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SHORT COMMUNICATION

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Survey for Hepatitis E virus infection in non-human primates in zoos in Spain

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Summary
Hepatitis E virus (HEV) is an emerging zoonotic pathogen that has been detected in different animal species. A survey study was carried out to assess HEV infection in non-human primates (NHPs) housed in zoos in Spain. Anti-HEV antibodies were detected in eight of the 181 NHPs tested (4.4%; 95%CI: 1.4–7.4). At least one seropositive animal was detected in five of the 33 species sampled (15.2%). This is the first report of seropositivity in black-and-white ruffed lemurs (*Varecia variegata*), common chimpanzees (*Pan troglodytes*), and Barbary macaques (*Macaca sylvanus*). Anti-HEV antibodies were found in six of the eight zoos included in the study (75.0%). Seroconversion was detected in one chimpanzee, which confirms HEV circulation in one zoo between 2015 and 2016. Seropositivity was significantly higher in hominids than in other NHP families. HEV RNA was not detected in any of the serum samples tested. The results indicate susceptibility of NHPs to HEV infection. Further studies are required to elucidate the role of these species in the epidemiology of HEV.

KEYWORDS
Hepatitis E, Non-human primates, Spain, Zoonotic

1 | INTRODUCTION

Hepatitis E virus (HEV; species *Orthohepevirus* A; genus *Orthohepevirus*; family *Hepeviridae*) is an important emerging pathogen and the most common cause of human acute hepatitis (Ricci et al., 2017). Genotypes 1 and 2 are restricted to waterborne human outbreaks in developing countries, whereas genotypes 3 and 4 are zoonotic and have been documented worldwide. Even though pig and wild boar (*Sus scrofa*) are recognized as the main reservoirs of genotypes 3 and 4 (Pavio, Doceul, Bagdassarian, & Johne, 2017), HEV RNA and anti-HEV antibodies have been detected in other mammal species, including captive wildlife animals (Zhang et al., 2008; Reviewed in Spahr, Knauf-Witzens, Vahlenkamp, Ulrich, & Johne, 2018a).

Non-human primates (NHPs) have been widely used as models in HEV studies, confirming their susceptibility to HEV infection by genotypes 1–4 (Reviewed in Spahr, Knauf-Witzens, Vahlenkamp, et al., 2018a; Kenney & Meng, 2019). Likewise, serological and molecular studies have evidenced natural exposure to HEV in NHPs (Hirano et al., 2003; Melegari et al., 2018; Spahr, Knauf-Witzens, Dähnert, et al., 2018b; Spahr, Knauf-Witzens, Vahlenkamp, et al., 2018a). Nevertheless, information on the role of these species in the epidemiology of HEV is still very limited. Hence, the aim of this study was to assess HEV infection in NHPs housed in zoos in Spain.

2 | MATERIALS AND METHODS

A total of 194 sera from 181 NHPs belonging to 33 different species were obtained from eight different zoos (A–H) in Spain between 2002 and 2018. Additionally, longitudinal samples were collected from nine of the 181 animals, including three common chimpanzees (*Pan troglodytes*), two De Brazza's monkeys (*Cercopithecus neglectus*), one Barbary macaque (*Macaca sylvanus*), one mangabey (*Cercocebus atys*), one mongoose lemur (*Eulemur mongoz*), and one red-bellied lemur (*Eulemur rubriventer*) (Table 1). Epidemiological information related to the sampled animals (zoo, species, Hominidae family, Parvorder (New World monkeys vs. Old World monkeys), age, sex, and sampling date) was gathered whenever possible. Sera were tested for the presence of anti-HEV antibodies using a commercial indirect ELISA (Wantai HEV-IgG ELISA®; Beijing Wantai Biological Pharmacy Enterprise© Ltd, Beijing, China). Following the manufacturer's instructions, samples with mean optical densities (OD) > mean OD negative control + 0.16 were considered to be positive. This commercial ELISA has been widely used in NHP studies (Huang et al., 2011; Li et al., 2005; Wang et al., 2019).

Hepatitis E virus RNA was simultaneously extracted from pools of 400 µl of serum using the QIAamp MinElute virus spin kit on the automated QIAcube platform (QIAGEN, Hilden, Germany). Sera from four different animals of the same species were included in each pool whenever possible. To detect HEV RNA, a real-time reverse transcription PCR (CFX Connect Real Time PCR System) was performed with the iTaq Universal Probes One-Step Kit (Biorad, Hercules, California, EEUU). The primers (15 µmol) used were sense HEV5260 (5´-GGTGGTTTCTGGGGTGAC-3´) and antisense HEV5330 (5´-AGGGGTTGGTTGGATGAA-3´), and the probe employed (20 µmol) was the HEV5283 (5´-FAM-TGATTCTCAGCCCTTCGC-TAMRA-3´), as described previously (Abravanel et al., 2012).

Associations between prevalence of anti-HEV antibodies and HEV RNA and explanatory variables (age, sex, Hominidae family and Parvorder) were analysed using the Fisher's exact test or Pearson's chi-square test. Variables with $p < 0.15$ in bivariate analysis were included for further analysis. Collinearity between pairs of variables was tested by Cramer's V coefficient. Finally, multiple logistic regression analysis was carried out. Values with $p < 0.05$ were considered statistically significant.

3 | RESULTS

Anti-HEV antibodies were detected in eight of the 181 NHPs tested (4.4%; 95% confidence intervals [95%CI]: 1.4–7.4). At least one seropositive animal was detected in five of the 33 species sampled (15.2%), and seropositivity values ranged from 12.5% (1/8) in

TABLE 1 Antibodies against hepatitis E virus detected in longitudinally sampled non-human primates

ID number	Species	Zoos	2007	2008	2009	2010	2011	2012	2013	2015	2016
284	Common chimpanzee	C	–	–	–	–	–	–	–	NEG	†POS; POS
288	Barbary macaque	C	–	–	–	–	–	–	–	POS	POS
329	Mangabey	C	NEG	–	–	NEG	–	–	–	–	–
332	De Brazza's monkey	G	–	–	–	–	‡NEG; NEG	–	–	–	–
335	De Brazza's monkey	G	NEG	–	–	NEG	–	–	–	–	–
340	Mongoose lemur	G	–	NEG	–	–	–	–	NEG	–	NEG
342	Red-bellied lemur	G	–	–	–	–	[a,c]NEG; NEG	–	–	–	–
364	Common chimpanzee	G	NEG	–	NEG	–	NEG	–	–	–	NEG
365	Common chimpanzee	G	–	–	–	–	–	–	NEG	–	NEG

Note. ID number: Identification number.Samples collected in:
[a]†March and April; [b]‡May and November; §February and August.

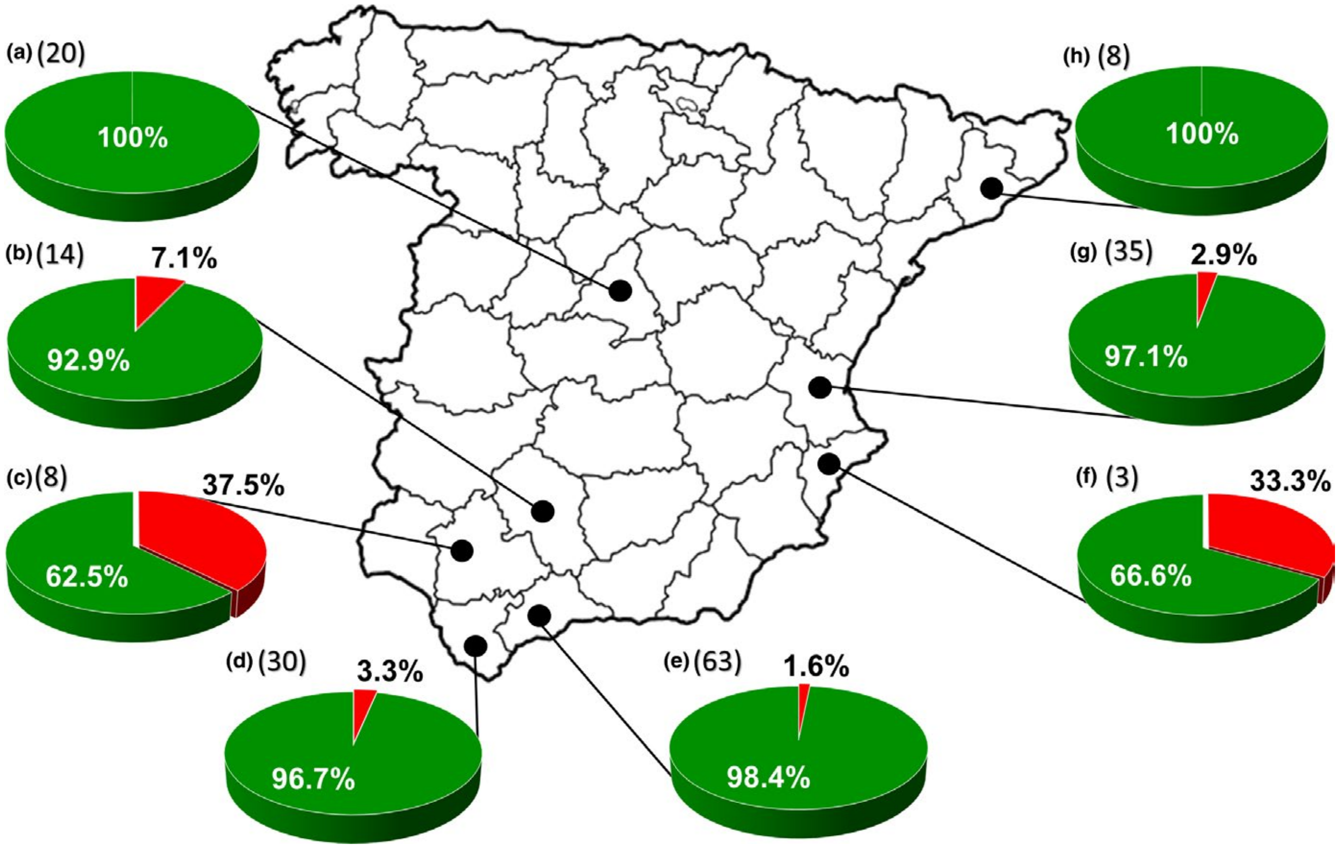

FIGURE 1 Seropositivity to hepatitis E virus in non-human primates (NHPs) in eight zoos in Spain. Pie charts indicate the frequencies of seropositive (red) and seronegative (green) animals. The total number of NHPs analysed in each zoo (A-H) is depicted in parenthesis

Western chimpanzees (*Pan troglodytes verus*) to 50.0% (1/2) in mona monkeys (*Cercopithecus mona*). Anti-HEV antibodies were also found in black-and-white ruffed lemurs (*Varecia variegata*) (1/7; 14.3%), common chimpanzees (3/17; 17.6%), and Barbary macaques (2/9; 22.2%) (Supplementary Material). Of the nine NHPs sampled more than once, seven were seronegative and one showed seropositivity at all samplings. In addition, one chimpanzee was seronegative in March 2015 but showed seropositivity in both March and April 2016 (Table 1).

Anti-HEV antibodies were found in six of the eight zoos included in the study (75.0%) (Figure 1). HEV seropositivity by age, sex, Hominidae family and Parvorder are shown in Table 2. Multivariate regression analysis identified hominids as a risk factor associated with HEV seropositivity. Frequency of seropositive hominids (4/41; OR = 6.8; p = 0.031; 95%CI: 1.2–39.0) was significantly higher than in rest of primate families (4/140). HEV RNA was not detected in any of the 194 serum samples tested.

TABLE 2 Explanatory variables included in the bivariate analysis of seroprevalence of hepatitis E virus in captive non-human primates in zoos in Spain

Variable	Categories	No. Positives/No. analysed (%)	*p*
Age	Adult	6/102 (5.9)	0.144
	Young	0/38 (0.0)	
Sex	Female	6/85 (7.1)	0.057
	Male	1/86 (1.2)	
Hominidae family	Yes	4/41 (9.8)	0.079
	No	4/140 (2.9)	
Parvorder	New World monkey	0/14 (0.0)	0.598
	Old World monkey	3/76 (3.9)	

4 | DISCUSSION

Our results confirm that NHPs are naturally exposed to HEV in zoos in Spain. Five of the 33 species tested presented at least one animal with anti-HEV antibodies. The seropositivity detected in our study (4.4%) was in accordance with that found in NHPs in zoos in Italy (4.6%; 4/86) and Germany (3.9%; 10/259) (Melegari et al., 2018; Spahr, Knauf-Witzens, Dähnert, et al., 2018b). In contrast, previous studies failed to detect anti-HEV antibodies in NHPs in zoos from China (0/2) and Korea (0/1) (Song et al., 2013; Zhang et al., 2008). Differences between studies should be carefully interpreted given the diversity of species analysed and the number of animals tested. To the best of the author's knowledge, this is the first report of anti-HEV antibodies in Barbary macaques, black-and-white ruffed lemurs and mona monkeys, which increases the number of species susceptible to this virus. The seropositivity observed in Barbary macaques (22.2%) is within the values (range 3.6%–36.7%) found previously in other NHP species of the *Macaca* genus in Asia (Arankalle, Goverdhan, & Banerjee, 1994; Hirano et al., 2003; Huang et al., 2011). Anti-HEV seropositivity was 6.8 times higher in primates of the Hominidae family than in other NHP families. Higher genetic susceptibility to HEV infection, differences in behaviour or interactions with other species are possible factors associated with the higher seropositivity detected in hominids. Additionally, since there is a direct relationship between volume of food ingested and size of animal, the probability of HEV infection could also be proportionally higher. Ingestion of contaminated water or meat products is considered the main route of HEV transmission in humans (Pavio et al., 2017) and probably also in NHPs (Spahr, Knauf-Witzens, Dähnert, et al., 2018b). However, since the diet of these species is mainly herbivore and the virus has also been detected in fruit and vegetables (Kokkinos et al., 2012; Terio et al., 2017), they should be considered possible sources of infection.

None of the NHPs were positive for active infection, which indicates absence of genotypes 1–4 of HEV in sera at the time of sampling. The duration of HEV in serum ranged between one and 11 weeks in experimentally infected NHPs and seroconversion generally occurs three to five weeks after infection (Tsarev et al., 1993, 1994). Even though the specificity of the ELISA used in this study has previously been shown to be high in both human and NHPs (Avellon, Morago, Garcia-Galera del Carmen, Munoz, & Echevarría, 2015; Li et al., 2005; Liu et al., 2013), cross reactions with other hepeviruses cannot be ruled out. In this context, divergent strains of HEV have previously been detected in chimpanzees in China (Zhou, Li, & Yang, 2014). Additional studies are required to assess the circulation of other hepeviruses in NHPs in the study area.

Anti-HEV antibodies were found twice in one NHP sampled after a time interval of seven months, which may be associated to a long-lasting humoral immune response. The persistence of anti-HEV antibodies in experimentally infected NHPs varies between less than 100 days and more than 7 years (Arankalle, Chadha, & Chobe, 1999; Li, Zhuang, Kolivas, Locarnini, & Anderson, 1994). Alternatively, the persistence of seropositivity found in this animal could be related with repeated exposure to the virus. In this context, the seroconversion detected in one chimpanzee confirms HEV circulation in zoo C between 2015 and 2016. Even though biosecurity measures are implemented in the facilities housing NHPs in the zoos sampled in this study, the transmission of HEV by direct or indirect contact with other NHPs, humans, or other sympatric species, such as wild rats and cats, cannot be ruled out (Huang et al., 2011; Spahr, Knauf-Witzens, Dähnert, et al., 2018b; Zhang et al., 2008). In this context, natural infection and transmission of HEV among NHPs housed together in the same facilities have been evidenced previously (Yamamoto et al., 2012).

In conclusion, the seropositivity found in 15.2% of the NHP species sampled indicates the susceptibility of these species to natural exposure of HEV or other related hepevirus. HEV infection in NHPs in zoos in Spain could be of public health and conservation concern. Control measures should be implemented to prevent transmission of this pathogen between NHPs and other sympatric species, including humans. Further studies are required to elucidate the role of these species in the epidemiology of HEV and to identify the sources of infection in NHPs housed in zoos.

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CONFLICT OF INTEREST

None of the authors of this study has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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SUPPORTING INFORMATION

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

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