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·动物及兽医生物技术·

中国西藏部分地区猪戊型肝炎病毒流行病学调查

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Gongga, Wang YF, Yixicuomu, et al. Epidemiological investigation of hepatitis E virus infection in Tibetan swine population. Chin J Biotech, 2018, 34(9): 1453–1459.

摘 要: 戊型肝炎病毒 (Hepatitis E Virus，HEV) 感染是一个重要的全球公共卫生问题，而猪被认为是 HEV 的天然宿主。HEV 可以跨种间传播，且已经证实生吃感染的猪肉会导致人感染。在中国西藏许多地区仍然有生吃猪肉、猪肝等的习惯，且不同种家畜混合饲养，极易造成 HEV 感染和传播。然而中国西藏地区猪 HEV 流行情况报道甚少。文中对中国西藏 5 个地区市 (拉萨、日喀则、山南、那曲和昌都) 猪血清进行 HEV Immunoglobulin-M (IgM) 和 IgG 抗体检测，并通过逆转录巢氏 PCR (RT-nPCR) 进行 HEV RNA 检测和定量 RT-PCR (qRT-PCR) 进行病毒拷贝计算，首次报道了藏猪血清 HEV RNA 阳性率。结果显示，在西藏猪中 HEV 有较高的流行趋势。猪血清 HEV IgM 抗体阳性率高达 7.6% (26/340)，HEV IgG 抗体阳性率为 1.8% (6/340)，HEV RNA 阳性率高达 7.6% (26/340)，血清中病毒拷贝高达 1.7×10^7 copies/mL，而且 5 个地区有不同的流行趋势。结果表明西藏猪 HEV 感染情况严重。有关部门应加强管理，以避免人与动物之间的交叉感染和暴发。

关键词: 戊型肝炎病毒，猪，中国西藏地区

Epidemiological investigation of hepatitis E virus infection in Tibetan swine population

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Abstract: Hepatitis E virus (HEV) infection is a main global public health issue. HEV can be zoonotically transmitted across species, and swine is recognized as a major reservoir of HEV. However, information is lacking on the prevalence of HEV infection in Tibet of China, where raw pork and mixed farming of different species of domestic animals are consumed

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traditionally. In this study, swine serum was collected for HEV IgM and IgG antibodies test from five regions in Tibet of China. Meanwhile, HEV RNA was detected in swine sera. HEV has a high prevalence trend in Tibetan swine. Swine serum anti-HEV IgM antibody positive rate was as high as 7.6%, the positive rate of anti-HEV IgG antibody was 1.8%, the positive rate of HEV RNA also was 7.6%, the virus titers in serum was above 1.7×10^7 copies/mL, and there were different epidemic trends in five regions. In conclusion, antibody detection and RNA detection showed that swine in Tibet had a higher incidence of HEV infection. HEV infection in Tibetan swine is more serious and management should be strengthened to avoid cross-infection between humans and animals and outbreaks in Tibet.

Keywords: Hepatitis E virus, swine, Tibet (China)

Hepatitis E virus (HEV) is a positive-sense, single-stranded RNA virus, which is the sole member of the *Orthohepevirus* genus in the *Hepeviridae* family[1-2]. The World Health Organization has been reported that approximately 20 million people are infected by HEV every year worldwide[3] and caused the high mortality rate of approximately 20% in pregnant women[1,4]. Moreover HEV also can cause chronic infection in immunocompromised people[5-6]. Recently, HEV has emerged as a global public health issue[7-8]. The main transmission route of the infection was fecal-oral route[9-10], food-chain and contaminated water are the main reason for the outbreak[11-14]. Besides, sporadic HEV infection is primarily caused by consumption of HEV contaminated meat in industrialized countries[15].

HEV mainly exists with seven genotypes (Gt1–7) and one serotype[2,16-17]. Gt1 and Gt2 are only infected to humans and lead to large outbreaks in developing regions[3,18-19]. Gt3 and Gt4 can be transmitted zoonotically through the ingestion of infected meat and cause infections in worldwide[10,20]. The Gt5 and Gt6 are isolated from wild boars in Japan[21-22]. Gt7 is isolated from camel in Japan, which also can be transmitted to humans[23]. And other genotypes have been found in several animal species, including rat (HEV C1)[24-25], ferret (HEV C2)[26], chicken (avian HEV)[27], bat (bat HEV)[28] and trout (trout HEV)[29].

Swine are recognized as the main natural reservoirs of HEV[30-31], and many research has reported that the HEV infection is highly prevalent in swine in many countries[32-33], including China[1,34-37]. Tibet Province is located in southwestern China, where mixed farming of domestic animals is a common practice. But the prevalence of HEV in swine was rarely reported in Tibet, and the only one report being restricted to serological surveys[36]. In this study, we aimed to assess the prevalence of HEV in Tibet swine by detecting anti-HEV IgG and IgM in Tibetan swine serum. At the same time, we are the first to report the positive rate of HEV RNA in Tibetan swine serum**.**

1 Materials and methods

1.1 Sample collection

Serum samples (*n*=340, age=1±0.2 years old, male, *n*=209, female, *n*=131) were collected from the five cities from November 2016 to Mary 2017, including Shigatse city (*n*=140, male, *n*=87, female, *n*=53), Lhasa city (*n*=55, male, *n*=38, female, *n*=17), Lhoka city (*n*=54, male, *n*=35, female, *n*=19), Chamdo city (*n*=51, male, *n*=39, female, *n*=12), Nakchu city (*n*=40, male, *n*=28, female, *n*=12). The samples were stored at –40 °C until use.

1.2 Detection of anti-HEV IgG and IgM antibodies

Serum samples were tested for the presence of anti-HEV IgG and IgM antibodies using commercial enzyme-linked immunosorbent assay kits (WanTai Beijing, China) containing recombinant ORF2 peptides from the HEV genome as well as both positive and negative controls. Samples were tested in duplicate according to the manufacturer's instructions, with cut off values for IgG and IgM assays set at 0.22 and 0.24, respectively, which were determined based on the mean optical density 450 values from the negative controls.

1.3 Detection of HEV RNA in serum

Total RNA was extracted from the serum using the AxyPrepTM Body Fluid Viral DNA/RNA

Miniprep Kit (Jiangsu, China) according to the manufacturer's instructions. Reverse-transcription was performed using a reverse transcriptase kit (AMVXL for real-time polymerase chain reaction RT-PCR; TaKaRa, Japan) according to the manufacturer's directions. A 348-nucleotide amplicon from HEV open reading frame 2 (ORF2) was amplified by nested RT-PCR as previously described[1, 32].

1.4 Viral titer in serum

The viral titer of HEV in sample was quantified using SYBR green-based quantitative RT-PCR (qRT-PCR) with the specific primers as previously described[1,32]. In brief, 200 μL serum was subjected to RNA isolation. Isolated RNA was used to synthesize the first-strand cDNA, and cDNA was added as a template for qRT-PCR. qRT-PCR was performed under the following conditions: 95 °C for 30 s, followed by 39 cycles of 95 °C for 5 s and 60 °C for 31 s. The procedure was conducted using the BIO-RAD CFX Connect Real-Time System.

1.5 Statistical analysis

Prism software (GraphPad Software) was used for statistical analysis. Comparisons between two groups were performed with Wilcoxon matched pairs test. Differences were considered significant at a *P*-value less than 0.05.

2 Results

2.1 Seroprevalence of HEV in swine in Tibet of China

To investigate the seroprevalence of HEV in Tibetan swine, we collected 340 serums from five cities in Tibet (China) to detect the anti-HEV antibody. To our surprise, 26 out of 340 (7.6%) serum sample were positive to anti-HEV IgM antibody, and the Lhoka city has highest positive rate of anti-HEV IgM antibody up to 18.5% (10/54), Shigatse city was 5.0% (7/140), Lhasa city was 3.6% (2/55), Chamdo city was 5.9% (3/51) and Nakchu city was 10.0% (4/40), which were higher than the seroprevalence of anti-HEV IgM antibody in pigs in Shandong province of China (1.6%, 16/980) in 2014[38] and was lower than in Yunnan province of China (9.1%, 1/11) in 2009[39], but similar to that in Bavaria, Germany (7.0%, 36/516) in 2012. The difference analysis showed that the positive rate of anti-IgM antibody in Lhoka city was significantly different from the other three cities (Shigatse, Lhasa and Chamdo) (P<0.05). But there was no significant difference between other cities (P>0.05) (Fig. 1A).

And 6 out of 340 (1.8%, 6/340) serum sample were positive to anti-HEV IgG antibody, the Lhasa city has highest positive rate of anti-HEV IgG antibody up to 3.6% (2/55), and the Shigatse city

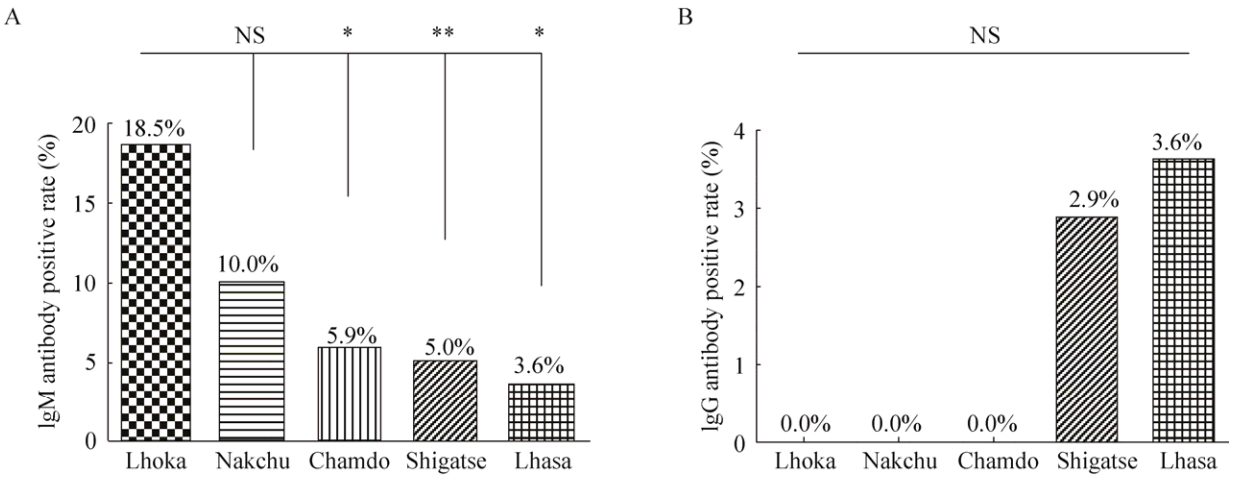

Fig. 1 Seroprevalence of HEV in Tibetan pigs of five cities in Tibet, China. (A) The positive rate of anti-HEV IgM in serum of Tibetan swine. (B) The positive rate of anti-HEV IgG in serum of Tibetan pigs. NS (not significant), P>0.05, *P<0.05, ** P<0.01.

was 2.9% (4/140), but the Lhoka, Nakchu and Chamdo cities were not tested in this research, which was lower than the seroprevalence of anti-HEV IgG antibody in pigs in Shandong province of China (66.4%, 651/980) in 2014[38], in Yunnan province, China (78.9%, 490/621) in 2011[39], in Tibet, China (42.4%, 92/453) in 2015 and in Punjab, India (60.5%) in 2017[40]. The difference analysis showed that there was no significant difference in the serum prevalence rate of anti-HEV IgG antibody between different five cities (P>0.05) (Fig. 1B).

2.2 High prevalence of HEV infection in swine in Tibet of China

Up to date, there is no report about the positive rate of HEV RNA in Tibetan swine serum. In this study, the swine serum HEV RNA was detected by RT-nPCR (Fig. 2A). And we found that 26 out of 340 (7.6%, 26/340) serum was positive to HEV RNA, those positive samples also were all the positive of anti-HEV IgM antibody, which was lower than Yunnan province of China (9.1%, 1/11) in 2011[39] and in South Brazil (20.0%) in 2017[41], but similar to that in Punjab, India (8.7%) in 2017[40].

And the five cities have different HEV RNA positive rate, the Lhoka city has highest positive rate of HEV RNA up to 18.5% (10/54), Nakchu city was 10.0% (4/40), Chamdo city was 5.9% (3/51), Shigatse city was 5% (7/140) and Lhasa city was 3.6% (2/55). The difference analysis showed that the positive rate of HEV RNA in Lhoka city was significantly different from the other three cities (Shigatse, Lhasa and Chamdo) (P<0.05). However, there was no significant difference between other cities (P>0.05) (Fig. 2B).

2.3 The HEV viral titers in serum of swine in Tibet of China

The viral titers in positive serums (n=26) were quantified by qRT-PCR. The viral titers of HEV in serum ranged from 5.5×10^5 copies/mL to 1.7×10^7 copies/mL. The high HEV titers in serum of swine indicate the high risk of HEV infection in swine in Tibet of China (Fig. 3).

3 Discussion

Zoonotic transmission of HEV has caused a major potential public health problem in the world[9-10]. In this paper, we focused on the Seroprevalence of HEV in swine in Tibet of China and we found the highly positive rate (7.6%) of anti-HEV IgM antibody in swine in Tibet of China. The positive rate of anti-HEV IgM antibody in different regions was different, and the difference analysis showed that the positive rate of anti-HEV IgM antibody in Lhoka region was significantly higher in other three cities. And we also found the highly positive rate (1.8%) of anti-HEV IgG antibody in swine in Tibet of China. The positive rate of anti-HEV IgG antibody in different regions was also different. These results showed that the HEV infected in swine was different in Tibet, which may be related

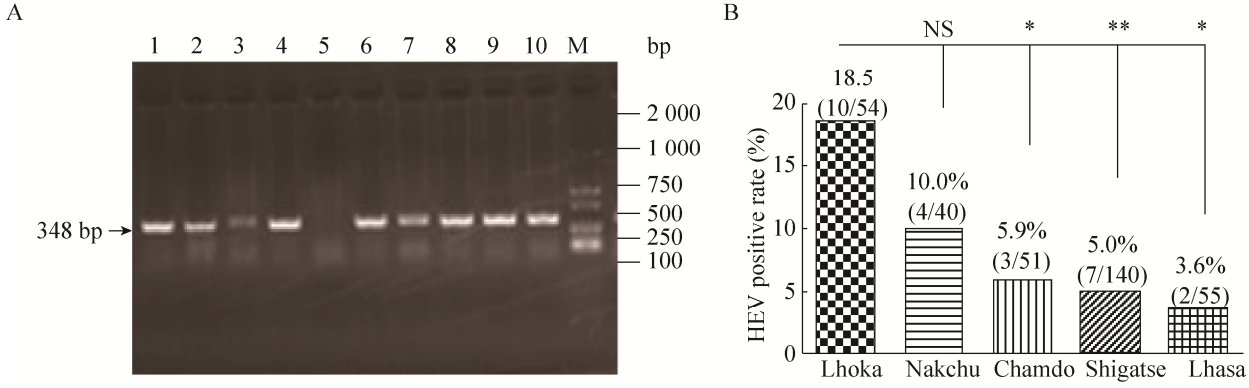

Fig. 2 Prevalence of HEV infection in Tibetan swine of five cities in Tibet, China. (A) The serum sample HEV RNA was detected by RT-nPCR. Lanes 1–10 is part of the sample number. M, DNA ladder. (B) The positive rate of HEV RNA in serum samples of Tibetan pigs of five cities in Tibet, China. NS (not significant). P>0.05, *P<0.05, **P<0.01.

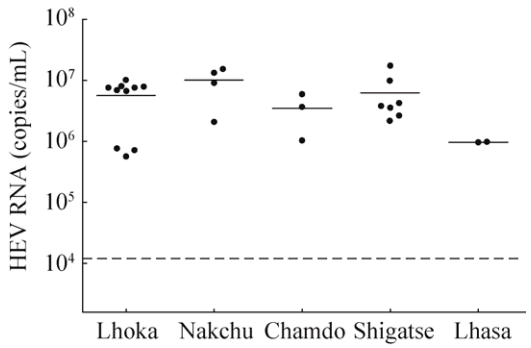

Fig. 3 The HEV viral titers in serum of Tibetan pigs of five cities in Tibet, China. Quantification of HEV genomic RNA from serum samples of Tibetan pigs. Unit for serum samples: copies/mL.

the local elevation, feeding methods and the environment. And we also reported high positive rate of HEV RNA (7.6%, 26/340) in Tibetan swine serum, which is the first report in Tibet of China. We found that anti-HEV IgM antibody and HEV RNA positive rate were higher in this study, and different regions of swine serum had high virus copies, which showed that pigs in Tibet are in the acute phase of infection and with the risk of outbreak in Tibet. So management should be strengthened to avoid cross-infection and outbreaks.

Compared with the previous studies[36,39-41], the high positive rate of anti-HEV IgM antibody and HEV RNA were found in serum in Tibetan swine, but the positive rate of anti-HEV IgG antibody was low. At the same time, the positive rate in different cities were divergent, which may be attributed to the different elevation, feeding ways of Tibetan swine and climate. However, compared with the results of swine HEV infection in Tibet reported in 2017 by Li et al[36], our survey involves more regions but relatively fewer samples in each region, which may explain why our positive rate is lower than Li et al. In order to further investigate the epidemic situation of HEV in swine in Tibet of China, we will expand sampling areas and sampling quantities in the next research.

Consuming raw (or uncooked) or undercooked meat has been confirmed to be associated with HEV infection[42-43], more importantly, consuming raw pork meat, liver remain widespread in Tibet of China, which may increase the risk of HEV infected. In addition, it has been reported that veterinarian closely contacted with swine was found with a higher anti-HEV IgG antibody titers[44], and mixed farming of different species of domestic animals also caused the contact infection. However, due to various factors, such as conditions and economics, the different species of domestic animals are still in mixed farming and grazing in Tibet of China, which also increases the risk of cross-infection between animals and humans and that may be the reason why the swine has high positive rate of anti-HEV IgM antibody and HEV RNA in Tibet of China.

4 Conclusions

In summary, high anti-HEV IgM and IgG antibody positive rate was detected in Tibetan swine serum. In addition, high HEV RNA positive rate and high HEV viral titers were also detected to prove that Tibetan swine are being infected with HEV. Management should be strengthened to avoid cross-infection between humans and animals in Tibet of China.

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