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Hepatitis E vaccine immunization for rabbits to prevent animal HEV infection and zoonotic transmission

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ABSTRACT

Hepatitis E virus (HEV) infection has become a significant global public health concern as increasing cases of acute and chronic hepatitis E are reported. HEV of animal origin was proved to be a possible source of human infection and a previous study showed that the recent licensed HEV 239 vaccine can serve as a candidate vaccine to manage animal sources of HEV infection. However, previous immunization strategy for rabbits was the same as that for human, which is too costly to conduct large-scale animal vaccination. In an effort to reduce the costs, three vaccination schemes were assessed in the present study. Forty specific pathogen-free (SPF) rabbits were divided randomly into five groups with eight animals for each and inoculated intramuscularly with different doses of HEV 239 and placebo, respectively. All animals were challenged intravenously with swine HEV-4 and rabbit HEV of different titers 7 weeks after the initial immunization and then fecal virus excretion was monitored for 10 weeks. The results indicated that immunizing rabbits with two 10 μg doses of the vaccine is superior to vaccination with two 20 μg doses or a single 30 μg dose, which can protect rabbits against homologous and heterologous HEV infection. These findings could enable implementation of large-scale animal vaccination to prevent rabbit HEV infection and zoonotic transmission.

1. Introduction

Hepatitis E virus (HEV) infection is a major cause of acute viral hepatitis in areas with poor sanitation, and recently became a significant global public health concern as increasing cases of hepatitis E have been reported in developed countries [1]. HEV is a non-enveloped, positive sense single-stranded RNA virus that is transmitted mainly through the fecal-oral route. Most cases of hepatitis E are self-limited and the mortality rate is about 1% in the general population [2]. However, HEV infection can lead to poor outcomes in the context of preexisting liver diseases or pregnancy [3]. The mortality rate rises substantially to approximately 20% in pregnant women infected with HEV [4]. Moreover, chronic hepatitis E has been identified in immunocompromised individuals, including organ transplant recipients [5], patients receiving chemotherapy [6], and HIV-infected individuals [7]. Additionally, nonhepatic complications associated with HEV infection, such as neurologic disorders and pancreatitis, have been reported [8–11].

HEV belongs to the genus *Hepevirus* in the *Hepeviridae* family, which includes at least four major genotypes that can infect humans. Genotypes 1 and 2 infections are restricted to human and are the major cause of sporadic cases and waterborne epidemic acute viral hepatitis in resource-limited regions. Genotypes 3 and 4 are responsible for sporadic cases of hepatitis E in both developing and developed areas and are thought to be zoonotically transmitted from animal reservoirs to humans via the consumption of uncooked or undercooked infected meat or by direct contact with infected animals [12–18]. In the last decade, the sporadic cases of autochthonous HEV infection caused by genotype 3 and 4 reported in industrialized countries have increased, which are mainly zoonotic and transmitted by ingesting uncooked or poorly cooked pork or game meat [1,12,19–27]. Moreover, in recent years, considerable cases of fulminant hepatitis E and chronic hepatitis E have been increasingly reported after eating undercooked meats and entrails of pigs, wild boars, and deer [18,28–31]. Therefore, it is essential to manage HEV zoonotic transmission from animal

Abbreviations: rHEV, HEV strain isolated from rabbit; sHEV-4, HEV-4 strain isolated from swine.

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reservoirs to humans. However, prevention of human infection remains challenging as the target population is currently uncertain and possible transmission routes are not fully understood. Therefore, preventing the spread of HEV from animal hosts seems to be a practical measure. Strategies might include avoiding uncooked or undercooked meat products, ensuring the proper disposal of animal feces, and vaccinating animal reservoirs. However, the first two ways are too complicated to control, especially for people who in general likes eating something uncooked or undercooked. In addition, soft fruits or vegetables irrigated with contaminated surface water may also constitute potential sources of zoonotic transmission [32,33], which are not easy to control. Hence, vaccination of primary hosts could be the optimal option, but this would be a costly undertaking with the same vaccine program as for humans. Thus, it is of interest to explore an immunization strategy for animal reservoirs of HEV to prevent animal HEV infection and zoonotic transmission as well.

HEV was isolated from rabbits in China, USA, and France recently [34–36]. Phylogenetic analysis showed that the rabbit HEV isolates are most closely related to genotype 3 HEV, but sequences of the full-length genome have <80% identity to the genotype 1–4 HEV. Besides, rabbit HEV is characterized by an insertion of 31-aa in the X domain of ORF1, which does not exist in genotype 1–4 HEV, but is present in all rabbit strains isolated worldwide. Thus, some researchers have suggested that it represents a new genotype [34,37], while others considered it as a distant member of the zoonotic genotype 3 [35,38]. Accumulative lines of evidence have indicated that rabbits are sensitive to HEV infection and seem to constitute an animal reservoir of HEV [34,39]. The fact that rabbits can be experimentally infected by sHEV-4 [40] or human HEV-4 [41] and that rHEV is transmissible from rabbits to cynomolgus macaques [42] both suggest that rabbits may be a new source of human HEV infection. Interestingly, a HEV strain in rabbits closely related to human HEV strain has been identified in France [36], and recently, a HEV strain closely related to a human HEV sequence was identified for the first time in the pet house rabbit [43]. These findings might shed new light on the potential of zoonotic HEV transmission from rabbits to humans. Furthermore, HEV infection in rabbits could lead to adverse outcomes in pregnancy and vertical transmission [44]. In order to explore the immunization strategy for rabbits to prevent animal HEV infection and zoonotic transmission, we tested in the present study the protective efficacy of HEV vaccine with various dosages and immunization times on rabbits challenged by HEV strains with different titers and genotypes.

2. Materials and methods

2.1. Ethics statement

The animal experiments were approved by the Committee of Laboratory Animal Welfare and Ethics, Peking University Health Science Center. This study was performed in strict accordance with the protocol for the review on Laboratory Animal Welfare and Ethics, Peking University Health Science Center.

2.2. Vaccines

The HEV 239 vaccine (Hecolin; Xiamen Innovax Biotech, Xiamen, China) is a bacterially expressed recombinant peptide corresponding to amino acid residues 368–606 of ORF2 of HEV-1, which contains 30 μg of the purified antigen in 0.5 ml buffered saline adsorbed to 0.8 μg aluminum hydroxide [45]. The licensed hepatitis B vaccine (10 μg of Engerix-B, GlaxoSmithKline, Belgium) was used as the placebo.

2.3. Specific pathogen-free rabbits

Forty 7-week-old, specific pathogen-free (SPF) Japanese white rabbits (1–1.5 kg) were obtained from the Department of Laboratory Animal Science of Peking University Health Science Center. Prior to vaccination, all rabbits were confirmed negative for anti-HEV antibodies by an enzyme-linked immunosorbent assay (ELISA) and negative for HEV RNA in fecal/serum by RT-nPCR [40]. Each rabbit was placed in a separate cage and fed twice a day.

2.4. Challenge viruses

A rabbit HEV strain of CHN-BJ-R14 (rabbit feces, JX109834, and rHEV) and a swine HEV genotype 4 strain of SD-sw2 (pig feces, KP284140, and sHEV-4) isolated from feces, which were prepared as suspension in phosphate-buffered saline (PBS, pH 7.4), were used as the inocula in this study. The viral suspension was centrifuged at 5000 rpm at 4 °C for 30 min and filtered sequentially through 0.45 and 0.22 μm filters before inoculation. The viral load of each HEV strain was adjusted to both 6×10^4 and 6×10^6 copies/ml, according to their original titers determined by TaqMan RT-qPCR as described previously [46].

2.5. Immunization and challenge

All rabbits were divided randomly into five groups (A–E) with eight rabbits per group. The rabbits in group A, B, and C were inoculated intramuscularly in the thigh on week 0 with a 10-, 10-, and 20-μg dose of the vaccine in 0.5 ml, respectively, and exactly the same vaccination procedure was repeated on week 4. The rabbits in group D were only vaccinated intramuscularly in the thigh on week 0 with a 30 μg dose in 0.5 ml. Similarly, 10 μg dose of the hepatitis B vaccine were administered twice to rabbits in group E on week 0 and 4. On week 7, eight vaccinated rabbits in each group were further divided into two subgroups of four rabbits, which were then challenged intravenously with 1 ml of inocula (the rabbits in subgroup A1 and A2 were challenged with 6×10^4 copies/ml of rHEV or sHEV-4, and those in other subgroups were challenged with 6×10^6 copies/ml of rHEV or sHEV-4, Fig. 1). At the same time, eight placebo-vaccinated rabbits were divided into four subgroups with two rabbits each, which were also challenged intravenously with 1 ml of inocula (the rabbits in subgroup E1 and E2 were challenged with 6×10^6 copies/ml of rHEV or sHEV-4, and those in subgroup E3 and E4 were challenged with 6×10^4 copies/ml of rHEV or sHEV-4, Fig. 1).

2.6. Sample collection and processing

Samples of serum and feces were collected prior to inoculation and weekly for 10 weeks post challenge. The amount of HEV RNA in feces was determined by TaqMan RT-qPCR [46]. Anti-HEV antibody was detected using standard methods [40]. Antibody level was expressed in titer, which was defined as the maximum dilution of the test sample that yields an average OD value that exceeded the cutoff value. The anti-HEV levels were also calibrated against a World Health Organization (WHO) reference standard for anti-HEV (National Institute for Biological Standards and Control (UK), catalog no. 95/584), which was from Wantai, Biopharmaceutical, Beijing, China and had been assigned an arbitrary value of 100 U/ml [47].

2.7. Statistical methods

All data was processed by SAS 9.1. The mean antibody levels of different groups were compared using two sample independent *t*-tests with the assumption that the mean antibody levels of each

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