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SHORT COMMUNICATION

Monitoring of hepatitis E virus in zoo animals from Spain, 2007–2021

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Abstract

Hepatitis E virus (HEV, family *Hepeviridae*) is an important emerging and zoonotic pathogen. In recent decades, the number of human cases of zoonotic hepatitis E has increased considerably in industrialized countries and HEV has been detected in an expanding range of mammal species. Although domestic pigs and wild boar are considered the main reservoirs of zoonotic HEV genotypes, the role of other susceptible animals in the epidemiology of the virus is still poorly understood. A large-scale, long-term study was carried out (1) to assess HEV exposure in captive zoo animals in Spain and (2) to determine the dynamics of seropositivity in individuals that were sampled longitudinally during the study period. Between 2007 and 2021, serum samples from 425 zoo animals belonging to 109 animal species (including artiodactyls,

carnivores, perissodactyls, proboscideans and rodents) were collected from 11 different zoological parks in Spain. Forty-six of these animals at seven of these zoos were also longitudinally sampled. Anti-HEV antibodies were detected in 36 (8.5%; 95% CI: 5.8–11.1) of 425 sampled zoo animals. Specific antibodies against HEV-3 and HEV-C1 antigens were confirmed in ELISA-positive animals using western blot assay. Two of 46 longitudinally surveyed animals seroconverted during the study period. Seropositivity was significantly higher in carnivores and perissodactyls than in artiodactyls, and also during the period 2012–2016 compared with 2007–2011. HEV RNA was not detected in any of the 262 animals that could be tested by RT-PCR. To the best of the author's knowledge, this is the first large-scale, long-term surveillance on HEV in different orders of zoo mammals. Our results indicate exposure to HEV-3 and HEV-C1 in zoo animals in Spain and confirm a widespread but not homogeneous spatiotemporal circulation of HEV in captive species in this country. Further studies are required to determine the role of zoo species, particularly carnivores and perissodactyls, in the epidemiology of HEV and to clarify the origins of infection in zoological parks.

KEYWORDS
hepatitis E, emerging, zoonoses, zoological parks, epidemiology

1 | INTRODUCTION

Hepatitis E virus (HEV) (family *Hepeviridae*) is the most common cause of acute viral hepatitis in humans worldwide, with at least 20 million infections annually (Nimgaonkar et al., 2018). The virus has a positive-sense single-stranded RNA genome, which is divided into at least three open reading frames (ORF), named ORF1, ORF2 and ORF3. Four genera have been recognized so far (Purdy et al., 2022), although *Paslahepevirus*, and particularly the species *Paslahepevirus balayani*, is the most important in terms of public health concern. Eight different genotypes (HEV-1 to HEV-8) of this species have been confirmed, of which HEV-3 to HEV-8 infect animals, including HEV-3, HEV-4 and HEV-7 that also affect human beings. While HEV-4 and HEV-7 are mainly distributed in Asia and/or Northeast Africa (Rasche et al., 2016; Velavan et al., 2021), HEV-3 is the only one with global distribution and the most prevalent among animals (Izopet et al., 2019; Mulder et al., 2019). In industrialized countries, human cases of HEV-3 have increased sharply during the last decade (Aspinall et al., 2017), mainly associated with the consumption of animal products but also with the contact with infected species. Indeed, animal handlers, such as veterinarians, farmers, and forestry workers have been shown to be at increased risk for HEV infection (Mrzljak et al., 2021). While domestic pigs and wild boar are the main reservoirs of HEV-3, this genotype has the widest host range (Meng, 2016). HEV-3 has been detected in other artiodactyls and in different lagomorph, non-human primate, perissodactyl, rodent and carnivore species (Spahr et al., 2017a). In addition, rodents and wild carnivores have traditionally been considered the only reservoirs of the species *Rocahepevirus ratti* (genotypes HEV-C1 and HEV-C2, respectively) (Purdy et al., 2022; Wang et al., 2020). However, several mammal species susceptible to this hepevirus have also been reported in the last few years, with infections being confirmed in the Syrian brown bear (*Ursus arctos syriacus*) and also human beings (Spahr et al., 2017b; Sridhar et al., 2018). Exposure to this emerging species has also recently been confirmed in dogs and cats (Caballero-Gómez et al., 2022).

Due to the high diversity of animal species and routine veterinary control, zoo animals are considered useful species for obtaining insights into the epidemiology and distribution of emerging zoonotic pathogens (Caballero-Gómez et al., 2020; Robinette et al., 2017). However only a very few studies have assessed HEV circulation in zoo animals worldwide to date, most of which have focused on certain animal species and/or analysed a limited number of zoos or animals (Table 1). The main aims of the present large-scale study were (1) to assess HEV presence and circulation in captive zoo animals in Spain, and (2) to determine the dynamics of seropositivity in individuals that were sampled longitudinally during the study period.

2 | MATERIAL AND METHODS

2.1 | Sampling

A total of 425 zoo animals belonging to 109 species were sampled at 11 different zoological parks (A-J) in Spain between 2007 and 2021 (Table S1). Samples were obtained from serum banks or from animals subjected to health programs, surgical interventions or medical check-ups during the study period. Serum samples were stored frozen at −20°C until shipment to the Animal Health Department at the

TABLE 1 Seroprevalence and RNA detection rate of hepatitis E virus in zoo animals worldwide

Country	No. of captivity centres	Sampling period	Order(s) analysed	No. seropositives/ no. analysed(%)	No. RNA positives/no. analysed (%)	Reference
Germany	3 (most samples from 1)	2006–2016	Afrosoricida	0/1 (0.0)	NA*	Spahr et al. (2017b)
			Artiodactyla	16/167 (9.6)	0/98 (0.0)	
			Carnivora	10/37 (27.0%)	3/37 (8.1)	
			Chiroptera	0/4 (0.0)	0/4 (0.0)	
			Diprodontia	0/2 (0.0)	0/1 (0.0)	
			Perissodactyla	2/24 (8.3)	0/20 (0.0)	
			Proboscidea	0/6 (0.0)	NA	
			Rodentia	0/2 (0.0)	0/3 (0.0)	
Germany	9	2015–2016	Primates	10/259 (3.9)	0/256 (0.0)	Spahr et al. (2017c)
Italy	1	2001–2017	Primates	4/86 (4.7)	0/86 (0.0)	Melegari et al. (2018)
China	1	2006	Artiodactyla	NA	7/23 (30.4)	Zhang et al. (2008)
			Carnivora		2/3 (33.3)	
			Casuariiformes		0/1 (0.0)	
			Primates		0/2 (0.0)	
			Rodentia		0/1 (0.0)	
			Galliformes		1/2 (50.0)	
			Psittaciformes		0/1 (0.0)	
			Gruiformes		1/3 (33.3)	
			Struthioniformes		0/1 (0.0)	
Korea	1	2005–2010	Anseriformes	0/2 (0.0)	NA	Song et al. (2013)
			Artiodactyla	38/168 (22.6)		
			Carnivora	1/14 (7.1)		
			Ciconiformes	0/1 (0.0)		
			Diprodontia	0/3 (0.0)		
			Falconiformes	0/2 (0.0)		
			Gruiformes	0/3 (0.0)		
			Perissodactyla	1/4 (25.0)		
			Primates	0/1 (0.0)		
			Rodentia	0/2 (0.0)		
			Struthioniformes	0/1 (0.0)		
Spain	8	2002–2018	Primates	8/181 (4.4)	0/181 (0.0)	Caballero-Gómez et al. (2019b)
Spain	11	2007–2021	Artiodactyla	6/195 (3.1)	0/262 (0.0)	
			Carnivora	25/171 (14.6)		
			Perissodactyla	5/28 (17.9)		Present study
			Proboscidea	0/14 (0.0)		
			Rodentia	0/17 (0.0)		
Thailand	1	2009	Artiodactyla	NA	0/19 (0.0)	Wiratsudakul et al. (2012)

*Not analysed.

University of Cordoba (Spain) for laboratory analysis. Whenever possible, epidemiological information, including species, age, zoo provenance and sampling date, was gathered from each animal, whenever possible. Longitudinal samples (between two and six samplings per animal) were also retrospectively gathered from 46 of the 425 analysed animals at seven of the sampled zoos. The median (interquartile range Q1–Q3) of the period between consecutive samplings during follow-up was 36 months (12–60).

2.2 | Laboratory analyses

The presence of antibodies against HEV was determined using a commercial double-antigen multispecies sandwich HEV ELISA 4.0v (MP Diagnostics, Illkirch, France), following the manufacturer's instructions. The plates of this ELISA are coated with the recombinant ET2.1 protein, which is highly conserved in HEV genotypes (Hu et al., 2008) and can detect the presence of total antibodies in sera from a wide range of mammals. This ELISA has previously been used in humans, artiodactyls and carnivores and has been reported to be highly sensitive and specific in detecting anti-HEV antibodies (Caballero-Gómez et al., 2022; Carpentier et al., 2012; Kukielka et al., 2015).

Whenever possible, samples from seropositive zoo animals were further investigated by western blot (WB) analysis to confirm exposure to *Paslahepevirus balayani* and/or *Rocahepevirus ratti* species, including HEV-3 and HEV-C1 genotypes, respectively, in zoo species. Carboxy-terminal segments of the capsid proteins of HEV-3 and HEV-C1 and a nucleocapsid protein derivative (amino acid residues 1–39/213-433) of *Puumala orthohantavirus* strain Vranica/Hällnäs, as negative control, were produced as His-tagged recombinant proteins in *Escherichia coli* and purified by nickel-chelate affinity chromatography (Dremsek et al., 2011; Lundkvist et al., 2002). Then, purified proteins were run in a 12% SDS-PAGE and transferred to a polyvinylidene difluoride (PVDF) membrane and analysed for control by anti-His tag and HEV capsid protein cross-reactive monoclonal antibodies (Merck, Darmstadt, Germany; Kubickova et al., 2021). Serum samples were diluted 1:100 in 5% skimmed milk in phosphate-buffered saline (PBS)-0.1% Tween 20 (PBS-T) and the antigen–antibody reaction was detected by adding purified recombinant protein A/G conjugated with horse-radish peroxidase (HRP) (Thermo Scientific, Schwerte, Germany), diluted 1:50,000 in 5% PBS-T. The immunoreaction was detected using Clarity Western ECL Substrate (Biorad, Feldkirchen, Germany) and documented in a VersaDoc 4000MP (Bio-Rad) with an exposure time between 1 and 60 s. Seropositivity was confirmed by WB when blotted bands matching either HEV-3 and/or HEV-C1 antigens were observed. The presence of specific antibodies against HEV-3 or HEV-C1 was considered when serum samples reacted against only one of these genotypes. On the other hand, taking into account the cross-reactivity among these genotypes (Kubickova et al., 2021), if blotted bands were observed, regardless of the intensity, for both HEV-3 and HEV-C1, the result was considered as indeterminate (Figure S1).

Whenever possible, RNA was extracted from 400 μl pools of serum using the QIAamp MinElute Virus Spin Kit (QIAGEN, Hilden, Germany), following the manufacturer's instructions. Each pool contained sera from four different individuals (100 μl of each sample). The purified RNA was eluted in a total volume of 50 μl. For detection of *Paslahepevirus* RNA, real-time RT-PCR (CFX Connect Real Time PCR System) targeting a 70-nucleotide sequence of ORF3 was performed, using 25 μl of RNA template and the QIAGEN One-Step RT-PCR kit, as previously described (Frias et al., 2021). The detection limit was set at 21.9 IU/ml (95% confidence interval [95% CI]: 17.4–34.3). A nested broad-spectrum RT-PCR, that amplifies a conserved 280-nucleotide segment of ORF1 (RdRp-coding sequence), was also carried out according, in accordance with Johne et al. (2010). This assay was designed and validated to detect strains belonging to the genera *Paslahepevirus*, *Avihepevirus* and *Rocahepevirus*. The first round was performed using the QIAGEN One-Step RT-PCR kit and the nested PCR was carried out with a premixed 2X solution Taq DNA polymerase, dNTPs and reaction buffer kit (Promega). The second PCR products were examined on 1.5% agarose gel stained with RedSafe™ Nucleic Acid Staining solution.

2.3 | Statistical analyses

Associations between prevalence of anti-HEV antibodies and HEV RNA and explanatory variables (age, sex, order, zoo provenance and sampling period [2007–2011, 2012–2016 and 2017–2021]) were analysed using the Pearson's chi-square or Fisher's exact tests. Variables with $p < .05$ in bivariate analysis were included for further analysis. Collinearity between pairs of variables was assessed by Spearman's Rho test. Finally, a multivariable analysis was carried out using a generalized estimating equations (GEE) model. The number of seropositive animals was assumed to follow a binomial distribution and zoo provenance was used as the subject variable. Given the absence of seropositivity in individuals of the orders *Proboscidea* and *Rodentia*, these species were excluded from the multivariable analysis. Forward selection of variables was used, starting with the variable with the lowest p value in bivariate analysis. Values with $p < .05$ were considered statistically significant. Statistical analyses were performed using SPSS 25.0 software (Statistical Package for Social Sciences, Inc., Chicago, IL, USA).

3 | RESULTS AND DISCUSSION

A total of 36 (8.5%; 95% CI: 5.5–11.1) of the 425 sampled zoo animals showed anti-HEV antibodies using ELISA (Tables 1 and 2; Table S1). Seropositivity was confirmed in 14 of the 19 ELISA-positive animals that could be analysed by WB (Table S1). Of them, the presence of specific IgG antibodies against HEV-3 and HEV-C1 was detected in four and three animals, respectively. These findings indicate circulation of both *Paslahepevirus balayani* and *Rocahepevirus ratti* in zoo animals in Spain. On the other hand, seven reacted against both HEV-3 and HEV-C1 antigens (Figure S1), which may indicate exposure to both genotypes. However, given that cross-reactivity among members of the *Hepeviridae* family has previously been observed (Kubickova et al.,

TABLE 2 Seropositivity to hepatitis E virus in zoo animals in Spain and results of the bivariate analyses

Variable	Categories	No. positives/no. analysed (%)*	*p*
Order	Artiodactyla	6/195 (3.1)	<.001
	Carnivora	25/171 (14.6)	
	Perissodactyla	5/28 (17.9)	
	Proboscidea	0/14 (0.0)	
	Rodentia	0/17 (0.0)	
Age	Young	2/50 (4.0)	.143
	Adult	18/179 (10.1)	
Gender	Male	16/141 (11.3)	.119
	Female	12/174 (6.9)	
Zoo	A	0/5 (0.0)	.051
	B	10/59 (16.9)	
	C	3/40 (7.5)	
	D	2/27 (7.4)	
	E	11/66 (16.7)	
	F	1/36 (2.8)	
	G	4/68 (5.9)	
	H	0/20 (0.0)	
	I	0/5 (0.0)	
	J	0/16 (0.0)	
	K	5/83 (6.0)	
	2007–2011	5/88 (5.7)	.006
Sampling period	2012–2016	18/119 (15.1)	
	2017–2021	10/188 (5.3)	

*Missing values omitted.

TABLE 3 Generalized estimating equation analysis of potential risk factors associated with hepatitis E virus seropositivity in zoo animals in Spain

Variable	Categories	*p*	OR (95% CI)
Order	Artiodactyla	[a]	[a]
	Carnivora	<.001*	6.2 (2.2–16.9)
	Perissodactyla	.006*	6.4 (1.7–24.0)
Sampling period	2007–2011	[a]	[a]
	2012–2016	.022*	2.9 (1.2–7.3)
	2017–2021	.931	1.0 (0.4–2.4)

[a]Reference category; *p value < .05.

2021), exposure to other known or to a hitherto unknown virus of a putative novel genotype in zoo animals cannot be ruled out.

The overall seroprevalence of HEV detected in the present study (8.5%) is of the same magnitude as that observed in zoo animals in Germany, where 11.5% of 244 individuals tested presented anti-HEV antibodies (Spahr et al., 2017b), but lower than the seropositivity found in captive animals in Korea (19.9%; 40/201) (Song et al., 2013). Comparisons between studies should be made with caution given the differences in the numbers of animals and species analysed, study design and diagnostic assays employed. Nevertheless, our result confirms that animals in zoo parks are naturally exposed to this virus in Spain, which could be important for public health since zoonotic HEV transmission through direct or indirect contact with infected captive zoo animals could occur, particularly to zoo staff.

Seropositivity was detected in 23 (21.1%) of the 109 species analysed (Table S1). We report for the first time HEV exposure in thirteen carnivore species, four artiodactyla and two perissodactyla. Of these, the presence of specific antibodies against HEV-3 was confirmed in the serval (*Leptailurus serval*) and the sun bear (*Helarctos malayanus*), and specific anti-HEV-C1 antibodies were detected in the Iberian wolf (*Canis lupus signatus*), white rhinoceros (*Ceratotherium simum*) and South American sea lion (*Otaria byronia*) (Table S1). Previous studies have reported exposure to HEV-C1 in humans, captive Syrian brown bears, pet dogs and stray cats (Caballero-Gómez et al., 2022; Spahr et al., 2017b; Sridhar et al., 2018). Our results increase the range of species susceptible to HEV-3 and HEV-C1. Further studies are warranted to assess the role of these species in the epidemiology of HEV.

Seropositive animals were detected in orders Perissodactyla (17.9%; 5/28), Carnivora (14.6%; 25/171) and Artiodactyla (3.0%; 6/195), but not in orders Proboscidea (0/14) and Rodentia (0/17) (Table 2). The absence of seropositivity against HEV in rodents, which are one of the main reservoirs of *Rocahepevirus ratti*, may be related with the species sampled in the present study (Table S1). Although six different rodent species have been analysed, none of them belongs to *Muridae* and *Soricidae* families, the only ones in which rodents positive to genotype HEV-C1 have been detected to date (Reuter et al., 2020). Multivariate regression analyses identified the 'order' as a potential risk factor associated with HEV exposure in zoo animals in Spain. Seropositivity was significantly higher in perissodactyls (5/28; OR = 6.4; p = .006; 95% CI: 1.7–24.0) and carnivores (25/171; OR = 6.2; p < .001; 95% CI: 2.2–16.9) than in artiodactyls (6/195) (Table 3). It should be noted that anti-HEV antibodies were only found in three of the eight perissodactyl species tested (Malayan tapir (*Tapirus indicus*), Przewaslki's horse (*Equus caballus przewalskii*) and white rhinoceros). Further studies with a higher number of species belonging to this order are needed to better explain the higher seroprevalence observed in perissodactyls. On the other hand, the differences observed between captive carnivores and artiodactyls could be associated with diet. The diet of captive artiodactyl species is mainly based on commercial feed for equines/ruminants, fruits and vegetables, whereas carnivores in the sampled zoos are fed with meat products from different animal species. Although fruits and vegetables have been recognized as potential sources of HEV, the consumption of products derived from infected animals is considered the main transmission route of HEV in humans and probably also in other mammals (EFSA, 2017; Spahr et al., 2017a). However, given that zoo animals live in limited enclosures

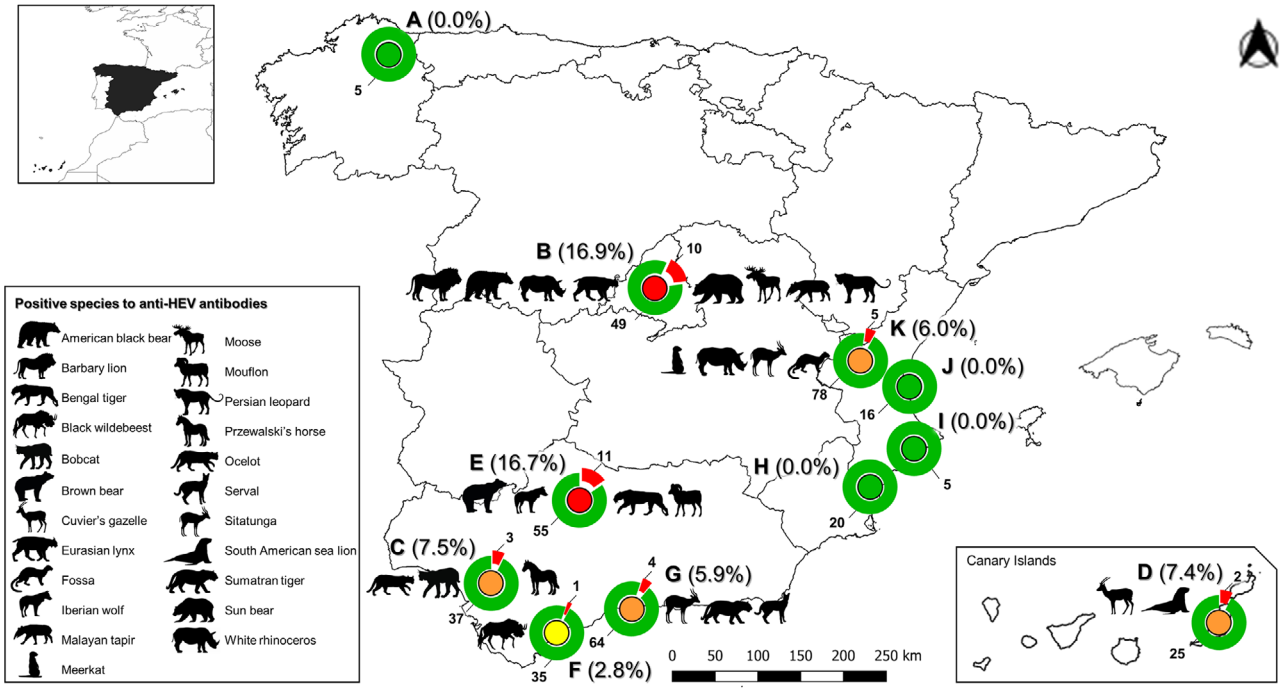

FIGURE 1 Distribution of the zoos (a–k) sampled in Spain. The number of positive (red) and negative (green) animals tested by ELISA at each zoo park is represented in a pie chart. Coloured dots indicate the frequency of seropositivity (yellow: lesser than 5.0%; orange: between 5.1% and 10.0%; red: between 10.1% and 25.0%; green: absence).

which may favour frequent interactions between individuals, the contact with infected sympatric animals might be other source of infection. In any case, additional studies are needed to clarify the sources of HEV transmission in animals kept in captivity in zoo parks.

Seropositive individuals were detected at seven of the 11 zoos tested, with within-zoo seropositivity ranging between 2.8% and 16.9% (Figure 1). This finding may be associated with differences in management measures and/or the number of animals and species analysed in each zoo. Seropositivity was also found every year between 2008 and 2021, except for 2013. Significantly higher seroprevalence was detected during 2012–2016 (18/119; OR = 2.9; $p = .022$; 95% CI: 1.2–7.3) compared to 2007–2011 (5/88) (Table 3). These results indicate an endemic but not homogeneous spatiotemporal circulation of HEV in zoo animals in Spain.

Of the 46 animals sampled longitudinally, 44 were seronegative at all samplings. One fossa from zoo K showed seropositivity in both March and November 2009 (Table 4), which could be related to the life-long persistence of anti-HEV antibodies, as has been previously suggested for other animal species (Caballero-Gómez et al., 2019a), although repeated exposure to HEV cannot be ruled out. Interestingly, a Malayan tapir from zoo B seroconverted between 2014 and 2019, and an ocelot from zoo C that tested seronegative in 2008 showed seropositivity in 2019, which indicate that these individuals were exposed to the virus between those years. These results, coupled with the presence of anti-HEV antibodies in a yearling Iberian wolf in zoo E in 2014, suggest HEV circulation in zoo animals from these zoos during the study period. Previously detected seroconversion in a common chimpanzee from zoo E between 2015 and 2016 supports this hypothesis (Caballero-Gómez et al., 2019b).

Information about HEV viraemia in animal species is still very scare. In non-human primates, rabbits and swine, the duration of HEV in sera has been shown to be limited, usually between one and four weeks (Ma et al., 2010; Meester et al., 2021; Tsarev et al., 1994). Previous studies have confirmed active HEV infection in sera from different animal species kept in captivity (Spahr et al., 2017b; Yamamoto et al., 2012). Spahr et al. (2017b) detected closely related rat HEV (HEV-C1) RNA sequences in a captive Syrian brown bear and Norway rats (pests) from the same zoo in Germany, suggesting that infection in the bear originated from the rodents. In addition, cross-species transmission of HEV-4 was suspected in a zoo-like location involving different avian and mammal species in China (Zhang et al., 2008) and HEV-3 transmission was confirmed among captive macaques in Japan (Yamamoto et al., 2012). In our study, HEV RNA was not found in serum samples of the 262 animals tested, which indicates the absence of active infection in those individuals at the sampling point.

In conclusion, the results obtained in the present study indicate widespread but not homogeneous spatiotemporal circulation of HEV (and other hepeviruses) in zoo animals in Spain. Although active HEV infection was not detected, the frequency and dynamics of seropositivity provide evidence that animal zoo species are naturally exposed to this emerging virus, which could be of public health concern. Further studies are required to determine the role of these species in the epidemiology of HEV and to clarify the sources of infection in animals housed in zoo parks.

TABLE 4 Antibodies against hepatitis E virus in longitudinally sampled zoo animals

Species	Zoo	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Malayan tapir	B								●					●	
Ocelot	C		●											●	
Fosa	K			● Mar; ● Nov											
Harbor seal	A								●				●		
Harbor seal	A									●			●		
Harbor seal	A									●			●		
Sun bear	B											●			●
Giant panda	B											●		●	
Giant panda	B											●		●	
Bengal tiger	B										●			●	
Black wildebeest	B										●				●
Malayan tapir	B											●		●	
Dama gazelle	C			●				●							
Przewaslki's horse	C			●					●						
Cape mountain zebra	C			●					●						
Asiatic elephant	E												●	●	
Iberian wolf	E											● Nov; ● Dec			
Iberian wolf	E											●	●		
Iberian wolf	E										●	●			
Jaguar	E						●					●			
Binturong	G									●					●
Malayan tapir	G		●		●		●		●		●	●			
Malayan tapir	G			●		●	●			●					
Malayan tapir	G								●	●	●				
Philippine spotted deer	G						●					●	●		
Philippine spotted deer	G										●	●			
Sri Lankan leopard	G									●	●				
South American coati	G						●							●	
Spotted hyaena	K	●				●									
Spotted hyaena	K				● Jun; ● Oct										
Lion	K	●			●										
Lion	K			●	●										
Sri Lankan leopard	K				● Sep; ● Dec										
Common eland	K		●		●	●									
Bongo	K							●		●					●
Blesbok	K					●	●								
Blesbok	K		●		●										
Impala	K			●					●		●				

(Continues)

TABLE 4 (Continued)

Species	Zoo	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020
Impala	K								●	●					
African elephant	K								●		●				
White rhinoceros	K						●[Feb]; ●[Jun]			●					●
Thomson's gazelle	K				●	●									
Thomson's gazelle	K								●		●				
Katanga lion	K								●					●	
California sea lion	I													●	●
Grey seal	I														●[Feb]; ●[May]

Note: Coloured dots indicate antibodies to HEV (red: positive; green: negative). When two samplings were carried out in the same year, the sampled months are indicated in superscript.

AUTHOR CONTRIBUTION

Study concept and design: JCG, IGB, ARJ. Sample collection: JM, RG, RMV, EMN, MAQM, CSA, JP, NCG. Sample collection and procedures: JCG, DCT, ABB. Analysis and interpretation of the data: JCG, IGB, ARJ. Drafting of the manuscript: JCG, IGB, ARJ. Critical revision of the manuscript for important intellectual content: All authors. Statistical analysis: JCG, IGB, ARJ. Obtained funding: IGB, ARJ, AR.

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CONFLICT OF INTEREST

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the authors upon reasonable request.

ETHICAL APPROVAL

All samples were collected from serum banks or from animals subjected to health programs, medical check-ups or surgical interventions during the study period. Therefore, no ethical approval was necessary.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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