

Seroprevalence of hepatitis E virus in zoo animal species in Korea

Young-Jo Song¹, Bo-Sook Kim², Woo-Jung Park¹, Byung-Joo Park¹, Seul-Kee Lee¹, Jong-Il Shin¹,
Nak-Hyung Lee¹, Joong-Bok Lee¹, Seung-Yong Park¹, Chang-Seon Song¹, Kun-Ho Seo³, In-Soo Choi^{1,4*}

Departments of ¹Infectious Diseases, and ³Public Health, and ⁴Veterinary Science Research Institute,
College of Veterinary Medicine, Konkuk University, Seoul 143-701, Korea
²Seoul Zoo, Gwacheon 427-702, Korea

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Abstract : Hepatitis E virus (HEV) can infect not only human but also several animals. This study has been conducted to evaluate the comprehensive anti-HEV seroprevalence in zoo animals in Korea. Anti-HEV antibodies were identified in 14 of 64 zoo animal species. HEV antibodies were detected for the first time in Eurasian Lynx, Setland Pony, Fallow Deer, Ezo Sika, Formosa Deer, East Wapitis, Barasingha, Corriedale, American Bison, Guanacos, Reticulated Giraffe, and Saanen. These results indicate that the several zoo animal species were exposed to HEV.

Keywords : hepatitis E virus, seroprevalence, zoo animal species

Hepatitis E virus (HEV) is an emerging zoonotic agent. HEV is the only member of the genus *Hepevirus* in the family *Hepeviridae*. HEV is a non-enveloped virus that has an approximately 7.2 kb single-stranded genome comprised of positive-sense RNA [7]. HEV strains have been classified into four major genotypes based on phylogenetic analysis of full genome sequences or highly conserved partial sequences [7]. HEV infections by genotypes 1 and 2 are restricted to humans and cause mainly waterborne outbreak of hepatitis in developing countries. However, HEV genotypes 3 and 4 are detected in both humans and animals, and induce sporadic cases of acute hepatitis in developed countries. Human infections by HEV genotypes 3 and 4 are possibly transmitted from animal species including pigs and wild boar [8]. Previous serological studies in human, pig, and cat populations in Korea reported seroprevalence of anti-HEV antibody as 18%, 15%, and 8.1%, respectively [3, 12]. However, no reports have addressed the seroprevalence study in zoo animal species. This study was conducted to evaluate the comprehensive anti-HEV seroprevalence status in zoo animal species in Korea.

This study was conducted to evaluate comprehensively the anti-HEV seroprevalence in zoo animal species. A total of 201 serum samples were obtained from zoo animals at a Seoul Zoo, Korea from 2005 to 2010. The samples were stored at -80°C until analysis. The samples were all tested in duplicate for anti-HEV antibody with a direct sandwich enzyme immunoassay kit (Wantai Biopharmaceutical, China). The assay was carried out by following the manufacturer's

instructions. Briefly, 50 μL of the serum sample was added to each well containing 50 μL of the diluent, and the microplate was incubated for 30 min at 37°C . The microplate was washed five times with 350 μL of a wash solution. 100 μL of horseradish peroxidase-conjugated recombinant HEV antigen was added to each well, and the microplate was incubated for 30 min at 37°C . The microplate was washed five times with 350 μL of a wash solution. 50 μL of chromogen A and chromogen B solution were added into each well, and the microplate was incubated at 37°C for 15 min by avoiding light. The color-developing reaction was stopped by adding 50 μL of the stop solution to each well. The absorbance for each well was determined at 450 nm. The cutoff value was calculated to be 0.12 plus the mean absorbance of negative control according to the manufacturer's instructions. Serum samples showing an absorbance value greater than the cutoff value were determined to be positive.

Among the zoo animal species tested in this study, HEV antibodies were found in 1 of 1 Eurasian Lynx, 1 of 1 Setland Pony, 6 of 14 Fallow Deer, 2 of 8 Ezo Sika, 5 of 7 Formosa Deer, 7 of 11 Red Deer, 9 of 13 East Wapitis, 1 of 8 Barasingha, 1 of 5 Corriedale, 2 of 3 Domestic Goats, 2 of 2 American Bison, 1 of 5 Guanacos, 1 of 3 Reticulated Giraffe, and 1 of 7 Saanen (Table 1). Previous studies reported that anti-HEV antibodies were detected in different animal species including pigs, cattle, dogs, cats, horse, rabbits, and rodents [1, 12]. These results indicate that these animal species might be exposed to HEV or a HEV-like agent. These studies also suggest that several animals such as pigs may play an impor-

*Corresponding author

Tel: +82-2-2049-6055, Fax: +82-2-3436-5880

E-mail: ischoi@konkuk.ac.kr

Table 1. Prevalence of anti-HEV antibodies in zoo animal species

English name	Scientific name	No. of tested samples	No. of positive samples (%)
Order Diprotodontia			
Wallaroo	<i>Macropus robustus</i>	1	0 (0)
Parma Wallaby	<i>Macropus parma</i>	1	0 (0)
Red Kangaroo	<i>Macropus rufus</i>	1	0 (0)
American Black Bear	<i>Ursus americanus</i>	1	0 (0)
Ezo Brown Bear	<i>Ursus arctos yesoensis</i>	1	0 (0)
Asian black Bear	<i>Ursus thibetanus</i>	1	0 (0)
European Brown Bear	<i>Ursus arctos arctos</i>	1	0 (0)
Polar Bear	<i>Ursus maritimus</i>	1	0 (0)
Red Fox	<i>Vulpes vulpes</i>	1	0 (0)
Fennec Fox	<i>Vulpes zerda</i>	1	0 (0)
Leopard	<i>Panthera pardus</i>	2	0 (0)
Siberian Tiger	<i>Panthera tigris altaica</i>	1	0 (0)
Eurasian Lynx	<i>Lynx lynx</i>	1	1 (100)
Caracal	<i>Caracal caracal</i>	1	0 (0)
Eurasian Badger	<i>Meles meles</i>	1	0 (0)
Harbor Seal	<i>Phoca vitulina</i>	1	0 (0)
Order Perissodactyla			
Africa Pony	<i>Equus caballus</i>	1	0 (0)
Setland Pony	<i>Equus przewalskii caballus</i>	1	1 (100)
Grant's Zebra	<i>Equus burchellii</i>	1	0 (0)
Grevy's Zebra	<i>Equus grevyi</i>	1	0 (0)
Order Artiodactyla			
Fallow Deer	<i>Dama dama</i>	14	6 (42.9)
Hog Deer	<i>Axis porcinus</i>	3	0 (0)
Japanese Sika	<i>Cervus nippon nippon</i>	6	0 (0)
Ezo Sika	<i>Cervus nippon yezoensis</i>	8	2 (25)
Yak Sika	<i>Cervus nippon yakusimae</i>	11	0 (0)
Formosa Deer	<i>Cervus nippon taiouanus</i>	7	5 (71.4)
Red Deer	<i>Cervus elaphus</i>	11	7 (63.6)
East Wapiti	<i>Cervus canadensis</i>	13	9 (69.2)
Pere David's Deer	<i>Elaphurus davidianus</i>	6	0 (0)
Korean Water Deer	<i>Hydropotes inermis argyropus</i>	2	0 (0)
Barasingha	<i>Cervus duvaucelii</i>	8	1 (12.5)
Rocky Mountain Big-horn Sheep	<i>Ovis canadensis</i>	1	0 (0)
Corriedale	<i>Ovis aries</i>	5	1 (20)
Thinhorn Sheep	<i>Ovis dalli</i>	5	0 (0)
Nyala	<i>Tragelaphus angasii</i>	2	0 (0)
Sitatunga	<i>Tragelaphus spekeii</i>	4	0 (0)
Markhor	<i>Capra falconeri</i>	6	0 (0)
Himalayan Tahr	<i>Hemitragus jemlahicus</i>	14	0 (0)
Barbary Sheep	<i>Ammotragus lervia</i>	1	0 (0)
Ibex	<i>Capra ibex</i>	1	0 (0)
Domestic Goat	<i>Capra hircus</i>	3	2 (66.7)
European Mouflon	<i>Ovis ammon musimon</i>	2	0 (0)
Scimitar-horned Oryx	<i>Oryx dammah</i>	3	0 (0)
Domestic Water Buffalo	<i>Bubalus bubalis</i>	1	0 (0)

Table 1. continued

English name	Scientific name	No. of tested samples	No. of positive samples (%)
American Bison	<i>Bison bison</i>	2	2 (100)
Sable Antelope	<i>Hippotragus niger</i>	1	0 (0)
Dromedary	<i>Camelus dromedarius</i>	3	0 (0)
Guanaco	<i>Lama glama</i>	5	1 (20)
Llama	<i>Lama glama</i>	8	0 (0)
Roe Deer, Chinese Roe	<i>Capreolus pygargus</i>	1	0 (0)
Springbuck	<i>Antidorcas marsupialis</i>	1	0 (0)
Reticulated Giraffe	<i>Giraffe camelopardalis reticulata</i>	3	1 (33.3)
Saanen	<i>Capra hircus</i>	7	1 (14.3)
Order Rodentia			
Patagonian mara	<i>Dolichotis patagonum</i>	2	0 (0)
Order Primates			
Sooty Mangabey	<i>Cercocebus torquatus</i>	1	0 (0)
Order Anseriformes			
Mute Swan	<i>Cygnus olor</i>	1	0 (0)
Australian Black Swan	<i>Cygnus stratus</i>	1	0 (0)
Order Falconiformes			
Andean Condor	<i>Vultur gryphus</i>	1	0 (0)
European Black Vulture	<i>Aegypius monachus</i>	1	0 (0)
Order Gruiformes			
Manchurian Crane	<i>Grus japonensis</i>	1	0 (0)
White-necked Crane	<i>Grus vipio</i>	1	0 (0)
Sarus Crane	<i>Grus antigone</i>	1	0 (0)
Order Ciconiformes			
Oriental White Stork	<i>Ciconia ciconia boyciana</i>	1	0 (0)
Order Struthioniformes			
South African Ostrich	<i>Struthio camelus australis</i>	1	0 (0)
Total		201	40 (19.9)

tant role in the transmission of HEV to humans [8].

In this study, we determined the comprehensive anti-HEV seroprevalence in zoo animal species in Korea. Anti-HEV antibodies were detected in 14 of 64 animal species. Twelve of 14 animal species belonged to Order Artiodactyla. Previous reports indicated that 5% of red deer of Netherlands were positive for anti-HEV antibodies [11]. In the current study, 64% (7/11) of red deer had HEV antibodies indicating that a much higher exposure or infection of HEV in red deer than previously studied. In addition, 67% (2/3) of goat sera were positive for HEV. Similar result was reported in Turkmenistan with 67% of sero-positivity for HEV [9]. In contrast, several studies demonstrated no anti-HEV antibodies in goats in Western India [1]. These discrepancies need to be clarified by conducting more studies about HEV infection status in different regions. HEV antibodies were also found in Eurasian Lynx, Setland Pony, Fallow Deer, Ezo Sika, Formosa Deer, East Wapitis, Barasingha, Corriedale, American Bison, Guanacos, Reticulated Giraffe, and Saanen. In our knowledge, this is the first study to demonstrate anti-HEV antibod-

ies in these animals. We further investigated to detect HEV RNA in anti-HEV positive serum samples and in fecal samples of anti-HEV antibody positive animal species. However, we could not detect HEV RNA from the serum and fecal samples (data not shown). Although HEV RNA was not detectable in any of the serum and fecal samples of zoo animal species, serological evidence indirectly indicated that the several zoo animals were already exposed to HEV. Recent studies reported HEV infection in rabbits [4]. These data suggest that HEV infection would be possible in new host animals. It is already known that the transmission of HEV is mediated by a fecal-oral route in humans and pigs [2]. Therefore, other animals including zoo animals would also be infected with HEV through the same route as demonstrated in humans and pigs. A considerable portion HEV infection in humans is mediated by consumption of raw or under-cooked animal products or by direct contact with HEV-infected animals [5, 6, 10]. Therefore, zoo animal species are implicated as another risk factor for transmission of HEV to humans.

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