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Rapid communications

THE FIRST CASE OF LASSA FEVER IMPORTED FROM MALI TO THE UNITED KINGDOM, FEBRUARY 2009

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This is the first case of Lassa fever to be imported from Mali to the United Kingdom. This paper discusses the investigations, the virological analysis, the surveillance and management of contacts undertaken following a case of Lassa fever.

In February 2009, the twelfth recorded case of Lassa fever, since surveillance records are available, was imported to the United Kingdom (UK). This is the second case to be imported to the UK in 2009 and the first reported case to have acquired infection in Mali. Risk assessment of 117 UK healthcare contacts with potential direct exposure to the patient's body fluids was undertaken. Seven contacts are considered to be at high risk of infection and are being actively monitored for 21 days.

Background

Lassa fever is caused by an arenavirus and is an acute illness of between one and three weeks duration. The incubation period is usually seven to 12 days but may range between three and 21 days. About 80% of human infections in endemic areas are asymptomatic. The overall case fatality rate is 1%, although it is reported to be 15%-20% in hospitalised patients [1,2].

The natural host of Lassa virus is the multimammate rat (*Mastomys spec.*) which sheds the virus in urine and droppings. Transmission of the virus to humans usually occurs via direct or indirect contact with rodent excreta. Person-to-person transmission occurs through direct contact with blood, saliva, urine, faeces or semen [1].

Lassa fever is known to be endemic in parts of West Africa, with most cases reported from Guinea, Liberia, Sierra Leone and Nigeria. People living in rural areas of West Africa are most at risk of Lassa fever. Imported cases to the UK are rare and occur almost exclusively in individuals who have worked in endemic areas in high risk occupations such as medical or development workers [4]. Although there is some evidence of endemicity in neighbouring countries [1,3-5], this is the first case of imported Lassa fever from Mali into the UK.

Clinical case description

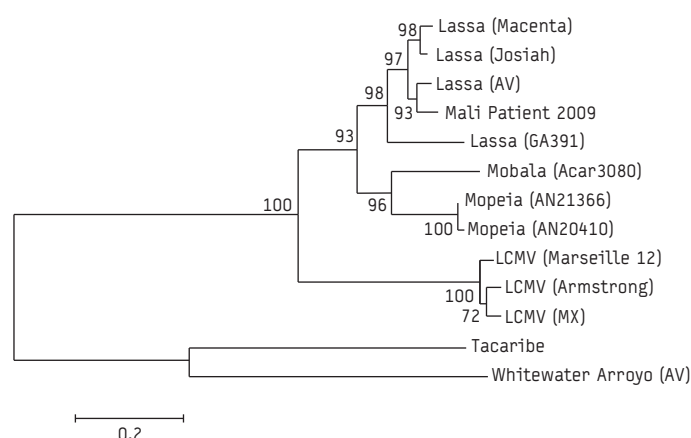
In February 2009, a man in his twenties was admitted to University College Hospital in London (UCLH) having been

medically evacuated from Mali with a 10-day history of fever and a diagnosis of falciparum malaria that did not respond to treatment. He had been in a village in southern Mali for four weeks, where he was working in remote rural conditions on the border with the Ivory Coast. He had travelled directly from the UK to Bamako, Mali and then travelled overland to southern Mali. Although precise details of possible exposure to rodents are not known, rodents including rats were seen regularly in the village.

On arrival the patient was alert and able to give a clear report on his medical history. However, he deteriorated rapidly and was transferred to a negative pressure room in the intensive care unit. He died of multi-organ failure later the same day. His malaria blood

FIGURE

Phylogenetic analysis of Arenaviruses, including the Lassa virus imported to the United Kingdom from Mali in February 2009



The tree was calculated by analysis of the 291 amino acids from the N-terminus of the lassavirus glycoprotein C using the neighbour-joining method. Bootstrap support (%) is indicated at each branch point. The virus from the patient described in this article (Mali patient 2009) is most closely related to the AV strain from Ivory Coast (Lassa AV) [7].

film and rapid antigen test were negative and a diagnosis of Lassa fever was confirmed the same night by PCR.

The patient was originally considered at low risk of Lassa fever because the disease has never been reported in Mali and is thus not considered to be endemic there. However, as he became more unwell his status was upgraded. Standard universal infection control precautions were followed and visors, but not full body protection, were worn during the attempted resuscitation.

Virological analysis

The diagnosis was confirmed in two different reverse transcription PCR (RT-PCR) assays targeting different regions of the genome and by sequencing of the 291 amino acids at the N-terminus of the Lassa virus glycoprotein C [6]. The detection of Lassa virus in two different RT-PCRs together with the characterisation of a unique part of the Lassa virus genomic sequence constituted a definitive diagnosis. Further studies including virus culture are in progress, and sequencing of the entire genome of the isolate is planned.

TABLE 1

Risk assessment for contacts of patients with Lassa fever

Risk Category	Description	Action and advice
Unclear	Not sure of contact	Reassure
		Inform to contact the infection safety officer should they recall any contact
		Give general factsheet
No risk (Category 1)	No direct contact with the patient or body fluids	Inform of absence of risk
	Casual contact e.g. sharing a room with the patient, without direct contact with body fluids	Advise to call if concerned following reading fact sheet
		No further action.
Low risk (Category 2)	Direct contact with the patient (e.g. routine medical/nursing care, handling of clinical/laboratory specimens), not handling body fluids or wearing personal protective equipment appropriately	Give category 1 (general) factsheet
		Self-monitor* for fever and other symptoms compatible with Lassa fever
		Report to the safety officer if temperature $\geq 38.0^{\circ}\text{C}$, with further evaluation as necessary
High risk** (Category 3)	Unprotected exposure of skin or mucous membranes (e.g. mucosal exposure to splashes, needlestick injury) to potentially infectious blood or body fluids, including unprotected handling of clinical/laboratory specimens	Give category 2 factsheet
		Record own temperature daily for 21 days following your last contact with the patient and report this temperature to the safety officer by 12 noon each day, with further evaluation as necessary
		Give category 3 factsheet
		Inform Health Protection Unit immediately if contact reports symptoms compatible of Lassa fever and further risk assessment is required

*Level of risk according to exposure and action and advice by category.

*Contacts to be monitored for 21 days from last possible exposure to case

** Within this group, consider ribavirin prophylaxis if any extreme exposure, e.g. percutaneous injury

TABLE 2

Categorisation of contacts in the United Kingdom, Lassa fever importation, February 2009

Contacts classification	Risk category			
	Category 1 (no risk)	Category 2 (low risk)	Category 3* (high risk)	Total
Intensive care unit staff	3	14	2	19
Accident and emergency (A&E) staff	17	12	0	29
Laboratory staff	21	45	3	69
Family	1	2	2	5
Colleague in Mali	0	1	0	1
UK ambulance service**	2	0	0	2
Totals	44	74	7	125

*Within this group, ribavirin prophylaxis was considered in the case of any extreme exposure e.g. percutaneous injury

** The air ambulance medics were still in attendance whilst transferring the patient to University College Hospital in London. The patient was wrapped in a tarpaulin sheet and the UK ambulance crew adopted universal barrier precautions, hence both were considered casual contacts and not at risk (category 1).

Phylogenetic analysis showed that the virus was distinct from other Lassa virus strains but grouped most closely with a strain of Lassa virus (Lassa (AV)) isolated from a case reported from Germany in 2000 [7] (Figure). The German patient had travelled through Ivory Coast, Ghana and Burkina Faso during the incubation period and the investigations could not determine where he had acquired the virus. The British case reported here had been working close to the border with Ivory Coast.

Surveillance and management of contacts

An Incident Control Team (ICT) meeting was called by UCLH early the following day to discuss risk assessment of contacts, safe decontamination of the environment and management of the body.

Risk assessment

The ICT identified 123 people who could have come into direct contact with the Lassa virus either through contact with the case or exposure to body fluids. Almost all of these contacts were UCLH emergency care and laboratory staff. All UK based contacts were assigned to one of three categories depending upon their level of risk (no risk, low risk or high risk, see Table 1) and were managed as reported recently [8]. Contacts will be monitored for 21 days from exposure.

International contacts

The German air ambulance crew are being followed up and managed by German authorities, and the World Health Organization (WHO) is supporting health authorities in Mali in conducting field investigations and in the implementation of control measures.

Risk assessment outcome and follow up

The outcome of the UK risk assessment is shown in Table 2. None of the category 3 contacts received ribavirin prophylaxis. The evidence base for the use of ribavirin prophylaxis is limited, but category 3 contacts were given information explaining its possible benefits and side effects and were left to make an informed choice.

Discussion

In the case described here, the reported diagnosis of malaria and the fact that Mali has not been considered endemic for Lassa fever made the clinical diagnosis difficult. As a consequence, the initial risk of Lassa fever was considered low. Only when the patient developed multi-organ failure six hours after admission was the risk of Lassa fever upgraded. Universal barrier precautions were used throughout, but not the high levels of protection currently recommended for viral haemorrhagic fevers [9]. As a result, 76 hospital staff were put at risk in the space of eight hours, and three of seven category 3 contacts were laboratory staff. Although transmission to healthcare workers from imported Lassa fever cases is very rare, this can cause considerable anxiety among contacts. There is only one reported case of transmission in a hospital setting in an industrialised country, and this was a seroconversion without clinical illness in Germany [10].

This is the first Lassa virus to be characterised from Mali. The virus is closely related to isolates from neighbouring countries and was amplified using a widely used diagnostic PCR test [6]. There is serological evidence that Lassa virus is present in Mali [3,5], but this is the first proven imported case and has implications for current risk assessment in travellers returning from this area.

Acknowledgements

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Rapid communications

WHERE IS WEST NILE FEVER? LESSONS LEARNT FROM RECENT HUMAN CASES IN NORTHERN ITALY

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West Nile disease in humans has been detected for the first time in Italy in two regions, Emilia-Romagna and Veneto. Surveillance subsequently set up in Veneto detected a case of West Nile neuroinvasive disease and a few asymptomatic infections, but no case of West Nile fever. We conclude that also West Nile fever cases should be specifically targeted by surveillance.

Background

The first equine outbreak of West Nile virus (WNV) infections in Italy was reported in 1998 in Tuscany (province of Florence) [1]. Subsequent active human surveillance of stable workers identified four individuals who were seropositive but did not report any symptoms [2]. To our knowledge, no human passive surveillance (for ill people) was carried out in Italy at that time.

For the purpose of this paper, we define active surveillance as the active search for infected workers in stables who reported equine disease, while we define passive (rapid) surveillance as the screening for WNV disease among patients who seek medical care and fulfil the case definition (of suspected cases) outlined below.

Since 2001, a national veterinary surveillance system based on periodic testing of sentinel chickens and horses has identified sporadic seroconversions [3] without further occurrence of actual WNV disease until 2008 [4]. In August 2008, an equine WNV outbreak was detected in areas surrounding the Po river delta, involving the regions Emilia-Romagna (provinces of Bologna and Ferrara) and Veneto (province of Rovigo) (Figure 1). Subsequently, active and passive human surveillance was started in both regions [5].

Case definition

West Nile fever is the most common clinical presentation of West Nile disease, and is characterised by a influenza-like, usually self-limiting febrile illness, usually accompanied by headache and joint and/or muscular pain, and sometimes by other non-specific symptoms including a maculopapular rash. West Nile neuroinvasive disease is a rarer, severe presentation characterised

by meningitis, encephalitis or infection of spinal motor neurons, with a high frequency of transient to permanent neurologic sequelae in survivors [6].

FIGURE 1

West Nile virus outbreaks in Italy, 1998 (horses) and 2008 (horses and humans)



The case definition of suspected cases (passive surveillance) was the same in both regions and specifically applied to West Nile neuroinvasive disease, including patients aged 15 years and older, with fever ≥ 38.5 °C and neurological symptoms such as encephalitis, meningitis, Guillain-Barré syndrome or acute flaccid paralysis.

Cases were further classified as:

- possible: clinical symptoms and clear (aseptic) cerebrospinal fluid (CSF);
- probable: clinical symptoms and at least one of the following laboratory criteria: ELISA detection of IgM antibodies against WNV; seroconversion; fourfold increase of IgG antibodies in acute- and convalescent-phase serum samples (preferably with 15-20 days between the two samples);
- confirmed: clinical symptoms and at least one of the following laboratory criteria: isolation of WNV from blood or CSF; ELISA detection of IgM antibodies in CSF; positive RT-PCR in blood and/or CSF; ELISA detection of increasing levels of IgM and IgG antibodies against WNV, confirmed by neutralisation testing carried out at the reference laboratory of the Istituto Superiore di Sanità in Rome [5].

Asymptomatic WNV infection was defined by positive serology in individuals identified through active surveillance and reporting no clinical symptoms.

West Nile virus in the Emilia-Romagna region, 2008

In September 2008, the first human case of West Nile neuroinvasive disease in Italy was reported in an 83 year-old female resident of rural Emilia-Romagna (province of Bologna) [7]. In October, another two neuroinvasive cases were reported in the same region (province of Ferrara). To the best of our knowledge, no case of WNV fever was reported in this region (L. Venturi, personal communication).

West Nile virus in the Veneto region, 2008

Passive surveillance

In October 2008, human passive (rapid) surveillance in Veneto identified an 81 year-old female resident of the province of Rovigo who was hospitalised with viral meningoencephalitis. As of 11 March 2009, the patient remains hospitalised in critical condition.

Active surveillance

Four WNV asymptomatic infections were identified by active surveillance (see Table and Figure 2). All serologically confirmed cases were WNV infections reported retrospectively by active surveillance: when WNV disease in horses was known to have occurred in a given stable, all stable workers were contacted by the

regional health teams, asked to complete a clinical questionnaire, and serum samples were taken for laboratory analysis as outlined above.

Other

In November 2008, a 48 year-old female resident of the province of Rovigo spontaneously presented at the Centre for Tropical Diseases in Negrar (Verona) and reported a fever episode three months previously, accompanied by maculopapular rash, adenopathy and severe headache, the latter still incompletely resolved as of January 2009. The patient had fully recovered in February.

At the time of the acute episode she had not been tested. Serologic testing in November 2008 (ELISA and neutralisation test) confirmed her as a case of WNV fever. As this case was identified neither by active nor by passive (rapid) surveillance, it is classified in the Table below as "other".

In February 2009, a second, probable human case of West Nile neuroinvasive disease in the same province in Veneto was identified retrospectively (highly positive IgG, ELISA test), although a positive PCR for Epstein-Barr virus in the CSF casts some doubts on this case. Laboratory confirmation is still pending, and the case is not included in the Table.

Discussion

In both Emilia-Romagna and Veneto, human surveillance was initiated once veterinary surveillance had identified the first cases in horses in August, 2008. Active surveillance targeted workers of infected stables, while passive (rapid) surveillance concerned suspected cases.

Not surprisingly, surveillance (in both Veneto and Emilia-Romagna) only detected either asymptomatic cases (active surveillance) or neurological cases (passive surveillance). West Nile fever cases were not reported, although this is by far the most frequent presentation of the disease. According to the literature, about 80 of 100 WNV infections are virtually asymptomatic, 20 are cases of West Nile fever, while the neuroinvasive disease accounts for less than 1% [6].

A major shortcoming of the current surveillance system is the concentration on neurological disease. Once animal or human cases have been detected, passive surveillance in humans should be broadened to include not only neurological disease, but also West Nile fever cases, in order not to miss the true extent of an outbreak.

The clinical picture of West Nile fever consists of fever with a variable combination of accompanying symptoms [6]. The case definition of a suspected case should therefore include any

TABLE

Individuals screened by active and by passive (rapid) surveillance for West Nile virus in the Veneto region, Italy, August 2008-March 2009

Type of surveillance	Screened	Positive asymptomatic	Positive for West Nile fever	Positive for West Nile neuroinvasive disease	Negative
Active	231	4			227
Passive (rapid)	7			1	6
Other	6		1		5
Total	244	4	1	1	238

unexplained fever, with or without rash, headache, adenopathy or joint pain, occurring in areas where veterinary disease is reported. Any more specific definition would not be sensitive enough to detect most cases.

Although West Nile fever is a benign disease, many reasons would justify making passive human surveillance as sensitive as possible during an outbreak. Firstly, the potential risk involved for blood transfusions [8] and transplantations [9] is not negligible: although viraemia roughly coincides with fever, and febrile patients would be suspended from blood or transplant donation in any case, effective surveillance of fever would better identify the geographic areas where human cases occur and where precautionary restrictions should be introduced. Secondly, West Nile fever itself may be a more severe illness than had previously been thought and recovery may require more than two months [6]: although there is no specific treatment to be offered, it is important to reassure the patients with regard to the cause of their complaints.

In summary, experience from the recent outbreaks highlights a need to review the current surveillance system for WNV. Timely diagnosis and reporting not only of cases of West Nile neuroinvasive disease, but also of West Nile fever cases would allow a more accurate assessment of the geographic distribution of WNV infection and guide control measures [10].

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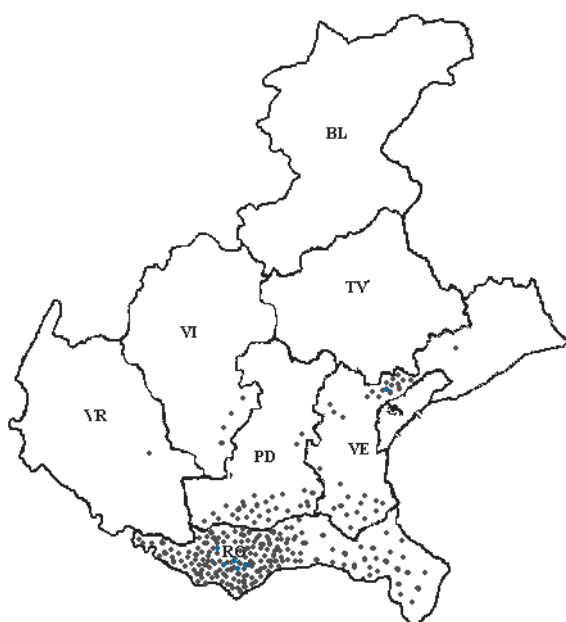
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FIGURE 2

Serology results (positive in blue, negative in gray) in the provinces of Veneto, Italy, August 2008-March 2009



RO: Rovigo; VR: Verona; VI: Vicenza; PD: Padova; VE: Venezia; TV: Treviso; BL: Belluno

Surveillance and outbreak reports

AN OUTBREAK OF *SALMONELLA* TYPHIMURIUM INFECTIONS IN DENMARK, NORWAY AND SWEDEN, 2008

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In November-December 2008, Norway and Denmark independently identified outbreaks of *Salmonella* Typhimurium infections characterised in the multiple-locus variable number of tandem repeats analysis (MLVA) by a distinct profile. Outbreak investigations were initiated independently in the two countries. In Denmark, a total of 37 cases were identified, and multiple findings of the outbreak strain in pork and pigs within the same supply chain led to the identification of pork in various forms as the source. In Norway, ten cases were identified, and the outbreak investigation quickly indicated meat bought in Sweden as the probable source and the Swedish authorities were alerted. Investigations in Sweden identified four human cases and two isolates from minced meat with the distinct profile. Subsequent trace-back of the meat showed that it most likely originated from Denmark. Through international alert from Norway on 19 December, it became clear that the Danish and Norwegian outbreak strains were identical and, later on, that the source of the outbreaks in all three countries could be traced back to Danish pork. MLVA was instrumental in linking the outbreaks in the different countries and tracing the source. This outbreak illustrates that good international communication channels, early alerting mechanisms, inter-sectoral collaboration between public health and food safety authorities and harmonised molecular typing tools are important for effective identification and management of cross-border outbreaks. Differences in legal requirements for food safety in neighbouring countries may be a challenge in terms of communication with consumers in areas where cross-border shopping is common.

Introduction

The endemic level of salmonellosis in three Nordic countries Norway, Sweden and Finland is low. The majority of cases are acquired during travel abroad, and among domestic cases, *Salmonella* Typhimurium is the most common serovar [1]. In Norway approximately 70-80% of notified cases of salmonellosis are acquired abroad, and among patients infected in Norway, *S. Typhimurium* is the most common serovar with generally 5-15

domestic cases reported monthly [2]. In Sweden the situation is similar, with the majority (70-80%) of human cases being travel-related and *S. Typhimurium* being the most common domestic serovar [3]. Norway, Sweden and Finland have special rules concerning trade of meat and meat-products within the European Union (EU), requiring that each consignment produced in another EU Member State and destined to be sold in these countries must be accompanied by a certificate stating that the product has been analysed for the presence of *Salmonella* according to a defined procedure [4]. In Denmark, both the epidemiological situation and legal requirements regarding *Salmonella* in meat is different. An estimated 40-50% of *Salmonella* cases are travel-related [5] and *S. Typhimurium* is traditionally the second most frequent serotype after *S. Enteritidis*. However, a series of large *S. Typhimurium* outbreaks occurred in Denmark in 2008 [6], making *S. Typhimurium* the most frequent serotype in 2008 [7].

In order to rapidly identify possible outbreaks, Norway and Denmark routinely genotype all *S. Typhimurium* patient isolates with the multiple-locus variable number of tandem repeats analysis (MLVA) method [8]. The reference laboratory in Norway receives isolates from human cases as well as from animals, foods and feed. The database on MLVA-typed isolates currently comprises MLVA profiles from more than 3,000 isolates from both human and non-human sources, collected since 2004. In Denmark, human *S. Typhimurium* isolates are routinely MLVA-typed and phage-typed, while food and animal isolates are only phage-typed. In outbreak investigations food isolates with matching antibiograms and matching or related phage types are MLVA-typed. The Danish database currently comprises more than 4,000 human and non-human isolates collected since December 2004. If clusters of cases with a specific MLVA profile are detected, an investigation is initiated to verify and control the outbreak. In Sweden, isolates of *S. Typhimurium* from human, animals and feed are routinely phage-typed, and food isolates are phage-typed upon request. Until early

2009, MLVA-typing was only performed on clusters of various phage types of particular interest from an epidemiological point of view.

Outbreak detection

On 7 November 2008, the Danish Statens Serum Institut (SSI) registered a cluster of eight recent cases of *Salmonella* Typhimurium with the same new distinct MLVA profile. On the same day the Zoonosis Laboratory at the Danish National Food Institute identified two isolates from pork products with the same MLVA profile.

On 4 December 2008, the Norwegian Institute of Public Health (NIPH) also registered a cluster of six cases with *S. Typhimurium*-infection with a new, distinct MLVA profile, all submitted during the previous month.

In both countries independent outbreak investigations were initiated in order to identify the source of these infections and prevent further spread. Investigations in Sweden were undertaken later, following information from Norway on the possible source of infection in meat purchased in Sweden.

Methods

Case definition

For the purpose of outbreak investigation, a common case definition was used in all three countries. A case was defined as having a laboratory-confirmed *Salmonella* Typhimurium infection with the distinct MLVA-outbreak profile, and with illness onset after 1 September 2008. The MLVA profile was assigned as 3-12-4-13-2 (using allele numbers suggested by Lindstedt et al.); sizes of fragments were STTR9: 181 bp, STTR5: 275 bp, STTR6: 319 bp, STTR10: 370 bp, STTR3: 490 bp [8].

Patient interviews

In Denmark, seven among the initial cases were interviewed using a trawling questionnaire with focus on consumption of pork and pork products, and remaining cases were interviewed using a short standard questionnaire. In Norway, all cases were interviewed with a detailed standard trawling questionnaire for food-borne outbreaks. In Sweden, the cases were interviewed regarding general risk exposure for *Salmonella* with additional questions on supermarkets visited and travel history to either Norway or Denmark, as well as a focus on pork products consumption.

Microbiological investigation

In Denmark, due to a large ongoing outbreak of *S. Typhimurium* U292 [6], a temporarily intensified programme for surveillance of *Salmonella* isolates from food production facilities was set up in September 2008. As a result of this programme, a number of isolates were referred to the Danish National Food Institute for analysis. Isolates were phage-typed, and all isolates with phage types matching the human isolates were MLVA typed.

In Norway, food products from patients' homes, identified to be at risk, were sampled and tested. When preliminary results indicated presence of *Salmonella*, the isolates were sent from the local microbiological laboratories to the reference laboratory at NIPH for verification and MLVA-typing.

In Sweden, *Salmonella* Typhimurium RDNC and later also U302 isolates (due to relatedness with the phage type reactions in the Norwegian and Danish isolates) collected from patients and food products during late 2008 and early 2009 were typed with MLVA.

Environmental investigation

In all countries, detailed information regarding place and date of purchase of suspected products was collected from the

patients in order to trace the contaminated consignment. In Denmark, the Food Safety Authority obtained detailed information regarding distribution of contaminated batches. In Sweden, the local environmental health authority visited the relevant shops, checked their hygiene routines, traced the origin of suspected meat products and checked the *Salmonella* certificates on imported meat consignments.

Results

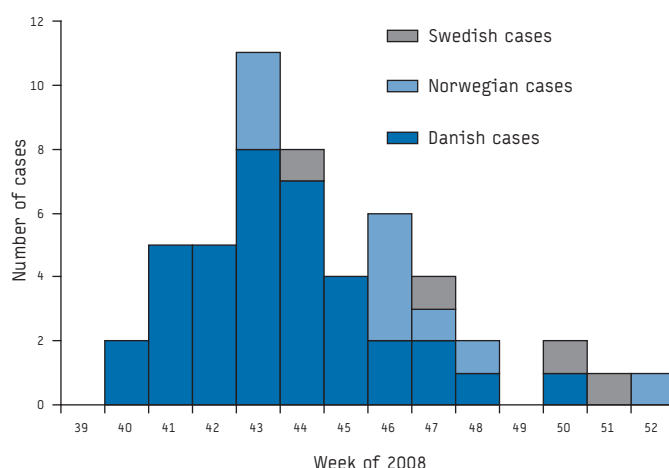
The investigation in Denmark

A total of 37 cases were confirmed. The outbreak strain was fully sensitive to all antibiotics tested and determined to be phage type U288 or RDNC. The majority of patients became ill in October and November (Figure 1). The median age of the cases was 54 years (range 1-86 years) and 15 were female. Four patients died, all were older than 75 years, and suffered from underlying illnesses. The precise causes of death could not be established, and it remains unclear to what degree the *Salmonella* infection contributed as a cause of death.

Within two weeks following the detection of the outbreak (on 7 November), the outbreak strain was identified among *S. Typhimurium* isolates from Danish pork meat (6 times) and pork products (4 times; raw pork sausage, raw pork roulade and twice in minced pork). The pork and pork products originated from 6 different companies. Of these, one company (in which most samples with the outbreak strain were found) was a cutting plant that supplied meat to the other five companies all of which were wholesalers. In addition, the outbreak strain was found in samples from a sow herd in December 2008. In the period during which the outbreak took place, pigs originating from the sow herd, but reared at other farms, were mainly slaughtered at two different slaughterhouses. Subsequently, it was also recognized that during 2008 there was an increased *Salmonella* seroprevalence in some of these slaughter pig herds. This was detected through the Danish serological *Salmonella* surveillance programme [5,9]. One of the slaughterhouses supplied meat to the incriminated cutting plant.

FIGURE 1

Cases of *Salmonella* Typhimurium in an international outbreak affecting Denmark (n=37), Norway (n=10) and Sweden (n=4), October-December 2008, by week of onset of illness (n= 51)



The majority of cases (30) were from Zealand (Figure 2); relatively many from the less densely populated south-western part of the island. The culture-positive meat processing plant, the source of the outbreak. The particular MLVA-pattern was found for the first time in Denmark in three patients with onset dates in June and July, 2008. They were not counted among the outbreak cases, though it remains possible that their infections also originated from the same pig herds.

The results of the patient interviews were compatible with the hypothesis that fresh pork meat and different pork products originating from the two above mentioned slaughterhouses were the source of the outbreak. The particular MLVA-pattern was found for the first time in Denmark in three patients with onset dates in June and July, 2008. They were not counted among the outbreak cases, though it remains possible that their infections also originated from the same pig herds.

The incriminated cutting plant and one of the two slaughterhouses had been selling pork and beef to a number of Swedish establishments. No direct trade link between the Danish

cutting plant and the slaughterhouse on one hand and the Swedish shops on the other was evident in the sales register from the Danish establishments. However intermediary establishments in Sweden were involved in distributing the meat. Links from Denmark to the Swedish shops were thereby established (Figure 3).

The investigation in Norway

Ten cases were verified with the outbreak strain. The outbreak strain was fully sensitive to all antibiotics tested and determined to be phage type RDNC. The patients were all adults (21-80 years) living in the south-eastern part of Norway and their illness onset was between the end of October and the end of December (Figure 1 and 2). Eight patients reported that during the week before illness onset, they had consumed minced meat purchased at shopping centres located across the border in Sweden. Four of them remembered having eaten raw, rare or undercooked minced meat. Several had tasted raw minced meat while preparing food. The minced meat was either a mix of pork/beef or only beef, but most said they were not sure about this. The outbreak strain, with the rare MLVA profile, was isolated from samples of minced meat from the homes of two patients, but since the product had been repacked in patients' households, the original wrapping with product information was not available. However, one of the patients provided a bank printout that confirmed the exact place (retail outlet) and date of purchase, thereby facilitating further traceback along the food chain.

International alerts

On 15 December, after receiving completed questionnaires from five Norwegian patients, all of whom reported consumption of meat bought in Sweden the week before illness onset, NIPH notified the Swedish Institute of Infectious Disease Control (SMI) about the outbreak and asked if they had seen similar isolates of *S. Typhimurium*. In reply, SMI reported no findings of the specific RDNC phage pattern. On 19 December, an urgent inquiry was sent through the Food- and Waterborne Diseases network at the European Centre for Disease Prevention and Control (ECDC), and in response, Denmark reported the ongoing phage type U288 outbreak with identical MLVA-profile.

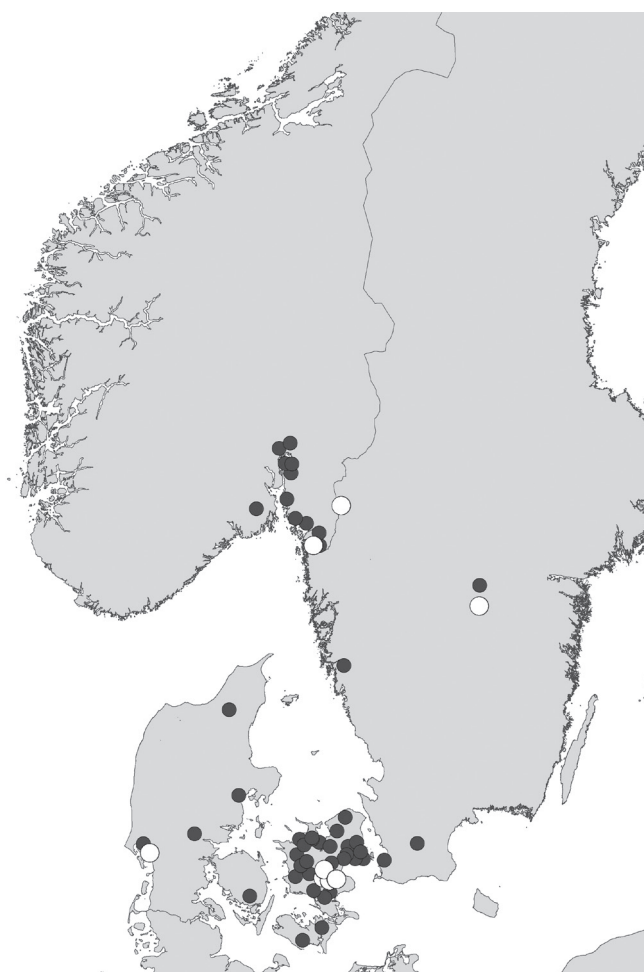
The investigation in Sweden

In Sweden four cases were confirmed with the outbreak MLVA profile. The patients were all adults, and three were in their 50s. They fell ill between October and December (Figure 1) and were from three different counties in the south of Sweden (Figure 2). One patient had been living and working in Copenhagen before disease onset and was most likely infected there. These cases were identified following MLVA-typing of recent patient-isolates belonging to phage type U302.

On 23 December, the Swedish National Food Administration found that the shops the Norwegian patients had visited, were selling pork from three Danish companies, one of which was the cutting plant incriminated during the investigations in Denmark. The two positive minced meat samples from the Norwegian patients contained only beef according to information from the shops. However, cross-contamination from other sources could have occurred during mincing in the shops. The Swedish environmental health authority could not identify any faults or breaches in the routines of the shops, and there was no meat from the relevant time period available for sampling. They could also confirm that the companies had thoroughly checked the *Salmonella* certificates of all consignments from other countries.

FIGURE 2

Place of residence of human cases of *Salmonella Typhimurium* (dark grey, n=51) and companies/shops (white, n=8) where meat was bought by cases or found positive with the outbreak strain of *Salmonella Typhimurium*, Denmark, Norway, Sweden, 2008



Sweden found two isolates with the outbreak strain from minced meat. The first minced meat sample was taken in November from a grocery store in the south of Sweden (Figure 2). A follow-up sample taken from the meat-grinder in the shop one week after the first, was also positive for *S. Typhimurium*, and both had the outbreak MLVA profile. This indicates that there was a persistent contamination of the grinder. This grocery store had been selling pork from the incriminated Danish cutting plant on some occasions during October and November. None of the four Swedish patients had been to this store nor to the shops visited by the Norwegian patients. However one of them had bought meat in another Swedish shop receiving meat from the above mentioned Danish slaughterhouse.

Product tracing

Product trace investigation revealed the trade route for meat from the Danish cutting plant to shops in Sweden, both to shops near the border where the Norwegian cases had bought meat and to another one where minced meat samples had tested positive for *Salmonella*, thus, confirming the link between Danish meat and positive findings of *Salmonella* in the environment and minced meat samples. Furthermore tracing of products from the second Danish slaughterhouse revealed a link to yet another Swedish shop indicating a possible second route of dissemination of contaminated meat to Sweden (Figure 3).

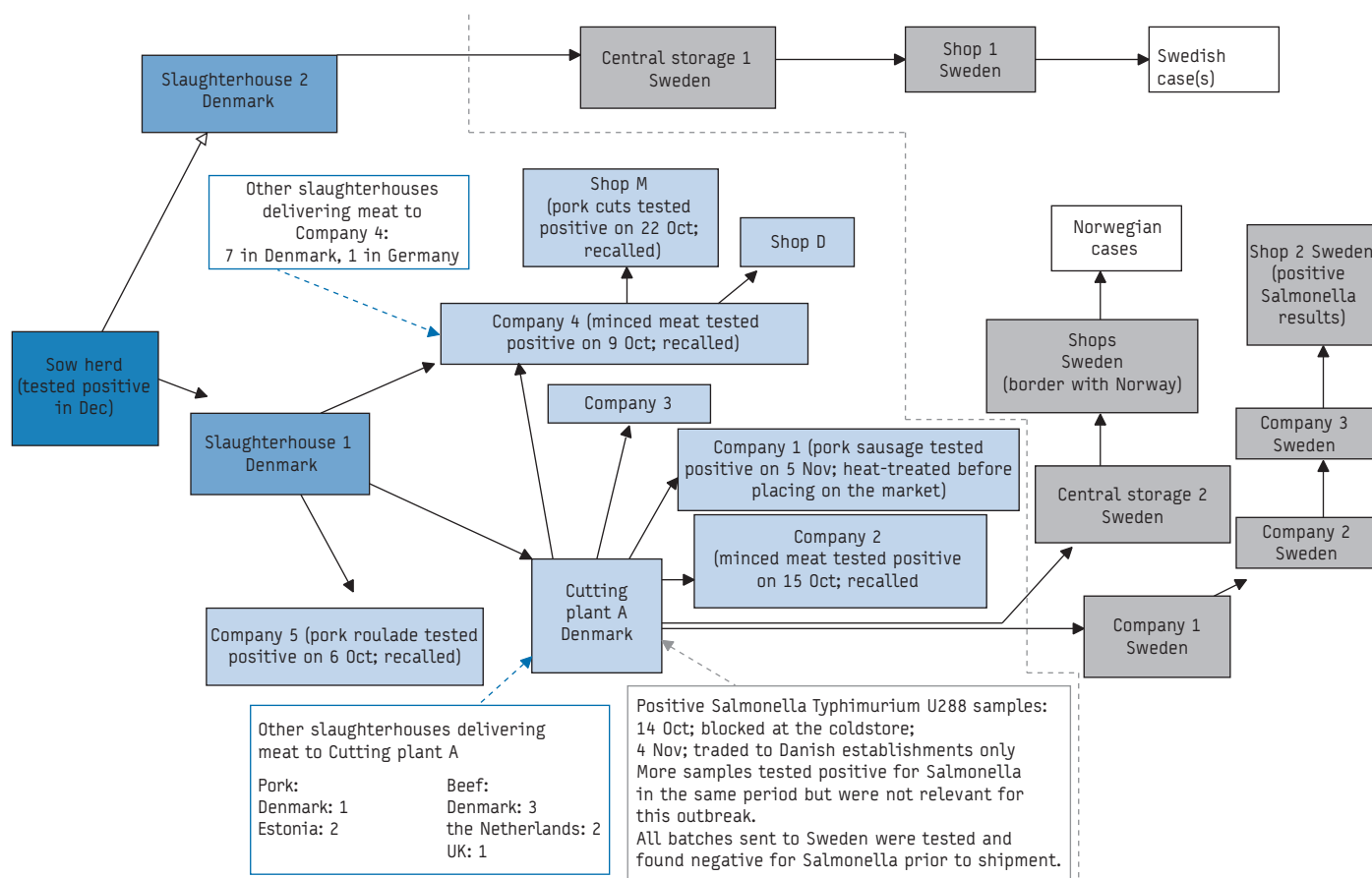
Control measures

In Denmark, following the multiple findings of the outbreak strain in food products, investigations were undertaken at the facility producing the raw pork sausage, as well as the cutting plant and the slaughterhouses that supplied meat to the cutting plant. Samples were taken for analysis from the cutting plant and the sausage-producing facility. The microbiological analysis did not identify *Salmonella*, which suggests that these facilities did not harbour persistent infections in their production environments. Furthermore, all analytical reports concerning batches of meat sent to Sweden from the cutting plant and one of the two slaughterhouses, were reviewed for sampling consistency according to the legal requirements. No known positive batches were put on the Swedish market, and the requirements concerning sampling and analyses were fulfilled.

In Norway, on 7 January, NIPH and Norwegian Food Safety Authority (NFSA) published an Internet update on the outbreak, in which the Norwegian public was informed that the *Salmonella* outbreak strain had been detected in minced meat bought in Sweden, and consumers were advised about safe handling of meat. This was followed by international alerts by the NFSA through the Rapid Alert System for Food and Feed (RASFF) [10] on 8 January, and by NIPH through the Early Warning and Response System (EWRS) on 9 January.

FIGURE 3

Trade route diagram for establishments involved in the outbreak of *Salmonella* Typhimurium in Denmark, Norway and Sweden, 2008



In Sweden, no further measures were taken, since the contaminated meat was not available in the shops anymore, and all environmental control samples from the shops were now negative.

Discussion

We report an outbreak of *S. Typhimurium* affecting three Nordic countries. The link between the outbreaks was established thanks to cross-border information exchange. Outbreaks that seem local may have international connections, and therefore early alerts are important for efficient investigation and management of such events. In the example described here, MLVA typing was instrumental in identifying and defining the outbreaks, in revealing the possible food/animal sources, and in establishing the link between the outbreaks in Denmark and Norway, and subsequently Sweden. We note with interest that the outbreak strain was communicated between the countries as belonging to three different phage type assignments: U288 in Denmark, RDNC in Norway and U302 in Sweden. Thus, in this outbreak, it would have been misleading if only phage type information had been exchanged between the countries. MLVA-typing has previously been successfully used in outbreak investigations in the Nordic countries [11-14].

In Sweden four human cases with the same MLVA profile were identified in addition to isolates from minced meat samples from a grocery store. No link between the cases and this grocery store or the shops identified by the Norwegian cases, could be established. However one of the Swedish patients reported buying meat in another Swedish shop, which was receiving meat from the incriminated Danish slaughterhouse. This shop belongs to a retail chain and is supplied from a central storage facility, and a possible connection to two more of the Swedish cases is likely. The fourth case was most probably infected during a stay in Denmark.

In Sweden, MLVA typing of human *S. Typhimurium* isolates had only been performed when epidemiologically relevant. However, as a result of this outbreak, as well as the Swedish experience with analysis of several clusters of other phage types of *S. Typhimurium* during 2008, it has now been decided to use MLVA to type all domestic human and other relevant *S. Typhimurium* isolates.

This outbreak also calls attention to some aspects concerning trade between countries with different endemic situation and regulations regarding *Salmonella*. Due to the endemic situation in Sweden and Norway (and also Finland) with a very low prevalence of *Salmonella* in domestic food, additional sampling for *Salmonella* is required on all fresh meat consignments sold to these countries from other EU/EEA countries [4].

The EC regulation on microbiological criteria for foodstuffs, says that when testing against set food safety criteria gives unsatisfactory results, the product or batch of foodstuffs shall be withdrawn or recalled [15]. However, products placed on the market, which are not yet at retail level and which do not fulfil the food safety criteria, may be submitted to further processing by a treatment eliminating the hazard in question provided that this use does not pose a risk for public or animal health and that this use has been authorised by the competent authority [16].

In Sweden, meat processing plants can get permission to heat-treat fresh meat contaminated with *Salmonella*, thereby eliminating the health hazard. However it is very uncommon that companies apply for this. Norway is due to implement EU harmonised

legislation in this area, thus in near future the same option to sanitize contaminated meat applies.

In this outbreak, the source of the Norwegian cases could be traced back to shops located in Sweden close to the border with Norway, selling meat from Danish producers. These shops target Norwegian consumers. Information about the outbreak and the source was made public in Norway, since several of the cases in Norway reported consuming raw meat. This illustrates some of the challenges regarding food safety advice to consumers in areas where cross-border shopping is common, as the consumers may not be aware of information about or recalls of products in neighbouring countries.

In conclusion, this outbreak illustrates that good international communication channels, early alerting mechanisms, inter-sectoral collaboration between public health and food safety authorities and harmonised molecular typing tools, are important for effective identification and management of cross-border outbreaks.

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Surveillance and outbreak reports

AN OYSTER-ASSOCIATED HEPATITIS A OUTBREAK IN FRANCE IN 2007

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Following the notification of nine hepatitis A cases clustered in the Côtes d'Armor district in northwestern France, epidemiological, environmental and microbiological investigations were set up in order to identify the source and vehicle of contamination and implement control measures. In total, 111 cases were identified in the outbreak, all of whom lived or had stayed as tourists in the Côtes d'Armor district. Of the cases, 87% had eaten raw shellfish, and 81% specifically oysters. Traceback investigations carried out on raw shellfish consumed by the cases showed that the raw shellfish originated from a single shellfish farm. The shellfish were probably contaminated either in the submersible tanks or in a depuration land-based tank where they were stored. The source of contamination was not identified but shellfish could have been tainted by sewage overflows or by wastewater releases from a polluted storm sewer close to the shellfish farm or from on-site sanitation facilities. To prevent future hepatitis A outbreaks due to shellfish consumption from this area, hazards specific to each farm should be analysed. Timely information on sewage overflows should also be part of communities' efforts regarding sewage collection and treatment.

Introduction

Hepatitis A virus (HAV) is transmitted via the faecal-oral route by either person-to-person contact or consumption of contaminated food or water. The incubation period ranges from 15 to 50 days with a mean of 30 days. The disease is usually diagnosed by detection of immunoglobulin M antibodies to hepatitis A (IgM anti-HAV) in the serum.

In France, surveillance of acute hepatitis A has been based on mandatory notification since November 2005. The notification form collects information on socio-demographic, clinical, biological characteristics and main at risk exposures to HAV infection. The incidence of reported cases of hepatitis A (notification rate) was 2.2/100,000 in 2006 and 1.6/100,000 in 2007 [1].

Between 14 and 21 August 2007, nine hepatitis A cases were notified to the district health services of the Côtes d'Armor (Brittany). Eight of them lived or had recently stayed in the northwestern area of the district, including four who were closely clustered near the same seaside resort - Paimpol bay - and seven who reported having eaten oysters. An investigation was carried out to confirm the outbreak, to assess its size, to identify the source and vehicle of contamination, and to implement appropriate control measures.

Methods

Epidemiological investigation

A case was defined as a person with IgM anti-HAV detected in the serum between 1 July and 15 October 2007 who had stayed in the Côtes d'Armor district in the six weeks before the onset of symptoms, whether as resident or as tourist.

The cases were identified through mandatory notification. In addition, in the Côtes d'Armor district, biologists, general practitioners, paediatricians, gastroenterologists and emergency physicians were informed about the current outbreak and asked to notify HAV cases.

The cases were interviewed by telephone using a standardised questionnaire about date and symptoms, place of residence, date and place of stay in the Côtes d'Armor district for tourists and possible exposures defined as food consumption and place of purchase, school or child care center attendance, travel, household contact with a case, participation in specific events or activities and bathing in recreational water.

Microbiological analysis

The biologists were asked to send the cases' sera to the National Reference Centre (CNR) for genotyping and phylogenetic analysis. A 452 base-pair fragment encompassing the VP1/2A junction was amplified and the phylogenetic tree was constructed with the MEGA software. The HAV genotype was determined using referent

sequences whose GenBank accession numbers were X75215, ABO20264, AF357222 for genotype IA, M14707 and M20273 for genotype IB, AY644676 for genotype IIA, AY644670 for genotype IIB, AY644337 and AJ299464 for genotype IIIA, D00924 for genotype V. Two other sequences, published by the CNR in the Event (Enteric Virus Emergence, New Tools) database were added for genotype IIIA: 2004-AUV-SEF-GIII and 2004-PB-CL-GIII.

Traceback investigations

Traceback investigations were carried out on suspected contaminated food for cases who had stayed briefly (less than 15 days) in the Côtes d'Armor district during the estimated at risk period and for cases included in clusters with common meals (as at family events). For these cases, places and dates of purchase and consumption of the suspected food could be determined precisely.

Environmental investigation

In order to determine the origin of the contamination of the suspected shellfish, the functioning of the sewage system and wastewater treatment plants located around Paimpol bay during June and July 2007 was investigated.

Laboratory testing for HAV was performed on shellfish samples collected between 24 August and 24 October from shellfish beds of Paimpol bay and on storage tanks located on the foreshore. Wild oysters around the bay, storm sewage, sludge, raw and treated sewage were also sampled for microbiological analyses. Viruses were extracted from shellfish tissues or concentrated from 40 ml of water samples before extraction and purification of nucleic acids [2,3]. All steps were controlled by adding a mengovirus at the first step of the extraction (extraction efficiency control) or external control RNA (inhibitors removal controls) in the real-time RT-PCR mix. Real-time RT-PCR was done as described [4].

Results

Epidemiological investigation

One hundred and eleven cases were identified. The symptoms occurred between 25 July and 9 October (weeks 30 to 41), mainly during weeks 32 and 33 (Figure 1). One hundred and six cases were interviewed.

Fifty-seven cases were tourists, either French or foreigners, including six cases who were living abroad: in Germany (1), the Netherlands (1) and Switzerland (4). The date of stay was collected for 53 tourists: 39 (74%) were present in the Côtes d'Armor district on 13 July, 46 (87%) on 14 July and 43 (81%) on 15 July. Twenty-six tourists were present in the area exclusively during the 7 to 22 July period.

The fifty-four (51%) remaining cases were living in the Côtes d'Armor district (Figure 2). The places of residence or stay in the district were clustered in the northwestern area near the towns of Paimpol and Lannion (Figure 3).

Among the 106 interviewed cases, 54 were men and the median age of cases was 40 years (range: 4 to 82 years). Eighty-eight cases (83%) reported jaundice and 28 (26%) were hospitalised. No death was reported.

At risk exposures were documented for 89 cases that occurred between 25 July and 2 September (weeks 30 to 35). All cases had eaten molluscan shellfish in the Côtes d'Armor district. Seventy-seven (87%) had eaten bivalve molluscs that are usually eaten

raw (oysters, warty venus, carpet shells, european bittersweets); 72 (81%) cases including the 26 cases who stayed briefly in the Côtes d'Armor district had eaten oysters (Table). Moreover, three clusters with common meals were identified among seven cases:

FIGURE 1

Cases of hepatitis A among residents and tourists in the Côtes d'Armor district outbreak, France, 2007, by week of onset (n=108)

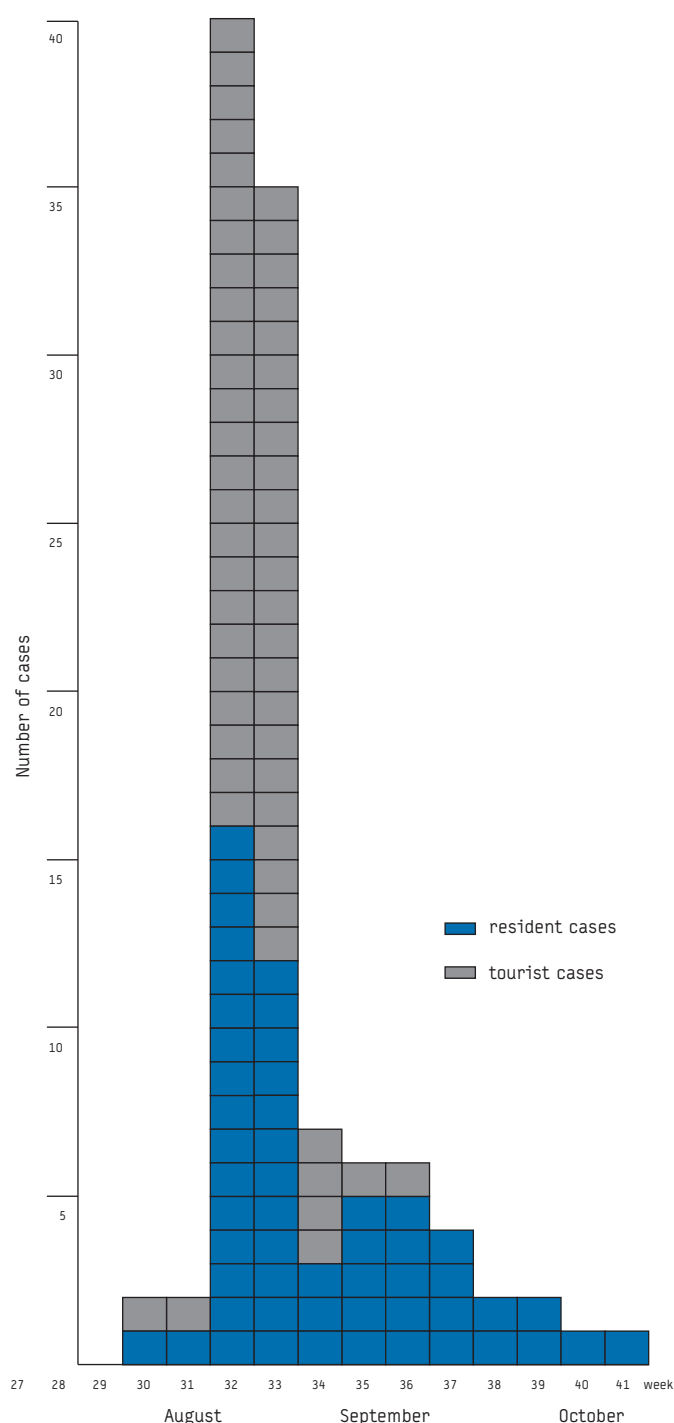


FIGURE 2

Place of permanent residence for cases of hepatitis A in the Côtes d'Armor district outbreak, France, 2007 (N=111)

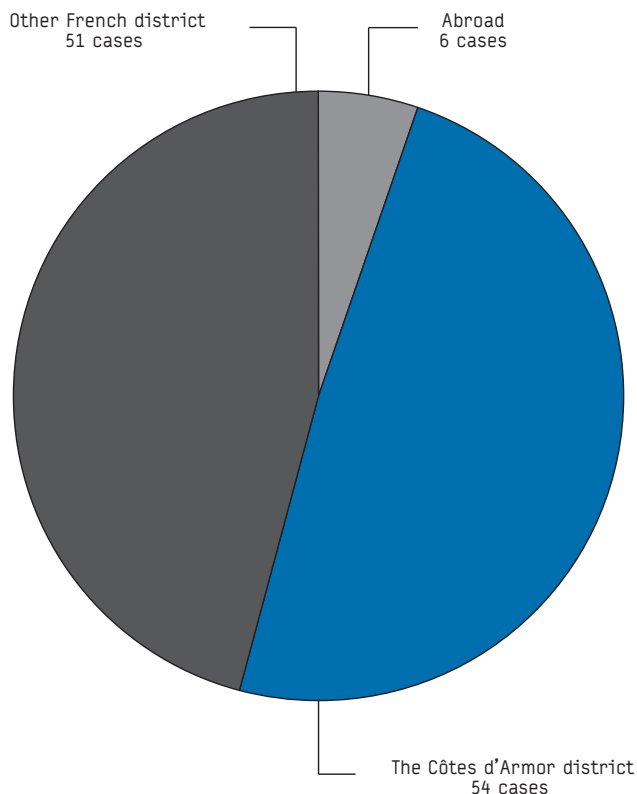
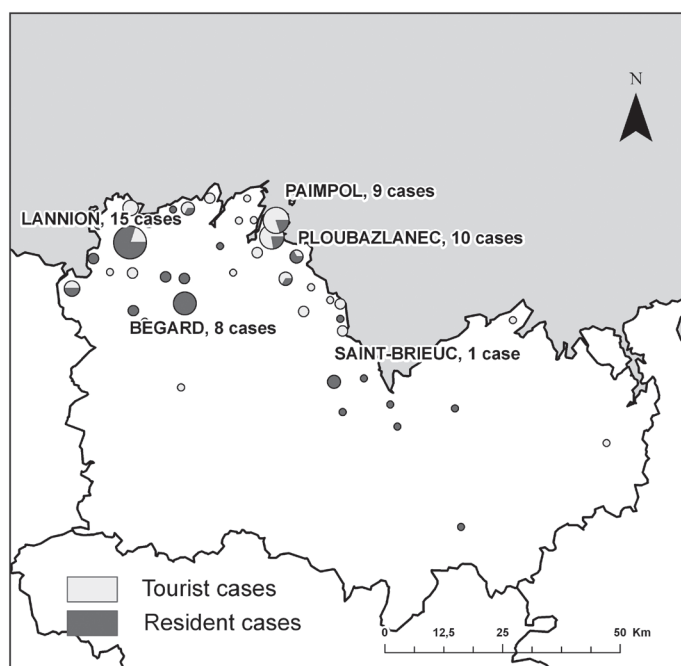


FIGURE 3

Place of residence or stay for cases of hepatitis A in the Côtes d'Armor district outbreak, France, 2007, by municipality (n=107)



two cases were linked to a meal on 13 July, two cases to a meal on 15 July and three cases to another meal on 15 July. Six of the seven cases had eaten raw shellfish.

The consumptions of raw vegetables, herbs and unpeeled fruits were documented for 80 cases. Tomatoes and lettuce had been consumed by 74 (92%) and 72 (90%) cases respectively. The other at risk exposures concerned less than 60% of the cases.

Microbiological analysis

Among the 71 sera received at the CNR, viral RNA was detected for 68 sera; 66 sequences were identical over an analysable 425 base-pair fragment and were clustered with genotype IIIA strains. The two other sequences differed only by one nucleotide change.

Traceback investigations

Considering the epidemic curve, the incubation period and the dates of stay of the affected tourists, it was estimated that the contaminated shellfish were probably consumed between 7 and 22 July. Traceback investigations were carried out for 20 of the 26 cases who had eaten oysters and stayed in the Côtes d'Armor district exclusively during this period. Seventeen cases had bought oysters from one farm located at the north of Paimpol bay, partly on the farm itself and partly through restaurants, supermarkets or fish shops. Although there were seven farms in the bay at the time of the outbreak, 13 of the 17 cases had exclusively consumed oysters originating from this particular farm. Among the three cases who had not consumed the oysters from the suspected farm, two had eaten other raw shellfish from the same farm and the last one had consumed wild oysters picked up near the farm. The raw shellfish consumed by six of the cases linked to the three clusters were exclusively originating from the previously mentioned suspected farm. On this farm, the shellfish from different production areas had been stored in submersible storage tanks up to 10 days and then depurated during 48 hours in a land-based tank before being sold. The farm was located near a storm sewer outlet at the north of the bay.

Environmental investigation

Sanitation of the bay

A separate sewage system with 16 pumping stations collects the wastewaters of Paimpol and Ploubazlanec in the north and northwest areas of the bay. The treatment plant is an activated sludge plant; a buffer tank is used to regulate and adapt the sewage inflow to the plant's capacity (22,000 inhabitant equivalents). There is no disinfection treatment. The disposal is located near Paimpol harbor entrance at the very far end of the bay. Local streams disperse the treated effluents towards the north seashore of the bay. This seems to have an impact on the bathing water quality at the two beaches closest to the disposal and each has once been classified C (water liable to be temporarily polluted) during the 2001 and 2006 summers. The months of June and July 2007 were much rainier than the same months of the 1997-2006 period: 85.4 mm vs 40.2 mm in June and 88.2 mm vs 51.5 mm in July. The monitoring of the sewage collecting and treatment installations revealed sewage overflows due to heavy rains: 300 m³ of diluted raw sewage discharged from the buffer tank on 24 June which was a neap tide day, and overflows from eight different pumping stations on 23 July. At the north of the bay, 40 houses, whose connection to the sewerage system is scheduled in the next few years, were served by on-site sanitation systems. Whether the facilities were working or not was not known at the time of the

investigation. A storm sewer outlet was also identified in the vicinity of the suspected shellfish farm.

Microbiological results

For viral investigations, a total of eight shellfish samples, four sludge and 24 water samples were analysed. All these samples were negatives for HAV RNA using the primer set and probe located in the 5'NC region. All the controls such as extraction efficiency and absence of inhibitors were verified, eliminating false negative result option.

Discussion and conclusion

We described a large hepatitis A outbreak which was the largest reported since the beginning of the mandatory notification in November 2005. Previously only two larger outbreaks had been reported in France, in 1992 and 1997 [5,6].

The results of the investigations indicated that it was a common point source outbreak due to the consumption of raw shellfish between 7 and 22 July 2007. The shellfish - mainly oysters - originated from a single farm located at the north of Paimpol.

The consumption of raw shellfish and especially oysters was frequently reported among the cases. The proportion of the cases who consumed oysters (81%) was much higher than in the CALIPSO

study carried out on a population selected for its heavy sea product consumption (61%) [7]. The consumption of raw shellfish among the cases was similar to those observed in previous hepatitis A outbreaks that occurred in Brittany in the Côtes d'Armor district in 1999 (oyster consumption: 88%) and in the Morbihan district in 1992-1993 (raw shellfish consumption: 81%) [6].

Raw seafood, and oysters in particular, are a well-known source of HAV outbreaks in France [5,6,8] and abroad [9,10].

Although more than 90% of the cases reported having eaten lettuce and tomatoes, the diversity of the purchasing places, the ban on using sewage sludge for market gardening, and the absence of wastewater reuse in French agriculture ruled out the hypothesis that raw vegetables might have been the vehicle for HAV in the outbreak described here.

Trace-back investigations revealed that the raw shellfish consumed by cases originated from only one farm and one site of Paimpol bay. During the period of suspected contamination, the French Research Institute for Exploitation of the Sea (Institut français de recherche pour l'exploitation de la mer - Ifremer) shellfish surveillance network (Réseau de Contrôle Microbiologique - Remi) had no evidence of faecal contamination of the shellfish ground areas (data not shown). The investigations suggested that the shellfish were probably contaminated on the farm, in the

TABLE

Molluscan shellfish consumptions during six weeks prior to symptoms onset in cases of hepatitis A in the Côtes d'Armor district outbreak, France, 2007 (n=89)

	Cases who stayed exclusively in the Côtes d'Armor district during the period at-risk (7-22 July, 2007), n (%)	Other cases, n (%)	All cases, n (%)
Raw bivalve molluscs:	26 (100)	51 (81)	77 (87)
Japanese oysters (<i>Crassostrea gigas</i>)	26 (100)	46 (73)	72 (81)
Warty venus (<i>Venus verrucosa</i>)	8 (31)	17 (27)	25 (28)
Grooved carpet shells (<i>Ruditapes decussates</i>), Japanese carpet shells (<i>Ruditapes philippinarum</i>)	9 (35)	13 (21)	22 (25)
Common european bittersweets (<i>Glycymeris glycymeris</i>)	4 (15)	8 (13)	12 (13)
Gastropod and other bivalve molluscs			
Mussels (<i>Mytilus edulis</i>)	11 (42)	42 (67)	53 (60)
Periwinkles (<i>Littorina littorea</i>)	9 (35)	25 (40)	34 (38)
Whelks (<i>Buccinum undatum</i>)	10 (38)	22 (35)	32 (36)
Common scallops (<i>Pecten maximus</i>)	6 (23)	14 (22)	20 (22)
Limpets (<i>Patella vulgata</i>)	0 (0)	2 (3)	2 (2)
Common cockles (<i>Cerastoderma edule</i>)	2 (8)	7 (11)	9 (10)
Hard shell clams (<i>Merceneria merceneria</i>)	1 (4)	0 (0)	1 (1)
All gastropod and bivalve molluscs	26 (100)	63 (100)	89 (100)

submersible tanks or in a depuration land-based tank where they had been stored temporarily before being sold. The date of the shellfish contamination was difficult to assess precisely because of the storage periods. It could have occurred anytime between mid-June and the second week of July.

We did not identify the source of the shellfish contamination. Shellfish could have been tainted by sewage overflows or by wastewater releases from polluted storm sewers or from on-site sanitation facilities. The location of the farm near a storm sewer outlet increased the vulnerability of the farm's shellfish storage tanks and of its depuration tank's water supply. Heavy rains may also have contributed to the shellfish contamination as well as the neap tides which hinder the dispersion of effluents.

HAV was not isolated from any of the different samples (shellfish, sewage, storm sewage, sludge, raw and treated sewage). However, the environmental contamination was limited in time and possibly restricted to a small area. Indeed, the sampling was late with respect to the date of the estimated shellfish contamination. The epidemic curve indicated a common point source. However its right skewed shape suggested a person-to-person transmission for the few cases occurring from week 36 onwards.

The number of persons affected by the outbreak was probably higher than reported due to asymptomatic or unrecognised infections. Under-notification of hepatitis A especially outside the Côtes d'Armor district and lack of information on foreign tourists diagnosed abroad could have also contributed to underestimating the outbreak burden.

The phylogenetic analysis attributed the outbreak to a strain belonging to HAV genotype IIIA. This genotype is endemic in South-East and Central Asia (India, Nepal, Sri Lanka and Malaysia) [11], and has also been associated with outbreaks among intravenous drug users (IDUs) in Nordic countries [12]. Before 2004, this was a rare genotype in France though it had been detected in a single patient in a previous outbreak in the Côtes d'Armor district in 1999 [13]. The strain identified in the present outbreak is closely related to a strain responsible for an outbreak in a primary school in Auvergne in 2004 [14] and distinct from previously published sequences.

The previous hepatitis A outbreak (33 cases) that occurred in the Côtes d'Armor district in the winter of 1999 was also linked to the consumption of raw oysters from the Paimpol bay. Raw sewage discharged from the treatment plant and sewage overflows were suspected as the source of contamination of oysters. Two additional outbreaks of hepatitis A due to shellfish consumption have been reported in other French regions [5,8]. The outbreak we investigated occurred during the summer contrary to the other French outbreaks that occurred in winter after the Christmas holidays when raw shellfish is heavily consumed.

Control measures taken by the district authorities included prohibition of recreational shellfish harvest in the bay from 24 August to 4 September. In order to prevent further outbreaks, measures should be implemented to improve the quality of the shellfish. The general improvement of the sewage collecting and treatment installations that has been implemented since the 1999 outbreak should be continued. The monitoring of these facilities should also be used to timely alert shellfish farmers, district health and veterinary services about sewage overflows. We also recommend

assessing specific risks on each farm of the bay to identify specific hazards and possible control measures. These recommendations may contribute to preventing not only hepatitis A [15] but also other food-borne infections.

Our results highlight the fact that in a country with low HAV endemicity, such as France, consumption of raw shellfish can cause a large community outbreak. Increasing susceptibility of the European general population either from low endemic countries or from countries in transition (from moderate to low) is an important public health issue as illustrated in 2008 by reported outbreaks in several European countries [16,17].

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Research articles

HEPATITIS A SEROPREVALENCE IN TUSCANY, ITALY

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Information regarding the current seroprevalence of hepatitis A virus (HAV) is useful for the control of HAV infections. The objective of our study was to evaluate the prevalence of anti-HAV antibodies among children (1-5 years old) and young adults (15-20 years old) in Tuscany, in central Italy. A total of 565 sera were collected in three years 1992, 1998 and 2004, equally distributed between the two age groups. The overall proportion of those that tested positive for anti-HAV antibodies was 8.3%. The proportion of immune children (1-5 years old) statistically significantly increased over the years. The percentage of immune subjects among 15-20-year-old young adults varied over the years, not showing a significant statistical trend, nevertheless our findings indicate that in a low endemicity area, adolescents and young adults are becoming increasingly susceptible to HAV infection. On-going monitoring of immunity to HAV is necessary for detecting trends over time.

Introduction

Hepatitis A is generally an acute, self-limiting liver infection transmitted through the faecal-oral route by a picornavirus, hepatitis A virus (HAV) that occurs worldwide and causes about 1.5 million cases of clinical hepatitis each year [1]. The degree of endemicity is closely related to hygienic and sanitary conditions, the socio-economic level and other development indicators [2]. In recent decades Italy has experienced a declining trend of HAV prevalence [3], probably related to improved health and sanitary conditions which have been responsible for the progressive decline in the infection rate among children under 14 years of age and a major shift towards the highest incidence in susceptible teenagers and young adults [4]. Nevertheless, Italy is considered to be an area with low/intermediate endemicity of hepatitis A. Data from the national surveillance system for acute viral hepatitis infection (Sistema Epidemiologico Integrato dell'Epatite Virale Acuta, SEIEVA), suggest a steady decrease in the incidence of reported cases of HAV infection over the past few years (from 10 per 100,000 inhabitants in 1985 to 3.6 per 100,000 in 2004) [5].

However, the epidemiological situation varies from region to region within Italy [4]. The practice of consuming contaminated raw seafood still causes outbreaks, especially in southern Italy. In particular, Puglia and Campania, two regions in the south, experienced a large outbreak during 1996-7 with approximately 11,000 cases of HAV infection reported, accounting for an annual incidence rate of approximately 130 per 100,000 population [6,7]. Moreover, in 2004 another outbreak, involving 882 cases, was described in Campania [8].

The availability of a safe, highly immunogenic vaccine that provides long-term protection against HAV has been proven to be useful in the containment of hepatitis A in endemic areas [9]. However, high costs and the limited availability of the HAV vaccine have raised some concerns regarding mass vaccination [4,10]. In Italy the current National Vaccination Plan (Piano Nazionale Vaccini PNV 2005-2008), recommends vaccination against HAV only for specific population groups (travellers to endemic areas, drug users, men who have sex with men (MSM), soldiers, sewage workers, patients presenting with liver disease, recipients of liver transplants and HAV-negative haemophiliacs) [11,12]. Since 1998, after a large epidemic of hepatitis A, the Puglia region (south-eastern Italy) has introduced a free-of-charge mass vaccination program (the first ever in Italy since safe and highly effective hepatitis A vaccines became available in 1995) for newborns (15-18 months of age) and adolescents (12 years of age), as part of the routine immunisation schedule, in order to reduce transmission [9]. For this reason, since 2001, when the Italian National Health System was decentralised, the regional health authorities have implemented vaccination strategies according to their own judgment. However, the region of Tuscany does not include hepatitis A vaccination in the regional infant and adolescent immunisation calendar. Preventive hepatitis A vaccination, however, is considered, in Tuscany and all other Italian regions, for close contacts of clinical cases as control measure in case of an epidemic.

Moreover, although hepatitis A is usually a self-limited disease, the likelihood and severity of symptomatic illness are age-related. In a low endemicity area the highest frequency of HAV infection is observed in adults, who are more likely to have clinical symptoms since the infection causes significant morbidity, along with absenteeism, hospitalisations and occasional mortality, while infants and young children are usually asymptomatic [13,14]. In the last decade, in Italy, a progressive reduction of the prevalence of the infection in children, teenagers and young adults has been described. However, the symptomatic/asymptomatic ratio and the percentage of patients with a more severe clinical presentation have progressively increased [15].

The objective of the present study was to determine the prevalence of anti-HAV antibodies in children and young adults in Tuscany, in central Italy, and to present epidemiological data on HAV infection in this area.

Methods

Serum samples

Serum samples from individuals aged 1-5 and 15-20 and collected during three different years: 1992, 1998 and 2004, in the region of Tuscany, were tested. Serum specimens were obtained by using leftover serum from specimens taken for diagnostic purposes and submitted to the Laboratory from individuals who received care in the University Hospital of Siena. The sera collected from the two ranges of age were randomly selected from the laboratory stock. We excluded samples from individuals known to be HIV-seropositive. Samples were collected anonymously and only the age of the subject and the date of the sample were recorded.

A total of 565 sera (283 for the age group 1-5 years and 282 for the age group 15-20 years, approximately 100 samples for each year) were collected and stored at -20°C. Of the 565 serum samples collected all were tested for the anti-HAV-IgG antibody: 47 (8.3%, 95% CI: 6.03-10.57) were positive, 509 (90.1%, 95% CI: 87.64-92.56) were negative and 9 (1.6 % CI: 0.57-2.63) gave ambiguous results. Thus, 556 samples were included in the statistical analysis, as the nine giving ambiguous test results were excluded (Table).

Serological test

Serological testing was performed at the Department of Physiopathology, Experimental Medicine and Public Health of the University of Siena. All serum samples were tested for detection of anti-HAV-IgG antibodies. The antibody index (calculated as sample OD/cut-off serum mean OD x 10), was determined by a commercial enzyme immunoassay test (Enzywell HAV Antibody, DIESSE) following the manufacturer's instructions. According to the manufacturer, the test has a sensitivity and specificity of 100%.

Samples with an index of < 0.8 were considered positive, samples with an index > 1.2 were considered negative, and samples with an index ranging from 0.8 to 1.2 were considered equivocal. Equivocal sera were retested for confirmation.

A positive result for total anti-HAV IgG antibodies is not able to distinguish recent infection from a previous one, thus a test for determining IgM antibodies is necessary for correct detection of current infection. However, the test for determining total anti-HAV IgG antibodies is important since this indicator allows for the identification of cohorts of subjects that are still at risk of infection.

There also is no way of determining whether the positive test results are due to past infection or due to vaccination. Detection of hepatitis A IgG antibodies indicates either past infection or vaccination.

Statistical analysis

Age-specific (1-5 years and 15-20 years) seroprevalence rates were calculated, along with the corresponding 95% confidence intervals (CI). The statistical analysis was performed by Epi Info version 6 program using the chi-squared (χ^2) test, as well as χ^2 test for trend to evaluate possible tendencies. Differences were regarded as significant when $p < 0.05$.

Results

Of the 565 serum samples collected, all were tested for the anti-HAV-IgG antibody: 47 (8.3%, 95% CI: 6.03-10.57) were positive, 509 (90.1%, 95% CI: 87.64-92.56) were negative and 9 (1.6 % CI: 0.57-2.63) gave ambiguous results.

The nine specimens with equivocal results were eliminated from the statistical analysis. Among these, five were taken from 1-5 years old children (four males, one female) and four were from patients aged 15-20 years (two males, two females). Thus, 556 samples were suitable for calculating the results.

Of the 278 serum samples from 1-5-year-old children 26 were positive (9.4%, 95% CI: 6.20-13.40) and of the 278 serum samples from the age group 15-20 years 21 were positive (7.6%, 95% CI: 4.73-11.31).

The percentage of immune children (1-5 years) increased from 2.7% (95% CI: 0.33-9.5) in 1992, to 6.2% (95% CI: 2.30-13.0) in 1998, and to 16.7% (95% CI: 10.19-25.05) in 2004 (Figure). The annual trend was statistically significant ($\chi^2 = 8.9$, p value = 0.0027).

The percentage of immune young adults, aged 15-20 years, varied from 7.5% (95 % CI: 2.80-15.61) in 1992, to 11.3% (95% CI: 5.0-21.0) in 1998, and to 5.5% (95% CI: 2.24-11.03) in 2004 (Figure). In this case, the yearly trend was not statistically significant.

Discussion and conclusion

The results of this study showed an increasing trend of the seroprotection rate in children aged 1-5 years with a particularly high antibodies titre in 2004 (χ^2 for linear trend=10.7; $p=0.0011$). The high anti-HAV antibodies rate among children in 2004 is likely to have been related to extensive vaccination of this age group in consequence of a small epidemics that occurred in communities of children in central Italy during that period [16]: children attending primary schools were contaminated by infected schoolmates who had contracted hepatitis A by eating raw seafood in endemic areas (Campania and Puglia) during Christmas holidays.

One case-control study conducted during an outbreak of hepatitis A which occurred in 2004 in southern Italy and affected different municipalities of Campania region, in the municipality with the highest attack rate showed that raw seafood consumption, particularly when it was illegally stored in water, was strongly associated with HAV infection [8]. The major role played by shellfish consumption in HAV transmission in Italy is supported by data from the surveillance system for type-specific acute viral hepatitis (SEIEVA) [5].

TABLE

Samples tested for the prevalence of antibodies against hepatitis A virus, Tuscany, Italy, by patients' age group, gender and the year of obtaining the specimen

Year of obtaining the sample	Age group (gender: male - female)	
	1-5 years n (m - f)	15-20 years n (m - f)
1992	73 (42-31)	80 (21-59)
1998	97 (44-53)	71 (22-49)
2004	108 (54-54)	127 (65-62)
Total	278 (140-138)	278 (108-170)

On the other hand, the prevalence of anti-HAV antibodies in young adults (15-20 years old) was higher during 1992 and 1998 (7.5% and 11.3%), followed by a decrease to 5.4% in 2004 (χ^2 for linear trend=0.40; $p=0.53$). However, this is not enough to support the presumption that there is an increasing trend in the risk of infection in young adults.

These results must be considered cautiously because the samples studied cover only three years (1992, 1998 and 2004) during a period of eight years (from 1992 to 2004). Nevertheless, these findings confirm the shift of the seroprevalence of hepatitis A virus infection in younger age groups, as observed in the urban population of India [17], in an area with low endemicity where adolescents and young adults are becoming increasingly susceptible to HAV infection.

In the past few years similar studies have been conducted in Italy but only two described the situation in the whole country. However, direct comparisons with these studies are difficult due to the differences in the age groups considered. One, conducted in 1990, showed an anti-HAV immune prevalence of 2.3% among 3 to 5 year-old children, and of 16.3% in teenagers aged 17-19 [18]. The other study performed on sera collected in 1997-1998 showed a prevalence of 34.9%, 12.9% and 14.6% in age groups of 0-1, 2-5 and 12-19 years respectively [19].

Other seroepidemiological investigations have only been conducted in specific areas or among certain risk groups. In 1994 the seroprevalence of HAV antibodies was tested in north-east Italy: the prevalence obtained was 0.7% among the group aged 10-19 years old and 6.0% in the group of over 19 years. Anti-HAV antibodies prevalence in army recruits was 66% in 1981, 30% in 1990 and 5% in 2003 (ranging from 2% in the north to 8% in the south) [20]. Furthermore, a specific report on the prevalence of hepatitis A virus (HAV) in a group of drug users in Italy showed an overall seroprevalence of 28.7% [21].

In conclusion, information regarding the current status of hepatitis A immunity, including the seroepidemiological survey described here, is crucial for providing new and timely parameters useful for mathematical modelling, such as the one proposed and investigated for temporal trends in southern Italy [22]. A

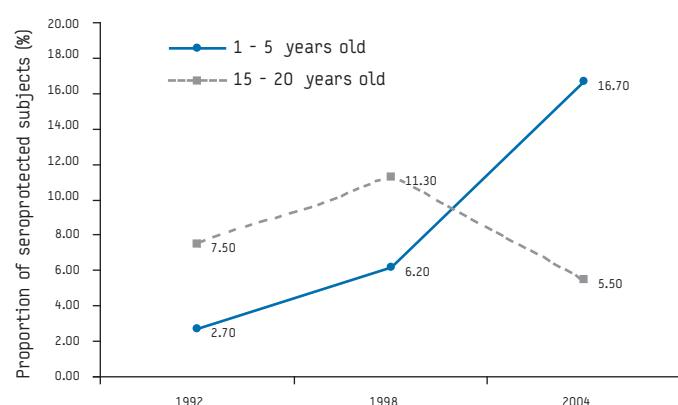
vaccination program for risk groups, travellers and food handlers would be suitable for the control of this virus at present, however, the current epidemiological situation in Italy does not suggest the need for mass vaccination of newborns and adolescents outside the endemic areas, such as Puglia and Campania. The same is true in other southern European countries, such as Spain, where universal immunisation seems economically ineffective and sufficient evidence for targeted vaccination has not yet been gathered [4].

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FIGURE

Seroprevalence of antibodies against hepatitis A virus, in age groups of 1-5 and 15-20 years, Tuscany, Italy, 1992, 1998, 2004



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EXECUTIVE AGENCY FOR HEALTH AND CONSUMERS LAUNCHED CALL FOR PROPOSALS IN THE FIELD OF HEALTH

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On 26 February 2009, the Executive Agency for Health and Consumers has launched a call for proposals for financial contribution to specific actions in the field of health such as: projects, conferences, joint actions, operating grants and other activities like organisation of workshops and expert meetings, including seminars, publications and various communication initiatives.

This call for proposals is open to non-governmental organisations, public sector bodies, public administrations, universities, higher education establishments, and commercial firms established in a European Union (EU) country or in EFTA countries members of the European Economic Area (Iceland, Liechtenstein, and Norway) or, under certain circumstances, in Croatia.

The final deadline for the submission of proposals is 20 May 2009.

An Information Day on the 2009 Calls for proposals will be held in Luxembourg on 18 March 2009. In some countries, the National Focal Points will also organise national information days.

Within this call for proposals, preference will be given to actions with a significant added value at European level in the following areas: improving European citizens' health, reducing health inequalities in and between EU Member States and regions, building capacity for development and implementation of effective public health policies particularly in areas of high need, involvement of new (non-traditional) actors for health in sustained, cooperative and ethically sound actions, both at regional or local level and across participating countries.

For the work programme 2009, the indicative amount of the operating budget is EUR 48,261,000, from which the amount of EUR 24,130,500 is reserved for the call for proposals for projects. Up to 60 percent of the project costs can be covered by the Community contribution but projects can receive up to 80 percent co-financing of eligible costs in cases of exceptional utility.

More information is available on the website of the Executive Agency for Health and Consumers at the following address: <http://ec.europa.eu/eahc>.

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