Epidemiol. Infect. (2002), **129**, 113–117. © 2002 Cambridge University Press
DOI: 10.1017/S0950268802007069 Printed in the United Kingdom

An outbreak of hepatitis A among homosexuals linked to a family outbreak

K. STENE-JOHANSEN[1]*, P. A. JENUM[2,3], T. HOEL[4], H. BLYSTAD[3], H. SUNDE[5] AND K. SKAUG[1]

[1] *Department of Virology, National Institute of Public Health, Oslo, Norway*
[2] *Fürst Medical Laboratory, Oslo, Norway*
[3] *Department of Bacteriology, National Institute of Public Health, Oslo, Norway*
[4] *Department of Vaccination, Ullevål University Hospital, Oslo, Norway*
[5] *Hedmark Central Hospital, Elverum, Norway*

(*Accepted 4 February 2002*)

SUMMARY

Several outbreaks of hepatitis A occurred in Norway in 1995–8. Molecular epidemiology was used to follow the spread of hepatitis A virus in the population. Distinct strains of hepatitis A virus (HAV) were detected by reverse transcriptase–polymerase chain reaction (RT–PCR) and subsequent sequencing in serum from patients in different communities at risk of infection. Two HAV strains were detected in an outbreak among 26 men having sexual contact with other men. One of these strains was also detected in a geographically limited family outbreak. The family outbreak was first believed to be acquired abroad. The sequence information linked the two outbreaks, and epidemiological and serological analyses revealed the transmission route. This study demonstrates the importance of molecular epidemiology in outbreak investigation, surveillance and monitoring of hepatitis A in the population.

INTRODUCTION

Hepatitis A is an endemic disease primarily transmitted by the faecal-oral route. Normally 100–150 cases are reported every year in Norway primarily among travellers to endemic regions. In 1995–8 outbreaks of hepatitis A among intravenous drug users (IVDU) caused an increase of hepatitis A, with 350 notified cases annually [1, 2]. The use of molecular epidemiology in surveillance and monitoring of hepatitis A in this period showed that two distinct HAV-strains caused outbreaks in the IVDU communities [2], whereas other strains were associated with travel to endemic regions [1–3]. The presented outbreak is the first documented outbreak in Norway among men having sexual contact with other men. Sequence information and biocomputing enabled us to distinguish between related strains of viruses and to deduce the relationship between viruses from different outbreaks and individual patients. In endemic regions HAV strains form discrete closely related clusters [4]. The introductions of hepatitis A from these regions to non-endemic regions usually cause geographically limited outbreaks that are caused by the same HAV strain. In this study we show that molecular epidemiology is a valuable tool to distinguish between independent outbreaks occurring at the same time and place, as well as linking geographically separate outbreaks.

* Author for correspondence: Department of Virology, National Institute of Public Health, P.O. Box 4404 Nydalen, N-0403 Oslo, Norway.

METHODS

Hepatitis A is a nominative notifiable disease in Norway. The notifications system is administrated by the National Institute of Public Health and includes

clinical and epidemiological data, as well as the laboratory results of anti HAV-IgM positive patients. In 1997–8 two separate clusters were defined among the notified cases.

Outbreak among men having sexual contact with other men

In the period from October 1997 to March 1998 26 men having sexual contact with other men were notified with hepatitis A in Oslo. Serum samples were obtained from 23 of the 26 cases from the local medical microbiological laboratories in Oslo and the surrounding regions. These samples were examined by RT–PCR.

Family outbreak

During December 1997 and January 1998 a family outbreak of hepatitis A occurred in a small community in Hedmark County in the eastern part of the country. The mother and father's mother were hospitalized during Christmas due to acute hepatitis A infection. Both had on separate occasions been on a vacation in Madeira in November/December. The three oldest children were slightly ill around Christmas with elevated values of alanine-aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transpeptidase (γGT). The oldest child had fever, abdominal discomfort and nausea, while the father and the youngest son were in good health at this time. However, the youngest son had vomiting prior to his parents' trip to Madeira. There were 21 family members celebrating Christmas at their home. Following the confirmation of hepatitis A infection in the mother, all members of the party were given prophylactic intramuscular injections of human immune globulin. In total 23 serum samples were collected from 12 family members and analysed for HAV-specific IgM antibodies. Samples from four family members were selected for further analysis by RT–PCR, based on a positive anti HAV-IgM result, the level of serum ALT, AST and γGT and the date of blood sampling.

HAV-sequencing

HAV RNA was isolated from 120 μl serum using the QIAamp Viral RNA kit (Qiagen) and analysed by nested RT–PCR comprising 348 bp within the VP1/2PA junction of the HAV-genome [3]. Positive PCR products were sequenced within this 348 bp region.

Sequence analysis was performed with the data programmes; Fasta3 (EMBL), FastA and Distances (Wisconsin Package Version 10.1, Genetics Computer Group). Accession numbers for Strain I (NOR-25) and Strain II (NOR-26) are AJ299460 and AJ299461 respectively.

RESULTS

Outbreak among men having sexual contact with other men

HAV RNA was detected by RT–PCR in serum from 18 (78%) of the 23 examined cases associated with the outbreak of hepatitis A among men having sexual contacts with other men in Oslo (Table 1). PCR products from 17 of the 18 samples were sequenced successfully within the 348 bp region (Table 1). Sequence comparisons revealed two different hepatitis A-virus strains within genotype IA. The divergence between Strain I and Strain II was about 7%. Strain I (NOR-25) was detected in 12 cases, all being identical except one sequence that showed 2/348 bp (0·9%) divergence. Strain II (NOR-26) was detected in five of the cases, all identical. In addition two cases, one case with a negative PCR result and one case where no sample was obtained were epidemiologically linked to each of the strains.

Family outbreak

HAV-specific IgM antibodies were detected in serum from 7 of the 12 family members indicating present or recent infection (Table 2). The father's father had previously been infected with hepatitis A. Of the four samples selected for PCR-analysis HAV RNA was only detected in serum from the mother (Table 1). Sequence comparison showed that this was identical to Strain II, associated with men having sexual contact with other men.

Outbreak investigation

The cause of the family outbreak was initially believed to be due to the parents and the father's parents stay in Madeira. However, the children's immune status at Christmas time indicated that they must have been

https://doi.org/10.1017/S0950268802007069 Published online by Cambridge University Press

Table 1. *Results of RT–PCR and sequence analysis of HAV infected homosexual men and the secondary infected family outbreak*

		PCR-analysis		Sequencing analysis	
Outbreak	Notified cases	POS	NEG	Strain I	Strain II
Homosexual	26	18	5	12*	5*
Family	7	1	3	—	1

* One of the 18 PCR positive samples could not be sequenced successfully.

Table 2. *IgM anti-HAV test results of the family members*

Patient	Day of sampling after index case	IgM
Mother	0	POS
Father	19	NEG
	32	POS
Daughter 1	11	POS
Daughter 2	11	POS
Daughter 3	11	POS
Son (index case)	11	POS
Father's mother	3	POS
Father's father	3	NEG*
Aunt	3	NEG
	21	NEG
Uncle	3	NEG
Mother's mother	3	NEG
Mother's father	4	NEG
	29	NEG

* The father's father was IgG anti HAV positive.

infected earlier and not secondary to their mother or grandmother. Further investigation revealed that the family's neighbour had an hepatitis A infection in October and during this time had leakage from his full septic tank. However, contamination of the family's well by the spill over was impossible. The father, who performed camera-inspection of sewerage, was called by the neighbour for the repair work. The son had directly been in contact with the sewage when helping his father with the repair work. There were no further social contact between the neighbour and the family members. The examination also revealed that the son had been ill with symptoms that could be a possible hepatitis A infection in late November just prior to his parents' trip to Madeira. While the parents were away all the children were looked after first by the mother's parents and later the father's parents. It also turned out that the neighbour was associated with an HAV outbreak among men having sexual contact with men in Oslo. The sequence analysis showed that the

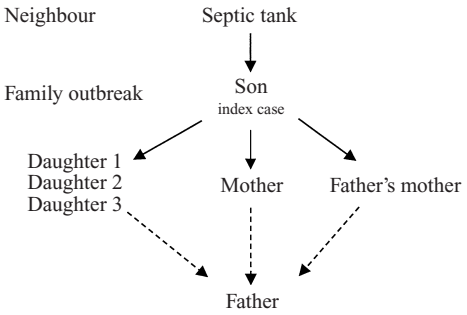

Fig. 1. Transmission route of hepatitis A virus in the family outbreak.

mother and the neighbour were infected with the same strain.

The youngest son was most probably infected with hepatitis A in late October through contact with sewage from his neighbour's septic tank. When he became slightly ill in late November he probably infected his three older sisters, his mother and his father's mother, who all fell ill during Christmas. The appearance of HAV-specific IgM antibodies in the fathers serum first in late January showed that he probably got infected during Christmas by the other family members (Fig. 1). The clinical manifestation may have been diminished by human immune globulin he was given.

DISCUSSION

Outbreak among men having sexual contact with other men

Two different HAV-strains were detected during the outbreak among men having sexual contact with other men. These results imply that there were in fact two intertwining outbreaks in the same period and region, which could not be epidemiologically distinguished. We have tried to trace the source of infection by comparing these sequences to previously characterized HAV-strains in the EMBL-database,

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and to characterized strains in our laboratory from 1995–8. Both HAV-strains could clearly be distinguished from the strains causing outbreaks in the IVDU communities in Oslo at this time. However, Strain I, with a 0·9% variation from consensus, was identical over the 348 bp sequenced to a hepatitis A virus detected during an outbreak among IVDUs in Finland from June 1994 to February 1995 (P. Leinikki, personal communication). Faecal contaminated drugs were suspected to be the cause of this Finnish outbreak [5]. We have not been able to find any epidemiological link to the Finnish outbreaks nor to the outbreaks in the IVDU communities.

Strain II showed close sequence similarity to four strains from previously characterized sporadic cases in Norway [1]. Two of these cases were homosexual men infected during the summer of 1995 who claimed to be infected abroad, in Turkey and Great Britain respectively. The other two patients were reported in October 1996. One of them claimed to have been infected in Athens or London. These cases were all geographically separated and no epidemiological link has been found. However, an outbreak of hepatitis A was reported in London among homosexual men over a 12-month period from April 1994 [6].

HAV populations that circulate in endemic regions seem to form discrete, closely related clusters [4]. The introduction of HAV from the same endemic population at several occasions would give a similar pattern of closely related clusters of HAV in non-endemic regions. However, as Strain II seems to be associated with men having sexual contact with other men during visits within Europe, there is likely to be a circulating strain of HAV in these communities in Europe.

Family outbreak

The son's immunological response against hepatitis A at Christmas indicated that the vomiting in November was probably associated with hepatitis A, making him the most probable index case in the family outbreak. His time of infection correlates with the time of contact with the neighbour's sewage, and the time when the neighbour was infectious. Faecal-oral transmission from the son to the other family members, except the father, is the likely route of transmission in this outbreak, as this correlates with the onset of symptoms, as well as the incubation period of hepatitis A. Our experience with monitoring HAV by molecular epidemiology is that different strains are detected among sporadic cases and smaller outbreaks associated with travel to endemic regions, and that these are different from the strains associated with IVDU- and homosexual communities [1–3]. Further, HAV strains found among cases within epidemiologically defined outbreaks are identical or very closely related within the genome region we use for molecular epidemiology. We therefore conclude that the family members, with positive HAV IgM antibodies in serum, most probably have been infected by the same HAV strain. The appearance of identical HAV strains in samples from the mother and the neighbour also indicates that sewage probably was the primary source of transmission in the family outbreak.

By comparing epidemiological and serological data the probable route of transmission within the family has been solved. What initially was believed to be an outbreak cluster caused by an imported strain from Madeira was shown to be due to domestic transmission, which was traced by sequence analysis to the outbreak among men having sexual contact with men.

In conclusion, in the period 1995–8 there were outbreaks of hepatitis A mainly associated with IVDU in Norway. Using molecular epidemiology we found different strains of hepatitis A among the IVDUs and homosexuals, indicating several outbreaks at the same time, rather than one big outbreak in the population. These outbreaks occurred coincidentally probably due to low immunity against hepatitis A in the population. We have differentiated the outbreak among homosexual men from the ongoing outbreaks in the drug user community, as well as other outbreaks and sporadic cases at this time. We have also found the probable source and route of transmission in a local outbreak and linked it to a geographically distinct outbreak. These findings demonstrate the importance of molecular epidemiology in outbreak investigation, surveillance and monitoring of hepatitis A.

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