



# Nationwide survey of hepatitis E virus infection among wildlife in Japan

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**ABSTRACT.** In Japan, hepatitis E virus (HEV) causes hepatitis in humans through the consumption of raw or undercooked meat, including game meat. In the present study, nationwide surveillance of HEV infection among a total of 5,557 wild animals, including 15 species, was conducted in Japan. The prevalence of anti-HEV antibodies in wild boar was 12.4%, with higher positive rates in big boars (over 50 kg, 18.4%) than in small individuals (less than 30 kg, 5.3%). Furthermore, HEV RNA was more frequently detected in piglets than in older boars. Interestingly, the detection of HEV among wildlife by ELISA and RT-PCR suggested that HEV infection in Sika deer was a very rare event, and that there was no HEV infection among wild animals except for wild boar, Sika deer and Japanese monkeys. In conclusion, wild boar, especially piglets, are at high risk of HEV infection, while other wild animals showed less risk or no risk of HEV transmission.

**KEYWORDS:** hepatitis E virus, seroprevalence, Sika deer, wild animal, wild boar

*J. Vet. Med. Sci.*

84(7): 992–1000, 2022

doi: 10.1292/jvms.22-0237

Received: 13 May 2022

Accepted: 23 May 2022

Advanced Epub:

7 June 2022

Worldwide, hepatitis E virus (HEV) is the causative agent of acute viral hepatitis. HEV outbreaks have been described in developing countries, with mortality rates reaching 20–30% in pregnant women [38, 40]. In industrialized countries, zoonotic food-borne transmission due to the ingestion of infected animal meat is considered to be the main route of infection, but solid organ transplantation and blood transfusion routes have also been described [15, 60]. Although HEV infection is generally self-limited, it can cause chronic hepatitis in immunocompromised patients [15].

HEV is a non-enveloped, single stranded positive sense RNA virus with a genome length of approximately 7.2 kb, which encodes 3 opening reading frames (ORFs). HEV belongs to the family *Hepeviridae* with two assigned genera: *Othohepevirus* with four species (A to D) and *Piscihepevirus* with a single species [42, 54]. Until now, *Othohepevirus A* has been classified into 8 genotypes. Genotypes 1 and 2 are found exclusively in humans, genotypes 3 and 4 are zoonotic and have been reported from various mammals [7, 41, 63]. Genotypes 5 and 6 were detected in Japanese wild boar, while genotypes 7 and 8 were described in dromedary and bactrian camels, respectively [50, 66].

In Japan, HEV genotypes 3 and 4 are predominant in human and animal populations, and domestic pigs are the main reservoir [36]. Food-borne transmission from game meat was first reported by the consumption of Sika deer meat (*Cervus nippon*) in 2003 [61]. Since then, HEV infections in humans were mainly linked to the consumption of pork and wild boar meat and several epidemiological surveys suggested that wild boar are highly susceptible to HEV infection [21, 50, 55, 58]. Furthermore, the HEV genome and antibodies have been found in companion, feral and wild animals, showing that the other species are also exposed to HEV infection by unknown route(s) [14, 27, 32, 67].

Despite increasing reports of zoonotic transmission from game meat, the distribution, and characteristics of HEV strains circulating among wild animals, including wild boar and Sika deer populations have not been fully understood. In addition, the shedding of the virus in wild boar feces has been observed [51], indicating that cohabiting wild animals are at risk of HEV infection.

In this study, a nationwide survey was conducted in order to assess the risk of zoonotic HEV infection among wild animals as well as to characterize the genotypes of the HEV strains in Japan.

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## MATERIALS AND METHODS

### Serum samples

A total of 5,557 serum samples were collected from wild boar (*Sus scrofa*), Sika deer (*Cervus nippon*), raccoons (*Procyon lotor*), mice (*Apodemus speciosus*, *Apodemus argenteus* and *Myodes smithii*), Japanese monkeys (*Macaca fuscata*), raccoon dogs (*Nyctereutes procyonoides*), Japanese badgers (*Meles anakuma*), mask palm civets (*Paguma larvata*), nutrias (*Myocastor coypus*), weasels (*Mustela itatsi* and *Mustela sibirica*), Japanese black bears (*Ursus thibetanus*), Japanese martens (*Martes melampus*), Japanese hares (*Lepus brachyurus*), red foxes (*Vulpes vulpes*) and Reeve's muntjac (*Muntiacus reevesi*).

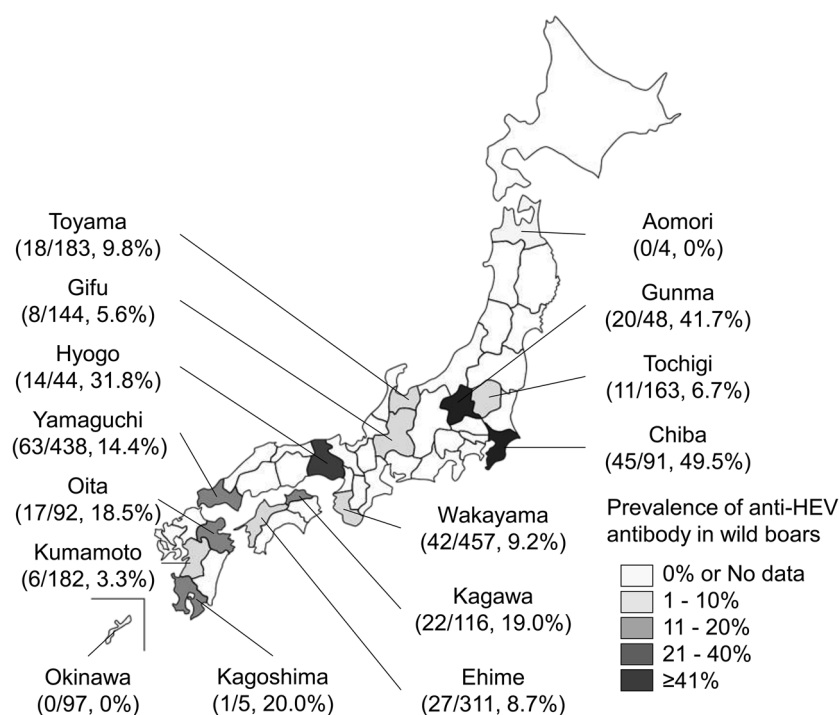
A total of 2,375 serum samples from wild boar were collected in Aomori (n=4), Chiba (n=91), Ehime (n=311), Gifu (n=144), Gunma (n=48), Hyogo (n=44), Kagawa (n=116), Kagoshima (n=5), Kumamoto (n=182), Oita (n=92), Okinawa (n=97), Tochigi (n=163), Toyama (n=183), Wakayama (n=457), and Yamaguchi (n=438) prefectures (Fig. 1). From Sika deer, 2,250 serum samples were collected from Aomori (n=39), Chiba (n=107), Ehime (n=45), Gifu (n=339), Gunma (n=106), Hokkaido (n=49), Kagawa (n=65), Kagoshima (n=29), Nagano (n=47), Oita (n=12), Wakayama (n=347), Yamaguchi (n=1,000) and Yamanashi (n=65) prefectures. In addition, 275, 160, 149, 115, 110, 36, 24, 22, 13, 13, 8, 6 and 1 serum samples were collected from raccoons, mice, monkeys, raccoon dogs, badgers, mask palm civets, nutrias, weasels, bears, martens, hares, foxes and muntjac, respectively. The collection and location are described in Fig. 1 and Table 1. These wild animals were found dead (roadkill or unknown reasons) or were mainly captured as countermeasures under the official population control program. All collected serum samples were stored at -20°C until use. There was no overlap in examined animals between this study and our previous reports [13, 67].

### Detection of anti-HEV antibodies among animal sera

Anti-HEV antibodies in wild animal sera were detected using our established ELISA [67], with a minor modification. ELISA antigen was prepared from HEV capsid protein expressing cells by transfection with the expression plasmid [67]. Peroxidase Conjugated Purified Recomb<sup>®</sup> Protein AG (Thermo Fisher Scientific, Waltham, MA, USA) was added as a secondary antibody. Following three washes with PBS-T, 100 µl of substrate agent (ABTS Microwell Peroxidase Substrate, Sera Care Life Sciences, Milford, MA, USA) was added to each well and the plates were gently shaken for 30 min at room temperature. Finally, the enzymatic reaction was stopped by adding 100 µl per well of 1% sodium dodecyl sulfate and the absorbance at a wavelength of 405 nm was measured using a spectrophotometer (Bio-Rad, Hercules, CA, USA). According to our previous report, the cut-off value was set at 0.437 for wild boar sera. For the other mammals, the cut-off value was tentatively set at 0.500.

### Detection of HEV RNA in wild animals

A total of 3,489 samples were screened for the presence of HEV genomes. RNA was extracted from 140 µl of each serum



**Fig. 1.** Geographic distribution of wild boar sampling areas in Japan. The number of anti-hepatitis E virus antibody-positive animals, the number of examined animals and percentage of positive animals are indicated by prefecture. The percentages are indicated by shading.

**Table 1.** Seroprevalence of hepatitis E virus (HEV) infection among wild animals in Japan

Species	Prefecture	Year	Percentage of positive animals (Number of HEV-positive animals/ Number of examined animals)
Wild boar	Aomori	2021	0% (0/4)
	Chiba	2015–2019	49.5% (45/91)
	Ehime	2016–2021	8.7% (27/311)
	Gifu	2014–2018	5.6% (8/144)
	Gunma	2015–2019	41.7% (20/48)
	Hyogo	2015–2016	31.8% (14/44)
	Kagawa	2016–2021	19.0% (22/116)
	Kagoshima	2016	20.0% (1/5)
	Kumamoto	2017–2018	3.3% (6/182)
	Oita	2012–2019	18.5% (17/92)
	Okinawa	2019–2020	0% (0/97)
	Tochigi	2011–2012	6.7% (11/163)
	Toyama	2014–2021	9.8% (18/183)
	Wakayama	2015–2021	9.2% (42/457)
	Yamaguchi	2016–2021	14.4% (63/438)
Deer	Aomori	2019–2021	0% (0/39)
	Chiba	2014–2020	0% (0/107)
	Ehime	2016–2019	0% (0/45)
	Gifu	2014–2021	0% (0/339)
	Gunma	2015–2021	0% (0/106)
	Hokkaido	2012	0% (0/49)
	Kagawa	2016–2021	1.5% (1/65)
	Kagoshima	2015–2017	0% (0/29)
	Nagano	2015–2016	0% (0/47)
	Oita	2008–2012	0% (0/12)
	Wakayama	2020–2021	0% (0/347)
	Yamaguchi	2010–2021	0% (0/1,000)
	Yamanashi	2014–2015	0% (0/65)
Raccoon	Gunma	2013–2014	0% (0/5)
	Hyogo	2015–2016	0% (0/23)
	Wakayama	2009–2015	0% (0/247)
Mouse	Ehime	2016–2018	0% (0/63)
	Yamaguchi	2015–2019	0% (0/97)
Monkey	Fukuoka	2014–2016	6.3% (2/32)
	Wakayama	2012–2017	0% (0/50)
	Yamaguchi	2018–2019	0% (0/67)
Raccoon dog	Gunma	2013–2014	0% (0/9)
	Wakayama	2014–2015	0% (0/88)
	Yamaguchi	2016–2019	0% (0/18)
Badger	Kagoshima	2016–2017	0% (0/13)
	Wakayama	2008–2020	0% (0/94)
	Yamaguchi	2018	0% (0/3)
Masked palm civet	Gunma	2013–2014	0% (0/3)
	Kagoshima	2017	0% (0/1)
	Wakayama	2012–2015	0% (0/32)
Nutria	Yamaguchi	2015–2016	0% (0/24)
Weasel	Wakayama	2007–2015	0% (0/21)
	Yamaguchi	2018	0% (0/1)
Bear	Akita	2017	0% (0/13)
Marten	Wakayama	2008–2015	0% (0/13)
Hare	Wakayama	2008–2019	0% (0/7)
	Yamaguchi	2018	0% (0/1)
Fox	Wakayama	2008	0% (0/2)
	Yamaguchi	2017–2018	0% (0/4)
Muntjac	Chiba	2015	0% (0/1)

sample using the QIAamp Viral RNA Mini kit (QIAGEN, Hilden, Germany) in accordance with the manufacturer's instructions. Nested reverse transcription (RT)-polymerase chain reaction (PCR) was performed for the detection of HEV RNA using OneStep RT-PCR Kit (QIAGEN) and KOD-Plus-NEO (Toyobo, Osaka, Japan) according to the kit protocols. Primers to detect ORF 2 gene of HEV genotypes 1, 3 and 4 [21] were used for HEV detection [27]. The 378 bp amplicon was purified using a QIAquick Gel Extraction Kit (QIAGEN) and the sequence was determined using BigDye Terminator v.3.1 technology (FASMAC, Atsugi, Japan). The obtained sequences were deposited in the DNA Data Bank of Japan (DDBJ accession number: LC706485-LC706506).

### Sequence analysis of mitochondrial DNA from sera

DNA extraction was performed using 100 µl of deer serum samples that were found to be positive for HEV RNA or anti-HEV antibodies, using the DNeasy Blood & Tissue Kit (QIAGEN). To determine the host genome, we used a set of primers (Mammalian-1 and Mammalian-2) targeting the cytochrome b gene region of the mitochondrial DNA, as described previously [17].

### Phylogenetic analysis

The phylogenetic analysis was performed by using the MEGA7 software program [20] based on the partial ORF2 sequences (338 bp) and the phylogenetic tree was generated by the neighbor-joining method based on 1,000 replicates, using the Kimura's two-parameter model. Updated HEV subtype reference strains were included for comparison [54].

### Statistical analysis

Pearson's  $\chi^2$  analysis was performed to evaluate the associations among HEV seroprevalence, HEV genome detection, and the variables of sex and body weight. *P* values of <0.05 were considered to be statistically significant.

## RESULTS

### Prevalence of anti-HEV antibodies among wild boar

Sera were obtained from 2,375 wild boar captured in 15 prefectures in Japan between 2012 and 2021 and were tested for the presence of anti-HEV antibodies (Fig. 1). The overall seroprevalence of anti-HEV antibodies in the wild boar population was 12.4% (294/2,375) and the prevalence by prefecture ranged from 0% to 49.5% (Table 1). The seroprevalence in wild boar of >50 kg in body weight (18.4%) was significantly higher in comparison to that among wild boars of <30 kg in body weight (5.3%) (*P*<0.001). No significant difference was observed between males (12.1%) and females (13.6%) (Table 2).

*Prevalence of anti-HEV antibodies among Sika deer*

Serum samples from 2,250 Sika deer captured in 13 prefectures between 2008 and 2021, were screened for antibodies against HEV. The total seroprevalence was 0.04% (1/2,250). The seropositive deer was captured in Kagawa Prefecture (1/65) and was negative for HEV RNA (Table 1). The anti-HEV antibody-positive serum was confirmed to have originated from deer by a sequence analysis of mitochondrial DNA in the serum.

*Prevalence of anti-HEV antibodies in other wild animals*

Sera of various wild animals collected between 2008 and 2020 were tested for the presence of anti-HEV antibodies. Two of 149 Japanese monkeys (1.4%) possessed anti-HEV antibodies and both were captured in Fukuoka Prefecture, resulting in a local prevalence of 6.3% (2/32). Sera collected from 275 raccoons, 160 mice, 115 raccoon dogs, 110 Japanese badgers, 36 mask palmed civets, 24 nutrias, 22 weasels, 13 Japanese black bears, 13 Japanese martens, 8 Japanese hares, 6 red foxes and 1 Reeve's muntjac were negative for anti-HEV antibodies (Table 1).

*HEV RNA detection in sera of wild boar, deer and other wild animals in Japan*

HEV RNA was detected from 21 wild boar serum samples (1.2%). The prevalence in the different prefectures ranged from 0% to 5.5% (Table 3), and a significant difference was observed between males (1.8%) and females (0.6%) ( $P < 0.05$ ). In addition, the prevalence of HEV RNA in wild boars of <30 kg in body weight (2.2%) was significantly higher than that in wild boars of >50 kg in body weight (0%) ( $P < 0.001$ ) (Table 4).

The HEV genome prevalence among Sika deer was 0.06% (1/1,688). The HEV-positive deer was captured in Yamaguchi

**Table 2.** Prevalence of anti-hepatitis E virus (HEV) antibodies in wild boar in Japan

	Sex			Body weight (kg)				Total
	Male	Female	No record	<30	30–50	>50	No record	
No. of examined animals	1,151	1,027	197	637	772	538	428	2,375
No. of positive animals	139	140	15	34	115	99	46	294
Percentage of anti-HEV antibody-positive animals	12.1%	13.6%	7.6%	5.3%	14.9%	18.4%	10.7%	12.4%

**Table 3.** Detection of hepatitis E virus (HEV) genome in wild animals in Japan

Species	Prefecture	Year	Percentage of positive animals (Number of HEV-positive animals/ Number of examined animals)
Wild boar	Aomori	2021	0% (0/4)
	Chiba	2015–2019	5.5% (5/91)
	Ehime	2016–2019	0% (0/115)
	Gifu	2014–2018	0% (0/140)
	Gunma	2015–2019	2.1% (1/48)
	Hyogo	2009–2011	2.6% (2/77)
	Kagawa	2016–2021	0.9% (1/116)
	Oita	2012–2019	2.9% (2/68)
	Toyama	2014–2021	0% (0/183)
	Wakayama	2020–2021	0% (0/354)
	Yamaguchi	2012–2021	1.7% (10/582)
Deer	Aomori	2019–2021	0% (0/39)
	Chiba	2014–2020	0% (0/108)
	Ehime	2016–2019	0% (0/45)
	Gifu	2014–2021	0% (0/339)
	Gunma	2015–2021	0% (0/106)
	Kagawa	2016–2021	0% (0/65)
	Yamaguchi	2010–2021	0.1% (1/986)
Bear	Akita	2017	0% (0/12)
Raccoon dog	Yamaguchi	2018–2019	0% (0/7)
Fox	Yamaguchi	2018	0% (0/2)
Badger	Yamaguchi	2018	0% (0/1)
Hare	Wakayama	2019	0% (0/1)

**Table 4.** Detection of hepatitis E virus (HEV) RNA in wild boar in Japan

	Sex			Body weight (kg)				Total
	Male	Female	No record	<30	30–50	>50	No record	
No. of examined animals	873	826	79	460	614	519	185	1,778
No. of positive animals	16	5	0	10	8	0	3	21
Percentage of HEV RNA-positive animals	1.8%	0.6%	0.0%	2.2%	1.3%	0.0%	1.6%	1.2%

prefecture. This HEV-positive serum was confirmed to have originated from Sika deer by a sequence analysis of mitochondrial DNA.

HEV RNA was not detected in serum samples from 12 Japanese black bears, 7 racoon dogs, 2 red foxes, 1 Japanese badger and 1 Japanese hare.

### Phylogenetic analysis of HEV

The phylogenetic analysis of the 338 bp amplicons showed that 9 strains of HEV in wild boar and 1 strain of HEV in Sika deer belonged to genotype 4, while 12 belonged to genotype 3 (Fig. 2, Table 5). The Sika deer strain formed one cluster together with the other wild boar strains collected in the same area, and the cluster was tentatively classified as cluster 4j (Fig. 2).

## DISCUSSION

In this study, the prevalence of HEV infection among wild mammals was compared, indicating that HEV mainly circulated among wild boar populations, while the other mammals showed less or no susceptibility to HEV infection.

In Japan, the prevalence of the anti-HEV antibody-positive wild boar was 12.4% (294/2,375). The prevalence of anti-HEV antibodies in the wild boar population ranged from 4.9% to 57.6% in Europe [1, 2, 6, 11, 18, 19, 43, 56, 68], and from 4.5% to 38.1% in Asian countries [8, 22, 46]. In Japan, HEV seropositivity rates among wild boar varied by prefecture, ranging from 4.5% to 42% [13, 29, 31, 46]. In this study, the prefectures of Chiba and Gunma, which are located in the Kanto region showed higher rates of seropositivity in comparison to other regions (Fig. 1). This HEV geographical distribution was similar to the one observed in human patients in Japan [36, 48, 60]. Nonetheless, wild boars have not been found in Hokkaido prefecture, which is a highly endemic area for HEV infection [47]. Therefore, the relationship between seropositivity in wild boar and the number of HEV patients remains unclear.

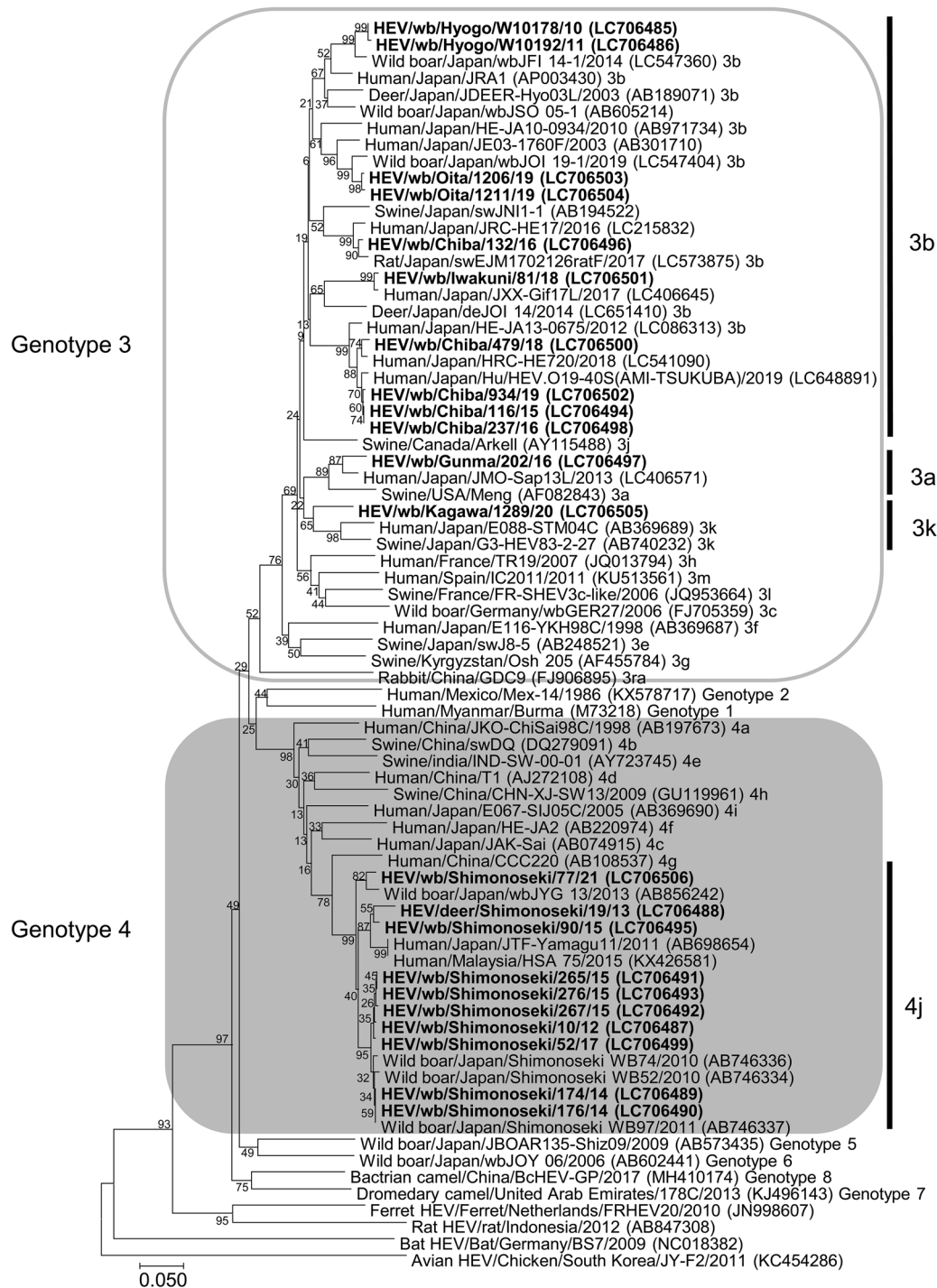
In this study, small wild boar (<30 kg) were infected with HEV, while heavy wild boar (>50 kg) possessed anti-HEV antibodies. These results indicated that small wild boar were infected with HEV and that big boar developed antibodies after recovering from HEV infection. The previous studies in wild boar also showed an association between body weight and the prevalence of anti-HEV antibodies or with HEV RNA detection [5, 33]. These results are similar to those in domestic pigs [28]. On the other hand, there was no significant difference in detection of anti-HEV antibody between male and female, similar to our previous reports [67]. However, HEV RNA was detected in male more than in female. In the previous reports, there was significant association between sex and HEV infection in human, but not in pigs and wild boar [10, 25, 30, 39, 52, 62]. Further study will be required to resolve this discordance in detection between HEV RNA and anti-HEV antibody.

In Sika deer, the seroprevalence was only 0.04% (1/2,250), which was consistent with previous reports showing a low prevalence of anti-HEV antibodies, ranging from 0% to 6.8% in Sika deer, roe deer (*Capreolus capreolus*), red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) populations [1, 24, 34, 44, 59, 64, 65, 70]. We confirmed that this antibody-positive deer serum originated from a Sika deer by sequencing of mitochondrial DNA and reperforming the ELISA. In this study, the HEV gene was also detected in one Sika deer, indicating the risk of HEV infection by consumption of deer meat. Therefore, it seems likely that Sika deer can be infected with HEV, but that such events must be very rare. In addition, other studies reported higher HEV seroprevalence in moose (*Alces alces*), reindeer (*Rangifer tarandus*) and red deer, which showed seroprevalence rates of 9.1–19.5%, 12–23.1% and 10–12.85%, respectively [3, 4, 23, 45, 53]. The variation in seroprevalence among the family *Cervidae* may be influenced by animal behavior or cohabiting species.

Genotypes 3 and 4 are predominant in Asia [7, 41]. Moreover, subtypes 3a, 3b, 3e, 3f and 3k and subtypes 4c, 4d, 4f and 4i have been detected in humans, pigs, wild boar, and deer in Japan [35, 49, 54]. Our results showed that genotype 3 has a wide distribution among wild boar populations. Ten of our strains formed a cluster (subtype 3b) with Japan-indigenous strains from swine, rat, wild boar and human origin. One strain from Gunma Prefecture and one from Kagawa Prefecture were more closely related to subtypes 3a and 3k, respectively. The genotype 4 strains, detected from 9 wild boar and 1 Sika deer in Yamaguchi Prefecture, formed one cluster with previously reported strains of wild boar and a human zoonotic case in Yamaguchi prefecture [13, 37]. The continuous circulation of similar strains in this area since 2011 and the formation of a cluster distinct from previously reported genotype 4 subtypes, suggests the presence of an endemic subtype, which was tentatively named 4j.

Until now, only genotype 3 strains have been detected from deer [1, 12, 35, 57]. Two genotype 4 human cases linked to consumption of deer meat were reported in South Korea and Japan [9, 16], but HEV RNA in the meat was not analyzed. Therefore, this study is the first to report genotype 4 infection in deer.





**Fig. 2.** Phylogenetic analyses based on the partial open reading frame (ORF) 2 sequences (338 bp). Hepatitis E viruses from 21 wild boars and one deer (bold) were compared to the reference strains proposed by Smith *et al.* [54] and the closest strains available in GenBank. The phylogenetic tree with 1,000 bootstrap replicates was generated by the neighbor-joining method. Reference sequences were labeled as “host/country/strain/year (GenBank accession number) subtype”.

Some wild animals have been shown to be susceptible to HEV infection [36, 63]. In this survey, we found seropositive rates of 1.4% (2/149) in Japanese monkeys, which was in consistent with previous reports in non-human primates that showed HEV circulation among macaques [14, 26, 69]. However, the other wild animals were negative for anti-HEV antibodies or HEV RNA. The primers used in this study could detect strains belonging to the *Orthohepevirus A* species, so we cannot deny the circulation of other species, like *Orthohepevirus C*. Overall, our findings, which were based on a nationwide survey of wild animals, indicated that the wild boar population is the dominant reservoir of HEV in Japan.

**Table 5.** Information on the hepatitis E virus (HEV) strains detected in the sera of wild animals

Species	Prefecture	Year	Isolate	Accession number	HEV genotype	Sex	Body weight (kg)
Wild boar	Hyogo	2010	HEV/wb/Hyogo/W10178/10	LC706485	3b	♂	32
	Hyogo	2011	HEV/wb/Hyogo/W10192/11	LC706486	3b	♂	16
	Yamaguchi	2012	HEV/wb/Shimonoseki/10/12	LC706487	4j	♂	41
	Yamaguchi	2014	HEV/wb/Shimonoseki/174/14	LC706489	4j	♀	10
	Yamaguchi	2014	HEV/wb/Shimonoseki/176/14	LC706490	4j	♂	10
	Chiba	2015	HEV/wb/Chiba/116/15	LC706494	3b	♂	50
	Yamaguchi	2015	HEV/wb/Shimonoseki/265/15	LC706491	4j	♂	27
	Yamaguchi	2015	HEV/wb/Shimonoseki/267/15	LC706492	4j	♂	23
	Yamaguchi	2015	HEV/wb/Shimonoseki/276/15	LC706493	4j	♂	16
	Yamaguchi	2015	HEV/wb/Shimonoseki/90/15	LC706495	4j	♂	25
	Chiba	2016	HEV/wb/Chiba/132/16	LC706496	3b	♂	30
	Chiba	2016	HEV/wb/Chiba/237/16	LC706498	3b	♀	35
	Gunma	2016	HEV/wb/Gunma/202/16	LC706497	3a	♀	15
	Yamaguchi	2017	HEV/wb/Shimonoseki/52/17	LC706499	4j	♂	40
	Chiba	2018	HEV/wb/Chiba/479/18	LC706500	3b	♂	26
	Yamaguchi	2018	HEV/wb/Iwakuni/81/18	LC706501	3b	♂	31
	Chiba	2019	HEV/wb/Chiba/934/19	LC706502	3b	♂	40
	Oita	2019	HEV/wb/Oita/1206/19	LC706503	3b	♂	No record
	Oita	2019	HEV/wb/Oita/1211/19	LC706504	3b	♀	No record
	Kagawa	2020	HEV/wb/Kagawa/1289/20	LC706505	3k	♀	10
	Yamaguchi	2021	HEV/wb/Shimonoseki/77/21	LC706506	4j	♂	No record
Deer	Yamaguchi	2013	HEV/deer/Shimonoseki/19/13	LC706488	4j	♀	40

In conclusion, the wild boar population is the dominant reservoir for HEV infection in the field in Japan. In addition, young wild boars were more frequently infected with HEV, suggesting the risk of HEV infection from piglets. Sika deer were rarely infected with HEV, indicating that there is a low, but not zero, risk of HEV infection from Sika deer.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

ACKNOWLEDGMENTS. Sampling of wild animal sera was performed by Dr. Tsutomu Takeda in Utsunomiya University, Dr. Masako Ando in Kagoshima University and Dr. Mayumi Yokoyama in University of Hyogo, many hunters in Yamaguchi and Wakayama prefectures, and the Japan Livestock Industry Association (Ehime, Kagawa, Gifu, Toyama, Chiba, Aomori). This research was funded by AMED Grant (JP20wm0225009, JP22fk0108634), and Health Labour Sciences Research Grant (21HA2006, 21KA1003, H30-shokuhin-ippan-004). MVM is supported by MEXT scholarship.

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