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Japanese encephalitis virus RNA detected in Culex pipiens mosquitoes in Italy

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Mosquitoes collected in northern Italy were screened for flavivirus RNA. Positive amplicons were sequenced and found most similar to insect flavivirus (ISF), Usutu virus (USUV) and surprisingly also to Japanese encephalitis virus (JEV). The sequence (167 bp), obtained from one pool of Culex pipiens, was found identical to JEV strains from bats in China. Unfortunately additional sequence data or virus isolations were not obtained in this study. Confirmation of potential introduction of JEV to Italy and other European countries is urgently needed.

In the course of a small-scale preliminary study screening for the presence of flavivirus RNA in mosquitoes in Italy, we obtained sequences of three different flaviviruses; an insect-specific flavivirus (ISF) related to cell fusing agent virus, Usutu virus (USUV) and, to our surprise, also of Japanese encephalitis virus (JEV). While ISF and USUV have been documented previously in Italy and several other European countries [1,2], JEV has, to our knowledge, not been detected in mosquitoes in Europe so far. JEV is a mosquito-borne flavivirus known to be endemic in Asia, extending to India and Pakistan in the west, where it is a leading cause of encephalitis. Although commercial inactivated vaccines are available against JEV, it causes an estimated annual number of 30,000–50,000 cases worldwide [3]. The majority of the infections are subclinical, but up to 30% of symptomatic patients die, and 30% of the survivors have persistent neurological sequelae [3]. The life cycle of JEV includes *Culex* spp. mosquitoes and water birds or pigs, but JEV also infects a wide range of other vertebrates. In addition to humans, horses may develop encephalitis and are considered dead-end hosts for JEV transmission [4].

Sample collection

Following the active circulation of WNV and USUV, the recent detection of novel ISFs in Italy and elsewhere in Europe, and the detection of dengue virus in southern France and Croatia [1,7,10,11], the aim of this study was to screen mosquitoes for flavivirus RNA using a system allowing the detection of all flaviviruses. Female mosquitoes were collected in late summer of 2010 and 2011 in rural areas near Modena and Bologna in Emilia-Romagna region (Figure 1), using CO_2-baited traps.

Mosquitoes were identified using morphological characteristics [5], pooled by species (identification at subspecies level was not done), date and site of collection (with a maximum of 27 individuals per pool) and stored at -80°C until processed. The mosquito species collected included mainly *C. pipiens*, and additionally *Aedes albopictus*, *A. caspius* and *A. vexans*. A total of 62 pools were studied; 52 had been collected in 2010 (all *C. pipiens*) and 10 in 2011 (five *C. pipiens*).

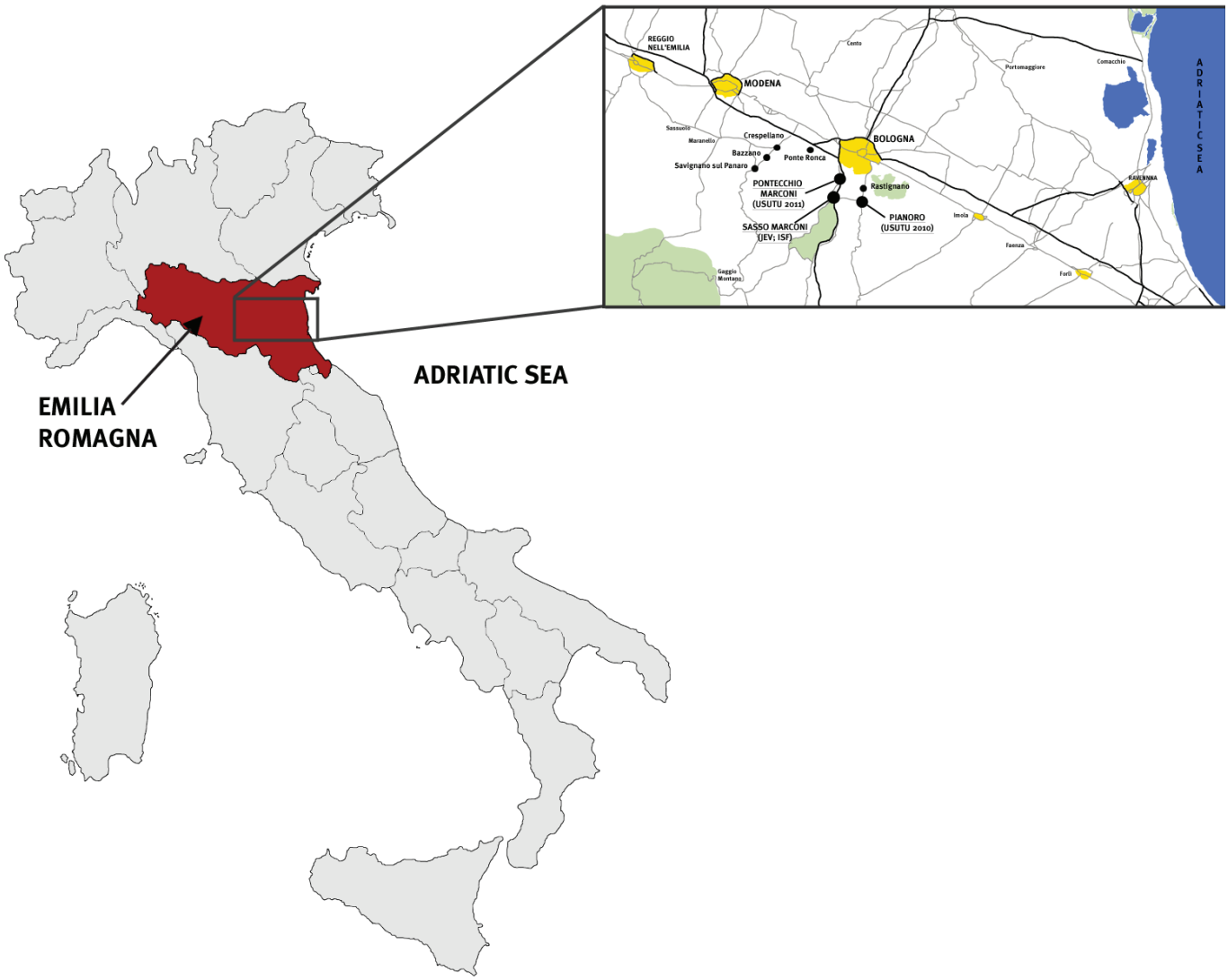
Molecular analysis

The mosquitoes were ground manually using sterile sand and Dulbecco's phosphate-buffered saline. Nucleic acids were extracted using EasyMag (bioMérieux) and examined by RT-PCR targeted to a conserved region of the flavivirus NS5 gene [6]. The PCR products were sequenced directly and cloned when necessary (CloneJET PCR Cloning Kit, Fermentas). The obtained sequences were identified using BLAST (blast.ncbi.nlm.nih.gov/Blast.cgi).

Of the 62 pools, five were found positive for flavivirus RNA. The sequences (Box) were identified as (i) ISF from *A. albopictus*, 2011 (two sequences; lengths 133 and 87 bp; identical in the overlapping region), (ii) USUV from *C. pipiens*, 2010 and 2011 (two sequences; lengths 133 and 167 bp; identical in the overlapping

Figure 1

Mosquito collection locations, Italy, summer 2010 and 2011



The black dots represent the eight collection sites near Bologna and Modena.

region) and (iii) JEV (one sequence; 167 bp) from one pool of *C. pipiens*, 2010 (Figure 2). The ISF (collected in Sasso Marconi) and USUV (collected in Pontecchio Marconi and Pianoro) sequences were identical to other sequences previously reported from Italy [1,7]. The JEV sequence (genomic position: 9,109–9,275) was obtained from mosquitoes collected in Sasso Marconi. It showed 100% similarity to four sequences in Genbank, all of them representing JEV genotype III viruses isolated from bats in China between 1986 and 2009 (JN711458, JN711459, JF706285, JF185036).

The PCR product yielding the NS5 sequence related to JEV was amplified from the original material twice and sequenced in three separate laboratories. Additional sequence data would be needed for detailed characterisation of the viral strain and sequence analysis, but unfortunately the attempts to amplify longer sequences from the JEV-positive pool using primers targeted to E, NS5 and NS3 regions in nested and semi-nested protocols remained negative. Attempts to isolate the virus from the JEV- and USUV-positive pools on Vero and on C6/36 insect cells were not successful.

Discussion

While the potential risk of JEV spreading to Europe has been acknowledged before [8], and despite the active surveillance for flaviviruses such as WNV and USUV, to the best of our knowledge, this is the first report of a JEV-like sequence in mosquitoes in Europe. The JEV-like sequence was detected within a small scale preliminary study, and some details of the field work along

Box

Viral nucleotide sequence fragments obtained from mosquitoes collected in Italy, summer 2010 and 2011 (n=3)

JEV_pool_M20 167 bp

TCATGTGGCTTGGAGCACGGTATCTAGAGTTTGAAGCTTTGGGGTTCCT
GAATGAAGACCATTGGCTGAGCCGAGAGAATTCAGGAGGTGGAGTGG
AAGGCTCAGGCGTCCAAAAGCTAGGATACATCCTCCGTGACATAGCAGG
AAAGCAAGGAGGGAAAATGTAC

USUV_pool_M7 167 bp

TCATGTGGCTAGGCGCCAGATTCCTGGAGTTTGAAGCTCTGGGCTTTCT
GAATGAGGACCATTGGTTAGGAAGAAAGAATTCTGGAGGAGGTGTTG
AAGGACTTGGTGTCCAAAAACTTGGTTACATTCTGCGTGAGATGAGCC
ACCATTCAGGTGGGAAAATGTAC

ISF_ pool_M2B 133 bp

CTCGGAAGTCGTTTTCTGGAATTTGAGGCCTTGGGGTTCCTAAAT
GCTGATCACTGGGTCAGTCGTGAAAACTTTCCTGGGGGCGTGGGT
GGAGTGGGTGTCAATTACTTTGGCAACTACCTAAAGGAAATTT

with mosquito subspecies identification were unfortunately not documented in detail. Laboratory contamination as the source of the obtained JEV sequence was highly unlikely, as no JEV virus, RNA or PCR products had ever been handled in the laboratories where the mosquitoes were processed or where the RT-PCRs were performed. Interestingly, Mani et al. have reported detecting in 1996-97 JEV antibodies and RNA in Italian birds [9], but unfortunately no further information is currently available about the sequences found in that study.

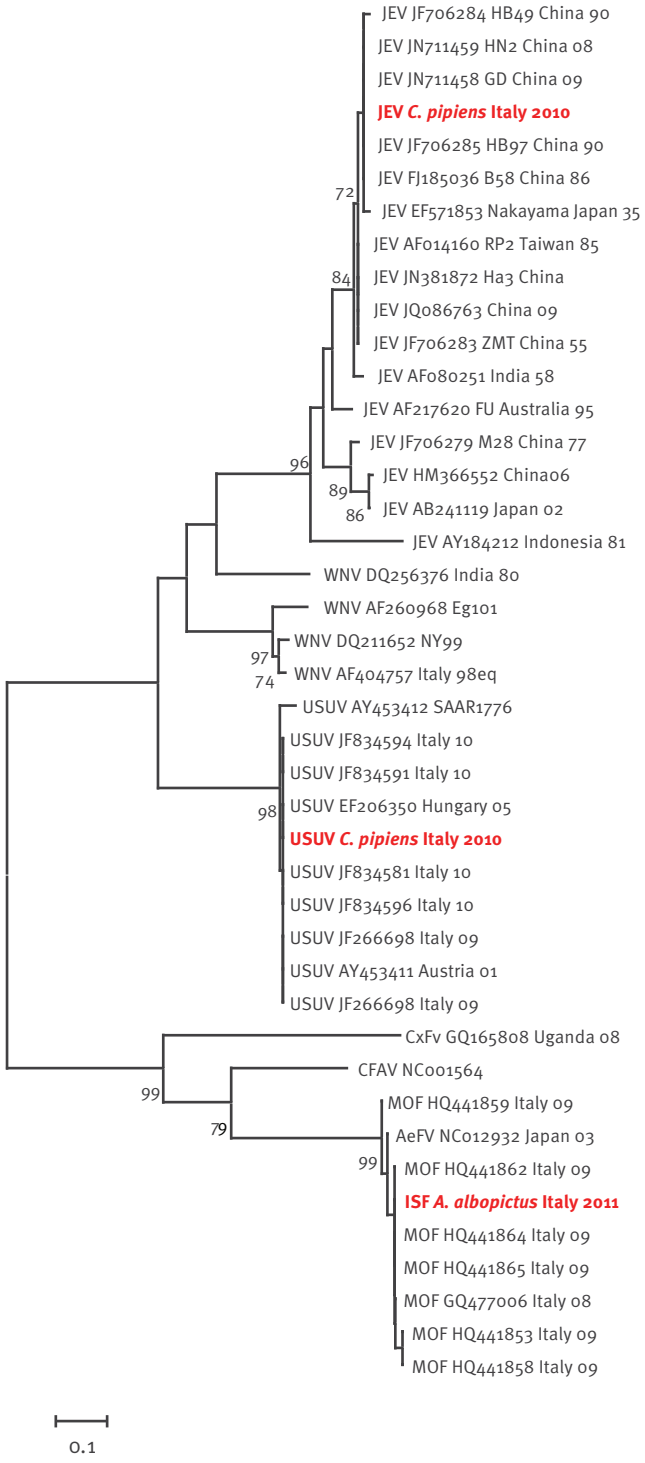
Recently, autochthonous dengue virus (DENV) infections have been detected in France [10] and Croatia [11]. While these viruses are most likely to have been imported there from endemic regions, most probably through viraemic travellers or via materials harbouring infected mosquitoes, eggs or larvae, JEV could have been introduced to Italy through waterfowl or wild waterbirds. Future arbovirus surveillance should include JEV-specific or pan-flavivirus detection methods, and it should be noted that due to cross-reactions, serological assays with the exception of seroneutralisation are probably unable to differentiate an immune response to JEV from one to WNV and USUV.

Conclusions

A partial genomic sequence of JEV was detected in Italian *C. pipiens* mosquitoes for the first time, but confirmation of the finding by additional sequence data or virus isolation has not yet been successful. The authors are aware that these findings are preliminary, and confirmation of the results is necessary. Further evidence of JEV circulation is required for evaluating the possible need for precautionary measures against JEV transmission in Italy and other European countries.

Figure 2

Phylogenetic tree based on a 122 bp region of flavivirus NS5 sequences obtained from mosquitoes collected in Italy, summer 2010 and 2011 (n=3)



ISF: insect-specific flavivirus; JEV: Japanese encephalitis virus; USUV: Usutu virus.

Mosquito pools positive for JEV and ISF were collected at Sasso Marconi, and pools positive for USUV from Pontecchio Marconi and Pianoro. The neighbour-joining phylogenetic tree was estimated using maximum composite likelihood model, including 1,000 bootstrap replicates using programme Mega.

Acknowledgments

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Public health response to an outbreak of Legionnaires' disease in Edinburgh, United Kingdom, June 2012

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We report an outbreak comprising 50 confirmed cases of Legionnaires' disease in Edinburgh, Scotland, June 2012. In addition, there were 49 suspected cases. Epidemiological evidence suggests that a common outdoor airborne exposure occurred over south-west Edinburgh. This probably emanated from cooling towers in the north-east of the affected area, although not yet clearly linked by scientific evidence. The co-ordinated public health, environmental and clinical response helped prevent ongoing exposure and mitigated associated mortality and morbidity.

In Scotland 15 to 40 cases of Legionnaires' disease occur annually, with approximately half travel-associated [1]. On 31 May 2012, a single case of Legionnaires' disease was reported and investigated in Edinburgh. After further notifications on 2 and 3 June, the number of reported cases of *Legionella pneumophila* infection in Lothian was four confirmed and four suspected. An incident management team (IMT) was convened on 3 June, in line with the Scottish Government framework for managing public health incidents [2].

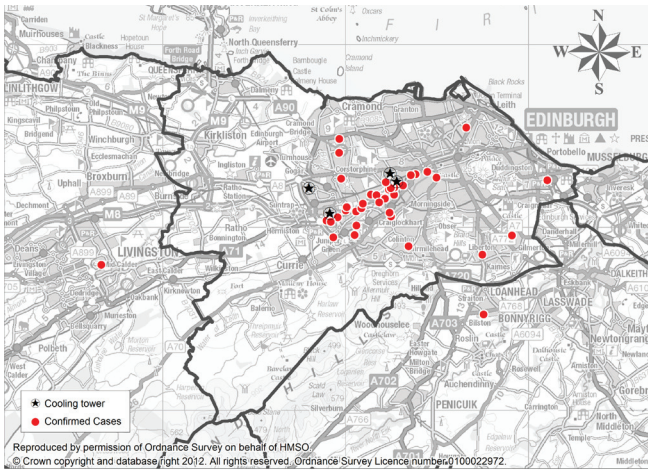
During June 2012 the IMT met twelve times. The Scottish Government established their Resilience Room, a coordination facility activated in cases of crisis, on 5 June following an increase in cases. A helpline was established via NHS 24 (http://www.nhs24.com/) on 6 June and regular updates sent to clinicians and information leaflets distributed to affected areas of Edinburgh from 7 June onwards. The Chair of the IMT provided five television, radio and newspaper interviews on 6 June and twenty in total over the following four weeks.

Epidemiological investigation

Mapping of cases (Figure 1) indicated that all were resident in or linked to south-west Edinburgh. Case definitions were based on European Union guidelines [3]. A confirmed case was defined as an individual with community-acquired pneumonia, microbiologically confirmed *Legionella pneumophila*, disease onset from 14 May 2012, based on the first case being notified 31 May and taking into account the incubation period, and with links to south-west Edinburgh. Based on clustering of cases, location of cooling towers and the prevailing wind, the IMT hypothesised that the most likely source of infection was the cooling towers to the north-east of the area. Immediate action was taken to sample and disinfect potential sources under the Public Health etc. (Scotland) Act 2008 [4], implement active case finding and inform public health agencies across the United Kingdom (UK). The public were informed of symptoms of Legionnaires' disease on 3 and 4 June and advised to contact primary care services if unwell. Further epidemiological investigations were undertaken by the Lothian public health team and Health Protection Scotland. All cases were interviewed after notification to obtain 'travel diaries' for 2 weeks prior to onset of symptoms and ascertain place of residence and work. Descriptive epidemiology determined

Figure 1

Confirmed cases of Legionnaires' disease by place of residence, Edinburgh, United Kingdom, as of 1 July 2012 (n=50)



HMSO: Her Majesty's Stationery Office

Not included in the map are 5 of the 50 cases who were resident in other NHS boards.

dates of onset of illness, disease status and age, sex and spatial distribution. Wind conditions were modelled between 14 May and 5 June and 'travel diaries' analysed to determine the association between cases and likely exposure.

Environmental and microbiological investigations

All cooling towers are required by law to be registered with the local authority [5] and management of water-associated Legionella risks is measured against the standards in the approved code of practice [6]. In addition to the four sites with cooling towers identified on day one of the outbreak (see Figure 1), the City of Edinburgh Council (CEC) and the Health and Safety Executive (HSE) [7] identified a further 60 sites including cooling towers, sprinkler systems and industrial washing facilities. These sites were assessed for risk based on their location and nature, and visited by staff from the CEC and the HSE. Where appropriate, water samples were taken and any potential sources disinfected. Samples were tested for Legionella species by the CEC Scientific Services, the National Reference Laboratory and the Health Protection Agency with the intention to match any environmental isolates with human isolates.

Results

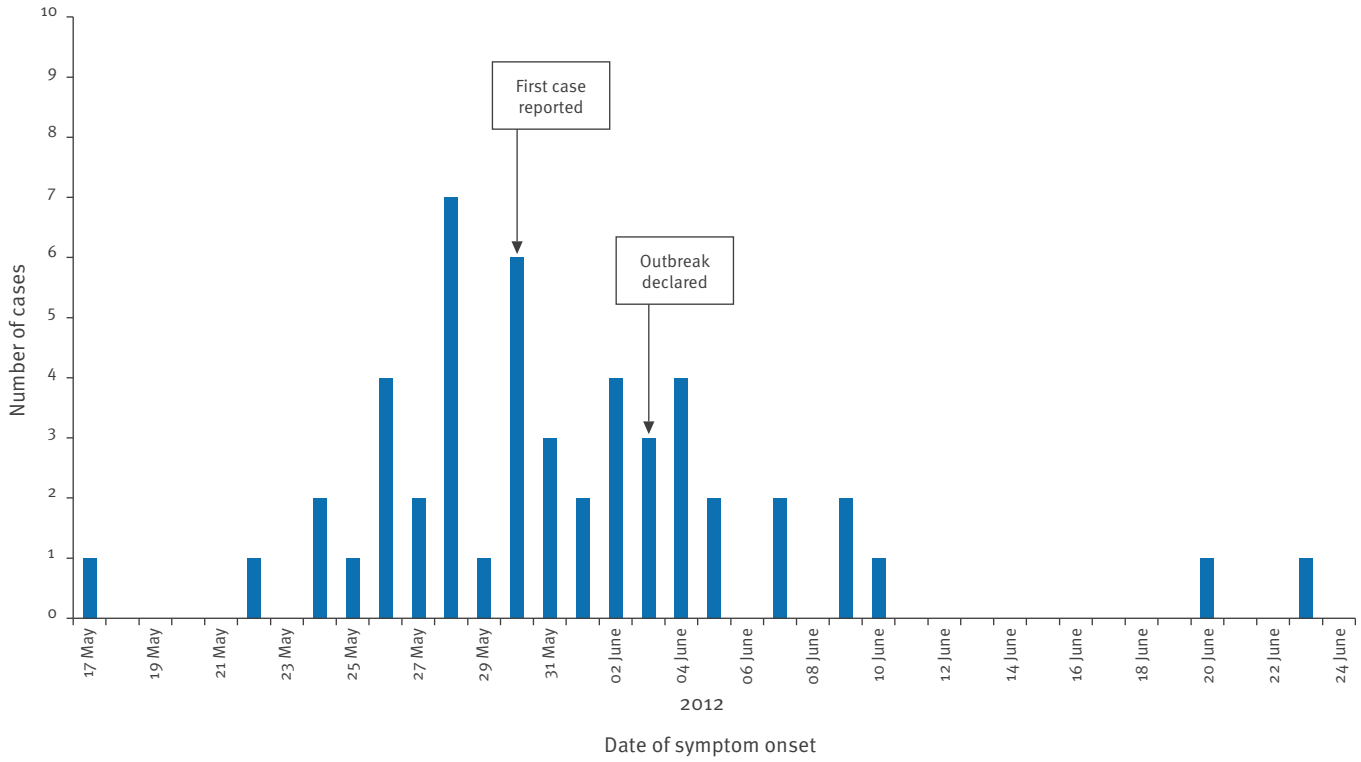
As of 1 July 2012, 50 confirmed cases have been identified. (see Figure 1) The average age was 56 years (range: 32–85 years), 72% were male and with two deaths amongst those confirmed to have Legionnaires' disease the mortality rate is 4.25%. A third death in a suspected case has also been reported. The confirmed cases were typical of Legionnaires' disease, predominantly males, smokers, aged over 50 years and with underlying health problems. There were also 49 suspected cases. Of the confirmed and the 49 suspected cases, 19 patients have received treatment in critical care and 52 patients on general wards. In addition, a large number of symptomatic individuals were assessed in the community.

The first date of onset was reported as 17 May. The epidemic curve of confirmed cases (Figure 2) shows a peak date of onset on 28 May. The peak in reporting was on 5 June. This pattern may reflect the combination of incubation period, increased case finding and increased public awareness.

The response from all clinical services was exceptional. Figure 3 shows the number of calls received by the NHS 24 helpline. Many of these callers would have

Figure 2

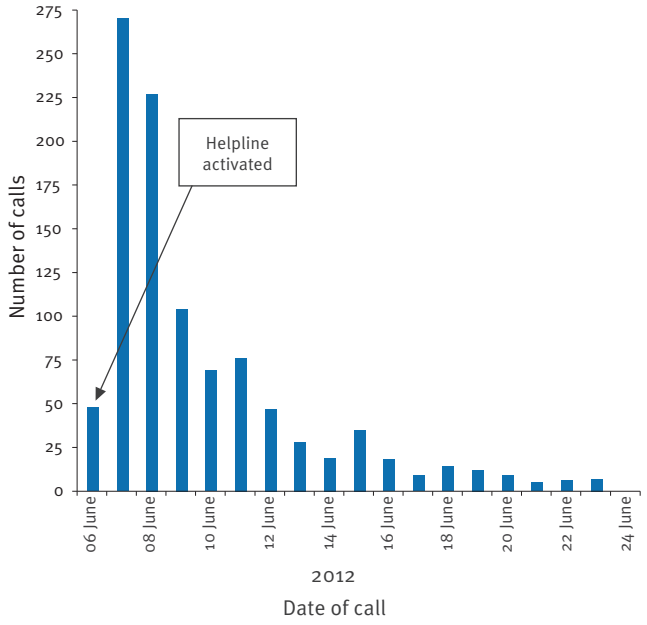
Confirmed cases of Legionnaires' disease by onset date, Edinburgh, United Kingdom, 17 May–1 July 2012 (n=50)



Source: NHS 24, 25 June 2012

Figure 3

Calls to the NHS 24 helpline, Edinburgh, United Kingdom, 6–23 June 2012 (n=1,003)



Source: NHS 24, 25 June 2012

been directed to consultations of general practitioners. The peak in demand on 7 and 8 June coincides with the first day the information leaflets were distributed to the public.

Epidemiological and meteorological evidence suggests that a common outdoor airborne exposure occurred over south-west Edinburgh, most probably emanating from the cluster of cooling towers in the north-east of the affected area between 23 May and 6 June 2012.

Microbiological results from 50 cases showed the presence of *Legionella pneumophila* serogroup 1. An additional 1,444 urine samples tested negative, as well as 557 sputum samples (some patients submitted both samples). After extensive environmental testing there is, as of 1 July, no current microbiological evidence to confirm the presence of *Legionella pneumophila* in any of the samples taken from potential sources. However, voluntary closure of cooling towers was attained from 7 June and since then eight improvement notices have been served to companies in the area by the HSE and CEC under the Health and Safety at Work etc. Act [8]. There is an ongoing joint investigation by Lothian and Borders Police and the HSE into the three deaths, with the HSE also investigating compliance with legal standards.

Discussion

The outbreak reported in Lothian is the largest in Scotland to date. In the last ten years, significant community outbreaks of Legionnaires' disease associated with cooling towers have occurred in the UK and elsewhere in Europe [9]. Other outdoor sources include industrial air scrubbers (Norway) [10], decorative fountains (Wisconsin) [11] and hot water systems (Denmark) [12]. The largest recorded European outbreak was in Murcia, Spain (449 cases) [13] and the largest in England was Barrow in Furness (185 cases) [14].

The current outbreak in Edinburgh occurred in a densely populated area of the capital city and the cluster of cases was well demarcated. This may be due to population density, the north-easterly wind and the topography as the area (Figure 1) is built up in a valley creating 'urban canyons' which can channel air flow. A significant proportion of Edinburgh's population may have been exposed to the plume because a main route from the city centre towards the two major motorways and the airport goes through the affected area.

Whilst expected mortality is often in excess of 10%, the low mortality observed (4.3% in the confirmed cases) may suggest that the timing and quality of care plus the proactive communication strategy may have mitigated the impact. The clustering of the dates of onset indicates a point source exposure which has now stopped and suggests that the potential sources were correctly identified and effectively treated at the beginning of the outbreak. Ongoing epidemiological investigations include sero-prevalence and case–control studies. The aim is to assess the extent and gradient of disease in the community, and the characteristics of those who developed Legionellosis.

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Duncan McCormick, Consultant in Public Health Medicine, NHS Lothian; Simone Thorn, Lead Health Protection Nurse Specialist, NHS Lothian; Colin Sibbald, Food Health and Safety Manager, City of Edinburgh Council; Andy Campbell, Food Health and Safety Officer, City of Edinburgh Council; Robbie Beattie, Scientific and Environmental Services Manager, City of Edinburgh Council; Stuart Wilson, Director of Communications, NHS Lothian; John Healy Team Leader, FOD SG Occupational Hygiene Team, Health and Safety Executive; Alistair McNab, Head of Operations (Scotland) Field Operations Directorate, Health and Safety Executive; Garry Stimpson, HM Principal Inspector of Health and Safety, Health and Safety Executive; Martin Donaghy, Medical Director, Health Protection Scotland; Alison Potts, Epidemiologist, Health Protection Scotland; Alison Smith-Palmer, Epidemiologist, Health Protection Scotland; Lynn Cree, Environmental Health Adviser, Health Protection Scotland; Jim McMenamin Consultant Epidemiologist, Health Protection Scotland; Michael Gillies, Clinical Director of Critical Care, NHS Lothian; Sian Tucker Acting Clinical Director, Lothian Unscheduled Care Service, NHS Lothian; Carol Harris, Communications Manager, NHS Lothian; Mary Hanson, Consultant Microbiologist, NHS Lothian; Johnathon Mills, Specialty Registrar, Medical Microbiology, NHS Lothian; Jane Lindsay, Reference Laboratory, Glasgow; Giles Edwards, Reference Laboratory, Glasgow;
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Investigations and actions taken during 2011 due to the first finding of *Echinococcus multilocularis* in Sweden

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Echinococcus multilocularis is a parasite that can cause alveolar echinococcosis disease. After the first positive finding of *E. multilocularis* in Sweden in 2011, a consulting group with representatives from relevant authorities was summoned. In this group, all relevant information was shared, strategies for information dissemination and any actions to be taken due to the finding of *E. multilocularis* were discussed and decided. The present paper describes the actions taken during 2011 and the results thereof, including surveillance in animals, risk assessment for humans to become infected and recommendations given to the public. Further discussion about whether the parasite was introduced, and if so, how, as well as possible future development of the infection in animals and humans in Sweden and future actions are included.

Introduction

Alveolar echinococcosis (AE) is a disease in humans caused by the larval stage of the tapeworm *Echinococcus multilocularis* (EM). It is considered to be the most serious parasitic disease in humans in Europe [1].The parasite develops with a tumour-like growth almost exclusively in the liver and the disease is characterised by a long incubation period, between five and 15 years, followed by a subsequent chronic course [2]. Although a serious disease, in Europe, the reported prevalence in humans is low, up to 1.4 per 100,000 population [2]. During the last decades, the known range of the parasite in Europe has extended and, although data is not comprehensive, it is assumed that the parasite is present over most of Europe with the exception of the British Isles and the Mediterranean region [1]. It is however unclear whether this extension corresponds to its true range or whether it reflects previous absence of surveillance [1]. In Sweden, Norway and Finland, surveillance in animals from 2000 to 2009 had shown that in 2009, using a design prevalence of 1%, these countries were most probably free from the parasite [3]. However, in February 2011, EM was identified in a red fox (***Vulpes vulpes***) in Lanneröd, Sweden for the first time [4]. The fox was shot within the routine surveillance programme in 2010. After this finding, a consulting group, lead by the National Board of Health and Welfare (SoS), was summoned. The group consisted of representatives of the Swedish Board of Agriculture (JV), the Swedish Institute for Communicable Disease Control (SMI), the National Food Agency (NFA), National Veterinary Institute (SVA), the Swedish Work Environment Authority and the relevant county medical- and county veterinary officers. Regular teleconferences were usually held every 1–2 weeks, during which information concerning EM and the situation in the country was shared, and strategies for information dissemination and actions to be taken were discussed and decided.

The aim of the present paper is to describe the actions taken due to this finding and the results thereof, i.e. surveillance in animals, risk assessment for humans to become infected and recommendations given to the public. Further discussion about whether the parasite was introduced, and if so, how, as well as possible future development of the infection and future actions are included.

Methods

Surveillance in animals

Immediately after the finding of EM, increased surveillance in foxes was started [4]. Hunters were requested to submit foxes primarily from southern Sweden because it was considered that EM was most probably introduced in this area. The aim was to analyse 3,000 foxes with segmental sedimentation and counting technique (SSCT) [5], thereby detecting a prevalence

of 0.1% on country basis. Furthermore, faecal samples from hunting dogs (n=119) in the four municipalities around Lanneröd were examined at SVA by egg flotation [6] and an in-house real-time polymerase chain reaction (PCR). A non-random sampling of potential intermediate hosts was also started in an area within a 50-km radius surrounding Lanneröd. During March–April, 2011, a total of 236 rodents were collected, mainly ***Arvicola amphibius*** followed by ***Myodes glareolus***, ***Microtus agrestis***, ***Apodemus sylvaticus***, and ***Apodemus flavicollis***. The rodents were autopsied and liver or other organs with lesions (n=72) were tested by an in-house PCR. As extensive sampling of rodents is probably needed to identify the intermediate host species in an area with very low prevalence of EM, sampling of rodents continues.

Risk assessment

By 3 March 2011, the Swedish government gave a mandate to JV and SoS to, in cooperation with relevant authorities and organisations, clarify necessary actions to protect public health as a consequence of the finding of EM. Within the government mandate, a qualitative risk assessment about the probability of humans becoming infected with EM was performed in the spring of 2011 by SMI and NFA.

Recommendations and public health measures

To ensure that relevant and harmonised information concerning what was known as well as what was not known was given to the public, this issue was continuously discussed in the consulting group. Furthermore, optimal ways of dissemination of this information was also investigated.

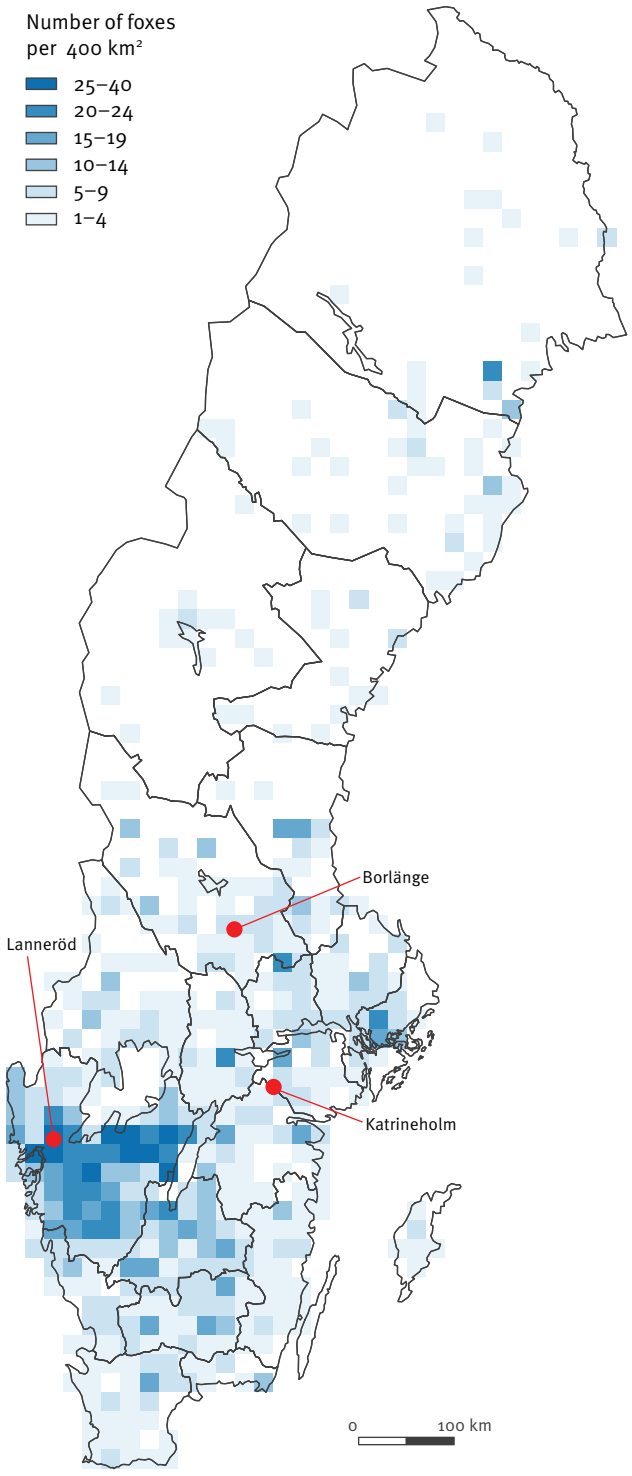
Results

Surveillance in animals

Shortly after the first fox testing positive for EM was found, the prevalence of EM in foxes seemed to be very low in Sweden, probably well below 1%. Surveillance of red foxes during 2000–2009 (n=2,962) had yielded negative results [3] and after the first positive finding, several hundred foxes, shot within the increased surveillance, were analysed with no further animals testing positive. Analysis of all faecal samples from hunting dogs in the four municipalities around Lanneröd did not yield any positive results and none of the rodents tested within the 50 km radius surrounding Lanneröd were found positive. The question was raised whether it could be possible to control and even eradicate EM. It was considered most probable that EM had been introduced to Sweden in recent years by infected dogs [4] and therefore the spread of EM could be geographically restricted. Besides Rebun Island, Japan, where EM was eradicated [7], the parasite had previously only been successfully controlled in geographically limited areas. However, based on advice from international experts and literature research, it was concluded that it might be possible to eradicate EM. A preliminary cost–benefit

Figure 1

Geographical distribution of all georeferenced foxes shot and analysed for *Echinococcus multilocularis*, Sweden, January–June 2011 (n=2,900)



The three locations where the three *Echinococcus multilocularis* positive foxes were respectively shot are indicated on the map. Foxes were georeferenced with the coordinate system RT90.

analysis showed that if eradication was possible, benefits would exceed the costs [8].

By 31 March 2011, when a total of 1,140 foxes (shot in 2011) had been analysed for EM, a second infected fox was found. This fox was shot in the Lanneröd region at the same location and by the same hunter as the first infected fox (shot in 2010). This finding confirmed the presence of the infection in this region but did not change the interpretation of the situation. By 27 April, when 1,758 foxes had been analysed, a third case was found nearby Katrineholm, more than 200 km northeast of Lanneröd. Although the probability that EM was spread to other parts of Sweden increased, investigation into ways to eradicate EM continued and the deworming recommendation was extended to include dogs at risk in this area as well. However, by the end of May, when 2,525 foxes had been analysed, a fourth infected fox was found outside Borlänge about 200 and 300 km respectively north of the previous findings (Figure 1). Thus it was concluded that EM was probably not restricted to only the few known infected areas in Sweden and that eradication was not feasible. By the end of June, the increased surveillance of foxes was completed and had resulted in the finding of a total of three positives of 2,985 analysed foxes (0.1%). The geographical distribution of foxes with georeferences (n=2,900) is illustrated in Figure 1.

Risk assessment

Humans become infected by ingesting eggs from the parasite and several modes of transmission are plausible, such as consuming contaminated food or water, inhaling eggs from contaminated environments or by letting contaminated hands or objects come in contact with the mouth. However, due to the long incubation period and the low incidence of AE there is little evidence in the literature to help discriminate the relevance of the different modes.

Evidence for direct food transmission is the observation that monkeys and pigs became infected by consumption of grass probably contaminated with fox faeces [9]. One epidemiological study identified consumption of unwashed strawberries as well as chewing on grass as risk factors, but not picking berries, eating unwashed herbs or vegetables [10]. In another study, consumption of strawberries, mushrooms, blueberries, herbs, parsley or cranberries were not identified as risk factors [11]. In contrast, using well water rather than tap water [12] or using water from certain lakes [2], was identified as a risk factor.

The results of the literature search were similarly inconsistent for risk factors regarding farming, gardening and hunting [11-13]. Many risk factors regarding environmental exposure are hard to separate from the consumption of food. One of the studies related two-thirds of the cases to farming or similar activities, probably reflecting contact with a contaminated environment [10]. The only garden activity more common among cases than controls was growing (not consuming) leaf or root vegetables, supposedly due to the amount and intensity of care required for annual compared to perennial plants [10].

Interaction with animals, regarding the risk of humans getting infected, has been investigated and inconsistent results have been presented. Two of five case–control studies identified dog ownership as a risk factor for acquiring AE [10,14], especially if the dog was left unattended in the garden or if it was killing game, whereas in the three remaining studies dog ownership was not found to be a significant risk factor [11-13]. The two studies on cat ownership as a potential risk factor, both found an association between being an AE case and owning a cat [10,11]. However, in one study the risk was small and much smaller compared to owning a dog [10].

A correlation between the prevalence in foxes and in humans has been found. However, although the prevalence in fox populations in some countries is high, the reported number of cases in humans is relatively low [15,16]. This may indicate that the actual risk of becoming infected is not only linked to exposure to the pathogen, but also to individual susceptibility, perhaps because of immunological differences [17].

In conclusion, risk factors most often identified in epidemiological studies are associated with living, working or other activities in rural environments, which makes it difficult to distinguish between environmental, food, soil, and other routes of transmission. With the evidence available, contact with contaminated environment, is considered to be an important risk factor and farmers, hunters and dog owners, whose dogs eat rodents were considered to be the group at highest risk.

Due to the current low prevalence in foxes and since no cases of AE have been reported in Sweden, the competent authorities concluded that the risk to humans in Sweden of developing AE was considered to be small. It was estimated that about one person among the nine million Swedes would be infected and develop AE every fifth year. Moreover, if the probability of infection in humans were to become similar to Switzerland this figure could increase to 20–30 cases yearly. As the prevalence of EM in the fox population could change over time, it was considered important to repeatedly monitor the fox population to be able to assess a possible increase of EM prevalence, and the risk that this may pose to humans

Recommendations and public health measures

Initially, recommendations to prevent human infection were kept general, but emphasised the importance of proper hand hygiene after contact with free running pets in risk areas. After finalisation of the risk assessment, it was concluded that the importance of food and drinking water for the transmission of AE to

humans could not be assessed and that there were no documented risk-reducing effects of washing vegetables and berries. Based on these knowledge gaps and the low number of reported AE cases even in areas in mainland Europe where the prevalence of EM in foxes is high, and taking the benefits of outdoor activities including harvesting and consuming berries and vegetables into consideration, the NFA and SMI concluded that it was not appropriate to issue any specific recommendations about EM and food. However, consumers were informed that good hygienic practices when handling food apply also with regard to EM. To consumers who do not accept any risk, information was given that boiling food is the only effective way to inactivate EM. Recommendations were communicated by authorities via the internet and also by a common information site (www.krisinformation.se).

After the first positive finding of EM in a Swedish fox in Lanneröd, JV issued recommendations that dogs at risk, i.e. dogs that could catch rodents, in the four surrounding municipalities should be dewormed monthly. Later, when another fox tested positive near Katrineholm, the deworming recommendation was extended to also include dogs at risk in this area. However when results of the surveillance indicated that EM was endemic at a very low prevalence in Sweden, recommendations to dog owners in the country were withdrawn. For worried dog owners, whose dogs eat rodents, deworming the dogs monthly was nevertheless suggested to prevent infection.

For the particular case of pet dogs entering the country from abroad, it was decided that dog owners should be informed, that dogs coming from endemic regions of mainland Europe need be dewormed before entry in Sweden. It is important to highlight that the risk of dogs becoming infected is greater in many European countries where the prevalence of EM is much higher compared to Sweden. In Sweden the prevalence in foxes appears so far to be very low, about 0.1%, but in certain areas in Europe 50% of foxes or more may be infected [2]. Deworming will reduce the risk not only for the individual dog owners, but also prevent introduction to areas where EM may not yet be present.

It was concluded that should the prevalence of the EM within the Swedish fox population remain very low, no further recommendations to the public would be given. Monitoring the fox population, however, was considered important to be able to reassess information campaigns to the public if an increase of EM would be observed. In addition, increased monitoring was considered necessary as the geographical spread of EM as well as the prevalence in different areas is not well known. There is also a need for more information on the fox population density in different areas of Sweden and how the population changes over time. Of special interest are urban foxes as they, due to closer contact with people, are considered to pose a greater risk. It was therefore concluded that increased and repeated monitoring of EM in foxes as well as monitoring of the fox population is needed.

If high population densities of urban foxes with a high prevalence of EM were found in Sweden, this would increase the risk to humans. Because control strategies applied locally, such as deworming dogs and baiting strategies for foxes can reduce this risk [16,18] it was concluded that an action plan should be prepared in case such high risk areas were found in Sweden. The action plan should also clarify how relevant information is provided to the public and groups most at risk.

Finally it was concluded that there is a need for research. More knowledge about the epidemiology of EM in Sweden is also needed, such as which intermediate hosts are involved in the life cycle of EM and what the present and expected future distribution and prevalence of EM in the country may be. More knowledge is needed on risk factors for developing AE as well as what can be done to prevent infection.

Discussion

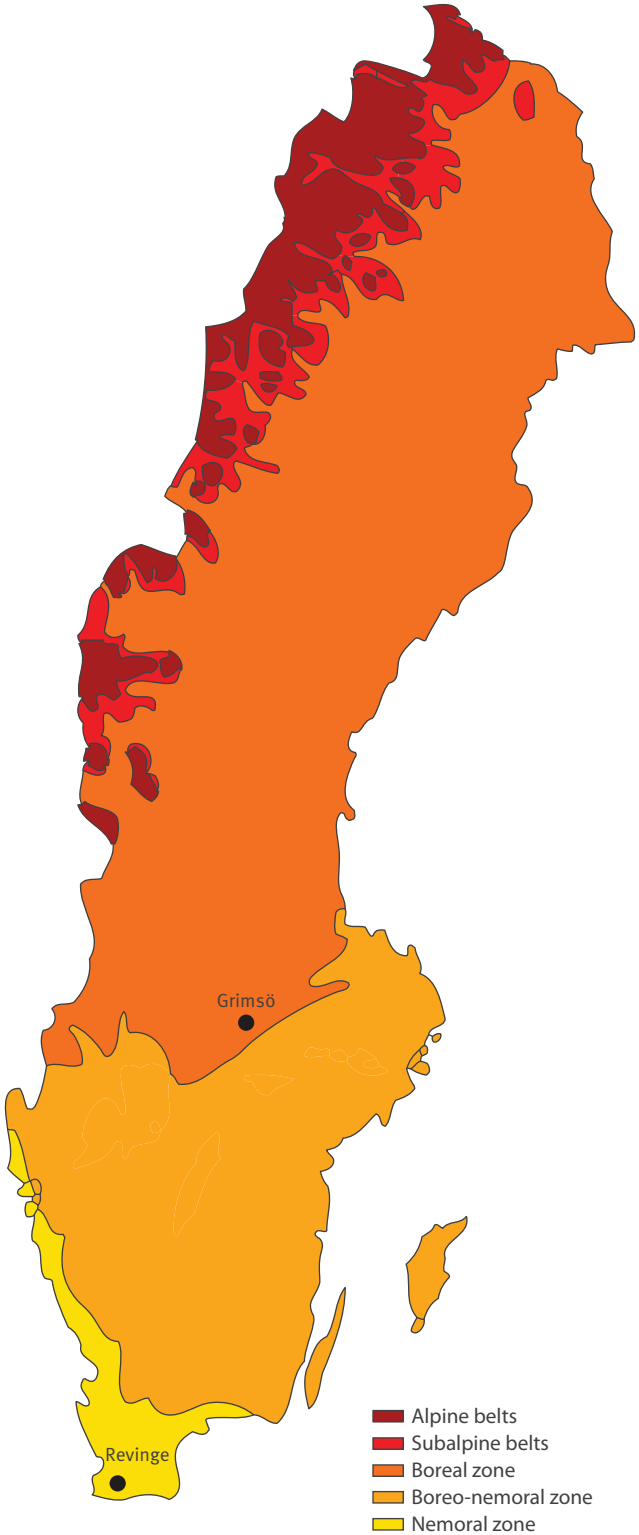
It is not known when EM was introduced to the Scandinavian Peninsula. However, if introduction was recent, unlawful admission of dogs from mainland Europe is the most probable explanation. Risk assessments have shown that without a very high compliance with import requirements, introduction of dogs from endemic areas constitutes a risk of introduction of EM [19,20]. Compliance with import requirements has decreased and the number of imported dogs has increased substantially in Sweden since 1994 (personal communication, Maria Cedersmyg, January 2012). Prior to 1994, all dogs were dewormed in quarantine prior to entry to Sweden. In 1994, for dogs from certain European countries, this was replaced by a requirement that a veterinary deworming certificate should be shown at the border. Furthermore, in 1995, border control was restricted as Sweden joined the European Union (EU), thereby prohibiting routine control of deworming certificates of dogs.

Another possible explanation for the present findings of EM in foxes is that the parasite has been endemic for a long time but escaped detection due to limited surveillance. According to the negative binomial distribution and assuming a test with 100% sensitivity, 3,000 foxes have to be analysed to have a 95% probability of detecting EM given a prevalence of 0.1%. In the routine surveillance in Sweden, started in the year 2000, more than 2,900 samples were analysed before the first case was detected. This highlights that extensive surveillance is needed to detect a low prevalence of EM. Introduction by foxes from Finland was considered unlikely as, despite intensive surveillance [3], the parasite has not been found in this country.

The present and future spread of EM in Sweden is unknown. The epidemiology of EM depends on the fox population density as well as the interaction with

Figure 2

Vegetational zonation in Sweden, 1999



Locations where fox population densities have been estimated, Grimsö and Revinge, are shown. Vegetation data is reproduced with permission from Acta Phytogeographica Suecica [35].

intermediate hosts. For non-urban mainland Europe fox population densities have been reported to be 0.5–3 foxes/km² [21-25]. In Sweden, the corresponding figures (during the 1970s) were 0.8 (Revinge, nemoral zone) and 0.2–0.4 foxes/km² (Grimsö, southern boreal zone) [26,27] (Figure 2). During the 1980s an epizootic of sarcoptic mange struck the Swedish fox population and the density of foxes declined considerably especially in southern Sweden [28]. However, the population recovered to the levels of the 1970s in the early 1990s, and monitoring has not revealed any dramatic change after this recovery [29,30]. The fox population density varies, from relatively high and stable in the nemoral and boreonemoral zones (south) to a lower density with a much higher degree of fluctuation in the boreal zone (north) [26,27,31,32] and the fluctuations in the north follow those of vole populations [33]. The three areas where EM has been found have suitable fox habitat characterised by a mixture of forest and agricultural land. It is concluded that although the fox population density in Sweden is lower compared to mainland Europe, it is sufficient to maintain the lifecycle of EM. Perhaps besides northern Sweden, where the decreased fox density during the lowest phase of the population fluctuation may be too low for EM to prevail, there is no reason to believe that EM could not be established in the rest of Sweden. In urban areas, the fox populations in mainland Europe have been reported to be high and may exceed 10 foxes/km² [34] and these fox populations play an important role in the transmission of human AE [18]. However, although foxes are present in cities also in Sweden, information on the urban fox population densities are lacking.

Furthermore, it is not known which intermediate host species are involved in the life cycle of EM in Sweden. Based on previous knowledge on EM prevalence among intermediate host species [36-38], known and expected food preference by the red fox in Sweden and Norway [39], and the occurrence of different small rodents in the identified EM-infected areas in Sweden, the most likely intermediate host candidates should be ***Arvicola amphibius, Microtus agrestis and Myodes glareolus***; all common and distributed throughout Sweden [40]. ***Microtus arvalis***, one of the principal intermediate hosts in mainland Europe does not occur in Sweden.

It was concluded that the risk of developing AE in Sweden is low. However, it might be argued that the risk of being infected by EM could be higher in Sweden than in other countries with similar prevalence. One reason is the unique legislation on Right of Public Access to land, which gives the public right to roam freely in the countryside. Outdoor activities such as hiking, camping and berry- and mushroom picking are long standing traditions in Sweden. Hunting is a widespread activity that adds to the number of people in close contact with nature. Still, there is a lack of scientific studies comparing behaviour in different countries, making it not possible to assess whether the risk is higher in Sweden due to particular behaviours, such

as outdoor activities. Another reason for the risk being hypothetically higher in Sweden is that EM was only recently detected, so there is no tradition of how to minimise risk of exposure. It has not been shown that information will reduce the risk, but there are studies reporting differences between countries in Europe in terms of knowledge and perception of the risk of AE [41]. In some other countries in Europe, where EM is endemic, there are recommendations to rinse and/or cook berries and vegetables before eating them and to wash the hands thoroughly after contact with soil or vegetation, to avoid being infected with EM. For dog and cat owners there are recommendations to regularly deworm the pets in case they roam outdoors and eat wild rodents.

After concluding that eradication was not possible, the only preventive action taken by the authorities was issuing recommendations. However, due to lack of knowledge, the recommendations given were quite general. In this situation, there was a requirement from the general public and especially from hunters to at least try to prevent further spread of EM. The question was raised whether increased fox hunting could be beneficial. However, because hunting may increase the immigration rate and lower the age distribution of the fox population [26], hunting may increase the spread of EM especially if the prevalence of EM is higher in adjacent areas. Hunting may also increase the EM biomass if the proportion of young foxes increases as, apart from one recent study in Lithuania [42], the worm burden has been reported to be higher in younger foxes [43,44]. A hunting pressure high enough to influence spring density of reproducing animals is probably seldom attained. It was concluded that intensified hunting in infected areas and especially in hot-spots may be beneficial however, increased fox hunting in areas where EM has not been found is not recommended.

According to the authorities, more knowledge about the prevalence of EM in different areas is needed. Although an extensive surveillance was performed after the first finding, there is a need for additional sampling especially in areas where the sampling intensity was lower. Furthermore, there is a need for long term monitoring to follow any future changes in prevalence. It is also important to extend the current monitoring of the population density of small rodents [45,46] and to also involve the south of Sweden. At present there is no suitable method for large scale surveillance of EM. Until now surveillance in Sweden has been based on foxes shot by hunters. The latter foxes were analysed with coproantigen enzyme-linked immunosorbent assay (ELISA) [47] or in-house PCR, and after the first positive finding with SSCT [5]. However, collection of foxes shot by hunters is cumbersome, costly and associated with a risk of exposure to EM. Sampling of fox faeces is expected to lower the costs and also the risk of exposure but none of these are considered suitable for large scale surveillance. However, earlier modeling results have indicated that, depending on the expected prevalence of EM infections in wild boars and the sensitivity of the test, surveillance of EM-lesions or antibodies in wild boars could be used to monitor EM in areas with a dense wild boar population [3]. Investigations are ongoing to evaluate whether surveillance in wild boars could be appropriate for the southern half of Sweden where 57,300 wild boars were shot during the hunting season 2010/11 [48].

Finally, the need for more research was identified by the authorities. Most important, more knowledge about risk factors for becoming infected with EM is needed so that relevant recommendations can be given to minimise risk of infection. Risk factor studies using diagnostic tools such as serology may have the potential to improve knowledge about risk for exposure to EM. The most important knowledge gaps identified in the risk assessment of transmission of EM via food were the importance of the risk of consumption of berries, fruits and other vegetables and how much the risk can be reduced by careful washing/rinsing of berries and vegetables. There is also a need for a cost effective surveillance that could be implemented on a large scale to estimate the level of contamination in different geographical regions and also assess future trends. Furthermore, from a Swedish point of view, there is a need for scientific studies comparing human behaviour in different countries, so it can be investigated whether the Swedish Right of Public Access to land (allowing people to roam freely in the country side and for example pick berries and mushrooms) and the present use of it, affects the risk of becoming infected by EM. Finally, there is a need to increase our understanding of the epidemiology of the disease in Sweden by efforts such as increased surveillance to identify the intermediate host species for EM.

Conclusions

The present risk to humans of becoming infected with EM and developing AE is considered to be small. It is most probable that EM is already spread within Sweden. Increased surveillance is needed to enhance knowledge about present and future prevalence of EM. An action plan will be developed to handle a potential future increased risk for humans, if the prevalence of EM increases. There is a need for more research about the epidemiology and surveillance of EM.

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Surveillance of tularaemia in Kosovo*, 2001 to 2010

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Tularaemia, caused by *Francisella tularensis*, had not been registered in Kosovo* before an outbreak in 1999 and 2000. A national surveillance system has been implemented in Kosovo* since 2000 to monitor a number of diseases, including tularaemia. Antibody detection in human sera was used for laboratory diagnosis of tularaemia and *F. tularensis* lipopolysaccharide antigen was used as a marker of infection. The purpose of this study is to describe the incidence of tularaemia in Kosovo* after the 1999–00 outbreak. In 2001 and 2002, a second outbreak occurred, with 327 serologically confirmed cases. From 2001 to 2010, 25–327 cases were registered per year, giving a mean annual incidence of 5.2 per 100,000 population. The most likely sources of infection were contaminated drinking water and food. The dominant clinical manifestations were the glandular (79%) and ulcero-glandular (21%) forms. By 2010, the disease had spread throughout Kosovo*. Presumably as a result of war and subsequent environmental disruption, mass population displacement and breakdown of sanitation and hygiene, the two major outbreaks of tularaemia resulted in the establishment of an active endemic area of tularaemia in Kosovo*.

Introduction

The causative agent of tularaemia, a relatively rare zoonotic disease, is *Francisella tularensis*. The bacterium is widely distributed in the northern hemisphere and is found in Europe [1]. Most countries in central and southern Europe reported single cases over the past decades. The disease is a more serious public health problem in Balkan countries and has also been reported in Turkey [2-4].

The cells of this Gram-negative, non-motile, capsule-forming, facultative intracellular bacterium are pleomorphic, typically appearing as short rods or coccoid forms [5]. Two subspecies of *F. tularensis* cause tularaemia in man. Biovar A1 of the subspecies *tularensis* (or type A) is the most virulent type of *Francisella* bacteria and can be associated with lethal pulmonary infections in humans [6]. Subspecies *holarctica* (or type B) is assumed to be less virulent. *F. tularensis* ssp. *tularensis* has been isolated almost exclusively in North America, whereas *F. tularensis* ssp. *holarctica* could be found in the entire northern hemisphere [5]. Interestingly, a second biovar of *F. tularensis* ssp. *tularensis*, A2, has a lower morbidity and mortality in humans than ssp. *holarctica* [6].

Depending on the site of entry of the pathogen, tularaemia can occur as ulcero-glandular and glandular forms, as well as oculo-glandular, oropharyngeal, respiratory or typhoidal [5]. Humans acquire the bacterium through contact with infected animals and/or vectors, by inhaling contaminated dust or aerosols, or by consumption of contaminated food or water. Human-to-human transmission is unlikely [5].

Francisella is capable of infecting a large number of animal species such as hares, rabbits, mice, lemmings and even fish [5]. Birds are known to be carriers, but probably do not develop the disease themselves [5]. Various vectors could play a role in transmission of *F. tularensis* from animals to humans: in Scandinavia, mosquitoes probably play a major role, while in North America, ticks are considered to be the most important vector [5].

F. tularensis is able to survive in the environment under cool and humid conditions, probably for weeks, and has been found in water and soil [7]. The mechanisms for survival of the bacteria are not yet well understood. Protozoa and nutrient conditions could play a role in protecting the bacteria [8-10].

History shows that tularaemia outbreaks are associated with poor hygienic conditions, especially in war and post-war situations, accompanied by a large increase in rodent populations and subsequent mass death of these animals [1,11-13]. Natural outbreaks occur in various endemic areas and can involve several hundred patients [1]. *F. tularensis* has been listed as a potential biowarfare agent [13,14].

Tularaemia had not been recorded in Kosovo* until an outbreak of the disease in 1999–2000 [13,15]. An intensive case investigation was carried out during the first outbreak [15]: specific antibodies against *F. tularensis* were detected in 247 serum samples from 912 suspected cases. Kosovo*, in south-east Europe, covers an

area of 10,908 km2, with a population of approximately 2.1 million inhabitants and population density of 192 per km2. It proclaimed its independence in 2008.

In January 2000, the national Institute of Public Health in Pristina implemented a surveillance system for 20 communicable diseases, based on syndromic approaches and clinical diagnoses. Timely reporting of disease syndromes from the different municipalities (administrative regions) allows a number of the most important infectious diseases in Kosovo* to be monitored simultaneously.

This study aims to provide a follow-up on the incidence of tularaemia in Kosovo* after the first outbreak in 1999–00 until 2010.

Methods

Case definition

A suspected case of tularaemia was defined as a person with fever and enlargement of cervical lymph nodes. The following indicators could also be present: skin ulcers and perspiration; weakness, body pain and headache; and throat pain and ingestion problems. Confirmed cases were individuals with the above clinical picture in whom laboratory confirmation of the infection was obtained.

To ensure that all individuals with the tularaemia were identified, the case definition was rather broad. The clinical manifestation of *F. tularensis* infection can be quite similar to that of tuberculosis, brucellosis or mumps – all of these diseases have a relatively high prevalence and incidence in Kosovo* (data not shown). Therefore, laboratory conformation by detecting specific antibodies or the pathogen itself is required for the final diagnosis of tularaemia.

Collection of epidemiological data

All outpatient clinics and medical centres are obliged to fill in official reporting forms every week to record aggregated and individual data on the 20 specified communicable diseases, including tularaemia. The forms are sent to the regional Institute of Public Health, which subsequently passes them on to the national institute. Double reporting is prevented by checking the personal data of reported cases. At the national institute, the data are entered into a central database, to be regularly analysed by means of Epi-Info software.

Diagnostic sera were obtained by local physicians from suspected cases and sent for analysis to the national Institute of Public Health. The sera were analysed for specific antibodies to *F. tularensis*, routinely using a microagglutination assay [16]. Positive sera and additional sera from persons suspected to be infected with *F. tularensis* were checked by a highly specific enzyme-linked immunosorbent assay (ELISA) and western blot analysis, as described below.

Microagglutination

The live vaccine strain of *F. tularensis* biovar *holarctica* (ATCC 29648) was grown for 2–3 days at 37 °C in a 5% CO2 humid atmosphere on heart-cysteine-blood agar and harvested into sterile distilled water or isotonic sodium chloride. Bacterial concentrations were adjusted photometrically at 580 nm to an optical density of 1.0. The suspension was inactivated with paraformaldehyde and prepared as agglutination antigen as described elsewhere [16]. The assay was adjusted by the optimal antigen concentration and evaluated on the basis of a titre of 1:16 or higher being considered positive.

Antibody detection in human sera by ELISA and western blot

The ELISA was used for screening and western blot for confirmation. Both have been described elsewhere [16-18] and were used with some modifications. Briefly, a 96-well microtitre plate (Polysorb, Nunc, Germany) was coated with purified lipopolysaccharide (LPS) from the live vaccine strain as antigen. Bound human antibodies to *F. tularensis* were detected by polyvalent goat anti-human IgA-IgM-IgG horseradish peroxidase-conjugated secondary antibody (dianova, Germany) and subsequent substrate reaction. For the western blot, the soluble fraction of formalin-inactivated live vaccine strain was separated using sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred to polyvinylidene difluoride (PVDF) membranes (ImmobilonP, Millipore, United States). Using polyvalent horseradish peroxidase-conjugated secondary antibodies, the typical LPS ladder revealed the presence of specific anti-*F. tularensis* antibodies. The final results were given after confirmation of the ELISA results by western blot: 'positive' denoted strong bands, 'negative' – almost no bands and 'borderline' – weak but clearly visible bands).

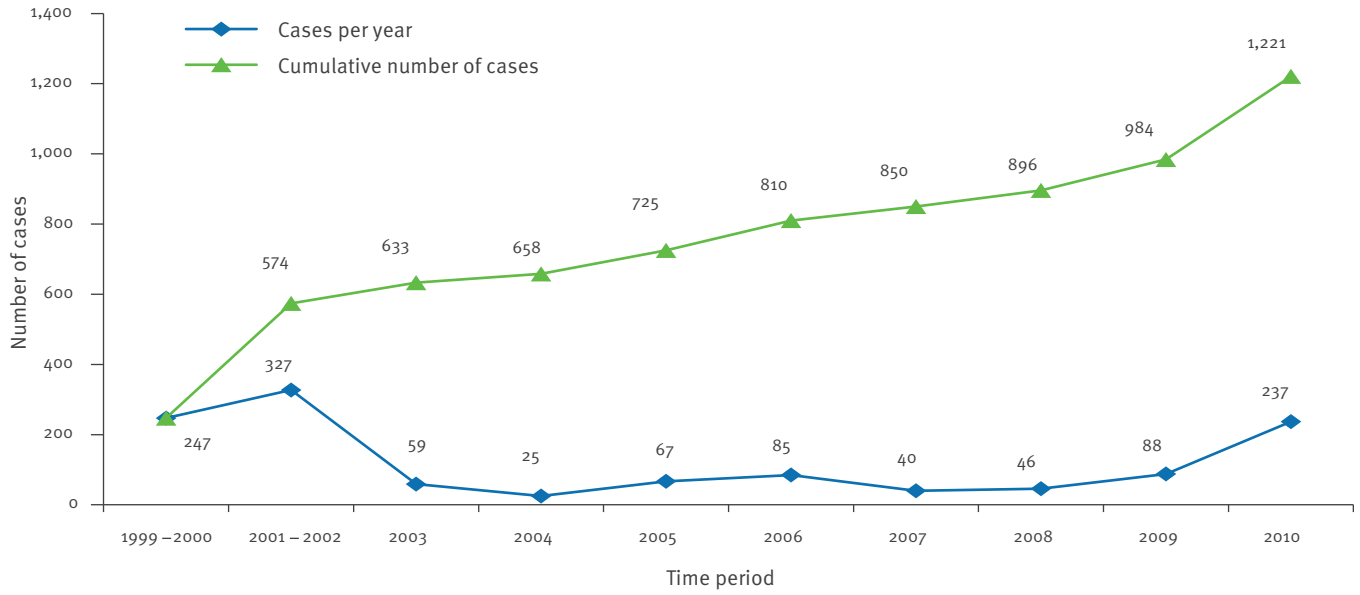
Samples for antigen detection and capture ELISA

Environmental samples (faeces from rabbits and mice, water samples), tissue samples (spleen, liver) from dead mice, rats and rabbits, as well as clinical samples from serologically confirmed tularaemia patients were analysed using a capture ELISA. It was essentially performed as described previously, using the *F. tularensis* LPS-specific murine monoclonal antibody 11/1/6 as capture antibody bound to the solid phase [19-21].

Faeces from pathogen-free inbred and outbred mice and rabbits were kindly provided by the National Research Centre for Environment and Health (GSF) in Munich, Germany, which were used as negative controls. Samples were homogenised and pre-treated with LPS-extraction buffer containing chenodeoxycholic acid in phosphate buffered saline/ethylenediaminetetraacetic acid (PBS/EDTA). Large particles were allowed to sediment for 5 minutes and the supernatant was analysed for the presence of *F. tularensis* LPS using the cELISA.

Figure 1

Reported confirmed cases of tularaemia, Kosovo*, 1999–2010 (n=1,221)



* This designation is without prejudice to positions on status, and is in line with United Nations Security Council Resolution 1244/99 and the International Court of Justice Opinion on the Kosovo declaration of independence.

Results

Tularaemia outbreak in 2001 and 2002

After the tularaemia outbreak in 1999–00, a second outbreak occurred from November 2001 to June 2002, which was investigated by a Kosovar/German team from the Bundeswehr Institute of Microbiology, Munich, Germany. This outbreak has not been previously described in the scientific literature. During this period, 1,168 serum samples from suspected cases were tested; 327 cases laboratory confirmed by ELISA and western blot (Figure 1). Although the second outbreak started in other parts of Kosovo* (east and south-east), the affected areas generally overlapped those of the previous outbreak.

The epidemiology of the second outbreak was quite similar to that of the first [15]. It can be assumed that the main reason for the spread of the disease seemed to be again the bad sanitary conditions, especially in rural areas of Kosovo*. As described for the first outbreak [15], housewives (37%) and farmers (27%) were the most affected occupational groups. Similarly, cases were mainly female (60%) and the age group 20–40 years (52%) were also most affected.

It was characteristic of both outbreaks that people in affected regions reported an enormous increase in the rodent population, especially field, forest and domestic mice before the outbreak among humans.

The investigation of animal and environmental specimens by the capture ELISA, which is highly specific for *F. tularensis* [19], showed that the antigen was detected mainly in mouse and hare faeces (Table). During the outbreaks, faeces of small rodents were regularly found by the investigation teams in products stored in food stores of affected households and showed the most striking positive results in antigen detection of *F. tularensis*. During the first and second outbreaks, 145 and 220 samples were collected respectively from similar sources, of which 10 and 22, respectively, were positive. We could not detect *F. tularensis* antigen in a very limited number of available clinical specimens: throat swabs (n=18), pus and wound secretions (n=4). At that time, a more sensitive polymerase chain reaction (PCR) was not available.

As in the first outbreak, the predominant manifestation of the disease during the second was oropharyngeal. The main route of transmission leading to this oropharyngeal form was probably ingestion of contaminated food or water [15]. More than 90% of the patients (305/327) had as the leading symptom enlarged cervical lymph nodes, whereas the other patients had enlarged lymph nodes in other locations such the axilla or inguinal region. This clinical manifestation of oropharyngeal tularaemia was dominant throughout the study period.

Table

Francisella tularensis antigen detection in animal and environmental specimens during tularaemia outbreaks in Kosovo*, 1999–2002 (n=365)

Sample type	Source	1999–00		2001–02		Total without controls	
		Number of samples	Number positive	Number of samples	Number positive	Number of samples	Number positive
Faeces	Mice	55	7	58	9	**113**	**16**
	Control: pathogen-free mice	NT	NT	100	0	–	–
	Hares	NT	NT	104	12	**104**	**12**
	Control: pathogen-free rabbits	NT	NT	57	0	–	–
Animal tissue	Mice, rats, hares	63	3	35	1	**98**	**4**
	Control: pathogen-free mice	NT	NT	25	0	–	–
Water	Wells, ponds	27	0	23	0	**50**	**0**
Total without controls	–	**145**	**10**	**220**	**22**	**365**	**32**

NT: not tested.

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Surveillance of tularaemia from 2001 to 2010

After the first outbreak, the surveillance system revealed the presence of cases every year (Figure 1). From 2003 to 2010, the annual number of tularaemia cases was between 25 and 237, with a mean annual incidence of 3.9 ±3.2 standard deviation (SD) per 100,000 population. During this period, a total of 647 cases were reported.

In 2010, more than 200 tularaemia cases were registered. The reason for this high number of cases is unknown: no specific source of infection nor a specific outbreak scenario could be identified. The cases were distributed equally throughout Kosovo* and throughout the year. This could indicate a high epizootic and zoonotic activity in that year, for some unknown reason.

Housewives and farmers were the most affected occupational groups, representing about 33% (n=216) and 24% (n=153) of cases, respectively. This is also reflected in the sex distribution of all cases during this period: 57% (n=372) of cases were female and 43% male (n=275) (Figure 2). Most cases (n=309) were in the age group 20–40 years, of whom 61% (n=188) were female and 39% (n=121) male. In addition, quite a high proportion, about 20% (n=128) of children and teenagers (aged under 20 years) were infected.

Since 2001, the clinical manifestation in the 974 patients was oropharyngeal or glandular tularaemia: about 93% (n=906) of the cases had unilaterally enlarged cervical lymph nodes or swollen axillar or inguinal lymph nodes; 7% (n=67) had the ulcero-glandular form.

Since the first outbreak, tularaemia has spread to other parts of Kosovo*. As a result, all municipalities participating in the surveillance system have reported human tularaemia cases (Figure 3). The three municipalities marked in grey in Figure 3B were not participating in the surveillance system.

Discussion

By mid-1999, more than 10 years of political crisis and warfare in Kosovo* had resulted in environmental disruption, mass population displacement and a breakdown of sanitation and hygiene [15]. Many essential public health functions, such as disease surveillance and outbreak response, had collapsed [15]. It

Figure 2

Age and sex distribution of confirmed tularaemia cases, Kosovo*, 1999–2010 (n=1,221)

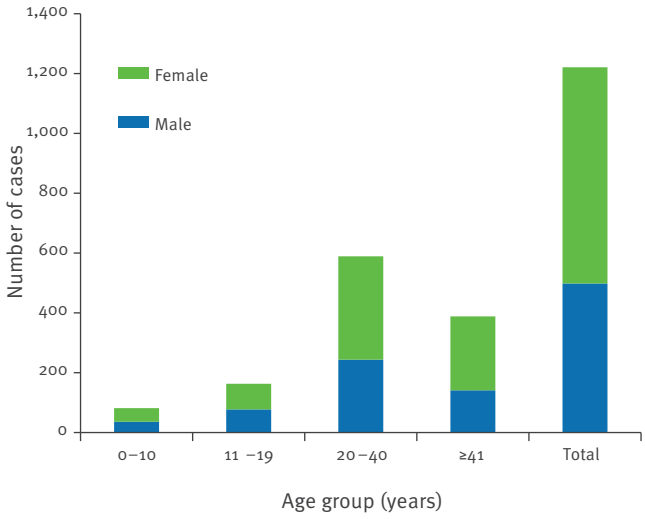

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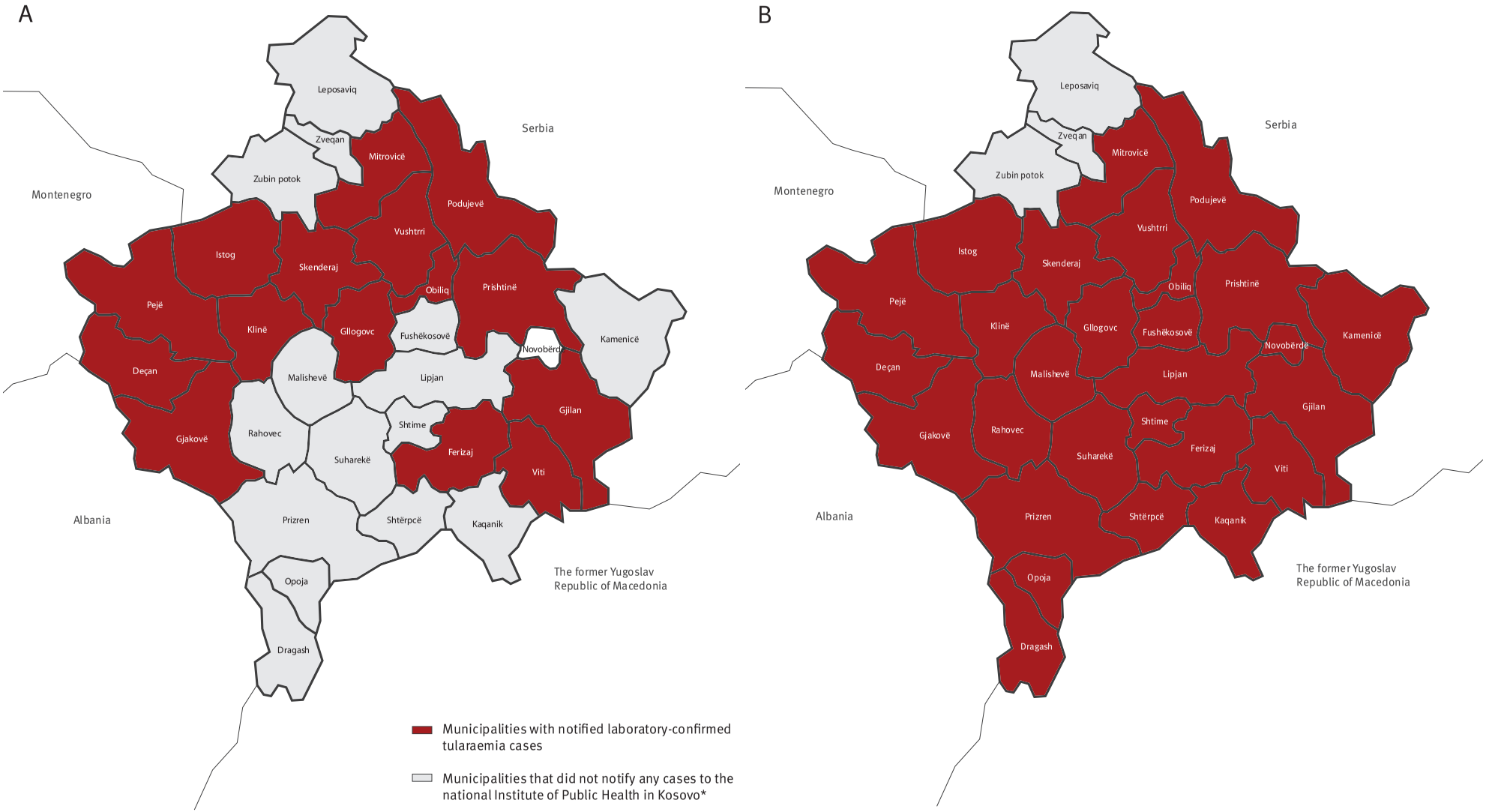
Figure 3

Distribution of confirmed tularaemia cases, Kosovo*, Panel A: 1999–2000 (n=247), Panel B: 1999–2010 (n=1,221)



The three municipalities marked in grey in Panel B were not participating in the surveillance system.

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is suspected that tularaemia had been present in the region during and/or after the Second World War, but there are no official data about the disease in Kosovo* during that time. Although the disease was notifiable in the former Yugoslavia, local representatives stated that no cases of tularaemia had been detected before the war in Kosovo* in 1998 to 1999 [13].

The number of laboratory-confirmed cases of tularaemia in the first outbreak in 1999–00 was unexpectedly high, given that Kosovo* had been considered non-endemic for the disease at that time. An even larger number of cases was seen during the second outbreak in 2001–02. The circumstances of a typical post-war situation in the autumn of 1999 were probably responsible for the outbreaks: people left their homes and did not harvest the fields, which led to an oversupply of food for rodents [13]. Consequently, an unusually large increase in the rodent population was observed until January 2000. It is known that an increased density of the rodent population can facilitate the spread of zoonotic infectious pathogens including *F. tularensis* among animals and induce an epizootic spread to man [22]. We found *F. tularensis* antigen in a relatively high percentage of samples from collected rodents and hares. However, for further identification of the infectious source and characterisation of the causative agent, attempts should still be made to obtain isolates of *F. tularensis* from the samples collected between 1999 and 2002.

As almost all the tularaemia patients during 2001 to 2010, as in the first outbreak [15], had the oropharyngeal form with fever and a unilateral cervical lymph node enlargement as the main symptoms, obviously the main route of infection was alimentary ingestion of *F. tularensis*. It was rather surprising that during the first outbreak, cases were spread over a large part of Kosovo*. It can be speculated that either the pathogen had already been present in these regions in spite of not having been observed or it was spread by human and animal migration as a consequence of the war. It was assumed by the national Institute of Public Health and the outbreak investigation team at that time that an emerging or re-emerging endemic region with periodic outbreaks of tularaemia might develop in Kosovo*. In fact, the data for 2003 to 2010 indicate a continuous activity of tularaemia after the initial outbreaks.

The clinical manifestation in 2010 was similar to that during the outbreaks in 1999–2002, which suggests that the routes of transmission have remained the same. In comparison, in other parts of the world, the ulcero-glandular form of tularaemia is primarily detected, which can arise due to direct exposure of the patients' skin to infected animals, carcasses, water or other materials, or to arthropod vectors [1,5]. Climate change is believed to influence the spread of vectors and therefore of tularaemia [23]. Obviously alimentary ingestion of the pathogen has been the major route of infection in Kosovo*. Given the situation, ingestion of contaminated food and water arising from the poor hygiene conditions seem to be the most likely risk factors for the infection.

Interestingly, the mean incidence of tularaemia in Kosovo* from 2001 to 2010 (5.2±4.6 SD per 100,000 population) is comparable to that in Sweden (3.2±2.08 SD per 100,000 population, calculated for the same time period from data from the Swedish Institute for Communicable Disease Control [24]), which is known to be endemic for tularaemia, and about 100 times higher than that in Germany (0.013±0.012 SD per 100,000 population; calculated for the same time period from SurvStat data from the Robert Koch Institute in Germany [25]). Tularaemia in Germany is less evident than in some other countries, but little is known about the epizootic activity of the disease in Germany. Thus, low numbers of reported human cases in Germany may not reflect the actual prevalence of the pathogen in nature and the potential risk of epidemics [26].

In 2010, Kosovo* represented an emergent endemic region for tularaemia. The reasons for this development are not fully understood. More recent data are in the process of being evaluated at the national Institute of Public Health in Pristina. Further surveillance of this disease is important in order to detect possible outbreaks in a timely manner and to take adequate measures to prevent the further spread of the disease. The main reason for ongoing activity of the disease seems to be the still poor sanitary conditions, especially in rural areas of Kosovo*. In addition, animal control and surveillance, including that of rodents, should be carried out to prevent further outbreaks. Further field investigation is required to obtain *Francisella* isolates for clarification of the subspecies prevalent in Kosovo* and to further identify reservoirs and routes of transmission of the pathogen. Additional resources are required to manage this serious health problem, although other infectious diseases may have an even higher impact on public health in Kosovo*.

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The European Centre for Disease Prevention and Control launches a public consultation. Send your comments!

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The European Centre for Disease Prevention and Control (ECDC) is launching a public consultation today on the draft technical report entitled *Prevention of norovirus infection in schools and childcare facilities*.

The draft technical report synthesises current international recommendations and review findings related to the prevention and control of gastroenteritis outbreaks in schools and childcare facilities. The focus is on norovirus, which is one of the most common causes of childhood gastroenteritis and is characterised by high rates of infectivity and transmission. Furthermore, the report seeks to identify the key facts that can support message development for the implementation of health communication activities in childcare settings.

The purpose of the consultation is to give members of the scientific community, as well as all other interested stakeholders, the opportunity to provide their comments on the draft document, in a spirit of openness and transparency.

The draft technical report, as well as relevant information on how to submit comments to ECDC can be found on the ECDC website: http://ecdc.europa.eu/en/publications/Publications/Forms/ECDC_DispForm.aspx?ID=923.

Interested parties are invited to provide their written comments by **31 August 2012**.

European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) 2012 – call for abstracts closes 13 July

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Last chance: the call for abstracts for the European Scientific Conference on Applied Infectious Disease Epidemiology (ESCAIDE) closes on Friday 13 July 2012 at 23.00.

For information about ESCAIDE, please consult www.escaide.eu. ECDC also regularly send out information about ESCAIDE via Twitter, on ECDC_EU, # ESCAIDE