HEPATITIS E VIRUS INFECTION AS A MARKER FOR CONTAMINATED COMMUNITY DRINKING WATER SOURCES IN TIBETAN VILLAGES

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Abstract. In April–May 2001, a study was conducted to determine the prevalence of antibodies against hepatitis E virus (HEV) among 426 persons 8–49 years of age randomly selected from two groups of rural villages in central Tibet. Group 1 villages were assessed in 1998 as having poor quality water sources; new water systems were then constructed prior to this study. Group 2 villages had higher quality water and were not designated as priority villages for new systems prior to the study. No participants tested positive for IgM; only IgG was detected in the analyzed samples. Overall, 31% of the participants had ever been infected with HEV (95% confidence interval [CI] = 26.7–35.7%). The rate was higher in men (36.6%) than women (26.3%) and highest in those 30–39 years of age (49.1%). The rate of past infection was higher in group 1; the risk ratio was 2.77 (95% CI = 1.98–3.88). This difference is most likely the result of the poor quality of the original water sources in these villages. In resource-poor countries, HEV may be a useful health indicator reflecting the degree of contamination in village water sources. This may be especially important in rural areas (such as Tibet) where maternal mortality ratios are high because HEV may be an important cause of deaths during pregnancy in disease-endemic areas.

INTRODUCTION

Shigatse municipality is a county-size area at the center of Shigatse Prefecture, a large prefecture in the Tibetan Autonomous Region of China that borders Lhasa Prefecture in the north and Nepal in the south. The municipality contains both the urban area of Shigatse City and the surrounding rural area comprising 165 villages. The population of the rural villages is approximately 54,000. Although there has been some urban migration in central Tibet, the population of these rural villages has been relatively stable over the past two decades. The Tibet Primary Health Care and Water Supply Project was a technical cooperation project of the Australian and Chinese governments, implemented between December 1997 and September 2001. The project focused on the rural villages and was implemented through the Shigatse Municipal Council, Shigatse Municipal Public Health Bureau, and Shigatse Municipal Water Resources Bureau. The water component of the project consisted of the installation of piped systems that delivered water from protected springs to 54 villages.

Epidemiologic studies indicate that hepatitis E virus (HEV) is almost exclusively transmitted through drinking water contaminated with feces. Major animal reservoirs with antibodies to HEV include pigs, rats, lambs, and chickens. The rate of positive test results for antibodies to HEV in pigs in China has been reported as high as 79%. Infection with HEV causes a disease similar to that caused by infection with hepatitis A virus. The viral agent was only identified in the early 1990s. The main difference between illness caused by the hepatitis A and E viruses is that hepatitis E causes high mortality in women infected by the virus during pregnancy (between 10% and 25%). Hepatitis E is common in India, Nepal, central Asia, western China, and eastern Africa. No studies of HEV prevalence in Tibet have been published. The prevalence of antibody to HEV in suspected or documented disease-endemic regions has been lower than expected (3–26%) and higher than expected in non-endemic regions such as the United States (1–3%).

Since the project had a goal of improving the health status of the population in the target villages and given that HEV is mainly transmitted through drinking contaminated water, it was desirable to establish the baseline prevalence of the disease. An attempt was made in 1999 to conduct a prevalence survey; however, an accident destroyed all the blood samples. When the project was extended in 2001 to continue work on water system construction, it was agreed to conduct a HEV prevalence study. The objectives of the study were to 1) determine the overall prevalence of antibodies against HEV (ever-infected) among the populations of villages participating in the water supply component of the project, and 2) compare the prevalence of HEV in villages where the original water supplies were assessed as being of poor quality and requiring urgent construction of new water systems in 1998, and in villages where the original water supplies were assessed as being of higher quality such that new water system construction was not initially required.

METHODS

The study was conducted in April and May 2001 (early spring). It was based on a stratified random sample of residents between 10 and 49 years of age in 20 villages. The villages were chosen on the basis of baseline assessments (in 1998) of the quality of their water supplies and, thus, their priority for new water system installation. Ten villages had heavily contaminated supplies (ponds and open ditches) and had new systems installed in 1998 (group 1). The other 10 villages had higher quality original supplies (springs and wells) and were not designated as requiring new systems until the project was extended into 2001, after this study took place (group 2).

The study was reviewed and approved by the local health authorities. All participants provided informed consent after being given a standard written description of the study in the Tibetan language. A sample size of 400 was chosen based on
an expected HEV prevalence of 20%, a 95% level of confidence, a precision of ± 5%, and an expected design effect of 1.5 due to the clustering effect of the method used to select participants within each village.

The sample selection began at the center of each village where a random direction was chosen. A house in that direction was randomly chosen as the first house. Participants in the target age group were recruited sequentially in each nearest household until the desired sample size of 20 was reached. Because some villages were very small, less than 20 participants were selected and extra participants were chosen in the other villages to make up a target sample size of at least 400. An informed consent form was read in Tibetan to each participant. A standard questionnaire was used to collect basic information on sex, age, history of jaundice, duration of residence in village, source of drinking water, and whether drinking water was always boiled.

A lancet was used to obtain a finger prick specimen of blood from each subject. Several drops of blood were transferred to filter paper, stored at room temperature (approximately 10–15°C), and then transported to the Department of Microbiology of the Peking University Health Science Center in Beijing where enzyme-linked immunosorbent assays (ELISAs) were conducted for past infection (IgG) and recent infection (IgM). This ELISA was developed by the Burnet Institute and is based on the open reading frame 2.1 protein expressed in *Escherichia coli*. It has been assessed to be a sensitive and specific test (sensitivity and specificity were approximately 98%) in countries with both high and low endemicity.

The findings were entered and analyzed using Epi-Info version 6 software (Centers for Disease Control and Prevention, Atlanta, GA). We did not seek to isolate HEV from water sources because we were not investigating a current outbreak; moreover, villagers in group 1 were using relatively new improved water systems.

RESULTS

The actual sample size was 426 and the age of participants ranged from 8 to 49 years. There were 194 males and 232 females. There was 1 subject less than 10 years of age, 227 (53.3%) between 10 and 19 years of age, 138 (32.4%) between 20 and 29 years of age, 53 (12.4%) between 30 and 39 years of age, and 7 (1.6%) between 40 and 49 years of age. Most participants (371) had lived in their village for their entire lives. There were 213 participants in each of the two groups based on the quality of original water supplies. The locations of the sampled villages are shown according to whether they were in group 1 or group 2 in Figure 1.

The overall prevalence of IgG to HEV (evidence of past infection) was 31% (95% confidence interval [CI] = 26.7–35.7%). No participant was positive for IgM, which appears approximately three weeks after exposure and persists for approximately six months. IgG appears at approximately the same time and persists for at least 10 years, possibly for life.

The prevalence was 45.5% in group 1 participants and 16.4% in group 2 participants. The difference between the two groups was significant with a risk ratio of 2.77 (95% CI = 1.98–3.88). Thus, people living in a village where the original water supply had been assessed as heavily contaminated and requiring urgent improvement were almost three times more likely to have ever been infected with HEV than people living in villages where the water supply had been assessed as better quality. There was significant clustering of HEV within both groups of villages. For example, among group 1 villages, there were three in which the prevalence was less than 25% and four where it was greater than 60%. Among group 2 villages, there were two with a prevalence greater than 40% and three where it was 0% (Figure 2). However, the median prevalence
of HEV infection was 42.6% in group 1 villages and 13.7% in group 2 villages.

A history of jaundice was reported by 29 participants (7%), of whom seven (24%) had an HEV infection. However, there was no significant association between a history of jaundice and having IgG to HEV. There was a significantly higher prevalence of past HEV infection among men (36.6%) than women (26.3%), with a risk ratio of 1.39 (95% CI 1.05–1.85). There was a significant trend (P = 0.002) in past infection based on age. The prevalence increased from 23.8% among persons 10–19 years of age to 37% among persons 20–29 year of age and to 49.1% among persons 30–39 years of age. The prevalence decreased to 14.3% among persons 40–49 years of age; however the sample was only seven persons in this age group. Stratified analysis by age group and village group indicates that there is a significantly lower prevalence among people living in group 2 villages in each age group (Figure 3). There was no difference in past infection with HEV between those respondents who reported always drinking boiled water and those who did not.

The findings were analyzed according to the current source of drinking water for group 2 villages. This analysis could not be carried out for group 1 villages because new water systems had been constructed in 1998, two years prior to the study, which confounded the analysis. Residents of these villages were unlikely to have recently changed drinking water source. The findings in group 2 villages (Figure 4) suggest that the rate of infection with HEV does have a negative correlation with water quality, as assessed by type of drinking water source. Those participants who reported the use of piped water were all from Xialu village, a larger village that had had a functional piped water system, albeit of inferior quality, constructed some years prior to the project. Of all the villages in the sample, this village has the highest standard of living. Only 11.1% of the residents there were positive for IgG to HEV.

**DISCUSSION**

The overall prevalence of past infection with hepatitis E of 31% found in this study is high by global standards and indicates that the disease is an important endemic problem in central Tibet. No participants in this study tested positive for IgM, which reflects recent infection. However, our study had only one participant less than 10 years of age, which limits our ability to make conclusions about infection rates in the previous decade.

Hepatitis E is a major cause of epidemic enterically transmitted hepatitis in southern and central Asia. These epidemics occur every 7–10 years during the wet season in countries where the virus is endemic, including northwestern China. The largest recorded outbreak of acute HEV occurred in Xinjiang, China in 1986–1988 with more than 119,000 documented cases.\(^6\) Hepatitis E virus has also been identified as a common cause of sporadic cases of infectious hepatitis in China. For example, a study in Guangzhou in southern China found HEV-specific IgM (evidence of recent infection) in 52% of patients with acute hepatitis.\(^7\) The same study found that 18% of 77 healthy subjects had hepatitis E-specific IgG (evidence of past infection).

A 1997 study conducted by the Burnet Institute, using the same ELISA as in this study, found that 33% of the population in the Kathmandu Valley, Nepal, had serologic evidence of past HEV infection.\(^8\) This rate is comparable to the overall prevalence found in the Tibet study; however, no significant clustering was observed in the populations studied in Nepal. Additional studies in Nepal using this ELISA have found HEV-specific IgM in 75% of 72 patients with acute hepatitis in a non-outbreak setting.\(^5\)

The higher prevalence of past infection among males than females is consistent with unpublished studies in Nepal. It is possible that men in rural Tibet drink water from contaminated surface water sources when away from home more often than women, who usually drink tea or boiled water in the home. The direct relationship between past infection and age...
(until the age of 39 years) is also consistent with the results of a separate study in Nepal. The decrease in prevalence in those 40–49 years of age may be an artifact due to the small number of persons of this age sampled in the study, or may reflect the loss of antibody among people who were infected when they were young adults. We did not find an association between past HEV infection and drinking boiled water. Other relevant behavioral factors such as hand washing and use of soap were not studied.

The prevalence among study participants in group 1 villages (45.5%) was significantly higher than in group 2 villages (16.4%). Local government authorities selected group 1 villages for water supply improvement by the project because they were perceived to have the poorest water supplies in Shigatse municipality and the greatest needs for improvement. The 10 group 2 villages where water systems were constructed in 2001 (after the study) were not included in the original list of priority villages because their water sources were considered to be relatively lower in risk. They were added as a result of a decision to extend and expand the project. This suggests that local assessments that water supply is of poor quality correlated with increased prevalence of previous infection with HEV. Although other factors, including food preparation and consumption habits, may increase the risk of HEV transmission, all the participants in this study had a similar cultural background and our study did not measure other potential risk factors.

The fact that some group 2 villages did have a prevalence of HEV greater than 40% may be explained by the variable quality of water sources both within and between villages, even though they were generally assessed to be superior to those in group 1 villages. Figure 4 shows that the prevalence of past infection with HEV varies significantly according to current drinking water source. Those people using wells or the piped system constructed by local authorities had the lowest prevalence of past HEV infection, a finding consistent with other observations suggesting that such sources provide the best quality drinking water. Other analyses carried out in this environment assessed wells, piped systems, and springs as being of better quality than streams and canals. In the context of this study, the terms stream and canal also include small, dirty ditches and ponds within village confines. These appear heavily contaminated and are not preferred by most rural inhabitants. They are used for drinking water because of the long distances involved in traveling to springs and streams higher in the hills. Surface water sources have long been known to be more heavily contaminated than wells and protected springs. It is interesting to note that even the poorly constructed piped system at Xialu (described as such by both local officials and international advisers) was associated with lower prevalence of past HEV infection.

This study shows that hepatitis E infection is a major chronic public health problem in the rural areas of Shigatse municipality in central Tibet. This virus is likely to be responsible for a high proportion of morbidity and mortality associated with enterically transmitted hepatitis in the region. This may also contribute to the high mortality associated with pregnancy and childbirth experienced by rural Tibetan women.

Hepatitis E virus is mainly transmitted by contaminated drinking water. Thus, the findings of the study highlight the need for further improvements in rural water supplies in Tibet. Health education training and materials should include discussions about HEV and the need to drink water from safe sources, to protect existing water supplies, and to drink boiled water where feasible. Although secondary spread of HEV is believed to be uncommon, it does occur. Therefore, personal hygiene should be promoted vigorously, including hand washing with soap after defecation and before cooking and eating.

Hepatitis E virus may be a useful long-term marker of improved water supplies. However, current knowledge is inadequate regarding the length of time that infected persons retain antibodies (IgG). Some studies have shown antibody loss as soon as six months after infection; however, those studies were conducted using tests that are less sensitive for the detection of past infection than the one used in this study. Consequently, it may take five or more years after the quality of drinking water is improved before decreases in IgG prevalence (past infection) can be detected. Therefore, the preferred method of monitoring would be a comparison of the prevalence of IgG to HEV based on surveys before and after improvements in water supplies. This was the intended approach in Shigatse; however, the blood specimens from the first survey were inadvertently destroyed. Children ≥ 5 years of age should be included in such surveys.

Conversely, the most striking finding of this study is the significant clustering of HEV prevalence in different villages, which is most likely due to the variable quality of water supplies. This suggests that the incidence of HEV-specific IgG may be a useful indicator of the historic level of water contamination in communities, and would allow more efficient use of resources in improving water engineering and management. This may be especially important in rural areas where maternal mortality ratios are high (such as Tibet) because HEV may be an important cause of deaths during pregnancy in disease-endemic areas.

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