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Increase in malaria cases imported from Pakistan to Germany in 2012

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A significant increase of malaria cases imported to Germany from Pakistan was observed in 2012. As of 14 November, Pakistan was the country of infection in 32 out of 434 malaria cases in 2012, compared to zero to eight annual malaria cases (out of over 500 cases) in previous years. Physicians and public health authorities should consider malaria in febrile patients returning or migrating from Pakistan.

In the second half of 2012, there was a significant increase in malaria cases imported from Pakistan among cases of malaria reported in Germany.

Malaria is a major travel-associated disease, and is mandatorily notifiable in Germany [1]. In national surveillance, in addition to the laboratory diagnosis (*Plasmodium* species) information is routinely available for the majority of cases on the country of infection and on several demographic variables such as age, sex, country of origin, the individual's status in Germany (e.g. migrant, employee), chemoprophylaxis. Since 2005, between 526 and 633 imported cases of malaria were annually reported to the Robert Koch Institute (RKI, German National Public Health Institute) (2011: 562 cases) [2,3]. In the vast majority of these cases, *Plasmodium* infection had been acquired in African countries (2011: 89%).

As of 14 November 2012, 32 cases of malaria imported from Pakistan were reported to RKI (out of a total number of 434 cases). Case notifications peaked in late summer with 10 cases reported in July, six in August and eight in September. This contrasts with previous years (2001–2011) where between zero and eight cases (median five cases) had been imported from Pakistan annually. Interestingly, over the same period, the number of malaria cases imported from India has remained stable. As of 14 November 2012, 13 malaria cases were reported from India and from 2001 to 2011 between six and 17 cases (median 10 cases).

Of the 32 cases reported in 2012 so far, 30 were persons originating from Pakistan (23 migrants/asylum seekers/refugees, three employees, two students, two persons with unknown status). The majority of them

have been living in Germany since the end of 2011 or the beginning of 2012. For five persons originating from Pakistan it was reported that they had travelled to Pakistan and stayed there between four and eight weeks. Cases were predominantly male (24 males, seven females, one case without information on sex), and 21 were aged between 20 and 39 years (range 9 to 73 years). In the majority of cases (n=23) *Plasmodium vivax* was detected. Of the remaining nine cases, three were classified as Malaria tertiana (i.e. classical microscopic characteristics for *P. vivax*/*P. ovale* but no further differentiation between these two *Plasmodium* species reported), two were notified as *P. falciparum* infections, two as mixed infections, and two cases were reported with *Plasmodium* detection but no further specification of the species was provided. None of the malaria patients had taken chemoprophylaxis.

Discussion and conclusion

The increase in malaria cases from Pakistan seen in Germany in 2012 may reflect changing patterns of malaria risk in the country, or a growing number of migrants to the country from Pakistan. In Pakistan, there is a countrywide malaria risk in areas below 2,000 m altitude, and about 70% of the malaria incidence is due to *P. vivax* [4]. According to the World Health Organization, malaria has re-emerged in recent years as a major cause of morbidity in Pakistan. The disease mainly affects the districts with suboptimal healthcare service delivery along the international borders with Afghanistan and Iran. Mass population movements, low immune status of the population, climatic changes, poor socioeconomic conditions, declining health infrastructure, and mounting drug and insecticide resistance in parasites and vectors, all contribute to the huge disease burden [5].

It would be interesting to know if other countries in Europe have been observing a similar increase in malaria cases from Pakistan in 2012. Pakistan is not a common destination for tourists or professionals from European countries. However, persons of Pakistani origin may travel back and forth (e.g. to visit relatives and friends), and soldiers from non-endemic countries on mission in Afghanistan may also be exposed to malaria

while travelling through Pakistan. Therefore, our findings are relevant to travel medicine. Persons travelling to Pakistan should be counselled on the potential risk of acquiring malaria and on adequate prevention measures.

In Greece, in the context of the autochthonous malaria outbreaks in summer 2011 in the Evrotas region, *P. vivax* infection had been diagnosed in a number of migrant workers mainly from Pakistan. Their malaria importation status remained unclear but it is possible that they had acquired the infection already in the country of origin [6,7]. In the 2012 autochthonous outbreak in Greece also a high proportion of cases concerned migrants from malaria-endemic countries [8].

Physicians and public health experts in non-endemic countries should be aware of the possibility of malaria in patients with fever returning or originating from countries such as Pakistan where malaria has re-emerged in recent years but which are often not considered as classical malaria endemic countries. In such patients timely testing for malaria is necessary because it facilitates timely and adequate treatment. This is of high benefit to the individuals affected by malaria and it could also reduce the risk of local outbreaks in areas where vector abundance and climatic conditions may make transmission possible.

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Malaria in Greece, 1975 to 2010

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Malaria, which was endemic in Greece in the past, was officially eliminated in 1974. Since that time and up to 2010, a number of imported cases (ranging from 19 to 76) have been annually reported. The total number of reported laboratory-confirmed cases between 1975 and 2010 was 1,419. *Plasmodium falciparum* was identified in 628 (44%) of these cases, while *P. vivax* was found in 524 (37%). Of the total cases, 1,123 (79%) were male (ratio males vs. females: 3.78). Age was only available for 490 cases, of which 352 (72%) belonged to the 18–40 year-age group. Of the 382 malaria cases reported from 1999 to 2010 for which the region/country of acquisition was known, 210 (55%) were from Africa and 142 (37%) from Asia. The massive introduction of economic migrants, in the period from 1990 to 1991 and from 2006 onwards, mainly from countries where malaria is endemic, resulted in the appearance of introduced sporadic cases. In Peloponnese, Central and East Macedonia, Thrace and East Attica, mosquitoes of the genus *Anopheles* (e.g. *Anopheles sacharovi*, *A. superpictus* and *A. maculipennis*) that can act as plasmodia vectors are abundant and during the summer of 2011, 27 *P. vivax* cases were reported in Greek citizens residing in the agricultural area of Evrotas in Lakonia and without travel history. As further *P. vivax* malaria cases occurred in the Lakonia and East Attica areas in 2012, it is becoming urgent to strengthen surveillance and perform integrated mosquito control that will help eliminate the potential risk of malaria reintroduction and reestablishment.

Introduction

Almost a century has passed, since Ronald Ross [1] and other scientists [2] combined their efforts to eliminate malaria in Greece. From 1905 to 1940, apart from the mountainous areas and the big urban centres, malaria was present in mainland Greece, and on the island of Crete [2] and had become a serious problem up to 1937 with about one to two million cases occurring annually (1 million from 1905–21 and 1–2 million from 1921–37). This had remarkable socioeconomic consequences [2,3]. In 1958 and 1959 malaria control centres were organised and operated in several parts of the country, such as Skala in the Evrotas, the Lakonia

region, Lamia in Fthiotida and Provatas in the Serres region in Macedonia. The national malaria control and elimination programmes that were applied especially from 1946 to 1960 resulted in the official recognition of Greece as a malaria-free country in 1974 [4,5].

Malaria is notifiable in Greece since the 1930s [6] and from that time malaria cases have been diagnosed microscopically by local laboratories, by detecting *Plasmodium* in the blood. Since the 1960s, blood samples of all suspected cases or cases characterised as positive are also sent to the Malaria Reference Laboratory (MRL) of the National School of Public Health (NSPH) for verification, where thick and thin blood smears are examined. Since 1999, blood samples of suspected positive cases, are also routinely screened by a multiplex polymerase chain reaction (PCR) specific for *P. falciparum* and *P. vivax* [7]. It is always attempted to test the initial sample that triggered suspicion of malaria infection.

After malaria elimination, a surveillance system of laboratory-confirmed malaria cases was established by the Public Health Division of the Greek Ministry of Health (MoH). From 1974 to 1997, all laboratory-confirmed cases were reported to MoH and related files kept in the ministry's archives. In 1995, however, the responsibility of data collection was officially transferred to the Hellenic Centre for Disease Control and Prevention (HCPCP), but MoH nevertheless continued recording malaria cases in 1995 and 1996. During these two years, MRL cooperated with MoH and HCDCP and contributed to cross-check the existing data. Since 1997 to date, all laboratory-confirmed malaria cases are reported to HCDCP where data is maintained in an electronic database.

Surveillance is based on mandatory notification of confirmed cases and passive case detection is performed through hospitals after *Plasmodium* detection in the hospital laboratories. Doctors in private practice or from non-governmental organisations refer patients to the MRL for laboratory confirmation and subsequently report positive cases to the surveillance authority

(MoH before 1997 or HCDCP after 1997). Since HCDCP has taken over the collection of data on confirmed malaria cases, active surveillance additionally occurs and on a weekly basis, HCDCP contacts local hospital laboratories to verify that the database is completely updated. In areas of Greece where possible domestic transmission is suspected, enhanced surveillance is implemented by tracing reported cases, visiting homes and performing personal or telephone interviews.

Following a short transition period after malaria elimination, malaria in Greece was limited to imported cases, with rare sporadic cases not related to travel [8,9]. *Anopheles* mosquitoes, which are potential *Plasmodium* vectors, nevertheless persisted in the country. *Anopheles sacharovi*, *A. superpictus*, and members of *A. maculipennis* complex are among the species that have been identified in Greece [10-14]. Entomological surveillance was performed occasionally in several parts of the country, however not on a regular basis.

In 2011, 27 autochthonous cases of malaria were detected in Evrotas, Lakonia [15]. Since then, an active surveillance system has been implemented in areas where malaria cases are reported in order to increase awareness among residents as well as to conclude on the control measures that should be taken and where.

The aim of this paper is to present a comprehensive report of malaria cases in Greece from 1975 to 2010, prior to the occurrence of the 2011 autochthonous cases [15].

Methods

Data on malaria laboratory-confirmed cases were collected for the period from 1975 to 2010. For the whole period, information on cases occurring via blood transfusion as well as on fatal cases was available from the MoH or HCDCP records. Information on confirmed cases' original clinical records and treatments, kept in the local hospitals, was also provided to MoH and then to HCDCP. It should be noted that during the 1975 to 1997 period, when cases were notified to MoH, data on confirmed malaria cases also included information about the prefectural region where a case was recorded, sex, *Plasmodium* species and the type of infection (introduced, autochthonous, induced, relapse) [16]. During the period from 1975 to 1997 the age of the patient, profession, hospital, place of residence and travel information were only available for autochthonous or hospitalised cases.

In order to characterise a malaria case as introduced, the World Health Organization (WHO) guidelines were followed. The exact definition refers to a case that comes from transmission from imported case in an area where malaria does not occur regularly. According to WHO, 'a case in which it can be proved that the infection is a first step (first generation) of local transmission subsequent to a proved imported case i.e. in

which the mosquito was infected from an imported case' [16].

From 1997 onwards, when HCDCP took over the surveillance and data collection of laboratory-confirmed malaria cases, additional information was recorded such as travel history, country of origin, and visiting friends and relatives. Age information is routinely recorded since 2002.

Regarding the location of acquisition, data is provided only for the period starting from 1999 when the cooperation of MRL with Roll Back Malaria Office became more intense. HCDCP since 1999, interviews all patients characterised as malaria cases to collect and confirm information.

P. falciparum malaria cases were classified and recorded as severe if they presented with anaemia, acute respiratory distress syndrome with metabolic acidosis, shock, abnormal bleeding and/or disseminated intravascular coagulation, jaundice, parasitaemia >5% or cerebral malaria [17].

Results

From 1975 to 2010, a total of 1,419 laboratory-confirmed malaria cases were diagnosed by MRL and initially reported to MoH and, after 1997, to the HCDCP. The mean annual number of reported cases was 39.4, ranging from 16 (in 1997) to 79 cases (in 1978 and 1982). From 1975 to 1991, the mean annual number of cases was 49.8 (range: 28 in 1990 to 79 in 1978), but decreased to 27.8 in the 1992 to 2008 period (range: 16 in 1997 to 43 in 2003). During 2009 and 2010, a new increase in the total number of malaria cases was recorded (in 2009 the total annual number was 51 and in 2010 the total annual number was 48). All patients received appropriate anti-malarial treatment. Depending on the *Plasmodium* species being recognised and the patient's clinical status, quinine, mefloquine, chloroquine and primaquine were the anti-malarials of choice. In two severe malaria cases, artesunate was used.

One fatal case was recorded in 2003, in a man from Albania in his thirties, who had travelled to Cameroon for occupational reasons. Three days after his return to Greece, he developed malaise, chills and headache that lasted two days. On the sixth day he developed high fever and presented to his general practitioner who noticed altered mental status and he was admitted to hospital the same day. The diagnosis of malaria was made on the second day of his hospitalisation. Examination of peripheral blood showed mixed infection by *P. falciparum* and *P. vivax*. After his admission to the hospital, he showed pronounced anaemia, impaired renal function and developed acute respiratory distress syndrome. Treatment started immediately after diagnosis with intravenous quinine and doxycycline. His condition deteriorated and five days after his admission to the hospital, he died.

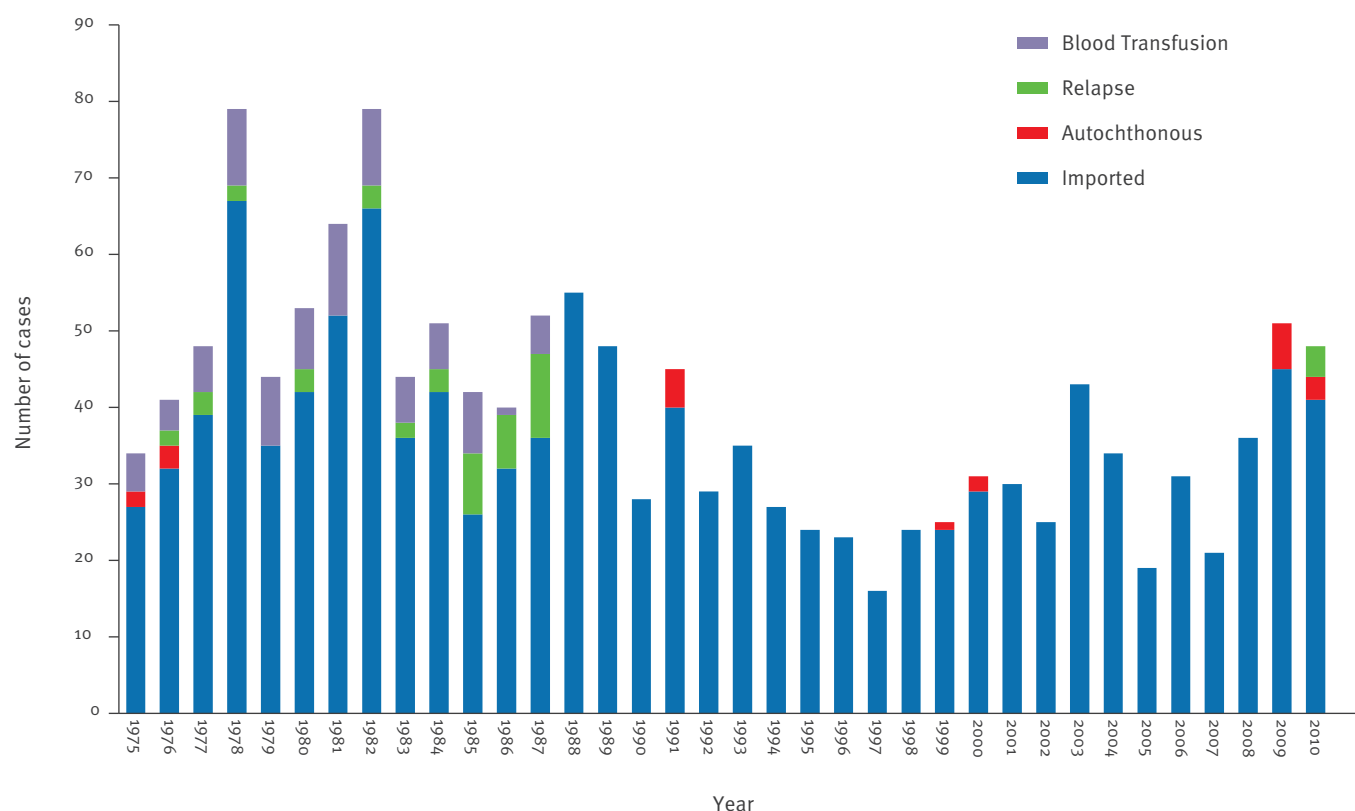
TABLE

Number of malaria cases by *Plasmodium* species, Greece, 1975–2010 (n=1,419)

Year	<i>Plasmodium vivax</i> n (%)	<i>P. falciparum</i> n (%)	<i>P. malariae</i> n (%)	<i>P. ovale</i> n (%)	<i>P. vivax, P. malariae</i> n (%)	<i>P. vivax, P. falciparum</i> n (%)	Not identified n (%)	Total n
1975	14 (41)	13 (15)	5 (6)	0 (0)	0 (0)	0 (0)	2 (0)	34
1976	19 (46)	11 (27)	9 (22)	0 (0)	0 (0)	0 (0)	2 (5)	41
1977	18 (37)	16 (33)	6 (17)	0 (0)	0 (0)	0 (0)	8 (13)	48
1978	32 (40)	32 (41)	11 (14)	0 (0)	0 (0)	0 (0)	4 (5)	79
1979	16 (36)	15 (34)	10 (23)	0 (0)	0 (0)	0 (0)	3 (7)	44
1980	22 (41)	19 (36)	10 (19)	0 (0)	0 (0)	0 (0)	2 (4)	53
1981	22 (42)	27 (34)	14 (22)	0 (0)	0 (0)	0 (0)	1 (2)	64
1982	30 (41)	32 (38)	16 (20)	0 (0)	0 (0)	0 (0)	1 (1)	79
1983	16 (39)	17 (36)	10 (23)	0 (0)	0 (0)	0 (0)	1 (2)	44
1984	21 (47)	24 (41)	6 (12)	0 (0)	0 (0)	0 (0)	0 (0)	51
1985	13 (38)	16 (31)	11 (26)	0 (0)	0 (0)	0 (0)	2 (5)	42
1986	16 (57)	23 (40)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4)	40
1987	14 (57)	30 (27)	5 (10)	0 (0)	0 (0)	0 (0)	3 (6)	52
1988	10 (66)	36 (18)	5 (9)	0 (0)	0 (0)	0 (0)	4 (7)	55
1989	8 (17)	29 (60)	3 (6)	0 (0)	0 (0)	0 (0)	8 (17)	48
1990	8 (29)	16 (56)	1 (4)	0 (0)	0 (0)	0 (0)	3 (11)	28
1991	16 (36)	20 (44)	3 (7)	0 (0)	0 (0)	0 (0)	6 (13)	45
1992	7 (24)	9 (31)	2 (7)	0 (0)	0 (0)	0 (0)	11 (38)	29
1993	11 (55)	19 (31)	5 (14)	0 (0)	0 (0)	0 (0)	0 (0)	35
1994	8 (30)	10 (37)	3 (11)	0 (0)	0 (0)	0 (0)	6 (22)	27
1995	13 (54)	6 (25)	0 (0)	0 (0)	0 (0)	0 (0)	5 (21)	24
1996	9 (39)	8 (35)	0 (0)	0 (0)	0 (0)	0 (0)	6 (26)	23
1997	5 (31)	6 (38)	0 (0)	0 (0)	0 (0)	0 (0)	5 (31)	16
1998	9 (38)	10 (41)	1 (4)	1 (4)	0 (0)	0 (0)	3 (13)	24
1999	11 (44)	9 (36)	0 (0)	0 (0)	0 (0)	0 (0)	5 (20)	25
2000	10 (32)	14 (46)	0 (0)	1 (3)	0 (0)	0 (0)	6 (19)	31
2001	4 (13)	16 (53)	0 (0)	2 (7)	0 (0)	0 (0)	8 (27)	30
2002	7 (28)	14 (56)	0 (0)	1 (4)	1 (4)	1 (4)	1 (4)	25
2003	15 (35)	26 (61)	0 (0)	1 (2)	0 (0)	0 (0)	1 (2)	43
2004	14 (41)	19 (56)	1 (3)	0 (0)	0 (0)	0 (0)	0 (0)	34
2005	9 (48)	9 (47)	1 (5)	0 (0)	0 (0)	0 (0)	0 (0)	19
2006	9 (29)	20 (65)	1 (3)	1 (3)	0 (0)	0 (0)	0 (0)	31
2007	10 (47)	9 (43)	1 (5)	0 (0)	0 (0)	0 (0)	1 (5)	21
2008	27 (75)	8 (22)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3)	36
2009	23 (45)	24 (47)	1 (2)	0 (0)	0 (0)	2 (4)	1 (6)	51
2010	28 (55)	16 (31)	0 (0)	1 (2)	1 (2)	1 (2)	1 (2)	48
Total	524 (37)	628 (44)	141 (10)	8 (1)	2 (0)	4 (0)	112 (8)	1,419

FIGURE 1

Number of malaria cases according to the source of malaria infection, Greece, 1975–2010 (n=1,419)



To our knowledge, there were three severe cases of malaria in Greek citizens. These occurred in the period from 1992 to 2010, and concerned two adults who had travelled to north-east Asia and an 11 year-old child who had travelled to Tanzania. In all these cases, chemoprophylaxis was either incomplete or absent. The first two individuals sought medical assistance with delay, while the child received insufficient doses of the antimalarial drug.

Sex and age distribution

Of all 1,419 malaria cases, 1,123 (79%) were male and 296 (21%) female. Of the 490 cases for whom age was known, the age distribution was between six months and 89 years with a median age of 31 years. The 18 to 64 year age group comprised 416 cases (85%) and 352 cases (72%) were aged between 18 and 40 years. Ten cases (2%) were over 65 years-old, while 14 cases (3%) were less than five years-old, 16 (3%) were aged between five and 12 years and 34 (7%) were aged between 13 and 17 years giving a total of 64 cases (13%) in the category below and up to 17 years of age.

Plasmodia species

Most cases were infected with *P. falciparum* (n=628, 44%), 524 (37%) with *P. vivax*, and 141 (10%) with *P. malariae*, while only eight cases (<1%) were due to *P. ovale*. Four cases (<1%) presented with a mixed

P. falciparum/*P. vivax* infection, two (<1%) acquired a mixed *P. vivax*/*P. malariae* infection and in 112 cases (8%) the species was not identified. Mixed *P. falciparum* and *P. malariae* infections were never detected. The highest number of *P. falciparum* infections (n=36) was reported in 1988 and the lowest (n=6) in the years 1995 and 1997, whereas for *P. vivax* the highest number (n=32) was recorded in 1978 and the lowest (n=4) in 2001 (Table).

Source of malaria infection

Of all 1,419 reported malaria cases, 1,259 (89%) were classified as imported. Ninety cases (13%) of the 671 malaria cases during the period from 1975 to 1987 were due to blood transfusion, with the last one reported in 1987, according to MoH records. From 1975 to 2009, among a total 1,371 malaria cases, 44 cases (3%) of *P. vivax* relapse were found, while in 2010, four cases of relapse occurred among migrants who lived and worked for a few months in Greece. The latter cases reported previous episodes of malaria in the country of origin, thus suggesting, according to the official definition, that their infection was due to relapse [16]. The majority of cases reported from 1988 onwards were imported (731/748, 98%) and only 17 (2%) of the cases were not related to travel and possibly introduced (Figure 1).

Region of acquisition of infection

Of all 382 imported and relapse cases reported since 1999, 133 (35%) were in Greeks and 249 (65%) in migrants.

A total of 210 migrants from Africa as well as individuals travelling to and from Africa accounted for 55% of malaria cases (mainly from sub-Saharan countries of Central West Africa). In addition, 142 (37%) cases, including four cases of relapses, acquired their infection in Asia (mainly in Pakistan, India and Bangladesh). The World Health Organisation European Region accounted for five cases (1%) without further information on their travel history. According to their declaration, these cases were of European origin, specifically from eastern Europe, Russian Federation, Georgia and Kazakhstan. While from 1999 to 2005, the majority of imported cases originated from Africa, the pattern has changed since 2006, with cases originating from Asia accounting for the majority of imported cases (Figure 2).

Autochthonous cases

In 1975 and 1976, two and three cases without travel history to a malarious country, were reported respectively, but no other autochthonous cases were reported until 1991. In 1991, five introduced autochthonous cases were reported from the prefectural regions of East Attica and Viotia in central Greece (3 and 2 cases, respectively) (Figure 3), where a large population of non-documented migrants originating from malaria endemic countries worked seasonally. All cases were recorded between 26 of July and 27 of August 1991 and were hospitalised in Athens.

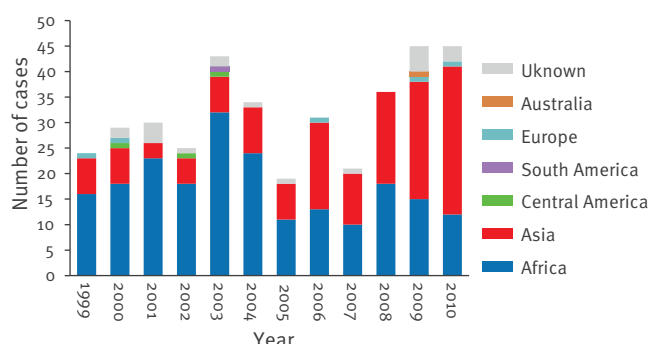
The first of the two cases in Viotia was caused by *P. vivax* and involved a girl who was treated with chloroquine. The second case was due to *P. falciparum* and concerned a man in his sixties, who had agricultural-related activities. He was treated with quinine and doxycycline. This autochthonous *P. falciparum* case was directly reported to the related Department of the Ministry of Health, examination of the sample was performed, but the sample was not sent to the MRL for verification.

In East Attica, the three reported cases were infected with *P. vivax*. The first was a teenager who was a student and had visited the Rafina area, the second, a girl, who was in the Avlaki area on vacation and the third, a male student in his teens who had repeatedly visited the area of Schinias. All cases were successfully treated with chloroquine and primaquine.

Another case was detected in 1999 in the prefectural region of Fthiotida (central Greece). In 2000, two cases were detected in a tourist resort area in the prefectural region of Chalkidiki, northern Greece. These two malaria cases concerned German tourists, who were diagnosed with malaria after returning to their country. The epidemiological investigation revealed that

FIGURE 2

Number of malaria cases according to the continent of acquisition of infection, Greece 1999–2010 (n=382)



they both stayed in the same hotel as a tourist from Mozambique who had at least six recent malaria episodes. In 2009, a cluster of six cases without travel history to a malarious country occurred in the municipality of Evrotas (prefectural region of Lakonia, Peloponisos) in southern Greece. Four of those cases were Greek Roma, residing in a camp.

In 2010, three new autochthonous cases were reported, two in the prefectural region of Viotia, and one, in a Greek Roma from the same area as the 2009 cluster (prefectural region of Lakonia). The cases in Viotia were two Greek Roma children aged less than seven years. Regarding the Roma population, after several discussions with the local authorities, we were informed that they had travelled within the Greek borders and not in malaria endemic countries. In addition, four individual cases of autochthonous malaria in the prefectural region of Evros, northern Greece have been diagnosed, but they have never been verified nor reported to the health authorities [8].

Discussion

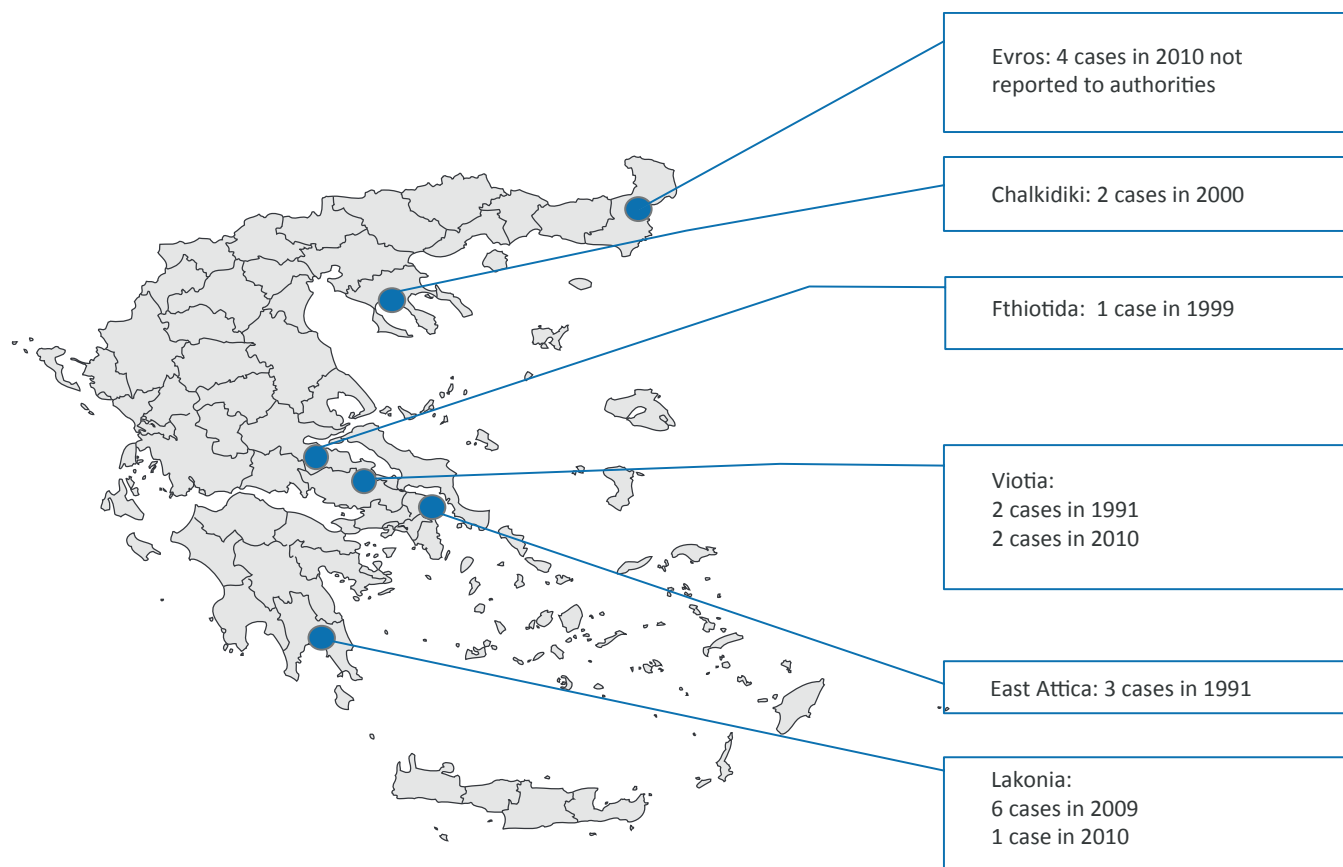
The anti-malarial campaign in Greece was labour-intensive and long lasting. Local and foreign authorities, in collaboration with scientific centres, such as the Rockefeller Institution, most contributed to achieving the main goal of eliminating malaria [18–20].

This effort, although interrupted during World War II, subsequently continued intensively even with limited available resources. Finally, at the end of the 1960s, Greece was considered as a malaria-free country, with the official recognition for elimination occurring in 1974 [4]. The first following years were characterised by the loosening and insufficient application of surveillance measures, together with abolishment of the existing malaria control centres during the 1980s.

Nevertheless, since 1974, a considerable number of imported cases have been reported in Greece, as also

FIGURE 3

Prefectural regions with autochthonous introduced malaria cases in Greece, 1991–2010 (n=21)



in several other European countries such as France, Germany, Italy, and the United Kingdom [21–24].

Malaria cases due to blood transfusion were not observed in Greece after 1987. This can be attributed to the implementation of a strict policy on blood management, mainly based on careful selection and exclusion of donors and where necessary, laboratory testing of donated blood. One death from malaria was reported in Greece during the study period. A plausible explanation for the very low case fatality rate may be the early malaria suspicion that was critical for confirmation of the diagnosis. Another reason might be the fortunate coincidence of the sensitivity of the *P. falciparum* strains to quinine, which was always available immediately from the public health authorities of the MoH. It would be difficult to assume non-reporting of malaria deaths. Of course this cannot be excluded, however only in non-diagnosed cases.

As it already has been mentioned, in Greece, large populations of *Anopheles* mosquitoes, which are competent *Plasmodium* vectors, have been recognised (*A. maculipennis*, *A. sacharovi*, *A. superpictus*). In certain parts of the country, due to the presence of rice fields and suitable habitats, mosquito populations are abundant. As far as the vectorial competence of local *Anopheles* to carry *P. falciparum*, it should be mentioned that since

the period when malaria was endemic in Greece, the competence of several species (*A. sacharovi*, *A. maculipennis*, *A. superpictus*) to carry the four *Plasmodium* species was proven, with *A. sacharovi* being the most prevalent species. Studies from the past [25] have shown on the basis of over 50,000 dissections, that *A. sacharovi* is the most competent vector with a sporozoite rate of 1.3 %, followed by *A. superpictus* with 0.8% and *A. maculipennis* with 0.07%. During the 1930s the distribution of malaria parasites among human cases, was 49% *P. falciparum*, 27% *P. vivax* and 22% *P. malariae* [26].

Moreover, where autochthonous malaria cases were recorded, *Anopheles* mosquitoes of the species *sacharovi* and members of the *maculipennis* complex have been recognised occasionally. However, data on their behaviour, habits and host biting preferences is missing. So far, there is no documented study concerning other *Anopheles* species present in Greece, such as *A. hyrcanus* and *A. claviger*, which are also considered to be potential malaria vectors.

As between 1977 and 1990 no autochthonous cases had been recorded in Greece, the occurrence of domestic cases of malaria after 1990 could be attributed to the presence of imported cases from malaria endemic countries. This is also supported by the fact that

autochthonous cases appeared during periods of mass entry of undocumented economic migrants, especially from countries where malaria is endemic. After extensive investigation, five autochthonous cases in 1991, one in 1999, two in 2000, six in 2009 and three in 2010 were reported in areas that can be considered as potentially prone to malaria. A few other cases in the Prefectural region of Evros, close to the borders with Turkey were reported, which were never confirmed from the authorised healthcare providers [8]. All but one of the autochthonous cases of malaria were due to *P. vivax*. A genotyping study of plasmodia is currently in progress.

Autochthonous malaria cases in other European countries were also sporadically reported (Italy 1997, Bulgaria 1995 and 1996, Germany 2001, France 2007) [27,28]. In Spain, the first autochthonous case of *P. vivax* malaria was diagnosed 40 years after elimination; nevertheless, the source of infection could not be identified [29]. Since 2000, a rather impressive outbreak in Turkey and the former Soviet Union countries caused by *P. vivax* and *P. falciparum* has been documented [30]. The prompt implementation of malaria control measures reduced dramatically the number of malaria cases to 176 (in Azerbaijan, Kyrgyzstan, Tajikistan, Turkey and Uzbekistan), as opposed to 90,712 in the year 1995 [30].

In Greece, the frequency of identified *Plasmodium* species in human malaria cases is almost the same as in other Mediterranean countries [22,24,28,29]. Initially, the majority of imported cases originated from Africa, but since 2009 most originate from Asia. In the United States, from 2000 to 2008, a similar increase in malaria cases originating from Asia was also observed [31,32]. The problem of individuals who became cases while visiting friends and relatives in an endemic area also exists in Greece but to a lesser extent. Travellers of this group, rarely visit the pre-travel health services before their trip. Furthermore, many of them do not receive proper anti-malarial chemoprophylaxis as they believe that they are protected by life-long immunity against malaria [33].

All cases but one were successfully treated based on existing experience and recommendations of WHO and Centers for Disease Control and Prevention (CDC) [34-36].

On the basis of the data presented and considering the recent *P. vivax* malaria cases of 2011 in the Lakonia area as well as the *P. vivax* malaria cases of 2012 in the Lakonia and East Attica areas [15,37], it is clear that surveillance and vector control programmes should be strengthened and rapidly intensified. In this respect, a coordinated effort has begun with the collaboration of Greek authorities, the European Centre for Disease Prevention and Control (ECDC) and WHO experts, to prevent potential malaria reestablishment.

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Ensuring safety of home-produced eggs to control salmonellosis in Poland: lessons from an outbreak in September 2011

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Implementation of control measures in line with European Commission regulations has led to a decrease in salmonellosis in the European Union since 2004. However, control programmes do not address laying hens whose eggs are produced for personal consumption or local sale. This article reports an investigation of a salmonellosis outbreak linked to home-produced eggs following a family event held in a farm in September 2011 near Warsaw, Poland. In the outbreak, 34 people developed gastroenteritis symptoms. Results from a cohort study indicated a cake, prepared from raw home-produced eggs, as the vehicle of the outbreak. Laboratory analysis identified *Salmonella enterica* serotype Enteritidis (*S. Enteritidis*) in stool samples or rectal swabs from 18 of 24 people and in two egg samples. As no food items remained, we used phage typing to link the source of the outbreak with the isolated strains. Seven *S. Enteritidis* strains analysed (five from attendees and two from eggs) were phage type 21c. Our findings resulted in culling of the infected laying hens and symptomatic pigeons housed next to the hens. *Salmonella* poses as a public health problem in Poland: control measures should not forget home-produced eggs, as there is a risk of infection from their consumption.

Introduction

In Europe, infection with *Salmonella* is a common cause of gastroenteritis [1]. *Salmonella enterica* serotype Enteritidis (*S. Enteritidis*) remains frequently reported, accounting in 2009 for 57% of all *Salmonella* serotypes [1]. Infection with *S. Enteritidis* is primarily linked to ingestion of contaminated meat or egg products [2]. Phage typing is a tool that is used to establish links between poultry flocks infected with *S. Enteritidis* and outbreaks among humans [3]. It can also be used to assess strains currently in circulation [4] and identify temporal trends [5]. It is not, however, routinely performed in many European countries, including Poland [4].

To prevent *Salmonella* infections, a number of control programmes have been implemented in the poultry industry within the European Union (EU) [1], including screening of laying hens, as recommended by the European Commission [6]. The introduction of programmes targeting laying hens has resulted in an overall decrease in the number of cases of *Salmonella* infection in the EU since 2004 [7]. However, under current regulations of the European Commission on the control of *Salmonella*, private farms producing table eggs (i.e. eggs produced or used for human consumption) for their own consumption or for sale to local retailers are exempt from current screening processes [8].

Despite the overall decrease in outbreaks of *Salmonella* infection in the EU, surveillance data since 1991 in Poland have shown that egg products play a pivotal role in the occurrence of salmonellosis outbreaks in humans, with 63% of the outbreaks between 2005 and 2010 being linked to this source [9]. The most striking increase has been in the proportion of outbreaks due to *S. Enteritidis* infection linked with the consumption of home-produced eggs: surveillance data show an increase from 76% of all *S. Enteritidis* outbreaks in 2004 to 82% in 2010 [9].

On 7 September 2011, a physician alerted the public health authority in Otwock, Poland, reporting that five people had fallen ill with gastroenteritis following a family event held three days earlier at a farm in the suburbs of Warsaw. The aim of our outbreak investigation was to stop the occurrence of cases, identify the source and explore ways to improve salmonellosis control in the long term at the household level.

Methods

Outbreak case definition

We defined a probable case of gastroenteritis as a person who took part in the family event on 4 September 2011 or who ate food that was brought home from the event and then developed diarrhoea, vomiting, stomach ache or temperature higher than 39 °C within 72 hours after consumption.

We defined a confirmed case of gastroenteritis as a probable case with laboratory confirmation of infection with *S. Enteritidis*.

Descriptive epidemiology

The hosts of the family event provided a list of addresses and contact numbers of all attendees of the family event. The public health authority collected information regarding demographics and health status of all the attendees and those identified during the outbreak investigation, in addition to a comprehensive list of all food and beverages consumed during lunch, dinner and dessert at the event. Initially, probable cases completed an open-ended, hypothesis-generating paper questionnaire: the majority were administered via telephone by the local authorities four days following the event. The questionnaire was a routine outbreak form, mainly focused on food consumption, with some additional questions regarding animal contact and travel within the previous two weeks. The National Institute of Public Health - National Institute of Hygiene then prepared a closed-ended questionnaire to be completed by all people on the list provided by the hosts and those identified during the investigation, to collect information on food/beverage items consumed during or after the family event and symptoms experienced. These close-ended questionnaires were administered, mainly via telephone, nine days after the event by each local health authority involved. Laboratory test results were added to the questionnaire results by the local health authorities or, in the case of phage typing, by the microbiology department at the National Institute in Warsaw.

Analytical epidemiology

We conducted a retrospective cohort study among attendees of the family event only. We calculated the risk of illness among people who had consumed food items and beverages, comparing this with the risk in people who had not consumed the same items. This yielded food/beverage-specific attack rates (ARs), relative risks (RRs) and 95% confidence intervals (CIs). We checked for effect modification and then established factors associated with becoming a case using multiple logistic regression. We included variables in the model if they had a *p* value of less than 0.2. We excluded attendees identified as asymptomatically infected and those for whom basic information (e.g. age and items consumed at the event) was missing. Stata 10 was used for the statistical analysis [10].

Laboratory investigation

Human samples

Physicians collected stool specimens or rectal swabs from symptomatic and healthy people who consented (from 17 attendees and four people who ate food after the event). Local laboratories tested these samples for adenovirus, rotavirus and norovirus and cultured for *Salmonella*.

Antibiotic susceptibility profiles of *Salmonella* strains from four specimens were determined for treatment purposes [11,12]. These strains were from the first four cases, who lived in the farmhouse or surrounding houses on the premises.

The laboratory at the National Institute of Public Health - National Institute of Hygiene phage typed these same *Salmonella* strains cultured from stool specimens of the first four cases [13]. We identified phage types (PTs) using reference phages provided by the Health Protection Agency, Colindale, United Kingdom.

In addition, the public health authority collected stool specimens from two attendees who had been involved in the preparation of food for the event who lived on the premises where the event had been held (one of whom was a professional food handler). The strain from the stool culture of this professional food handler was also phage typed and the antibiotic susceptibility profile was determined. The health authorities also collected a stool specimen from an additional food handler who attended the family event, but did not take part in the food preparation. The reason for collection of specimens from these persons was to identify any asymptomatic infections that could have constituted sources of secondary infections or clusters.

Extended laboratory and environmental investigations

The public health authority also requested information from the hosts regarding where the food had been prepared and stored, as well as details of the cold chain. In addition, the people involved in food preparation were asked about how the meals were served and the cutlery used during serving (e.g. knives).

Swabs were not taken from kitchen equipment at the event. As the event was held at a private residence, the taking of swabs in Poland is not currently required by law. In addition, the food safety authority visited the premises three days after the event, at which point the equipment had been cleaned. No left-over food items from the event were available for testing, thus phage typing of environmental and clinical isolates was used to identify the potential source of the outbreak. Raw eggs from the laying hens that resided on the farm had been used in food preparation for the event. As there were no samples available from the eggs used, fresh eggs from the flock were taken for laboratory analysis.

No samples were taken from pigeons that lived in an aviary situated next to the laying hens.

Once the public health authority had received initial notification of the outbreak, they informed the local veterinarian, a legal requirement in Poland.

Results

An overview of the inclusion/exclusion criteria for the cohort analysis and the results of the laboratory analysis are shown in Figure 1.

Descriptive epidemiology

The event, held on 4 September, took place on the hosts' farm, where two families plus tenants lived (n=11), in the farmhouse or surrounding houses on the premises. The hosts of the event kept 17 laying hens and 80 pigeons in adjacent aviaries.

A total of 57 people attended the event, of which 26 were symptomatic. Food taken home from the event was eaten later by eight people (all developed symptoms). Two attendees were involved in preparing food for the event (one of whom was a professional food handler). An additional food handler attended the

event but was not involved in food preparation. All of those living on the farm premises (n=11) attended the event.

In the open-ended (trawling) questionnaires administered to probable cases, we found that numerous names were used for the cakes at the event, making it hard for us to compare the answers. Thus there was an intentional overlap between the open-ended and closed-ended questionnaires, to clarify any ambiguity.

The closed-ended questionnaire was administered to 65 people linked with the outbreak: 57 attendees (including the two people who prepared the food for the event) plus eight who ate food from the event later. We obtained information on sex and health status (healthy vs symptomatic) from all 65 people: if the person did not complete the questionnaire, the information was provided by the hosts or family members. The 65 people resided in six districts located in two provinces: 33/65 were female.

Of the 65, 18 were probable and 16 were confirmed cases. Symptoms of the 34 cases included diarrhoea (n=32), temperature >39 °C (n=26), vomiting (n=22),

FIGURE 1

Inclusion/exclusion criteria for cohort analysis and results of laboratory analysis, *Salmonella* outbreak related to a family event, Warsaw, Poland, September 2011

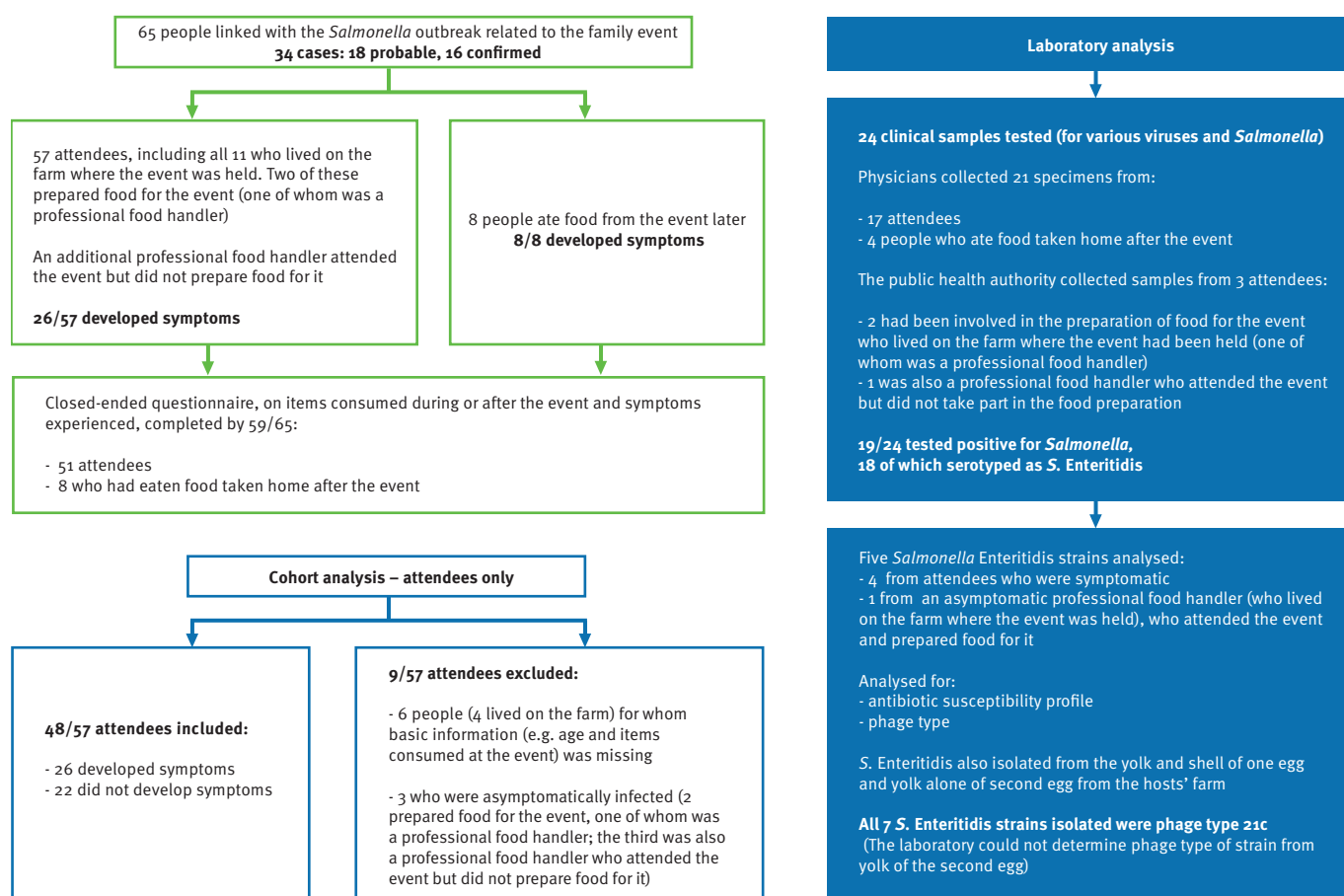


TABLE 1

Demographic details of people who attended^a or ate food from a family event later, Warsaw, Poland, September 2011 (n=65)

Demographic information	Cases ^b	Total	AR (%)
Age (years)			
<19	9	14	64
20–39	12	22	55
40–59	7	13	54
≥60	6	10	60
Unknown	0	6	0
Sex			
Female	17	33	52
Male	17	32	53

AR: attack rate.

^a Includes two attendees who prepared food for the event (one of whom was a professional food handler).

^b Probable (n=18) and confirmed (n=16).

stomach cramps (n=13) and nausea (n=2). Four of the 34 were hospitalised.

The overall attack rate was 52% (34/65); the rates were similar in all age groups and by sex (Table 1).

A total of 59 people completed the close-ended questionnaires: 51 attendees (including the two food handlers), eight who had eaten food brought home after the event. Information on age was missing for six people: of the 59 who provided the information, the median age was 36 years (range: 4–88).

The outbreak began on 4 September (the day of the event); the number of cases peaked between midnight and noon the following day and subsequently decreased (Figure). Time of symptom onset was provided by 29 cases: the remaining five could not recall the exact or approximate time. Of these 29 cases, 19 reported symptoms within 24 hours of the event (range: 3.5–69 hours).

Analytical epidemiology

The cohort study comprised 48 attendees; nine attendees were excluded, in accordance with the criteria: six did not provide information on food consumption (four of whom lived on the farm) and three were asymptotically infected with *S. Enteritidis* (two of whom lived on the farm). Of the 48 attendees, 26 developed symptoms (14 probable and 12 confirmed cases). People who ate angel cake (RR: 3.2; 95% CI: 1.7–6.2), cream cake (RR: 1.9; 95% CI: 1.4–2.6) and caramel cake (RR: 1.8; 95% CI: 1.4–2.4) were more likely to become ill (Table 2). Angel cake was eaten by most people in

the cohort: 19 out of the 26 people who became ill ate it. Furthermore, three of four people who ate cream cake and/or caramel cake and became ill also ate angel cake. The remaining seven people who became ill but did not eat angel cake ate the following cakes: éclairs and cream cake (n=1), éclairs only (n=5) and a cake not on the list of food items collected (n=1).

To explore whether cream cake or éclairs also acted as vehicles of infection, we examined the risk of developing symptoms for people who had eaten these cakes, according to whether they had eaten angel cake (Table 3). People who ate cream cake, but not angel cake, were four times more likely to become ill (RR: 4.00; 95% CI: 2.00–8.00). Consumption of éclairs was associated with a higher risk of illness, but this was not significant (RR: 4.71; 95% CI: 0.66–33.61). Stratification by caramel cake consumption was not possible because no one had been exposed to this cake alone.

Taking the results from our initial analysis into account, our multivariable model indicated consumption of angel cake as the only factor associated with illness (aOR: 192; 95% CI: 7–5,200) (Table 2). Cream cake was not included in the model as a result of colinearity.

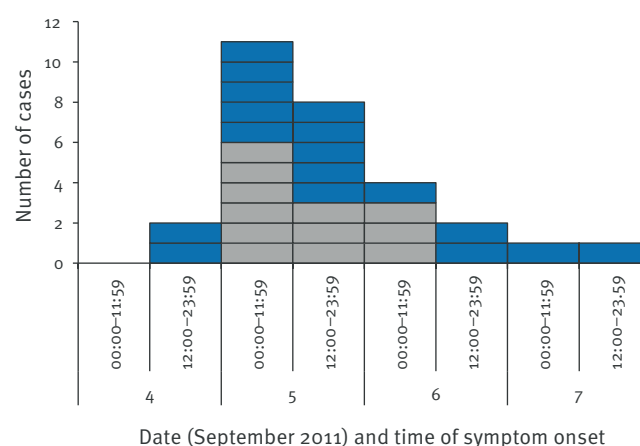
Laboratory investigation

Human samples

A total of 24 rectal swabs/stool specimens were collected: physicians collected samples from 21 people (17 attendees and four from people who ate food after the event) and the public health authority collected samples from the three food handlers/people involved

FIGURE 2

Cases of gastroenteritis by time of symptom onset, Warsaw, Poland, September 2011 (n=29)^a



Blue boxes represent probable cases (17 of 18 identified), grey boxes represent confirmed cases (12 of 16 identified).

^a Time of symptom onset was unknown for five of the 34 cases.

TABLE 2

Attack rate of gastroenteritis according to consumption of food items and beverages among attendees at a family event, Warsaw, Poland, September 2011 (n=48)

Type of meal/ beverage	Exposure ^a	Consumed			Did not consume			RR	95% CI	p value	aOR	95% CI
		Cases ^b	Total	AR (%)	Cases ^b	Total	AR (%)					
Lunch	Chicken soup	9	14	64	17	32	53	1.2	0.7–2.0	0.48	–	–
	Pork chop	9	15	60	17	31	55	1.1	0.7–1.8	0.74	–	–
	Tripe	16	32	50	10	14	71	0.7	0.4–1.1	0.18	0.1	0.01–1.7
	Cabbage salad	4	11	36	22	35	63	0.6	0.3–1.3	0.12	0.1	0.004–2.8
	Potatoes	8	21	38	18	25	72	0.5	0.3–0.9	0.02	0.1	0.01–1.3
Dinner	Chicken	2	2	100	24	44	55	1.8	1.4–2.4	0.21	–	–
	‘Bigos’ (meat stew)	5	6	83	21	40	53	1.6	1.0–2.5	0.16	82	0.7–10,290
Dessert	Angel cake	19	21	91	7	25	28	3.2	1.7–6.2	0.01	192	7–5,200
	Cream cake ^c	4	4	100	22	42	52	1.9	1.4–2.6	0.07	–	–
	Caramel cake	2	2	100	24	44	55	1.8	1.4–2.4	0.21	–	–
	Apples in jelly	5	7	71	21	39	54	1.3	0.8–2.3	0.39	–	–
	Eclairs	14	23	61	12	23	52	1.2	0.7–1.9	0.55	–	–
Alcohol	Vodka	4	9	44	3	12	25	1.8	0.5–6.0	0.35	–	–

aOR: adjusted odds ratio; AR: attack rate; CI: confidence interval; RR: relative risk.

The shaded rows represent items for which $p < 0.2$ and were therefore included in the multiple logistic regression model.

^a Food items and beverages provided by the hosts.

^b Probable (n=14) and confirmed (n=12).

^c Not included in the multiple logistic regression model due to colinearity.

in food preparation who also attended the events. Of the 24, 19 tested positive for *Salmonella*, 18 of which serotyped as *S. Enteritidis* (the 19th sample – from an attendee who did not live on the farm – was reported as *Salmonella* spp.). The laboratory did not identify any other pathogens in the samples, 17 of which were from attendees and four from people who ate food after the event.

Of the 18 serotyped strains, three were from attendees who did not have symptoms, two of whom were professional food handlers, one of whom was involved in food preparation for the event alongside the third asymptomatic individual. The public health authority exempted the two professional food handlers from work until they provided three consecutively negative samples.

Antimicrobial susceptibility profiles were determined for the same five strains that were phage typed. All were fully sensitive to ciprofloxacin, furazolidone, trimethoprim/sulfamethoxazole and had medium sensitivity to ampicillin and amoxicillin/clavulanic acid.

These same five strains of *S. Enteritidis* were phage typed as 21c. Apart from the strains isolated from the egg samples (described below), no other strains were phage typed due to financial constraints.

Extended laboratory and environmental investigations

S. Enteritidis was also isolated from the yolk and shell of one egg and the yolk alone of a second taken from the event hosts' laying hens. Two *Salmonella* strains isolated from the first egg were phage typed as 21c. The laboratory could not determine the phage type of the strain isolated from the yolk of the second egg.

The hosts used raw eggs, collected four days before the event from their laying hens, in the preparation of a quadruple cream-layered angel cake. During inspection by the public health authority, the hosts explained that all cakes had been stored at room temperature alongside other dishes on a warm autumn day. All cakes had been set out on the table on the same serving plate along with one knife.

During the outbreak investigation, the hosts informed the public health authority that half of their pigeons had fallen ill. The pigeons were then diagnosed as being infected with *Salmonella* by a veterinarian on the basis of their symptoms (the veterinarian did not conduct any laboratory tests on the pigeons). Due to the timing of the symptoms, it was assumed that the pigeons were also infected with *Salmonella*.

Given the laboratory results from the egg samples from the hosts laying hens and recommendation from the

TABLE 3

Cases of gastroenteritis among people who ate different types of cake at a family event, Warsaw, Poland, September 2011 (n=48)

Exposure to angel cake	By consumption of cake type	Cases ^a	Total	AR (%)	RR	95% CI
Yes	Cream cake	3	3	100	1.13	0.96–1.32
	No cream cake	16	18	89		
No	Cream cake	1	1	100	4.00	2.00–8.00
	No cream cake	6	24	25		
Yes	Caramel cake	2	2	100	1.12	0.96–1.30
	No caramel cake	17	19	89		
No	Caramel cake ^b	0	0	0	–	–
	No caramel cake ^b	7	25	28		
Yes	Eclairs	8	9	89	0.97	0.73–1.29
	No éclairs	11	12	92		
No	Eclairs	6	14	43	4.71	0.66–33.61
	No éclairs	1	11	9		

AR: attack rate; CI: confidence interval; RR: relative risk.

^a Probable (n=14) and confirmed (n=12).

^b No RR yielded due to zeros present in this stratum.

veterinarian, the hosts took action: they agreed to cull the entire flock of 17 laying hens and the 40 ill pigeons assumed to be infected, remove their bodies from the premises and disinfect the entire area where the animals lived. The veterinarian provided treatment for the remaining 40 non-symptomatic pigeons.

Discussion

This gastroenteritis outbreak, which affected just under half (26 of 57) attendees of a family event, was linked to infected home-produced eggs from laying hens. Results from the outbreak investigation identified a number of factors that could have contributed towards its occurrence. These included the use of an unprocessed contaminated ingredient, inappropriate storage, cross-contamination and infection of food handlers [14].

The distribution of probable and confirmed cases suggested a point source food-borne outbreak. Epidemiological investigations pointed to angel cake as the vehicle of infection. *S. Enteritidis* is frequently isolated in products made using raw eggs [15–19]. The hosts used raw eggs knowing their hens had not been screened for *Salmonella*. The general public often consider organic [20] or free-range chickens more likely to be *Salmonella* free [21]. However, unless poultry are subject to checks, such assumptions cannot be made. The frequency with which such checks should be carried out and the methods that should be used are issues that raise a number of challenges. Any decision would have to take into account the following

points: whether the outcome would result in a recommendation or a regulation, whether the focus should be on villages or individual farms; the age of the laying hens; and whether ‘new’ hens had been introduced into existing flocks, as well as the presence of other animals on the premises. Recommendations on the frequency of testing should be carefully evaluated especially in terms of cost-effectiveness and acceptability. They should also consider who would pay for screening or regulation. The debate would benefit from a panel of experts being called together, including those from the European Food Safety Authority, European Centre for Disease Prevention and Control and others involved in work with poultry and *Salmonella*. Such discussions would be pertinent, as use of raw eggs from flocks that are regularly screened for *Salmonella* could potentially have prevented the outbreak. Infections with *Salmonella* traced back to unscreened home-produced eggs emphasise the importance of screening laying hens in private residences.

The high attack rate (52%, 34/65) in this outbreak could be attributed in part to the lack of refrigeration of dishes as they were stored at room temperature on a warm day. Lack of refrigeration allowed *S. Enteritidis* to grow, as previously documented in other outbreaks [18,22,23]. Had the dishes been refrigerated, the attack rate may have been reduced.

People who only ate cream cake from the desserts offered were four times more likely to develop symptoms (Table 3). This, along with the fact that the cakes

were placed together on the same serving platter with a single knife, supports the hypothesis of cross-contamination. Furthermore, the hosts left all the cakes out on the table throughout the course of a warm afternoon, where the cakes could soften and mix with others. Cross-contamination is frequently reported in *S. Enteritidis* outbreaks [23,24], particularly in places where large quantities of food are served [17,25]. We could not document a significant association between consumption of éclairs and illness; however, similar cross-contamination may explain why five cases who ate only éclairs for dessert also became ill.

Given the absence of left-over food specimens, we used phage typing to establish a link between consumption of angel cake and symptoms of *Salmonella* infection. *S. Enteritidis* PT21c was found, documenting its presence in Poland. This phage type is rare [22]. Phage typing is a key tool, used in outbreaks and as part of surveillance to assess the strains currently in circulation [4]. However, as this technique is not routinely performed in Poland, we cannot determine whether PT21c is in frequent circulation in the country. Phage typing needs to be encouraged, particularly during outbreak investigations, in countries where case reports of *Salmonella* infection remain high. Other tools for subtyping *S. Enteritidis* include pulsed field gel electrophoresis (PFGE) [26] and multiple-locus variable-number tandem-repeat analysis (MLVA) [27]. PFGE is considered to have low discriminatory power for *S. Enteritidis*, especially in outbreak settings [27]. MLVA has been shown to have better discriminatory power than phage typing or PFGE [28]; however, this technique for *S. Enteritidis* typing is not currently used at the National Institute of Public Health- National Institute of Hygiene in Warsaw.

Study limitations

Physicians did not take stool specimens from all people in the cohort. We therefore cannot rule out the possibility that other members of the cohort, particularly those who lived on the premises where the event was held, were asymptotically infected with *Salmonella*. If they had been infected with the same strain, they should have been excluded from the cohort, as consumption of cake would not have affected them. In our cohort, we excluded all known asymptomatic infected people who had been tested (as they were food handlers or involved in food preparation for the event). Lack of identification and exclusion of any additional asymptomatic infected people would result in an underestimation of the strength of association we calculated. Thus, this limitation does not prevent us from concluding that the angel cake was the vehicle in this outbreak.

The small numbers in this outbreak limited interpretation of results from the analytical study, due to lack of power. Its findings, however, were indirectly supported and strengthened by the microbiological results.

Conclusions

Salmonella control activities, such as screening of laying hens, have helped decrease the number of cases of *Salmonella* infection reported in Poland; however, this outbreak points to gaps that still exist. Identifying home-produced eggs as the source of the outbreak indicates that privately owned hens are not adequately covered by measures in place. Furthermore, the high attack rate observed demonstrates the impact use of unscreened home-produced eggs can have in the population. This – along with surveillance data suggesting that home-produced eggs remain a common cause of outbreaks of *Salmonella* infection among humans in Poland [9] – calls for action. On the basis of our results, we suggest areas where changes to everyday practices could be of benefit. To bridge this gap in salmonellosis control, we need to actively engage the general public. First, the general public should ensure safety of their food through the use of screened eggs in dishes that require raw ingredients. Second, food items containing raw products should be kept and served separately. Third, products requiring refrigeration should be kept at low temperatures prior to consumption. In the context of outbreaks, phage typing is one of the tools that can be used to establish links between cases and sources, for example, in the absence of food items.

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Two outbreaks of diarrhoea in nurseries in Norway after farm visits, April to May 2009

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During a 2009 nationwide outbreak of sorbitol-fermenting *Escherichia coli* O157 in Norway, the Norwegian Institute of Public Health was notified of diarrhoea outbreaks in two nurseries. A link to the nationwide outbreak was suspected and investigated, including retrospective cohort studies. Both nurseries had recently visited farms. Faecal specimens were obtained from symptomatic children as well as from the farm animals and tested for *Campylobacter*, *Salmonella*, *Yersinia*, *Shigella* and pathogenic *E. coli*, and isolates were further characterised. Nursery A had 12 symptomatic children, and we found the same strain of *C. jejuni* in faeces from children and lambs. Nursery B had nine symptomatic children, including one child with bloody diarrhoea carrying enterohaemorrhagic *E. coli* (EHEC) O26. EHEC O26 with a similar multiple-locus variable number tandem repeat analysis (MLVA)-profile was found in sheep. Five children had enteropathogenic *E. coli* (EPEC) O76. Animals were not tested for EPEC O76. We found no significant association between illness and risk factors for either nursery. The isolated pathogens differed from the one involved in the nationwide outbreak. In each nursery outbreak, the pathogens isolated from children matched those found in farm animals, implicating animal faeces as the source. Hygiene messages are important to prevent similar outbreaks.

Introduction

There are several reports from around the world of sporadic cases as well as outbreaks of zoonoses, especially among children, after farm visits [1,2]. The most commonly described pathogens in these incidents are different strains of *Escherichia coli* [3-12], but other pathogens including *Campylobacter* are reported as well [1,13].

Enterohaemorrhagic *E. coli* (EHEC) are known to cause infections that can lead to serious complications such

as haemolytic-uraemic syndrome (HUS), especially in children, immuno-compromised persons and the elderly. The proportion of patients diagnosed with EHEC who develop HUS is around 10% [14,15], but varies by host factors and type of EHEC. In Europe, more than 50% of patients diagnosed with sorbitol-fermenting (SF) EHEC O157 (SF O157) develop HUS [14,16].

In the spring of 2009 there was a national outbreak of SF EHEC O157 in Norway, affecting 13 children, including nine HUS cases of whom one died [17,18]. This outbreak attracted a lot of media attention, reinforced by the public's memory of the first large EHEC outbreak in Norway in 2006, that affected 17 children including 10 HUS cases of whom one died [19].

In May 2009, as the Norwegian Institute of Public Health (NIPH) was investigating the national outbreak of EHEC SF O157, additionally the chief medical officers of two distinct Norwegian municipalities each notified an outbreak of diarrhoea in a nursery in their respective municipalities: On 12 May we received the notification from Nursery A in Rogaland County in south-western Norway, while on 14 May we received the notification from Nursery B in Akershus County in the eastern part of Norway. A stool specimen from a child with bloody diarrhoea from Nursery B was positive for *stx2*, a gene encoding one of the EHEC toxins. We also had information that children attending both nurseries had participated in farm visits. During the visits children had cuddled the farm animals. Nurseries in Norway function as pedagogical daycare facilities for children under the age of six years.

We initiated investigations of the two nursery outbreaks. Our aims were to decide whether they were associated with the concomitant national outbreak of EHEC SF O157, and to identify the source or sources of infection in order to stop the current outbreaks and

prevent similar outbreaks in the future. In order to reach our aims, we wanted to test the following hypotheses: (i) The pathogen causing the nursery outbreaks was EHEC SF O157. (ii) The nursery children who participated in the farm visit had a higher risk of becoming ill than those who did not.

Coincidentally with initiating the investigations we took preliminary measures to control the outbreaks by excluding ill children from attending the nurseries, as recommended in the NIPH guidelines for infection control in nurseries [20].

Materials and methods

Epidemiological investigation

The investigations were conducted by the NIPH in cooperation with the chief medical officers in the affected municipalities and the Norwegian Food Safety Authority (NFSA). We performed a retrospective cohort study in each nursery. We collected information on each child's nursery attendance, travel history and participation in gatherings preceding the outbreaks, symptoms of disease (if any), food consumption, participation in the farm visit, and animal contact at the farm. Questions about food consumption were based on menu lists of food and beverages served in the nurseries, collected by the local NFSA offices.

We collected this information from the nursery staff, using detailed questionnaires based on the NIPH's standardised outbreak questionnaire [21], adjusted for the respective nurseries. For Nursery A the farm visit took place on 5 May, the questionnaires were filled out on 15 May, and the questions covered the period from 4 May. The time frame for travel history was 1–7 May. The question about gatherings was not included in the questionnaire for each child, but the nursery staff were asked if they were aware of anyone in the nursery group participating in any gatherings during the week before the outbreak. For Nursery B the farm visit took place on 29 April, the questionnaires were filled out on 19 May, and the questions covered the period from 27 April. The time frame for travel history and gatherings was 27–30 April.

We collected information on gastrointestinal illness for each child in the query period. For those who were ill, we asked about specific symptoms including diarrhoea, vomiting, nausea, abdominal pain, fever, bloody stools and joint pain.

Case definitions

We defined a case for Outbreak A as a child that attended Nursery A in April and May 2009 and a case for Outbreak B as a child that attended Nursery B in the period from April 29 to May 19, with the following additional criteria: Suspected cases were those who showed symptoms of gastroenteritis in the query period (general gastroenteritis, vomiting and/or diarrhoea). Because the microbiological results later

indicated that the outbreak in Nursery A was caused by *Campylobacter jejuni* and the outbreak in Nursery B by *E. coli*, the definition for confirmed cases was chosen accordingly as: those who tested positive for *C. jejuni* in Nursery A and those who tested positive for pathogenic *E. coli* in Nursery B.

Statistical analyses

We conducted descriptive statistics and univariate analyses using Stata (version 11). In the univariate analyses we calculated the relative risk (RR) with 95% confidence intervals (CI) for association between illness and different risk factor exposures such as participation at farm visit, sex and age of the child as well as consumption of diverse food items and beverages. We performed the analyses both for confirmed cases only, and for suspected and confirmed cases combined.

Microbiological investigations

Human specimens

We aimed to collect faecal specimens from all children with symptoms. The initial analyses were performed at the regional medical microbiological laboratories, and included testing for *Campylobacter*, *Salmonella*, *Yersinia*, *Shigella* and pathogenic *E. coli* according to the standard protocols of the respective laboratories. The specimens from the children in Nursery A were also tested for rotavirus and adenovirus by immunochromatography [22]. The specimens from the children in Nursery B were not tested for viruses after we had identified pathogenic *E. coli* as the pathogen of the outbreak in this nursery. For the bacterial isolates that we suspected as possible causative infecting agents, we conducted further verification and typing (described below) at the reference laboratory at the NIPH.

From children who tested positive for pathogenic *E. coli*, we collected specimens repeatedly, until we considered them not to be contagious anymore and hence allowed them to attend nursery again. According to NIPH guidelines [20], for EHEC this requires five consecutive negative tests of faecal specimens collected a minimum of 24 hours apart.

Animal specimens

The district offices of the NFSA collected faecal specimens from animals and transported them at ambient temperature to the laboratory at the Norwegian Veterinary Institute (NVI) for examination within 24 hours.

From the farm visited by Nursery A, specimens from six lambs were collected and tested for *Campylobacter* according to an ISO-method [23]. In addition we evaluated the bacterial flora by plating out on non-selective media, and performed standard bacteriological testing. From the farm visited by Nursery B, we collected 36 specimens from sheep and 17 from cattle (one test per animal), and tested them individually for *E. coli*

O26 by automated immunogenic separation (AIMS) as described previously [24]. We did not perform standard bacteriology on these animal specimens, and as adequate methods were unavailable we did not analyse them for *E. coli* O76.

Typing and comparisons of human and animal isolates

At the DNA-analysis laboratory of the Department of Foodborne Infections at the NIPH, we typed and compared all animal isolates with the human isolates from the corresponding outbreak. We ascertained the DNA profiles of *C. jejuni* isolates by combining three different methods: clustered regularly interspaced short palindromic repeat (CRISPR) polymorphism, single nucleotide polymorphism (SNP) typing and binary gene typing (BGT) [25-28]. We assessed the DNA profiles of *E. coli* by multi-locus variable number of tandem repeats analysis (MLVA). We implemented a generic *E. coli* MLVA assay for all non-O157 isolates as detailed previously [29,30]. For the *E. coli* O26 isolates, we examined virulence, including detection of *eae* and the *stx* genes, as described elsewhere [31,32].

Environmental investigations

The district offices of the NFSA inspected the farms and the nurseries and collected specimens of food and drinks from Nursery A on 13 May. We also asked for water specimens from both nurseries. On 15 May the district office of the NFSA inspected and collected specimens of food and garbage from the kitchen in the home of the child with bloody diarrhoea in Nursery B.

Nursery A brought its own water from a water processing plant approved by the NFSA to the farm visit. Nonetheless, on 26 May we collected a water specimen from the farm, which was served by groundwater from a well. Eurofins Environment Testing Norway AS, Stavanger, analysed this specimen for total bacterial count at 22°C, coliform bacteria, generic *E. coli*, *Campylobacter*, and *Clostridium perfringens*.

We also collected faecal specimens from the floor of two lamb pens at the farm visited by Nursery A. We investigated these specimens in the same way as the faecal specimens taken from the animals.

Results

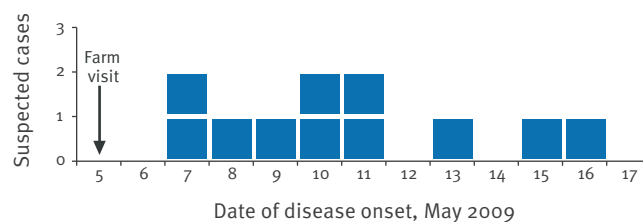
Descriptive epidemiology

Nursery A

Of the 24 children attending Nursery A, 12 met the definition of a suspected case (attack rate (AR): 50%). The suspected cases were all between three and six years of age with median age four years, as for the nursery group in general. Ten of the suspected cases were girls. The first child became ill on 7 May. The 12 suspected cases included one child that became ill on 16 May, the day after the end of the query period. We included the information on this child's disease upon later notification from the chief medical officer of the

FIGURE 1

Number of suspected cases in Nursery A by date of onset of disease, Norway, May 2009 (n=11)



Note: One of the 12 suspected cases is not shown as illness onset date was not available.

municipality. Of the 24 children in the nursery, three did not participate in the farm visit. One of these had symptoms defining her as one of the 12 suspected cases (date of onset 11 May).

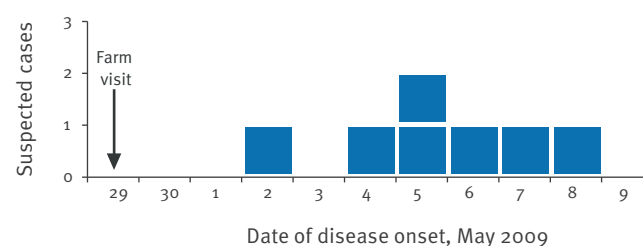
We aimed to exclude all children with symptoms of gastroenteritis from nursery attendance until 48 h after cease of symptoms, as recommended by the NIPH guidelines for infection control in nurseries [20].

Nursery B

Of the 16 children attending Nursery B, seven met the definition of a suspected case (AR: 44%). The suspected cases were all between one and five years of age, as for the nursery in general. The median age for the suspected cases was two years, compared with three years for the nursery in general. Three of the suspected cases were girls. The first child became ill on 2 May, while the latest reported illness onset was on 8 May. One of the 16 children did not participate in the farm visit. This child did not become ill.

FIGURE 2

Number of suspected cases in Nursery B by date of onset of disease, Norway, April–May 2009 (n=7)



Microbiological results

Nursery A

We sampled and analysed four of the 12 suspected cases. These four specimens all yielded *C. jejuni* with identical DNA-profiles, and no other pathogens.

Specimens from four of the lambs on the visited farm were positive for *C. jejuni* with the same DNA-profile as the human isolates. We detected no other pathogens in the specimens from the lambs.

Nursery B

We analysed specimens from the seven suspected cases in Nursery B. In addition, two further children were sampled, who are not considered in the epidemiological analysis above because they had more general symptoms not included in the final suspected case definition. From specimens of the suspected case with bloody diarrhoea, we isolated EHEC O26, *stx1* negative, *stx2a* positive and *eae* positive. In addition we identified atypical enteropathogenic *E. coli* (aEPEC) O76, all with an identical MLVA-profile, from the specimens of a further five children, including one who did not fulfill the suspected case definition.

We aimed to exclude all children with faecal specimens positive of EHEC or EPEC from nursery attendance until they had repeated negative faecal specimens, as recommended for EHEC cases by the NIPH guidelines for infection control in nurseries [20].

We identified EHEC O26, with the same virulence genes as the human isolate, from two specimens of cattle and four specimens of sheep on the farm visited by Nursery B. The MLVA-profiles of the human and animal isolates were almost identical, differing in one locus only.

Environmental results

Nursery A had visited a farm with about 290 sheep and 430 lambs. The children were allowed to enter lamb pens. There was a sink in the barn, but the children did not use it to wash their hands. The staff from Nursery A brought hand disinfection that the children used prior to their meal. They ate outside in the yard sitting on

the ground on seating pads. The analyses of the water samples from this farm did not yield positive results. Faecal specimens from the floor of the lamb pens tested positive for *C. jejuni*.

Nursery B had visited a farm with around 60 cattle and 90 sheep. The children had close contact with cows and lambs in the barn and did not wash hands before their meal, which they ate outside in the yard. The water supply both in the nursery and at the farm was a water processing plant approved by the NFSA, with no reports from other recipients indicating contamination of the water. Therefore the local NFSA office regarded the water supply to be of good quality and did not collect any water specimens.

As the microbiological results incriminated farm animals as the source of infection in both outbreaks, we did not analyse the food specimens taken from Nursery A, or the food and garbage specimens taken from the kitchen in the home of the child with bloody diarrhoea and EHEC in Nursery B.

Analytical epidemiology

Nursery A

In total we examined 69 risk factors. By univariate analysis we found that children who ate carrots during the farm visit were more likely to become ill (RR: 2.1; 95% CI: 1.4–3.2), but it has to be noted that this result is based on a single child who ate carrots. We found no other exposure significantly associated with disease. Table 1 shows examples of the risk factors examined for nursery A and their association with being a case (suspected or confirmed).

Nursery B

In total we examined 55 risk factors. By univariate analysis we found no exposure among the children increasing the risk of becoming ill. Table 2 shows examples of the risk factors examined for nursery B and their association with being a case (suspected or confirmed).

The exposures shown in Table 1 and Table 2 are chosen to illustrate the different categories of risk factors

TABLE 1

Selected results from univariate analysis for Nursery A, Norway, May 2009 (n=24)

Exposure		Exposed		Unexposed		Risk ratio	95% Confidence interval
		Cases/total	%	Cases/total	%		
Farm visit 5 May	Participation	11/21	52	1/3	33	1.6	0.30–8.2
	Close contact with lambs	11/21	52	1/3	33	1.6	0.30–8.2
	Eating carrots	1/1	100	11/23	48	2.1	1.4–3.2
	Eating fish cakes	11/21	52	1/3	33	1.6	0.30–8.2
Food and beverages consumed in the nursery 4–7 May	Mutton sausage	12/23	52	0/1	0	-	-
	Cucumber	12/23	52	0/1	0	-	-
	Tap water	12/23	52	0/1	0	-	-

TABLE 2

Selected results from univariate analysis for Nursery B, Norway, April–May 2009 (n=16)

Exposure		Exposed		Unexposed		Risk ratio	95% Confidence interval
		Cases/total	%	Cases/total	%		
Farm visit 29 April	Participation	8/15	53	0/1	0	-	-
	Eating grilled minced steaks	8/15	53	0/1	0	-	-
Food and beverages consumed in the nursery 27–30 April	Honey dew melon	8/16	50	0/0	0	-	-
	Saveloy	0/0	0	8/16	50	-	-
	Liver paste	6/14	43	2/2	100	0.43	0.23–0.78
	Mayonnaise	8/16	50	0/0	0	-	-
	Margarine	4/12	33	4/4	100	0.33	0.15–0.74
	Gherkin	6/11	55	2/5	40	1.4	0.41–4.5
	Tap water	8/16	50	0/0	0	-	-

examined. Some were included because they had been pointed out as sources of earlier outbreaks in Norway (for instance mutton sausage [19]) or abroad.

Overall, the staff in both nurseries had given very similar answers on the questionnaires for all children. As univariate analysis did not yield any positive associations between exposures and illness, and as the number of subjects was low, we considered multivariate analysis not appropriate.

Discussion

We found that the causative pathogens of the outbreaks were *C. jejuni* in Nursery A, and *E. coli* O26 and O76 in Nursery B. Thus, we excluded an association with the concomitant national outbreak of *E. coli* SF O157.

In each outbreak, we found the same pathogens in faecal specimens from farm animals and from the sick children, implicating the animals as source of the outbreaks, directly or indirectly. The association between illness and eating carrots at the farm visited by Nursery A could only explain one of the 12 cases, and is therefore not plausible as the source of the outbreak.

Earlier publications of outbreaks in Norway due to transmission of zoonoses by animal contact are scarce. However, in 2005 a small outbreak of cryptosporidiosis among students and workers at a farm used for training by the Norwegian School of Veterinary Science was traced to contact with calves [33]. Two other outbreaks of cryptosporidiosis, which occurred in March 2009 and March 2012 among schoolchildren staying in a wildlife reserve, have also been attributed to animal contact [34,35]. In addition, animals were discussed as the cause of an outbreak of *E. coli* O145 in a third nursery [36] in September and October 2009. Generally, animal health in Norway has been regarded as good for many years. For example, the national surveillance programme did not detect any *Salmonella* among domestic animals in 2009 [37]. In contrast, a recent study identified Norwegian sheep flocks as an important

reservoir for potentially human-pathogenic *E. coli* O26 [31,38]. Our findings are especially relevant in light of the popularity of visiting farms with children; similar outbreaks might occur again.

It is possible that some of the sick children were secondary cases who acquired the infection from nursery mates. Such secondary transmission of zoonotic agents has also been described after visits to a petting zoo in Canada [9] and is likely in a nursery environment due to the difficulty of ensuring good hand hygiene among young children. The incubation period of campylobacteriosis ranges between one and 10 days [39], indicating secondary transmission for the child in Nursery A that became ill on 16 May. The incubation period of EHEC ranges between two and 10 days, but is probably shorter for EPEC [40], not excluding the possibility of secondary transmission for the children in Nursery B with later disease onset. Both nurseries aimed to exclude children from the nursery while they were symptomatic, but possible failure to achieve this completely could explain secondary transmission.

In two earlier campylobacteriosis outbreaks related to farm visits, the reported ARs for *Campylobacter* ranged from 0.5% [1] to 53% [13], whereas the AR in the outbreak in Nursery A was 50%. In previously described outbreaks of pathogenic *E. coli* infection after farm visits, the ARs ranged from 0.06% to 18% [8,10,12], whereas the AR in the outbreak in Nursery B was 44%. The AR depends on the dose of ingested organisms, but for both pathogens the infectious dose is low [39,40]. The fact that the children in Nursery B did not wash their hands after close animal contact and before their meal, suggests that many of the children could have ingested an infective dose of the bacteria. This can explain the high AR seen in this outbreak compared with previously described farm-related outbreaks of pathogenic *E. coli*. However, ARs are subject to substantial variation in small cohorts and should be interpreted with caution.

Limitations

The small number of affected children hampered the epidemiological investigations. For example, we could not assess the risk ratio of participating in the farm visit, since almost all children participated. It is possible that we could have obtained more conclusive indications of appropriate prevention measures if we had included more detailed questions about the children's behavioural pattern on the farm, as has been described in a Swiss study [41].

Recall problems probably influenced the nursery personnel's answers to the questionnaires, reflected by the similarity between their answers for the different children in each nursery. It is conceivable that the nursery personnel had problems remembering details about food consumption and behaviour of each child. A possible differential recall of exposures by case status is also understandable.

For Nursery A we applied a combined method for DNA typing of *C. jejuni* that is as yet unpublished. However, the basic work has been described in several publications [25-28]. As we received faecal specimens from only four of the 12 suspected cases in Nursery A, we had to use the suspected case status together with the confirmed case status for the epidemiological analyses, rather than the confirmed case status alone.

For Nursery B, we did not examine the animal faeces for EPEC O76. The DNA profile of human and animal EHEC O26 isolates differed in one locus. When employing methods with large discriminatory power like MLVA, it is not unexpected for such small variations in DNA-profiles to occur within the short time frame of an outbreak. They reflect recent evolutionary divergence from a common ancestor, and do not preclude our conclusion regarding the source of infection. However, genotyping results must always be seen in context with the other results from the outbreak investigation.

Conclusion

The outbreaks affecting Nursery A and B were not part of the concomitant national outbreak of *E. coli* SF O157. This was an important finding, since the EHEC SF O157 outbreak caused nine HUS cases of whom one died, and identification of the source was a major priority at the time. Furthermore, we concluded that the nursery outbreaks were caused by contact with animal faeces during the farm visits. This is only the third time an outbreak in Norway has been traced to animal contact.

Recommendations

Increased popularity of petting farms may lead to the occurrence of similar outbreaks in the future. Consequently, authorities in Norway as well as in other countries need to enforce hygienic measures when visiting farms with children. We did not advise the farms and nurseries described here to stop arranging farms visits with children, but we recommended letting only the oldest children enter the animal pens, and keeping

them away from animals with diarrhoea, in addition to focusing on hand hygiene. Studies have reported that there is room for improvement concerning farm visitors' information on hygiene and hand washing in general [41-43]. To reduce human exposure to livestock faeces, several studies recommend a strict separation between picnic areas and animals, and to reinforce the importance of providing hand-washing facilities [2,42]. Previous findings suggest that active rather than passive interventions are more effective for increasing compliance [43].

The NIPH has published guidelines for farm visits with children [44]. In light of our findings, we recommend further efforts to spread and implement these guidelines among farmers and nursery staff.

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The '2012 European guideline on the diagnosis and treatment of gonorrhoea in adults' recommends dual antimicrobial therapy

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The '2012 European guideline on the diagnosis and treatment of gonorrhoea in adults' was launched on November 15, 2012 on the website for European STI Guidelines and is also accepted for publication in *International Journal of STD & AIDS* [1]. The newly launched guideline, an updated version of the 2009 European (IUSTI/WHO) guideline on the diagnosis and treatment of gonorrhoea in adults [2], provides up-to-date guidance on:

- broader indications for testing and treatment of gonorrhoea;
- the introduction of dual antimicrobial therapy. The recommended treatment for uncomplicated *Neisseria gonorrhoeae* infections of the urethra, cervix, rectum and pharynx, when the antimicrobial sensitivity is unknown, is a combination of ceftriaxone 500 mg and azithromycin 2 g;
- recommendation of test of cure in all gonorrhoea cases to ensure eradication of infection and identify emerging resistance;
- recommendations to identify, verify and report failures with recommended treatment regimens [3-5].

Further details regarding recommended diagnostics, recommended and alternative treatment regimens, treatment of ceftriaxone-resistant gonorrhoea, management of additional types of gonococcal infections or complications and of specific patient groups are also available in the newly launched guidelines [1].

Gonorrhoea remains a major public health problem. In 2008, the World Health Organization (WHO) estimated 106 million cases among adults worldwide (3.4 million in the WHO European region) [3]. In the European Union (EU), gonorrhoea is the second most commonly reported bacterial sexually transmitted infection (STI) after chlamydia [4]. However, the incidence is underestimated because of suboptimal diagnostics, case reporting and surveillance.

During recent years, resistance has been identified to the recommended extended-spectrum cephalosporins cefixime and ceftriaxone, the mainstay of first-line antimicrobial monotherapy for gonorrhoea [2]. Several treatment failures with cefixime and a few with ceftriaxone were recently verified in Europe, together with the first three extensively drug-resistant (XDR) *N. gonorrhoeae* strains with high-level ceftriaxone resistance [5,6]. In this developing situation, the WHO [3] and the European Center for Disease Prevention and Control (ECDC) [4] published global and regional action/response plans, respectively, to combat and mitigate the spread of multidrug-resistant gonorrhoea. This emergence of resistance has prompted revision of national and international treatment/management guidelines.

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