greece [4]. In addition, many CCHF cases have been reported in Turkey this year, but none of them in the European part of the country [5].

The strain identified in our case was similar to those found in the Balkan peninsula, Russia and Turkey, but differed greatly from the Greek strain AP92, isolated from ticks in 1976, which has been suggested to cause inapparent infections in humans [3]. CCHF is endemic in the Balkan peninsula. However, it has never been reported in Greece before and the anti-CCHFV antibodies detected in 1% of the human population were most probably produced against the strain AP92 [3]. Further phylogenetic studies may show possible relations between CCHFV strains circulating in the region.

Whether climatic and environmental changes played any role in providing the favourable conditions for CCHF emergence in Greece has to be further investigated. It is not possible to predict the future occurrence of CCHF in Greece. However, clinicians have to include CCHF in the differential diagnosis of febrile hemorrhagic syndromes, even in non-endemic regions, as coincidence of factors benefit the emergence of new pathogens in an area, especially when neighbouring countries with similar landscape are endemic.

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A preliminary report on Crimean-Congo haemorrhagic fever in Turkey, March - June 2008

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Introduction

Crimean-Congo haemorrhagic fever (CCHF) is a disease caused by a virus belonging to Bunyaviridae family. CCHF virus isolation and/or disease have been reported from more than 30 countries in Africa, Asia, south-eastern Europe, and the Middle East [1]. The main transmission routes of the virus are tick-bite and contact with tissues, body fluids and blood of infected animals [1-4]. Nosocomial transmission is another important route of infection [1]. The incubation period is generally described as 1-3 days after tick-bite and 5-6 days after exposure to infected animal or human blood or body fluid, but it can be longer. Fever, chills, headache, fatigue and myalgia are the most common symptoms in the pre-haemorrhagic period. The disease progresses to haemorrhagic form in severe cases [1]. The fatality rate of disease is reported between 7.5-50% in hospitalised patients [4-7]. This wide range may due to phylogenetic variation of the virus, transmission route and different treatment facilities [4-7].

Epidemiological situation in Turkey

Although confirmed CCHF patients or serological evidence of the virus were being reported from neighboring countries, there had been no evidence of CCHF case before 2002 in Turkey. The first cases were detected in the town of Tokat in Kelkit Valley region in northern Turkey (Figure 1) in 2002 [8].

Between 2002 and 2007, a total of 1,820 confirmed cases, including 92 deaths, were reported to the Ministry of Health (MoH) of Turkey, showing an increasing trend over the years (Figure 2). The majority of cases (95%) were reported from middle and eastern Anatolia, particularly from the cities of Tokat, Sivas, Yozgat, Çorum, and Erzurum [9]. Most of the cases were diagnosed between March and October with peak levels in June and July, which corresponds with the tick season. The average case fatality rate between 2002 and 2007 was 5%, (range 4.5%-6.2) [9]. Seventy percent of the cases had a history of tick contact, while most of the remaining 30% had a history of contact with livestock, and three cases were attributed to nosocomial transmission [9].

Studies on ticks performed in areas where human cases had been reported found CCHF in *Hyalomma marginatum marginatum* pools (10,11).

Since December 2003, CCHF is a notifiable disease in Turkey. Cases with epidemiological risk factors, clinical symptoms and laboratory findings compatible with CCHF are reported to the Ministry of Health (MoH) as probable cases.

The case definition for probable cases includes:

Epidemiological risk factors: Tick-bite or tick contact; work in animal husbandry or farm; contact with the body fluid of a CCHF patient; work at a laboratory; close contact with a CCHF case.

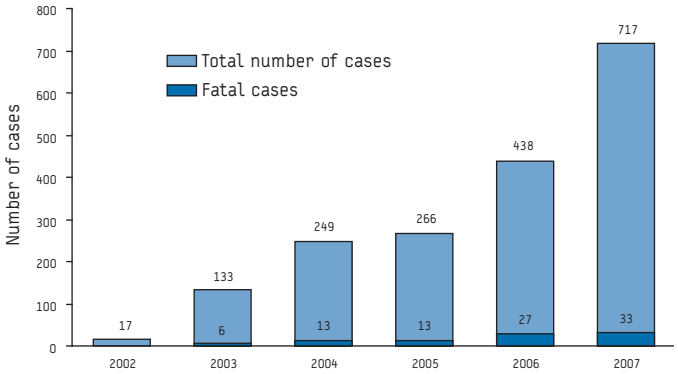
Clinical symptoms: Fever, haemorrhage, headache of acute onset, myalgia/arthralgia, lethargy, nausea/vomiting, or abdominal pain/diarrhea.

Figure 1

Kelkit valley region in Turkey where most of the cases of Crimean-Congo haemorrhagic fever have been reported from (2002-2008)

Figure 2

Number of cases of Crimean-Congo haemorrhagic fever reported in Turkey in 2002-2007 (n=1,820)



Laboratory findings: Thrombocytopenia (platelet <150.000/mm3) and/or leucopenia (WBC <4000/mm3), elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH) and creatine phosphokinase (CK).

Cases with confirmed CCHF virus RNA in the blood or body fluid samples through RT-PCR evaluation or IgM positivity through ELISA are considered confirmed CCHF cases. The laboratory diagnostics for CCHF is done on the national level in the Virology Laboratory of Refik Saydam Hygiene Center in Ankara.

Preliminary results in 2008

The first CCHF case in 2008 was detected and notified to the MoH on 24 March. As of 30 June, 688 confirmed cases have been reported: four in March, 57 in April, 282 in May and 345 in June (Figure 3). Of these, 41 patients have died due to CCHF, corresponding to a case fatality of 5.96 %.

As in previous years, most of the cases were from Middle and Eastern Anatolia region (91%). Sporadic cases (9% of the total) have been reported from south-eastern and western parts of Turkey, as well.

The male to female ratio was 1.07. The mean age of the patients was 44.3 ±19.5 years (range: 2-93 years). The proportion of cases was highest among patients of working-age, especially adults from rural areas. The distribution of patients according to occupations was 51.8% farmers followed by 18.9% homemakers (who in rural areas generally work in agriculture and animal husbandry), and 16.5% those working in animal husbandry sector.

Regarding possible modes of transmission, 71% of the cases had a history of tick bite; 21.9% reported unprotected contact with blood or body fluids of domestic animals. Five healthcare workers exposed to patients' blood and body fluids by mucosal contact have been diagnosed as nosocomial CCHF cases until the end of June. None of them died.

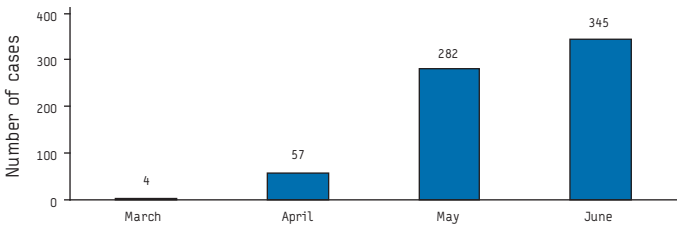
Control measures

A scientific advisory commission was set up by MoH in 2003. This commission meets regularly and its recommendations regarding treatment options, isolation measures, suggestions for disinfection, and approach in handling the deceased have been put in action.

In 2004, MoH in collaboration with the Ministry of Agriculture and Rural Affairs (MARA) initiated a surveillance and control programme including education regarding the disease and its transmission routes, tick removal, handling tick-bite cases, protected contact with animals, prevention of nosocomial infections and early detection of cases. This programme has been conducted throughout the whole country, and especially intensively in the epidemic region. It has been updated in 2007.

Figure 3

Number of cases of Crimean-Congo haemorrhagic fever reported in Turkey in 2008 (n=688; as of 30 June 2008)



In 2008, brochures, posters and TV spots informing about the risk of CCHF infection were updated and distributed to educate the public and the health-care workers. In the epidemic area, education programmes have been conducted door to door by provincial health directorates under the MoH. These included information regarding inspecting body for ticks, removing ticks as soon as possible, limiting exposure to body fluids or blood of livestock and using permethrine repellent 0.5% for treating clothes. The MoH collaborates closely with the MARA regarding tick combat in livestock on the central and provincial level.

Conclusion

Cases of CCHF have been reported in Turkey since 2002, mostly in spring and summer and in middle and eastern Anatolia. This has been associated with factors such as climatic features (temperature, humidity, etc.), changes of vector population, geographical conditions, flora, wild life and animal husbandry sector [12]. The number of cases has been increasing over the years, which may also be due to better awareness of health care personnel and public about the disease in addition to the above factors [9].

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Large ongoing Q fever outbreak in the south of The Netherlands, 2008

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Background

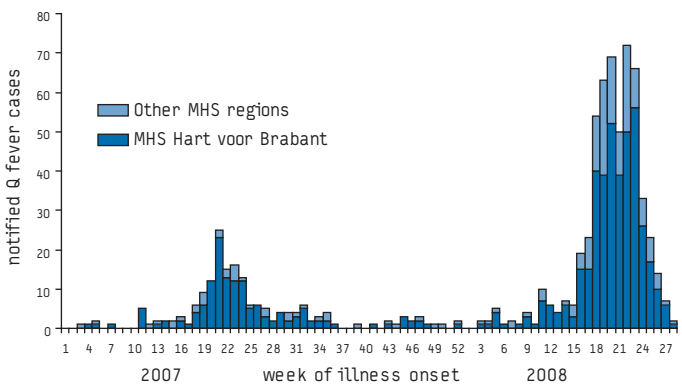
Q fever is a worldwide zoonosis caused by the bacterium *Coxiella burnetii* which is common in a wide range of wild and domestic animals. Cattle and small ruminants, in particular sheep and goats, have been associated with large human outbreaks. Humans become infected primarily by inhaling aerosols that are contaminated by *C. burnetii*. Most infections remain asymptomatic but in about 40% lead to a febrile disease, pneumonia and/or hepatitis. Chronic infections, mainly endocarditis, are observed in 3 to 5% of cases, with an increased risk for pregnant women and persons with heart valve disorders or impaired immunity. Q fever in pregnancy, whether symptomatic or asymptomatic, may also result in adverse pregnancy outcomes [1]. Q fever in humans is a notifiable disease in The Netherlands. The notification criteria for a confirmed case of acute Q fever are clinical symptoms consistent with Q fever and a positive serology defined by immunofluoresence assay (IFA) test or a *C. burnetii* complement fixation test [2]. Also clinical patients diagnosed by PCR are considered as confirmed cases. Between 1997 and 2006, Q fever was notified rarely with an average of 11 (range 5-16) cases annually [3]. In 2007, we reported in this journal the first community outbreak of Q fever in the south of The Netherlands [4].

Current situation

We report a second large outbreak of Q fever that started in the first half of 2008. Since the spring of 2008, a marked increase in notified Q fever cases has been observed with a total of 677 cases notified up to 24 July 2008 in OSIRIS, an internet-based reporting system for notifiable infectious diseases in The Netherlands. Of these cases, 17 had illness onset in 2007, 546 in 2008, while for the remaining 114 recently notified cases the date of illness onset is still unknown. The majority of cases reported illness onset between week 18 and 24, similar to the outbreak in 2007 (Figure 1). The overall female to male ratio is 1:1.7. The age distribution in 2008 ranges between 7 to 87 years (IQR 41-60 years, median 51 years) and is similar to the age distribution in 2007 (Figure 2). The preliminary hospitalisation rate of cases in

Figure 1

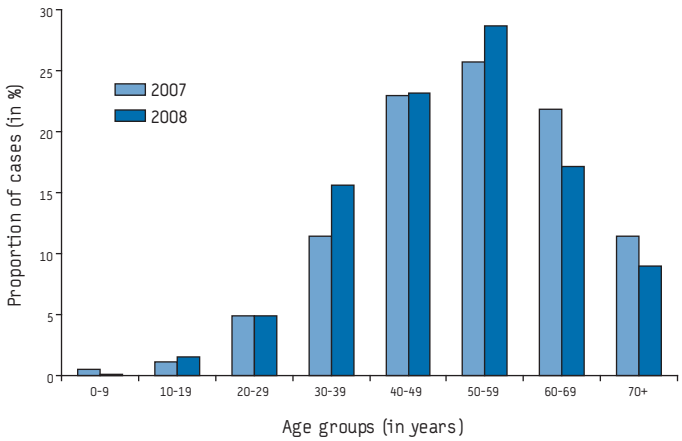
Notified cases of Q fever by week of illness onset, municipal health service (MHS) 'Hart voor Brabant' and all other MHS, 1 January 2007-24 July 2008, the Netherlands (n=182 in 2007 and n=546* in 2008); Source: OSIRIS



* for further 114 cases notified in 2008 the date of illness onset is still unknown

Figure 2

Age group distribution of Q fever cases in 2007 (1 January - 31 December, n=182 cases) and 2008 (1 January - 24 July, n=660 cases); source: OSIRIS



2008 was 22% compared to 43% during the same period (week 1-28) in 2007.

Of the cases with illness onset in 2008 for whom information on symptoms was available (n=300), 94% reported fever, followed by fatigue (89%), night sweating (78%), headache (71%) and general malaise (63%). Sixty-five percent of the cases with known symptoms had pneumonia. So far, no pregnancy has been reported among notified cases.

Although the 2008 epidemic is located in the same part of the country as the outbreak in 2007, it is more widespread in the province of Noord-Brabant and expanded to the adjacent province of Gelderland (Figure 3). This area is known for its large density of dairy goats. Seventy-five percent of the cases notified in 2008 reside in one municipal health service region 'Hart voor Brabant'. Within this region, several distinct clusters of Q fever have been observed in rural municipalities with cumulative incidences as high as 14 acute *C. burnetii* infections per 1,000 inhabitants (Figure 3). The outbreak is ongoing but the numbers seem to decrease in the entire Q fever affected area.

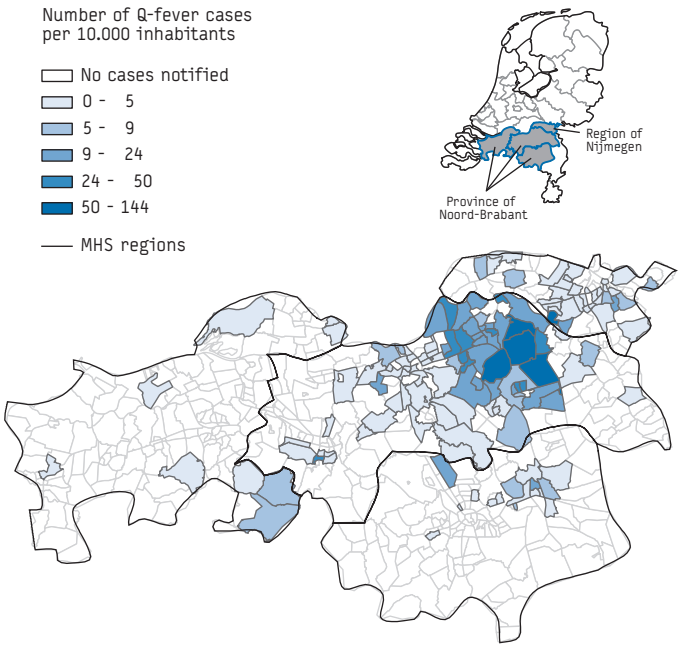
Control measures and new legislation

Since 2007, Q fever has become an important public health problem in The Netherlands, warranting a continuous enhanced surveillance. Efficient data sharing between public health institutions and veterinary health partners on regional and national level is a prerequisite for timely and thorough source tracing and identification. Following the 2007 outbreak, an informal agreement was made that the veterinary and the public health sectors would exchange information on farms with newly diagnosed animal cases of Q fever to allow for an adequate response and control. On 3 June 2008, an outbreak management team was convened and recommended a mandatory notification of Q fever in ruminants. In the same month this recommendation was implemented by the Dutch Ministry of Agriculture and the Ministry of Health; farmers and veterinarians have to report symptoms compatible with Q fever, usually abortion waves, in small ruminants held in deep litter houses. In addition, a ban to spread manure during the three months following the detection of Q fever at the farm and a restriction for visitors at the farm were imposed [5,6].

The current situation has also led to public health questions about the need for screening of pregnant women for Q fever and exclusion of blood donations from individuals in affected regions. On 22 July, an international expert meeting was organised by the National Institute for Public Health and the Environment (RIVM) and the Health Council of The Netherlands, with participation of the European Centre for Disease Prevention and Control (ECDC), to address these important issues. The outcome of this meeting will be reported separately.

Figure 3

Q fever notifications per 10,000 inhabitants by four-position postal code areas*, 1 January-23 July 2008, 3 MHS regions in Noord-Brabant province and MHS region Nijmegen in Gelderland province (n=628)



* In The Netherlands six-digit postal codes (e.g. 4000 AB) indicate areas at street level. The Figure is based on larger districts defined by the first four digits of the postal code (e.g. 4000)

Discussion

This is by far the largest community outbreak of Q fever ever reported in the literature. Other European countries such as Denmark and Germany have also reported a changing epidemiology of Q fever and an increase in cases in 2008 but not to the same extent as in The Netherlands [7,8]. The sharp increase in cases in the spring and the widespread pattern of this community outbreak with more than 600 cases reported in 2008 is alarming. This high number of notified cases is partly explained by an increased awareness of Q fever among general practitioners (GP), specialists and medical microbiological laboratories, especially in the region where the 2007 outbreak occurred. We hypothesize that this has also led to a different diagnostic approach and earlier diagnosis of suspected cases, leading to less hospital admissions in the notified cases. Signals from rural GP practices indicate, however, that there is an unprecedented marked and striking increase in pneumonia and signs and symptoms associated with Q fever in their patient population [personal communication].

To date there has been no conclusive evidence as for the source(s) of the epidemic. Although a single animal source can cause many human Q fever cases [9], the larger geographic area in which cases occur in 2008, compared to 2007, points at multiple sources. Several studies to assess the risk factors for Q fever in the general population, high-risk groups, and in ruminants are ongoing or starting in the near future, including source investigations focusing mainly at small ruminant farms and pet farms.

We hope through this paper to raise awareness of this problem and inspire colleagues from other European countries to report whether they have observed similar increase in Q fever case numbers and share their experience.

Acknowledgements

We would like to acknowledge all public health physicians and nurses from municipal health services and medical microbiologists from the regional laboratories in Noord-Brabant province and the region Nijmegen in Gelderland province. Figure 3 was compiled by Cindy Deuning (RIVM) based on four-digit post code data provided by the municipal health services.

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Tularaemia outbreak in Castilla y León, Spain, 2007: an update

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An outbreak of tularaemia occurred in Castilla y León in northwestern Spain between June and December 2007, as previously reported by Martín et al. [1]. The scope of the present article is to describe in more detail, and update the results of, the outbreak investigation.

An increased incidence of cases diagnosed as "fever of unknown origin" was detected in late June 2007 by the Castilla y León Epidemiological Surveillance Network based on a series of notifications from a rural area in the province of Palencia and from Leon city. Subsequent epidemiological investigation confirmed a tularaemia outbreak.

Outbreak investigation

After the initial cases were confirmed as tularaemia, the regional Epidemiology Service launched an active search for cases, both prospective and retrospective. Primary and specialised healthcare professionals were informed about the situation and asked to notify all suspected cases and to take clinical specimens for laboratory investigation.

Most collected samples were sent to the National Reference Laboratory in Madrid, where cases were confirmed by means of serological techniques (microagglutination and tube agglutination), culture or PCR.

All cases were first interviewed face-to-face by clinicians and then over the phone by epidemiologists. The questionnaire collected information about the patient, clinical symptoms and potential exposures associated with the risk of infection. Follow-up information on the conclusion of the treatment and disappearance of the symptoms were available for 73.5% of the cases.

The cases, as reported by clinicians, were classified by epidemiologists by means of the case definition and confirmation criteria agreed jointly with the National Epidemiological Surveillance Network and the European Centre for Disease Prevention and Control [2].

The clinical symptoms compatible with one of the different forms of tularaemia included:

- Ulceroglandular (cutaneous ulcer with regional lymphadenopathy),
- Glandular (regional lymphadenopathy with no ulcer),
- Oculoglandular (conjunctivitis with preauricular lymphadenopathy),
- Oropharyngeal (stomatitis or pharyngitis or tonsillitis and cervical lymphadenopathy),
- Intestinal (intestinal pain, vomiting, and diarrhoea),
- Pneumonic (primary pneumonic disease),
- Typhoidal (febrile illness without early localising signs and symptoms).

The laboratory criteria for diagnosis included:

- Isolation of *Francisella tularensis* from a clinical specimen,
- Detection of *F. tularensis* genome by PCR,
- Demonstration of a specific antibody response in paired serum samples.

Classification of confirmed case: a clinically compatible case confirmed by laboratory diagnosis.

Additional criteria for cases associated with the outbreak included: a person resident in Castilla y León who between mid-May and December 2007 met the above criteria.

Results

A total of 507 cases were laboratory-confirmed, of these 91.5% using serological techniques (microagglutination and tube agglutination), 5% by culture and 3.5% by PCR. *F. tularensis holarctica* was identified as the agent causing the outbreak.

The outbreak was focused in the northwest quadrant of Castilla y León (in five of the nine provinces) and has not spread to either neighbouring areas of Castilla y León or other Spanish regions.

Cases were reported with the onset of symptoms between week 20 (earliest 15 May) and week 52 (latest 31 December) of 2007.

Ninety percent of cases occurred from week 25 (starting 18 June) to week 43 (starting 22 October) of 2007, with a peak (59.5% of the cases) between weeks 26 and 33 (24 June – 18 August) (Figure 1).

The majority of cases (80.1%) were male. Patients aged 41 to 70 years accounted for 69.2% of cases, although all age groups were affected (Figure 2).

The most frequent clinical form reported was the typhoidal one (59.0%), followed by the ulceroglandular, glandular and pneumonic forms (14.6%, 12.6% and 7.9%, respectively) (Table 1).

The majority of cases (71.1%) were treated by general practitioners, 25.0% were hospitalised, while the remaining 3.9% attended specialist out-patient facilities.

Ciprofloxacin (750 mg every 12 hours) and doxycicline (100 mg every 12 hours) for 10 to 14 days were the most frequently used antibiotics, although other fluoroquinolones and tetracyclines were also administered.

Patients' responses to the prescribed antibiotic treatment were favourable in most cases, with only a few complications and no fatal cases reported. The most frequently observed complications were: asthenia (8 cases), suppurative adenopathies (7 cases), persistent arthralgias (6 cases), persistent adenopathies (4 cases) and allergic reaction to prescribed treatment (2 cases).

Information on possible exposures revealed that 34.9% of cases were farm workers or people whose jobs involve contact with gardens or natural environments (e.g. gardeners, rangers or reserves and lakes maintenance staff). Contact with rodents (24.3%) or domestic animals such as dogs or cats (19.7%), handling crayfish (13.2%) or frequent walks through the countryside (11.8%) were reported in a significant number of cases. Other possible infection routes reported were recent arthropod bites (10.9%), contact with livestock (9.5%) or with manure, straw or alfalfa hay (4.9%), or having handled and/or skinned hares (6.5%). Patients could indicate more than one possible exposure and these are neither exclusive nor exhaustive (Table 2).

Discussion

The first known tularaemia outbreak in Castilla y León, with 534 reported cases, took place in 1997 [4,5,7]. Another, smaller one,

Figure 1

Confirmed cases of tularaemia by week of onset of symptoms, outbreak in Castilla y Leon, Spain, 2007 (n=507)

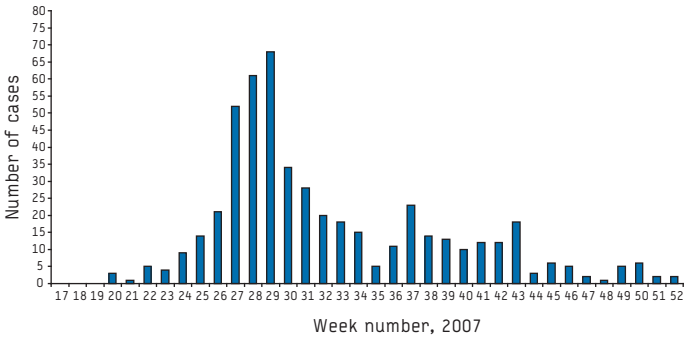

Figure 2

Age distribution of confirmed cases of tularaemia, outbreak in Castilla y León, Spain, 2007 (n=507)

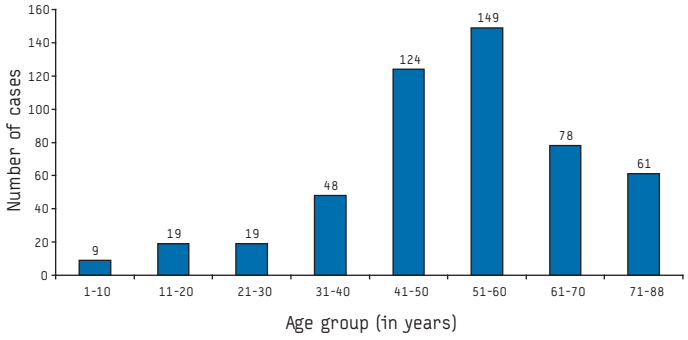

Table 1

Clinical form of tularaemia reported in confirmed cases identified in the outbreak in Castilla y León, Spain, 2007 (n=507)

Clinical form	Number of cases	Proportion of all cases (%)
Typhoidal (febrile illness with no early localisation of sings or symptoms)	299	59.0
Ulceroglandular (cutaneous ulcer with regional lymphadenopathy)	74	14.6
Glandular (regional lymphadenopathy with no ulcer)	64	12.6
Pneumonic (primary pleuropulmonary disease)	40	7.9
Oropharyngeal (stomatitis or pharyngitis or tonsillitis with cervical lymphadenopathy)	14	2.8
Intestinal (abdominal pain, vomiting and diarrhoea)	10	2.0
Oculoglandular (conjunctivitis with regional lymphadenopathy)	6	1.2
Total	**507**	**100.0**

Table 2

Possible exposures associated with the risk of tularaemia infection reported by cases in the outbreak in Castilla y León, Spain, 2007 (n=507)

Exposures	Number of cases	Proportion of all cases (%)
Farm work or jobs related to gardens or natural environments	177	34.9
Contact with rodents	123	24.3
Contact with other animals such as dogs or cats	100	19.7
Having handled crayfish	67	13.2
Walks through the countryside	60	11.8
Recent arthropod bites	55	10.8
Contact with livestock	48	9.5
Exposure to untreated water	41	8.1
Having handled and/or skinned hares	33	6.5
Manure, straw or alfalfa hay	25	4.9
Raw to medium cooked meat consumption	1	0.2

Note: Cases could indicate more than one possible exposure, hence the percentages do not add to 100%

with 13 cases, occurred in 2004 [6]. Several sporadic cases were also notified in the interim. The prevailing modes of transmission for these earlier outbreaks were contact with leporids in 1997 [4,5,7] and crayfish in 2004 [6].

In the 2007outbreak, the most frequent clinical presentation of the disease, the typhoidal form, together with the potential risk factors indicated by cases suggest two different means of transmission responsible for the outbreak: mainly by inhaling the bacteria, a pattern seen in just over half the cases (pneumonic and probably many of the typhoid forms), and, secondly, through direct contact, with local manifestations of the disease (ulceroglandular and ganglionar forms).

At the time of the outbreak, harvesting and related farm works were being conducted, which may have caused aerosols capable of transporting the bacterium. Unusual climatic and environmental circumstances (mild winter and dry spring) might have contributed to this outbreak, together with the significant diversity of illness reservoirs and infection sources that usually take part in transmission (leporids, sheep, rodents, canids and haematophagus vectors). All these factors have probably aided the proliferation of *Francisella tularensis*, a bacterium that can survive for long periods in water, mud and animal carcasses.

Different studies are being conducted in order to improve our knowledge of this outbreak and its causes: a) a spatial analysis to evaluate the possible correlation with either environmental or animal factors; b) a case-control study to identify the potential risk factors associated with infection sources and modes of transmission; and c) a seroprevalence study of *F. tularensis* in asymptomatic people in the case-control study area. A collection of cases' sera was created to allow further investigation.

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West Nile fever in a patient in Romania, August 2008: case report

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On 25 August 2008, the National Institute of Research Development for Microbiology and Immunology (the "Cantacuzino" Institute) in Bucharest, Romania reported the detection of IgM antibodies against West Nile virus in the serum of a male patient in his mid forties, from Braila town (Braila county, south eastern part of Romania).

Case report

Clinical data

On 3 August 2008 the patient fell ill with fever between 38° and 39°C, severe headache, macula-papular exanthema, vomiting, diarrhea, ocular aches. His symptoms worsened and five days later he was admitted to the infectious disease section of the local hospital with moderate clinical symptoms of meningitis. A possible rickettsiosis was diagnosed and he received doxycycline and symptomatic treatment. The patient fully recovered and was discharged on 15 August. Patient history revealed that he had gone fishing two weeks before the onset of disease, in Gropeni village in Braila county, on the shores of the Danube river where IgG against West Nile virus had been detected in horses in 2007.

Laboratory findings

Cerebrospinal fluid (CSF) sampled at time of admission was clear, the cell count was 20 per mm3 with 100% lymphocytes. Leptospirosis was considered as differential diagnosis but the slide agglutination test was negative. Samples were sent to "Cantacuzino" Institute on 18 August for further testing for *Rickettsia conorii*, however, the immunofluorescence test was negative. Although the samples had been sent only for the diagnosis of a rickettsial disease, they were also tested for antibodies against West Nile virus, according to the requirements of surveillance system for West Nile fever [1]. IgM antibodies against West Nile virus in the patient's serum were detected on 25 August, the positive result of the CSF sampled at the time of hospital admission was obtained on 3 September. The case was thus confirmed according to the European Centre for Disease Prevention and Control (ECDC) case definition.

Epidemiological investigations

The local public health authority (PHA) of Braila sampled mosquitoes in the village where the patient had been fishing. The samples sent on 4 September to the "Cantacuzino" Institute tested negative.

Epidemiologists checked the consultation registry at the infectious disease hospital in Braila and at the general practitioner (GP) clinic in the village where the patient had been fishing, searching for patients presenting with the symptoms "fever and exanthema" between 15 July and 26 August. This led to the detection of a female patient who had been hospitalised at the infectious disease centre on 20 August and reported to the Centre for Prevention and Control of Communicable Diseases, Public Health Institute of Bucharest on 2 September. Her blood was tested for antibodies against West Nile virus and *R. conorii*. The test results were negative for IgM antibodies.

Information about the mortality in birds and horses in the area as well as results from surveillance for the presence of West Nile virus in birds and animals, performed in 2008, was requested from the local (Braila Sanitary-Veterinary Direction - SVD) and the national veterinary authorities (National Sanitary-Veterinary Authority and Food Safety) and the Diagnostic Institute for Animal Health. All veterinary institutions were also notified about the human case.

Immediate control measures on local level

Doctors at the infectious disease hospital in Braila and the village GP were informed about the case and asked to perform serum investigation for West Nile virus in patients presenting with fever associated with exanthema, without a known cause.

The administrative authorities of the two localities were also notified about the case, as they are responsible for specific control measures against mosquitoes.

Health education campaigns for the general population included messages about informing a physician in case of sickness (fever and rash) and taking protective measures (clothing, repellents) for mosquito bites and sanitary measures in and around their living space.

Risk assessment and implications for the future

A risk analysis of the current situation performed by the specialists of the Centre for Prevention and Control of Communicable Diseases (CPCCD) on 1 September concluded that Braila county is one of the counties in Romania with a risk for the occurrence of West Nile virus. Climatic conditions, temperature, humidity (rain, soil humidity, natural water reservoirs such as Danube delta) and the presence of migratory and indigenous wild birds and horses favour

the existence and multiplication of the *Culex* spp. mosquitoes. Considering this and the recent detection of a human case of West Nile virus infection several measures were proposed by the CPCCD specialists:

- In the area of Gropeni which is currently the only remaining area at risk, regular surveillance of the mosquito population will continue and samples will be sent for analysis to "Cantacuzino" Institute.
- A serum survey in the human population is needed in order to identify the infection among the population of the Gropeni area.
- The County Haematological Centres are not equipped to detect the West Nile virus in donated blood, therefore a temporary suspension for blood donation from people of the village of Gropeni was recommended until the end of October 2008.
- A decision to prolong this period/ to extend temporary suspension of blood donation might be taken on the basis of monitoring climatic conditions and mosquito population from Gropeni area.
- Serum testing of random samples from the serum deposits of the Braila Haematological Centre from blood donated in August should be undertaken to collect additional information regarding the current situation.

West Nile virus surveillance in Romania

The vector for West Nile virus present in Romania is *Culex* spp. (*molestus / pipiens*), which is active from May to October each year. Since 1997, active surveillance for West Nile virus in humans, has been performed between the months of May and October in all counties along the river Danube, including Bucharest. Furthermore, surveillance is ongoing in wild birds and horses. Humans with clinical symptoms of meningitis and clear CSF are tested for the presence of IgM antibodies against West Nile virus. Suspected and positive cases are mandatorily notifiable.[1] From the start of active surveillance in the current season only six probable meningitis cases with clear CSF have been reported, however, all were negative for West Nile virus antibodies. No systematic serosurveys have been undertaken neither from patients presenting with what might have been atypical symptoms of West Nile fever, nor from the general population in Braila county. No systematic surveillance exists regarding the presence of West Nile virus in mosquitoes.

Results from Braila county

In the last ten years there were two confirmed human cases with West Nile fever symptoms in the county of Braila, one in 1997 and the other in 2001. In both cases the examination of the CSF showed clear liquor and signs of meningitis.

Serology studies undertaken in 2007 in horses demonstrated the presence of West Nile virus infection (unpublished data, communication by SVD Braila). Braila county was among the counties included in the studies. Serum samples were taken from horses in five towns, two of them neighboring Gropeni village where the patient had gone fishing. Out of 23 serum samples taken, 13 were positive showing IgG antibodies against the West Nile virus (unpublished data). According to experts of the Braila SVD bird mortality in 2007 was not higher compared to past years.

Conclusion

Three cases of West Nile virus infection detected in Braila county in the past decade together with animal data demonstrate that there is a risk of infection in humans resulting from mosquito bites in this area. In the current case the probability that the patient had acquired the infection in the town where he resided was considered to be low because there mosquito control measures had been carried out twice in 2008. Therefore he was thought to have been infected while fishing in an area where there is a high density of mosquitoes and measures for mosquito extermination are not practised. This highlights the need for systematic vector control measures in the affected area and for education of the population regarding the necessary mechanical (such as long sleeved shirts and pants) and/or chemical protection (repellents) while fishing or pursuing other recreational or occupational activities.

Acknowledgements

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Detection of West Nile virus infection in horses, Italy, September 2008

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Six confirmed and five suspected cases of West Nile virus infection in horses have been reported in the vicinity of Ferrara in Italy. To verify the diffusion of viral circulation and to prevent the spread of disease, the regional authorities of Emilia-Romagna adopted a special plan of West Nile fever surveillance.

Detection of cases

As of 22 September 2008, 12 horses with neurological symptoms indicating the possibility of West Nile virus infection have been reported. The notifications were made in accordance with the already existing national surveillance of West Nile disease. In six of these cases the diagnosis was confirmed by laboratory analysis performed at the national reference centre (Centro di Referenza Nazionale per le Malattie Esotiche – CESME), for five the initial ELISA test was positive but the confirmation is still pending, and one tested negative.

The infected horses belong to eight different stables, seven in the province of Ferrara and one in the province of Bologna at the border with Ferrara. There are about 220 horses kept in these stables and all are to be tested for West Nile virus infection. The blood sampling and laboratory testing is currently ongoing.

West Nile virus has also been recently detected in wild birds in the area. Although no anomalous mortality has been signalled, surveillance of wild birds conducted between 19 August and 14 September in the framework of a general monitoring of the regional wild fauna resulted in detection of West Nile virus in six crows and seven magpies, all from the province of Ferrara.

To date there have been no human cases of West Nile fever reported in Italy. Active surveillance of cases of meningoencephalitis (with clear cerebrospinal fluid [CSF]) was started on 16 September and is ongoing. So far one suspected case was notified in a patient resident in the province of Bologna near the border with Ferrara. However, the results of laboratory analysis are still pending.

Control measures

The public health authorities in Emilia-Romagna are closely monitoring the situation and adapting the action plan to the evolving epidemiological situation. Currently, the following measures are in place or planned:

Veterinary surveillance

The veterinary surveillance which started on 15 September comprises passive surveillance (until 31 October) and active surveillance (until 31 December) of cases of West Nile fever in horses. It is also foreseen that samples collected from cattle in the region as part of sentinel surveillance for bluetonque disease will be tested for West Nile virus. Furthermore, a national plan for surveillance of wild birds (other than corvids) is under preparation.

Human surveillance

The surveillance of human cases ongoing since 15 September includes rapid detection and reporting of cases with neurological symptoms compatible with of West Nile disease (until 31 October), as well as active surveillance among employees of stables where cases of infection in horses have occurred, to promote the awareness on this disease, preventive measures and early detection of West Nile fever.

The case definition used includes patients >= 15 years old, with fever >= 38.5ºC and neurological symptoms: encephalitis, meningitis or Guillain-Barré syndrome or acute flaccid paralysis. Cases are classified as:

a) possible: clinical symptoms and clear CSF;
b) probable: clinical symptoms and at least one of the following laboratory criteria: presence of IgM antibodies against West Nile by ELISA; seroconversion by ELISA; fourfold increase of IgG antibodies against West Nile in two consecutive (>5 days, preferably 15-20 days) samplings by ELISA;
c) confirmed: clinical symptoms and at least one of the following laboratory criteria: isolation of West Nile virus in blood or CSF; presence of IgM antibodies in CSF (by ELISA); detection of nucleid acid specific for West Nile virus by RT PCR in blood or CSF; detection of increased levels of IgM and IgG antibodies against West Nile by ELISA confirmed by neutralisation testing.

At the moment, considering the surveillance measures adopted, as well as the example of other countries especially France [1], the Italian authorities decided not to introduce any restrictions on blood donations. However, the situation is monitored closely and should a human case be confirmed, this decision will be reconsidered.

Vector surveillance and control

In addition to surveillance, vector control measures are being implemented in the area affected, i.e. the province of Ferrara and the border zones of the provinces of Ravenna, Bologna and Modena. In these areas samples of mosquitoes (Culex spp. and Aedes spp.) are being collected; 10,000 catchments divided into pools are going to be analysed (by PCR). In addition to larvicide disinfestations in every potential breeding site, adulticide interventions are planned to be undertaken in every urban areas and on the occasion of open-air public gatherings, e.g. fairs and festivals, especially held outside the urban centres and in the vicinity of water reservoirs.

Conclusion

This event illustrates the necessity of a coordinated strategy plan combining surveillance in domestic animals, wild fauna and in humans for assessing the magnitude of the outbreak and for an efficient management.

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http://www.moh.gov.cy

CZECH REPUBLIC

Zpravy CEM (Bulletin of the Centre of Epidemiology and Microbiology)
Centrum Epidemiologie a Mikrobiologie Státního Zdravotního Ústavu, Prague.
Monthly, print and online. In Czech, titles in English.
http://www.szu.cz/cema/adefaultt.htm

EPIDAT (Notifications of infectious diseases in the Czech Republic)
http://www.szu.cz/cema/epidat/epidat.htm

DENMARK

EPI-NEWS
Department of Epidemiology, Statens Serum Institut, Copenhagen.
Weekly, print and online. In Danish and English.
http://www.ssi.dk

FINLAND

Kansanterveys
Department of Infectious Disease Epidemiology, National Public Health Institute, Helsinki.
Monthly, print and online. In Finnish.
http://www.ktl.fi/portal/suomi/julkaisut/kansanterveyslehti

FRANCE

Bulletin épidémiologique hebdomadaire
Institut de veille sanitaire, Saint-Maurice Cedex.
Weekly, print and online. In French.
http://www.invs.sante.fr/beh/default.htm

GERMANY

Epidemiologisches Bulletin
Robert Koch-Institut, Berlin
Weekly, print and online. In German.
http://www.rki.de/DE/Content/Infekt/EpidBull/epid__bull__node.html

HUNGARY

Epinfo (az Országos Epidemiológiai Központ epidemiológiai információs hetilapja)
National Center For Epidemiology, Budapest.
Weekly, online. In Hungarian.
http://www.oek.hu/oek.web?to=839&nid=41&pid=7&lang=hun

ICELAND

EPI-ICE
Landlæknisembættið
Directorate Of Health, Seltjarnarnes
Monthly, online. In Icelandic and English.
http://www.landlaeknir.is

IRELAND

EPI-INSIGHT
Health Protection Surveillance Centre, Dublin.
Monthly, print and online. In English.
http://www.ndsc.ie/hpsc/EPI-Insight

ITALY

Notiziario dell'Istituto Superiore di Sanità
Istituto Superiore di Sanità, Reparto di Malattie Infettive, Rome.
Monthly, online. In Italian.
http://www.iss.it/publ/noti/index.php?lang=1&tipo=4

Bolletino Epidemiologico Nazionale (BEN)
Istituto Superiore di Sanità, Reparto di Malattie Infettive, Rome.
Monthly, online. In Italian.
http://www.epicentro.iss.it/ben

LATVIA

Epidemiologijas Bileteni
Sabiedribas veselibas agentura
Public Health Agency, Riga.
Online. In Latvian.
http://www.sva.lv/epidemiologija/bileteni

LITHUANIA

Epidemiologijos žinios
Užkreciamuju ligu profilaktikos ir kontroles centras
Center for Communicable Disease Prevention and Control, Vilnius.
Online. In Lithuanian.
http://www.ulpkc.lt/ulpkc.laikrastis.php

NETHERLANDS

Infectieziekten Bulletin
Rijksinstituut voor Volksgezondheid en Milieu
National Institute of Public Health and the Environment, Bilthoven
Monthly, print and online. In Dutch.
http://www.rivm.nl/infectieziektenbulletin

NORWAY

MSIS-rapport
Folkehelseinstituttet, Oslo.
Weekly, print and online. In Norwegian.
http://www.folkehelsa.no/nyhetsbrev/msis

POLAND

Meldunki o zachorowaniach na choroby zakazne i zatruciach w Polsce
Panstwowy Zaklad Higieny,
National Institute of Hygiene, Warsaw.
Fortnightly, online. In Polish and English.
http://www.pzh.gov.pl/epimeld/index_p.html#01

PORTUGAL

Saúde em Números
Ministério da Saúde,
Direcção-Geral da Saúde, Lisbon.
Sporadic, print only. In Portuguese.
http://www.dgsaude.pt

ROMANIA

Info Epidemiologia
Centrul pentru Prevenirea si Controlul Bolilor Transmisibile,
National Centre of Communicable Diseases Prevention and Control, Institute of Public Health, Bucharest.
Sporadic, print only. In Romanian.
http://www.cpcbt.ispb.ro

SLOVENIA

CNB Novice
Inštitut za varovanje zdravja, Center za nalezljive bolezni, Institute of Public Health, Center for Infectious Diseases, Ljubljana.
Monthly, online. In Slovene.
http://www.ivz.si

SPAIN

Boletín Epidemiológico Semanal
Centro Nacional de Epidemiología, Instituto de Salud Carlos III, Madrid.
Fortnightly, print and online. In Spanish.
http://www.isciii.es/jsps/centros/epidemiologia/boletinesSemanal.jsp

SWEDEN

EPI-aktuellt
Smittskyddsinstitutet, Stockholm.
Weekly, online. In Swedish.
htpp://www.smittskyddsinstitutet.se/publikationer/smis-nyhetsbrev/epi-aktuellt

A selection of report titles from the national epidemiological bulletins in the European Union and Norway is translated and published online once a month: http://www.eurosurveillance.org

UNITED KINGDOM

England and Wales
Health Protection Report
Health Protection Agency, London.
Weekly, online only. In English.
http://www.hpa.org.uk/hpr

Northern Ireland
Communicable Diseases Monthly Report
Communicable Disease Surveillance Centre, Northern Ireland, Belfast.
Monthly, print and online. In English.
http://www.cdscni.org.uk/publications

Scotland
Health Protection Scotland Weekly Report
Health Protection Scotland, Glasgow.
Weekly, print and online. In English.
http://www.hps.scot.nhs.uk/ewr/index.aspx

OTHER JOURNALS

EpiNorth journal
Norwegian Institute of Public Health, Folkehelseinstituttet, Oslo, Norway
Published four times a year in English and Russian.
http://www.epinorth.org

OTHER LINKS

European Union
"Europa" is the official portal of the European Union. It provides up-to-date coverage of main events and information on activities and institutions of the European Union.
http://europa.eu

European Commission - Public Health
The website of European Commission Directorate General for Health and Consumer Protection (DG SANCO).
http://ec.europa.eu/health/index_en.htm

Health-EU Portal
The Health-EU Portal (the official public health portal of the European Union) includes a wide range of information and data on health-related issues and activities at both European and international level.
http://ec.europa.eu/health-eu/index_en.htm

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