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, AB020264, 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 form 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:

Cases of hepatitis A among residents and tourists in the Côtes d'Armor district outbreak, France, 2007, by week of onset (n=108)
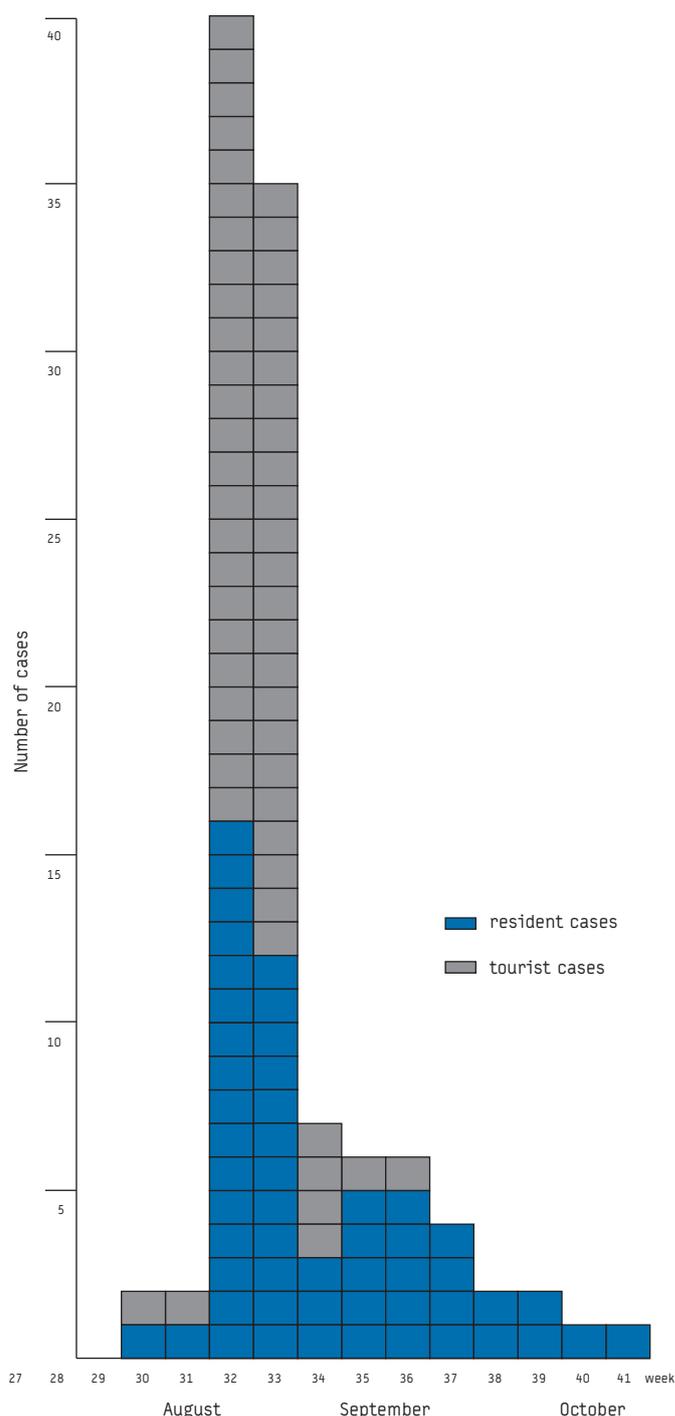
Place of permanent residence for cases of hepatitis A in the Côtes d'Armor district outbreak, France, 2007 (N=111)

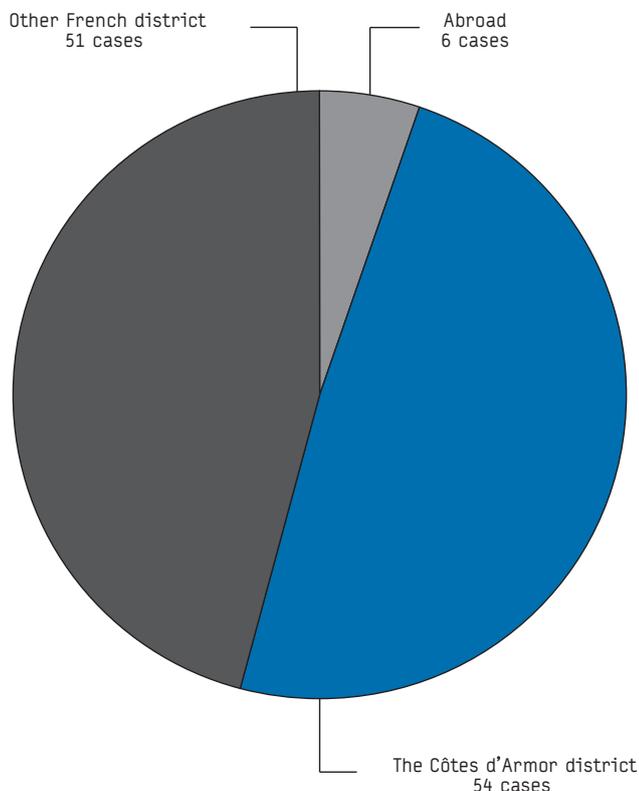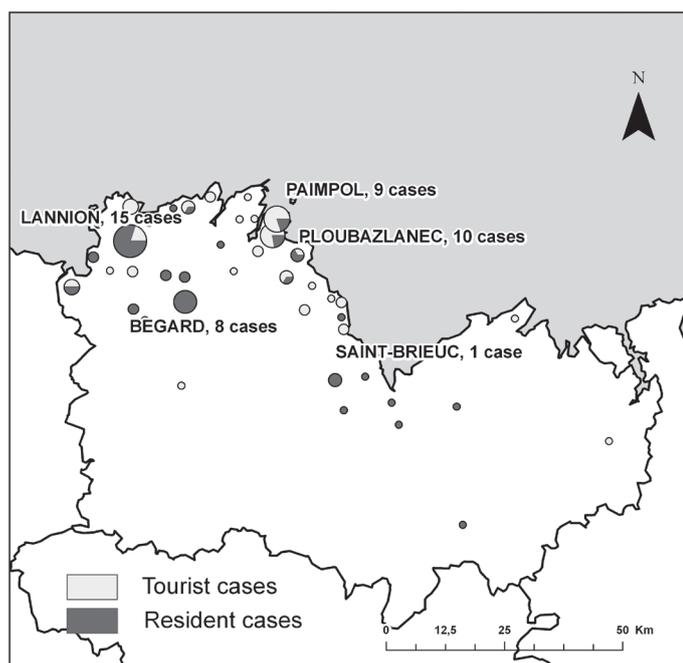**Place of residence or stay for cases of hepatitis A in the Côtes d'Armor district outbreak, France, 2007, by municipality (n=107)**

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 (Crassostrea gigas)	26 (100)	46 (73)	72 (81)
Warty venus (Venus verrucosa)	8 (31)	17 (27)	25 (28)
Grooved carpet shells (Ruditapes decussates), Japanese carpet shells (Ruditapes philippinarum)	9 (35)	13 (21)	22 (25)
Common european bittersweets (Glycymeris glycymeris)	4 (15)	8 (13)	12 (13)
Gastropod and other bivalve molluscs			
Mussels (Mytilus edulis)	11 (42)	42 (67)	53 (60)
Periwinkles (Littorina littorea)	9 (35)	25 (40)	34 (38)
Whelks (Buccinum undatum)	10 (38)	22 (35)	32 (36)
Common scallops (Pecten maximus)	6 (23)	14 (22)	20 (22)
Limpets (Patella vulgata)	0 (0)	2 (3)	2 (2)
Common cockles (Cerastoderma edule)	2 (8)	7 (11)	9 (10)
Hard shell clams (Merceneria merceneria)	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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