Increasing Contact with Hepatitis E Virus in Red Deer, Spain

Mariana Boadella, Maribel Casas, Marga Martín, Joaquín Vicente, Joaquim Segalés, José de la Fuente, and Christian Gortázar

To describe the epidemiology of hepatitis E virus (HEV) in red deer in mainland Spain, we tested red deer for HEV RNA and antibodies. Overall, 10.4% and 13.6% of serum samples were positive by ELISA and reverse transcription–PCR, respectively. The increasing prevalence suggests a potential risk for humans.

Hepatitis E virus (HEV) is the only member of the Hepa-erviridae family (1). Four major genotypes of HEV have been recognized: genotypes 1 and 2 are restricted to humans and associated with epidemics in developing countries; genotypes 3 and 4 are zoonotic in developing and industrialized countries. Wild and domestic animals are being identified as potential HEV reservoirs (1–3).

Studies on wild sika deer (Cervus nippon) have detected low prevalence rates for HEV, which suggests that sika deer are accidental hosts for the virus (4,5), despite the transmission link discovered between them and HEV in Japan (3) that raised awareness of the possibility that game animals transmit HEV (2). In Europe, information about HEV infection in wild ruminants is limited to reports suggesting that roe deer (Capreolus capreolus) and red deer (Cervus elaphus) can act as HEV hosts (6–8). Except for these limited studies, no large-scale surveys have been conducted of HEV epidemiology in wild cervids. In Spain, the relatively high HEV seroprevalence detected in domestic pigs and wild boar suggests that HEV infection is probably widespread (9).

Red deer density, distribution, and hunting harvest are increasing throughout Europe (10). In Spain, the high densities recorded (11) indicate that red deer are an important source of game meat. This scenario emphasizes the need for a better understanding of the epidemiology of HEV in game populations in Spain.

Our goals were to describe the epidemiology and time trends of HEV in red deer in peninsular (mainland) Spain by serologic testing and PCR. On the basis of previous results on wild boar (9), we hypothesized that red deer would show widespread contact with HEV in Spain.

The Study

Serum samples from 968 Iberian red deer were collected during 2000–2009. These samples came from hunter-harvested red deer in 21 wild or semifree ranging populations (892 deer) and from 2 farms (76 deer). Sampling sites were representative of a variety of habitats and climates, which can be simplified into 5 different bioregions (Figure) (12). Sampling sites were grouped into 7 areas and 2 red deer farms (Table; Figure). Sex and age of deer were recorded. Management conditions of red deer were classified as open (no fencing and no management, 9 sites), fenced (fencing and artificial feeding, 12 sites), and farmed (livestock-like management, 2 farms). To analyze time trends, we classified samples collected during 2000–2005 as time 1 and those collected during 2006–2009 as time 2. Only sites where sampling occurred in both periods and with comparable sampling sizes were included in the time-trend analysis.

Serum samples were tested for HEV immunoglobulin (Ig) G by using ELISA as described (4,13), except for including protein G horseradish peroxidase (Sigma Chemical, St. Louis, MO, USA) as a conjugate, as in previous
controls were added.

PCR as described (instructions. HEV was detected by using a seminested RT-
mL of serum with Nucleospin RNA virus kit (Macherey-
selected and analyzed. Viral RNA was extracted from 150
values >100% were considered positive.

Results were expressed as the percentage of optical density
(%) OD by using the formula \[\% \text{ OD} = 100 \times \frac{\text{sample OD}}{\text{sum of negative controls OD}}.\] Serum samples with % OD

For the RT-PCR, 81 serum samples were randomly
selected and analyzed. Viral RNA was extracted from 150
mL of serum with Nucleospin RNA virus kit (Macherey-
Nagel, Düren, Germany) by following the manufacturer’s instructions. HEV was detected by using a seminested RT-
PCR as described (14). In each run, negative and positive controls were added.

Eight HEV RT-PCR–positive samples were sequenced.
HEV sequences were identified by using the BLAST algo-

Stereine exact method was used to estimate apparent
prevalence confidence intervals (CIs). \( \chi^2 \) tests were used to
analyze the association of age, sex, sampling site, and manage-
ment conditions with serologic and RT-PCR results.
Association between seropositivity and HEV RNA in the
serum was also analyzed by using Pearson \( \chi^2 \) test. Differ-
ences were considered statistically significant at \( p<0.05 \).

Overall, 101 (10.4%, 95% CI 8.6–12.5) serum sam-
ple were positive for IgG (Table). HEV seroprevalence
did not differ significantly between sex \( (\chi^2 = 0.894, 1 \text{ df,}
\ p=0.39) \) and age classes \( (\chi^2 = 12.436, 3 \text{ df, } p=0.05) \). Ser-

In contrast, the highest seroprevalence rates were reported in
red deer farms, where densities were the highest and red
doer had no contact with wild boar or domestic swine. In
contrast, the highest seroprevalence rates were reported in
open areas where contact with suids may have occurred.
However, wild boar densities also are high in fenced hunt-
ing estates \( (15) \), and HEV antibody prevalence rate was in-

Conclusions

Our findings of HEV infection confirm that HEV cir-
culates actively among red deer in the Iberian Peninsula, as
described for wild boar \( (9) \). Red deer can be infected with HEV
\( (7,8) \), and the results of our large serosurvey in this
species in Europe show an increasing prevalence trend in
the last decade.

de Deus et al. found higher IgG seroprevalences in es-
states with higher wild boar densities \( (9) \). However, in the
present study, mean seroprevalence rates were lowest in
red deer farms, where densities were the highest and red
doer had no contact with wild boar or domestic swine. In
contrast, the highest seroprevalence rates were reported in
open areas where contact with suids may have occurred.

Presence of HEV RNA in 13% of deer serum implies that
deer represent a risk for zoonotic transmission, and conse-

Cantábrico Occidental  3  122  21  17.2 (11.4–24.9)  2/14
Cantábrico Oriental  1  29  0  0.0 (0.0–11.5)  0
Sierra Central  1  16  0  0.0 (0.0–20.8)  0
Montes de Toledo  7  366  19  5.2 (3.2–8.0)  2/18
Valle del Guadiana  2  86  22  25.6 (17.3–35.9)  5/13
Sierra Morena  4  203  15  7.4 (4.3–11.9)  1/14
Dofiana  3  70  22  31.4 (21.3–43.5)  1/21
Cádiz†  1  50  1  2.0 (0.1–10.6)  0
Navarra†  1  26  1  3.8 (0.2–18.8)  0
Total  23  968  101  10.4 (8.62–12.53)  11/81

* Ig, immunoglobulin; HEV, hepatitis E virus; CI, confidence interval; RT-PCR, reverse transcription–PCR.
† Red deer farms.
tential source of HEV infection in humans. Further studies are needed to fully elucidate the epidemiology of HEV in wildlife and the foodborne zoonotic transmission risks.

Acknowledgments

We thank Bibiana Peralta for providing the HEV antigen protein for ELISA and RT-PCR–positive controls. We also thank our many colleagues at Instituto de Investigación en Recursos Cinegéticos who helped in field and laboratory work.

This study was financed by FISACAM PI-2007/56. The study also benefited from agreements of IREC with MARM-OAPN, Castilla-La Mancha and Principado de Asturias. Funding for sampling was also granted by AGL2008-03875. CRESA thanks CONSOLIDER (CSD-2006007).

Ms. Boadella is a PhD student at the Instituto de Investigación en Recursos Cinegéticos. Her research deals with various aspects of shared wildlife diseases, including time trends and risk factors in diseases of wild ungulates.

References


Address for correspondence: Mariana Boadella, Instituto de Investigación en Recursos Cinegéticos, Consejo Superior de Investigaciones Científicas, CSIC-Universidad de Castilla-La Mancha, Junta de Comunidades de Castilla-La Mancha, Ronda de Toledo s/n, 13005 Ciudad Real, Spain; email: mariana.boadella@uclm.es