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From national to international – challenges in crossborder multi-country, multi-vehicle foodborne outbreak investigations

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This issue of *Eurosurveillance* features two articles by Horvath et al. and Scavia et al. reporting the findings of the investigations of an unusual increase in Salmonella Goldcoast infections in Hungary and Italy respectively, in 2009 [1,2]. The increase in notified cases started around June and July in both countries. While the magnitude remained limited, it persisted over a period of several weeks and therefore, in early October, Hungary reported this increase to the European Epidemic Intelligence Information System for food- and waterborne diseases in the European Union (EU) [3], to check if other EU/European Economic Area (EEA) Member States had experienced a similar increase. In response to this, within four weeks, five countries: Denmark, Italy, Norway, Spain, and United Kingdom, reported recently diagnosed laboratory-confirmed S. Goldcoast cases possibly linked to the cases in Hungary [4].

S. Goldcoast is a rare serovar, with between 150 and 200 cases reported annually in the EU/EEA from 2007 to 2010, excluding 2009 (unpublished data). In 2009, 314 cases were reported, representing a significant increase compared to the background level in the years 2007 to 2010.

The investigations reported in this issue concluded that a contamination of meat had probably occurred along the pork production chain, had persisted over several months and resulted in multiple vehicles derived from the pork production chain causing an unusually high number of sporadic and clustered cases.

The two articles highlight challenges in the investigation of potential cross-border multi-country outbreaks where multiple contaminated vehicles originating from the similar type of food production chain may serve as a source of infection of sporadic cases and may cause point source outbreaks at local, regional or national level. Firstly, such outbreaks do not usually result in a large number of cases accumulated over a limited time period and associated with a single vehicle. Therefore, the delay in the recognition of a potential common origin impairs the ability to investigate timely and precisely the food items consumed during the incubation period of the cases. Secondly, as the contamination may involve several products, pork meat containing products in the outbreaks described in this issue of Eurosurveillance, epidemiological investigations may remain inconclusive. In such instances investigating household outbreaks can be of high value. Even though often small and limited, household outbreaks may reveal a common exposure to pork meat or a single vehicle in a family cluster and family clusters may indicate the presence of contaminated raw food of animal origin on the retail market. It is noteworthy that both outbreaks described reported the involvement of homemade products from pork meat. Households have been reported as the most important setting for Salmonella outbreaks; 55.6% of notified Salmonella foodborne outbreaks with strong evidence (n=341)occurred in single households in 2010 [5].

The high similarity of the molecular typing results from samples of animal, food and human origin in the Italian investigation further strengthened the evidence supporting the origin of the contamination in the pork production chain. The high genetic homology based on pulsed-field gel electrophoresis (PFGE) analysis between the Hungarian and Italian outbreak profiles allowed linking the increase in the number of cases in the two countries to a probable common source of contamination. In addition, the simultaneous occurrence of the increase as well as the strong suspicion of pork containing products as a source of infection in both countries, further strengthened this hypothesis. The timely epidemiological and microbiological investigation along the food chain was however not possible and was largely hampered by the difficulty in tracing back the meat used in salami production.

The two *S*. Goldcoast outbreaks stress the importance of collaboration between public health and veterinary authorities and the need to share samples and data from human, animal, food and feed across sectors when investigating such complex outbreaks. In addition, it is crucial to ensure the comparability of molecular typing methods used in both sectors so that a microbiological link between human and food/animal samples, which should be confirmed through an epidemiological link, can be ascertained in a timely manner. The limitations of the epidemiological investigation in a context of sporadic cases and small clusters prevent establishing firm evidence of an association with a particular vehicle. In such situations, the concordance of molecular and epidemiological evidence pointing towards a source of contamination is crucial.

Similarly, linking cases and clusters that occur at the same time in several countries allows the coordination of investigations through the use of standard questionnaires adapted to cultural differences and the sharing of results. This in turn may increase the statistical power of the investigation by enrolling more cases in the analytical studies. However, the sharing of information among countries only after an alert from a single country, does not allow for early recognition of a similar pattern occurring simultaneously in several Member States. This is why the European Centre for Disease Prevention and Control (ECDC) has started, together with EU/EEA countries, a pilot project for molecular-typing-based surveillance. The aim of this project is to enable immediate sharing of molecular typing information for Salmonella serovars, Shiga toxin/verocytotoxin -producing Escherichia coli and Listeria monocytogenes to allow early recognition of such patterns through regular analysis of molecular typing profiles [6]. This will ensure that even small multi-country outbreaks will be recognised early on in the future, and permit timely investigation of potential sources and vehicles of infection and implementation of control measures. The establishment of similar type data collection in the food sector will further enhance the possibilities to timely control the spread of foodborne pathogens.

Conflict of interest

None declared.

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Evidence of person-to-person transmission within a family cluster of novel coronavirus infections, United Kingdom, February 2013

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In February 2013, novel coronavirus (nCoV) infection was diagnosed in an adult male in the United Kingdom with severe respiratory illness, who had travelled to Pakistan and Saudi Arabia 10 days before symptom onset. Contact tracing identified two secondary cases among family members without recent travel: one developed severe respiratory illness and died, the other an influenza-like illness. No other severe cases were identified or nCoV detected in respiratory samples among 135 contacts followed for 10 days.

On 8 February 2013, the Health Protection Agency (HPA) in London, United Kingdom (UK), confirmed infection with novel coronavirus (nCoV) in a patient in an intensive care unit, who had travelled to both Pakistan and Saudi Arabia in the 10 days before the onset of symptoms [1]. This patient (hereafter referred to as Case 1) was the 10th confirmed case reported internationally of a severe acute respiratory illness caused by nCoV. Two secondary cases of nCoV were subsequently detected. We describe the public health investigation of this cluster and the clinical and virological follow-up of their close contacts.

The nCoV was first described in September 2012 in a Saudi Arabian national who died in June 2012 [2,3]. The UK detected its first case of nCoV infection in a male foreign national transferred from Qatar to London in September 2012 [4]. By February 2013, a total of two clusters had been described globally: one cluster (n=2) among staff in a hospital in Jordan and a family cluster (n=3) in Saudi Arabia [5]. No clear evidence of personto-person transmission was documented in either cluster [6].

Index case exposure history and laboratory investigations

The index case was a middle-aged UK resident, who had travelled to Pakistan for five weeks. He then travelled directly to Saudi Arabia on 20 January where he remained until his return to the UK on 28 January 2013. During his stay in Saudi Arabia, he spent time in Mecca and Medina on pilgrimage. On 24 January, while in Saudi Arabia, he developed fever and upper respiratory tract symptoms (Figure 1). No direct contact with animals or with persons with severe respiratory illness was reported in the 10 days before the onset of illness.

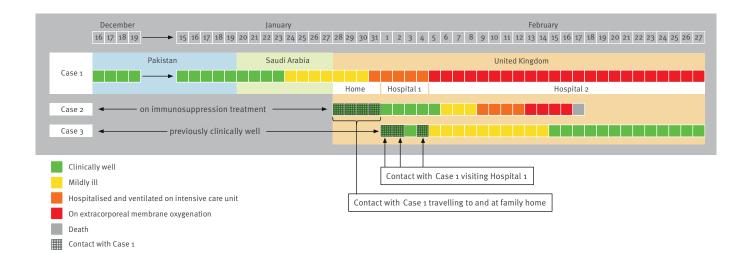
When back in the UK, the patient's respiratory symptoms worsened and he visited his GP on 30 January; he was admitted to hospital on 31 January. He rapidly deteriorated and required invasive ventilation for respiratory support. Due to further deterioration, he needed extracorporeal membrane oxygenation (ECMO) and was thus transferred to a tertiary centre on 5 February, where he remains severely ill on ECMO as of 1 March.

Initial laboratory investigation included a respiratory virus screen, with confirmation of influenza A infection on 1 February. This was subsequently characterised as influenza A(H1N1)pdmo9. As the patient's clinical condition failed to improve following administration of influenza-specific antiviral drugs, he was subsequently investigated for nCoV infection in line with HPA guidance [7]. On 7 February, nCoV was detected initially in a throat swab with a real-time PCR assay at a local laboratory, and nCoV was confirmed on 8 February by the HPA Respiratory Virus Reference Unit.

Public health management

Following the confirmation of this imported nCoV case, the UK public health authorities implemented enhanced infection control measures to minimise possible onward transmission of infection: identification and follow-up of contacts to investigate whether transmission had occurred and prompt diagnosis and appropriate management of any further cases. The HPA protocol for investigation of nCoV cases and their close contacts was used [8]. For the purpose of the investigation, a close contact was defined as:

Timeline of three novel coronavirus cases, United Kingdom, December 2012 to February 2013



- Aeroplane setting: the aircraft passengers in the same row and the two rows in front and behind a symptomatic case;
- Household setting: any person who had prolonged (>15 minutes) face-to-face contact with the confirmed case(s) any time during the illness in a household setting;
- Healthcare setting: either (i) a worker who provided direct clinical or personal care to or examined a symptomatic confirmed case or was within close vicinity of an aerosol-generating procedure AND who was not wearing full personal protective equipment (PPE) at the time; or (ii) a visitor to the hospital who was not wearing PPE at the bedside of a confirmed case; full PPE was defined as correctly fitted high filtration mask (FFP3), gown, gloves and eye protection;
- Other setting: any person who had prolonged (>15 minutes) face-to-face contact with a confirmed symptomatic case in any other enclosed setting.

Identification and follow-up of individuals who had close contact with the index case from entry into the UK at any time during his symptomatic period was rapidly initiated by the HPA together with staff from the two hospitals the patient had attended (including the Infection Prevention and Control Teams and Occupational Health).

Close contacts were followed up for a minimum period of 10 days after last exposure to the index case. Following the identification of two secondary nCoV cases among symptomatic family contacts of the index case, contact tracing was initiated for their respective additional contacts. Follow-up included collection of information on the date and setting of contact with the index case, PPE use (healthcare workers) and any symptoms of respiratory infection in the 10 days after last exposure. Contacts who developed any symptoms of acute respiratory infection in this period were asked to self-isolate in their homes (or were isolated in hospital if admitted) until asymptomatic.

The airline provided details of passengers to the HPA to allow follow-up of those persons in the same row as the case and the two adjacent rows to the patient as per World Health Organization (WHO) guidance for severe acute respiratory syndrome (SARS) [9]. Passengers who were in the UK were followed up by the HPA to inform them of the potential exposure and determine whether they had developed symptoms of acute respiratory illness in the 10 days post exposure. UK authorities informed relevant overseas national authorities directly about non-UK resident contacts on the flight through International Health Regulation mechanisms.

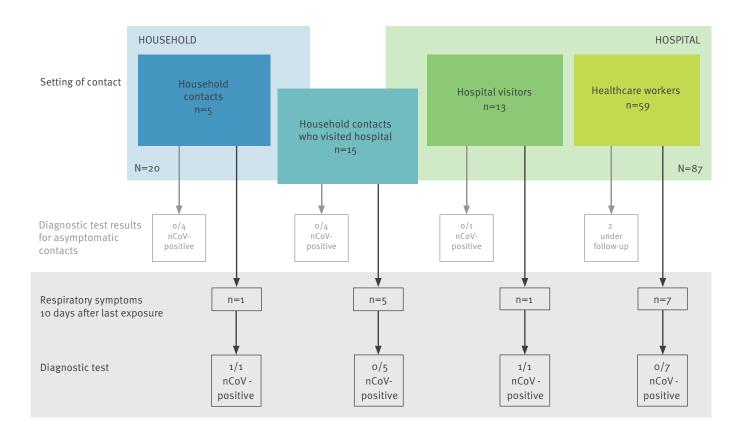
Laboratory investigation

Symptomatic contacts had respiratory samples taken (nose and throat swab, and sputum if they had a productive cough) for testing for a panel of respiratory viruses (influenza virus, respiratory syncytial virus, parainfluenza virus types 1,2,3 and 4, adenovirus, rhinovirus, human metapneumovirus) and for nCoV. Criteria for laboratory confirmation of nCoV were Up E real-time PCR detection in two different laboratories [3] and detection of two other regions of the nCoV genome [3, HPA unpublished data].

In addition, nose and throat swabs were taken from a group of asymptomatic contacts of the three confirmed cases for nCoV testing to determine if there was evidence of asymptomatic carriage.

Paired serum samples are being taken from all household and healthcare contacts regardless of symptoms

Outcome of contact^a follow-up for 10 days after last exposure to index case for respiratory illness and nCoV infection, after entry to the United Kingdom, February 2013 (n=92)



^a Excluding flight contacts.

with the initial sample taken within seven days of last exposure and the second at least 21 days after the first. Once collected, samples will be tested for serological reactivity to nCoV.

Initial epidemiological investigation of cluster

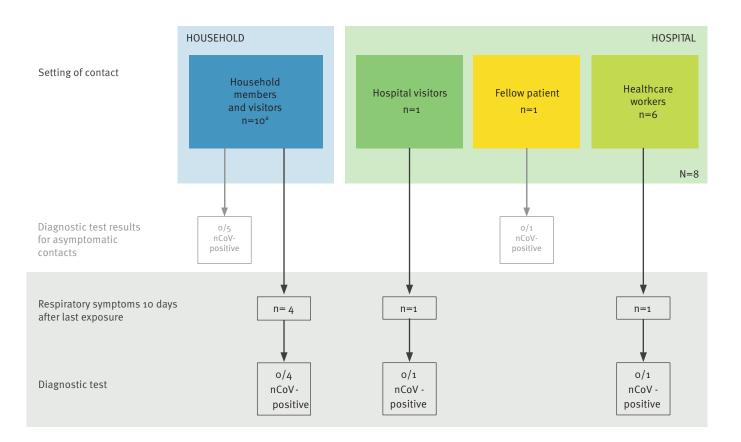
By 28 February, tracing of contacts of the index case (Case 1) had identified 103 close contacts in the UK, including 59 healthcare workers in the two hospitals, 20 household contacts of whom 15 also visited him at the hospital, 13 family and friends who visited the case in hospital, and 11 contacts during the flight who were UK residents or nationals. In addition there were nine non-UK flight contacts.

Based on available information, a number of healthcare workers with direct contact with Case 1 did not have full PPE, e.g. were not wearing an FFP3 mask. Seven of 59 healthcare workers developed mild, self-limiting respiratory symptoms in the 10 days after last contact. The nCoV was not detected by PCR in the respiratory samples of any of these seven symptomatic contacts (Figure 2).

Six of the 20 household contacts of the index case developed acute respiratory symptoms in the 10 days since last exposure, of whom one progressed to severe illness requiring hospitalisation. This single hospitalised contact was subsequently confirmed to have nCoV infection (hereafter referred to as Case 2), and was also positive for type 2 parainfluenza virus. The remaining five symptomatic household contacts had mild self-limiting disease, and nCoV was not detected from their respiratory samples nor in any of the asymptomatic household contacts of Case 1 that were tested (Figure 2).

One of the 13 non-household contacts visiting Case 1 at the hospital, hereafter referred to as Case 3,

Outcome of contact follow-up for 10 days after last exposure to Case 2 (secondary case) for respiratory illness and nCoV infection, United Kingdom, February 2013 (n=18)



^a 10/10 household members and visitors also had contact with Case 1, 2/10 also had contact with Case 3.

developed an acute mild, respiratory illness, and nCoV was detected in a respiratory sample, as was type 2 parainfluenza virus.

Two of the 11 UK-based passengers reported respiratory symptoms: one had recovered by the time of interview and did not have respiratory samples taken. In the other, nCoV was not detected from respiratory samples.

The periods of exposure of Case 2 and Case 3 to Case 1 and the timelines of their illnesses are represented in Figure 1.

Case 2 and his contacts

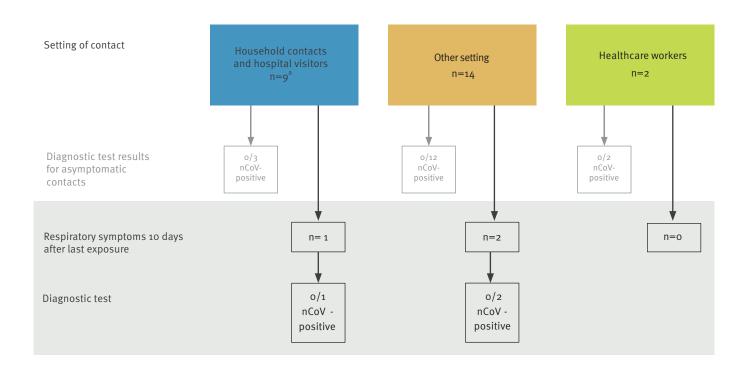
Case 2 was a male household member, who had an underlying malignant condition, the treatment of which is likely to have resulted in immunosuppression. He had not travelled overseas. Contact with the index case in a household setting occurred from the arrival of Case 1 in the UK until Case 1 was admitted to hospital on 31 January. Case 2 reportedly became unwell on 6 February and was admitted to hospital on 9 February. He required intensive care and ECMO treatment. In a nose and throat swab taken on 10 February, nCoV and type 2 parainfluenza virus were detected. His respiratory condition deteriorated and he died on 17 February.

A number of household contacts (four of 10), hospital visitors (one of one) and healthcare contacts (one of six) of Case 2 developed mild self-limiting respiratory illness in the 10 days after last exposure. In addition, case 2 had one neighbouring patient contact in the hospital, who did not develop symptoms. None had nCoV detected in respiratory samples (Figure 3).

Case 3 and her contacts

Case 3 is an adult female family member of Case 1 who lived in a different household and had not recently travelled abroad. She was exposed to Case 1 only while visiting him in hospital on three separate occasions from

Outcome of contact follow-up for 10 days after last exposure to Case 3 (secondary case) for respiratory illness and nCoV infection, United Kingdom, February 2013 (n=25)



^a 2/9 household members and visitors also had contact with Case 2.

1 to 4 February for a cumulative period of 2.5 hours, during which full PPE was not worn. During these visits Case 1 was intubated on a closed ventilator circuit. Case 3 had no contact with Case 2 while he was unwell. Case 3 developed a self-limiting influenza-like illness starting on 5 February, one day after her last contact with Case 1. She did not require medical attendance for her illness and fully recovered after nine days. She tested positive for nCoV on a single sputum sample taken on 13 February and positive for type 2 parainfluenza virus on a nose and throat swab taken on 15 February. Serology results are awaited.

A total of 25 close contacts of Case 3 were identified (nine household contacts, 14 other contacts, and two healthcare workers) of whom three developed mild self-limiting respiratory illness in the 10 days post exposure. None of these, nor the asymptomatic contacts that were tested, were found to have nCoV in respiratory samples (Figure 4).

Of the 44 contacts of Cases 1, 2 and 3 who were swabbed, 11 had another respiratory virus detected in respiratory samples: rhinovirus (n=7), influenza A(H3) and type 2 parainfluenza virus (n=1), type 2 parainfluenza virus (n=1), type 3 parainfluenza virus (n=1) and metapneumovirus (n=2).

Public health implications

We present evidence of limited person-to-person transmission of nCoV following contact with an index case returning to the UK from travel to Pakistan and Saudi Arabia. Neither of the two secondary cases that were detected had recently travelled and must therefore have acquired their infection in the UK. Both were extended family members and reported contact with the index case. One probably acquired the infection in a household setting and the other while visiting the index case in hospital. The nCoV was not detected among an additional 92 close contacts of the index case, or among the close contacts of the two secondary cases. These findings suggest that although personto-person infection is possible, there is no evidence at present of sustained person-to-person transmission of nCoV in the UK in relation to this cluster. The limited transmissibility is consistent with the data available to date, with only two other reports of small, self-limited clusters of severe disease in the Middle East: one in a healthcare setting and the other in a household setting [5]. Furthermore, intensive follow-up of close contacts of two other cases imported to European countries has failed to demonstrate onward transmission [10,11].

We found that the index case in this cluster was coinfected with influenza. Type 2 parainfluenza virus was detected in the two secondary cases. This raises questions about what roles these other infections might play in relation to nCoV transmissibility and/or the severity of the illness. In addition, as the index case was diagnosed initially with influenza, this lead to a delay in recognition of nCoV. This highlights the importance of considering a diagnosis of nCoV in atypical cases (in this case the poor response to antiviral drugs), even if a putative alternative diagnosis has already been made. HPA guidance has been adapted accordingly [7].

Although the transmissibility patterns of nCoV and SARS have been different to date, confirmed cases of nCoV reported globally have suggested a clinical picture similar to SARS, in particular the presentation with severe respiratory illness, with nine of the 15 cases reported globally to date having died [12]. Two of the three cases we describe fit this clinical picture: two required ECMO treatment and one of them died. However, the third case presented with an acute selflimiting respiratory infection that did not require hospitalisation or medical attention. This first reported case of a milder nCoV illness raises the possibility that the spectrum of clinical disease maybe wider than initially envisaged, and that a significant proportion of cases now or in the future might be milder or even asymptomatic. This highlights the importance of intensive contact tracing and virological and serological follow-up around all confirmed cases of nCoV. The application of recently developed serological assays in one case--contact study did not provide evidence of asymptomatic infection, although the contacts investigated were exposed late in the case's illness, when the viral load might be lower [11]. Paired sera are being gathered from contacts in this current investigation to determine whether there may have been more widespread mild or asymptomatic infection.

The fact that the two secondary cases acquired their infection from an imported sporadic case has enabled a preliminary estimation of the incubation and serial intervals. The timing of onset of symptoms in the index and the two secondary cases and of exposure suggests a putative incubation period ranging from one to nine days and a serial interval (time between onset of illness in index case and secondary case) of 13 to 14 days. Although the data are extremely limited, the observed upper range of the incubation period is perhaps more similar to that seen for SARS (usual range: two to 10 days) rather than seasonal coronavirus infection (usual range: two to five days) [13]. It is therefore not possible to ascertain with certainty whether the index case acquired his infection in Saudi Arabia or in Pakistan, although previous nCoV cases have been linked to the Middle East. This highlights the importance of gathering more information to determine risk factors for acquisition of infection.

All confirmed nCoV cases detected to date, apart from the two secondary cases in the UK cluster, spent time in the Middle East during the putative incubation period. This, together with our observations of limited secondary transmission, highlights the importance of ongoing vigilance and rapid investigation of cases of severe respiratory illness in residents of and travellers from that area. Further work is required to determine how widely nCoV is circulating globally. In particular serological investigations are needed on the extent of recent infection in various populations, as well as virological investigation of cases of severe undiagnosed respiratory illness in settings both in and beyond the Middle East.

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Conflict of interest

None declared.

Authors' contributions

The HPA HPU and regional teams and NHS hospital teams were responsible for the collection of data and samples

on cases and their contacts. The HPA Microbiology service teams were responsible for testing and interpretation of results from respiratory samples. National co-ordination of the investigation including design, data collation and analysis was undertaken by the HPS Colindale team in collaboration with other team members. HPS Colindale were responsible for the initial draft of the article. All co-authors provided comments and approved the final version.

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Nosocomial outbreak of disseminated orf infection in a burn unit, Gaziantep, Turkey, October to December 2012

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We report the first outbreak of nosocomial orf infection in a hospital burn unit in Gaziantep, Turkey. The outbreak lasted from October to December 2012 and involved a total of thirteen cases. It demonstrates the risk of introduction of orf virus to a burn unit, and the potential for extensive transmission among patients with compromised skin integrity. The importance of hygiene measures and infection control are highlighted and possible transmission routes of the virus discussed.

On 30 October 2012, a patient was admitted to the burn unit of Dr. Ersin Arslan Community Hospital, Gaziantep, Turkey, after the Islamic feast of the sacrifice (el eid adha), which had started on 25 October 2012. The patient had been hospitalised in another local hospital before being transferred to the burn unit. Upon admission in the burn unit, the patient presented granulation at a burned skin site on the forearm. The lesions, that resembled a possible fungal infection, were not present on intact skin. On the way to recovery, all the epithelising burn injured areas of the patient were covered with papules, sparing the intact skin. Following the patient's hospitalisation, 12 patients subsequently admitted to the burn unit between 31 October and 25 November 2012 developed similar skin lesions and, unlike the first patient, also fever (>38 °C). The skin lesions occurred after a mean of 15 days (range: 8-26 days) from time of burn injury, and appeared on epithelising areas, sparing intact skin. Papules first developed at wound sites, which then progressed to pustules, weeping nodules, and finally to crusted lesions (Figure 1). Autologous skin grafts (originating from other sites of the same patient) were completely covered with the lesions, whereas intact skin areas remained unaffected. All patients had lymphadenomegaly, and disseminated skin lesions.

Gaziantep is the sixth biggest province of Turkey and located in the southeast part of Turkey, with a population of one and a half million. The Dr. Ersin Arslan Community hospital burn unit has 14 beds. The population it serves includes patients coming from rural areas and recently refugees from Syria. The hospital has an active infection control team which is responsible for appropriate surveillance and preventive measures.

The symptoms of the patients were compatible with orf disease, a zoonotic infection caused by a dermatotropic parapoxvirus that infects sheep and goats. Orf virus is transmitted to humans through contact with an infected animal or fomites. In humans, orf usually manifests as a solitary ulcerative skin lesion sometimes

FIGURE 1

Weeping nodules of orf disease in a patient of a burn unit, Gaziantep, Turkey, November 2012



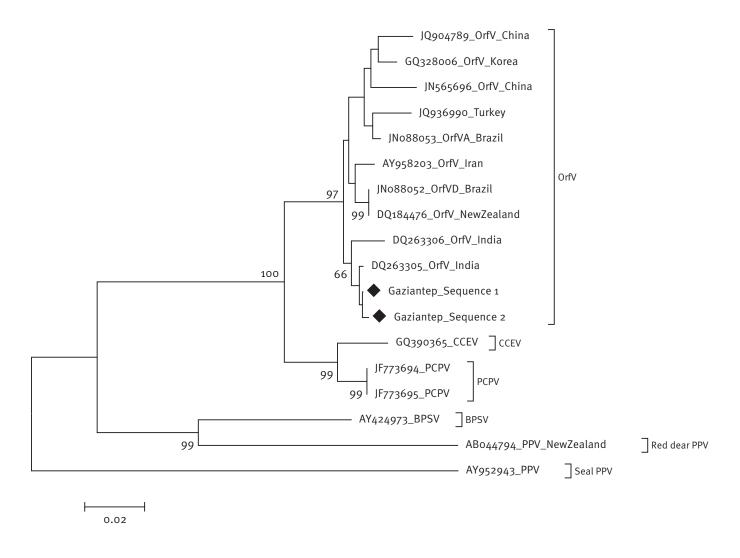
Time of symptom onset of cases of orf disease in a hospital burn unit as well as hospitalisation days, Gaziantep, Turkey, October-December2012 (n=13)

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Light gray shadowed boxes indicate days when the cases were hospitalised in the burn unit. Red shadowed boxes show the day of symptom onset.

^a Case 1 is the index case and was symptomatic upon first day of admission on 30 October 2012.

Phylogenetic analysis of partial B2L sequences derived from cases of orf disease in a burn unit, Gaziantep, Turkey, October–December2012



BPSV: bovine papular stomatitis virus; CCEV: camel contagious ecthyma virus; OrfV: orf virus; PCPV: pseudocowpox virus; PPV: parapoxvirus. The tree is based on partial B2L sequences (462 bp). Bootstrap values (>50% only) are displayed above branches. Diamond shapes indicate the sequences of the cases in this study. Except for the sequences derived from the cases in this study, all OrfV sequences included in the phylogenetic tree are derived from infected animals.

resembling bacterial infection or neoplasm. The incubation period is three to seven days [1]. In Turkey, sporadic or small clusters of zoonotic cases have been described previously [2,3]. Human infection typically is acquired through animal contacts during occupational activities [4], or following the Islamic feast of the sacrifice (eid el adha) in Islamic communities [2,5-7].

Because the first symptomatic patient in the burn unit had been admitted shortly after the Islamic feast of the

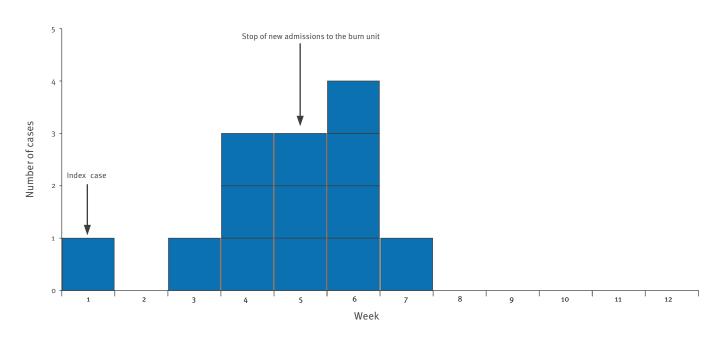
sacrifice, an outbreak orf disease was suspected and this prompted an investigation.

Outbreak investigation and results

The outbreak investigation included patients from the burn unit of Dr. Ersin Arslan Community Hospital, Gazantiep, who were hospitalised between 30 October 2012 and 2 January 2013.

The institutional review board (IRB) of Koç University approved the study.

Number of cases of orf disease in a hospital burn unit as a function of time, Gaziantep, Turkey, October 2012–January 2013 (n=13)



The week numbers are relative to the outbreak with week 1 ending on 31 October 2012.

Thirteen patients were included in total (Figure 2). Eleven of the 13 (85%) patients were male, the mean age was 37 years (standard deviation: 19; minimum: 14, maximum: 69). All the patients were from Gaziantep, except three, who were from Syria. The median proportion of surface area burned was 20% (range: 5%-60%).

Swabs (from 9 patients) and/or biopsy specimens (from 13 patients) were taken from the lesions of case patients. Three environmental samples were collected including one from a water tank, one from a pair of scissors, and one from an ointment box in the wound dressing room. The patient samples and environmental samples were transported on ice to the laboratory and stored at -70 °C until processing. Nucleic acids were isolated with a commercial kit (High Pure Viral Nucleic Acid Extraction Kit, Roche, Germany). A nested polymerase chain reaction (PCR) protocol was used for detection of parapoxvirus DNA in samples [8]. The PCR products were purified by using EZ-10 Spin Column Gel Extraction kit (Bio Basic, Ontario, Canada) and sequenced bi-directionally on ABI-PRISM 310 Genetic Analyzer, using BigDye chemistry (Applied Biosystems, CA, USA). The sequences were edited and analysed using the SeqMan software (DNAStar Package Madison, USA) and two representative sequences were submitted to GenBank under accession numbers KC776922 and KC776923*. The two representative outbreak sequences were subjected to phylogenetic analysis with the Molecular Evolutionary Genetics Analysis (Mega 5.1) software programme. A Neighbourjoining phylogenetic tree with 1,000 replicates using Kimura-2 parameter distance matrix was inferred from the outbreak sequences and 16 reference sequences obtained from GenBank.

Biopsy samples obtained from 13 patients and wound swabs from nine were positive for orf virus DNA. All patient sequences were identical except for sequences of two samples belonging to the same patient which differed at two positions among 462 bases. These nucleotide substitutions did not result in amino acid changes. The sequences from the outbreak cases clustered with Indian strains, but not with a strain previously reported from Turkey [9].

Orf viral DNA was detected from all three of the environmental samples. Patient and environmental samples were studied separately, and negative controls were included to each PCR batch to exclude the possibility of any cross-contamination.

Control measures

In our case, all infected patients were isolated, cohorted, and new patient admissions in the burn unit were stopped after 25 November (Figure 4). Since all environmental samples were positive for viral DNA, all surfaces were cleansed with hypochloride solution. The healthcare workers were educated on probable routes of transmission, with emphasis on patient to patient cross contamination and on appropriate use of personal protective equipment. Hands of caregivers were not screened. Poly-hexanide solutions were used as an antiseptic during wound care. No further case of orf infection was detected after 6 December 2012.

Discussion and conclusions

This is the first report of a nosocomial outbreak of orf infection, to our knowledge. Orf disease is usually known to have a benign course, but it can cause a serious problem in burn units because the skin integrity of patients is compromised on large surfaces and, utensils and the environment can be easily contaminated. Poxviruses can survive in animate and inanimate surfaces for years [10]. This property increases these viruses' capacity for nosocomial outbreaks. Parapox virus infections are usually zoonotic and nosocomial infections of poxviruses are rarely reported. A nosocomial buffalo poxvirus infection that spread between five burns units in Karachi, Pakistan was reported in 2007 [11]. The outbreak was hypothesised to be related to movement of patients between units. Control measures reduced transmission, but sporadic cases continued due to the admission of new patients with community-acquired infections [11].

All the environmental samples collected as a part of the current outbreak investigation were positive. Spillage of virus containing droplets during wound caring could be an explanation for such an extensive dissemination, however, transmission via the hands of caregivers might have taken place.

Nine of 13 (79%) patients involved in the outbreak had secondary infections. *Acinetobacter* spp., *Pseudomonas* spp., *Staphylococcus aureus*, and *Candida* spp. were responsible for the superficial secondary infections. Two of 13 (15%) patients died. Overall fatality rate of the unit within the last five years was around 2%. Although the fatality rate during the outbreak was 15%, the attribution of orf viral infection to the high fatality was not clear. The two fatal cases had both *Pseudomonas* and *Acinetobacter* infections, and probably died because of sepsis. *Acinetobacter baumannii* was isolated from blood culture of one of these cases. For the other patients, disease was self-limiting and symptoms disappeared within six weeks.

The sequences derived from orf virus infected patients in this outbreak did not cluster with a previously reported orf virus sequence from Turkey.

Although orf virus infection is a benign and self-limited disease, it can cause serious problems in burn units. This particular outbreak highlights the importance of strict hygiene in such settings. Infection control measures such as isolation, cohorting, and appropriate use of personal protective equipment should be carefully implemented. In this outbreak, after such measures were taken, as well as a temporary suspension of patient admissions to the burn unit, no further case was detected after 6 December 2012.

*Addendum:

The GenBank accession numbers were added on 15 March 2013.

Conflicts of interest

None declared.

Authors' contributions

Kenan Midilli: laboratory work, manuscript preparation; Ahmet Erkılıç: clinical work, infection control, manuscript preparation; Mert Kuşkucu: laboratory work, analysis; Harun Analay: clinical work; Suna Erkılıç: laboratory work; Nur Benzonana: manuscript preparation; M.Sait Yıldırım: laboratory work; Kamil Mülayim: clinical work; Hakan Acar: manuscript preparation; Önder Ergönül: manuscript preparation, analysis of data.

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Investigation into an unusual increase of human cases of Salmonella Goldcoast infection in Hungary in 2009

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We describe the outbreak investigation associated with an unusual increase in Salmonella Goldcoast cases in Hungary observed in autumn 2009, which included descriptive and analytical epidemiological studies and microbiological and veterinary investigations. Sixty cases were identified between 1 January 2009 and 1 March 2010, 50 of them from late July 2009 to January 2010. Of 50 S. Goldcoast isolates, 44 showed an indistinguishable pulsed-field gel electrophoresis profile. We conducted a matched case-control study that indicated a statistically significant association between S. Goldcoast infection and the consumption of pork cheese. The majority of cases (seven of nine) reporting consumption of this product belonged to a single family cluster. After removing six cases of this cluster, pork cheese still showed an elevated but non-significant risk for being a case in the univariable analysis (Mantel-Haenszel odds ratio (MH OR): 3.87, 95% confidence interval (CI): 0.38-39.47). A single S. Goldcoast isolate was identified during routine veterinary surveillance activities in 2009 in minced beef from a butcher's shop, originating from an abattoir where also pigs were slaughtered. We conclude that the outbreak was probably due to multiple sources of contaminated meat, probably pork, released on the market over a period of several months in 2009.

Introduction

Salmonella enterica serovar Goldcoast is rarely reported to cause outbreaks in the European Union (EU). Previous outbreaks have been reported in the United Kingdom and Germany and were found to be associated with the consumption of French paté, watercress, cheddar cheese and raw fermented sausage [1-4]. In a multi-country outbreak due to Salmonella Goldcoast in 2004, the majority of reported cases had travelled to Mallorca in Spain in the week before their disease onset, although no common source of exposure could be identified [5-7].

In Hungary, between 2004 and 2008, an average of 12 cases of S. Goldcoast was reported annually (ranging from three in 2007 to 21 in 2005). An

unusual increase to 28 cases of diarrhoea due to S. Goldcoast was observed in Hungary between late July and September 2009. In order to verify whether this occurred only in Hungary, the Hungarian National Center for Epidemiology (NCE) sent an urgent inquiry to the European Food and Waterborne Diseases and Zoonoses (FWD) network on 7 October 2009. Italy responded that they had observed a similar unusual increase in S. Goldcoast reports in 2009.

In order to understand the scale of the outbreak, to compare S. Goldcoast isolates and to identify possible risk factors for these infections, we embarked on a microbiological and epidemiological investigation of all S. Goldcoast cases identified in Hungary between 1 January 2009 and 1 March 2010. The investigation conducted by our Italian counterparts is described in this edition of the journal [8].

Methods

Descriptive epidemiology

We defined a case as a person resident in Hungary with a positive laboratory diagnosis for S. Goldcoast. We included all cases of infection with S. Goldcoast registered in the national database of notifiable diseases between 1 January 2009 and 1 March 2010.

Human isolates of S. Goldcoast from cases in 2009 were sent to the National Reference Laboratory for Salmonella at the NCE for confirmation. At the Department of Phage Typing and Molecular Epidemiology, pulsed-field gel electrophoresis (PFGE) and antibiotic resistance testing were performed using streptomycin, chloramphenicol, tetracycline, kanamycin, ampicillin, gentamicin, nalidixic acid, ciprofloxacin, cefotaxime, sulphonamide, sulphamethoxazole/trimethoprim as agreed by the EU surveillance panel [9]. The PFGE analysis was performed according to the PulseNet standardised protocol using Xbal DNA digestion and 50 µM thiourea was added to the running buffer [10,11]. Salmonella enterica serotype Braenderup H9812 strain was used as the molecular size marker [12]. DNA profiles differing by

maximum one band were considered as indistinguishable patterns.

For veterinary isolates, we reviewed all events of contamination of animals and food items by *S*. Goldcoast reported at the national level during 2009. These events are detected as part of routine surveillance through serotyping of *Salmonella* isolates at the Veterinary *Salmonella* Reference Laboratory in order to monitor the incidence of *S*. Enteritidis, *S*. Typhimurium and other serotypes in the framework of the EU Directive 2003/99/EC [13].

Analytical epidemiology

The epidemiologists at NCE conducted explorative telephone interviews in Hungarian with the 16 cases identified between 9 October and 14 November 2009 (the time when the unusual increase was observed). For this purpose, a standardised trawling questionnaire was used in order to generate hypotheses on which consumed food items could pose a risk of infection with *S*. Goldcoast. The questionnaire collected demographic information and details on food items, including typical Hungarian dishes and foods, consumed in the seven days before symptom onset.

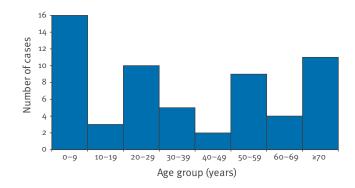
A matched case-control study was conducted to identify those of the food items mentioned in the trawling questionnaire for which consumption was associated with becoming a *S*. Goldcoast case in Hungary after 1 October 2009. A case was defined as a person of any age resident in Hungary and registered either in the national database of notifiable diseases or the electronic database of mandatory notification of the regional laboratories with laboratory-confirmed infection by *S*. Goldcoast since 1 October 2009. This time period was chosen in order to reduce the problems of recall in the epidemiological interviews which only started during December 2009. Cases who had been interviewed using the trawling questionnaire were also included in this study.

A control was defined as a person within five years of the matched case's age who resided in the same administrative region as a case. Controls were not to have experienced any of the following gastrointestinal symptoms during one month before the interview: either diarrhoea (three or more loose stools per day) or vomiting. Controls were randomly selected from the national database of notifiable diseases and the electronic database of mandatory notification of the regional laboratories among those reported for any disease other than gastroenteritis in the two-week period from one week before to one week after the onset of symptoms of their matched case.

For the sample size calculation we assumed that 70% of the Hungarian population consumed pork-containing food products at least several times a week [6]. We sought two controls per case and calculated that

FIGURE 1

Notified *Salmonella* Goldcoast cases by age group, Hungary, January 2009–March 2010 (n=60)

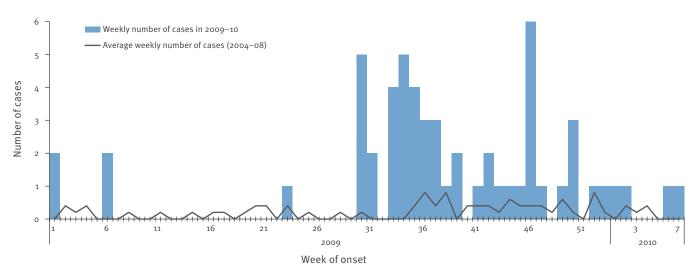


a sample size of 44 cases and 88 controls (2:1) would have 80% power to detect a minimum significant odds ratio (OR) of 5.0 with exposure in controls of 70%.

The questionnaire used for interviews of cases and controls was specifically designed for this investigation in Hungary and adapted from the trawling questionnaire; and contained questions about demographics, clinical symptoms, laboratory examinations, travel history and the history of consumption of the food items most frequently mentioned in the trawling questionnaire in the seven days before getting sick (for cases) and before the interview (for controls). This also included guestions about the way the food item had been prepared (consumed raw, insufficiently heated or well heated) and whether the product was homemade, locally produced or a commercial product. Questions about cheese as a possible risk factor were discarded for the case-control questionnaire as the majority of cheeses consumed by the respondents to the trawling questionnaire were produced by only a few manufacturers and commercially distributed in large numbers nationwide. Interviews with cases and controls were conducted by telephone by the Department of Communicable Disease Epidemiology at the NCE in Hungary.

The proportion of cases who consumed certain food items was compared with the proportion of their matched controls for the same exposure. Matched odds ratios and their respective 95% confidence intervals (CI) and p values were calculated for each exposure and food item using the Mantel–Haenszel method. Sensitivity analysis was conducted to take into account any potential cluster effect. Conditional logistic regression was used for multivariable analysis. The null hypotheses were tested at the 0.05 significance level. Data analysis was done using STATA version 10.

Notified Salmonella Goldcoast cases (only symptomatic) by week of onset, Hungary, January 2009–March 2010 (n=57)



Date of onset of symptoms was not available for three cases.

FIGURE 3

Distribution of Salmonella Goldcoast cases, Hungary, January 2009–March 2010 (n=60)



Results

Descriptive epidemiology

Identified cases

A total of 60 cases of S. Goldcoast were notified between 1 January 2009 and 1 March 2010 in Hungary. Thirty-four of these cases were male, and 16 were children under the age of 10 years (Figure 1). The majority of them, 50 cases, occurred from late July 2009 to January 2010; five cases occurred before week 30 in 2009, and two cases after week 2 in 2010 (Figure 2). Cases were distributed widely throughout the whole country [14]. All seven regions of Hungary were affected (Figure 3). The majority of cases (n=16) occurred in the capital city, Budapest. The incidence (cases/per 100,000 population) was two to four times higher than the national average (0.6/100,000) in three counties (Csongrád: 2.6, Győr-Moson-Sopron: 2.2, and Tolna: 1.3). None of the cases reported international travel before becoming ill.

Seven of 10 cases that occurred in November 2009 were part of a family cluster from two cities in southern Hungary. The investigation by the local health authorities revealed that they had bought pork meat, pork lard, pork internal organs and pig's head from different local supermarkets between late October and early November to prepare sausage, black pudding (blood sausage) and pork cheese (known as disznósajt in Hungarian). Pork cheese is a special type of Hungarian food that consists of cooked pig's organs and meat, stuffed into a pig's stomach. The family consumed the home-made pork cheese on a day in mid-November (except for one case who ate it on the following morning). The dates of onset of symptoms were between one and four days later. Two cases from this cluster were hospitalised. The family did not eat from the sausage and black pudding prepared at home before their onset of symptoms.

Microbiological findings

Laboratory investigation of 50 *S*. Goldcoast isolates revealed that 35 of them were sensitive to all 12 antimicrobials used in the agreed EU surveillance panel. The majority (n=44) of the isolates were indistinguishable by PFGE and belonged to the pattern named SCG *Xba*³ (personal communication, Ida Luzzi, June 2010). The PFGE profiles for all seven cases from the family cluster also belonged to SGC *Xba*³. Leftover food items from the household of the family cluster tested negative for *Salmonella* spp. contamination.

Veterinary findings

No increase in the occurrence of *S*. Goldcoast in any of the routinely investigated animals or animal products was observed during 2009 by the Hungarian Veterinary Authorities. The Hungarian Agriculture Office informed the National Public Health and Medical Officer Service that following routine meat inspection in 2009, only one of 5,000 tested samples was positive for

Analytical epidemiology

Trawling questionnaire

Sixteen cases with dates of onset of disease between 9 October and 14 November were interviewed using the trawling questionnaire. They came from six different regions and their age ranged between two and 88 years (mean age: 52 years), the male/female ratio was 9/7.

Fifteen cases reported frequent consumption of a variety of pork-containing items in the seven days before onset of symptoms. Furthermore, during this same exposure period, only two cases had consumed beef, which had been well cooked. The results from the trawling questionnaire along with the literature review suggested that pork-containing products were the potential risky food items transmitting *S*. Goldcoast.

Case-control study

Cases and controls: A total of 23 cases (mean age: 41 years; range: 0–88 years) and 36 controls (mean age: 30 years; range: 0–75 years) were identified to be eligible for the matched case–control study. The male/ female ratio was 13/10. The reported dates of symptom onset were between 9 October 2009 and 16 February 2010. Four cases were discarded from the final analysis because no appropriate controls could be identified for them. Also, two controls were discarded after their matched case was not interviewed. Therefore a total of 19 cases and 34 controls were included in the statistical analysis.

Clinical presentation: The most frequent symptoms reported by the 23 interviewed cases were diarrhoea (the passage of three or more loose or liquid stools in a 24 hour period, 18 cases), abdominal cramps (10 cases), fever (10 cases) and fatigue (nine cases). Only two cases were hospitalised for their symptoms. No deaths occurred in the study population.

Food consumption history: The food items most frequently consumed by cases were pork chop (nine cases), pork cheese (nine cases) and salami (five cases). In the univariable analysis, a statistically significant association was only identified between *S*. Goldcoast infection and the consumption of pork cheese (Mantel-Haenszel odds ratio (MH OR): 11.29; 95% CI: 1.38–92.32). However, as noted above, the majority of cases (seven of nine) reporting consumption of this product pertained to a single family cluster. After removing the six cases of the family cluster, pork cheese still showed an elevated risk for being a case in the univariable analysis (MH OR: 3.87), but it was not significant (p=0.25; 95% CI: 0.38–39.47).

TABLE

Matched univariable analysis of consumption of various pork food items, *Salmonella* Goldcoast case-control study, Hungary, January 2009–March 2010 (n=53)

Food item	Cases	(n=19)	Control	s (n=34)	MH OR	o=9/_Cl
rood item	Exposed	%	Exposed	%	(p value)	95% CI
Pork cheese	9	47.4	3	8.8	11.29 (0.02)	1.38 - 92.32
Raw and other sausages	4	21.1	3	8.8	1.51 (0.60)	0.31-7.17
Salami	5	33.4	9	26.5	1.08 (0.91)	0.29 - 3.93
Liver paté	1	8.4	4	12.1	1.00 (1.00)	0.09-11.03
Liver sausage	1	5.0	1	2.9	1.00 (1.00)	0.06-15.98
Ham smoked	1	7.1	3	8.3	0.67 (0.73)	0.07-6.40
Smoked sausage	3	20.0	12	35.3	0.65 (0.60)	0.13 - 3.16
Pork crackling	2	10.0	5	14.7	0.64 (0.60)	0.12-3.38
Liver paste	2	18.2	8	23.5	0.60 (0.58)	0.11-3.49
Pork chop	9	64.3	27	79.4	0.49 (0.36)	0.11-2.28
Pork lard	1	5.9	6	17.7	0.35 (0.04)	0.04-3.02

CI: confidence interval; MH OR: Mantel-Haenszel odds ratio.

Only food items are listed for which discordant pairs of cases and controls could be formed for matched analysis.

'Other sausages' refer to sausages that are not totally raw but treated to some extent.

The food consumption frequencies by cases and controls are listed in the Table. The multivariable analysis using conditional logistic regression did not implicate any food item being independently associated with *S*. Goldcoast infection.

Discussion

S. Goldcoast is rarely identified as the implicated pathogen in persons with gastrointestinal disease in Hungary. Previous surveillance data has shown that on average 12 human cases were reported annually between 2004 and 2008. The increase in reported cases observed from July 2009 onwards was therefore noteworthy and merited further epidemiological and microbiological investigation.

Both our epidemiological and microbiological results suggest that the unusual increase in S. Goldcoast cases in Hungary between 1 January 2009 and 1 March 2010 was part of a national outbreak with multiple sources. The majority of the human S. Goldcoast isolates showed indistinguishable PFGE profiles and were sensitive to the same panel of antimicrobial drugs, which suggests that they belonged to the same bacterial clone and thus shared a common origin earlier in the food chain. None of the cases reported travel outside of Hungary in the days leading up to their illness, and therefore they had domestically acquired infections. Additionally, the country-wide distribution and the presence of confirmed cases in all age groups suggests that the potentially contaminated food ingredient was consumed by all groups of people and that it was available throughout the country. The higher number of reported cases from Budapest is simply a reflection of the population density in the capital city. As confirmed cases were reported over a period of 24

weeks, the presence of contaminated food items in the food chain was continuous for at least six months. A continuous source of contaminated food could point to a product with a long shelf life or to a source in animal populations (i.e. pigs or cattle) that carry the infection and may result in a prolonged introduction of contaminated meat into the retail market. Pork cheese, which was significantly associated with being a case in the univariable analysis, was probably identified because of the family cluster in which cases had consumed the food item together before developing disease symptoms. That pork cheese was prepared at home makes it possible that it was improperly cooked and/or became cross-contaminated. Unfortunately, the analyses were not able to implicate any food item being independently associated with *S*. Goldcoast infection due to the small sample size. However, the family outbreak suggested that pork-containing products were a likely source of infection. The sample from minced beef, which was found positive for S. Goldcoast in the veterinary surveillance, could have originated from a truly positive cow, but cross-contamination, either at the abattoir or at the butcher's shop, cannot be excluded since the meat originated from an abattoir where pigs were also slaughtered.

This outbreak investigation encountered several limitations which might explain the absence of concrete epidemiological evidence to identify the potential source(s) of infection for the *S*. Goldcoast cases in Hungary between 1 January 2009 and 1 March 2010. According to the epidemic curve, the peak of the unusual increase occurred between late July and September 2009. As this outbreak investigation only started in December 2009, the majority of the cases were not included in the matched case–control study. In addition, the cases that were included in the case-control study reported symptom onset as far back as the beginning of October 2009. They might therefore have been unable to recall all food items consumed and answer the questions accurately in the questionnaire. The small sample size (19 cases and 34 controls) reduced the actual power of the study to 55% to detect a minimum significant odds ratio of 5.0 with exposure in controls of 70%. The sample size could not be increased further due to the matching control selection procedures and criteria as well as waning of the outbreak after March 2010. The lack of statistical power in the case-control study reduced our ability to identify clear risk factors.

The case-control questionnaire was exclusively focused on pork products based on explorative interviews and background published evidence. Other possible sources of contamination might have remained undetected. Cheese as a possible risk factor was discarded assuming that the outbreak would have been much larger if the common source of *S*. Goldcoast infection had been a widely distributed commercial cheese product. Also, certain exposures which have been identified as potential confounders in previous studies, such as drinking alcohol during the meal, were not included in the questionnaire and therefore were not controlled for in the final analysis of the data [15].

We continue to believe that the national outbreak of S. Goldcoast in Hungary in 2009 and early 2010 was likely to be related to the consumption of pork meatcontaining products due to the following reasons. Firstly, two earlier large outbreaks of S. Goldcoast outbreaks in the EU were due to pork-containing products (fermented sausage and minced pork) [16,17]. Secondly, the results from the investigation of a simultaneous unusual increase in S. Goldcoast cases in Italy in 2009 suggested that the consumption of porkcontaining food, in particular salami could have been a potential risk factor for becoming a case [8]. Thirdly, the results from the trawling questionnaire with the Hungarian cases showed that most cases (except one) had consumed pork meat products and very few other meat products in the seven days before their disease onset. Finally, recent data from Salmonella surveys in pig holdings throughout the EU suggest that S. Goldcoast is one of the most common serotypes identified in pig breeding and production holdings outside Hungary [18]. This last piece of information allows us to hypothesise that, rather than a single contaminated food item, pigs from a number of holdings were contaminated, partly exported to other countries where they were raised and slaughtered and released to the national markets during several months. Fusce eget velit sapien. Donec et eros diam.

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Field epidemiologists of regional and local levels of the National Public Health and Medical Officer Service (Hungary) and Hungarian Veterinary Authorities for their contribution to the study. Fernando Simon and Alicia Barrasa (National Centre for Epidemiology, Madrid, Spain) for critical review of the study protocol and the results. Celine Gossner (ECDC, Stockholm, Sweden) for the preparation of the map.

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A multistate epidemic outbreak of *Salmonella* Goldcoast infection in humans, June 2009 to March 2010: the investigation in Italy

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After an urgent inquiry into a suspected international outbreak of Salmonella Goldcoast infection was launched by Hungary in October 2009 a nationwide multidisciplinary investigation was carried out in Italy. The aims were to verify whether the higher than expected number of cases of S. Goldcoast infection that had occurred in Italy in the previous months were linked to the outbreak in Hungary and to determine their origin. Between June 2009 and March 2010, 79 confirmed cases of S. Goldcoast infection were identified. Of these, 17 were part of three different point-source outbreaks probably associated with the consumption of salami. Eating salami was also reported by 20 of the 39 sporadic cases that could be interviewed. Fifteen strains of S. Goldcoast isolated from the cases were typed by pulsed-field gel electrophoresis. They shared more than 90% homology with the Hungarian epidemic strain and were also highly similar to S. Goldcoast strains that had been isolated in Italy from pigs and pork-containing food items in 2009 and 2010. Although the origin of the outbreak and the common source linking the Hungarian and the Italian cases could not be definitively identified, our results suggest a possible zoonotic connection of the outbreak cases with the pork production chain.

Introduction

Salmonella enterica serotype Goldcoast is a pathogen of zoonotic origin which causes clinical disease in humans, primarily acute gastrointestinal illness (AGI). *S*. Goldcoast infection in humans is rare and is usually acquired through the consumption of raw or undercooked food of animal origin. Although most *S*. Goldcoast infections are reported as sporadic cases, a few epidemic outbreaks have been described in the international literature [1-4]. In 2005 an outbreak of *S*. Goldcoast infection involved tourists from the United Kingdom (UK), Ireland, Denmark, Norway and Sweden who had travelled to Spain, but could not be linked to any specific source [2]. In 2001, at least nine people in Germany became infected due to the consumption of fermented sausages of pork origin [1]. The specific association of this *Salmonella* serovar with swine emerged also from the baseline surveys on the prevalence of Salmonella spp. in different food-producing animals in the European Union (EU) [5-8]. Compared with other Salmonella serovars that have been shown to occur in many animals species and categories already surveyed in the EU (hens, broilers and turkeys), S. Goldcoast was the only serovar exclusively associated with pigs [9]. S. Goldcoast has also been reported as one of the most common Salmonella serovars identified from cattle in Germany, the United Kingdom (UK) and Spain in 2007 and 2008 [10,11].

In Italy, besides the national official surveillance system for human cases of Salmonella spp. infections based on the identification of clinical illness, the laboratory-based surveillance network for enteric pathogens Enter-net Italia (www.iss.it/ente) provides information on the microbiological characteristics of Salmonella spp. strains isolated from humans [12]. The data are gathered through a network of regional reference laboratories which characterise the strains isolated from the peripheral diagnostic laboratories. It is important to mention that due to a certain local variability of the number of peripheral laboratories and their compliance to surveillance, the sensitivity of the Enter-net Italia may differ between Italian regions, as a recent paper has shown for some regions of northern Italy [13].

The Enter-net Italia network is coordinated by the National Reference Laboratory for *Salmonella* infection in humans at the Istituto Superiore di Sanità (ISS). It is strictly interfaced with the homologous Enter-Vet

surveillance network for food and animals, coordinated by the Veterinary National Reference Laboratory for *Salmonella* at the Istituto Zooprofilattico Sperimentale delle Venezie, with whom they share protocols and databases [12].

On 7 October 2009, Hungary launched an urgent inquiry through the Food- and Waterborne Diseases and Zoonoses Network (FWD network), which is coordinated by the European Centre for Disease Prevention and Control (ECDC), reporting an unusual increase in S. Goldcoast human infections in the country. The Enter-net Italia database indicated that a higher than expected number of *S*. Goldocoast isolates, clustering in time and in space at regional level, had occurred also in Italy, particularly since June 2009. Moreover, the molecular characteristics of the S. Goldcoast strains isolated from the case-patients, analysed by pulsedfield gel electrophoresis (PFGE), showed a genetic similarity of more than 90% with those from Hungary. Since Spain, Denmark, Norway, and the UK had also reported a higher than expected number of cases of S.Goldcoast infection in the same period (personal communication Celine Gossner, 8 December 2009), it was hypothesised that the Italian cases could be part of a larger multistate outbreak. In this article we report the results of the investigation carried out in Italy with the aim of identifying the origin of the Italian cluster of S. Goldcoast cases and the possible epidemiological link with cases that occurred in other EU Member States.

Methods

To coordinate the investigation activities, a multidisciplinary team was set up in November 2009 including the coordinators of the Enter-net and Enter-Vet surveillance networks, the health authorities of those regions where human cases had been passively reported, and the veterinary regional laboratories. Active case finding was carried out by sending an alert to the official health authorities of all the Italian regions requesting information on any further laboratory-confirmed cases of *S*. Goldcoast infection that had occurred in 2009. The same alert was sent to the Enter-Vet network in order to obtain information about the origin and the characteristics of any *S*. Goldcoast strains isolated from animals and food in the last five years.

Epidemiological investigation

The following definition of a *S*. Goldcoast epidemic case was adopted: a person who had a confirmed laboratory diagnosis of *S*. Goldcoast in Italy, after 1 June 2009. This date was chosen because a higher than expected number of cases was reported starting from 1 June. Patients fulfilling these criteria were traced and interviewed, upon consent, using a standardised questionnaire. For patients younger than 18 years the interview was conducted with the parents. The questionnaire was based on one designed by ECDC for the *S*. Goldcoast multistate outbreak investigation, and modified to better fit the Italian context (especially

concerning food exposures). We collected information on the clinical course of AGI associated with *S*. Goldcoast infection, food consumption, contact with other people reporting AGI symptoms and contact with animals, in the week before the onset of symptoms. The cases were also asked about recent travel abroad. Cases who had already been interviewed by the local health authority were not interviewed again.

Cases were categorised as clustered or sporadic cases depending on whether a clear epidemiological link with other *S*. Goldcoast cases in Italy could be established. An epidemiological link included persons that attended the same social event as a confirmed case or those living with a household member with a confirmed *S*. Goldcoast infection.

A descriptive study was conducted with the sporadic cases in order to describe the travel and food consumption history as well as contacts with animals or people with AGI, in the week before the onset of illness. To investigate a single point-source outbreak that occurred on 9 June 2009 associated with S. Goldcoast infection, a cohort study was performed aimed at detecting the association between the consumption of food and the occurrence of AGI. Overall, the cohort study included 34 people, resident in the Lombardia region, who had participated in a day trip to Tuscany, and provided information on food consumption. The following case definition for AGI was adopted: a person who developed diarrhoea or had a positive culture for S. Goldcoast within seven days after the trip to Tuscany. For every food item, specific attack rates in exposed and unexposed individuals were calculated. The food-specific risk ratio (RR) with 95% confidence intervals (CI) and p values were calculated by univariate analysis, with being or not an AGI-case as the outcome variable and the consumption of each food item as the explanatory variable.

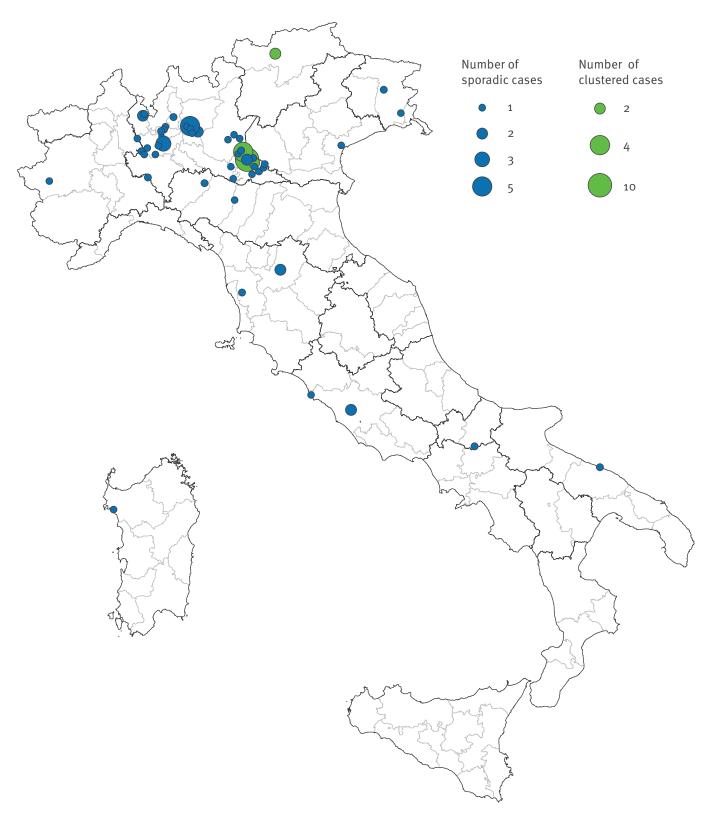
Microbiological and molecular investigation

S. Goldcoast human isolates from case-patients reported since 1 June 2009 were sent to ISS for sero-typing confirmation, antibiotic resistance testing and molecular characterisation by PFGE. The antimicrobial resistance was assessed by the disk diffusion method using the Enter-net reference panel [14] which includes 12 antibiotic disks (Becton Dickinson).

The PFGE analysis was performed according to the PulseNet standardised protocol [15] using *Xba*l as the restriction enzyme (New England Biolabs, Ipswich, MA). To avoid degradation of DNA samples 50μ M thiourea was added to the running buffer and agarose gel [16]. *S. enterica* serotype Braenderup H9812 strain was used as the molecular size marker [17].

Dendrogram and cluster analysis were performed using algorithms available in the BioNumerics software package v.6.0 (Applied Maths, Sint-Martens-Latem, Belgium). Per cent similarity between different

Geographical distribution, by place of residence, of *Salmonella* Goldcoast case-patients, Italy, 1 June 2009 to 31 March 2010 (n=76)



For three cases the place of residence was unavailable.

chromosomal fingerprints was scored by the Dice coefficient. The unweighted pair group method with arithmetic means (UPGMA), with a 1.00% tolerance limit and 1.00% optimisation, was used to obtain the dendrogram. DNA profiles differing by one or more DNA fragments were considered as distinct patterns. Strains with a coefficient of similarity of at least 90% were considered as genetically closely related. *S*. Goldcoast strains isolated in 2009 from the pig production chain and one PFGE profile from an isolate belonging to the Hungarian outbreak were also included in the cluster analysis.

Results

Between 1 June 2009 and 31 March 2010, a total of 79 *S*. Goldcoast cases were identified across Italy. The majority of them (n=60) were reported from northern Italy, particularly the Lombardia region (Figure 1), with the remaining cases affecting 10 additional regions (Italy has 20 regions in total). More cases were male (n=48) than female (n=31), and more cases were adults of at least 18 years of age (n=56) than children under the age of 18 years (n=23). The median age of casepatients was 50 years (range: 10 months–93 years).

The distribution of the cases by week of onset of symptoms (Figure 2) showed that cases peaked in week 23 (June 2009), which coincided with the time of the outbreak related to the trip to Tuscany. Sixty-two cases had no apparent link with other cases with a laboratory-confirmed diagnosis of *S*. Goldcoast and were classified as sporadic cases. In the following, these are described separately from the remaining 17 cases that were part of three different clusters.

The sporadic cases of S. Goldcoast infection

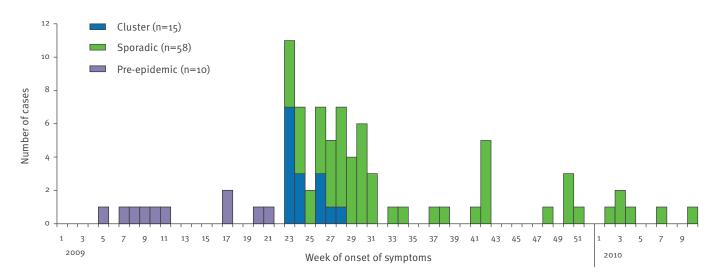
The investigation of sporadic cases took place between 1 December 2009 and 31 March 2010. The questionnaire on clinical symptoms associated with *S*. Goldcoast infection and exposures to potential sources of infection could be administered only to 39 of the 62 sporadic cases. In 22 cases the interview could not be completed due to one of the following reasons: refusal to participate, death, failure to trace the patient or reporting date after 31 March 2010. One case who had been interviewed was later excluded from the analysis because the information had been provided by a relative of the patient.

Thirty-seven of the 39 interviewed cases reported clinical symptoms. These included watery diarrhoea, abdominal cramps, fever, fatigue, vomiting and nausea (Table 1). The duration of illness ranged between two and 20 days (mean: 7±5.2 days; median: 5 days). Sixteen cases were hospitalised. Two patients (66 and 77 years of age) with underlying chronic disease, died following the *S*. Goldcoast infection.

In the seven-day period before the onset of symptoms 15 cases reported exposure to risk factors for *S*. Goldcoast infection not related with food-consumption: travelling abroad (n=2), visiting farms (n=2), and contact with companion animals (n=11), poultry (n=6) or food-producing animals (cattle) (n=1). Contact with household members with gastrointestinal symptoms in

FIGURE 2

Distribution, by week of onset of symptoms, of *Salmonella* Goldcoast case-patients, Italy, 1 January 2009 to 31 March 2010 (n=83)



Purple boxes refer to pre-epidemic cases, blue and green boxes refer to outbreak cases. Two cases in the cluster and four sporadic cases were asymptomatic and are not shown in the figure.

TABLE 1

Frequency and duration of clinical symptoms reported by sporadic *Salmonella* Goldocoast case-patients, Italy, 1 June 2009 to 31 March 2010 (n=37)

Symptom	S. Goldcoast cases reporting	Cases who answered the	Duration o	fsymptoms
Symptom	the clinical symptoms	question on the symptom	Range (days)	Mean (days)
Watery diarrhoea	28	33	2-20	6
Bloody diarrhoea	4	33	1-10	4
Abdominal cramps	25	33	1-20	6
Fever	22	33	1-10	3
Fatigue	21	31	2-20	7
Vomiting	16	33	1-6	2
Nausea	13	33	1-6	3
Headache	8	32	1-5	3
Body ache	6	30	1-10	5

the seven days before the onset of illness was reported from six patients. The description of food items consumed in the week before the onset of AGI symptoms is reported in Table 2.

Microbiological investigation

During the period from June 2009 to February 2010, 15 *S*. Goldcoast strains from human sporadic cases were received at ISS. Nine were from cases that occurred in northern Italy, five were from central Italy and one from southern Italy. All of them were confirmed as *S*. Goldcoast with specific antisera and susceptible to all drugs tested, when tested for antimicrobial susceptibility.

All but three strains were typeable by PFGE. Cluster analysis was performed including PFGE profiles of 12 Italian human outbreak strains, one isolate from a Hungarian case, representative of 43 strains with identical PFGE pattern isolated from patients involved in the outbreak, five strains isolated in Italy from food (pork minced meat, pork sausages and fish), and one from rendered animal proteins of pork and beef origin.

The cluster analysis of the PFGE profiles revealed that all the strains of human and animal origin had a high genetic homology ($\geq 90\%$) with the Hungarian representative strain.

The clustered cases

Three different clusters of *S*. Goldcoast infections, all from the same area of the Lombardia region, could be identified. The largest cluster included 11 cases from the same town, who had participated in a day trip to Tuscany in early June 2009. Of the whole group of participants, 34 persons (21 female and 13 male) could be traced and interviewed about the occurrence of symptoms and food items consumed in the restaurant where

the group had had lunch, as well as food they had brought from home. The median age of the respondents was 61 years (range: 3-91 years). A total of 19 people (all but one older than 18 years) reported AGI symptoms, which included watery diarrhoea (n=19), fever (n=12), vomiting (n=6) and abdominal pain (n=10). Of those, one patient needed hospitalisation. The mean incubation time was 30 ± 19 hours (median: 23 hours; range: 12n-64 hours). Fifteen patients submitted stool samples for laboratory investigation, from which *S*. Goldcoast was isolated in 11 cases.

Eating a sandwich with a traditional salami (*Salame Mantovano*) taken from home was the only item with a statistically significant association with AGI (RR: 1.98; p=0.048). AGI occurred in 14 of 20 people reporting and in five of 14 people not reporting consumption of salami. The salami sandwiches had been prepared at home by some of the participants, using various types of *Salame Mantovano* (including both commercial and home-made products), all purchased or produced for domestic consumption in the Mantova province.

The second cluster involved a family and was also linked to the consumption of a salami. *S*. Goldcoast was isolated from all four members of the family (both parents and their teenage children) resident in a village located 10 km from the place of residence of the cases in the first cluster. The family members showed AGI symptoms in early July 2009, two days after having eaten a *Salame Mantovano* purchased from a local retailer. The food trace-back showed that two different brands of *Salami Mantovano* had been sold in the supermarket. They were manufactured by two different factories located in the province of Mantova.

The third cluster included an adult and teenage child, both resident outside the Lombardia region, who had

TABLE 2

Food consumed in the seven days before the onset of clinical symptoms by sporadic *Salmonella* Goldcoast case-patients, Italy, 1 June 2009 to 31 March 2010 (n=37)

Food	S. Goldcoast cases reporting the consumption of the food item	S. Goldcoast cases who answered the question on the food item
Pork-containing food items		
Cooked ham	25	36
Dry-cured ham	27	37
Salami	20	37
Bacon	11	35
Sausages	10	36
Frankfurter sausages	8	34
Other pork- containing food	3	32
Meat		
Beef	28	33
Pork	7	36
Dairy		
Matured cheese	23	32
Cream cheese	24	33
Raw milk	0	34

travelled at the beginning of July 2009 to Mantova province, where they had consumed a sandwich with *Salame Mantovano* in a bar. The adult showed clinical symptoms of AGI two days later and was positive in culture for *S*. Goldcoast. The child also tested positive for *S*. Goldcoast but did not have any clinical symptoms.

Investigation of food and the food chain

Due to a delay of more than four months between the occurrence of cases and the *S*. Goldcoast outbreak investigation, specimen of the suspected food could not be collected and examined for *S*. Goldcoast. Even for the cluster on the trip to Tuscany, which was promptly investigated after it was reported, neither leftover food nor samples of the same batches of salami could be taken and tested for *S*. Goldcoast, since the preliminary investigation omitted to include in the analytical study any food taken from home but focused on the food items consumed in the restaurant.

Data for *S*. Goldcoast from the Enter-Vet database indicated that six strains had been isolated in 2009 in Italy from food and matrices of animal origin, mostly from the pork food chain. In particular the isolates were obtained from various batches of pork minced meat (n=2) and pork sausages (n=2), sampled in different cutting and manufacturing plants in northern and central Italy, from a sample of rendered animal protein of pork and beef origin intended for use as agricultural fertiliser, sampled in a rendering plant, and from a sample of fish organs. These last two sources were the only ones sampled in Mantova province. All isolates but one had been sampled between 10 June and 28 October 2009, whenever most human cases were observed. Trace-back of the *S*. Goldcoast strains isolated from the pork minced meat led to a single pig farm, located in Lombardia region, that raised pigs only for the fattening production-cycle. No further trace-back was possible for the other isolates.

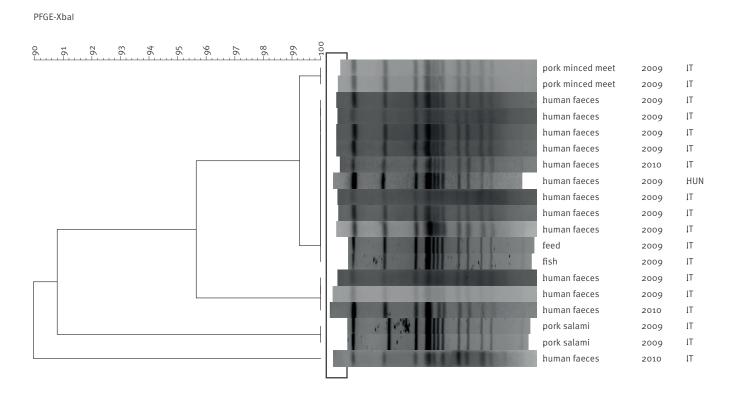
Overall 28 of the 31 strains of *S*. Goldcoast of animal origin isolated in Italy between 2007 and 2009, were sampled along the pig production chain either at farm level (n=4), slaughterhouse (n=10), rendering plant (n=1), or in the post-harvest production chain such as processing plants (n=2), salami factories (n=10) and retail (n=1). Of the *S*. Goldcoast strains isolated in the post-harvest stage, six were from ground raw pork meat and four were from finished salami at different points in the curing process.

Unfortunately details on the reasons for sampling were not available, except for the *S*. Goldcoast strains from farms and slaughterers that had been sampled in 2007 and 2008 in the context of the EU baseline surveys on the prevalence on *Salmonella* spp. in slaughter and in breeding pigs, respectively [8,9].

Discussion

Our investigation revealed that a community-wide outbreak of *S*. Goldcoast probably associated with a continuous source of infection occurred in Italy between 2009 and 2010. It cannot be excluded that the real burden of this outbreak and its geographical distribution

Cluster analysis of Salmonella Goldcoast strains of human and animal origin, Italy, June 2009-March 2010 (n=19)



Human strains include isolates from 12 Italian cases and one Hungarian case. Strains of animal origin include isolates from pork minced meat (n=2), pork sausage (n=2), rendered animal protein (n=1) and fish (n=1) isolated in Italy in 2009 and 2010.

were wider than what was identified. Possible reasons for that include a general tendency to submit only a small proportion of stool samples for diagnostic investigation in patients with AGI [18], especially when they are characterised by mild symptoms, and the heterogeneous availability of typing methods for Salmonella spp. in peripheral laboratories across the country. During the study period, the majority of cases were reported from the Lombardia region. This region has a well-established surveillance system for enteric pathogens causing AGI, including Salmonella spp., which is more sensitive than systems in other Italian regions [13]. It can therefore be argued that the outbreak may not have been limited to Lombardia, but that other epidemic cases may have occurred elsewhere in the country and remained undetected and/or unreported.

The consumption of pork-containing products has previously been described as associated with *S*. Goldcoast outbreaks. Our investigation therefore concentrated on the possibility that pork-containing products could be the main cause of this outbreak. This hypothesis was strongly supported by the investigation in food and animals, which indicated a close relationship between S. Goldcoast and the pork production chain, and by the microbiological characterisation of the S. Goldcoast strains, including PFGE typing, which showed a high genetic homology ($\geq 99\%$) between strains of human and pig origin.

Epidemiological and microbiological results failed to implicate a definitive source of the outbreak. Nonetheless the consumption of different types of pork-containing food, in particular salami remains a very possible source, as it was frequently reported by the sporadic cases and also emerged from the investigation of all three clusters of *S*. Goldcoast infection. Besides dry cured and cooked ham, which are considered products with a low-risk of *Salmonella* spp. infection, salami was the most frequent food exposure among the pork products, even if the consumption of salami alone would not explain all *S*. Goldcoast cases in this outbreak.

Salami has been implicated in several *Salmonella*related food-borne outbreaks in Italy [19,20] as well as other countries [21-23]. Salami are dry fermented sausages traditionally considered safe due to low pH, low water activity and high salinity, but Salmonella can survive fermentation and drying steps if the manufacturing process or fermentation periods are inadequate. The main reasons for contaminated salami are Salmonella contamination at the initial manufacturing stages and/or failures during the fermentation process [24]. Observations from a recent study on the survival of Salmonella in different types of Italian salami, demonstrated that the Salmonella population declined during the experimental period but surviving organisms were always detected at the end of that period [25]. Several studies aiming at estimating the magnitude of reduction of the *Salmonella* population in Italian salami, using different techniques of preparation and storage conditions, have yielded varying results. A model developed by Pin et al. [26], predicted one order of magnitude reduction of the Salmonella population in salami during the storage period. Higher levels of reduction were reported by Nightingale et al. [27] and Porto-Fett et al. [28] in experimental studies in fermented and dried Italian-style and Genoa salami, respectively. Conversely, Messier et al. [29] did not detect surviving *Salmonella* organisms after 11 days in Genoa salami inoculated with 10³ cfu/g. These data demonstrate that although salami manufacturing processes generally lead to appreciable reductions in the levels of Salmonella, they do not always result in a reduction of the initial pathogen loads, adequate to avoid possible transmission to human.

The delay between the occurrence of the majority of the epidemic cases and the time of the investigation, represented the most critical limitation of our study. Had both the epidemiological and microbiological investigations been conducted immediately after the peak of the outbreak in 2009, more concrete risk exposures may have been identified. The possibility of sampling and testing the suspected food items were also strongly hampered by the poor timeliness of the outbreak investigation, which limited the possibility to detect the outbreak source. Even the trace-back activity for suspected food, which can be of crucial importance for the identification of the source of infection, or at least for identifying where a contamination of the food-production chain could have occurred (e.g. preharvest or post-harvest), was limited by the difficulties of tracing back the salami. Salamis are usually made from minced meat of various species and animals (usually either pork or pork and beef) that may originate from various batches and/or carcasses. This makes the trace-back of these products, even on a small scale, extremely challenging.

These limitations of our investigation may also explain why a common link between cases of *S*. Goldcoast infection in Italy and Hungary could not be clearly established, although evidence from the molecular characterisation of *S*. Goldcoast strains and the epidemiological findings (temporal pattern connection, characteristics of the cases, suspected food) suggested an evident epidemiologic relationship between them. In conclusion, this report shows that the outbreak of AGI associated with the rare *S*. Goldcoast serotype that occurred in Italy in 2009 and 2010 was probably part of a larger multistate outbreak with a continuous source. Our results highlight how crucial the exchange of information was, at EU, national or regional level, for both the outbreak detection and investigation. Epidemiological and microbiological information on cases, collected in a surveillance system for AGI associated with enteric pathogens, should be aimed at detecting and promptly investigating community-wide outbreaks. Whenever such data are disconnected, as in the case of Italy, it is essential to efficiently combine the information, in order to avoid delay in outbreak detection and investigation. Similarly, sharing of protocols for *Salmonella* spp. strain typing between human and veterinary laboratory networks is critical in order to generate and confirm hypotheses on possible sources of infection.

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Conflict of interest

None declared.

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Outbreaks associated to large open air festivals, including music festivals, 1980 to 2012

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In the minds of many, large scale open air festivals have become associated with spring and summer, attracting many people, and in the case of music festivals, thousands of music fans. These festivals share the usual health risks associated with large mass gatherings, including transmission of communicable diseases and risk of outbreaks. Large scale open air festivals have however specific characteristics, including outdoor settings, on-site housing and food supply and the generally young age of the participants. Outbreaks at large scale open air festivals have been caused by Cryptosporium parvum, Campylobacter spp., Escherichia coli, Salmonella enterica, Shigella sonnei, Staphylococcus aureus, hepatitis A virus, influenza virus, measles virus, mumps virus and norovirus. Faecal-oral and respiratory transmissions of pathogens result from non-compliance with hygiene rules, inadequate sanitation and insufficient vaccination coverage. Sexual transmission of infectious diseases may also occur and is likely to be underestimated and underreported. Enhanced surveillance during and after festivals is essential. Preventive measures such as immunisations of participants and advice on-site and via social networks should be considered to reduce outbreaks at these large scale open air festivals.

Introduction

Mass gathering (MG) medicine is an emerging specialty [1] that includes effective strategies and planning to address health security and risks associated with MGs. The number of attendees that classifies an event as a 'mass gathering' is wholly arbitrary. Definitions vary greatly, with some sources specifying any gathering to be a MG when more than 1,000 individuals attend, whereas others require the attendance of as many as 25,000 people to qualify [2]. Outbreaks, however, occur irrespective of the size of a gathering and are described not only in settings such as private parties, weddings, and other events involving fewer than 1,000 attendees, but also in large MGs, such as religious MGs, attended by millions of people [3]. In addition to the number of people, MGs are events at a specific site for a definite time which may greatly vary between different MGs. These gatherings might be planned or unplanned and recurrent or sporadic.

Although large scale open air festivals have become important spring and summer activities and attract thousands of people, they are probably neglected, particularly music festivals, in terms of public health attention, as well as surveillance and prevention of infectious disease strategies, compared to other categories of MGs such as sport or religious events. Indeed, most of the literature on MGs in this respect, has addressed health issues related to very large MGs, including the Hajj [2] and the Olympic Games [4].

A characteristic of large scale open air festivals, including music festivals, may be that they are not always organised and managed by professionals and may involve inexperienced volunteers as staff members. Music festivals in particular have also specific characteristics, including outdoor settings, on-site housing and food supply, the young age of the participants, recreational motivations, and the potential for excessive alcohol or drug consumption, which may notably increase the risk of sexually transmitted infections (STIs) [5] and possibly of other infectious diseases.

A total of 858 music festivals were recorded worldwide in 2012 in choosefest database (www.choosefest.com/). The largest music festivals were in Europe where 13 of the 20 top international festivals occur (Table 1). Most festivals take place over a three to four day period with the longest lasting 11 days. Attendance for the top 20 festivals in terms of size ranges from 17,000 to 175,000 per day. According to a survey conducted in the United Kingdom (UK) during the year 2009, the total number of visits to UK music festivals was estimated to exceed 7.7 million attendees, including overseas and domestic participants combined (www.ukmusic.org/assets/media/UK%20Music%20 -Music%20Tourism.pdf). Music festivals, which may involve as many as 400,000 cumulated attendees, share the usual health risks associated with large MGs, including communicable and non-communicable diseases [6-8]. A mean percentage of 1.5% attendees at music festivals seek medical care during these events, and the highest proportion recorded of attendees seeking care was 10% [9-12]. This situation may have an impact on local healthcare facilities, especially

TABLE 1

Top 20 international music festivals by estimated attendance per day, 2012

Festival	Location	Duration (days)	Estimated attendance per day
Glastonburry	Worthy Farm, Pilton, Shepton Mallet, Somerset, United Kingdom	5	175,000
Roskilde	Roskilde, Denmark	4	110,000
Rock Werchter	Werchter, Belgium	4	110,000
Rock al Parque	Bogota, Colombia	4	88,600
T in the Park	Balado, Kinross-Shire, Scotland	3	85,000
Summerfest	Henry Maier Festival Park, Milwaukee, Wisconsin, United States	11	82,000
Exit	Petrovaradin Fortess, Novi Sad, Vojvodina, Serbia	4	75,000
Coachella	Coachella, California, United States	3	75,000
Reading-Leads Festival	Reading, Leads, United Kingdom	3	75,000
Sziget	Budapest, Hungary	6	65,000
Pukkelpop	Kiweit-Hasselt, Belgium	3	62,500
Pinkpop	Landgraaf, Netherlands	3	60,000
Big Day Out	Gold Coast, Sydney, Melbourne, Adelaide, Perth, Australia and Auckland, New Zealand	11	56,000
Burning Man	Black Rock Desert, Nevada, United States	7	50,000
Bonnaroo	Great Stage Park, Manchester, Tennessee, United States	4	40,000
Fuji Rock	Naebi Ski Resort, Japan	3	40,000
Hurricane	Scheeßel, Lower Saxony, Germany	3	40,000
Downlaod	Donington Park, United Kingdom	3	37,000
Benicassim	Benicassim, on the coast between Valencia and Barcelona, Spain	4	32,000
Wireless	Hyde Park, London, United Kingdom	3	17,500

Sources: http://www.wikifestivals.com/wiki/list-international-music-festivals-attendance, http://www.cnbc.com/id/42150834/The_Worlds_s_Biggest_Music_Festivals, http://en.wikipedia.org/wiki/Music_festivals.

in terms of the workload on local hospitals, as found in Punchestone Racecourse, Ireland in 2004 [13]. During the Oxegen festival, which had 80,000 attendees over three days, a 45% increase in admissions to the emergency department (mainly for trauma) was observed at Nass General Hospital, with 51% of these admissions treated as inpatients. Interestingly, 47% of these patients had consumed alcohol and/or drugs [13].

A comprehensive review on outbreaks in relation to large scale open air festivals is missing. The objective of this report is to summarise the evidence related to the substantial challenges posed by communicable diseases to the organisers of large scale open air festivals, including music festivals, and outline details of infections resulting from faecal-oral, respiratory and sexual transmission. Opportunities to control these outbreaks are discussed.

Methods

Definition of large scale open air festivals

Outbreaks in the setting of an open air festival, including a music festival or other art festival, village festival, cultural festival, university, religious events and large weddings were included.

Case reports and human/non-human experimental laboratory studies were excluded from the review. Outbreaks in relation with sport events, Hajj pilgrimage, food festivals, fairs or occurring in the setting of cruise ship, school, restaurant or hotel (including weddings at restaurants or hotels) were excluded because they were reviewed elsewhere [6-8].

Search strategy

To retrieve information on the transmission of infectious diseases and outbreaks during large scale open

air festivals, we first conducted a literature search using the MEDLINE database (www.ncbi.nlm.nih.gov/ pubmed), from 1980 to July 2012, cross-referencing the following terms: 'mass gatherings', 'festivals' or 'music festivals' and 'infection' or 'infectious diseases' or 'outbreak'. Only studies published in English, but one in Serbian, were included in this review. Relevant systematic and narrative reviews were also utilised for useful background information. Subsequently, the reference lists of the systematic reviews and other identified papers were scanned for potentially relevant primary studies that could be considered for inclusion in the review. Additional search was conducted using ProMED-mail (www.promedmail.org) cross-referencing the following terms: 'festivals' or 'music' and through Google (www.google.fr) and Yahoo (fr.yahoo.com) general search-engines cross-referencing the terms: 'festivals' or 'music festivals' and 'infectious' or 'outbreak'.

Results

Using our search strategy, 107 articles were retrieved through MEDLINE and scanning of reference lists, and 23 of these were relevant to our subject from a review of titles, abstracts, and full text of the articles obtained. Pro-MED mail search allowed identifying only three relevant outbreaks that were already retrieved through MEDLINE. Google and Yahoo search retrieved respectively 31,900,000 and 12,400,000 hits, using 'festival' and 'outbreaks' terms; 6,670,000 and 11,300,000 hits, using 'music festival' and 'outbreaks' terms and 558,000 and 200,000 hits, using 'music festival' and 'infectious' terms. Given the poor relevance of search results obtained through Google and Yahoo, no further analysis was conducted using these two search-engines.

Outbreaks and infectious diseases occurring during large scale open air festivals were classified into three categories: (i) faecal-oral transmission and gastrointestinal diseases, (ii) respiratory transmission and respiratory infections and (iii) blood-borne and sexually transmitted diseases.

Faecal-oral transmission and gastrointestinal diseases

A total of 10 outbreaks of gastrointestinal infections associated with faecal-oral transmission in the context of large scale open air festivals were retrieved and are summarised in Table 2. Of these five were linked to music festivals. Outbreaks of *Cryptosporium parvum*, *Campylobacter, Escherichia coli, Salmonella enterica, Shigella sonnei, Staphylococcus aureus*, hepatitis A virus and norovirus infections have been described over the last two decades in the United States (US), Canada, Europe and Japan and have been as well associated with events including 350 attendees, as with open air festivals involving as many as 80,000 participants. In some outbreaks, the attack rate was close to 50% of attendees, such as in Shigella outbreaks [14,15] or gastroenteritis outbreaks due to norovirus [16]. Failures of hand-washing hygiene among food handlers suspected to be infected by the infectious agent lead to the contamination of food and were an important route of transmission for gastrointestinal diseases at large scale open air festivals [14,17-19]. A lack of respect for food hygiene rules has also been observed during such festivals [17,18,20]. Factors that contribute to gastrointestinal outbreaks at these festivals include (i) excessive production of food beyond the safe food production capacity [17], (ii) preparation of meals by a large number of volunteer food handlers [14,18], and (iii) the sale of food prepared under unsanitary conditions, sometimes by vendors without a license [19-21]. Moreover, during a lunchtime concert in Cardiff, the most likely cause of the outbreak of norovirus infection was vomit contaminating inadequately cleaned and disinfected hard surfaces, carpets and soft furnishings [16].

Another important factor associated with the risk of gastrointestinal infections is the lack of adequate sanitation. Inadequate sanitation was the cause of the contamination of drinking water during the annual meeting of the Rainbow Family in the US in 1987, resulting in a large outbreak of shigellosis [15]. Inadequate sanitation associated with limited access to running water for hand washing has also been reported as the source of gastrointestinal outbreaks [14].

Unusual transmissions of gastrointestinal diseases have also occurred during large scale open air festivals. An outbreak of Escherichia coli O157 was reported during the Glastonbury music festival in England and was linked to mud contaminated by infected cattle. Heavy rain had turned the site into a quagmire, and attendees had high levels of contaminated mud on their hands and faces [22]. Additionally, a swimming pool at an accommodation during a dance festival in Canada was identified as the source of an outbreak of diarrhoea due to *Cryptosporidium parvum* [23].

Overall, the estimated incidence of gastrointestinal diseases per 100,000 attendees ranged from nine to more than 55,000 during the outbreaks included in this review.

Respiratory transmission and respiratory infections

Infections acquired by respiratory routes reported in the literature in association with nine large scale open air festivals are described in Table 3. Of these, three were music festivals.

Influenza outbreaks have been reported at music festivals [24-27], with the potential for the spread of new influenza viruses in some cases [27,28]. In Serbia in 2009, 40% of the pandemic influenza A(H1N1)pdm09 cases were linked to the Exit festival [26]. Interestingly, during 2009, influenza A(H1N1)pdm09 outbreaks were recorded at three of the top six music festivals (>100,000 cumulated attendees) in Europe (Rock **TABLE 2**Outbreaks of gastrointestinal diseases associated with large scale open air festivals, 1987–2008

Favouring factors	Inadequate sanitation	Limited access to soap and running water for hand washing and large number of non- professional food handlers	Lack of respect for food hygiene rules	Grazing of cattle on site two days before the event and heavy rain	Vomit inappropriately cleaned	Swimming pool in one accommodation	Exceeded food production capacity	Infected food handler	Unknown	Lack of respect for food hygiene rules
Mode of transmission	Food-borne, water-borne, person-to-person	Food-borne and person-to-person	Food-borne	Direct contact	Direct contact	Faecal-oral	Food-borne	Food-borne	Person- to-person, food-borne	Food-borne
Source of outbreak	Inadequate sanitation	Uncooked tofu salad	Unpasteurised milk	Mud (contaminated by cattle faeces)	Vomits inappropriately cleaned	Swimming pool	Pastry with vanilla cream	Coleslaw	Unknown	Crepes
Indication of patient age	Mean patient age: 27 years	Mean patient age: 31 years	Age range of the majority of patients: 20–30 years	Mean patient age: 21 years	Primary school students	Mean patient age: 23 years	Age range of 24% of patients: 1–24 years; of 35%: 25–44 years	Median patient age: 16 years	Median patient age: 23 years	ND
Estimated incidence per 100,000 attendees	55,118	45,244	103	6	24,410	13,111	QN	6,000	31-2,083	150
Case numbers	Estimated >7,000; nationwide dissemination (3 States)	3.175 primary cases (278 ill staff and 2,897 ill attendees); 182 secondary cases; 117 hospitalisations	72	7	٥٥٤	59 (2 hospitalisations)	1,435 (117 hospitalisations)	21	25	75 (staff and attendees)
Pathogen involved	Shigella sonnei	Shigella sonnei	Campylobacter	Escherichia coli 0157	Norovirus	Cryptosporidium parvum	Salmonella enterica	Hepatitis A virus	Hepatitis A virus	Staphylococcus aureus
Size (cumulated number of attendees) and type of attendees ^a	12,700	6,403 women	70,000	80,000	1,229 children	430 dancers, >450 spectators	QN	350	1,200-82,000	50,000
Year	1987	1988	1992	1997	1999	2001	2002	2003	2003	2008
Place	Nantahala National Forest, United States	Michigan, United States	South west England	South west England	Cardiff, England	Dauphin, Manitoba, Canada	Catalonia, Spain	Australia	United States (9 States)	Japan
Event	Annual meeting Rainbow Family	Michigan Women's Music Festival	Glastonbury music festival	Glastonbury music festival	Lunchtime concert	Ukrainian Dance Festival	San Juan Festival	Youth camp	Outdoor concerts 'jam bands'	Nagoya University Festival
Reference	[15]	[14]	[20]	[22]	[16]	[23]	[17]	[19]	[21]	[18]

ND: not determined. ^a When attendees were of a certain type.

TABLE 3

Outbreaks of respiratory transmissions and infections associated with large scale open air festivals, 2006-2010

Reference	Event	Place	Year	Size (cumulated number of attendees)	Outbreak type	Number of cases and description	Estimated incidence per 100,000 attendees	Age of patients as specified	Favouring factors/ consequence
[31]	Easter Youth Festival	Carinthia, Austria	2006	ND	Mumps virus	214 cases (143 confirmed, 71 probable)	QN	Age range of 80% of patients: 16–30 years	Occurrence in unvaccinated people
[32]	Annual village festival	Navarra, Spain	2006	> 4,500	Mumps virus	19 primary cases; 58 secondary cases	422	Age range: 18–37 years	Failure in immunisation by Rubini strain
[28]	World Youth Day (religious meeting)	Sydney, Australia	2008	500,000	Numerous influenza virus strains (A and B)	100 confirmed cases	20	Median age: 21 years	Community strains from Australia that were rarely isolated before World Youth Day 2008 were frequently identified among pilgrims; novel influenza viruses were introduced
[27]	Iztapalapa Passion Play (religious meeting)	Iztapalapa, Mexico	2009	2 million	Influenza A(H1N1) pdmog virus	38 cases; of 202 cases of influenza A(H1N1) pdmo9 in Mexico from 1 to 5 May 2009, 38 originated from the Iztapalapa Passion Play meeting	N	Age range of 32% of patients: 5–19 years; of 28%: 20–29 years	Local and national spread, start of the pandemic influenza A(H1N1)pdmog
[24]	Rock Werchter (music festival)	Belgium	2009	113,000	Influenza A(H1N1) pdmo9 virus	14 confirmed cases; 30 ILI cases	12	Mean age: 23 years	International spread
[25]	Sziget Festival (music festival)	Budapest, Hungary	2009	390,000	Influenza A(H1N1) pdmog virus	8 confirmed cases; 14 ILI cases	N	Mean age: 21 years (personal communication, Dr Peter Felkai, January 2010)	International spread
[26]	Exit Festival (music festival)	Novi Sad, Serbia	2009	165,000	Influenza A(H1N1) pdmog virus	49 confirmed cases	30	Age range of 42% of patients: 20–29 years	During 2009, 40% of influenza A(H1N1) pdmo9 cases in Serbia were linked to this festival
[29]	Taizé (religious meeting)	France	2010	3,500	Measles virus D4-Manchester	13 primary cases; 17 secondary cases; 7 tertiary cases	371	Median age for primary cases: 17 years; for secondary cases: 15 years; for tertiary cases: 13 years	Low vaccine coverage
[30]	Wedding	Andalucia, Spain	2010	ND	Measles virus	25 primary cases; 58 secondary cases	QN	97% of patients >16 years-old	Low vaccine coverage

ILI: Influenza-like illness; ND: not determined.

Werchter, Belgium; Sziget Festival, Hungary; and Exit Festival, Serbia).

In 2010, 13 primary measles cases were identified among unvaccinated persons aged between nine and 32 years-old in 11 districts in Germany. All cases had attended religious meetings in Taizé, France [29]. This outbreak illustrates the risk of long distance spread of infectious diseases associated with international large scale open air festivals. The same year, 25 primary cases of measles had been reported from Granada, southern Spain, of whom 22 were unvaccinated children under the age of 15 years [30]. This outbreak involved a subpopulation with low vaccination coverage and parents with ideological objections to vaccination participating to a large wedding reception [30]. Secondary cases were documented in both outbreaks.

An outbreak of mumps occurred in Austria in 2006 involving 214 individuals. Nearly half of the cases for whom vaccination status was known occurred in nonvaccinated persons, another 40% were vaccinated with one dose of vaccine. The majority of cases (80%) occurred in persons between 16 and 30 years of age with a peak in the age group of 21 to 25 years (42%). Considering the minimum incubation period of 10 to 14 days, the mumps outbreak probably originated with virus transmissions to susceptible individuals at a village Easter festival [31]. In 2006 too, a mumps outbreak including 19 primary cases was recorded in a village in Spain. Patients' ages ranged between 18 and 37 years and 94% of the patients reported attending the annual festival held in the village. 58 secondary cases were reported [32].

Overall, the estimated incidence of confirmed respiratory infections per 100,000 attendees ranged from two to more than 420 during the outbreaks included in this review.

Sexual and blood transmission of infectious diseases

During music festivals, the risk of transmission of sexual or blood-borne infections is considered important, but this consideration is mainly speculative. Indeed, this risk is difficult to assess. Some of these infections have long incubation periods, making it difficult to relate the infection to the event. Despite the lack of evidence for the transmission of such diseases at festivals, the consumption of drugs and alcohol during music festivals is known to be high and may lead to at-risk behaviours, particularly unprotected sexual behaviours [5,33,34]. To our knowledge, the sexualrisk behaviour of participants during music festivals has been evaluated at several times only during the Big Day Out, one of the biggest music festivals in Australia [33,35]. Among sexually active participants, 43% reported not using a condom because of alcohol use [33]. Knowledge of STIs was poor overall [33]. In this young population (89% in the age group 16-24 years), surveillance of STI risk behaviour between 2005 and

2008 indicated that reporting having had a recent STI test increased from 23% in 2006 to 32% in 2008 [35]. Attempts to test for Chlamydia infection during the Big Day Out in 2009 has not been effective, with only 21% of participants returning the test and only one diagnosed case of Chlamydia infection [36].

Discussion

We describe various outbreaks related to large scale open air festivals, highlighting the fact that at these gatherings of people where the majority of attendees are aged 15 to 30 years, transmission of infections occurs at local, national [15,17,21] and international levels [24,25,29], as found during larger MGs [8]. Within open air festivals, music festivals contributed a large proportion of the outbreaks.

The outbreaks published in MEDLINE and ProMED are probably a tiny and heavily biased subset of all outbreaks associated with large scale open air festivals. This constitutes a limitation of our study and may explain the geographical repartition of the outbreaks included in our review. Unfortunately, general searchengines like Google and Yahoo are not sufficiently accurate tools to further identify outbreaks. Even the US Centers for Disease Control and Prevention foodborne outbreak online database (wwwn.cdc.gov/ foodborneoutbreaks/Default.aspx) does not allow a distinction between festivals, fairs, and other temporary or mobile services.

During large scale open air festivals, respiratory infections can easily be transmitted due to overcrowded conditions which may contribute to the spread of new influenza viruses, as reported during the 2009 pandemic [25,26]. The risk of transmission and of the introduction of new strains of viruses in countries hosting MGs or large scale open air festivals has been documented at such events [8,27,28]. Therefore, risk assessment and the establishment of preventive strategies should be implemented in preparation for large scale open air festivals, as for other MGs. Measles is one of the most contagious human diseases, with a basic reproduction number ranging from 7.7 to 15 in a susceptible population [37]. Increased numbers of cases have recently been observed in young adults, notably in Europe [38]. Therefore, it is not surprising that measles outbreaks have occurred during youth festivals [29,30]. Interestingly, although immunisation programmes are required for certain MGs, such as the Hajj [8], there is no immunisation recommendation for large scale open air festivals.

Outbreaks of vaccine-preventable diseases, including hepatitis A, influenza, measles and mumps, have been reported in connection with large scale open air festivals [19,21,24,25,29-32] and could have been prevented by adequate vaccination coverage in the populations taking part. Although meningitis outbreaks have not been associated with large scale open air festivals, they have been described in the context of the Hajj [8]. Vaccination against meningococcus may be considered for young people planning to participate in a large scale open air festival. The use of drugs and alcohol is common at large open air festivals and may theoretically increase the risk of transmission of STIs and blood-borne diseases [5,13,33].

A high incidence of Shigella, norovirus, Cryptosporidium parvum and hepatitis A virus infections was found among attendees of large scale open air festivals where outbreaks were reported compared to their respective incidence in the general population, suggesting that increased transmission of gastrointestinal infections may also occur in these settings. In contrast, no evidence for substantial increased risk of outbreaks of gastrointestinal infectious diseases was found during large international sport events notably due to proper sanitary supervision of food preparation and of water sources in these events [39]. Among previous outbreaks of gastrointestinal diseases reported at large scale open air festivals, the role of inadequate sanitation has been highlighted [14,15]. Good sanitation is therefore critical even at the smallest gatherings, where the organisation is often non-professional. Respect for hygiene rules for food handling is also critical to avoid food-borne disease transmission. In particular, the role of volunteers in food preparation should be examined. They constitute a potential risk because they are not trained in the necessary hygiene measures [14].

The discrepancy between sport events and large scale open air festivals in terms of infectious diseases may also be the consequence of the relatively short duration of sport events which frequently last shorter than one day, with many participants moving to other locations at the end of the event and who do not live on site [39]. In contrast, large scale open air festivals last several days with most attendees staying on site during the event, sharing accommodations and sanitations. In an old study conducted in 1972, the overall incidence of communicable diseases was 75 per 100,000 attendees during a 2.5 day music festival and 580 per 100,000 during a seven day-long music festival, suggesting that the length of exposure is a contributing factor to transmission of infectious diseases in the specific setting [10].

To prevent infectious diseases, communication and on-site advice about infectious risks should be implemented and hand-washing hygiene, cough etiquette and condom use should be promoted. Reminding people before festivals to check whether they have been immunised for certain vaccine preventable diseases including measles, mumps and influenza should be promoted. To prevent STIs and blood-borne diseases, campaigns involving the free distribution of condoms and syringes for intravenous drug users may be proposed. Finally, surveillance after large scale open air festivals with participant recall may be implemented to better address infectious disease threats of international concern at the earliest possible stages. Social networks such as Twitter or Facebook can assist with active surveillance (e.g. public health outreach on Twitter or Facebook for people reporting health related issues in the host city during a festival and for people participating in a festival). Social media can function also as a method of passive surveillance (e.g. analysis of geographically tagged tweets). Mobile phones, particularly mobile internet use, facilitate the availability of real-time information at any time and nearly anywhere in the world to the general public and may be useful in this context [4]. Surveillance could also be operated through specialised networks reporting on travelassociated diseases, including GeoSentinel [40] and EuroTravnet [41]; however participation to large scale open air festival and MGs is not documented specifically at the moment in these databases.

Conclusions

Infections related to large scale open air festivals may be under-reported given the important number of these festivals worldwide. Alternatively these could also possibly be overestimated due to 'common sense' assumptions about conditions at festivals. We assume that most outbreaks probably remain unnoticed and underreported. The relationship between infections and festivals may be difficult to establish, especially for diseases with long-incubation periods and international events, as the participants spread worldwide. Cases could have been classified under 'travel-related' infections since participation to festivals or other MGs is usually not documented in surveillance networks records. We cannot give an estimate of the frequency of communicable diseases during large scale open air festivals, because of the relative paucity of published data. The creation of an online event and patient registry should overcome this gap [42].

During an event, syndromic surveillance associated with the use of adequate laboratory facilities may help to recognise the first cases of an outbreak although the value of syndromic surveillance during MGs is highly debated [43].

Preventive measures including immunisation of people participating in large scale open air festivals and targeted on-site advice about food hygiene, hand hygiene, cough etiquette and the use of condoms may be considered. However, the effectiveness of such preventive measures in the context of MGs has not been established and research is needed before this could be recommended [44]. Finally, strict adherence to food safety protocols and adequate sanitation should be promoted to prevent gastrointestinal diseases. The presence of on-site medical staff has proven to be cost-effective in improving the medical management of injuries and other health issues, such as infections [45].

Conflict of interest

The authors have no conflict of interest.

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