Investigation of an epidemic of Hepatitis E in Nellore in south India

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Summary

Objective To determine the incidence of acute hepatitis because of hepatitis E virus (HEV) and the source of the epidemic in Nellore in south India in 2008.

Methods Anti-HEV IgM ELISA and RT-PCR for HEV-RNA were carried out on blood and stool samples from patients with acute hepatitis presenting to different hospitals in the city. The city was divided into 33 clusters, and 20 families from each cluster were systematically interviewed to determine the incidence of hepatitis E in the city. The survey was conducted on 2685 residents of 673 households from 24th November to 4th December 2008.

Results The overall incidence of hepatitis was 5.7% (152/2685), i.e. an estimated 23 915 persons in the city were affected. There were two deaths because of acute hepatitis in the population surveyed translating to an estimated 315 deaths. Men had higher attack rates than women (7.8% vs. 3.5%) and young adults compared to children under 5 years (6.9% vs. 2.9%). Families drinking water from the pumping station at Bujjamarevu had the highest attack rate of 54.5% (39.8–69.2%). HEV IgM antibodies were present in 80/100 plasma samples tested. HEV-RNA was detected in 43/100 individuals tested, and isolates were characterized as genotype 1 by sequencing.

Conclusion Sewage draining into the river close to the pumping stations and broken pipelines crossing sewage drains may have triggered this large outbreak.

Keywords attack rate, epidemic, genotyping, hepatitis E virus, IgM anti-HEV, south India

Introduction

Hepatitis E virus (HEV) is a major cause of acute, self-limiting hepatitis. It is enterically transmitted and frequently results in epidemic, as well as sporadic, hepatitis. Epidemics of hepatitis E are generally restricted to developing countries where the disease is endemic because of poor sanitation and hygiene. Several outbreaks have been reported from countries in the Indian subcontinent, in south-east and central Asia, Africa and Mexico (Khuroo 1980; Wong et al. 1980; Velazquez et al. 1990; Naik et al. 1992). Contamination of drinking water by sewage has frequently been the cause of such outbreaks (Kane et al. 1984), which tend to occur both during rainy and summer seasons (Vishwanathan 1957; Naik et al. 1992; Corwin et al. 1997). They vary from single to multi-peaked epidemics, lasting for a few weeks to a year and affect thousands of people (Vishwanathan 1957; Bali et al. 2008).

Sporadic cases of hepatitis occur frequently in countries where epidemics of hepatitis E are also reported (Arankalle et al. 1993). In developed countries, hepatitis E occurs sporadically and infections are more often food-borne than water-borne (Teo 2007). HEV primarily affects young adults aged 15–40, and fewer cases are seen in children (Khuroo 1980). Person to person or secondary transmission is inefficient (Somani et al. 2003). Other modes of transmission are through blood transfusions (Khuroo et al. 2004) and vertical transmission (Khuroo et al. 1995). HEV results in increased mortality in pregnant women in certain geographic regions of endemic countries (Khuroo et al. 1981; Khuroo & Kamili 2003). In post-transplant patients who are immunocompromised, hepatitis E progresses to chronic hepatitis (Kamar et al. 2008).

The first epidemic of hepatitis E was from Delhi during 1955–1956 and was identified retrospectively (Vishwanathan 1957; Wong et al. 1980). Subsequently, further epidemics have been reported from India (Khuroo 1980;
Naik et al. 1992; Bali et al. 2008; Rai et al. 2008; Sailaja et al. 2009). Sera from each of several enterically transmitted non-A non-B hepatitis outbreaks between 1956 and 1985 showed presence of antibodies to HEV (Arankalle et al. 1988). However, a large number of pre-1980 outbreaks of hepatitis E have probably gone unreported, as Hepatitis A virus (HAV) was thought to be the cause of all waterborne epidemics because of the lack of sensitive assays for HEV (Khuroo 1980; Wong et al. 1980). Later specific serology to HEV and molecular methods specific to HEV were developed to establish the aetiology of such epidemics (Reyes et al. 1990).

In October 2008, we received information that several people were presenting to the hospitals with acute hepatitis in Nellore, a city in the state of Andhra Pradesh, India. The objectives of our study were to: (i) perform a detailed epidemiological investigation to determine the incidence of the disease in the population, (ii) to confirm the cause of the outbreak, (iii) to identify the source of the infection and (iv) to propose appropriate control and preventive measures.

Methods

Pilot survey

A patient with acute hepatitis from Nellore was admitted to Christian Medical College, Vellore in October 2008, and the referring gastroenterologist reported a large outbreak of acute viral hepatitis in Nellore. A team consisting of a gastroenterologist, an epidemiologist and a virologist from Vellore, visited Nellore in October and collected blood and stool samples from 100 patients with icteric hepatitis presenting to major hospitals in Nellore. The criteria for diagnosis of icteric hepatitis were defined as those cases that had icterus, dark coloured urine, fever of about 38 °C, elevation of alanine transaminase >2.5 times normal and/or bilirubin >1 mg/ml in serum and/or presence of bile salts and pigments in urine. To identify further cases and to determine the incidence of acute viral hepatitis in the city, an intensive door to door survey of all city wards was conducted to identify individuals in the household with signs and symptoms of jaundice from June 2008 onwards. The 100 hospital patients’ samples contributed to the determination of the cause of the outbreak but were excluded from the survey.

Selection of clusters

Maps of the city and the wards were obtained from the municipal authorities. Water sources, pumping stations and distribution systems were provided by the engineers in charge of the central water distribution. A cluster sampling method was used to determine attack rates for the city with reference to the drinking water source.

Nellore has 400,000 residents and is divided into 50 wards. The main source of drinking water is the Pennar river that runs through the northern part of the city and finally joins the Bay of Bengal. Sub-surface water from the river is collected in infiltration galleries and then pumped to collection wells, where it is chlorinated to 2 ppm. There are two pumping stations in the river; one is the main head water works (HWW) at Santhapet, the other is downstream at Bujjamarevu (Figure 1). From the HWW, the water is pumped to 16 overhead tanks and four underground sumps. The pumping station at Bujjamarevu supplies two overhead tanks. There are 10 areas not supplied with river water which pump ground water directly, and one area that is not supplied by the municipal water supply system. Based on the source of drinking water, we divided the city into 33 clusters. Using this categorization, areas supplied by each water source were represented.

Field survey

From 24 November to 4 December 2008, a door-to-door survey was carried out by a survey team of medical interns from Narayana Medical College and Hospital, Nellore. Information on the total number of members in the household, age, sex and drinking water source was obtained using a semi-structured questionnaire. Details of any jaundice in the household in the last 6 months (June–December) were also collected. Duration of illness and complications, if present, were recorded. In each cluster, the centre was identified and a street selected randomly by tossing a coin. On each selected street, every third house was surveyed. If the house was locked, the next house was surveyed and subsequently, from that house, every third house, until 20 houses had been completed.

Serological investigation

Plasma samples from all 100 patients were screened for anti-HEV IgM antibodies using ELISA. Antigen-coated plates (Genelabs Diagnostics, Singapore (presently MP Bio) which use four short recombinant proteins derived from 3’ termini of ORF2 (42aa) and ORF3 (33aa) from Burmese and Mexican prototype sequences were used. The manufacturer’s instructions for washing, detection and calculation of cut-off value were followed. The in-house anti-HEV IgM–positive control, included on each plate, was plasma from a patient who was icteric and positive for HEV-RNA by RT-PCR. The in-house negative control was serum from
a child previously tested and found to be negative for anti-HEV IgM and IgG.

Virological investigation

Viral RNA was extracted using the Trizol method (Invitrogen, Carlsbad, CA, USA), with 100 μl of plasma or stool suspension added to 1000 μl of the reagent. The RNA was reverse transcribed using random primers (hexamers) (Pd(N)6; Pharmacia Biotech, UK) to cDNA using 400 units of Moloney murine leukaemia virus reverse transcriptase (Invitrogen, Life Technologies, UK). Nested PCR was performed using the first round primers, MJC ESP 5’-CATGGTAAAGTGGGTCAGGGTAT-3 and MJC EAP 5’-AGGGTGCCGGGCTCGCCGGA-3. Second round primers MJC ISP 5’-GTATTTCGGCCTGGAGTAAGAC-3 and MJC IAP 5’-TCACCGGAGTGYTTCTTCCAGAA-3 amplified a 325-bp fragment which corresponds to the RNA-dependent RNA polymerase (RdRp) gene (Zhai et al. 2006). The amplified product was sequenced using ABI

Figure 1 Map of Nellore showing the Pennar river in the north, the two pumping stations and the 33 clusters. Clusters 6 and 18 received water from the pumping station at Bujjamarevu. Also seen are sewage canals draining into the river upstream of the pumping station at Bujjamarevu (denoted by arrows). All clusters other than 6 and 18 were supplied from the pumping station at Santhapet.

Phylogenetic analysis of nucleotide (nt) sequences was performed using the BioEdit software package (version 7.0.5.3). Dendrograms were constructed using MEGA (version 4.0), and genetic lineages were inferred by neighbour-joining algorithm using 1000 pseudoreplicates. Sequences from Nellore strains were compared with reference strains implicated in outbreaks and sporadic infections isolated from India.

Genotypes were assigned based on >90% homology at the nucleotide level with sequences from other published strains within a given genotype available at GenBank. Five nucleotide sequences isolated from the Nellore epidemic have been submitted to the GenBank (Accession numbers GU075688 to GU075692).

Water analysis
To assess the water quality in the city during the outbreak, water samples were collected from Navabpet, which was the worst affected area in Nellore city. Municipal water from street taps from six different locations and one bore water sample were collected and tested for presumptive coliform count using standard techniques (Ramakrishna et al. 1996).

Statistical methods
Data were entered in Epi-Info 2002 (CDC, GA, USA, 2002) and analyzed using STATA 10.0 (STATA Corp., TX, USA, 1984–2009) software. The overall and stratum-specific attack rates (with 95% CI) were calculated and compared by chi-square tests. To adjust for the effect of intra-cluster correlation because of the cluster sampling design, all analyses were performed using the survey design analysis (‘svy’) commands in STATA. The confidence intervals (95% CI) were calculated using the Taylor linearization method, which takes into account the complex survey design for the estimation of variance (Kreuter & Richard 2007).

Results
Survey findings
The sample survey covered 673 households consisting of 2685 individuals, with 1344 men and 1341 women. Of these, 152 (106 men and 46 women) reported jaundice between June and December 2008. There were 2 deaths because of hepatitis in the survey, with a case-fatality rate of 1.3% (95% confidence intervals, CI, 0–3.2%). The overall attack rate was 5.7% (95% CI, 4.2–7.2%). When extrapolated to the total population of Nellore city, an estimated 23 915 (95% CI, 16 837–28 992) individuals were affected, and the expected number of deaths in the city was 315 (0–765).

Men had a higher attack rate of 7.8% (5.7–9.9%) than women, 3.5% (2.1–4.9%), and were at 2.4 (1.6–3.6) times greater risk of getting infected than women ($P < 0.001$). Children under 5 years had a lower attack rate of 2.9% (0–6.3%), while attack rates in older age groups were 5.6 (2.8–8.3%) in children aged 5–15, 6.9% (5.1–8.7%) in those aged 16–45 and 2.8% (0.9–4.7%) in the age group older than 45 years.

Attack rates and water source
Families who received water from the pumping station at Bujjamarevu had the highest attack rate of 54.5% (39.8–69.2%). The difference in attack rates in comparison with families who received their drinking water from other pumping stations was significant, $P < 0.001$. In households, the highest attack rate of 22.3% (15.3–29.3%) was seen in families who drank municipal water, compared to other sources of water (Table 1).

Virological investigation
Of the 100 blood samples tested for anti-HEV IgM antibodies, 80 were positive for anti-HEV IgM. HEV-RNA
was detected in 43/100 samples tested using RT-PCR. The sequence data showed that the strains belonged to Genotype 1, subtype A, which is the predominant type circulating in India. The sequences from strains isolated from Nellore were nearly identical. The closest sequence to the Nellore strains was a strain isolated from a pregnant woman in Japan which was genotype 1A (Figure 2). The other closest strains were implicated in small outbreaks in India and strains isolated from sewage from western India (Aggarwal et al. 1999; Vaidya et al. 2002). All samples which tested positive for HEV-RNA were collected within 2 weeks post-onset of symptoms; however, when repeat samples from 9 patients were tested 2 weeks later, they were no longer viremic.

Water analysis data

All 6 water samples from the municipal taps tested were unsatisfactory with presumptive coliform counts >180/100 ml. However, the bore water was satisfactory. The residual chlorine level was zero in all water samples from municipal taps tested except one with 0.4 ppm of chlorine.

Discussion

An estimated 23 915 cases of acute viral hepatitis occurred in Nellore between June and December 2008. During hepatitis E outbreaks, the overall attack rates range from 1% to 15% (Khuroo 1980; Kane et al. 1984; Naik et al. 1992; Tsega et al. 1992). We report an attack rate of 5.7%, among the highest reported so far from India. Only one epidemic has previously been reported from southern India, where 1611 hospitalized cases were reported in Hyderabad in 2005 (Sailaja et al. 2009). All other outbreaks reported so far in India have been from the north. The largest epidemic reported was in Kanpur in 1991, which affected an estimated 79 000 individuals (Naik et al. 1992).

Many epidemiological features of this outbreak resemble previous hepatitis E epidemics. Men had higher attack rates than women, attack rates in children were low and young adults showed high attack rates (Naik et al. 1992; Tsega et al. 1992). It is still not known why men are at greater risk and children spared. In this report, families who received their water exclusively from the Bujjamarevu

Figure 2 Phylogenetic tree constructed by neighbour-joining method based on partial nucleotide sequence of gene encoding RNA-dependent RNA polymerase region of ORF 1 (4234–4560). Strains isolated from the Nellore epidemic are indicated in the box and their closest strain in a circle.
The pumping station had the highest attack rates. There were sewage canals draining into the river close to this station as well as at other points and leaking drinking water pipes running through sewage drains (Figure 1). Although the point source of contamination that led to this outbreak and the events that triggered it could not be determined, unsafe water collection and distribution systems with a high possibility of contamination with sewage were noticed at all points from the pumping station to the distribution system to the households.

It is interesting to note that the strain closest to the Nellore strains was isolated from a pregnant woman in Japan, given that human HEV circulating in Japan belongs to genotype 4 and the same genotype is found only in pigs and not humans in India (Arankalle et al. 2002). However, she was a tourist who had just visited India and may have acquired the infection during her stay.

There were no cases of hepatitis E in pregnant women in the survey population but one hospitalized pregnant woman, who was positive for HEV-RNA but recovered uneventfully, had been included in the pilot survey. This is contrary to reports from north India where a high incidence has been reported in pregnant women (Kumar et al. 2004; Patra et al. 2008). A study on sporadic disease conducted in Chennai, south India showed a very low incidence in pregnant women. It has been proposed that a subgenotypic shift in the northern Indian strains may contribute to virulence, while the strains in the south might be stable and less virulent (Rasheeda et al. 2008).

Our study used molecular methods in addition to serology, to confirm and genotype the cause of the epidemic. The higher attack rates in the areas supplied by the downstream pumping station which had more sewage outlets upstream indicate that the likely source of this large outbreak was from the municipal water source. This is supported by the lack of detectable chlorine and the high coliform counts. These findings suggest that better engineering of sewage canals and drinking water distribution systems and a continued process of maintenance and chlorination are needed. The study findings were reported to the city authorities who then directed repair of water pipelines to the worst affected areas. Daily newspapers reported our findings to educate the public about control measures. In a resource-limited setting, particularly when urban areas grow rapidly, provision for sewage and protected water supply is often neglected, with serious consequences for public health.

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References


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