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Situation of HIV infections and STIs in the United Kingdom in 2007

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In the United Kingdom (UK) in 2007, an estimated 77,400 persons were living with human immunodeficiency virus (HIV) of whom 28% are unaware of their infection. A total of 7,734 persons were newly diagnosed with HIV infection in 2007, of which 31% were diagnosed late. This highlights the need for wider HIV testing, especially in those areas with a high diagnosed prevalence, as recommended in recent national guidelines. Among newly diagnosed cases of HIV in 2007, 41% acquired their infection through sex between men (four in five of whom acquired their infection in the UK) and 55% through heterosexual contact (four in five of whom acquired their infection abroad, mainly in sub-Saharan Africa). Young persons aged 16 to 24 years are disproportionally affected by sexually transmitted diseases (STIs) accounting for 65% of genital chlamydia infections, 50% of cases of genital warts and 50% of cases of gonorrhoea that were diagnosed in 2007.

Human immunodeficiency virus infections

The Health Protection Agency's Centre for Infections, in the United Kingdom (UK) has recently released a series of four reports on HIV and STIs. The most recent ones provide an overview of HIV in the UK [1] and a focus on the continuing HIV and STIs epidemics among men who have sex with men (MSM) [2]. Two earlier reports describe the epidemiology of HIV and STIs among black African and black Caribbean communities in the UK [3] and among young people aged 16-24 years [4].

The number of people living with HIV continued to rise in 2007 with an estimated 77,400 persons living with both diagnosed and undiagnosed HIV infections, representing a rate of 127 persons living with HIV per 100,000 population (170 per 100,000 men and 84 per 100,000 women) [1]. Among the 73,300 (range 68,800-78,500) persons aged 15-59 years living with HIV, 28% (24%-33%) were unaware of their infection.

In 2007 there were 7,734 persons newly diagnosed with HIV in the UK. Importantly, almost one third (31%, 2,345/7,649) of adults were diagnosed late (defined as a CD4 cell count <200 per mm within three months of diagnosis), beyond the point at which treatment should have begun. The proportion diagnosed late was lowest among MSM (19%) and higher among heterosexual women (36%) and heterosexual men (42%).

HIV testing policy in the UK

The high proportions of individuals unaware of their HIV infection and being diagnosed late highlight the need for wider HIV testing to benefit both the individual, with access to earlier treatment and thus improved prognosis, and the community with reduced onward transmission.

The recently released new national HIV testing guidelines in the UK aim to promote HIV testing in a wide range of healthcare settings [5]. The guidelines recommend the routine offer of HIV testing to all those attending hospital services (e.g. genitourinary medicine, antenatal services, tuberculosis clinics etc) as well as wider HIV testing in those areas where the local diagnosed HIV prevalence exceeds two in 1,000 among the population aged 15-59 years. In these areas, HIV testing should be offered to all men and women registering in general practice and to all those who are admitted to general medical wards. With an estimated one undiagnosed HIV infection for every two diagnosed, these areas are likely to have an undiagnosed prevalence of one in 1,000, the threshold at which routine testing is assumed to be cost effective [6]. In 2007, the prevalence of HIV exceeded this threshold in 42 of the 152 primary care organisations in England, the majority in London, which serve nearly half of the UK population.

HIV testing in 2007

In 2007, approximately 800,000 HIV tests were carried out in genitourinary medicine (GUM) clinics in the UK. Data from unlinked anonymous serosurveillance in a network of 16 GUM clinics throughout the UK showed that the proportion of attendees accepting the offer of a HIV test has increased in recent years among all populations at risk. Overall among heterosexuals, the rate of uptake has increased from 66% in 2003 to 75% in 2007. Among black Africans and MSM in 2007 HIV test uptake was higher (85% and 86%, respectively). However, uptake of HIV testing varied with HIV status, with 65% of HIV positive MSM accepting an HIV test compared to 87% among HIV negative MSM. Among black Africans the uptake was 61% and 86% for HIV positive and negative individuals, respectively.

Unlinked anonymous testing also highlighted that among GUM attendees in 2007, 3.4% of MSM and 0.4% of heterosexuals had a previously undiagnosed HIV infection. The prevalence of undiagnosed HIV infection was higher among heterosexuals born

in sub-Saharan Africa (2.4%) than in those born in the UK (0.2%) and those born elsewhere (0.4%). Among HIV infected attendees, 30% left the clinic without an HIV test result either because they were not offered, or had declined testing.

HIV prevalence in the unlinked serosurveillance of women giving birth in the UK in 2007 was 0.21%, equivalent to one in every 468 women giving birth. Prevalence was highest among pregnant women born in sub-Saharan Africa (2.5%) and in Central America and the Caribbean (0.53%). The prevalence of HIV among UK-born women remained low (0.05%). HIV testing among pregnant women remained high in 2007, with 94% of women in antenatal care accepting a routine HIV test in 2007. As a result the estimated proportion of HIV-exposed infants who become infected has decreased from 17% in 1998 to less than 5% in 2007.

Among the 7,734 persons newly diagnosed with HIV in the UK in 2007, 41% acquired their infection through sex between men (four in five of whom acquired their infection in the UK) and 55% through heterosexual contact (four in five of whom acquired their infection abroad, mainly in sub-Saharan Africa). Although the majority of persons infected heterosexually acquired their infection abroad, the estimated proportion who acquired their infection within the UK has doubled since 2003, from 11% (540/4800) to 23% (960/4250).

MSM, however, remain the group at greatest risk of acquiring HIV infection in the UK [2]. An estimated 30,800 (range 28,700-33,700) MSM aged between 15 and 59 in the UK were living with HIV in 2007, of whom 25% (range 20%-32%) were unaware of their positive status. In 2007, there were 2,679 new HIV diagnoses among MSM (increasing to 3,160 if adjusted for missing data), a similarly high number as previous years, and the highest ever reported. With the exception of non-specific urethritis and gonorrhoea, which both declined in 2007, diagnoses of STIs among MSM have closely mirrored increases in HIV diagnoses. Of particular concern are the increasing proportions of MSM diagnosed with STIs who are already diagnosed with HIV, accounting for 32% of gonorrhoea, 40% of syphilis, and 78% lymphogranuloma venereum cases reported through enhanced surveillance systems

Black Africans accounted for 35% (2,691) of new HIV diagnoses in 2007, the majority of which were probably heterosexually acquired (94%), and in Africa (88%) [3]. It was estimated that in 2007 there were 25,900 (range 22,900-29,600) heterosexuals born in Africa aged between 15 and 59 who were living with HIV in the UK, of whom 24% (range 14%-34%) were undiagnosed with HIV. Of all new HIV diagnoses in 2007, 31% were made late, but among black Africans this figure was much higher at 42%. The estimated prevalence of diagnosed HIV among black Africans is 3.7% and among black Caribbeans 0.4% whereas, among the white population it is much lower at 0.09%.

Sexually transmitted infections

Young people (aged 16-24) in the UK, are disproportionately affected by STIs, with the exception of HIV [4]. Although young people represent 12% of the UK population, they accounted for 65% of genital chlamydia cases, 50% of cases of genital warts, and 50% of gonorrhoea cases seen in 2007. Black Caribbean communities are also a key prevention group for STIs as they are disproportionately affected by bacterial STIs. In a GUM clinic sample in 2007, black Caribbeans represented 26% of all heterosexually acquired gonorrhoea cases.

In 2007, more than a million sexual health screens (which include a test for both gonorrhoea and chlamydia) were performed in GUM clinics in the UK, representing a 61% increase since 2003. The National Chlamydia Screening Program (NCSP) offers sexually active young people in England screening for chlamydia infection and other sexual health promotion activities, mainly in community settings. In 2007, over 270,000 screenings for chlamydia were performed among young people through the NCSP among which 9.5% of women and 8.4% of men tested positive, with an overall positivity rate of 9%.

Conclusion

The recent increases in reported diagnoses of HIV and STIs in the UK have continued in 2007. The control of STIs requires easy access to sexual health services that can provide advice, screening and their treatment. HIV testing should be promoted extensively among prevention groups as well as in the general population living in high prevalence areas. Interventions to promote sexual health should be strengthened and expanded to meet better the needs of those at high risk of acquiring and STI, including HIV.

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The Russian influenza in Sweden in 1889-90: an example of Geographic Information System analysis

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Using data from a study of the 1889-90 Russian flu in Sweden, this article describes how the application of Geographic Information System (GIS) may improve analyses and presentation of surveillance data. In 1890, immediately after the outbreak, all Swedish doctors were asked to provide information about the start and the peak of the epidemic, and the total number of cases in their region and to fill in a questionnaire on the number, sex and age of infected persons in the households they visited. General answers on the epidemic were received from 398 physicians and data on individual patients were available for more than 32,600 persons. These historic data were reanalysed with the use of GIS, in map documents and in animated video sequences, to depict the onset, the intensity and the spread of the disease over time. A stack diagram with the observations grouped into one week intervals was produced to depict the spread in one figure only. To better understand how the influenza was disseminated, Thiessen polygons were created around 70 places reported on by the doctors. Having prepared GIS layers of the population (divided into parishes), estimations could be made for all the Swedish parishes on the number of infected persons for each of the 15 weeks studied. The described models may be useful in current epidemiological investigations, as well.

Introduction

The aim of this paper is to demonstrate how Geographic Information System (GIS) improves prospective surveillance and our knowledge on the diffusion of influenza epidemics. As an example we describe the application of this method to analyse historic data on the Russian influenza epidemic of 1889.

The cause of influenza was disputed in the 19th century. One old theory dating from the days of Hippocrates (460-377 BC), saying that diseases are disseminated from miasma (bad or polluted air), had many advocates. Miasma was believed to come from decomposition in the ground, and attack weak individuals and occasionally cause disease outbreaks [1]. In "The History of Epidemics in Britain (1891-1894)", the well known British epidemiologist, Charles Creighton, tried to prove the miasmatic theory [2]. When his works were published, England and the rest of the world had just suffered from an influenza pandemic more severe than its predecessors. It was named Asiatic or Russian influenza, as the first reports of incidences came from a small village in the Asian part of Russia. It was detected in May 1889, reached St Petersburg in October and spread all over the world within a year.

On 4 February 1890, the Swedish Society for Medical Doctors assembled to discuss how the Russian influenza, at that time peaking in Sweden, was disseminated. The Society decided to undertake an epidemiological study to find the answer. A survey in the form of postal cards and a questionnaire were sent to all Swedish doctors. The results were collected by Dr Klas Linroth (1848-1926), Sanitary Inspector in Stockholm, in cooperation with Dr Curt Wallis and Dr Fredrik Warfvinge. Linroth analysed the results and concluded that the disease was spread along the communication network from person to person and not by miasma [3]. Linroth's main conclusions concerning the disease are presented in Table 1, to provide a general background for the geographic analyses made in this study.

Using Linroth's data this work intends to show how modern GIS technology can be used to add a geographic dimension to epidemiological analysis in order to facilitate the evaluation of hypotheses, conclusions and decisions. We looked into the geographical aspects of Linroth's carefully conducted epidemiological surveillance and explored the GIS methods in combining epidemiological data with geographic and census data to extract new information. The use of standard formats

Table 1

Main findings of the epidemiological study conducted by K. Linroth during the Russian influenza epidemic in Sweden in 1889-90

Duration	3 months (end November 1889 - end February 1890)
Incubation time	1-3 days
Duration of the disease	2.3 – 9.4 days
Proportion of the population affected (men; women)	60.7% (60.0%; 61.1%)
Proportion of infected by age-groups (in years)	
<1	36.2% (153/450)
1-10	59.8% (4,938/2,956)
11-20	65.3% (3,379/5,170)
21-40	61.5% 6,162/10,014)
41-60	62% (2,896/4,666)
>60	47.2% (837/1,770)
Excess mortality, Stockholm	0.13% (300/235,000 inhabitants, Stockholm)

and techniques ensures that the results obtained can easily be shared between the authorities and communicated to the public. To understand the nature of a pandemic it is essential to study its progress in both space and time. Modern GIS has all the tools necessary to make this happen.

Methods and results

The data sets

The request from the Swedish Society of Medical Doctors to all Swedish doctors contained two forms; the first one was a postcard, with three questions:

1. When was the first influenza case detected in your district?
2. When do you consider that the epidemic in your district reached its peak?
3. How large, according to your opinion, was the percentage infected by the influenza?

The postcards (study 1) were returned by 398 doctors. From the answers a table was compiled and a map was drawn in 1890, indicating when the influenza first appeared at different locations. To support the contagiousness theory an analysis of the railway network was done in relation to the onset of the outbreak. In the first week in December 1889, 12 of the 13 affected places outside Stockholm had railway stations. In another table Linroth demonstrated that by 20 December, 82% of reporting places with a railway station and 47% without one had been affected. Sea ports with daily communications to and from Stockholm were also attacked early. There is, however, an uncertainty concerning these results, as it was never quite clear to the respondents whether it was the first locally infected patient or the first infected individual arriving by train or by boat that should be regarded as the first case in a particular location.

The second form (study 2) was designed to assess the number, age and sex of patients infected in the doctors' districts. The doctors were asked to fill in a questionnaire for each household they visited, providing information on the number, sex and age of all persons in the household and of those that had had influenza. They were also asked to communicate any observation that could add to the understanding of the characteristics and the spread of the influenza. In total 126 forms were returned with information on 32,683 individuals (0.68% of the total population in Sweden at the time) and 42 of the doctors added personal notes. Separately, Linroth received 115 letters with additional information. Linroth used these answers to compile a table giving detailed information on the development of the influenza at 69 locations.

GIS data and analysis

In the GIS study, both Linroth's tables were converted into Excel format. For unknown reason the first data set is in 5 days intervals, whereas the second (the more comprehensive one) is in one week intervals (Table 2). The tables were checked for inconsistencies and some of the place names were changed to the spelling of today, to enable interactive geocoding (giving geographical coordinates to address information). In ArcGIS (GIS software from ESRI Inc., US) the Excel tables could be spatially joined to a Multinet (geographic data from Tele Atlas NV, The Netherlands) layer, using the place names. New point layers were thus created to link Linroth's data to places with geographical coordinates.

A background map showing land and water and the communication network was obtained. In 1890, the railway network was the main communication system of the country. By courtesy of the Railway Museum in Gävle we received a digital copy of a map of the Swedish railroads of 1890. As the geometrical quality of this map is not very

Table 2

Part of K. Linroth's table with data on Russian influenza epidemic in 69 Swedish localities (study 2), converted into Excel format using ArcGIS

Object ID	Name	Before 1889/12/01	Week 89/49	Week 89/50	Week 89/51	Week 89/52	Week 90/01	Week 90/02	Week 90/03	Week 90/04	Week 90/05	Week 90/06	Week 90/07	Week 90/08	Week 90/09	After 1990/03/01
6	Karlskrona			4	10	21	31	30	21	7	14	3				
7	Eksjö	1		1	5	13	27	25	7	1	3	2	2	3	2	
8	Jönköping			1	1	7	4	4		1						
9	Västervik			2	2	30	73	67	42	18	6	6	1	2	3	
10	Värnamo					81	50	12	3		3	1	3		2	
11	Söderåkra				1	9	13	12								
12	Marstrand	1	2	5	7	23	36	27	6	2		1	4			4
13	Halmstad		1	8	13	39	61	57	20	12	6	4	2	3	3	1
14	Göteborg			12	123	526	3525	2226	767	279	138	75	39	28		
15	Skövde					20	24	29	3							
16	Hjo			7	13	54	133	169	102	5	23	16	11	2		
17	Motala				12	44	59	58	2	2		2				
18	Linköping				18	65	95	36	13	2	3			4		1
19	Eskilstuna			1	23	60	299	171	115	14						
20	Bettna			1	6	13	33	32	3	2	1					
21	Österlövsta			2	25	86	58	14	10	1	5	1	2			
22	Västerås			3	8	18	16	2	2	2						

good, we decided to use the modern, geometrically satisfactory, railroad data from Tele Atlas instead. From the current (as of 2007) Tele Atlas railroad layer all railroads not existing in 1890 were manually deleted, using the museum map as master.

Using data from study 1 (onset of first cases) as well as the railway network, maps were created to show, in five-day intervals, how the epidemic spread in the country.

Similar maps were created on the basis of study 2 data. For each of the 15 weeks in the spread sheet (Table 2) from Linroth's second study, GIS layers were created, showing the number of infected persons at each location. These layers can be displayed in many different ways. Figure 2 shows places in Sweden where influenza was reported with the weekly incidences indicated by the size of the dots.

The map series in Figure 1 (with data from Linroth's first study) can be compared with the map series in Figure 2. The time intervals are slightly different but comparing the two map series one can still see the same pattern along the railways.

Using the same technique as was demonstrated in a previous study [4], time layers can be organised to create video sequences. This was done for both data sets and video clips can be supplied upon request to the authors.

We also managed to create maps with bar charts showing in one map (Figure 3) the progress of the disease week by week. This gives an understanding, in one single map, of how, where and when the influenza was spread.

Other researchers [5] have chosen to visualise local peaks of influenza in maps where each specific colour represents a specific time period (typically a month). Compared to this technique our method has the benefit of showing peaks that may vary in length of time.

Modelling of spread, combining population and the epidemiological data

The available data on the number of infected persons (study 2) refer only to 69 locations and the 32,683 infected individuals. There is no information on the spread of the epidemic to other places. Thus, it is not known when and how many people in total were infected in Sweden.

A geodatabase of the Swedish population per parish and year has been created for another study, not yet published. The population data was gathered by Professor Lennart Andersson-Palm at the University of Gothenburg. By importing the parish boundaries along with population figures from 1890 into this study, the local impact of the influenza was geographically assessed, based on reported data from 69 locations.

Thereafter, to understand how the influenza was disseminated to all other places, the so called Thiessen (or Voronoi) polygons [6] were created around each of the 69 studied locations. All positions within a polygon are closer to the point location, around which the polygon was created, than to any other of the remaining 68 location points (Figure 4).

Furthermore, we assumed that influenza spread in the same way in all parishes.

Using a GIS tool called "Select by Location" [7] we can see, for example, that the Thiessen polygon surrounding Västerås has 90 parishes (the small polygons in the map in Figure 4) with their centroids within the surface of the polygon.

The 90 parishes were then merged into a new polygon. We called this polygon a "Thiessen area". This was repeated for each of the 69 Thiessen polygons, covering all of Sweden. It is important to note that the shorter the distances between the observation points are, the smaller the Thiessen areas get.

We then created an Excel sheet with the numbers of infected persons per Thiessen area (Table 3).

Next, this Excel sheet was spatially joined with the Thiessen area layer. Time layers were then extracted for each week. These layers were colour coded to show the magnitude of the influenza, per Thiessen area, for the week layer displayed (Figure 5). Using the ArcGIS Animation Manager [8], video sequences were created for the 15 weeks the influenza lasted. The video sequences can

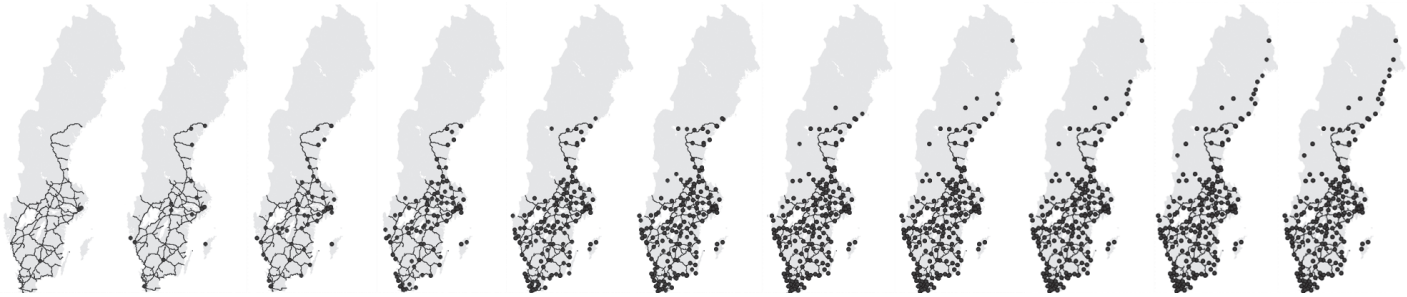
Table 3

Part of a spread sheet containing calculated numbers of infected persons per Thiessen area for each week during the influenza epidemic. Analysis of Russian influenza in Sweden in 1889-90

Name	Before 1889/12/01	Week 89/49	Week 89/50	Week 89/51	Week 89/52	Week 90/01	Week 90/02	Week 90/03	Week 90/04	Week 90/05	Week 90/06	Week 90/07	Week 90/08	Week 90/09	After 1990/03/01
Vaxholm	0	2292	24062	8021	1146	1146	0	0	0	0	0	0	0	0	0
Visby	0	2973	811	4865	8649	9730	2973	2162	1351	811	270	0	0	0	0
Vrigstad	0	0	0	5386	6155	20005	10003	3847	2308	0	0	0	0	0	0
Vålberg	0	0	0	0	0	3553	7105	21316	3553	0	0	0	0	0	0
Värnamo	0	0	0	0	21737	13418	3220	805	0	805	268	805	0	537	0
Västervik	0	0	283	283	4252	10345	9495	5952	2551	850	850	142	283	425	0
Västerås	0	0	5051	13469	30305	26937	3367	3367	3367	0	0	0	0	0	0
Åmål	0	272	0	272	1630	2445	7879	8694	4075	2445	1087	0	0	272	0
Örebro	342	856	1883	9242	10782	10611	4792	2054	1540	685	856	342	342	342	171
Österlövsta	0	0	311	3890	13383	9026	2179	1556	156	778	156	311	0	0	0
Överkalix	0	0	0	0	0	0	0	40	437	800	701	368	209	80	35
TOTAL	4908	33825	123653	190456	435529	718155	575811	348406	185867	111512	70479	44338	14825	5943	7786

Figure 1

Onset of Russian influenza epidemic in Swedish localities (data derived from study 1). Analysis of Russian influenza in Sweden in 1889-90.



Dots represent places where first infected cases had been reported to date, maps correspond to five-day intervals, starting from the last week of November 1889 (left) until 21 January 1890 (right). The railroad network is shown.

Figure 2

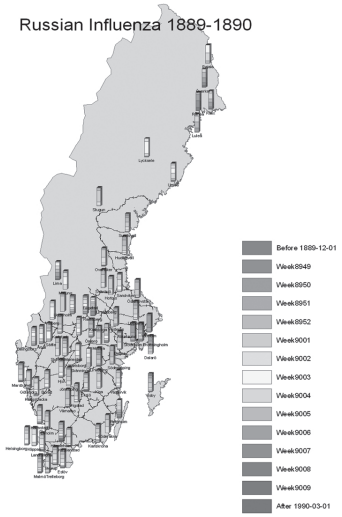
The numbers of infected patients reported by the local doctors within one-week intervals (study 2). Analysis of Russian influenza in Sweden in 1889-90.

The dots indicate the number of cases; each map represents one week, starting from the last week of 1889 (upper left) and ending with the week of 1 March 1890 (lower right). The railroad network is shown.

For higher resolution colour maps, see: http://www.eurosurveillance.org/public/public_pdf/GIS_colour.pdf

Figure 3

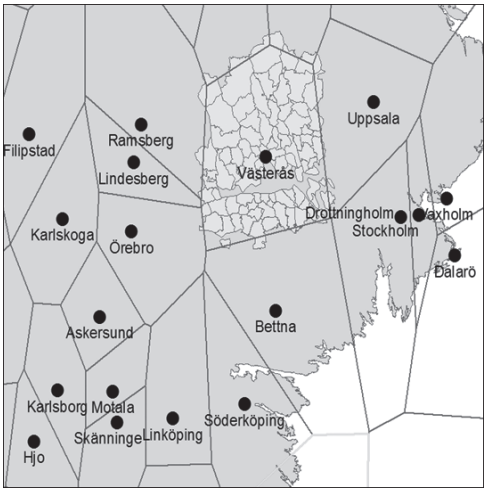
A map with bar charts showing the intensity of the pandemic, week by week. Analysis of Russian influenza in Sweden in 1889-90.



One colour (shade) represents one week. The heights of the respective coloured segments represent the relative intensity at each time period and location. The largest segments represent the local peaks in intensity. To interpret this map correctly it needs to be magnified at least 100%.

Figure 4

Thiessen polygons surrounding each of the observation points where cases had been reported by local doctors. Analysis of Russian influenza in Sweden in 1889-90.



The small polygons represent parishes.

Figure 5

The estimated cumulative total numbers of infected persons in Thiessen areas indicated by colour-coding. Analysis of Russian influenza in Sweden in 1889-90.

Darker colours mean more infected persons, varying from 0 to 50,000. The time difference between maps is one week, starting with the week of 1 December 1890 (upper left) and ending with the week of 1 March 1990 (lower right). The railroad network is shown. With fewer observation points in the north, the Thiessen areas there are accordingly larger in comparison with the southern parts of Sweden.

be displayed in ArcMap or saved as video clips that may easily be distributed.

Using the above material it is possible to estimate how many infected persons there were for each of the 2,390 Swedish parishes (according to the administrative division of the country at that time) during the whole period of the Russian influenza epidemic in Sweden.

For example, the 90 parishes surrounding Västerås had a total population of 143,105 persons in 1890. Taking into account Linroth's figures on the extent of the epidemic, the incubation time, dissemination speed and mortality, we conclude that 60% of the population of these parishes (85,683 persons) should have been infected by the Russian influenza and the number of deaths was approximately 260 (0.3% mortality).The epidemic was present in this area between week 50 of 1889 and week 4 of 1890, with a peak in the last week of 1889

Discussion

One of the basic reasons for K. Linroth and his colleagues to start their study was to determine whether the influenza was a miasmatic or a contagious disease. Many of the respondents commented on that issue. Linroth claimed that the disease basically followed the transportation network (the railways and, in some cases, the seaways) in "a way typical for contagious diseases" (Linroth's words) but also for those diseases that were called contagious-miasmatic. Linroth does not define the meaning of contagious-miasmatic. He could be referring to Peter Ludwig Panum who in 1847 discussed the possible miasmatic-contagious character of measles [9].

The dissemination was very fast and the local epidemics developed at a pace that in some cases were described as explosive. Due to the general susceptibility, the short incubation time and the difficulty to detect the very first cases, more proof were needed to scientifically verify that the influenza was indeed contagious. Linroth was however of the opinion that the many individual testimonies describing how the infection was transferred directly from infected persons justified the hypothesis: Influenza is a contagious disease

The effects and the spread of the Russian flu were studied in many countries [10] and it can be regarded as a watershed in the history of influenza. It was the first influenza epidemic after the breakthrough of bacteriology. The miasmatic theory was thereafter not much heard of for many years. Modern studies on bioaerosols, however, to some extent support the theory of contagious-miasmatic disease dissemination. A new influenza virus was found in bioaerosols in 1968 and large quantities of influenza virus are suggested to be spread over the world as a bioaerosol, and El Nino is said to have influenced the quick spread of bioaerosols in the atmosphere [11]. We believe that climate changes may influence the spread of influenza over the world, though this remains to be proven. Had GIS existed in the times of Linroth, addition of weather information to the study could have helped in verifying the theory of influenza being a contagious disease. Other modern explanations of the spread of influenza [12] claim that pathogens may be seeded in humans over a longer period of time and that host susceptibility varies in cycles.

Unfortunately it has not been possible for us to get access to the original data and answers from which Linroth compiled his tables. Also, there are obvious weaknesses in the Linroth study itself. There were no clear definitions of the cases. The doctors answered voluntarily and in some cases off the top of their heads. There was an obvious lack of control system and data provided by the doctors was not verified. Still, the fact that they made so many home visits gave them a very good insight into the effects of the influenza.

The various applications of our GIS model illustrate visually what had previously been difficult or impossible to demonstrate. Concerning the spread model, it can of course be disputed whether the same temporal pattern can really be applied to all of the parishes with their centroids within a certain Thiessen polygon. Linroth collected evidence that locally the disease first appeared close to the place containing the railway station. The population living at distant houses and farms were infected at a later stage. It is our opinion though, that ours is the best assumption you can make based on the existing data, as there is no reliable information on where the influenza started in each region (polygon). Thiessen polygons are frequently used in several GIS applications. One example is the empirical modelling of government health service use by children with fevers in Kenya [13]. Another technique for generalisation is Kriging as described in T. Sakai et al. [5].

Additional advantage of the method presented is that GIS analyses can be made on site. This study has been performed using desktop GIS. There are many ways to make the established geodatabase public. Using a GIS web server, maps and data can easily be accessed and interrogated from web readers over Internet or an Intranet. Interactive questions can then be put to the geodatabase via the map interface by anyone (authorised) having access to a web reader.

In our time, influenza data from sentinel doctors are continuously collected at the Swedish Institute for Infectious Disease Control [14]. No more than 500-1000 patients are reported yearly. So far this has been regarded as too little for GIS work on monitoring and prediction. This year however, an evaluation of the usefulness of data collected for GIS analyses will be performed. In an ongoing study, 3,500 persons from all over Stockholm (positions are defined with zip code) continuously submit self-reports on influenza-like symptoms using a web application or over the phone via an interactive voice response [15]. The number of reporting individuals will be extended in 2009. We believe that GIS applications as described in this article will be extremely useful not only for visualisation of spread, but also for prediction and for identification of still unknown factors that may contribute to influenza epidemics.

Conclusion

Combining epidemiological data with additional available geographically oriented data clearly shows the power of GIS in epidemiological research, also in a historic perspective. In the study presented in this paper it made it possible to display the spatial patterns in time in tables, in video sequences and in single map documents showing temporal variations. Using the interactive map as an interface to the epidemiological data we were able to extend the analysis to a level where everyone involved in disease prevention and crisis management may have easy access to vital information, presented in an intuitive way. This can also be applied in case of information to the public. The techniques we used in this study can of course be used in other studies in epidemiology or related disciplines. How to implement these methods in crisis management should be further studied.

Note: Lars Skog works for ESRI S-Group Sverige AB, provider of GIS and mapping software.

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Increased number of *Clostridium difficile* infections and prevalence of *Clostridium difficile* PCR ribotype 001 in southern Germany

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In recent years, *Clostridium difficile* infection (CDI) has emerged as an increasing problem, both in in- and outpatients. In a rural region of southern Germany, the annual number of *C. difficile* toxin (Tcd)-positive patients has increased from 95 to 796 in the period from 2000 to 2007. Simultaneously, the proportion of positive tests among all Tcd examinations has risen from 7.0% to 12.8%, indicating that the higher number of affected patients was not solely due to an increase in the number of assays. Elevated numbers of CDI have recently been associated with outbreaks of the ribotype 027 strain, particularly in North America. This strain has also been isolated in Europe, including in Germany. Ribotyping and PCR testing for binary toxin genes of *C. difficile* strains isolated from in- and outpatients demonstrate a predominance (59%) of *C. difficile* ribotype 001, which exhibits antibiotic resistance to erythromycin, ciprofloxacin, and moxifloxacin, but lacks binary toxin genes. In summary, in our region of Germany, the number of patients affected by CDI has increased, probably due to spread of *C. difficile* ribotype 001.

Introduction

Numbers of *Clostridium difficile* infections (CDI) are increasing in- and outside of Europe [1-5]. CDI in North America and partly also in western Europe have often been attributed to outbreaks caused by the hypervirulent strain NAP1/027 containing the binary toxin genes cdtA and cdtB [1,3,6]. Recently, this strain has also been isolated from patients in western Germany [7]. Different *C. difficile* strains are isolated in different European countries, suggesting a prevalence of particular strains in local settings [8-10].

CDI is usually regarded as a nosocomial infection that can be minimised by robust infection control practices and good antibiotic stewardship. In some hospitals in Europe it has become the most frequent nosocomial disease and consequently, analyses of *C. difficile* epidemiology were restricted to hospital outbreaks [11]. However, community-acquired cases of CDI have been observed for a few years now [12,13]. Interestingly, *C. difficile* strains associated with CDI in hospitalised patients were different from the ones isolated from community cases [13].

Our laboratory is located in a rural area in southern Germany. In this region, CDI is noticed as a growing nosocomial problem with sporadic fatal cases. However, the available information about the real extent of this apparent increase in CDI is limited. Furthermore, no studies have been done on distinct *C. difficile* strains in Germany or defined regions in Germany. We therefore collected data on the number of patients known to excrete *C. difficile* toxin (Tcd) in stool and on the number of patients analysed for Tcd. PCR was performed on *C. difficile* isolates from outpatients and from patients treated in two hospitals located in southern Germany, in order to gain knowledge about the epidemiological background of these regional strains.

Here we present data about the prevalence of a quinolone- and erythromycin-resistant *C. difficile* ribotype 001 strain in southern Germany.

Patients and methods

Laboratory and hospitals setting

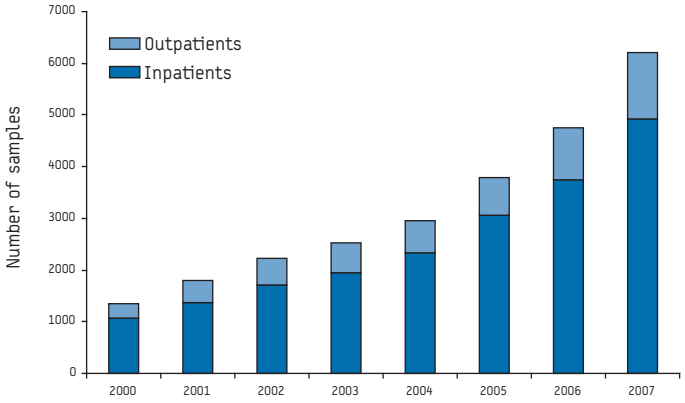
The Synlab Medical Care Service analyses laboratory samples from about 40 hospitals and more than 2,000 physicians serving outpatients. In 2006 a total of 161,000 microbiological samples were examined. *C. difficile* was isolated from Tcd-positive stool samples from patients diagnosed at two hospitals (A and B) and from outpatients. Hospital A is a primary health care hospital with 270 beds comprising two tertiary university hospital facilities (cardiology, gastroenterology). In 2006, 10,793 patients were admitted to that hospital (74,146 patient days). Hospital B is a primary health care hospital with 135 beds, and 4,886 patients (34,811 patient days) were admitted to that clinic in 2006.

Epidemiolologic analysis of *C. difficile* in South Germany

Numbers of Tcd-positve stool samples and numbers of Tcd-positive patients were evaluated by the Hybase system (Cymed AG, Bochum, Germany) linked to the laboratory data system "promed open" (mcs, Eltville, Germany). Hybase (http://www.cymed.de/download_hy.php) is a computer programme that supports the surveillance of bacterial pathogens, e.g. calculation and documentation of the number of notifiable bacterial species.

Figure 1

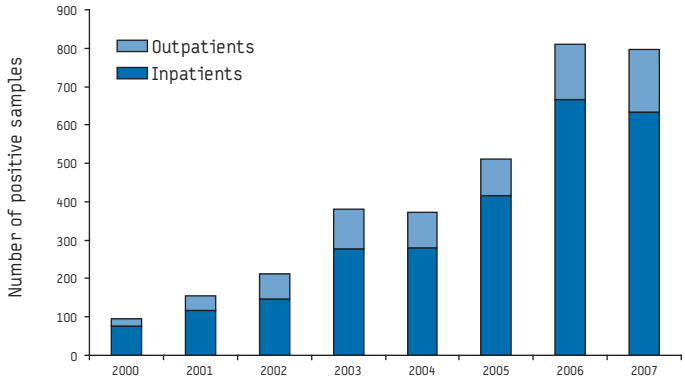
Number of patients examined by Tcd ELISA, southern Germany, 2000–2007



Tcd: *C. difficile* toxin; ELISA: enzyme-linked immuno-sorbent assay.

Figure 2

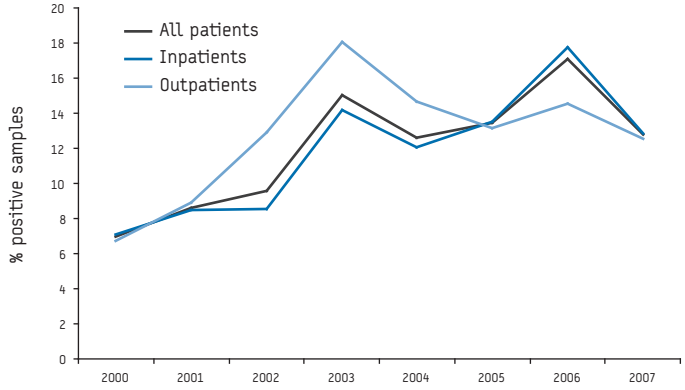
Number of Tcd-positive patients, southern Germany, 2000–2007



Tcd: *C. difficile* toxin.

Figure 3

Percentage of Tcd-positive patients, southern Germany, 2000–2007



Tcd: *C. difficile* toxin.

Data (age, sex, outpatients versus inpatients, taking into account where they were treated) from patients with Tcd-positive stool in 2006 were documented.

C. difficile toxin analysis, culture and antibiotic susceptibility testing

Stool samples from inpatients of hospitals A and B were collected between May 2006 and March 2007 and tested for *C. difficile*. Samples from outpatients were collected between March and April 2007.

Tcd was examined by using an enzyme-linked immunosorbent assay (ELISA) detecting toxin A and B (R-Biopharm AG, Darmstadt, Germany). Bacterial cultures were grown on *C. difficile*-selective agar containing cefoxitin and cycloserin (Heipha, Eppelheim, Germany; www.heipha.de/db/files/209e.pdf) under anaerobic conditions.

Identification of *C. difficile* was performed on rapid ID 32 A system (identification system for anaerobes, Biomerieux, Nürtingen, Germany). Antibiotic susceptibility was tested using ATB ANA strips (susceptibility test for strict anaerobic bacteria, Biomerieux, Nürtingen, Germany) according to the manufacturer´s instructions or alternatively in an E-test procedure (for erythromycin, ciprofloxacine, moxifloxacine, cefotaxime; AB-Biodisk, Solna, Sweden). E-test results were confirmed at the German consiliary laboratories for *C. difficile* (Mainz) or gastrointestinal infections (Freiburg). Presence of binary toxin genes was examined at the German consiliary laboratory for *C. difficile* (Mainz) according to Stubbs et al. [14].

Ribotyping of *C. difficile* strains

Ribotyping was performed at the German consiliary laboratory for gastrointestinal infections (Freiburg). PCR ribotyping was performed according to he protocol of Bidet et al. [15] resulting in so-called "ribotype Freiburg". In previous comparative analyses, representative isolates of each ribotype Freiburg had been sent to the Anaerobe Reference Laboratory in Cardiff for re-typing according to the "Cardiff" PCR ribotyping library in order to establish the correlation between ribotype Freiburg and the commonly used ribotype nomenclature of Stubbs et al. [16]. It was therefore possible to relate local PCR results not only to "ribotype Freiburg" but also to European C. difficle ribotypes.

Results

Over the past years, reported numbers of patients affected by *C. difficile* infection (CDI) have increased markedly in Germany [4]. Figures 1-3 show a comparison of the number of stool samples tested for *C. difficile* toxin (Tcd) with the number of Tcd-positive stool samples in the period between 2000 and 2007.

The number of patients analysed for Tcd increased by 458% (from 1,358 to 6,214; Figure 1), but the actual number of Tcd-positive samples increased by 838% in the same period of time (from 95 to 796; Figure 2). The percentage of Tcd-positive patients increased from 7.0% in 2000 to 12.8% in 2007, with two peaks in 2003 (15.0%) and in 2006 (17.1%; Figure 3). As demonstrated by Figure 3, the peak in 2003 predominantly resulted from a high proportion of Tcd-positive outpatients (18.0%). In contrast, the peak in 2006 was caused by Tcd-positive inpatients (17.8%).

In summary, these data indicate that the increasing numbers of CDI in this region are real and not simply a result of increasing

analysis efforts. Furthermore, not only hospitalised patients but also non-hospitalised patients were affected by CDI.

Previous reports have identified high age as an important risk factor for contracting CDI [4,5]. A representative list concentrating on the age and sex distribution of patients who had Tcd-positive stools in 2006 is shown in Table 1.

A total of 784 patients were registered in our database, 17.3% of which were outpatients. Looking at the median age, the majority were elderly patients. Interestingly, the median age of outpatients (69 years) was lower than that of inpatients (77 years). In addition, Tcd-positive women tended to be older than Tcd-positive men.

To assess the cause for the increasing numbers of Tcd-positive patients via spread of hypervirulent *C. difficile* 027, ribotyping of *C. difficile* was performed on isolates from Tcd-positive stool samples previously collected from outpatients and from patients treated in two different hospitals in southern Germany (Hospitals A and B).

As shown in Table 2, at least seven different *C. difficile* ribotypes could be identified. While *C. difficile* ribotype 001 was isolated from 11 patients, other types were only isolated once from a single patient. *C. difficile* ribotype 001 was isolated from inpatients of both hospitals and was also common in outpatients indicating a predominance of this strain in this region.

Ribotype 001 *C. difficile* lacked the binary toxin genes but was resistant to quinolone antibiotics (ciprofloxacine, moxifloxacine) as well as to erythromycin, cefotaxime (MIC >16 µg/mL) and clindamycin. However, ribotype 001 strains were susceptible to ampicillin/sulbactam, piperacillin, piperacillin/tazobactam, imipenem, vancomycin and metronidazole.

Discussion

Worldwide – as well as in Germany – there is a discussion about increasing case numbers of CDI-affected patients [1-5]. In this study we demonstrate that the number of Tcd-positive patients increased markedly in southern Germany in the period between 2000 and 2006. It was assumed that this might be a result of intensified examination efforts, as from 2000 to 2007, the total number of stool samples examined for Tcd per year increased, too. However, the percentage of Tcd-positive patients also increased markedly during this period (from 7.0 to 12.8%) showing a maximum in 2006 (17.1%). This higher ratio indicates that the increased number of Tcd-positive patients is a real phenomenon and not solely due to the fact that examination efforts were stepped up.

Between 2006 and 2007, the number of CDI-affected patients remained constant, although the number of patients examined for CDI increased. This finding suggests that the intensified infection control measures may have been successful in preventing the nosocomial spread of *C. difficile*. However, the possibility to separate between nosocomial and community acquired CDI is limited by the lack of patient data.

In agreement with earlier studies [4,5], Tcd-positive stool samples were mainly obtained from elderly patients. The fact that 136 of 784 Tcd-positive patients (17.3%) in 2006 were outpatients clearly shows that CDI was not restricted to hospitalised patients. On the other hand, the median age of Tcd-positive inpatients was higher than that of Tcd-positive outpatients, an indication that CDI in younger people has a milder course and does not require hospital admission.

The *C. difficile* 027 strain was detected in Germany for the first time in 2007 [7]. However, the ribotyping results presented here reveal that this strain was not prevalent in northern Bavaria. In contrast, multi-resistant *C. difficile* 001 were frequently found.

Table 1

Number and characteristics of in- and outpatients with Tcd-positive stool samples, southern Germany, 2006

	Outpatients	Inpatients (all hospitals)	Hospital A	Hospital B
Number of patients	136	648	45	34
CDI per 1,000 admissions			4.2	6.1
CDI per 10,000 patient days			7.0	9.0
Proportion of positive Tcd analyses (%)	14.6	17.8	16.4	13.9
Age distribution				
Age of patients, median (mean)	69.0 (62.3)	77 (73.1)	75 (72.6)	80.5 (76.6)
Number of patients <6 years	6	4	0	1
Number of patients <21 years	8	14	1	0
Number of patients 21-79 years	79	325	25	15
Number of patients >79 years	35	258	18	11
Number of patients >89 years	8	47	1	7
Sex distribution				
Number of female patients	77 (56.6 %)	373 (57.6 %)	27 (60 %)	19 (55,9 %)
Age females, median (mean)	68 (62.3)	79.0 (76.1)	79 (75,48)	81 (82.0)
Number of male patients	59 (43.4 %)	275 (42.4 %)	18 (40.0 %)	15 (44.1 %)
Age males, median (mean)	69,0 (63.3)	73.0 (69.2)	71 (68.39)	75 (69.8)

Tcd: *C. difficile* toxin

For this analysis, *C. difficile* were cultured from Tcd-positive stool samples from in- and outpatients. The hospitalised patients had been treated at two hospitals located about 200 km apart. Since *C. difficile* type 001 was also isolated from outpatients, it is obvious that this strain is predominant in southern Germany.

All tested ribotype 001 *C. difficile* proved to be resistant to erythromycin and moxifloxacine in the antibiotic susceptibility testing, a feature commonly observed for ribotypes 001, 027 and 106 [6,17]. Ribotyping and binary toxin gene analysis showed that all of these *C. difficile* strains were different from the NAP1/027 strain. Recently, it has been discussed whether ribotype 027 strains could be more virulent than other ribotypes [11,18]. Only scarce clinical information - reported anecdotally - is available about the death of several patients. Nevertheless, it is clear that severe courses of CDI in our region are not limited to ribotype 027 isolates.

Ribotyping further revealed that more than 50% of *C. difficile* isolates exhibited identical features, a possible indication of clonal spread within the local population. In the case of increased CDI case numbers due to admission of affected patients bearing predominantly ribotype 001, proven clonality of *C. difficile* isolates by ribotyping might erroneously suggest nosocomial spread. Under the given circumstances of many *C. difficile* isolates being clonally related, this typing method therefore provides only limited information for outbreak analyses in a defined hospital. Consequently, use of more discriminatory typing methods, e.g. multi-locus variable-number tandem repeat analysis (MLVA), may be better suited for future epidemiological studies, at least if ribotype 001 or other frequently occuring ribotypes are involved [19].

In summary, the present study shows an increase of Tcd-positive patient numbers in southern Germany. Multi-resistant *C. difficile* ribotype 001 is prevalent in southern Germany, and this strain is thought to be responsible for severe, if not fatal, cases of CDI. In due course, more discriminatory methods may be able to improve our understanding of the epidemiology of this successful strain.

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Table 2

Characterisation of *C. difficile* isolates obtained from Tcd-positive stool samples, collected between May 2006 and March 2007 in southern Germany

Source, Age, Sex	Typing		Binary toxin genes		MIC (µg/ml)		
	Ribotype Cardiff	Ribotype Freiburg	*cdtA*	*cdtB*	Ery	Moxi	Cipro
A, 87, m	001	45	-	-	>256	>32	>32
B, 73, f	001	45	-	-	>256	>32	>32
A, 83, m	001	45	-	-	>256	>32	>32
B, 78, f	001	45	-	-	>256	>32	>32
B, 81, f	001	45	-	-	>256	>32	>32
A, 75, m	n.d.	n.d.	+	+	n.d.	n.d.	n.d.
A, 66, m	078	40	+	+	0.75	2	>32
A, 73, m	049	22	-	-	1.0	1	>32
A, 67, f	014	1	-	-	0.5	1,5	>32
B, 14, f	015	8	-	-	0.75	1	>32
B, 75, f	001	45	-	-	>256	>32	>32
B, 88, f	n.d.	n.d.	-	-	>256	>32	>32
A, 83, f	n.d.	n.d.	-	-	0.75	1,5	>32
A, 89, f	001	45	-	-	>256	>32	>32
Out, 63, m	042	21	-	-	0.5	1,5	>32
Out, 89, f	001	45	-	-	>256	>32	>32
Out, 64, m	001	45	-	-	>256	>32	>32
Out, 56, f	081	16	-	-	0.5	1	>32
Out, 31, f	n.d.	n.d.	-	-	>256	1	>32
Out, 77, f	001	45	-	-	>256	>32	>32
Out, 82, m	001	45	n.d.	n.d.	>256	>32	>32
U	001	45	n.d.	n.d.	>256	>32	>32

Patients had been treated either at hospital A or B or had been outpatients (Out). Minimal inhibitory concentrations (MIC) of erythromycin (Ery), ciprofloxacine (Cipro) and moxifloxacine (Moxi) were determined by E-test. Only two isolates exhibited binary toxin genes (*cdtA*, *cdtB*). One strain obtained from a university hospital in south-western Germany (U) was also included.
n.d. = not determined; Tcd: *C. difficile* toxin.
Ribotype Cardiff represents the European ribotype.

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Surveillance of listeriosis in Navarre, Spain, 1995-2005 – epidemiological patterns and characterisation of clinical and food isolates

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We monitored the incidence of human listeriosis in Navarre, a region in north of Spain between 1995 and 2005, and carried out the characterisation of *Listeria monocytogenes* isolates obtained from clinical samples and ready-to-eat products (sliced cooked meat, smoked salmon and liver pate). The active surveillance requesting hospitals to notify all listeriosis cases (n=40) yielded higher incidence rates (average annual rate 0.65/100,000 inhabitants, range 0.18-1.18/100,000 inhabitants) than expected. Pregnant women were the largest group affected (n=13, 32.5% of the cases), with a peak in incidence during the last three years of the study period. From the 40 human cases we obtained 33 *Listeria* isolates. Serological and molecular characterisation by PFGE identified 20 different pulsotypes, which on three occasions enabled us to link sporadic cases into clusters. Although we could not identify the incriminated food product we found two clinical pulsotypes among smoked salmon and cooked meat isolates. Surveillance of listeriosis in Spain should be improved and coordinated with other European Union Member States in order to better estimate the burden of disease and to prevent foodborne outbreaks.

Introduction

Listeria monocytogenes has been recognised as a serious foodborne pathogen, with a case-fatality rate between 20% and 50% [1-3]. However, the important impact that this disease has on public health is not always recognised, particularly since listeriosis is a relatively rare disease compared with other common foodborne infections such as salmonellosis. Listeriosis is likely to be underreported due to its status as a non-notifiable disease in many countries, including Spain, and because of the absence of adequate surveillance programs. The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial resistance and Foodborne outbreaks in the European Union produced jointly by the European Food Safety Authority (EFSA) [4] reported an incidence between 0 and 10 cases per million in 2006 among the 24 countries that submitted listeriosis information. It is significant that incidence rates above 5 cases per million were reported in countries where listeriosis is statutorily notifiable, such as France, Germany, Finland and Switzerland. However, even in countries with obligatory notification of listeriosis and efficient surveillance systems, such as PulseNet in the United States [5], the number of cases could be greater than reported due to the occurrence of many sporadic cases and spontaneous miscarriages which are not investigated.

As pointed out by Kiss et al. and MacKenzie et al. [6,7], integrated food chain surveillance is necessary for all national and international authorities in order to achieve adequate information regarding the true impact of listeriosis in the population. Serotyping of *L. monocytogenes* has a low discriminatory power for subtype differentiation [8], however, when combined with molecular methods based on DNA macrorestriction pattern analysis [9] it becomes a useful tool in epidemiological investigation. Among the molecular typing methods, pulsed-field gel electrophoresis (PFGE) has been one of the most frequently used in epidemiological investigation of listeriosis because of its excellent discriminatory power and reproducibility [10-15].

In Spain, the current surveillance of listeriosis is based on voluntary reporting of cases to the Microbiological Information System of the National Reference Laboratory. The present study set out to evaluate the incidence of listeriosis in Navarre, a region in northern Spain, over a period of 11 years from 1995 to 2005, by implementing active surveillance in this geographical area. The objectives of this study were:

1) to obtain epidemiological data on cases of listeriosis reported by the three main hospitals of Navarre;
2) to compare *L. monocytogenes* isolates recovered from food products and human cases of listeriosis in Navarre over the same period of time, by serological and PFGE characterisation.

Methods

Listeriosis surveillance and epidemiological data

In order to determine the incidence of listeriosis, we asked the three main hospitals in Navarre to report cases of listeriosis. The case definition was based on the isolation of *L. monocytogenes* from a hospitalised patient with a clinically compatible illness. A case was considered perinatal in the following cases: infected pregnant woman, miscarriage, stillbirth or newborn less than one month old. When the pathogen was isolated from both the pregnant woman and her newborn child, this was considered to represent a single case. Information regarding sex, age, clinical symptoms, immunosuppressive treatment or underlying disease, and death or recovery of the patients, was reported when available.

In addition, patients diagnosed in 2005 were interviewed about their consumption habits with regard to high risk foodstuffs. The questionnaire (available upon request from the corresponding author) covered different aspects relating to the consumption of ready-to-eat (RTE) products (type, brand and store where purchased) during the two months preceding disease onset. Specific questions regarding high-risk RTE products sampled in the study were also included.

Collection of *L. monocytogenes* strains

A total of 87 *L. monocytogenes* isolates were obtained from food samples in a study which we performed in 2003-2005 [16]. Of these, 45 were isolated from a market sampling pool of 783 RTE high-risk food products that included sliced cooked meat products (pork, chicken and turkey), sliced smoked fish products (salmon and trout) and liver pate. Isolation and identification of *L. monocytogenes* was carried out using aseptic techniques following the NF EN ISO 11290-1 [17]. The remaining 42 food strains were obtained as a result of our earlier study on the occurrence of *L. monocytogenes* in the same type of RTE food products carried out in Navarre between 1995 and 2002 [18].

With respect to clinical strains, from the 40 human cases of listeriosis reported between 1995 and 2005, we were able to obtain only 33 isolates. They were isolated in the hospital microbiology laboratories and most of them originated from either blood or cerebral spinal fluid or placenta, while stool cultures for *Listeria* were not available. These isolates were subsequently submitted to our laboratory at the University of Navarre, where identification of strains was carried out by biochemical and serological methods.

All strains were stored at -80ºC in sterilised skimmed milk.

Serological characterisation

Serotyping was carried out using commercial specific antisera (Denka Seiken Co., Ltd., Tokyo, Japan) following the manufacturer's instructions. Both polyclonal anti-O antisera (O-I/II, O-V/VI, O-I, O-II, O-VI, OVII, O-VIII, Y O-IX) and anti-H (H-A, H-AB, H-C, H-D) were used in the determination of somatic and flagellar antigen, respectively. Interpretation of the results was carried out according to the serotyping scheme established by Seeliger and Höhne [19].

Molecular characterisation by pulsed-field gel electrophoresis (PFGE)

PFGE was performed according to Graves and Swaminathan [8], with minor modifications. Before performing PFGE, strains were revitalised by plating onto blood agar (Biomerieux, Marcy L'Etoile, France) and incubated at 37ºC for 18 h. DNA from a single *Listeria* colony was digested with ApaI (Roche Diagnostics, Barcelona, Spain) and separated at 6 V/cm for 19.5 h on a CHEF-DR II PFGE apparatus (Bio-Rad, Hercules, California, US) with switch time from 4 to 40 seconds at 14ºC. Staphylococcus aureus ATCC 29213 was used as a control for digestion. The obtained images were digitised and analysed using Gel Compar II® software (Applied Maths, Kortrijk, Belgium). Restriction patterns were analysed using the criteria described by Tenover et al. [20]. Similarity values of the patterns were calculated using the Dice correlation coefficient with a 1.0% band position tolerance and unweighted pair group method using arithmetic average (UPGMA). Clinical and food isolates were compared, and pulsotypes were numbered consecutively.

Statistical analysis

The statistical package used was SPSS v13.0. The contingency table analysis was based on the chi square distribution (Pearson's chi square test).

Results

Clinical and epidemiological data

A total of 40 cases of listeriosis were documented in Navarre during the 11-year surveillance study period (Figure 1). Between 1995 and 2005, the mean annual incidence was 0.65/100,000 inhabitants, ranging from 0.18/100,000 in 1998 (n=1) to 1.18 /100,000 in 2005 (n=7). Table 1 shows the epidemiological data of reported cases. From the available information, 26 cases (65%) correspond to non-perinatal infections, while perinatal infections (pregnant women and newborns) were described in 13 cases.

In total, 21 deaths were reported resulting in the average case fatality rate of 52.5%. These included eight foetal deaths in

Figure 1

Cases of listeriosis reported in Navarre, Spain, from 1995 to 2005 (n=40)

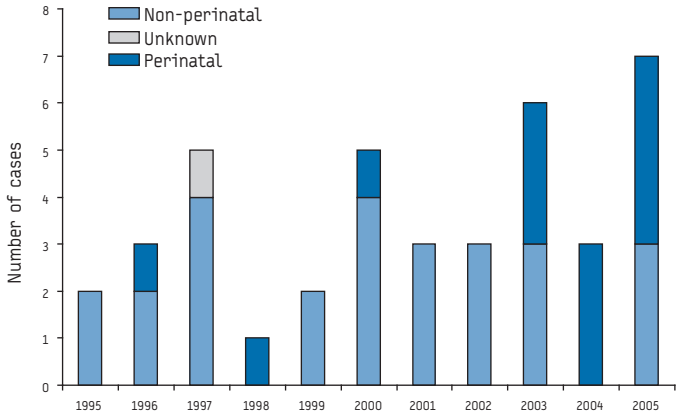


Table 1

Principal characteristics of listeriosis cases reported in Navarre, Spain, from 1995 to 2005 (n = 40)

Epidemiological data[a]	Number of cases (% of the total)	Number of deaths	Case-fatality rate (%) by group
Clinical form			
Perinatal	13 (32.5)	8[b]	61.5[b]
Non-perinatal	26 (65.0)	13	50.0
Risk factor (non-perinatal)			
No detectable pathology	5 (12.5)	1	20.0
Known risk[c]	15 (37.5)	8	53.3
Other conditions[d]	6 (15.0)	4	66.7
Age group (in years)			
<1	1	1	100
1-19	2 (5.0)	1	50.0
20-39	12 (32.5)	0	0
40-59	8 (20.0)	2	25.0
>60	12 (30.0)	8	66.7

[a] Information not available in all cases
[b] Only foetal death (all women recovered)
[c] Aged >60 or immunocompromised patients (cancer, HIV, organ transplantation).
[d] Chronic diseases

perinatal cases (all women recovered) and 13 deaths among the non-perinatal cases (case-fatality rate of 50%). Among this latter group, 15 patients (37.5% of the total) had an underlying listeriosis risk factor defined as age >60 years and/or immunosuppressive conditions such as cancer, HIV or organ transplantation. Further six cases had underlying chronic conditions, such as diabetes (n=1), addiction to alcohol (n=2) and other (n=3). The remaining five non-perinatal cases (12.5% of the total) were healthy people aged between 2 and 59 years.

Clinical symptoms most frequently reported were septicaemia (37.5%) and meningitis (15.0%) (Table 2). No cases of acute gastroenteritis caused by listeriosis were detected in the course of the study.

The most affected group at risk of listeriosis was pregnant women (n=13; two of them with underlying diseases). The number of perinatal cases was significantly higher in the years 2003-2005 compared with the previous period of 1995-2002 (10 vs. 3) (Figure 2). The clinical information available showed that all of the infected mothers recovered (one of them was diagnosed with meningitis). However, 61.5% of all pregnancy-associated cases resulted in miscarriage (n=5), stillbirth (n=2) or infant death within 24-48 hours of birth (n=1) (Table 2).

Serotyping results

Four serovars were determined among the 33 clinical isolates as shown in Table 3. In all of the different risk groups most of the isolates belonged to serogroup 4 (78.8%). Serotype 4b was the predominant (75.8%, n=25), followed by serotype 1/2a (18.2%, n = 6). With respect to the food isolates, the predominance was for serogroup 1 (77.0%). In contrast with the results obtained from clinical strains, serotype 1/2a was the most common (51.7%, n=45), followed by serotype 4b (23.0%, n=20), serotype 1/2c

Table 2

Clinical symptoms of listeriosis patients reported in Navarre, Spain, 1995-2005 (n=40)

Clinical symtoms[a]	Number of cases	Proportion of total (%)
Septicaemia[b]	15	37.5
Meningitis[c]	6	15.0
Miscarriage	5	12.5
Stillbirth	2	5.0
Premature	1	2.5
Endocarditis	2	5.0
Other[d]	5	12.5
Unkown	5	12.5

[a] Multiple responses were possible
[b] One case also with encephalitis
[c] Classified as meningitis (n = 4), meningoencephalitis (n = 1) and cerebral abscess (n = 1)
[d] Hormonal disorders, arthritis, osteomyelitis, kidney failure, heart attack

Figure 2

Incidence of listeriosis in Navarre, Spain in 1995-2005 and pregnancy-associated cases per 1,000 births in the same time period

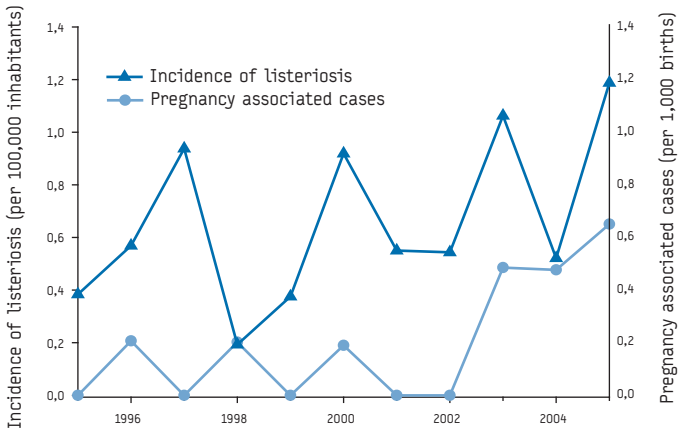


Table 3

Serotype distribution of *Listeria monocytogenes* isolated from ready-to-eat (RTE) food and clinical cases of listeriosis in Navarre, Spain, during the period 1995-2005

Source	Proportion of serogroup 1 (%)	Proportion of serogroup 4 (%)	Number of isolates of each serotype					Total number of isolates
			1/2a	1/2b	1/2c	4b	3b	
Clinical isolates	21.2	78.8	6	1	-[a]	25	1	33
Non condition associated with risk of listeriosis	20.0	80.0	1	-	-	4	-	5
Pregnancy	20.0	80.0	2	-	-	8		10
Transplantation	28.6	71.4	1	1	-	4	1	7
Cirrhosis/Alcoholism	0	100	-	-	-	4	-	4
Cancer	33.3	66.7	1	-	-	2	-	3
Others	25.0	75.0	1	-	-	3		4
RTE foods	77.0	23.0	45	3	19	20	-	87
Sliced cooked meat	94.6	5.4	32	3	18	3	-	56
Sliced smoked fish	43.3	56.7	13	-	-	17	-	30
Pate	100[b]	0	-	-	1	-	-	1

[a] No clinical isolates of this serotype detected
[b] Only one isolate obtained

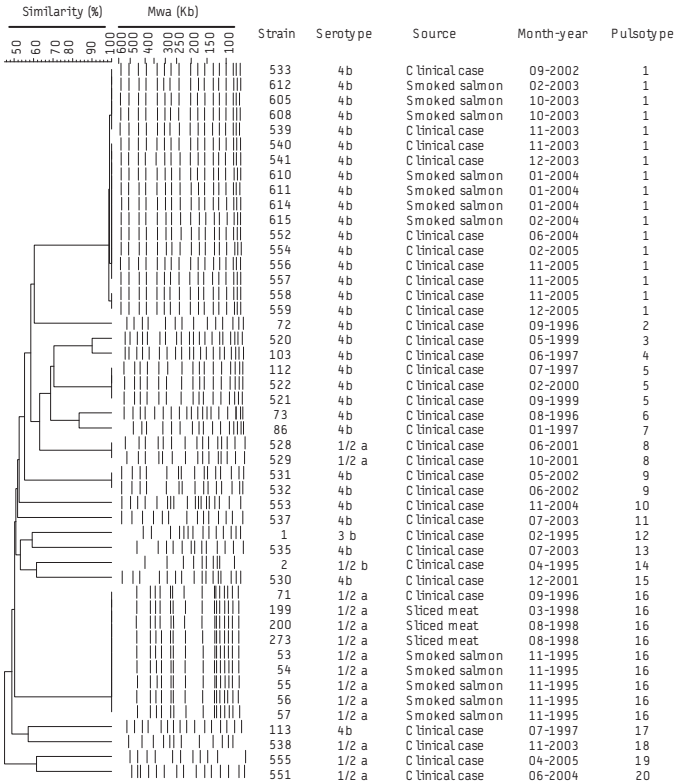
(21.8%, n=19) and finally by serotype 1/2b (3.5%, n=3). When food categories were examined according to serotype, we found that serotype 4b was the predominant in smoked fish (56.7%, n=17), while serotype 1/2a was the most frequent among sliced meat products (57.1%, n=32). The unique strain isolated in liver pate belonged to serotype 1/2c.

PFGE results

PFGE revealed a total of 20 different pulsotypes among clinical isolates, distinguished by one or more band differences ranging in size from 50 to 500 kb (Figure 3). Pulsotypes 1, 5, 8 and 9 contained two or more strains which remained indistinguishable from each other. While strains of pulsotype 5 (n=3) were recovered from different years, strains of pulsotype 1 (n=10) and pulsotype 9 (n=2) were related in time and geographical distribution, showing that possible outbreaks could have occurred. Among isolates with pulsotype 1, three strains corresponded to listeriosis cases diagnosed between November and December 2003, and four were isolated from four pregnant women affected between November and December 2005. However, the oral interviews about food intake in these patients did not give us information about a possible common food source. With respect to the 87 food isolates, we found 19 different pulsotypes (data not shown), but only two of them were similar to the previous clinical pulsotypes characterised. We found a cluster of seven strains isolated from smoked salmon showing pulsotype 1. Two of these were isolated in October 2003, 1-2 months before the isolation of three clinical strains with identical pulsotype. In addition, pulsotype 16 was shared by a clinical strain and seven food isolates (three isolates from sliced meat and four from smoked salmon).

FIGURE 3

Dendrogram for *Listeria monocytogenes* pulsotypes of all 33 isolates obtained from clinical cases and some isolates from food samples, Navarre, Spain, 1995-2005



Strain	Serotype	Source	Month-year	Pulsotype
533	4b	Clinical case	09-2002	1
612	4b	Smoked salmon	02-2003	1
605	4b	Smoked salmon	10-2003	1
608	4b	Smoked salmon	10-2003	1
539	4b	Clinical case	11-2003	1
540	4b	Clinical case	11-2003	1
541	4b	Clinical case	12-2003	1
610	4b	Smoked salmon	01-2004	1
611	4b	Smoked salmon	01-2004	1
614	4b	Smoked salmon	01-2004	1
615	4b	Smoked salmon	02-2004	1
552	4b	Clinical case	06-2004	1
554	4b	Clinical case	02-2005	1
556	4b	Clinical case	11-2005	1
557	4b	Clinical case	11-2005	1
558	4b	Clinical case	11-2005	1
559	4b	Clinical case	12-2005	1
72	4b	Clinical case	09-1996	2
520	4b	Clinical case	05-1999	3
103	4b	Clinical case	06-1997	4
112	4b	Clinical case	07-1997	5
522	4b	Clinical case	02-2000	5
521	4b	Clinical case	09-1999	5
73	4b	Clinical case	08-1996	6
86	4b	Clinical case	01-1997	7
528	1/2 a	Clinical case	06-2001	8
529	1/2 a	Clinical case	10-2001	8
531	4b	Clinical case	05-2002	9
532	4b	Clinical case	06-2002	9
553	4b	Clinical case	11-2004	10
537	4b	Clinical case	07-2003	11
1	3 b	Clinical case	02-1995	12
535	4b	Clinical case	07-2003	13
2	1/2 b	Clinical case	04-1995	14
530	4b	Clinical case	12-2001	15
71	1/2 a	Clinical case	09-1996	16
199	1/2 a	Sliced meat	03-1998	16
200	1/2 a	Sliced meat	08-1998	16
273	1/2 a	Sliced meat	08-1998	16
53	1/2 a	Smoked salmon	11-1995	16
54	1/2 a	Smoked salmon	11-1995	16
55	1/2 a	Smoked salmon	11-1995	16
56	1/2 a	Smoked salmon	11-1995	16
57	1/2 a	Smoked salmon	11-1995	16
113	4b	Clinical case	07-1997	17
538	1/2 a	Clinical case	11-2003	18
555	1/2 a	Clinical case	04-2005	19
551	1/2 a	Clinical case	06-2004	20

Discussion

A collaborative surveillance study of *Listeria* infections in Europe led by the French Institut de Veille Sanitaire and Institut Pasteur [21] described an incidence of 0.15 cases per 100,000 inhabitants in the year 2000 for Spain (based on information from voluntary reporting), whilst for that same year the incidence detected for Navarre in our study was 0.91 (five cases reported in our active surveillance). Similarly, for 2004, the EFSA report [22] described an incidence of 0.20 in Spain whereas the rate obtained in our study was 0.51 (three cases). Considering that the likelihood of listeriosis in Navarre was similar to the whole of Spain (similar proportion of people at risk and food intake habits), we think that the active surveillance carried out in our study was the reason for the higher incidence obtained in Navarre compared to that reported for the whole of Spain in the mentioned publications. Similar incidence rates to those we found in Navarre were described in countries with mandatory notification of listeriosis, such as Denmark, where Gerner-Smidt et al. [23] reported incidences ranging from 0.45 to 0.75 cases per 100,000 between 1994 and 2003, and increasing to 0.8. during 2004 [22]. It is interesting to note that the sensitivity of the Danish system for *Listeria* infection is thought to be almost 100% and is based on immediate notification to the National Surveillance Centre (Staten Serum Institute) of all patients from whom *Listeria* has been isolated. This corresponds to the methods used in our study. Likewise, active surveillance of *L. monocytogenes* infections in the Netherlands revealed an increase in incidence from 0.26 cases per 100,000 inhabitants in 2001 to 0.62 per 100,000 in 2005 [24].

It should be noted that the 52% case-fatality rate (foetal death included) obtained in our study is similar to that described in other Spanish reports [25] but higher than the average reported over recent years in the EFSA publication [4]. However, the EFSA report admits that the lower than expected reported case-fatality rate might be due to a lack of data on patient outcomes after the initial notification, which indicates the importance of clinical data recovery to assess the impact of listeriosis.

With respect to listeriosis associated with pregnancy, several authors have reported high incidences with case-fatality rates above 45% [6,26]. The high numbers of listeriosis cases we detected among this group in Navarre is due, in part, to the fact that we included investigation of tissue samples obtained from hospitalised women after miscarriage. However, it should be noted that testing for *L. monocytogenes* was performed in a similar way during the entire study period, without a systematic analysis of all miscarriage tissues. So, the increase of the number of pregnancy-associated cases in recent years can not be considered a surveillance artefact. Taking into account that bacterial cultures are not routinely obtained from spontaneously aborted foetus or stillborn neonates in a wide range of Spanish hospitals, we believe that the true incidence of listeriosis in this risk group may still be underestimated. In order to assess the rates more accurately, we recommend the routine investigation of *L. monocytogenes* in tissue after miscarriage and stillbirth in the whole of Spain and also at European level.

To assess the real impact of listeriosis in Spain and in the whole of EU, better harmonisation of data collection systems at national

level is required. Validated clinical and food questionnaires would be valuable in all diagnosed or suspected cases of listeriosis, providing more precise and complete information about symptoms, outcomes and food consumption habits of affected people. This way we would be able to conduct epidemiological studies (useful for outbreak investigations) and to provide dietary advice to high-risk individuals in avoiding specific foods. In our study, suspecting a possible outbreak in December 2005 after four cases had been detected in a short period of time, we decided to interview the patients using a food questionnaire but this oral survey failed to identify a possible common food source. Nevertheless, this does not exclude the possibility of a common source but rather reflects the limitations of this preliminary survey and the need for validation. Active surveillance in Italy [27] which involved distributing clinical and food questionnaires to the hospitals and the characterisation of all strains resulted in a higher number of cases of listeriosis than reported by mandatory notifications.

In addition to the accurate recovery of epidemiological information, there is a need to isolate and characterise *L. monocytogenes* clinical strains. Although most cases of listeriosis occur sporadically [28], serological and molecular analysis could help us to relate sporadic cases that are geographically and time-related, allowing the detection of possible outbreaks that perhaps go unnoticed if few people are involved. Serological characterisation is useful for rapid screening of strains during suspected outbreaks. In line with the findings of several authors [6,11,15,25,29] our results confirm the fact that most cases of human listeriosis are caused by serotypes 4b (75.8%). However, recent studies observed a variation of this classical distribution with an increase of serotype 1/2a [27,30].

The combination of serology and PFGE has provided us with the opportunity to link sporadic cases on three different occasions during the period 2002-2005. It should be noted that pulsotype 1 was the most frequent among the clinical strains isolated in the study (10 out of 33), and also one of the predominant profiles in the food isolates (seven isolates from smoked salmon). The profile of three patient isolates in November-December 2003 (pulsotype 1) was indistinguishable from that of two isolates obtained from smoked salmon a month before, but we had no available information about the food intake of these patients as the food questionnaire was not carried out at that time. The repeated isolation of pulsotype 1 in smoked salmon (2003-2004), and pulsotype 16 in smoked salmon and sliced cooked meat (1995-1998) suggests the persistence of specific subtypes in food processing plants. Considering that food products at risk of containing *Listeria* are often commercially available over a wide area, characterisation of food and clinical strains should be managed at a national level in order to trace probable sources of infection and to detect related cases occurring in other regions of Spain.

In conclusion, the present study shows that harmonised and active surveillance of listeriosis is needed in Spain in order to increase knowledge about real impact of this serious health problem. Accurate national surveillance should be based on the obligatory notification of listeriosis, the collection of epidemiological information by the application of a standardised food and clinical questionnaire and the sending of isolated strains to a reference laboratory for a serological and molecular characterisation. This active surveillance could be extended at European level, improving the available information to detect compatible cases and to trace probable sources of infection.

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