

Rapid communications

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Rapid communications

AN OUTBREAK CAUSED BY HANTAVIRUS IN THE BLACK SEA REGION OF TURKEY, JANUARY – MAY 2009

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We present a preliminary report of 12 laboratory-confirmed cases of haemorrhagic fever with renal syndrome (HFRS) in Turkey, diagnosed between January and May 2009 according to the clinical symptoms and serological confirmation. Studies are still ongoing to better understand the dynamics of the reservoir population as well as the epidemiological characteristics and risk factors among humans.

Background

Since the first hantavirus, Hantaan virus (HTNV), was isolated in 1976, many other hantaviruses have been identified, and at least 22 of them are pathogenic to humans. Hantaviruses are rodent-borne, enveloped RNA viruses with a diameter of 120 nm, belonging to the family Bunyaviridae. Each hantavirus is carried by a specific rodent species (subfamilies: *Murinae*, *Arvicolinae*, *Sigmodontinae*) or insectivore species and transmission to other species including humans is a “dead end” for the virus [1-4]. Transmission of hantavirus is believed to occur mainly through aerosols from infected animal excreta, i.e. saliva, urine and faeces. Although this is undoubtedly the most common route of transmission among rodents and from animals to humans, virus transmission by bite may also occur and result in both animal and human infection [1,4-6]. Hantaviruses have the potential to cause two different types of diseases in humans: haemorrhagic fever with renal syndrome (HFRS) and hantavirus pulmonary syndrome (HPS).

Outbreak investigation

In January 2009, the Ministry of Health in Turkey (MoH) was informed by the public health authorities of provinces Zonguldak (A) and Bartın (B) about a cluster of three suspected cases of HFRS with clinical symptoms. Both provinces share a common border and have similar natural vegetation and animal diversity (Figure 1).

A blood sample was taken from only one of the three first reported cases and the laboratory investigation confirmed an infection caused by hantavirus. This was the first laboratory-confirmed case of hantavirus infection in Turkey. Therefore, an epidemiological investigation was initiated to facilitate case-finding in the affected area.

For investigation purposes, the following case definitions were adopted:

- A suspected case of HFRS was defined as a patient:
- without any previously known blood or kidney disease;

- who has been in a location with suspected or confirmed cases of HFRS within the last two months before onset of illness;
- with an acute illness characterised by abrupt onset with at least two of the following criteria: fever, diarrhoea, nausea, myalgia, weakness, abdominal pain, chill, thrombocytopenia, impaired renal function.

A confirmed case was defined as a patient with IgM positive test result by using immunoblot technique in the serum sample.

In February 2009, all physicians and the local authorities in the two provinces affected were informed by the MoH about an increased risk of hantavirus infection. A case management flow chart was drawn and distributed to all healthcare facilities. It was requested that patients who meet the case definition criteria for suspected case of HFRS should be referred to the Zonguldak Karaelmas University Hospital and serum and urine samples should be sent to the Refik Saydam National Public Health Agency in Ankara.

Indirect immunofluorescence assay (IFA) (hantavirus mosaic-1 (Euroimmun, Germany)) was used as diagnostic test and performed according to the manufacturers' instructions. Result at a dilution $\geq 1:100$ was considered positive. All of the IgM IFA-positive cases were confirmed by immunoblot (Euroimmun, Germany). In addition, molecular analysis by generic hantavirus RT-PCR method was performed on samples (serum/plasma and/or urine) taken from 14 patients.

Preliminary findings

Between 22 January and 1 May 2009, a total of 25 suspected cases of HFRS were reported. Blood samples were taken from 23 patients and tested for hantaviruses. The remaining two patients had died before sampling, so they are considered as suspected cases. We confirmed that 12 out of 23 samples (52.2%) were positive for hantavirus in IFA and immunoblot. However, no positive result was found in the plasma/serum (n=14) and/or urine samples (n=6) by RT-PCR method.

The epidemic curve is shown in Figure 2. The mean age of laboratory-confirmed patients was 56 years (range 22-78), the male to female ratio was 6:1. All 25 suspected cases were admitted to hospital. The fatality rate among these hospitalised patients was 8%.

Seroprevalence study

From 18 to 20 March 2009, a seropositivity study for hantaviruses among the healthy population was carried out in province B. The aim of the study was to show the presence of hantavirus in the area and to identify the possible risk factors of infection. In the study, convenience sampling method was used, the study population consisted of six groups: four of these were at known risk for hantavirus infection (hunters, foresters, villagers involved in forestry, miners), subjects of the fifth group originated from the three villages where confirmed/suspected cases were living, and the last group was from an urban area of province B. A total of 306 sera were collected. A questionnaire was filled in for each person including demographic data, clinical symptoms (if any) and the date of onset of symptoms, diagnostic tests and treatment, and epidemiological data on housing conditions, travel history and animal exposure in the past two months.

The final results of this study are not yet available. To date, the laboratory testing has been completed but the statistical analysis is still being performed by the epidemiology unit. Preliminary results indicate that the overall seroprevalence was 5.2%.

Conclusion

We confirmed 12 cases of HFRS reported in Turkey in 2009 using IFA and immunoblotting techniques. Our results were serologically positive for Puumala subtype, but it should be considered that among the subtypes of hantavirus, cross-reactivity is frequently seen serologically. In addition, the generic hantavirus RT-PCR was not positive; hence, sequence analyses have not been performed.

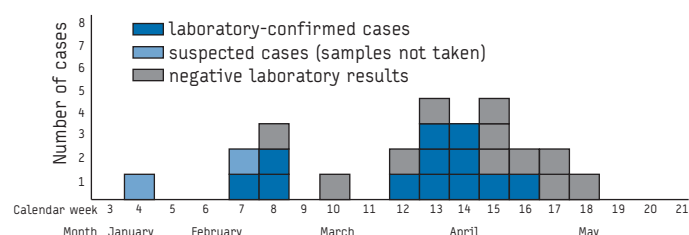
FIGURE 1

Map of Turkey indicating the area where human cases of hantavirus infection were reported in January - May 2009



FIGURE 2

Distribution of suspected cases of hantavirus infection reported in Turkey, from January to May 2009, by week of notification



The reason for this might be that viraemia is very short in hantavirus infections. Another limitation of the study was that neutralisation tests have not been performed.

We found a 5.2% seroprevalence of hantavirus antibodies amongst the healthy but at-risk population of one of the affected provinces. These preliminary data show that the virus is circulating in the area. Until now, asymptomatic or mild infections with non-specific symptoms may have been the cause for the underestimation of the real number of hantavirus infections. It is necessary to finalise the statistical analysis of the seroepidemiological study to plan further studies and surveys in Turkey. The plan is to inventorise the local rodent species, identify circulating hantavirus serotypes in rodents, perform molecular characterisation of strains isolated from rodents and humans and compare them with strains circulating in the neighbouring countries, and investigate transmission mechanisms and the time and space-distribution of human hantavirus infections.

Hantavirus causes a significant number of human illnesses, making it a global public health threat [7]. The presence of the virus in Turkey is not surprising because it is circulating in the neighbouring countries [1,4,7]. In the affected area, a comprehensive preventive strategy against hantavirus infection, including health education and promotion activities, rodent control and surveillance, has been implemented. For example, guidelines were distributed for public on rodent proofing and trapping in and around homes, and the careful disposal of dead rodents.

Acknowledgements

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INFLUENZA A(H5N1): AN OVERVIEW OF THE CURRENT SITUATION

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Influenza viruses continue to threaten the world with a new pandemic. While currently attention is focused on the newly emerged A(H1N1) virus, the avian influenza A(H5N1) virus is still a cause of concern. Extended research is focused on the genetic evolution of the viruses, as well as their susceptibility to available antiviral drugs. One of the major priorities of the World Health Organization is to develop candidate vaccines, four of which are already licensed for use in the European Union. Since the last influenza pandemic in 1968, our knowledge of the influenza virus and its biology has greatly increased, revealing new avenues in the research for antiviral strategies and the development of effective vaccines.

Introduction

Influenza viruses continue to threaten the world with a new pandemic. While currently attention is focused on the newly emerged influenza A(H1N1) virus spreading around the globe, the avian influenza A(H5N1) virus is still a cause for concern, not only as a threat in itself but also in combination with the new influenza A(H1N1) epidemic. The newly emerged influenza A(H1N1) strain is spreading rapidly to the human population, which indicates sustained human-to-human transmission, compared to the avian A(H5N1) strains which are highly pathogenic, but with limited ability for human-to-human transmission. No one can surmise the effect of an A(H1N1) spread to the countries where A(H5N1) is endemic. For this reason, continuous influenza surveillance and global monitoring of influenza infections is critical at this point.

Since the re-emergence of the A(H5N1) influenza virus in 2003 in Asia, Africa, the Pacific Region, Europe and the Middle East, the virus has become endemic in some countries, and continues to cause outbreaks in poultry. More importantly, it is now causing sporadic human infections that are associated with high morbidity and mortality rates. Evidently, should an avian influenza pandemic occur, the outcome is likely to be very severe. It is thus of great importance to monitor the emergence of such infections both in poultry and in humans, to isolate and characterise the circulating viruses and to invest in antiviral susceptibility testing and vaccine development.

The World Health Organization is coordinating the global response to human cases of H5N1 avian influenza and monitoring the corresponding threat of an influenza pandemic. The cumulative number of cases of A(H5N1) virus infections reported to WHO until 15 May 2009, was 424 with 261 subsequent deaths, accounting

for 61% mortality rate (Figure) [1]. 2006 was the year with the highest number of reported cases and a case fatality ratio of 63% [2]. The reported number of cases declined after that, probably reflecting the successful monitoring and detection of infections in poultry and humans. Fatality rates were high in all age groups, but were the highest in persons between 10 and 39 years of age, regardless of their sex. Cases occurred all year round.

Genetic characterisation of circulating viruses

The hemagglutinin sequences of circulating influenza A(H5N1) viruses are classified into distinct clades. Recent human clade 1 infections have been limited to Cambodia, Thailand and Viet Nam. Clade 2.1 viruses have continued to circulate in poultry and have caused human infections in Indonesia, while clade 2.2 viruses have the most diverse distribution, with outbreaks in birds in over 60 countries in Africa, Asia and Europe and human infections in Azerbaijan, Bangladesh, China, Djibouti, Egypt, Iraq, Nigeria, Pakistan and Turkey. Clade 2.3.4 viruses have been responsible for human infections in China, Lao People's Democratic Republic, Myanmar and Viet Nam. Since September 2008, human infections have been limited to China, Viet Nam, Cambodia, Egypt and Indonesia [3].

A number of recent reports highlight the importance of mutations in A(H5N1) avian influenza viruses, indicating that these genetic variations may increase the possibility of a new pandemic. Influenza viruses are inherently unstable, due to their segmented RNA genome and the lack of a genetic proofreading mechanism that allows undetected errors that occur during replication. Since the first documentation of human infection with the A(H5N1) avian influenza virus in 1997, the virus has undergone several changes. These changes have influenced the patterns of virus transmission and have spread amongst domestic and wild birds. Human infections are still considered a relatively uncommon event as the virus does not spread easily from birds to humans or from human to human. Trustworthy prediction of the evolution of influenza viruses cannot be made, as it is almost impossible to identify whether or when the A(H5N1) virus might obtain the characteristics needed to spread among humans and there is also a lack of knowledge as to which specific mutations will allow human-to-human transmission of the virus [4].

Fortunately, the A(H5N1) viruses have not yet demonstrated the capacity for efficient and sustained human-to-human transmission, although limited transmission is believed to be the cause of

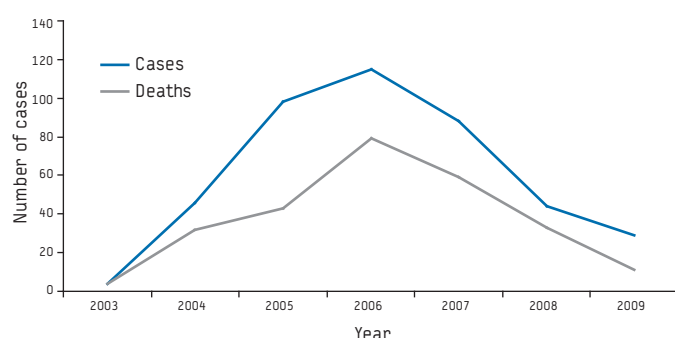
some family clusters of cases [5]. Since those sporadic family clusters of A(H5N1) cases may be the first suggestion of a viral or epidemiologic change, they are being thoroughly investigated in order to determine any direct human-to-human transmission of the virus [6]. Such clusters involving highly probable human-to-human transmission have been documented in Egypt, China, Thailand, Vietnam, Indonesia and Pakistan [7,8]. Studies have also shown a higher prevalence of A(H5N1) antibodies among healthcare workers exposed to A(H5N1) patients in comparison with the prevalence among non-exposed healthcare workers. These findings constitute the epidemiological evidence that A(H5N1) viruses were indeed transmitted from patients to healthcare workers, who then possibly had an asymptomatic infection [9]. Such unconfirmed cases have a potentially huge impact on the case fatality ratio and could indicate that the A(H5N1) virus is probably less lethal than currently assumed.

Furthermore, it was recently observed that undetected A(H5N1) cases may be occurring in Egypt, given the unusual age-specific and sex-specific case incidence and fatality rates, which can be partly attributed to the existence of undetected fatal or non-fatal atypical or asymptomatic human A(H5N1) infections [8]. Asymptomatic human infections with A(H5N1) have been also reported from China, Vietnam, Japan, Thailand, and Korea although limited investigations suggest that the frequency of asymptomatic or clinically mild A(H5N1) virus infection have been rare since 2003 [10]. Most human cases have demonstrated the increased pathogenicity of the A(H5N1) strains.

Tumpey and colleagues, who reconstructed the A(H1N1) virus of 1918, have identified a number of common points between the viruses of Spanish and the avian A(H5N1) influenza. It was concluded that it is especially the polymerase, the hemagglutinin (HA) and neuraminidase (NA) genes that caused the extreme virulence and that the sequences of the polymerase proteins (PA, PB1, and PB2) of the 1918 virus differ by only 10 amino acids from the avian influenza viruses [4]. Human forms of seven out of the 10 amino acids have already been identified in currently circulating influenza A(H5N1) viruses. It is likely that also the other mutations will eventually emerge and make the A(H5N1) virus better suited for human-to-human transmission.

FIGURE

Human cases (n=424) and deaths (n=261) caused by influenza A(H5N1) virus infection, 2003-2009



Source: World Health Organization. Cumulative number of confirmed human cases of avian influenza A(H5N1) reported to WHO. 15 May 2009 [1].

Another important factor is the change of the HA protein to a binding preference for alpha 2,6 sialic acid, which is the major form in the human respiratory tract. In avian viruses the HA protein preferentially binds to alpha 2,3 sialic acid, which is the major form in the avian enteric tract. It has been shown that only a single amino acid change can result in the change of this binding preference. Altogether it seems that only a few mutations are needed to make the A(H5N1) avian influenza virus a pandemic virus, with possible mortality rates resembling the rates of the Spanish flu, which killed over 40 million people worldwide. Taubenberger et al. have recently showed that the 1918 virus was initially an avian virus, like the A(H5N1) [11].

In February 2004 and May 2005, the influenza A(H5N1) virus was detected in pigs in Viet Nam and Indonesia, respectively, increasing fears of the emergence of new variant strains. Along with the continuing pattern of virus circulation in poultry, the occurrence in swine raised the level of concern about the possible evolution of the virus into a strain with pandemic potential, as pigs may act as a mixing vehicle, in which influenza viruses can recombine with genetic reassortment.

In order to detect any variations that might lead to the development of a potentially pandemic strain, WHO influenza reference laboratories, in cooperation with the national influenza centres of affected countries, are isolating circulating influenza viruses and monitoring their variations with molecular techniques.

Vaccine development

One of the major priorities of WHO is to develop candidate vaccines with representative A(H5N1) viruses from all currently circulating clades. As of February 2009, a number of A(H5N1) reassortants have completed the regulatory approval; these reassortants belong to clades 1, 2.1, 2.2, 2.3.4 and 4 and have been developed by: National Institute for Biological Standards and Control (NIBSC), United Kingdom; Centers for Disease Control and Prevention (CDC), United States (US); Food and Drug Administration (FDA), US; and a consortium of St Jude Children's Research Hospital US, University of Hong Kong, China and National Institute of Allergy and Infectious Disease, US (SJ/HKU/NIAID). A number of reassortant viruses that belong to clades 2.2, 2.3.2 and 7 are prepared and awaiting regulatory approval and there are also two viruses (clade 2.3.4 A/chicken/Hong Kong/AP156/2008-like and clade 7 A/chicken/Viet Nam/ NCDV-03/2008) that have been proposed by WHO for candidate vaccine preparation [3].

The procedure for licensing in Europe is centralised through the European Medicines Agency (EMA), although national authorisation may still occur at the level of individual countries. To date, there are four licensed pre-pandemic and pandemic vaccines in the European Union. The first approved pre-pandemic vaccine is Prepandrix; it is an A(H5N1) adjuvanted vaccine manufactured by GlaxoSmithKline (GSK) plc, that could potentially protect against a range of different emerging H5N1 strains. The second is Daronrix vaccine, also developed by GSK, which contains inactivated influenza viruses of the A/Viet Nam/1194/2004 (H5N1) strain. When the World Health Organization declares a pandemic, Novartis is approved by EMA to adapt Focetria vaccine to contain the pandemic strain. In addition, Baxter's A(H5N1) vaccine, Celvapan, is the first approved pandemic vaccine that is cell-cultured based. A number of other countries, including US, Australia, Japan and China, also have licensed products [12].

On 12-13 February 2009, the Department of Initiative for Vaccine Research (IVR) of WHO convened the 4th meeting on "Evaluation of pandemic influenza prototype vaccines in clinical trials". Among A(H5N1) vaccines that have been evaluated, the egg-derived split/subunit, oil-in-water adjuvanted vaccines have demonstrated dramatic antigen-sparing, cross-clade immune responses, and effective priming. The MF59-adjuvanted A(H5N1) vaccine developed by Novartis is being evaluated in phase II trials and Sanofi-Pasteur's AFO3-adjuvanted A(H5N1) vaccine is undergoing phase II trials. Other market-approved A(H5N1) vaccine formulations include egg-derived, alum-adjuvanted whole or split virus vaccines in Japan (Biken), China (Sinovac) and Australia (CSL) [13]. The safety and immunogenicity of several A(H5N1) vaccines have been confirmed for both children and the elderly, while the evaluation of prototype pandemic vaccines for these groups is in progress. However, more data need to be accumulated, especially for the very young age groups from six months to three years of age, as in the event of a pandemic, priority immunisations will target the young, the elderly and the individuals that belong to high risk groups [13].

The development, the clinical trials and the licensing process of A(H5N1) vaccines is progressing and it is the responsibility of national authorities to decide on the use of one or more of these for the production of pilot lots of vaccine, depending on the geographical spread, epidemiology and antigenic and genetic properties of A(H5N1) viruses that are circulating in the area. A number of countries have been stockpiling such vaccines. Clinical trials are under way to evaluate vaccination schedules and to detect cross-immunity by vaccines containing viruses from different clades.

Antiviral susceptibility

Until the production of vaccines for prophylaxis against influenza A(H5N1) virus infection is completed, antiviral drugs are the first line of defence. For the treatment of seasonal influenza, two drug categories are currently commercially available, the neuraminidase (NA) inhibitors: oseltamivir and zanamivir, and the matrix protein 2 (M2) inhibitors: amantadine and rimantadine. Early administration of these drugs can reduce the severity and duration of illness from seasonal influenza viruses [14].

Though clinical data related to A(H5N1) infections are limited, it has been shown that early administration of NA inhibitors can decrease the severity of the disease and increase the prospects of survival. In case of a pandemic, the A(H5N1) virus is expected to be susceptible to the NA inhibitors. M2 inhibitors could also be administered against pandemic influenza, however resistance to these drugs may occur rapidly thus reducing their efficacy against the virus. In addition, a high percentage of currently circulating avian influenza A(H5N1) strains is already fully resistant to those drugs [15].

Concerning the NA inhibitors, some of the limitations for many countries are the low production capacity and the economic restraint. Due to the complex and time consuming manufacturing process, the producer of oseltamivir has to build a manufacturing capacity to meet the demands of the global market.

WHO has reserved a certain amount of oseltamivir for use in the first areas affected by an emerging pandemic virus. Based on mathematical modelling studies, the drugs could be utilised

for protection purposes at the beginning of a pandemic in order to delay its international spread and gain time to complete the vaccine supply. Influenza surveillance in the affected areas needs improvement, especially regarding the detection of clusters of cases which are closely related in time and place, in order to increase the chances that WHO's rapid intervention will be successful [16,17].

As antiviral susceptibility profiles are changing in various affected areas, combined treatment with both available antiviral drug classes is also a possibility. It is important to clarify whether in a pandemic situation, highly pathogenic A(H5N1) influenza viruses that will have acquired affinity for human rather than avian respiratory epithelium, will also have altered susceptibility to NA inhibitors, which is considered the first line of defence. Relevant studies have not shown such a relation [18].

Resistance to antiviral drugs in influenza viruses can emerge following medication or may result from natural variation. The essential task of the recognition of influenza virus variants resistant to these drugs is accomplished by a select group of the global experts that are members of the Neuraminidase Inhibitor Susceptibility Network, organised by WHO [14,19]. Recent reports on the drug-resistance of the seasonal A(H1N1) virus strains from countries of the northern hemisphere, show a high percentage of strains resistant to oseltamivir. A total of 30 countries have reported resistance to oseltamivir in A(H1N1) viruses, whereas A(H3N2) strains seem to be susceptible to oseltamivir and resistant to adamantanes [20,21].

Basic research on influenza viruses provides a much better understanding of the biology of the virus and offers the possibility of the development of new antiviral drugs [22]. Antibodies against HA that can neutralise virus infection can be potentially developed into effective influenza prophylaxis. Several candidate antibodies against A(H5N1) have been identified, and have found to be effective in neutralising the virus infectivity in tissue culture and experimental animals. Furthermore, short interfering (si) RNAs, that are able to inhibit the expression of specific genes by inducing sequence-specific degradation of target mRNA, have been designed against conserved sequences in the influenza A virus nucleoprotein, polymerase and matrix genes. These siRNAs are able to suppress virus replication and significantly reduce virus yields in tissue culture, and in the lungs of infected mice [22]. In addition, molecules that mimic the structures of the double stranded RNA replicative intermediates, essential for replication, are also considered to be potential drugs against influenza. Such molecules are not produced in the host cell, and their presence in mammalian cells stimulates an antiviral response. Although the in vitro data obtained seem promising, it remains to be established if this approach will be effective in preventing influenza virus infection in humans. Similarly, treatment of cells with chloroquine elevates the endosomal pH, and previous studies have demonstrated its inhibitory effects on influenza virus replication [22].

Since the last influenza pandemic in 1968, our knowledge of the influenza virus and its biology has greatly increased, revealing new avenues in the research for antiviral strategies and the development of effective vaccines. It is clear that the development of vaccines will limit the spread of a pandemic strain and new antiviral strategies will provide new means in countering a new pandemic. However, it is likely that during a pandemic, people that live in many parts of the world will not be able to afford the costs of prevention and

treatment. One of the major challenges in a new pandemic will be the availability of anti-influenza virus vaccines and drugs that can be easily produced on a mass scale, and distributed to all parts of the world.

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Research articles

RAPID SPREAD OF DRUG-RESISTANT INFLUENZA A VIRUSES IN THE BASQUE COUNTRY, NORTHERN SPAIN, 2000-1 TO 2008-9

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A worldwide increase of adamantane-resistant influenza A(H3N2) and oseltamivir-resistant influenza A(H1N1) viruses has been observed in recent years. The aim of this study was to analyse the prevalence of antiviral drug-resistant influenza A in a region of northern Spain. Resistance to adamantanes was detected in 45.3% (68/150) of influenza AH3 viruses analysed for the period from 2000-1 to 2008-9. Adamantane-resistance was absent in our region during the 2000-1 to 2002-3 influenza seasons. However, after the first adamantane-resistant virus (characterised as A/Fujian/411/2002) was detected in the 2003-4 season, a rapid increase in the proportion of resistant strains was observed (4.9% [2/41], 80% [8/10] and 100% [53/53] in the 2004-5, 2006-7 and 2008-9 seasons, respectively). Four of the first five adamantane-resistant AH3 viruses detected were isolated from adult patients, but the subsequent spread was observed mainly among children. No resistance to adamantanes was detected among the 65 influenza AH1 viruses analysed throughout the study period. Among the 172 influenza A (76 AH1 and 96 AH3) viruses analysed, five strains (AH1 with mutation H274Y) showed oseltamivir resistance, and all were detected in the last season. Amantadine use was very scarce in our region, and oseltamivir was not used at all; therefore the increase of resistance was attributed to imported drug-resistant influenza viruses.

Introduction

Only four licensed influenza antiviral agents are currently available: the adamantanes - amantadine and rimantadine, and the neuraminidase inhibitors - zanamivir and oseltamivir. Amantadine hydrochloride was approved in the United States (US) in 1966 for chemoprophylaxis of influenza A(H2N2) and has been used to prevent and treat influenza A for more than three decades [1]. Although adamantanes, which block the function of the M2 protein, can reduce the severity and duration of influenza A infection in healthy adults, their use has been limited due to rapid selection of resistant viruses during treatment. In recent years, a high percentage of influenza A(H3N2) viruses circulating in Asia, America and eastern Europe have shown resistance to adamantanes [2]. Southern Europe seems to have escaped this problem but resistance to oseltamivir has been observed since the beginning of the 2007-8 influenza season among influenza A(H1N1) viruses [3].

The aim of this study was to determine the prevalence of resistance to adamantanes and neuraminidase inhibitors (oseltamivir and zanamivir) in influenza A isolates obtained during nine seasons (2000-1 to 2008-9) in the Basque Country, northern Spain, a region that borders the southwest of France.

Materials and methods

The study was performed in the Microbiology Department of Hospital Donostia, which is the Reference Laboratory for Influenza Infections in the Basque Country, and has been integrated in the Spanish Influenza Surveillance System since 1998. Of the available 587 respiratory samples that tested positive by cell culture for influenza A virus, 282 (48%) were selected for the susceptibility study. All the minority subtype strains and an unselected sample of the predominant subtype circulating in each season were included. Most of the strains included in the study were obtained from consecutive patients who had consulted physicians participating in the Spanish Sentinel Influenza Surveillance System; a smaller proportion (19%) of isolates studied were obtained from patients admitted to or treated in the emergency department of our hospital. The age and gender of patients included in this study represent the normal distribution of people with influenza in our region. An aliquot of 400 µL of all the original samples was conserved at -80°C until susceptibility studies were performed. The distinct number of clinical samples studied in each season was dependent on differences in the season-to-season dynamics of influenza A viruses. The exact numbers of isolates of AH1 and AH3 viruses tested in each season and for each drug class are listed in the Table. The resistant strains mentioned in this study have not previously been reported in any other publication, except for four AH1 oseltamivir-resistant strains reported to the European Influenza Surveillance Scheme (<http://www.eiss.org/>).

Genotypic resistance was detected by sequencing of viral genome fragments and identification of mutations previously associated with drug-resistance. Viral RNA was extracted from respiratory samples using the bioMérieux NucliSENS easyMAG system (bioMérieux, Marcy l'Etoile, France). Transcription of RNA to cDNA was performed with M-MuLVreverse transcriptase (Promega, Madison, WI, US) using random primers. An M2 gene fragment (330 bp) [4,5] and a neuraminidase gene fragment

(708 bp) [6,7] were amplified for analysis of adamantane and neuraminidase inhibitor susceptibility, respectively. Amplified gene fragments were sequenced in an ABI PRISM 3100 Genetic Analyser (Applied Biosystems, Foster City, CA, US) and amino acid-deduced sequences were obtained. M2 and neuraminidase sequences were analysed to identify mutations previously associated with antiviral resistance. The amino acid substitutions L26F, V27A, A30T, S31N and G34E in the M2 protein were associated with resistance to adamantanes [4,8], the amino acid substitutions H274Y, E119V and R292K in the neuraminidase protein were associated with resistance to oseltamivir, and substitutions Y155H and I222T with resistance to zanamivir [9]. Phylogenetic analysis of the haemagglutinin gene was performed at the Reference Center for Influenza Surveillance in Spain (Instituto de Salud Carlos III).

Results

Mutations conferring resistance to adamantanes were detected in 31.6% (68/215) of the influenza A viruses studied over nine seasons (2000-1 to 2008-9). Resistance to adamantanes was detected in 45.3% (68/150) of influenza AH3 viruses, while no influenza AH1 viruses with mutations conferring resistance were found (0/65) (Table).

The first case of adamantane-resistance was detected in a sample obtained in November 2003 from a 37-year-old woman with typical influenza symptoms (high temperature, headache, muscle ache and respiratory symptoms). Phylogenetic analysis of the haemagglutinin gene confirmed its similarity with the A/Fujian/411/2002 strain. The proportion of strains resistant to adamantanes among AH3 viruses was 0% (0/20) from 2000-1 to 2002-3 season, 7.9% (5/63) from 2003-4 to 2005-6 season and 94% (63/67) during the last three seasons (Table, Figure). Four of the first five cases of adamantane-resistant AH3 viruses were detected in young adults (25 to 47 years old), but the subsequent eight cases were detected in children. Throughout the study period, resistance to adamantanes was more frequently detected among children (32/80, 40%) than adults (36/135, 26.7%) ($P < 0.05$). All adamantane-resistant strains except one showed serine-to-asparagine (S31A) amino acid substitution at position 31. The

remaining strain showed glycine-to-glutamic acid (G34E) amino acid substitution at position 34.

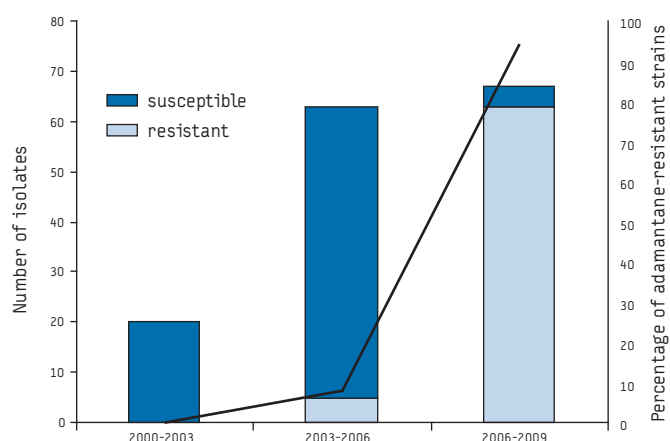
Among 172 influenza A (76 AH1 and 96 AH3) isolates analysed for resistance to oseltamivir, five strains (AH1 with mutation H274Y) were found resistant. All of these were characterised as A/Brisbane/59/2007 (H1N1)-like AH1. The first was isolated in November 2008 from an 18-year-old man with typical influenza symptoms. Resistance to zanamivir was not detected in any of the 172 isolates studied.

Discussion

The results of this study show that, unlike the situation before 2004-5, most of the influenza AH3 virus strains currently circulating in our region are resistant to adamantanes (100%

FIGURE

Triannual distribution of adamantane-resistant influenza AH3 virus strains detected between the 2000-1 and 2008-9 seasons in the Basque Country, northern Spain



TABLE

Number of influenza A viruses included in the genetic analysis of antiviral resistance during nine influenza seasons (2000-1 to 2008-9) in the Basque Country, northern Spain.

Season	Adamantanes		Oseltamivir*		Predominant virus in the season
	AH1	AH3	AH1	AH3	
	investigated/resistant	investigated/resistant	investigated/resistant	investigated/resistant	
2000-1	7/0	1/0	1/0	0/0	A/B
2001-2	3/0	18/0	0/0	7/0	A(H3N2)/B
2002-3	13/0	1/0	3/0	0/0	B/A(H1N1)
2003-4	0/0	18/1	0/0	2/0	A(H3N2)
2004-5	0/0	41/2	0/0	5/0	A(H3N2)
2005-6	19/0	4/2	8/0	0/0	A(H1N1)/B
2006-7	0/0	10/8	0/0	20/0	A(H3N2)
2007-8	18/0	4/2	59/0	9 /0	A(H1N1)/B
2008-9	5/0	53/53	5/5	53/0	A(H3N2)
Total	65/0	150/68	76/5	96/0	

* Resistance to zanamivir was not detected in any of the 172 influenza A viruses studied

resistance in the 2008-9 season). In 2005, genetic studies confirmed the emergence of adamantane-resistant influenza A(H3N2) strains in China and Hong Kong [10]. In the United States, the frequency of adamantane-resistance increased from 1.9% during the 2003-4 influenza season to 11% during the 2004-5 season [11]. Since then, a growing number of resistant A(H3N2) viruses have been reported in several countries, with 100% resistance reached in some Asian countries [2]. In most cases, the amino acid substitution detected (S31N) was the same as that detected in the present study. Anti-M2-resistant strains easily emerge during treatment with adamantanes [12]. Rimantadine is not licensed in Spain, while amantadine is available on prescription only, is not included in any over-the-counter cold remedies, and its use in our region is scarce. The number of defined daily doses per 1,000 inhabitants per day [13] of amantadine in 2007 in this region, which has approximately 2 million inhabitants, was 0.13 and was mainly limited to the treatment of Parkinson's disease. Therefore, the high resistance rate detected in our region is probably due to importation of resistant strains from other areas. Although the first few cases occurred in adults, the full spread across the region occurred mainly through children.

In January 2008, the emergence of resistance to oseltamivir among influenza A(H1N1) viruses was reported in Europe [3]. The results of analysis of early winter isolates revealed that 20% of the European strains were resistant to oseltamivir but retained sensitivity to zanamivir and adamantanes [3,14]. Up to June 2008, 52 countries worldwide reported similar results. The viruses carried a specific neuraminidase mutation (H274Y) that confers high-level resistance to oseltamivir in N1-containing influenza viruses [3,9,15]. Despite the spread of resistance across Europe, in Spain only two out of 108 (1.9%) A(H1N1) strains previously studied showed the H274Y mutation [14]. In our study, no mutations conferring neuraminidase inhibitor resistance were detected among the influenza A viruses (43 A(H3N2) and 71 A(H1N1) strains) analysed between the 2000-1 and 2007-8 seasons. However, during the 2008-9 season of the five influenza A(H1N1) strains isolated, all five showed the H274Y mutation conferring oseltamivir resistance. Neither the patients nor their closest contacts had received oseltamivir treatment, which suggested that, as occurred with the first adamantane-resistant viruses, these viruses were already resistant before infecting these patients. The north of our region flanks the border with France, where 46.6% of the A(H1N1) viruses studied during the 2007-8 season showed oseltamivir resistance [14].

The present study reveals, once again, how resistance can appear in a region without prior pressure from antiviral drugs and how resistant strains can rapidly disseminate among the population. In addition to promoting influenza vaccination among the general population, research into new anti-influenza agents that could counteract the effects of this resistance should be stimulated.

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Research articles

CLINICAL LABORATORY PRACTICES FOR THE DETECTION OF ROTAVIRUS IN ENGLAND AND WALES: CAN SURVEILLANCE BASED ON ROUTINE LABORATORY TESTING DATA BE USED TO EVALUATE THE IMPACT OF VACCINATION?

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Two rotavirus vaccines have recently been licensed in Europe. Rotavirus surveillance data in many European countries are based on reports of laboratory-confirmed rotavirus infections. If surveillance data based on routine laboratory testing data are to be used to evaluate the impact of vaccination programmes, it is important to determine how the data are influenced by differences in testing practices, and how these practices are likely to affect the ability of the surveillance data to represent trends in rotavirus disease in the community. We conducted a survey of laboratory testing policies for rotavirus gastroenteritis in England and Wales in 2008. 60% (94/156) of laboratories responded to the survey. 91% of reporting laboratories offered routine testing for rotavirus all year round and 89% of laboratories offered routine rotavirus testing of all stool specimens from children under the age of five years. In 96% of laboratories, rotavirus detection was presently done either by rapid immunochromatographic tests or by enzyme-linked immunosorbent assay. Currently, rotavirus testing policies among laboratories in England and Wales are relatively homogenous. Therefore, surveillance based on laboratory testing data is likely to be representative of rotavirus disease trends in the community in the most frequently affected age groups (children under the age of five years) and could be used to help determine the impact of a rotavirus vaccine.

Introduction

Two rotavirus vaccines with comparably good safety and efficacy profiles are now licensed for use [1,2]. In England and Wales the introduction of rotavirus vaccination is currently under consideration. However, some countries have already introduced them into routine childhood immunisation schedules with good effect [3,4]. In the United States, in February 2006, the Advisory Committee on Immunization Practices recommended “RotaTeq®”, a live, oral, human-bovine reassortant rotavirus vaccine for routine use in infants [5]. Preliminary analysis of the national surveillance data for 2007-8 indicated that during the rotavirus season (July 2007 to May 2008) there were fewer cases and that the timing of

the peak in incidence was delayed by two to four months compared to previous seasons [3]. This provides the first indication, post-licensure, that rotavirus vaccination reduces the burden of rotavirus disease in a large population and is consistent with the effects of vaccination seen for other childhood diseases [6].

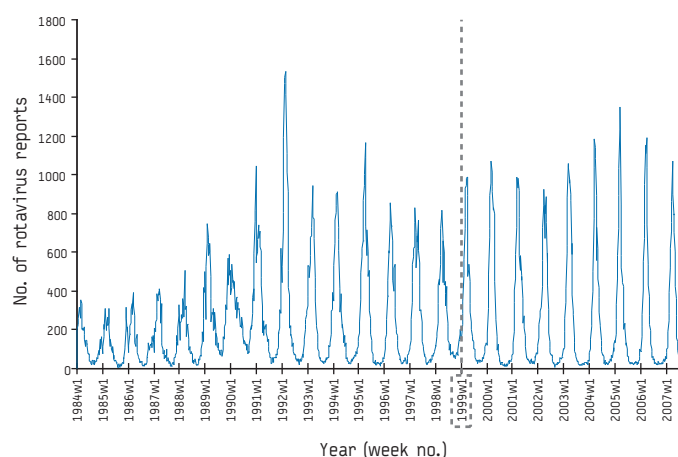
In England the estimated rate of rotavirus gastroenteritis in the community is 7.1 cases per 1,000 persons per year [7]. Though mortality is rare [8], rotavirus is recognised as a major burden on health services. The annual incidence of rotavirus hospitalisations in England is approximately 4.5 per 1,000 children under the age of five years [9,10]. Each year rotavirus is estimated to be responsible for 14,300 hospitalisations, 29,700 accident and emergency consultations and 90,600-133,400 general practice consultations in children under the age of five years in England and Wales [10]. The cost to the National Health Service is estimated to be GBP 14.2 million per year [10].

Current burden of disease estimates are, in part, generated using the national rotavirus surveillance data. Evaluating the need for and the impact of a rotavirus vaccine in the United Kingdom (UK) will rely partly on these surveillance data. At present, surveillance in England and Wales is based on reports of laboratory-confirmed rotavirus infections from over 150 clinical microbiology laboratories. Rotavirus reports show marked seasonality, currently peaking between February and March each year [9]. The majority of reported laboratory-confirmed rotavirus infections occur in children under the age of five years (94% of all reports in which the patient's age is recorded) [9].

However, only a fraction of community cases are reported to national surveillance. It has been estimated that for every rotavirus case reported to national surveillance in England there are 1.5 positive laboratory investigations, 11.3 cases who present to general practice, and overall 35 cases in the community [7]. Using the rotavirus national surveillance data to investigate

population disease patterns or potentially, to evaluate the impact of vaccination, requires that trends in laboratory-confirmed rotavirus infections are representative of trends in rotavirus gastroenteritis in the population. Variations in reporting practices, criteria for rotavirus testing and the diagnostic methods used, either between laboratories or from year to year, may create biases when using laboratory testing for surveillance data. If testing is only offered at certain times of year or in certain age groups, seasonal patterns of rotavirus disease in the population will be distorted in the national surveillance data. Understanding the effect of biases in laboratory testing and reporting practices on national data is fundamental to understanding the extent to which patterns observed in the surveillance data reflect underlying community trends. This study aims to examine how laboratory policies for rotavirus testing and reporting have affected rotavirus surveillance data since 1984.

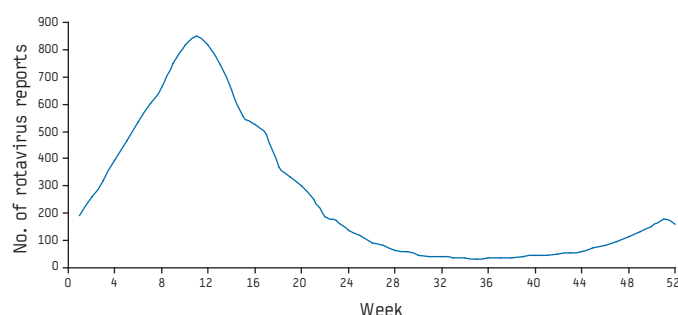
FIGURE 1
Weekly number of laboratory-confirmed rotavirus reports in England and Wales, 1984-2007



The dashed line indicates the start of 1999, the year in which most laboratories switched to using ELISAs or rapid immunochromatographic tests for rotavirus testing.

Source: Health Protection Agency rotavirus national surveillance data.

FIGURE 2
Average weekly number of laboratory-confirmed rotavirus reports in England and Wales, 1984-2007

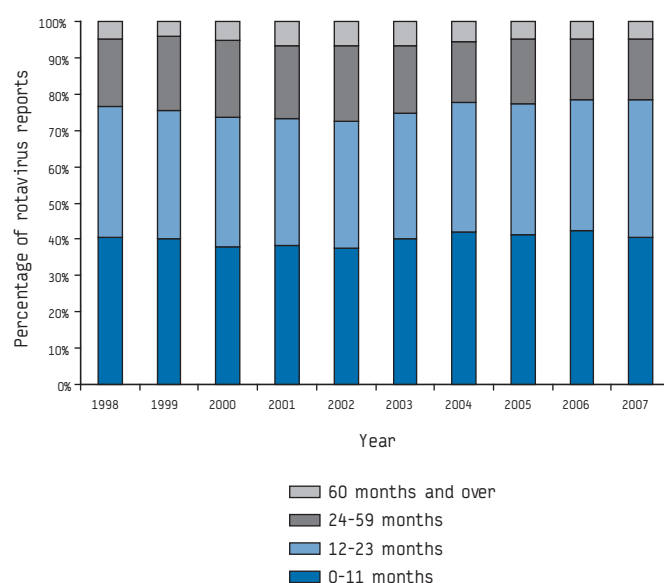


Source: Health Protection Agency rotavirus national surveillance data.

Methods

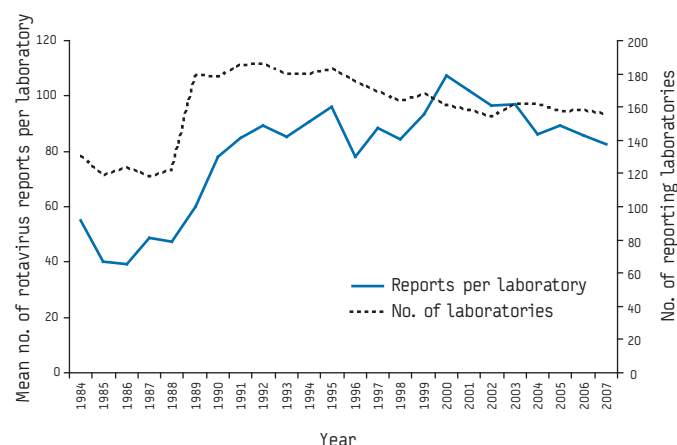
The Health Protection Agency (HPA) Centre for Infections receives reports of laboratory-confirmed rotavirus infections for England and Wales. Reporting is on a voluntary basis but is strongly encouraged. All reports have mandatory data fields for reporting laboratory, patient identifier, age, sex, pathogen, specimen type and specimen date. Laboratories feed reports into a set of database modules (some still send printed reports or paper report forms) and these are electronically transferred to regional HPA units which

FIGURE 3
Age distribution of laboratory-confirmed rotavirus reports, England and Wales, 1998-2007



Source: Health Protection Agency rotavirus national surveillance data.

FIGURE 4
Mean number of reported rotavirus infections per reporting laboratory and number of reporting laboratories in England and Wales, 1984-2007



Source: Health Protection Agency rotavirus national surveillance data.

collect the reports before transferring them to 'LabBase', the national laboratory reporting database at the Centre for Infections [11].

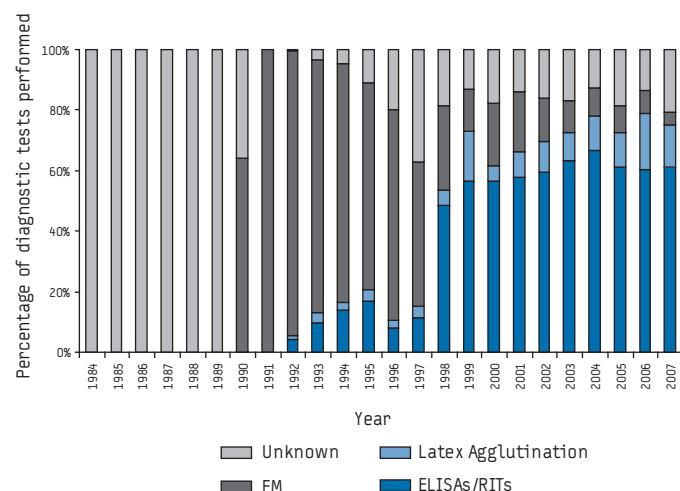
Medical microbiology laboratories reporting to the HPA include the National Health Service (NHS) and regional or collaborating HPA laboratories. These laboratories are mostly based within hospitals and all provide a clinical diagnostic microbiology service to both primary and secondary healthcare providers. Regional and collaborating HPA laboratories, in addition, provide specialist advice and support to other laboratories and microbiology services for health protection purposes. From a total of 208 NHS and HPA laboratories in England and Wales in 2007 [12,13], 156 were responsible for reporting cases of laboratory-confirmed rotavirus infections to national surveillance.

In May 2008 we distributed, by email, a structured questionnaire to the manager and consultant microbiologist (usually a medically-qualified doctor specialised in the diagnostics and management of infections) in each of these 156 laboratories. These laboratories were contacted directly using details available from the Department of Health [13], or via the regional consultant microbiologist who distributed the questionnaire to laboratories in their region. Two email reminders were sent if laboratories had not responded by August 2008. The survey included questions on the following (see Table):

1. The number of stools tested and positive for rotavirus in 2007,
2. Diagnostic tests used for rotavirus detection,
3. Policies on screening by age,
4. Months of the year in which routine rotavirus testing was performed,
5. Other indications for testing,
6. Dates and details of changes to testing policies over the period 1990-2007.

FIGURE 5

Diagnostic tests used for rotavirus detection in reported laboratory-confirmed rotavirus infections in England and Wales, 1984-2007



Source: survey answers.

'Routine' rotavirus testing was defined as rotavirus testing carried out on all stool specimens from gastroenteritis cases fitting a policy's inclusion criteria.

We used analysis of variance [14] to investigate whether certain testing policies were associated with higher positivity rates for rotavirus detection in stool specimens tested, and whether certain characteristics of a laboratory were associated with higher reporting efficiencies. Reporting efficiency was defined as the percentage of laboratory-confirmed rotavirus infections detected by a laboratory that were reported to LabBase. This was determined by dividing the number of rotavirus reports from a laboratory in LabBase in 2007 by the number of positive rotavirus specimens from that laboratory in the same year (survey question). This gives an indication of how efficient a laboratory was at reporting rotavirus diagnoses to national surveillance. For example, a reporting efficiency for a laboratory of 20% would mean that one in five rotavirus infections detected by that laboratory were reported or transferred to national surveillance.

To determine the effects of changes in diagnostic testing methods on long-term trends in national surveillance data, linear regression models were fitted to estimate whether the number of reports in a year were associated with the proportion of cases in that year diagnosed by a particular diagnostic test.

Results

The England and Wales rotavirus surveillance data (LabBase)

A total of 290,708 laboratory-confirmed rotavirus infections were reported in England and Wales between 1984 and 2007. Rotavirus reports showed marked seasonality that was regular and consistent over the surveillance period (Figure 1).

The rise in the number of rotavirus reports typically began in November and fell back to baseline in June. The peak in reported rotavirus infections was between February and April when 65%-70% of all reports occurred each year (Figure 2).

56% of laboratory-confirmed rotavirus infections were in male patients and 94% of all reports were in children under the age of five years. Information on age or date of birth of rotavirus cases was consistently recorded in LabBase from 1998 onwards. The age distribution of cases did not change over the surveillance period 1998-2007 (Figure 3) and cases in all age groups showed a similar seasonal pattern.

The number of rotavirus reports in England and Wales increased dramatically from the early 1990s (Figure 1). This sudden increase coincided with a rise in the average annual number of reports per reporting laboratory from 1989 (Figure 4) and an increase in the number of laboratories reporting each year from 1989 (Figure 4). While similar numbers of total annual reports have been received over the last 15 years, the number of contributing laboratories has declined slightly in the present decade compared with the 1990s (Figure 4).

In the surveillance data, basic information was also available on which type of diagnostic test was used in each reported laboratory-confirmed rotavirus infection. Prior to 1990 most laboratories did not report the method of rotavirus detection. Between 1990 and 1997 electron microscopy (EM) was the most frequent diagnostic test used. In 1998, there was a dramatic shift to enzyme-linked immunosorbent assay (ELISA) and rapid immunochromatographic tests (RITs), which subsequently predominated (Figure 5).

The Laboratory Survey of Rotavirus Testing Policies

Response

Ninety-four of 156 (60%) microbiology laboratories in England and Wales returned completed questionnaires.

Current diagnostic methods used

Most laboratories used RITs as their first line diagnostic method for rotavirus detection, either dual adenovirus/rotavirus RIT or single rotavirus RIT (Table). ELISAs were the second most common test used. Only 4% of laboratories currently used EM or latex agglutination to detect rotavirus in stool specimens.

TABLE

Routine laboratory testing policies for rotavirus in England and Wales in 2007 (survey of 94 laboratories)

Testing Policy	No. of Laboratories (%)
First line diagnostic method (n=94)	
ELISA	22 (23%)
Electron microscopy	2 (2.1%)
Latex agglutination	2 (2.1%)
Dual adenovirus/rotavirus RIT	34 (36%)
Single rotavirus RIT	34 (36%)
Seasonal policies for testing (n=94)	
All year	86 (91%)
All months except July	1 (1.1%)
October to May	4 (4.3%)
January to April	2 (2.1%)
July to December	1 (1.1%)
Age policies for testing, in years (n=94)	
< 3	6 (6.4%)
< 5	58 (62%)
< 6	4 (4.3%)
< 8	1 (1.1%)
< 10	1 (1.1%)
< 12	3 (3.2%)
< 16	8 (8.5%)
< 2 and ≥ 65	2 (2.1%)
< 5 and ≥ 60	1 (1.1%)
< 5 and ≥ 65	8 (8.5%)
≥ 65	2 (2.1%)
Other indications for testing (n=94)	
Clinician's request	94 (100%)
Diarrhoeal outbreak in ≥ 65 year-olds	35 (37%)
Diarrhoeal outbreak in paediatric ward	11 (12%)
Adult diarrhoeal outbreak when norovirus PCR-negative	4 (4.3%)
All liquid stools	1 (1.1%)
Stool specimens from immunocompromised patients	12 (13%)
Stool specimens from nursery workers	2 (2.1%)

ELISA: enzyme-linked immunosorbent assay; RIT: rapid immunochromatographic tests.

Seasonal policies for testing

91% (86/94) of laboratories routinely tested for rotavirus all year round. The exceptions were one laboratory which routinely tested in all months except July, four laboratories which routinely tested only from October to May, two laboratories which routinely tested only from January to April and one laboratory which routinely tested only from July to December (Table).

Age policies for testing

There was some variation in the age policies currently used for testing (Table). Complete testing for rotavirus in stool specimens from gastroenteritis cases in children under the age of five years was routinely performed in most laboratories (89%, 84/94). The two laboratories that routinely tested only in ≥65 year-olds served hospitals that did not have a paediatric department. Of the laboratories that routinely tested for rotavirus in children only (all age policies up to and including <16 year-olds), 43% had a policy whereby an institutional or hospital outbreak of diarrhoea in ≥65 year-olds would be an additional indication for rotavirus testing.

Other testing policies

Other indications for rotavirus testing included stool specimens sent from immunocompromised patients, nursery workers, outbreaks in paediatric wards, adult outbreaks when testing for norovirus was PCR-negative and all liquid stool specimens (Table). All laboratories tested for rotavirus in response to a specific clinical request, but 38% stated that the request would be referred to a Consultant Microbiologist if the patient from whom the stool specimen was collected did not meet any of the routine testing criteria.

Testing policies associated with higher positivity rates

No associations were found between the mean rotavirus positivity rates and the diagnostic method, seasonal or age policy currently used by laboratories (p values ≥ 0.1 for all testing policies investigated). The sample size for this analysis was small, as 38% of laboratories did not provide positivity rates. This resulted in wide confidence intervals for our estimates.

Laboratory reporting

All laboratories had a policy to report all rotavirus-positive specimens to the HPA Centre for Infections. On average, 71% (range 22-111%) of rotavirus infections detected by a given laboratory corresponded to a case report from that laboratory in LabBase in 2007. Reporting efficiencies over 100% could have resulted from errors during data input or delayed reporting. No associations were found between reporting efficiencies and rotavirus testing policies, affiliation of the laboratory to the HPA, whether a laboratory received specimens from more than one hospital or whether these hospitals were paediatric hospitals or had paediatric departments (p values ≥ 0.1 for all laboratory characteristics investigated).

Changes to laboratory practices

Thirty-nine of 94 (41%) laboratories provided data on whether testing policies changed over the last 15 years. Of the 32 laboratories (34% of all laboratories in the survey) reporting a change, 14 changed only the brand of the commercial assay they used and 18 changed the type of diagnostic method used, although only 11 of the 32 laboratories reporting changes could give the dates of when these changes occurred. Laboratories tended to switch from using ELISA, latex agglutination or EM to RITs from about 2000. These observations were consistent with information from the national database described above, which demonstrated

a national shift in diagnostic testing practices from using EM to ELISA or RITs after 1998 (Figure 4). If the surveillance data had been affected by this shift in diagnostic practice, one might have expected an artificial rise in the overall numbers of reported cases after the late 1990s as ELISA and RITs are more sensitive and less specific than EM for rotavirus detection [15]. However, we found no association between annual number of laboratory reports and the proportion of cases diagnosed by each diagnostic method (p values ≥ 0.1 for all diagnostic methods). Using LabBase and our survey results, we identified 59 laboratories that, from 1999 onwards, tested more than 90% of stool specimens for rotavirus each year by ELISAs or RITs.

Discussion

This study demonstrated that rotavirus testing policies in laboratories contributing to surveillance in England and Wales were reasonably consistent in 2007-8. The majority of laboratories were using RITs to detect rotavirus in stool specimens and were offering routine rotavirus testing all year round in children under the age of five years. These testing criteria for rotavirus are in accordance to those recommended in the National Standard Methods [16]. These are a set of standard operating procedures and guidance notes developed by the Standards Unit at the HPA to establish minimum best practice quality and efficiency in clinical microbiology laboratories in the UK.

No particular testing policy was found to be associated with higher positivity rates for rotavirus detection. This was unexpected, since laboratories testing only children under the age of five years might be expected to have higher positivity rates than those also testing older age groups. However, 38% of laboratories did not provide positivity rates. The resulting small sample size and wide confidence intervals may explain our failure to detect any associations. We reported that in 2007, on average, one in 1.4 (71%) rotavirus infections detected by a laboratory resulted in a case report from that laboratory to the national surveillance database "LabBase". This estimate is consistent with a previous study which reported that for one rotavirus case reported to national surveillance in England there were 1.5 laboratory-positive investigations [7].

In addition, we demonstrated how the number of rotavirus reports can be dramatically influenced by sudden changes in the number of laboratories reporting, and therefore why long term trends in the England and Wales rotavirus surveillance data must be interpreted with caution. Changes in the number of laboratories reporting and in the mean number of reports per laboratory both occurred around 1989. These changes coincided with a doubling of the number of rotavirus reports in England and Wales during the same period. During the late 1980s, developments in rotavirus vaccine research took place and there was a renewed interest in rotavirus epidemiology [17]. This could account for the changes in laboratory reporting practices seen at this time. A slight reduction in the number of reporting laboratories was observed towards the end of the study period. We attribute this decline to recent changes in the delivery of microbiology services in the UK that have resulted in the closing and merging of microbiology laboratories as well the sharing of services between laboratories. This would also explain why the fall in number of reporting laboratories did not coincide with a fall in the overall number of laboratory-confirmed rotavirus infections reported.

Our survey results are in contrast to the findings of a previous study which looked at policies for rotavirus testing in eight

laboratories in the East of England region between 1990 and 1998 [18]. That study reported marked differences in age and seasonal testing policies between laboratories. Due to the small sample size, their results are less likely to be representative of laboratories across England and Wales than ours. Our national survey may have failed to detect those earlier findings from 10 years ago because the laboratories previously studied may have closed or merged with other laboratories since then. It is also possible that changes in practices from 10 years ago or more were not reported because staff responsible for testing in the past and able to recall such a change may no longer work in the laboratory.

Our survey is subject to limitations. There was a poor response (41% of surveyed laboratories answered) to survey questions regarding changes to testing policies over the last 15 years. However, given the regularity of the seasonal pattern of laboratory-confirmed rotavirus reports, it is reasonable to assume that either few changes in policy took place or that the changes had little effect on the surveillance data. Our conclusions cannot be extended to laboratories that do not report cases of rotavirus to the HPA as we only surveyed reporting laboratories. Non-reporting laboratories will not influence surveillance data as they do not contribute any reports. Sixty-two of 156 (40%) laboratories did not respond to the survey. Differences between responders and non-responders might have resulted in bias. Non-responders may be laboratories that have little interest and testing experience in rotavirus disease. They may also be the laboratories with poor reporting efficiencies or inconsistent rotavirus testing policies, and therefore did not respond because they were unwilling to disclose this information.

Our survey of clinical laboratory practices for rotavirus testing in England and Wales suggests that it may be reasonable to assume that seasonal patterns in rotavirus surveillance data based on reports of laboratory-confirmed rotavirus infections are representative of patterns of rotavirus disease in children under the age of five years. Specifically, surveillance data are representative of cases for which a specimen is tested, not necessarily all rotavirus cases. As most laboratories do not test routinely in adults, the patterns of disease in this age group are less likely to be represented in the surveillance data. This is not likely to be a problem as vaccine policy questions relate primarily to children. If clinical testing policies remain as they are at present, the surveillance data could be used to assess the impact of rotavirus vaccination on the seasonality of rotavirus infections in England and Wales.

However, laboratory testing practices are not the only factor influencing how accurately the surveillance data reflect the epidemiological trends of rotavirus disease. Surveillance data represent only a fraction of cases occurring in the community as only a minority seek medical attention, and of these, stool specimens are investigated for only a fraction [7]. Therefore, surveillance data also reflect healthcare-seeking behaviour of parents of young children suffering from diarrhoea, and clinical practices regarding stool sampling of those children. If care seeking or stool sampling practices change with the advent of vaccination, there would be temporal biases in the laboratory-based data. This would limit its value in evaluating the impact of a vaccination programme, even if laboratory testing practices remain unchanged. In this respect, key additional data to be collected would be the number of negative tests, so that the proportion positive for rotavirus can be assessed. We recommend that this is collected nationally in the period following licensure of a new vaccine. It may also be possible

to introduce national guidelines for the sampling of children with diarrhoea to standardise practice.

Most laboratories in England and Wales started using ELISA and RITs for rotavirus testing after 1999. These tests have higher sensitivity but lower specificity than previously used diagnostics. Therefore, using data subsequent to 1999 would provide the most appropriate baseline information against which post-licensure trends can be assessed (see Figure 1). We have identified 59 laboratories that predominantly used ELISAs or RITs after 1999. Data from these laboratories would yield the clearest baseline information (i.e. secular trends independent of diagnostic testing issues). Assuming they continue to use these methods post-licensure, evaluations using data from these laboratories would minimise biases.

In order to assess the effectiveness of a rotavirus vaccine it will be crucial to link the surveillance data to vaccination history in child health records. If vaccination is introduced, those responsible for monitoring its effects should consider encouraging laboratories to broaden their age-based testing policies. Vaccination is likely to increase the age of infection [6] and this may be missed by the surveillance data if age policies remain restricted to the youngest age groups. Other national surveillance centres in Europe may benefit from performing a similar survey of laboratory practices for rotavirus testing to aid in the interpretation of their surveillance data and in anticipation of vaccination.

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