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Stepping up European measles surveillance

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For many years *Eurosurveillance* has made it a point to publish articles on measles outbreaks and measles prevention and control in Europe. The purpose has always been to increase awareness about this dangerous and potentially fatal infectious disease and highlight opportunities for preventive measures. Measles transmission has been firmly re-established in some European Union (EU) Member States [1]. It is astonishing to see that the EU has become an exporter of measles to the rest of the world, threatening to undermine years of efforts to eliminate endemic transmission of the measles virus. Visitors to Europe are now advised to immunise their infants as early as from six months of age [2,3] in order to protect them from a disease that can result in complications and lead to severe sequelae such as brain damage and death. All this happens despite the fact that measles can be prevented through vaccination with two doses of a measles-containing vaccine, optimally the measles-mumps-rubella vaccine, and that measles can be not only eliminated (less than one notified confirmed endemic case per million population) but also eradicated.

The conditions for eradication are favourable: humans are the only reservoir for the measles virus, the vaccine is safe, inexpensive and produces life-long immunity, diagnostic tests are both specific and sensitive, all infected people develop symptoms, and there are no chronic carriers. Eradicating measles would represent a major public health achievement, well worth the investment it requires. For the EU, the first step towards eradication of measles is effective control within its own borders. Finally, eradication will be the result of elimination of transmission on all continents.

However, given the current epidemiological situation, continued awareness and efforts are needed. Although measles transmission peaks during the winter and early spring in Europe, the many mass-gathering events that take place during the summer in Europe offer favourable conditions for the spread of the virus between countries here and to countries in other continents. Therefore all those who plan to attend mass gatherings in Europe, such as the World Youth Day on 16 to 21 August in Madrid, Spain, should ensure that they are protected against measles.

The European Centre for Disease Prevention and Control (ECDC) has taken initiative to step up measles surveillance in Europe. *Eurosurveillance* welcomes this initiative, which comprises the *European monthly measles monitoring* [4]. This online publication was launched on 13 July 2011 and promises to provide timely updates on measles outbreaks and endemic transmission in Europe based on the findings of active surveillance. This should help to raise public awareness, generate political will and increase public health resources for fighting an infectious disease that should long ago have been dispatched to the annals of infectious disease control.

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Outbreak of tularaemia in brown hares (*Lepus europaeus*) in France, January to March 2011

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We report an outbreak of tularaemia in brown hares (*Lepus europaeus*) in France, which occurred from January to March 2011 and was characterised by a high mortality rate in the local hare population. In France, hare tularaemia is usually sporadic and does not significantly affect hare populations. The epizootic form of the outbreak reported here led us to reconsider the potential associated risks for hare populations and public health.

Tularaemia is a cosmopolitan bacterial zoonosis caused by *Francisella tularensis*. This gram-negative bacterium contains several highly pathogenic subspecies, whose distribution is ubiquitous in the northern hemisphere [1,2]. Because of its pathogenic potential, tularaemia is a notifiable bacterial zoonosis in France, and is listed as potential bioterrorist weapon. The bacterium has very complex epidemiological cycles, including many wild species, whose epidemiological role is sometimes unclear. Namely, *F. tularensis* has been isolated from more than 250 species, including 190 mammals, 88 invertebrates, 23 birds, three amphibians as well as several species of reptiles and fishes, and two disease cycles, terrestrial and aquatic have been described [3,4]. The interaction between these two cycles remains not well known. In general, the disease cycle involves only few key species in a given region. In the terrestrial cycle in France, the European brown hare plays an important role in the ecology of tularaemia as amplifying host, and it may serve as a significant source of human infection [5]. Hare tularaemia has been reported in most of the departments in France, but endemic areas have been described in the northern part of France. A recent increase in cases in hares has been observed in 2007 and 2008 [6]. At the same time, during the winter, an excess of human cases has been reported (144 sporadic cases from 1 January 2007 to 31 December 2008, including 48 cases in 2007 and 96 in 2008, against a mean of 23 cases per year for the period 2003 to 2006), but the factors responsible for this increase have not been identified [7].

Although tularaemia is often fatal to hares, hare tularaemia in France is usually sporadic and does not significantly affect hare populations. We report here an outbreak of tularaemia in brown hares in France, which occurred in Pas-de-Calais from January to March 2011 and was characterised by a high mortality rate in the local hare population. The epizootic form of the outbreak reported here raised many epidemiological questions and led us to reconsider the potential associated risks for hare populations and public health.

Outbreak description

In March 2011, 51 tularaemia cases in hares, detected since January near Habarcq, Pas-de-Calais, were reported to the SAGIR network, an outbreak surveillance network that aims at determining the aetiology of wildlife mortalities [8]. About two thirds of the carcasses were recovered from the north-western part of a 110 hectares oak/ash wood, which was therefore considered as the epicentre of the outbreak. The mortality was quickly discovered as the wood is highly frequented by the public and regularly checked by the hunting managers. The outbreak occurred during the mating season in a high-density hare population (estimated at 2.3 per hectare in the wood). The two main reported waves of mortality seem to have coincided with sharp drops in temperature. The first wave occurred around 15 January, after the temperature had dropped by 10 °C in two days around 8 January and increased by 10 °C on 13 January. The second wave occurred around 1 March, after a drop of 8 °C within four days. An emergency investigation was set up to better understand the epidemiology of the outbreak. The timeline of the investigation is shown in the Figure. Information was provided to the local population after the first wave to prevent zoonotic transmission of the disease, which had not been reported in the commune since 1988 (personal communication C. Bethencourt, March 2011). To confirm that tularaemia was responsible of the epizootic, more than 10% of carcasses should be analysed. Eight of the 51 carcasses were found

unaltered, collected (see Figure) and sent for necropsy to the local veterinary laboratory. Infection with *F. tularensis* was confirmed by both bacterial culture and real-time PCR (polymerase chain reaction) [9]. All eight hares were in good body condition, with repleted stomach, confirming the acute nature of their death.

In all of them, macroscopic lesions typical of tularaemia such as splenomegaly, congestion and haemorrhagic lesions of several organs were observed, except that in addition, all hares had signs of tracheitis and sometimes bronchitis. European brown hare syndrome (EBHS) is suspected to be the cause of death when congestion and haemorrhagic lesions of several organs, mainly the tracheal mucosa and the lungs, and hepatitis or a discoloured liver are observed. EBHS, which is also an acute cause of death, was excluded by ELISA.

These findings raise the question of whether several different clinical pictures of tularaemia exist in infected hares, reflecting the route of infection, as is known for tularaemia infections in humans [1,10]. Hares are known to be infected by ticks or mosquitoes, but the upper respiratory tract affection suggests a respiratory route. In Hungary, histological studies lead to the same assumption that hares could become infected via airborne transmission [11].

Epidemiological investigation

Epidemiological investigations were performed on 14 March in order to collect information about the main potential sources of infection described in the literature, i.e. ticks [12,13], rodents [14] and other small animals, and water ponds [15,16]. Questing ticks were sampled by dragging a white cloth over a part of the ground in the epicentre and the immediate surrounding areas. Only few ticks (n=20) could be collected, which was not surprising because hard ticks do not

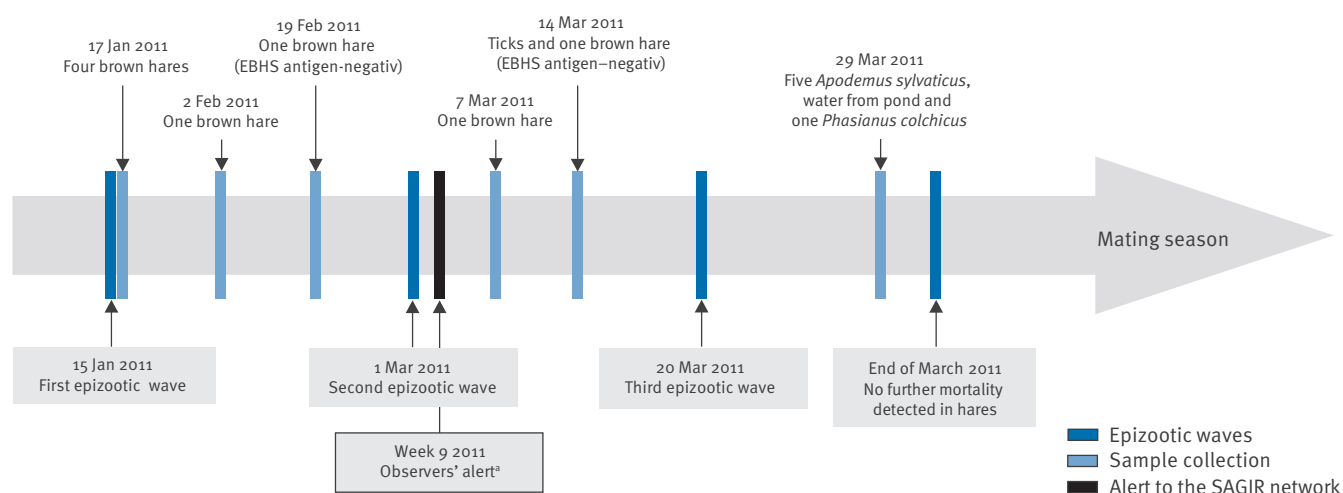
usually quest for hosts during the cold season. They all tested negative by real-time PCR. Conversely, several ticks collected alive on infected hare carcasses were positive, including one engorged tick and three ticks that did not seem engorged macroscopically but had probably started feeding on their hare host before it died. These ticks likely became infected after feeding on their bacteraemic host, either through their blood meal or through direct contact. A common pheasant (*Phasianus colchicus*) found dead close to the outbreak focus was collected and five wood mice (*Apodemus sylvaticus*) were captured alive and humanely killed. Infection by *F. tularensis* was investigated by testing the brain of pheasant and mice by real-time PCR and bacterial culture. All six animals were negative. Finally, muddy water was sampled from a permanent water pond present in the wood where hares were found dead. *F. tularensis* was not detected in these samples, neither by bacterial culture nor by real-time PCR.

Conclusions

The outbreak ended in late March and the epidemiological field investigations, performed in the middle of March have been unable to identify a source of infection, although they were conducted at a time when hare mortality was still observed. Molecular typing of the isolates recovered from collected hare carcasses and ticks will hopefully help us understand the origin of this outbreak by determining whether it was related to the focus (one case) reported at a distance of 10 km in 2007. Indeed, it is highly important to determine whether the epizootic form of this outbreak was due (i) to the emergence of a novel *F. tularensis* strain, (ii) to the fact that the hare population was unusually abundant, in which case the outbreak may have been a density-dependent phenomenon, or (iii) to particular ecologic circumstances favouring hare-to-hare transmission, since the outbreak occurred during periods of

FIGURE

Timeline of the epidemiological investigation into the tularaemia in hares, Pas-de-Calais, January–March 2011



EBHS: European brown hare syndrome; SAGIR: Network for the surveillance of wildlife in France

* The departmental correspondent of SAGIR alerts the national level when they observe abnormal mortalities.

frost (which may have led the hares to take refuge in the woods) and coincided with the mating season (when hares are immunosuppressed and in close contact with each other). Further studies are planned, including histology and surveillance of ticks and rodents, to gain knowledge on the transmission route and on the maintenance of a disease in the area.

The epizootic focus will be carefully surveyed this year and hunters, people walking in the forest, and physicians will be warned that a potential risk of disease transmission exists in the area, respectively by the departmental federation of hunters involved in the SAGIR network, local authorities, and the regional agency of health. We wish to warn wildlife managers that aggregated cases of hare mortality may be due to *F. tularensis* and that systematic precautions should be taken to make sure that humans do not contract the disease. Human tularaemia is usually sporadic in France even if aggregate cases have been described [17]. Infected ticks like those found alive on dead hares may become a reservoir in the area, thus the potential for human infection is now higher than it was. As the wood is highly frequented, tularaemia should be considered in the area as a diagnosis, particularly in people with contact with hares or ticks.

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* **Erratum:** The initial of the last author's name was corrected on 2 August 2011.

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Effective control of an acute gastroenteritis outbreak due to norovirus infection in a hospital ward in Athens, Greece, April 2011

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In April 2011, an acute gastroenteritis outbreak due to norovirus infection occurred in a hospital ward in Athens, Greece, affecting 28 people: 16 staff members, 10 inpatients and two relatives of symptomatic inpatients. The attack rate among the patients and staff was 16.4% (10/61) and 31.4% (16/51), respectively. The outbreak lasted eight days and the clinical symptoms were mild. Effective infection control measures prevented the spread of the virus to other hospital wards.

Outbreak description

Between 9 and 16 April 2011, a total of 28 cases of acute gastroenteritis occurred in an internal medicine ward of the Laikon General Hospital, in Athens, Greece. On 13 April, the Hellenic Center for Disease Control and Prevention was notified of the outbreak. Following an outbreak investigation set up by the hospital's Infection Prevention and Control committee, norovirus was found to be the causative agent of the outbreak.

Norovirus is highly contagious and important causative agent of epidemic gastroenteritis [1]. Outbreaks have been reported worldwide in diverse settings, including nursing homes, long-term care facilities and hospitals, as well as cruise ships, airplanes, military establishments and schools [2-6]. Unfortunately, infectious diseases are generally under-documented in Greece [7,8]. The only reported outbreak of confirmed norovirus infection in the country that we are aware of was in the town of Xanthi in 2005, in which 705 people were affected, due to waterborne transmission of the virus [9]. To our knowledge, the outbreak presented here is the first reported outbreak of norovirus infection in a Greek hospital.

Setting

Laikon General Hospital is a 487-bed, tertiary care university hospital. The 45-bed Internal Medicine Ward, located on the fourth floor, contained nine

four-bed, two three-bed, one two-bed and one one-bed hospital rooms. The ward staff comprised 51 staff members: 32 doctors (of whom 21 were trainee doctors), 13 nurses and six technical staff (cleaning and food-serving staff).

Epidemiological investigation

We collected epidemiological information on all inpatients and hospital employees who had been present in the department from 9 to 23 April 2011. We interviewed both groups on a daily basis to determine the date of symptom onset and variety and duration of symptoms. Detailed history was also taken concerning any hospital food that both groups had consumed. An infection control nurse made daily rounds throughout the hospital to look for similar cases in other departments, to assess whether the outbreak was likely to be due to contaminated food or water.

An outbreak case was defined as any inpatient or employee of the internal medicine ward, or relatives who visited inpatients (from 9 to 23 April 2011), with two or more episodes of vomiting and/or diarrhoea from 9 to 23 April, with or without other symptoms and with or without laboratory confirmation.

Between 9 and 16 April 2011, a total of 28 affected people were found to meet the case definition (Figure). The mean age of the cases was 51.6 years (range: 30–88) and half were male.

The main symptoms of cases were diarrhoea (n=21), abdominal pain (n=16), nausea (n=15), vomiting (n=13) and fever (38.0–38.5 °C) (n=11). The duration of illness ranged from 10 hours to three days, with a median of 35 hours.

The index case was found to be a 70-year-old male inpatient with an underlying condition, who had been

admitted on 7 April 2011 for other reasons. On 9 April, he reported multiple diarrhoeic episodes. Unfortunately, faecal specimens were not collected at that time. Two days later, all three male patients who had been hospitalised in the same four-bed room became symptomatic, as did another male patient who had been hospitalised in a different room. The following day (12 April), a female patient in a different room of the same ward and a trainee doctor also became symptomatic. Subsequently, the majority of the reported cases were members of the ward staff. The outbreak lasted eight days in total: the epidemic curve is shown in the Figure.

Of the 28 cases, 16 were hospital staff (five of whom were male): 13 were healthcare professionals (nine doctors and four nurses) and three were members of the technical staff (responsible for cleaning and serving food). In addition, 10 of the 28 cases were inpatients (eight of whom were male) and the remaining two cases were symptomatic patients' relatives who had stayed overnight in the ward.

The attack rate among staff members and inpatients was 31% (16 of 51) and 16% (10 of 61), respectively (Table 1).

Staff and patients had similar patterns of symptoms (Table 2). Interestingly, nine of the ten affected

inpatients were immunocompromised. Nevertheless, the illness was mild, with no substantial clinical deterioration.

Laboratory investigation

Faecal sampling started on 11 April 2011. Two specimens from each inpatient with diarrhoea, except for the index case – a total of 16 samples – were examined in the hospital's microbiological laboratory for *Shigella*, *Campylobacter*, *Salmonella*, *Escherichia coli* O157, as well as for *Clostridium difficile* toxin and the parasite *Giardia lamblia*, according to standard procedures. None of the samples were positive.

We then tested five consecutive samples, each from different inpatients, for norovirus, using an enzyme immunoassay (RIDA QUICK Norovirus Test, R-Biopharm, Germany) for genogroup I and II. Results were available within 24 hours. For financial reasons, we could not test the samples for all viruses that could be possible causes of the outbreak. We chose to look for norovirus, as this is considered to be a major cause of epidemic viral gastroenteritis and the most common cause of

TABLE 1
Attack rate in hospital inpatients and employees, gastroenteritis outbreak due to norovirus infection, Athens, Greece, 9–16 April 2011 (n=26)

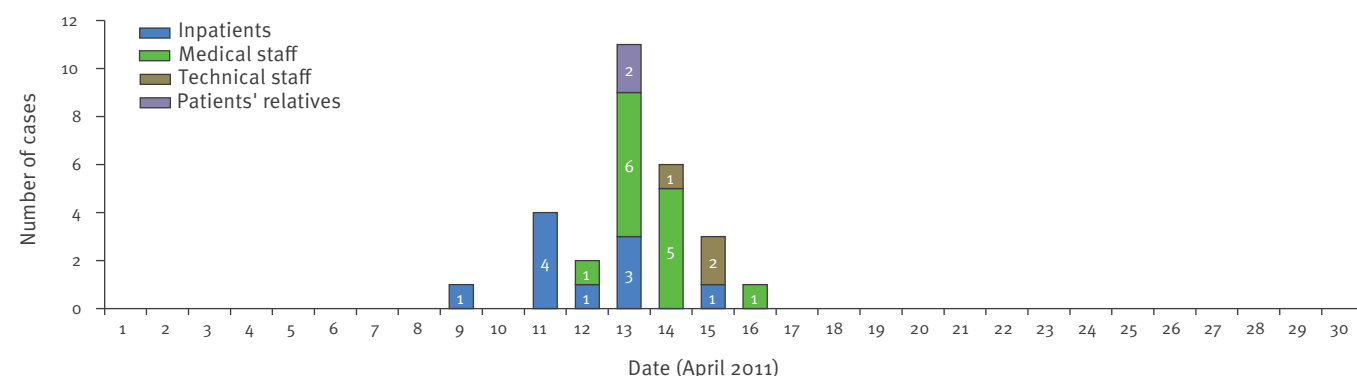
Type of person affected	Number	Attack rate (%)
Inpatients (n=61)	10	16
Employees (n=51)	16	31
Doctors (n=32)	9	28
Nurses (n=13)	4	31
Technical staff (n=6)	3	50

TABLE 2
Symptoms of affected hospital inpatients and employees, gastroenteritis outbreak due to norovirus infection, Athens, Greece, 9–16 April 2011 (n=26)

Symptom	Number of affected inpatients ^a (n=10)	Number of affected employees ^a (n=16)
Diarrhoea	9	10
Vomiting	3	10
Nausea	7	8
Abdominal pain	6	10
Fever	3	8
Mean duration of symptoms, in hours±standard deviation	38.4±17.3	32.8±11.7

^a Unless otherwise indicated.

FIGURE
Cases of gastroenteritis due to norovirus infection by date of symptom onset, Athens, Greece, 9–16 April 2011 (n=28)



all forms of gastroenteritis in adults worldwide [10]. Norovirus was detected in all five samples.

Similarly, financial constraints did not allow all inpatients to be tested for norovirus and no further typing of the virus could be carried out.

Contaminated food or water was not suspected during the outbreak as no other accumulated cases of acute gastroenteritis were identified concurrently in any other hospital ward; therefore no environmental sampling was carried out.

Infection control measures

The Hellenic Center for Disease Control and Prevention was notified early, on 13 April, four days after the start of the outbreak. Measures such as enhanced hand hygiene and cleaning with an appropriate disinfectant (1:50 dilution of sodium hypochlorite) of equipment, surfaces and rooms, as well as regular airing of premises were implemented on the same day. In addition, all symptomatic inpatients were isolated by keeping them in three consecutive four-bed rooms. They were allowed to use only designated toilets and bathrooms of the ward that were not used by unaffected individuals. The affected staff were told not to go work for at least three days, until they were no longer symptomatic, according to the standard policy of the hospital. Visits by friends and relatives to affected patients were not allowed. No new admissions to the ward were allowed in order to avoid spreading of the virus. An infection control nurse made daily rounds throughout the hospital to look for new cases and to monitor compliance with infection control measures. No other cases of acute gastroenteritis were reported in any of the other hospital wards.

Discussion

In this outbreak, the shape of the epidemic curve and the clustering of cases among inpatients and staff suggested that person-to-person transmission was the most likely mode of spread of the causative agent. The source that contaminated the index case was not identified, but given the dates of the appearance of cases, we consider that the virus was probably introduced by a single person and then it spread rapidly in the ward.

Interestingly, a higher attack rate was noted among staff compared with that among inpatients, although the difference was not statistically significant. This is in contrast to previous observations in hospital outbreaks of norovirus infection, which have shown a higher total number of cases, with higher attack rates among the inpatients compared with staff and more prolonged epidemic curve [11,12]. A possible explanation is that the infection control measures in the outbreak described in this report were implemented early, but the staff of the ward did not follow the precaution measures meticulously.

Early recognition of the outbreak and prompt implementation of effective infection control measures, including staffing restrictions and ward closure, was successful in containing the spread of the infection to just one ward and limiting the outbreak to a few days' duration. Although the higher attack rate among the staff probably implies that staff did not adhere strictly to infection control precautions, we hope that this outbreak has led to increased awareness of the importance of hand hygiene among hospital personnel.

The majority of the affected inpatients were immunocompromised. Although there is limited experience of norovirus infection in such individuals, recent studies have shown that in such hosts, noroviruses may be shed for prolonged periods of time and the patients may experience severe and life-threatening symptoms [13,14]. Although the outbreak described in our report was characterised by mild clinical manifestations, a more aggressive infection in such a setting could lead to severe morbidity, or even fatalities, if not dealt with early.

In conclusion, we report the first documented gastroenteritis outbreak due to norovirus in a Greek hospital: obviously such outbreaks should always be reported in the country. Successful control of the outbreak underlines the importance of the early detection of the causative agent and early and aggressive implementation of efficient control measures. This report underscores the possible role of medical staff in the propagation of outbreaks in healthcare settings if appropriate precaution measures are not taken.

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A venue-based HIV prevalence and behavioural study among men who have sex with men in Antwerp and Ghent, Flanders, Belgium, October 2009 to March 2010

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This venue-based, cross-sectional study reports on human immunodeficiency virus (HIV) prevalence and behaviour of 649 men who have sex with men (MSM) in Antwerp and Ghent, Flanders, Belgium, from October 2009 to March 2010. Using time-location sampling, we found that HIV prevalence in MSM who attended different types of venue ranged from a high of 14.5% (95% CI: 8.9–20.1; n=22 in cruising venues to 4.9% (95% CI: 1.9–7.9; n=10) in more general gay venues to 1.4% (95% CI: 0.0–3.6; n=3) at younger MSM venues. Of those who tested HIV positive (n=35, five were unaware of their HIV status or self-reported as being HIV negative. One in five respondents were of non-Belgian nationality. The results showed relatively high rates of testing for HIV (52.2%; 95% CI: 47.8–56.2; n=288) and other sexually transmitted infections (STIs) (57.4%; 95% CI: 52.6–62.0; n=248) in the last 12 months. A majority of the men (n=233) used condoms consistently during their last anal sexual contact with a casual partner; however, HIV-positive men who were aware of their serostatus (n=30) reported less condom use with casual partners. This is the first such study in Belgium and the results constitute the evidence base for local, targeted interventions. Furthermore, our findings underscore the need for European cross-border cooperation to prevent HIV infection and other STIs among MSM.

Introduction

In most western countries, the number of diagnoses of new human immunodeficiency virus (HIV) infections in men who have sex with men (MSM) continues to rise [1]. In France, for example, it has been estimated that each year an additional 1% of MSM become infected with HIV – an increase described as detrimental to the collective health of future MSM communities [2].

In Belgium, 1,135 new HIV infections in the general population were reported in 2009 to the Belgian Federal Institute for Public Health [3], the highest number ever in a year. About 82% (n=244) of the new

infections among men with Belgian nationality (n=297) occurred in MSM. An analysis by age group revealed that younger MSM (aged 15–34 years) were disproportionately affected [3]. An increased uptake of HIV testing cannot fully explain this phenomenon [4] – several factors may contribute to increased rates of new HIV infections among MSM, including sexual risk taking [5] combined with a high prevalence of HIV infection in some networks of MSM [6].

In Belgium, as in many western countries, trends in HIV incidence are derived from registered diagnoses of HIV. However, such data have their limitations (as there is no additional background information for up to a third of registered new cases of HIV infection [3]) and may not reflect the real scope of the epidemic. In addition to HIV incidence estimates, population-based HIV prevalence estimates are needed to assess the burden of disease in MSM and to make realistic projections for health-service needs and prevention planning. More data are also needed on different types of MSM settings, as they are important for targeting prevention efforts.

The only HIV prevalence estimates for MSM in Belgium to date have relied on self-reported HIV status. In Flanders, 5.6% (n=1,736) of MSM in 2007 self-reported as HIV positive; among French-speaking residents of Brussels, 9% (n=942) of MSM in 2006 reported being HIV infected [7,8]. However, data for both studies were mainly collected through a variety of MSM websites and therefore do not give representative prevalence estimates. For Wallonia, no data are available.

In order to address several gaps in data for Flanders, in 2008 the Flemish Ministry of Wellbeing, Public Health and Family commissioned a population-based study on the prevalence of HIV infection and behaviour of MSM in Antwerp and Ghent, Flanders. We report here on the results of the study carried out between October 2009 and March 2010.

Methods

Study design and estimated sample size

This study was carried out in the framework of 'Frequently Asked Questions' (FAQ), a series of behavioural and epidemiological research projects on HIV and other sexually transmitted infections (STIs) among MSM in Flanders [8]. The FAQ 2009 study design was a cross-sectional collection of blood samples and behavioural data, collected between October 2009 and March 2010. We set out to use time-location sampling to recruit men present at various venues in Antwerp and Ghent where MSM meet. This method has been shown to be successful in targeting hard-to-reach populations such as MSM [9].

Three main types or strata of MSM venues were defined on the basis of the age of the men who visited the venue and whether sexual contact was possible in the venue. The first stratum (cruising venues) comprised venues where sexual contact on site was possible, such as gay saunas, 'cruising' bars and sex clubs. The second (regular gay clubs/venues) consisted of more general MSM venues such as gay dance clubs or gay bars, where it is not possible to have sexual contact on site. The third (young MSM venues) consisted of settings where younger MSM meet, such as events organised by the regional organisation for lesbian, gay, bisexual and transgender youth, where sexual contact on site is also not permitted.

An inventory of all MSM venues in the Flemish cities of Antwerp and Ghent was then compiled from information obtained from the Internet and from community advisers, giving a total of 23 venues (10 cruising venues, nine regular gay bars/venues and four young MSM venues). Before the randomisation process, two types of enumeration were performed. Type I enumeration determined whether the venues gathered from the formative research were in fact venues that MSM attend. Next, type II enumeration was carried out to determine the number of eligible persons who attend a venue on a particular day and at a particular time period. On the basis of the enumeration data, 12 venues MSM venues (five cruising venues, five regular gay bars/venues and two young MSM venues) were randomly selected, without replacement, out of the list of 23, using STATISTICA v10. However, only seven owners or venue organisers agreed to collaborate: three cruising venues, two regular gay clubs/venues and two young MSM venues. The sample size for each stratum was calculated assuming a hypothetical prevalence of HIV infection of 15% for MSM at cruising venues and 5% in those at venues in the other two strata, as found in other studies [9-11]. In order to obtain a precision of 2.5%, it was estimated that a total of 684 MSM would be required: 292 from cruising venues, 196 from regular gay clubs/venues and 196 from young MSM venues.

For the data collection, 12 volunteers were recruited from organisations involved in the prevention of HIV

infection and other STIs and from community-based gay organisations. They received a half-day training on data collection procedures and ethical issues, to ensure optimal quality of data collection.

The volunteers were present at the venues on a Wednesday, Friday, Saturday or Sunday. Standardised time segments were used: three segments of three hours per location. Within the study period, time segments and days were randomised for data collection using STATISTICA v 10. A team consisting of a principal investigator and up to three volunteers (depending on the size of the venue) visited each selected venue.

Study population

Respondents were recruited according to the following inclusion criteria: being male, aged 18 years or older and having had more than one same-sex sexual contact in the previous 12 months. Exclusion criteria were being physically or mentally unable to give informed consent and/or complete the questionnaire used to collect behavioural data (described below), having already participated in the study or showing signs (in speech and movement) of excessive drug or alcohol use.

Procedures and data collection

According to the principles of time-location sampling, the selection of MSM was random. The volunteers at the venue approached every other person entering or passing by the volunteers. The potential respondent was asked to participate: if they agreed, written informed consent was obtained. MSM who approached the volunteers were given some information on the research project and on HIV/STI prevention, but the volunteers explained that self-selection was not possible. During the training of the volunteers, the issue of selection bias had been discussed. Also, time-location sampling does limit selection bias, as there is multi-level randomisation.

Respondents were asked to complete a self-administered paper questionnaire available in Dutch and French. The questions (n=32) were designed to gather data on socio-demographic characteristics, sexual orientation, partnership status, sexual contact according to partnership status, number of partners, condom use and position during anal sexual contact, sexual geography (i.e. places MSM frequent or strategies used to find partners), testing behaviour for HIV and other STIs, HIV status, history of other STIs and drug use. Questions on sexual activity were based on the United Nations General Assembly Special Session on HIV/AIDS (UNGASS) indicators [12]. The questions were pretested for clarity and feasibility among an MSM community test group during September 2009. Completing the questionnaire took the respondents about 10 minutes.

A different volunteer then collected a blood sample onto filter paper by means of a finger prick. As this method avoids using venous blood, it is therefore much less invasive – an important issue in the context

of venue-based studies to encourage participation. A similar sampling procedure was used successfully in Montreal, Canada, and Paris, France [10,13].

After each time segment of data collection, the samples and questionnaires were stored at the Institute of Tropical Medicine in Antwerp. All data were collected anonymously: a code linked a blood sample to the corresponding questionnaire. We checked for previous participation and the dataset was additionally checked for similarities in individual profiles (age and postal code) to exclude double entries.

The volunteers emphasised to the respondents that the HIV tests that would be carried out would not be used for diagnostic purposes. Respondents were given leaflets containing information on HIV testing and testing locations and an incentive was provided (a drink would be offered at the test, worth about three euros).

Laboratory testing

Blood samples were analysed at the AIDS Reference Laboratory of the Institute of Tropical Medicine, using Vironostika HIV Ag/Ab (bioMérieux), a fourth-generation test, and Enzygnost Anti HIV 1/2 Plus (Siemens), a third-generation test. If both tests were reactive, the sample was considered as HIV infected. Samples giving discordant results were considered as indeterminate and were not included in the analyses (n=4).

Statistical analysis

Data were analysed with SPSS v 18. Differences in proportions and means between different groups were tested for statistical significance using analysis of variance (ANOVA) and Tukey's post-hoc test.

Ethical approval

Ethical approval for the study was obtained from the Institutional Review Board of the Institute of Tropical Medicine, Antwerp, and the Ethics Committee of the University Teaching Hospital, Antwerp.

Results

Socio-demographic characteristics of study participants

A total of 649 MSM participated in the study: 167 at cruising venues, 219 at regular gay clubs/venues and 263 at young MSM venues. Of the 649 questionnaires received, three were invalid; 582 of those who completed the questionnaire agreed to have their blood taken. Participation rates were calculated as the total number of respondents that participated divided by the total number of men approached by a recruiter, expressed as a percentage. The participation rate for completing the questionnaire was 58% (n=167) at the cruising venues, 75% (n=219) at regular gay clubs/venues and 70% (n=263) at the young MSM venues. Because of the quantitative nature of the study, extra qualitative information on refusals was not obtained.

Mean age was highest among men recruited at the cruising venues (38.5 years) and, as expected, lowest in those recruited at the young MSM venues (26.9 years) (Table 1). At the cruising venues, 10.0% (n=17) of men were aged under 25 years; at the regular gay clubs/venues, this percentage was 25.0% (n=55) and at the young MSM venues, 40.0% (n=105).

Overall, 75.3% (n=489) of respondents were of Belgian nationality, 18.3% (n=119) were Dutch and 1.2% (n=8) were French nationals; the remaining men (n=99) had different nationalities. About 64% (n=415) had a degree. The vast majority of men (94.1%; n=611) were exclusively or primarily attracted to other men, while 2.6% (n=17) were equally attracted to men and women.

Prevalence of HIV infection and undetected HIV infections

At the cruising venues, 16.1% of men (n=26) reported that they were HIV infected; the corresponding percentages for the regular gay clubs/venues and the young MSM venues were 5.8% (n=12) and 3.2% (n=8), respectively (Table 2). The proportion of men who did not know their HIV status was similar at the cruising venues and the regular gay clubs/venues: 16.1% (n=26) and 17.5% (n=36), respectively. This was significantly lower than at the young MSM venues, where 26.0% of men (n=64) reported they did not know their HIV status.

The prevalence of HIV infection was highest among the respondents at the cruising venues, 14.5% (22 of 152). It was 4.9% (10 of 205) among those at the regular gay clubs/venues and 1.4% (3 of 221) at the young MSM venues (Table 2). The differences between strata were significant. Overall, 14.3% (n=5) of HIV-positive MSM thought they were HIV negative or were unaware of their status. This proportion was different in the different strata: at the cruising venues, 5.0% of men (n=1) were unaware of their HIV-positive status; at the regular gay clubs/venues, this percentage was 30.0% (n=3) and at the young MSM venues, 25.0% (n=1). These differences were not statistically significant, but the numbers were small. Five HIV-negative men self-reported being HIV positive. Four other respondents had discordant results between the two HIV tests.

Table 2 also presents HIV prevalence by age group in the different types of venue. Prevalence was highest among men at the cruising venues, in all age groups. The prevalence in men aged 25 years or younger and in men 40 years or older was similar in regular gay clubs/venues and in young MSM venues.

Testing for HIV infection and other STIs

Overall, 88.0% (n=531) had ever been tested for HIV and about half had been tested in the previous 12 months. Men at young MSM venues were least likely to have ever been tested (81.4%; n=184), while the percentage of men ever tested was similar at the cruising venues (92.6%; n=150) and the regular gay clubs/venues (90.2%; n=194) (Table 1).

TABLE 1

Characteristics and behavioural data of MSM study participants, Antwerp and Ghent, Flanders, Belgium, October 2009–March 2010 (n=649)^a

Item	All types of venue N1 + N2 + N3			Type of venue				ANOVA p value	Tukey's test						
	Cruising venues N1	Regular gay clubs / venues N2	Young MSM venues N3	Total	n ^c	%	Total			n ^c	%				
Total number of participants	649	219	263												
Participation rate (questionnaire completion), as percentage	67	75	70												
Participants' age															
Mean age in years (standard deviation)	31.3 (10.3)	30.6 (8.8)	26.9 (7.1)						N1 # N2 # N3						
Age range in years (interquartile range)	17 ^b –66 (13.8)	18–59 (11)	17 ^b –50 (9.5)												
Testing behaviour	Total	n^c	%	95% CI	Total	n^c	%	Total	n^c	%					
Ever tested for HIV	603	531	88.0	85.4–90.6	162	150	92.6	215	194	90.2	226	184	81.4	<0.001	N1 # N2, N2 # N3
Tested for HIV in last 12 months	552	288	52.2	47.8–56.2	150	81	54.0	194	97	50.0	208	110	52.9	NS	
Ever tested for another STI	580	416	71.7	68.1–75.5	160	134	83.8	210	158	75.2	210	119	56.7	<0.001	N1 # N2, N3
Tested for another STI in last 12 months	432	248	57.4	52.6–62.0	134	84	62.7	158	80	50.6	140	82	58.6	NS	
Sexual partnerships in last 12 months															
MSM with a steady male partner	589	260	44.1	40.1–48.1	147	67	45.6	196	96	49.0	246	93	37.8	<0.05	N2 # N3
MSM with a steady male partner plus casual partners	305	118	38.7	33.3–44.3	70	35	50.0	117	41	35.0	118	37	31.4	NS	
Median category of the number of partners	617	2–5	–	–	159	>10	–	207	2–5	–	251	2–5	–	<0.001	N1 # N2, N3
Anal sexual contact in last 12 months	623	529	84.9	82.1–87	157	132	84.1	212	189	89.2	254	207	81.5	NS	
Condom use during last anal sexual contact															
With casual partner	323	233	72.1	67.1–76.9	92	67	72.8	113	80	70.8	118	85	72.0	NS	
With steady partner	181	69	38.1	31.1–45.3	42	16	38.1	57	18	31.6	82	37	45.1	NS	
Drug and alcohol use in last 12 months															
Alcohol	565	262	46.4	42.3–50.5	144	63	43.8	192	78	40.6	230	121	52.6	NS	
Alkyl nitrites (poppers)	565	205	36.3	32.3–40.3	144	67	46.5	192	62	32.3	230	76	33.0	<0.05	N1 # N2, N3
3,4-methylenedioxymethamphetamine (ecstasy or XTC)	565	83	14.7	11.8–17.6	144	38	26.4	192	22	11.5	230	23	10.0	<0.001	N1 # N2, N3
Benzoylmethylcgonine (cocaine)	565	74	13.1	10.3–15.9	144	30	20.8	192	20	10.4	230	24	10.4	<0.05	N1 # N2, N3
Sildenafil citrate (Viagra)	565	69	12.2	9.5–14.9	144	41	28.5	192	18	9.4	230	10	4.3	<0.001	N1 # N2, N3
Gamma-hydroxybutanoic acid/gamma-butyrolactone (GHB/GBL)	565	55	9.7	7.3–12.1	144	28	19.4	192	17	8.9	230	10	4.3	<0.001	N1 # N2, N3
Cannabis (hashish)	565	48	8.5	6.2–10.8	144	15	10.4	192	14	7.3	230	19	8.3	NS	
Amfetamine (speed)	565	36	6.4	4.4–8.4	144	12	8.3	192	13	6.8	230	11	4.8	NS	
Methamfetamine (crystal meth)	565	8	1.4	0.4–2.4	144	4	2.8	192	2	1.0	230	2	0.9	NS	

ANOVA: analysis of variance; HIV: human immunodeficiency virus; NS: not statistically significant; MSM: men who have sex with men; STI: sexually transmitted infection.

denotes statistical significance.

^a For some variables, some data are missing, due to missing information on the self-reported questionnaire.

^b One 17-year-old did not tell the truth about his age during the recruitment of participants, but his age was reported on his questionnaire.

^c Unless otherwise indicated.

TABLE 2
HIV prevalence and undetected HIV infection in MSM study participants, Antwerp and Ghent, Flanders, Belgium, October 2009–March 2010 (n=649)^a

Item	All types of venue				Type of venue								ANOVA p value	Tukey's test				
	N1 + N2 + N3				Cruising venues N1				Regular gay clubs /venues N2						Young MSM venues N3			
	Total	n	%	95% CI	Total	n	%	95% CI	Total	n	%	95% CI			Total	n	%	95% CI
HIV test results																		
Positive	578	35	6.0	4.9–9.1	152	22	14.5	8.9–20.1	205	10	4.9	1.9–7.9	221	3	1.4	0.0–3.6	<0.001	N1 # N2 # N3
Unaware of HIV-positive status	35	5	14.3	2.5–26.1	20	1	5.0	–	10	3	30.0	–	4	1	25.0	–	NS	–
Discordant test result	578	4	0.7	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Prevalence of HIV infection in those tested, by age group																		
18–25 years	190	4	2.1	1.3–6.9	20	2	10.0	–	74	1	1.4	–	96	1	1.0	–	NS	–
26–40 years	261	17	6.5	4.6–11.2	61	10	16.4	–	96	6	6.3	–	104	1	1.0	–	NS	–
>40 years	126	15	11.9	5.3–16.1	70	10	14.3	–	35	3	8.6	–	21	2	9.5	–	NS	–
Self-reported HIV status																		
Positive	613	43	7.0	5.0–9.0	161	26	16.1	10.4–21.8	206	12	5.8	2.6–9.0	246	8	3.2	1.1–5.5	<0.001	N1 # N2 # N3
Unknown	613	121	19.7	16.6–23.0	161	26	16.1	10.4–21.8	206	36	17.5	12.3–22.7	246	64	26.0	20.5–31.5	NS	–

ANOVA: analysis of variance; HIV: human immunodeficiency virus; NS: not statistically significant; MSM: men who have sex with men.

denotes statistical significance.

^a For some variables, some data are missing, due to missing information on the self-reported questionnaire.

Testing rates ever for STIs were also high: 83.8% (n=134) for respondents at cruising venues, which was significantly higher than among those at regular gay clubs/venues (75.2%; n=158) and young MSM venues (56.7%; n=119). Overall, 57.4% (n=248) of respondents had been tested for STIs other than HIV in the previous 12 months (Table 1). Of the respondents who had been tested for other STIs, about 16.5% (n=41) had been diagnosed with one or more STIs; for men recruited at cruising venues, this was 28.6% (n=24).

The most frequently reported STIs among those tested were gonorrhoea (10.1%; n=29), chlamydial infection (9.1%; n=26) and syphilis (8%; n=23). There were 13 cases of hepatitis B (4.5%) and eight cases of hepatitis C (2.8%). Significantly more syphilis cases were reported in respondents in cruising venues (15.4%, Tukey's test $p < 0.05$) compared with the other two strata (4.8% in regular gay clubs/venues and 5.8% in young MSM venues, n=6). Chlamydial infection was significantly less likely to be reported by respondents from young MSM venues (in 3.9% (n=4) of respondents, whereas the percentage was 9.5% (n=10) in regular gay clubs/venues and 15.4% (n=12) in cruising venues; Tukey's test $p < 0.05$). During the previous 12 months, 24.0% (n=10) of the respondents diagnosed with an STI other than HIV were diagnosed with at least one other STI.

Sexual partnerships and sexual behaviour

A total of 260 (44.1%) of the respondents reported having had a steady male partner in the previous 12 months. For those in the younger MSM venues, this was 37.8% (n=93) (Table 1). Of all respondents who reported a steady relationship, about one third (n=118) had also had casual sexual partners in the previous 12 months; for respondents at cruising venues, this was 50.0% (n=35). The median category of number of sexual partners in the previous 12 months was two to five for respondents at regular gay clubs/venues and young MSM venues, which was significantly lower than that for respondents in cruising venues, where the median category was more than 10.

Overall, 84.9% (n=529) of the respondents reported having had anal sexual contact during the previous 12 months. One third acted almost exclusively as the receptive partner, slightly more reported insertive anal sexual contact and about one third reported both. A total of 61.9% (n=112) of the respondents had not used a condom with their steady partner at the last sexual encounter. There were no differences by respondent's HIV status in condom use during the last anal sexual contact. However, when having anal sexual contact with a casual partner or a sex-buddy (a sexual partner who was known to the person, but was not their steady partner), HIV status seemed to affect condom usage: among HIV-negative respondents (n=552), 412 (74.6%) consistently used a condom during their last anal sexual contact with casual partners, compared with 44.1% (n=15) among the HIV-positive respondents.

There were no significant differences between respondents by type of venue in the self-reported strategies that were used to search for sexual partners. Our results indicate that few MSM look for sexual partners in only one single type of venue or use only one strategy exclusively: respondents in all venue types found sexual partners through the Internet, via friends or at regular gay clubs or bars, although respondents in the young MSM venues were significantly more numerous at regular gay clubs or bars and had used the Internet more frequently to find sexual partners during the last 12 months. Overall, 76.6% of all respondents (n=464) reported having had sexual contact at their home after finding a sex partner through any strategy or at any of the venues. As expected, respondents from cruising venues were less likely to have sexual contact at home after finding a casual sex partner though this difference was not statistically significant.

Drug and alcohol use

The respondents reported using a range of different drugs in the previous 12 months, just before or during sexual contact (Table 1). The drugs included alkyl nitrites (poppers), 3,4-methylenedioxymethamphetamine (ecstasy or XTC), cannabis (hashish) and amphetamine (speed). Combinations of all of the above drugs were also reported. Significantly higher rates of use of alkyl nitrites (poppers), sildenafil citrate (Viagra), 3,4-methylenedioxymethamphetamine (ecstasy or XTC), gamma-hydroxybutanoic acid/gamma-butyrolactone (GHB/GBL) and benzoylmethylecgonine (cocaine) use were reported by respondents in cruising venues compared with those in the other venue types.

Excessive alcohol use was also reported. Respondents at young MSM venues used significantly more alcohol than those at regular gay clubs/venues.

Discussion

This is the first study in Belgium to estimate HIV prevalence among MSM visiting different types of venue. The lack of such research in the past may be due to the overall lack of knowledge of MSM populations and to difficulties in reaching these men for population-based research. The MSM sexual subculture is extremely diverse and caters for specific sexual desires. HIV prevalence research tends to focus primarily on cruising venues, where sexual contact on the premises is possible. By analysing three different types of venue, we were able to differentiate between specific venue types and our findings thus contribute to developing targeted prevention strategies.

Recruiting MSM from different settings and taking blood samples through a finger prick seemed feasible and was generally well accepted. However, a limitation of our study was that five of the 12 owners of the venues that were initially approached declined to participate in the study. They rarely gave a meaningful explanation for not participating. Further research could shed light on the venue owners' motivation for refusal. The exclusion of these venues limited the application of the

time-location sampling framework and the representativeness of the data for the MSM scene in Flanders. In addition, some of the men did not want to provide a blood sample as they said they feared the pain caused by a finger prick. Given the setting in which the samples were collected, this could have been said to hide the other reasons for refusing to be tested; however, this could not be explored in more detail in our study. Further, self-completion of the questionnaire in these venues resulted in some questions not being answered. Nevertheless, use of principles of the time-location sampling methodology means that our results should be representative for MSM present at the venues that were visited in the cities of Antwerp and Ghent, but the results cannot be generalised to the overall population of MSM in Flanders or the whole of Belgium. It is noteworthy, however, that analysis of the respondents' place of residence (by postal code) showed that the Belgian MSM in the study came from all over the country.

Our study found that the prevalence of HIV infection in men in the MSM venues that we analysed ranged from 14.5% in cruising venues, to 4.9% in regular gay clubs/venues, to 1.4% at young MSM venues. These differences in prevalence can be partly explained by differences in age. As the age of MSM at cruising venues was on average higher and HIV infection is more prevalent in older age groups, it is not surprising to find more HIV infections within these settings. However, in MSM aged 25 years and under who were present at cruising venues, the prevalence was 10%. These young men frequented a greater variety of settings and used diverse strategies to find sexual partners, while they reported less frequent HIV testing.

Our prevalence data are in line with other European venue-based research among MSM, which found a range of prevalence estimates. A study in the United Kingdom in 2007 found the prevalence of HIV infection in MSM to be 9.0% in Manchester, 12.0% in London, and 14.0% in Brighton, and about a third of infections were unknown by the respondents [14]. In Switzerland, the estimated prevalence of HIV infection among MSM in Geneva in 2005 was 11% [15]. More recently, in Barcelona, Spain, the figure was 17.0%; in Verona, Italy, 11.8%; in Bratislava, Slovakia, 6.1%; in Bucharest, Romania, 4.6%; in Ljubljana, Slovenia, 5.1%; and in Prague, Czech Republic, 2.6%. Among MSM aged under 25 years, the prevalence was 4.9% in Verona, Italy, and 12.5% in Barcelona, Spain, in 2008 to 2009 [9]. The most recent European study, among MSM in Paris, France, in 2009 found a prevalence of 17.7%, of whom 19.7% were unaware of their status [10]. However, caution has to be exercised when comparing these results as the data were collected in different urban contexts and time periods, using different methodologies. In our study, the percentage of men unaware of their HIV-positive status was lower across all settings (14.3%), although it was relatively high in the regular gay clubs/venues (30.0%) and young MSM

venues (25.0%). This finding could be explained by a lower uptake of HIV testing by younger MSM.

We found relatively similar but high rates of testing for HIV during the last 12 months, compared with results from a similar study in 2009 in France, a neighbouring country (where the rate of HIV testing was 63.0%) [10]. Respondents in the cruising venues in our study had been tested significantly more frequently for both HIV and other STIs than those from the other venue types. The higher uptake of lifetime testing in MSM in cruising venues corresponds to the higher risk of exposure within the settings they frequent. Further, the MSM present at cruising venues are generally older and may therefore have known about or experienced HIV screening for a longer time.

A majority of the men in our study reported condom use consistently during the last anal sexual contact with a casual partner, across all strata. However, HIV-positive men who were aware of their serostatus reported less condom use with casual partners and/or sex-buddies. Further, prevalence of HIV infection was highest among the participants from cruising venues – where sex in public is possible on site – even after stratifying by age group. Although it may appear that a certain group of HIV-positive MSM take no preventive measures at all, this may not always be the case. Research has shown that it is within the sexual networks of MSM that harm reduction strategies such as strategic positioning (HIV-positive men assume the receptive or 'passive' position, while HIV-negative men take the insertive or 'active' position) and serosorting (HIV-positive men have sex only with other HIV-positive men, while HIV-negative men only do so with other HIV-negative men) are widely practised and accepted as forms of preventive behaviour [6]. Within epidemiological research this process of seroadaptation has often been ignored [16]. However, as a substantial number of HIV-positive respondents in our study believed themselves to be HIV negative, these strategies cannot be considered as reliable.

The use of certain drugs was higher among participants in the cruising venues. Drug use and sexual risk behaviour among MSM attending these sex venues were reported to be high, as was reported in a study in 2009 on highly sexually active MSM attending cruising venues and parties in New York, United States [17]. From a public health perspective, prevention strategies for HIV/STIs and drug consumption are generally difficult to control in MSM venues, as they are privately owned. The relationship between drug taking, mental health and sexual risk behaviour is pivotal in understanding the HIV epidemic among MSM, not only among men attending these venues but for the whole community [18]. The use of poppers and Viagra has been shown to be associated with recent seroconversion [19] and sexual risk behaviour [20-23]. While the purpose of using these drugs is the enhancement of sexual experience [23], interventions tackling multiple drug use need to be

part of prevention strategies for the specific high-risk subgroup of users, including HIV-positive men [24].

With regard to venues frequented or strategies used by MSM to seek sexual partners, there appear to be differences according to age group. For instance, data from the Netherlands suggest that younger MSM seek out sexual contact more frequently through the Internet and friends, and at regular gay bars or parties, whereas older MSM prefer saunas, public places (e.g. public parks and car parks), 'darkrooms' (darkened rooms, sometimes located in a cruising bar, gay sauna or other place where sexual activity is possible), sex cinemas and sex clubs [25]. In our study, the role of the Internet appeared to be important. A majority of the respondents sought sexual partners on the Internet and then met in their homes. Therefore it is imperative that new interventions focus on providing information on HIV infection and other STIs on the Internet and work through digital interaction on changing sexual risk behaviour.

The complexity of the psychological, biological and social-structural elements that define the HIV epidemic among MSM requires a combination prevention solution. Our data show clearly that targeting only one element is not enough [6]. Our results constitute the evidence base for local targeted prevention, for policy changes directed at these specific settings and they form a baseline for analysing trends in HIV prevalence to inform prevention planning and monitor progress. We know from residence analysis that one in five men participating in the study came from neighbouring countries. Further, as the epidemiology of HIV infection among Belgian MSM mirrors developments in most western countries, there is a need for cross-border cooperation on research and development of interventions and policies [26]. Within such a framework, we could move towards a European-wide HIV prevention plan for MSM, as has been suggested [27].

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Investigation of a spatiotemporal cluster of verotoxin-producing *Escherichia coli* O157 infections in eastern England in 2007

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An outbreak of verotoxin-producing *Escherichia coli* O157 (VTEC O157) infections linked to an open farm occurred in eastern England in April and May 2007. This paper describes the investigation and highlights the importance of multidisciplinary collaboration for successful control of such outbreaks. There was a temporal cluster of 12 confirmed symptomatic cases of VTEC O157 and one asymptomatic carrier, from five families. The investigation revealed that four of these cases formed part of an outbreak involving two families who visited an open farm. The phenotypic and genotypic characteristics of the isolates from the two families and the putative farm animal contacts were indistinguishable, indicating that the animals were the source of the primary infections. No epidemiological link could be established between the remaining three families affected and the open farm or people having visited the farm. Control measures included improved hand washing facilities on the farm, information for visitors and staff, restricted access and suspended petting and feeding of animals, and thorough cleaning and disinfection of affected areas.

Introduction

The most important strains of verotoxin (VT)-producing *Escherichia coli* (VTEC) that cause diarrhoeal illness in the United Kingdom (UK) belong to serogroup O157 (VTEC O157) [1]. They produce VT1, VT2 or both toxins and are differentiated by phage typing and DNA-based techniques [2]. *E. coli* O157 is an important, although relatively uncommon, cause of infectious gastroenteritis in England and Wales. Between 2000 and 2008 the number of reference laboratory-confirmed isolates of VTEC O157 in England and Wales ranged from 595 in 2002 to 1,034 in 2009 and 793 in 2010 [3]. Of 948 strains in 2008, 73 came from the east of England [4]. In comparison, 55,609 *Campylobacter* strains were isolated in England

and Wales in the same year [5]. Several outbreaks of VTEC O157 have been recently reported in the UK [6-9]. Healthy domesticated animals such as cattle, sheep and goats are the natural reservoir for VTEC [1,10]. Spread to humans occurs through contaminated food or water, person-to-person spread or by direct and indirect contact with infected animals and their faeces [1].

The disease severity ranges from mild and self-limiting diarrhoea to serious and sometimes fatal illness, especially in young children or elderly people [11]. Haemorrhagic colitis develops in about half of the identified VTEC O157 cases in England and Wales [12]. Haemolytic uraemic syndrome (HUS) complicates about 2-7% of all cases of VTEC O157 gastroenteritis [13,14]. Its manifestations include renal failure, haemolytic anaemia, thrombocytopenia, and central nervous system symptoms. HUS has a fatal outcome in up to 17% of cases [15-17], while a substantial proportion of the survivors suffer renal or other long term residual impairments [18].

Between 18 April and 3 May 2007, the Norfolk, Suffolk and Cambridgeshire (NSC) Health Protection Unit (HPU) Norfolk office was notified of eleven cases of presumptive *E. coli* O157 infection and one asymptomatic carrier from four families in Norfolk. This represented an unusually high number of cases for this region within a period of a few weeks. The index case from the first family (A) had visited an open farm in eastern England. On 1 May 2007, the HPU was notified of a child from Lincolnshire (Family B) who was hospitalised with *E. coli* O157 gastroenteritis and HUS, and had visited the same farm. Norfolk and Lincolnshire are neighbouring counties. This report presents the epidemiological and microbiological investigations of this spatiotemporal cluster of VTEC O157 infections.

Methods

Epidemiological investigations

A primary case was defined as the first person in a household with gastrointestinal illness (three or more loose stools in a 24-hour period) that was microbiologically confirmed as caused by VTEC O157, and disease onset between 10 April and 1 May 2007. A secondary case was defined as a person with gastrointestinal illness, microbiologically confirmed as caused by VTEC O157, who had the second or subsequent such illness in the household and whose onset of illness was two or more days after the onset of the primary case.

The first meeting of the outbreak control team (OCT) took place on 30 April 2007. The Veterinary Laboratories Agency (VLA) was subsequently asked for assistance after epidemiological enquiries identified contact with animals on an open farm (rather than a food-borne infection) as the likely source for two index cases. Neighbouring Health Protection Units were alerted about the cluster of VTEC O157. General practitioners were alerted to report any cases of gastrointestinal illness and to send a sample for microbiological investigation. A structured questionnaire was administered per telephone to all probable cases (or to their parents) to record onset of illness and symptoms, and to explore possible risk factors such as visit to open farms and contact with animals, food and drink risk factors, travel, swimming, and close contact history.

The open farm was visited by the OCT to evaluate risks to health of staff and visitors. This was a typical open farm with ewes, lambs, goats, llamas, pigs, calves, chickens, rabbits and guinea pigs. There had been approximately 14,000 visitors to the farm during the Easter holiday period in April 2007. Environmental testing at the open farm was undertaken to evaluate possible sources of infection.

Microbiological investigations

All cases and household contacts provided stool specimens for laboratory investigation. In addition to considering isolates from the cases linked to the open farm, it was necessary to evaluate the laboratory typing of the strains from all human samples from the region to scrutinise possible links between cases. An additional 83 faecal specimens were collected from the animals on the open farm. Sampling was based on groups of animals sharing the same space, and concentrated on likely contacts: sheep, weaned and unweaned lambs, goats, kids, calves, pot-bellied pigs and pet rabbits.

Human and animal samples were cultured as described by Willshaw et al. [2] and presumptive VTEC O157 isolates were sent for phage typing to the central Laboratory for Enteric Pathogens at the Health Protection Agency (HPA) Centre for Infections. All isolates from human and animal sources were confirmed biochemically as *E. coli* and subsequently serotyped and phage-typed. They were tested for the presence

of VT1 and VT2 by polymerase chain reaction (PCR) and isolates from patients infected with the same phage type of VTEC O157 and animal isolates from the farm were compared by pulse field gel electrophoresis (PFGE) of fragments generated by the restriction enzyme XbaI [2].

Results

A total of 13 confirmed cases of VTEC O157, one of them asymptomatic, occurred in five families in the neighbouring counties of Norfolk and Lincolnshire in April and May 2007. The epidemiological curve for the 12 symptomatic cases is shown in Figure 1. Initially no epidemiological links were established between the four Norfolk families and the incident was investigated as a temporal cluster. After the case from Lincolnshire was identified, an outbreak was declared, consisting of Family A from Norfolk (one primary and two secondary cases) and Family B from Lincolnshire (one primary case only) who had visited the open farm. A narrower case definition for the outbreak was used which included only those persons with a link to the farm. The working hypothesis was that the outbreak was caused by direct transmission from contact with animals at the farm, followed by secondary transmission within the households.

The index case from Family A had visited the farm on 9 April 2007 and developed symptoms on 13 April 2007. It is possible that Case 2 (Table 1) of Family A is a co-primary case. She visited the farm on the 9 April 2007 and developed symptoms on 17 April 2007 (eight days later). The index case from Family B visited the farm on 20 April 2007 and developed symptoms on 22 April 2007. The main risk activities were identified from the questionnaires (100% response rate) as being bottle feeding of lambs and feeding and petting goats.

In addition to the outbreak cases, there were eight cases and one asymptomatic carrier from three other families (C, D and E) with no ascertainable links to the open farm or to Families A or B. These family groups were investigated and followed up as part of a

FIGURE 1

Cluster of verotoxin-producing *E. coli* O157 gastroenteritis, eastern England, April–May 2007 (n=12)

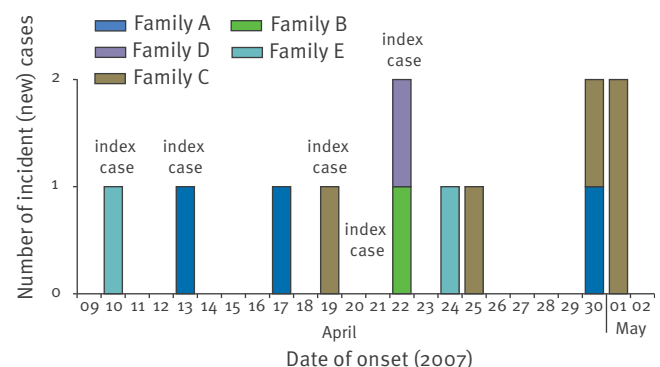


TABLE 1

Cases of verotoxin-producing *E. coli* O157 gastroenteritis, asymptomatic infections and family contacts, eastern England, April–May 2007

Family	Cases	Age group (years)	Area (County)	Symptoms	Date of onset	Date of positive specimen	Result	Relationship to index	Risk factors	Special control measures	Phage type
A	Case 1	5-10	Norfolk	Yes	13 Apr	17 Apr	Positive	Index case	Visited the open farm		PT2
A	Case 2	0-4	Norfolk	Yes	17 Apr	19 Apr	Positive	Sibling	Visited the open farm	Excluded from nursery	PT2
A	Case 3	35-40	Norfolk	Yes	30 Apr	1 Apr	Positive	Parent	Visited the open farm		PT2
A	Contact	50-54	Norfolk	No	NA	NA	Negative	Parent	Visited the open farm		
A	Contact	11-15	Norfolk	No	NA	NA	Negative	Sibling	Visited the open farm		
B	Case 4	5-10	Lincolnshire	Yes	22 Apr	26 Apr	Positive	Index case	Visited the open farm	Excluded from school (hospitalised with HUS)	PT2
B	Contact	35-40	Lincolnshire	No	NA	NA	Negative	Parent	None known		
B	Contact	30-34	Lincolnshire	No	NA	NA	Negative	Close relative	Visited the open farm		
B	Contact	35-40	Lincolnshire	No	NA	NA	Negative	Close relative	Visited the open farm		
B	Contact	60-64	Lincolnshire	No	NA	NA	Negative	Close relative	Visited the open farm		
C	Case 5	5-10	Norfolk	Yes	19 Apr	23 Apr	Positive	Index case	Lives next to another farm (private, not open to the public)	Excluded from school	PT2
C	Case 6	30-34	Norfolk	Yes	1 May	1 May	Positive	Parent	Lives next to another farm (private, not open to the public)		PT2
C	Contact	25-30	Norfolk	No	NA	25 Apr	Positive	Parent	Pig stockman, lives next to another farm (private not open to the public)		PT2
C	Case 7	0-4	Norfolk	Yes	25 Apr	25 Apr	Positive	Sibling	Lives next to another farm (private, not open to the public)	Excluded from nursery	PT2
C	Case 8	0-4	Norfolk	Yes	30 Apr	30 Apr	Positive	Sibling	Lives next to another farm (private, not open to the public)	Excluded from nursery	PT2
C	Case 9	50-54	Norfolk	Yes	1 May	3 May	Positive	Close relative	None known	Hospitalised	PT2
D	Case 10	30-34	Norfolk	Yes	22 Apr	25 Apr	Positive	Index case	Consumption of wild rabbit from private source		PT 21/28
D	Contact	35-40	Norfolk	No	NA	NA	Negative	Partner			
D	Contact	11-15	Norfolk	No	NA	NA	Negative	Child			
D	Contact	50-54	Norfolk	No	NA	NA	Negative	Parent			
D	Contact	50-54	Norfolk	No	NA	NA	Negative	Parent			
E	Case 11	0-4	Norfolk	Yes	10 Apr	20 Apr	Positive	Index case	Played with a friend who had diarrhoea and vomiting		PT8
E	Case 12	20-24	Norfolk	Yes	24 Apr	24 Apr	Positive	Parent			PT8
E	Contact	30-34	Norfolk	No	NA	NA	Negative	Parent			

HUS: haemolytic uraemic syndrome; PT: phage type.
 Outbreak cases are marked in blue.

separate cluster. One of these families (C) lived next to a farm where the father worked as a pig stockman. Five members of that family developed gastroenteritis caused by the same phage type (PT₂) of VTEC O157 but with a distinct PFGE profile (Table 1). The asymptomatic carrier was also a member of Family C and was found positive for the same strain of VTEC O157. Three of the six family members developed symptoms or were tested positive for VTEC O157 within a period of six days (between 19 and 25 April 2007), the others later. It could be assumed that these three cases were co-primary cases and had the same environmental exposure. Environmental sampling was not done because the farm was private and it did not present risk to the public. Consumption of a wild rabbit purchased from a private source was a suspected risk factor for family D. No food specimen was available to test this hypothesis however. Possible sources of infection for family E were unpasteurised cheese (which tested negative on culture) and a friend with diarrhoea and vomiting who had played with the five year-old index case.

Overall, this spatiotemporal cluster consisted of 12 laboratory-confirmed cases and one asymptomatic carrier from five families with isolates of *E. coli* O157 (Table 1). Four of the cases were linked to the open farm (Families A and B), while in eight cases and the asymptomatic

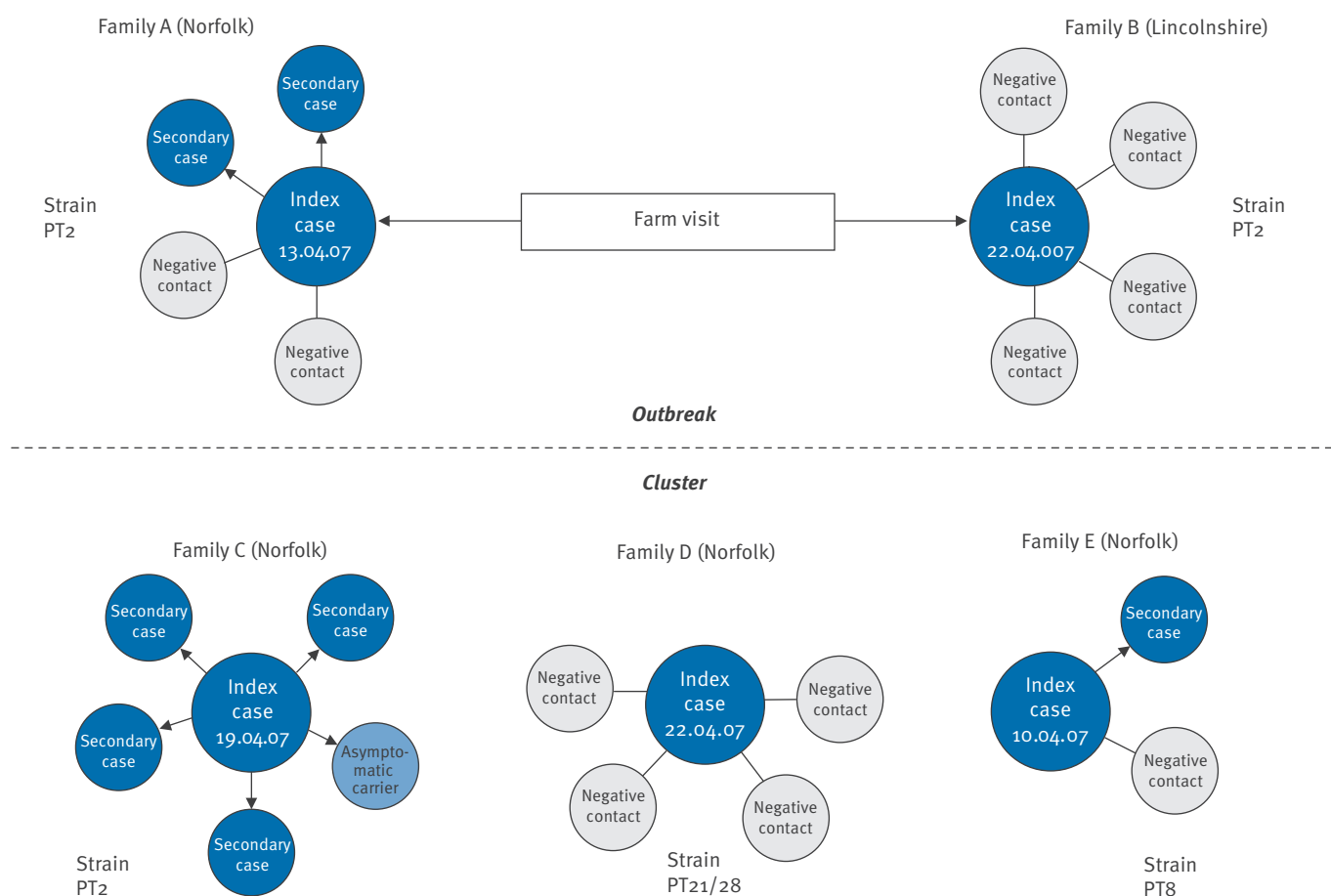
carrier (Families C, D and E) there was no epidemiological link to the farm (Figure 2). Nine of the cases were children aged five months to 13 years. Five cases were hospitalised: a six year-old child from Family B was admitted with HUS, a 53 year-old secondary case from Family C with severe diarrhoea, and three further cases were admitted to hospital for overnight stays.

Laboratory confirmation of epidemiological link with the open farm

Veterinary investigations yielded 17 presumptive *E. coli* O157 isolates from 83 samples taken. Fourteen isolates were from adult sheep or lambs, including lambs which had been bottle-fed by visitors. In addition, samples from one adult pig (out of three tested) and two 4–6 month-old cattle (out of 17 tested) were positive. Isolates from the two primary cases from Families A and B who had visited the farm and the two contacts from Family A were confirmed as *E. coli* O157 phage type PT₂ with genes for VT₂ but not VT₁. The veterinary isolates were also VTEC O157 PT₂, VT₂. The PFGE profiles of the strains comprised at least 20 XbaI fragments. A single profile was seen in the isolates from three human strains and all 17 animal isolates. The profile of the isolate from the first primary case had some evidence of one additional band in the profile that was not present in the strain from his

FIGURE 2

Spread of verotoxin-producing *E. coli* O157 infection within households, eastern England, April–May 2007



contacts (Table 2). The variation was not considered significant given that the strains were indistinguishable by other tests and their sources could be linked epidemiologically.

Isolates of VTEC O157, PT2, VT2 were also confirmed in six members of Family C in Norfolk. PFGE showed that the strain infecting this family differed by at least five fragment positions (out of 20) from the farm-associated cases. Evidence from the Laboratory for Enteric Pathogens database of profiles indicated that the strains were genotypically distinct from those isolated at the farm. This supported the view that these cases were not linked to the farm or to the families that visited it.

VTEC O157 of phage types other than PT2 were isolated from the other two Norfolk families (Table 1). Two isolates from Family E were confirmed as belonging to PT8, VT1 and VT2. A single isolate received from Family D was PT21/28, VT2. Given the discrimination by phage typing, none of the isolates was examined by PFGE.

Outbreak control measures

Initial outbreak control measures were instituted as soon as an outbreak was identified and included increased surveillance by raising awareness amongst primary care staff, hospital clinicians and laboratories, and informing Health Protection Teams and

Environmental Health Departments and general practitioners in neighbouring counties. All cases were followed up urgently by the Environmental Health Departments for the area. Affected families were given advice regarding hygiene measures to prevent secondary cases within households.

The OCT initially considered the option of temporarily closing the farm to the public pending investigations. However, any such formal enforcement action had to be based on a risk assessment of the threat to the health of the public. It was agreed at the time that the cases in two unrelated families did not constitute a significant risk, especially in view of the large number of visitors to the farm during the perceived exposure period. Furthermore, no immediate significant risks to public health were identified during the OCT's inspection of the farm. It was therefore decided not to close the farm, but following the advice of the OCT from 30 April 2007, the farm management (who were very cooperative), took immediate measures to improve hand washing facilities and signage around the site. Separate and identifiable areas were allocated for visitors to eat and drink. Staff were briefed about the need for increased hygiene and supervision of visitors, and the petting of animals and bottle-feeding of lambs was suspended voluntarily on a temporary basis. After confirmation of positive animal specimens, access of the public to the building housing most infected animals was restricted, and the areas were emptied of livestock and cleaned and disinfected thoroughly. These immediate measures were formalised on 4 May 2007 with the issuing by the local authority of a statutory notice under the Health and Safety at Work etc Act 1974 to require that health and safety risk assessments be carried out. The farm management complied with the notice by reviewing and updating the risk assessment relating to the visitor and animal contact activity, in accordance with the Health and Safety Executive guidance [19] and implementing the additional measures identified. The newly introduced measures included improving visitor information and leaflets. No further cases of VTEC O157 infection in visitors were reported that year.

TABLE 2

Typing of isolates of verotoxin-producing *E. coli* O157 linked to the open farm in Eastern England, April–May 2007

Case/source of specimen	Phage type	Presence of verotoxin gene by polymerase chain reaction	Pulse field gel electrophoresis
Family A, index case, visited farm (Case 1)	2	VT2	Profile 1 (possible extra small fragment; not significant)
Family A contact (Case 2)	2	VT2	Profile 1
Family A contact (Case 3)	2	VT2	Profile 1
Family B, index case, visited farm (Case 4)	2	VT2	Profile 1
Empty calf house	2	VT2	Profile 1
Calves 4–6 months-old	2	VT2	Profile 1
Pigs	2	VT2	Profile 1
Weaned lambs	2	VT2	Profile 1
Unweaned lambs (2–3 months-old)	2	VT2	Profile 1
Sheep house 1	2	VT2	Profile 1
Sheep house 2	2	VT2	Profile 1
Sheep house 3	2	VT2	Profile 1

VT: verotoxin.

Source: Laboratory of Gastrointestinal Pathogens, Centre for Infections, Health Protection Agency, UK.

Discussion and conclusions

Exposure to livestock on open farms continues to pose a threat to the general public and particularly to children, and a number of outbreaks have been reported from the UK [20–24]. In August and September 2009, an outbreak of *E. coli* O157 at Godstone Farm in England involved 93 visitors [7]. Seventeen of the cases (all of them children) were diagnosed with HUS. A review and analysis of open farm outbreaks in England and Wales over the period 1997–2007 has been presented by Pritchard et al. [6]: VTEC O157 was confirmed in 61.3% of the investigated premises containing animals of various species. *E. coli* O157 was isolated in 17.8% of all samples, and verotoxin genes were detected by PCR in 98.4 % of representative isolates. The main phage types were 2 and 21/28, which were also the most common types isolated from human cases during that 10

year period. The Health and Safety Executive guidance advises farmers to assume that their animals carry *E. coli* O157 and to put control measures in place to minimise the risk to visitors [19]. In the outbreak in 2007 described in this paper, results of phenotypic and genotypic typing of isolates from farm visitors and livestock supported the epidemiological evidence that contact with animals or their faeces was the source of the primary infections.

In August 2008, a further case of VTEC O157 with a link to the same open farm was reported to the Norfolk, Suffolk and Cambridgeshire HPU Norfolk office. The isolate was PT21/28, a different strain from the one isolated in the outbreak in 2007 described here. The index case was a seven year-old child who had most probably acquired the infection from her 11 month-old sibling whose stool was subsequently found positive for O157 and who had visited the open farm five days before onset of symptoms. A further site visit found that the recommendations from the previous year had been fully implemented. The only further recommendation to the farm was to additionally warn visitors that children aged two years or younger should be particularly protected, as enforcing hygiene measures and avoiding contact with the ground is particularly difficult in this age group.

This cluster of VTEC O157 infections also highlighted the risk of person-to-person spread of infection among family members. The need for follow-up of cases and enhanced advice on hygiene measures in the households to prevent secondary transmission should be stressed, particularly since transmission can also occur from asymptomatic cases [25]. The further case in 2008 highlights the difficulty of ensuring strict hygiene in very young children. Such children are likely to have direct contact with possibly contaminated ground, and are also unlikely to be able to follow hygiene guidance. They may also be in nappies, and contact with children in nappies is a known risk factor for gastrointestinal infection [13].

Multidisciplinary collaboration among the health protection, veterinary, environmental health and laboratory services was crucial for the prompt and successful control of this cluster of VTEC O157 infections. A particular issue faced by an OCT in this situation is the lack of clarity surrounding the availability and use of immediate formal action (if applicable) in the case of a perceived risk to the public posed by a commercial business. In this particular case, the OCT made a judgement based on an on-site assessment that the farm did not present a sufficient risk to the public to warrant temporary closure, and felt that the hazard from VTEC O157 could be controlled by risk management procedures involving cooperation by the farm management with immediate institution of improved safety measures. The Health and Safety at Work etc Act 1974, together with the associated guidance for open farms, gives a framework for a statutory response to situations where

employees and/or visitors may be exposed to risks of infection due to workplace activities. The outbreak at Godstone Farm in 2009 highlighted the importance of keeping public areas free from contamination with animal faeces, providing information to the public, and supervising children's handwashing [7].

There is a need to proactively re-assess ongoing health risks on open farms and ensure that control measures are in place at all times but particularly during peak holiday periods. Members of the public, particularly parents and children, should be kept well informed about the potential risks from zoonotic transmission of diseases such as VTEC.

It should be emphasised, however, that the risk of acquiring VTEC O157 infection from open farms is minimal compared with other hazards of daily life and that open farms serve an important educational role and are a major contributors to the tourism and leisure industries.

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ECDC introduces European monthly measles monitoring

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On 13 July 2011, European Centre for Disease Prevention and Control (ECDC) published the first issue of the *European monthly measles monitoring* [1]. The objective of this monthly online publication is to provide European Union (EU) Member States and European Economic Area (EEA) countries and other stakeholders with timely updates on the measles situation in Europe.

In 2010, the countries in the World Health Organization European Region committed to eliminate measles and rubella transmission by 2015 [2]. However, there are clear indications that the intensified circulation of measles virus in western Europe over the last few years will continue in 2011. ECDC's epidemic intelligence activities revealed six deaths and more than 21,000 measles cases in 23 of the 27 EU and four European Free Trade Association countries during the first six months of 2011. A high proportion of the infected were unvaccinated and the highest incidence was observed among children under one year of age who are too young to be vaccinated. The majority of cases result from transmission within and between EU Member States.

In response to the ongoing measles epidemic in the EU, ECDC will step up surveillance and establish a confidential communication platform for timely exchange related to vaccine preventable diseases, including outbreak reporting information between the Member States. In addition to this, the *European monthly measles monitoring* provides feedback to countries and decision makers with information compiled from multiple sources such as national websites, the EUVAC.NET database, the Early Warning and Response System (EWRS), validated media reports, and personal communication from national authorities. The interval for this report has been upgraded from quarterly to monthly in order to increase timeliness of the information provided.

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