Journal of Infection (2016) xx, 1–9

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Clinical and virological profiling of sporadic hepatitis E virus infection in China

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Accepted 8 June 2016
Available online ■ ■ ■

KEYWORDS
Hepatitis E;
Hepatitis E virus;
Clinical features;
Molecular epidemiology;
Zoonotic transmission

Summary *Objectives:* Recently, genotype 4 HEV (HEV-4) associated hepatitis E has overtaken HEV-1 infections in China, but few studies reported the clinical and virological features of HEV-4 infection.
Methods: Sixty-two patients with acute hepatitis E (AHE) were enrolled from three hospitals in China. Clinical profiles and epidemiological records were analyzed. Patients' serum samples were tested for anti-HEV IgM/IgG and fecal samples were screened for HEV RNA. Representative HEV isolates were partially sequenced and analyzed phylogenetically.
Results: A high median age (57.5 years) and an overwhelming proportion of males (51/62, 82%) were found. Most patients presented with symptoms of jaundice (56/62, 90%), malaise (44/62, 71%), anorexia (44/62, 71%) and nausea (41/62, 66%). Elevated mean values of total bilirubin (186 μmol/L), direct bilirubin (109 μmol/L), ALT (997 IU/L), AST (583 IU/L), ALP (159 IU/L) and GGT (170 IU/L) and reduced albumin level (32 g/L) were observed. The positive rate for anti-HEV IgM/IgG was 100% (62/62)/76% (47/62), for HEV RNA was 58% (25/43). Twelve HEV-4 isolates were obtained.
Conclusion: All HEV isolates belonged to HEV-4 and showed high sequence similarity to swine HEV-4. Most of the sporadic cases had typical clinical symptoms, signs of AHE, and elevated levels of serum bilirubin and liver enzymes.

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http://dx.doi.org/10.1016/j.jinf.2016.06.005
0163-4453/

Please cite this article in press as: Wang L, et al., Clinical and virological profiling of sporadic hepatitis E virus infection in China, J Infect (2016), http://dx.doi.org/10.1016/j.jinf.2016.06.005

Introduction

Hepatitis E virus (HEV), the pathogen causing acute hepatitis E (AHE), has become a worldwide public health concern. In large outbreaks, high morbidity and mortality have been documented, particularly in pregnant women, where mortality rates of up to 20% have been reported.[1] At least seven closely related genotypes of HEV (HEV-1-7) have been recognized to-date.[2] HEV-1 and HEV-2 have been associated with large waterborne epidemics throughout developing countries and has only been isolated from humans. HEV-3 and HEV-4 are zoonotic isolates that have been linked to sporadic cases occurring in developed countries.[1]

HEV infections are now thought to be indigenous in developed countries, with most cases being associated with HEV-3 or HEV-4 and characterized by icteric hepatitis or asymptomatic illness.[1] Consumption of contaminated meat and high-risk occupational exposures have been seen as the cause of these infections.[3] Recently, several reports have highlighted HEV-3 infection as being associated to chronic hepatitis and cirrhosis.[4] In addition, the recognition of such cases in the immunocompromised population has aroused broad concern globally.[1]

The first documented outbreak of HEV took place in India during 1955–1956, and involved 29,300 cases.[5] Large outbreaks involving thousands of affected individual have occurred subsequently in many countries including China, Mexico, Vietnam and Sudan. The most notorious of these, caused by HEV-1, took place in the Xinjiang Uyghur Autonomous Region of China in the late 1980s with approximately 120,000 people being affected.[6] In the subsequent 25 years, larger outbreaks of this type have only been rarely seen in China, with sporadic cases of indigenous HEV caused by HEV-4 having dominated.[7,8] In the last decade there has however been a rapid growth of AHE cases in China, with numbers almost doubling (16,444 to 26,988 cases) between 2004 and 2014 (http://www.nhfpc.gov.cn/). The patient profile in these sporadic cases has seen them occurring mostly in older men, but there have been few attempts to explain this infection pattern.[9,10] A high prevalence of anti-HEV antibodies together with HEV RNA has been detected in domestic pigs in China indicative of pigs being the primary reservoir for HEV.[8] In 2009, this group reported the first isolation of rabbit HEV-3 (rHEV-3) from farmed rabbits in China,[11] and subsequently showed that rHEV-3 can cross the species barrier to infect pigs[12] and a non-human primate.[13] These data suggest that rHEV-3 may present a new zoonotic threat for humans, although to-date no solid evidence has been forthcoming to show that humans have been infected with this virus.[14]

China is recognized as an endemic region for HEV and previous studies have focused on the clinical profiles of HEV-1 associated AHE.[15] By contrast the relatively low incidence of sporadic genotype 4 HEV has curtailed studies on the clinical features of the disease caused by this genotype. The involvement of cases from multiple regions of China is even rarer. In addition, in Chinese hospitals HEV associated disease is commonly diagnosed solely on the basis of serological assays of anti-HEV antibodies, precluding tabulation of genotype specific disease morbidity. Against this epidemiological background, the present study focused on obtaining a more comprehensive profile of AHE in patients from northern, eastern and northeastern China.

Materials and methods

Patients

Between January 2013 and December 2014 sixty two AHE patients were enrolled from three hospitals, 44 cases from Linyi City in Shandong province, 14 cases from Taiyuan City in Shanxi province and four cases from Harbin City in Heilongjiang province (Fig. 1). The Diagnostic Criteria for HEV (WS 301-2008, China) require patients to present with clear symptoms of hepatitis, exhibit elevation of liver enzyme levels, and have serological evidence of HEV infection. The latter involves detection of anti-HEV immunoglobulin M (IgM) and/or a spontaneous rise of anti-HEV immunoglobulin G (IgG). In addition to meeting these diagnostic criteria, the recent travel history and any other epidemiological risk factors were documented for all patients recruited to the study (Table 1).

Ethical approval and patient consent

This study was approved by the Ethics Committee of Peking University Health Science Center. All patients gave informed consent and permission for testing of clinical samples.

Collection and processing of samples

Serum and fecal samples were collected from all patients at the beginning of their hospitalization and immediately shipped to our laboratory in Beijing, where they were processed as previously described.[13]

Detection of anti-HEV antibodies and biochemical analyses

Anti-HEV IgM/IgG antibodies were detected using an enzyme-linked immunosorbent assay (ELISA) based on the E2 protein of HEV-1 (amino acids 394–606 bp of HEV ORF2) that was performed according to the manufacturer's instructions (Wantai, Beijing, China).[16] All the recruited patients were also tested for hepatitis A, B and C infection. The serum samples were also subjected to standard clinical biochemical profiling using a Hitachi Automatic Clinical Analyzer 7180.[13]

Detection of HEV RNA and phylogenetic analyses

RNA extraction and a nested reverse transcription polymerase chain reaction (RT-nPCR) for detecting HEV RNA were both performed as previously described.[13] Quantification of the HEV RNA was carried out using a TaqMan Real-time quantitative reverse transcription polymerase chain reaction (RT-qPCR).[17] Phylogenetic analysis was performed as previously described.[8]

Please cite this article in press as: Wang L, et al., Clinical and virological profiling of sporadic hepatitis E virus infection in China, J Infect (2016), http://dx.doi.org/10.1016/j.jinf.2016.06.005