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Serological evidence of hepatitis E virus infection in dromedary camels in Ethiopia

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ABSTRACT

The genome of dromedary camel hepatitis E virus (DcHEV) has been detected in stool and serum samples from dromedary camels, but the sero-epidemiological information of DcHEV infection remains unclear. A total of 246 serum samples collected from dromedary camels *(Camelus dromedarius)* in Ethiopia, and 40 serum samples from Bactrian camels *(Camelus ferus)* in Mongolia were examined for the detection of anti-DcHEV IgG antibody by a newly developed enzyme-linked immunosorbent assay (ELISA) by using DcHEV-like particles (DcHEV-LPs) as the antigen. The results revealed that 55 of the 246 (22.4%) dromedary camels were positive for anti-DcHEV IgG, whereas all 40 samples from the Bactrian camels were negative for DcHEV IgG antibody. A total of 98 serum samples from dromedary camels, including 25 anti-DcHEV-IgG positive samples, were used for the detection of DcHEV RNA by reverse transcription-polymerase chain reaction (RT-PCR), however, no positive samples were identified. These results suggested that the DcHEV infection occurred in the dromedary camels in Ethiopia. Further studies are required to determine whether Bactrian camels are susceptible to DcHEV infection. In addition, not only DcHEV-LPs, but also virus-like particles (VLPs) delivered from G1, G3 and G5 HEV are likely applicable for the detection of the anti-DcHEV IgG antibody.

1. Introduction

Hepatitis E virus (HEV) is a positive-sense single-stranded RNA virus that belongs to the genus *Hepevirus* in the family *Hepeviridae* (Meng et al., 2012), and is the causative agent of acute or fulminant hepatitis E, primarily transmitted by the fecal-oral route and blood transfusion. Hepatitis E is a public health concern in many Asian and African countries where sanitation conditions are insufficient (Balayan et al., 1983; Emerson and Purcell, 2003). The relatively high mortality rate in HEV-infected pregnant women (5%–25%) is unique among hepatitis viruses (Hussaini et al., 1997; Khuroo et al., 1981). Hepatitis E is caused mainly by genotypes 1 (G1)–4 (G4) HEV infection and genotype 3 (G3) and G4 HEV are responsible for sporadic and zoonotic infections in both humans and other animal species worldwide (Meng, 2010).

In addition to G1–G4 HEV, a novel dromedary camel HEV (DcHEV) has been isolated from dromedary camels and classified into genotype 7 (G7); DcHEV forms a separate species in *Orthohepevirus A,* which includes the G1–G6 and rabbit HEV (Smith et al., 2014; Woo et al., 2014). An HEV strain that belongs to G7 has also been isolated from a hepatitis E patient, suggesting that the DcHEV could cause zoonotic infection in humans (Lee et al., 2016). A recent study indicated that the DcHEV generated by a reverse genetic system resulted in HEV infection in cynomolgus monkeys, providing new evidence of zoonotic infection by DcHEV (Li et al., 2016b). A long-term epidemiological study confirmed that the partial DcHEV genome were detected in camel serum or fecal samples obtained in the UAE, Somalia, Kenya, and

Abbreviations: DcHEV, dromedary camel hepatitis E virus; G, genotype; VLPs, virus-like particles; DcHEV-LPs, virus-like particles of DcHEV; ELISA, enzyme-linked immunosorbent assay; OD, optical density

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Pakistan during the period 1983–2015, suggesting that DcHEV in dromedary camels is long established, diversified and geographically widespread (Rasche et al., 2016).

However, the sero-epidemiology of DcHEV is still unclear due to the lack of precise method for anti-DcHEV antibody detection. A previous study from our group demonstrated that an enzyme linked immunosorbent assay (ELISA) using virus-like particles of DcHEV (DcHEV-LPs) as the antigen could detect anti-DcHEV IgG and IgM in cynomolgus monkeys and rabbits (Zhou et al., 2015), raising the possibility of using DcHEV-LPs to detect anti-DcHEV antibodies in camels.

The aim of this study was to establish an ELISA for the detection of anti-DcHEV IgG antibody in dromedary and Bactrian camels using DcHEV-LPs as an antigen, and thereby provide a precise method for the sero-epidemiology of DcHEV infection in camels.

2. Materials and methods

2.1. Camel sera

Serum samples from 246 (26 males and 220 females) dromedary camels *(Camelus dromedarius)* were collected in Ethiopia in August 2013. The ages of the dromedary camels ranged from 1 to 13 years old. Serum samples were also collected from 40 (14 males and 26 females) Bactrian camels *(Camelus ferus)* in Mongolia in 2012 and 2013. The ages of the Bactrian camels ranged from 2 to 6 years old.

The serum samples were treated at 56 °C for 30 min before being transported and then stored at −20 °C until use. The serum samples were transported to Japan for laboratory analyses with the permission (Import Permit Nos. '25 douken 358' and '28 douken 160') of the Japanese government.

2.2. Detection of anti-DcHEV IgG antibody

Anti-DcHEV IgG antibody was detected by an ELISA using DcHEV-LPs as the antigen. Briefly, flat-bottom 96-well polystyrene microplates (Immulon 2; Dynex Technologies, Chantilly, VA) were coated with the purified DcHEV-LPs (1 μg/ml, 100 μl/well) and incubated at 4 °C overnight. Unbound DcHEV-LPs were removed, and the wells were washed twice with 10 mM phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBS-T), and then blocked at 37 °C for 1 h with 200 μl of 5% skim milk (Difco Laboratories, Detroit, MI) in PBS-T.

After the plates were washed 3 times with PBS-T, the camel serum (100 μl/well) was added in duplicate at a dilution of 1:200 in PBS-T containing 1% skim milk. The plates were incubated at 37 °C for 1 h, and then washed 3 times as described above. The wells were incubated with 100 μl of peroxidase-conjugated goat anti-camel IgG (H + L) (1:1000 dilution) (Alpha Diagnostic International, San Antonio, TX) in PBS-T containing 1% skim milk. The plates were incubated at 37 °C for 1 h, and washed 3 times with PBS-T. Then 100 μl of the substrate orthophenylenediamine (Sigma Chemicals, St. Louis, MO) and H_2O_2 was added to each well, and the plates were incubated in a dark room at room temperature for 30 min. The reaction was stopped with 50 μl of 4 N H_2SO_4 and the absorbance at 492 nm was measured with a microplate reader (Molecular Devices, Tokyo).

2.3. Nested broad-spectrum RT-PCR for detection of DcHEV RNA

The RNA was extracted using a MagNA Pure LC system with a MagNA Pure LC Total Nucleic Acid isolation kit (Roche Applied Science, Mannheim, Germany) according to the manufacturer's recommendations. Reverse transcription (RT) was performed with a high-capacity cDNA reverse transcription kit (ABI Applied Biosystems, Carlsbad, CA) under a protocol of 25 °C for 10 min, 37 °C for 120 min and 85 °C for 5 min in a 20 μl reaction mixture containing 1 μl reverse transcriptase, 2 μl of the random primer, 1 μl RNase inhibitor, 2 μl RT buffer, 0.8 μl 10 mM deoxynucleoside triphosphates, 8 μl RNA and 5.2 μl distilled water. Then 5 μl of cDNA was subjected to a nested broad-spectrum RT-PCR that targeting a portion of the ORF1 genome as described previously (Johne et al., 2010). The serum from a DcHEV-infected cynomolgus monkey was used as a positive control for the detection of DcHEV RNA (Li et al., 2016b).

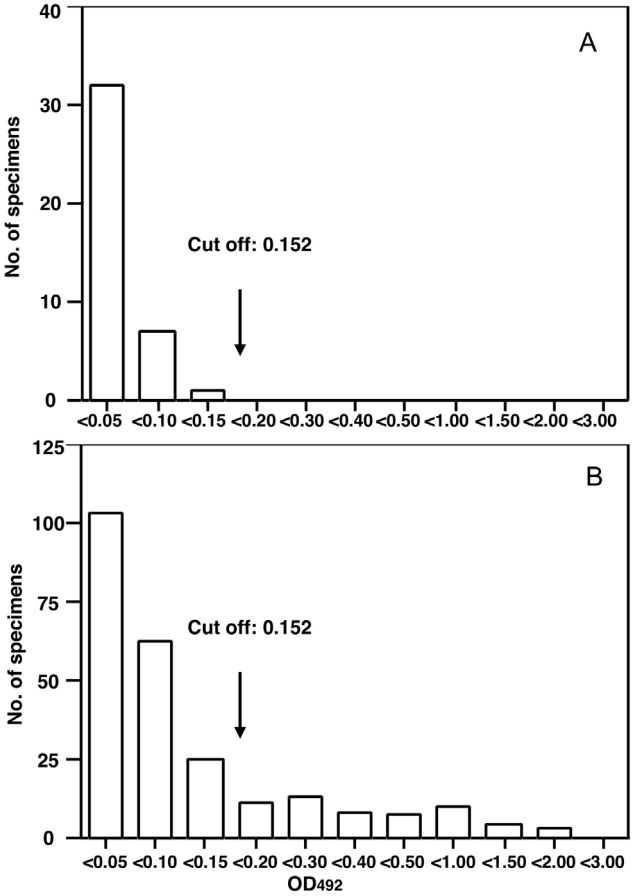

Fig. 1. Detection of anti-DcHEV IgG antibody in camels. Anti-DcHEV IgG antibody was detected in camel sera by an ELISA using DcHEV-LPs as the antigen. Serum samples from Bactrian camels (A) and dromedary camels (B) were tested, and the OD values were plotted as a frequency distribution. Arrows indicate the cut-off value.

3. Results

3.1. Detection of anti-DcHEV IgG antibody

A total of 246 serum samples from dromedary camels and 40 serum samples from Bactrian camels were used for the detection of anti-DcHEV IgG antibody at a dilution of 1:200. The distributions of the optical density (OD) values of anti-DcHEV IgG in Bactrian and dromedary camels are shown in Fig. 1A and B, respectively. The OD values of anti-DcHEV IgG of the sera from Bactrian camels ranged from 0.006 to 0.149, and no sample provided a notably large OD value. In contrast, the OD values of anti-DcHEV IgG detected in the dromedary camels ranged from 0.003 to 1.965. Therefore, these 40 serum samples from Bactrian camels were used to determine the cut-off value for the ELISA. The mean OD value of anti-DcHEV IgG in these serum samples was 0.036, with a standard deviation (SD) of 0.029. Therefore, the cut-off value for IgG was calculated as 0.152 on the basis of the mean OD values plus 4 times the SD (0.036 + 4 × 0.029). In addition, the anti-DcHEV IgG was also detected by a western blotting (data not shown), although only serum samples with an OD value over 0.353 could be detected by this assay, which was therefore less sensitive than the ELISA.