Article

Not peer-reviewed version

Prevalence and Risk Factors for Hepatitis E Virus (HEV) in Wild Boar (Sus scrofa) and Red Deer (Cervus elaphus) in Portugal

Humberto Pires , Luís Cardoso , Ana Patrícia Lopes , Maria da Conceição Fontes , Sérgio Santos-Silva , Manuela Matos , Cristina Pintado , Luís Figueira , Ana Cristina Matos , João Rodrigo Mesquita , Ana Cláudia Coelho *

Posted Date: 30 August 2023

doi: 10.20944/preprints202308.2008.v1

Keywords: ELISA, Hepatitis E virus, Portugal, red deer, risk factors, wild boar

Article

Prevalence and Risk Factors for Hepatitis E Virus (HEV) in Wild Boar (*Sus scrofa*) and Red Deer (*Cervus elaphus*) in Portugal

Humberto Pires [1], Luís Cardoso [2,3], Ana Patrícia Lopes [2,3], Maria da Conceição Fontes [2,3], Sérgio Santos-Silva [4], Manuela Matos [5], Cristina Pintado [1,6,7], Luís Figueira [6,7], Ana Cristina Matos [1,6,7], João Rodrigo Mesquita [4,8,9] and Ana Cláudia Coelho [2,3]*

1 Polytechnic Institute of Castelo Branco, Castelo Branco, Portugal; humberto.s.pires@gmail.com; cpintado@ipcb.pt; acmatos@ipcb.pt
2 Animal and Veterinary Research Centre (CECAV), and Department of Veterinary Sciences, University of Trás-os-Montes e Alto Douro (UTAD), Vila Real, Portugal; lcardoso@utad.com; aplopes@utad.pt; mcfontes@utad.pt; accoelho@utad.pt
3 Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Portugal
4 ICBAS – School of Medicine and Biomedical Sciences, Porto University, Porto, Portugal; up202110051@edu.icbas.up.pt; jmesquita@outlook.com
5 Centre for the Research and Technology of Agro-Environmental and Biological Sciences (CITAB), UTAD; mmatos@utad.pt
6 Research Center for Natural Resources, Environment and Society, Polytechnic Institute of Castelo Branco, Castelo Branco, Portugal; lmftfigueira@gmail.com
7 Researcher at Q-RURAL – Quality of Life in the Rural World, Polytechnic Institute of Castelo Branco, Castelo Branco, Portugal
8 Epidemiology Research Unit (EPIUnit), Instituto de Saúde Pública da Universidade do Porto, Porto, Portugal
9 Laboratório para a Investigação Integrativa e Translacional em Saúde Populacional (ITR), Porto, Portugal
* Correspondence: accoelho@utad.pt

Abstract: Hepatitis E virus (HEV) is a zoonotic foodborne virus with an annual infection prevalence of 20 million human cases, which seriously affects public health and economic development in both developed and developing countries. To better understand the epidemiology of HEV in the Centre of Portugal, a cross-sectional study was conducted from 2016 to 2023 with sera samples from wild ungulates. The seroprevalence and risk factors for HEV seropositivity were evaluated in the present study. Specifically, antibodies against HEV were determined by a commercial enzyme-linked immune-sorbent assay (ELISA). Our results show that in the 650 sera collected from 298 wild red deer and 352 wild boars from Portugal, 9.1% red deer (95% confidence interval [CI]: 6.3–12.9%) and 1.7% wild boar (95% CI: 0.6–3.3%) were positive for antibodies to HEV. Regarding age, the seropositivity in juvenile wild ungulates was 1.3% (95% CI: 0.27–3.72%) and 7.2% in adults (95% CI: 4.9–10.11%). Logistic regression models investigated risk factors for seropositivity. The odds of being seropositive was 3.6 higher in adults than in juveniles (95% CI: 1.72–18.11%) and the risk was 4.2 higher in red deer than in wild boar (95% CI: 1.64–10.69%). Both wild ungulate species were exposed to HEV. The higher seroprevalence in red deer suggests that this species may have a major contribution to the ecology of HEV in the Centre of Portugal. Further research is important to understand how wildlife affects the epidemiology of HEV infection in Portugal.

Keywords: ELISA; Hepatitis E virus; Portugal; red deer; risk factors; wild boar

1. Introduction

The most common cause of acute enterically transmitted hepatitis in developing countries is the hepatitis E virus (HEV) [1]. HEV is a foodborne zoonotic virus with a worldwide infection prevalence of 20 million human cases, causing severe implications for public health and economic progress in developed and developing nations [2,3]. In Europe, more than 20,000 confirmed hepatitis E cases

 doi:10.20944/preprints202308.2008.v1

were reported between 2005 and 2015 [4]. Besides humans, HEV infects various mammalian hosts [5]. HEV belongs to the family Hepeviridae, further subdivided into the subfamilies Parahepevirinae and Orthohepevirinae. Members of the Parahepevirinae subfamily infect salmon and trout. In contrast, mammals and birds are infected by members of the Orthohepevirinae subfamily, which is further divided into four genera: *Paslahepevirus*, *Avihepevirus*, *Rocahepevirus*, and *Chirohepevirus* [6].

HEV is a small virus quasi-enveloped with an icosahedral capsid enclosing the viral genome [7]. The positive-sense, single-stranded RNA comprising the genome is 6.4–7.3 kb long and has four partially overlapping open reading frames [8,9].

HEV is today referred to as *Paslahepevirus balayani*. Eight genotypes of HEV are known, namely HEV-1 through HEV-8, with genotypes 1 and 2 infecting exclusively humans, genotypes 3, 4, and 7 infecting both humans and animals, and genotypes 5, 6, and 8 infecting only animals [10]. HEV1 and HEV2 are prevalent in developing countries and can cause significant gastrointestinal symptoms/signs in humans and animals. These symptoms/signs are often attributed to consuming contaminated water and food [11,12]. Zoonotic genotypes 3 and 4 are transmitted primarily through consumption of contaminated pork and meat products, or contact with infected animals, particularly pigs [13]. In industrialized countries, HEV3 and HEV4 typically cause sporadic and pauci-symptomatic illnesses. In humans HEV typically manifests with mild clinical characteristics, involving increased liver enzyme levels, jaundice, and non-specific symptoms like loss of appetite, fatigue, and abdominal discomfort. These clinical signs often closely resemble symptoms observed in various other liver disorders, and they usually persist for a duration of 1 to 6 weeks. While most cases resolve on their own, instances of chronic hepatitis E infection have been documented in individuals with weakened immune systems, especially among organ transplant recipients using immunosuppressive drugs [14,15].

After being identified in domestic pigs in the United States in 1997, swine HEV strains have been recognized across the globe in both domestic and wild pig populations, showing considerable variations in their prevalence [16,17].

HEV3 and HEV4 genotypes have been detected in humans, as well as in *Sus scrofa domesticus* (domestic pigs) and *Sus scrofa* (wild boars) [12]. The HEV3 and HEV4 genotypes, found in various animal species such as wild ungulates exhibit significant similarities with the genotypes found in humans. These similarities support the zoonotic nature of these two genotypes [17-19]. Genotype 4 is predominantly common in China and is the predominant genotype [20], whereas genotype 3 is common in all other parts of the world and is the most common HEV genotype in Europe [21-24]. On the other hand, HEV genotypes 5 and 6 have only been identified in Japanese wild boar [22], and HEV genotypes 7 and 8 have recently been detected in camels in China and the Middle East [25,26].

The unequivocal link between the consumption of infected wild ungulate meat and human infection has been described [27]. Including, more specifically, the establishment that eating contaminated wild boar meat can result in human infection [28]. Cases of HEV have been directly connected to the consumption of raw deer meat by the presence of identical HEV strains in the consumed deer meat and the respective patients [29]. In immunocompetent human subjects, HEV causes sporadic self-limiting infections presenting a low mortality. Conversely, in immunocompromised patients, HEV can induce several and dangerous clinical signs [30,31]. It may also cause neurological manifestations [32] and is responsible for a self-limiting infection with an overall low mortality. However, mortality can reach higher levels in pregnant women [33]. The main HEV reservoirs are domestic pigs and wild boar [34,35]. However, zoonotic strains have also been identified in deer, rabbits, chickens, mongooses, rats, ferrets, fish and camels. This host range continues to expand over time, showcasing instances of infections crossing between different species [36,37]. The development of HEV antibodies in pigs occurs after the natural decrease in maternal antibody levels, typically around 8–10 weeks of age. Initially, IgM anti-HEV antibodies reach their highest levels alongside the peak of viral shedding through feces, followed by IgG anti-HEV antibodies peaking when the virus is being eliminated from the fecal matter. The liver is the targeted organ, from which HEV spreads to various tissues and organs through hematic diffusion [38].

 doi:10.20944/preprints202308.2008.v1

Although HEV infection has been detected on wild ungulates in many countries around the world [39-43], with only one seroepidemiological study on HEV in wild ungulates in Portugal, and only focusing on wild boar. As such, this study aimed to assess the seroprevalence, and risk factors associated with HEV in wild boar and red deer in Central Portugal.

2. Materials and Methods

From 2016 to 2023, a survey was conducted on serum samples randomly collected from free-ranging wild ungulates hunted and legally killed by hunters in east-central Portugal to investigate the presence of HEV. The first hunted animals were sampled up to a total of 10 per year at each side and this sampling process was repeated in each year.

Sampled municipalities included Alcafozes (n =16), Castelo Branco (n =30), Cegonhas (n = 8), Crato (n = 41), Fratel (n = 35), Granja (n = 10), Idanha-a-Nova (n = 29), Lousa (n = 44), Marvão (n = 31), Mata (n = 40), Monforte (n = 10), Monte Fidalgo (n = 76), Niza (n = 26), Ponte de Sor (n = 25), Portalegre (n = 49), Rosmaninhal (n = 39), Sarnadas do Ródão (n = 40), Tostão (n = 9), Vila Velha de Ródão (n = 64), Vale de Figueira (n = 6), and Vale Pousadas (n = 22).

These regions are home to the largest population of wild ungulates in Portugal. A veterinarian conducted a comprehensive examination of a total of 650 wild ungulates, representing two distinct species: 352 wild boar (*S. scrofa*) and 298 red deer (*Cervus elaphus*). Available data on age, sex, body condition, and capture location were utilized to provide insights into the distribution of seropositive individuals.

Blood samples were obtained from the heart or thoracic cavity of the animals during the hunting season. Blood was allowed to clot at environmental temperature and afterwards transported to the laboratory. Blood samples were then centrifuged at 1500×g for 10 min and the separated serum samples kept at −20°C until further testing. Sera were checked for the presence of antibodies to HEV in wild ungulates using an ELISA kit (ID Screen® Hepatitis E Indirect Multi-species ID.vet, Montpellier, France), under the manufacturer's recommendations and following the guidelines for the interpretation of results. Optical densities (OD) of the tested samples and positive and negative controls were measured by an ELISA plate reader at 450 nm. The OD ratio of the sample and positive control (S/P) was calculated for each sample as follows:

$$[(\text{OD sample}–\text{OD negative}) / (\text{OD positive}–\text{OD negative})] \times 100.$$

2.1. Statistical analysis

The outcome variable was dichotomized as positive versus not positive to identify any risk factor associated with seropositivity. The Chi-square test was used to assess significant differences among the groups. Multiple logistic regression was used to model the odds ratio (OR) and its 95% confidence interval (CI) of being seropositive related to the variables. Significant potential risk factors at $p < 0.05$ (two-tailed; alpha = 0.05) were then evaluated using stepwise regression to construct a multiple model (Wald test stepwise p-Wald value to enter $p < 0.05$). The multiple logistic model was developed using a stepwise approach. Backward elimination followed by a forward selection for each variable at a time was done using a likelihood ratio test at each step with 0.05 (two-tailed; alpha = 0.05) as a significant level for removal or entry. The fit of the models was assessed using the Hosmer and Lemeshow goodness-of-fit test [44]. The model was rerun until all remaining variables presented statistically significant values ($p < 0.05$). All statistical analyses were performed using SPSS® 25.0 software for Windows.

3. Results

The studied population of wild ungulates comprised 352 (54.2%) wild boar and 298 (45.8%) red deer. In age groups, adults represented 64.2% (n = 417) and juveniles 35.8% (n = 233). Following the European Regulations (EU Reg.), each animal received ante and post-mortem examinations, respectively performed by hunters (in agreement with the EU Reg. No. 853/2004) and by veterinary sanitary authorities (EU Reg. No. 625/2017).

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 30 August 2023 doi:10.20944/preprints202308.2008.v1

The global seroprevalence of HEV infection was 5.1% (n = 33; 95% CI: 3.5–7.1%).

Among the positive species, the prevalence in red deer (9.1%; 95% CI: 6.1-12.9%) was higher than in wild boar (1.7%; 95% CI: 0.6–3.7%), with a statistically significant value observed ($p < 0.001$).

Serologic reactivity data according to species, sex, age, and clinical signs examined are presented in Table 1. The seroprevalence values among males and females were 6.6 % (95% CI: 4.2-9.7%) and 3.3% (95% CI: 1.6-6.0%), respectively ($p = 0.054$) (Table 1). Regarding age, the lowest value of seroprevalence (1.3%; CI: 0.3–3.7%) was found in juveniles and the highest (7.2%; CI: 4.9–10.1%) in adults. Statistical significant differences were observed between these groups ($p < 0.001$). Furthermore, there was significant difference in seropositivity results among clinical signs related to the presence (1.7%; CI: 0.2–6.0%) or absence (5.8%; CI: 4.0–8.2%) in the studied species ($p = 0.037$) (Table 1).

Table 1. Screening for anti-HEV antibodies in free-ranging wild ungulates from the Centre of Portugal.

	Wild boar ***N* = 352**	**Red deer** ***N* = 298**	**No. anti-HEV pos./total (%; CI)** ***N* = 650**
Sex	$p = 0.524$	$p = 0.059$	$p = 0.054$
Male	4/190 (2.1%; 0.6–5.3%)	19/159 (11.9%; 7.4–18.0%)	23/ 349 (6.6 %; 4.2–9.7%)
Female	2/162 (1.2%; 0.015–4.4%)	8/139 (5.8%; 2.5–11.0%)	10/301 (3.3%; 95% CI: 1.6–6.0%)
Age	$p = 0.390$	$p = 0.016$	$p < 0.001$
Juvenil	2/178 (1.1%; 0.14–4.0%)	1/55 (1.8%; 0.0–9.7%)	3/233 (1.3%; 0.3–3.7%)
Adult	4/174 (2.3%; 0.6–5.8%)	26/243 (10.7%; 7.1–15.3%)	30/417 (7.2%; 4.9–10.1%)
Clinical signs	$p = 0.876$	$p = 0.286$	$p = 0.037$
Absence	4/245 (1.6%; 0.5–4.1%)	27/287 (9.4%; 6.3–13.4%)	31/532 (5.8%; 4.0–8.2%)
Presence	2/107 (1.9%; 0.2–6.6%)	0/11 (0.0; 0.0–2.9%)	2/118 (1.7; 0.2–6.0%)
Total	6/352 (1.7%; 0.6–3.7%)	27/298 (9.1%; 6.1–12.9%)	33/650 (5.1%; 3.5–7.1%)

Regarding distribution according to municipalities, anti-HEV antibodies were found in eight of them: three red deer from Castelo Branco (10.0%; 3/30 wild ungulates), four red deer from Crato (9.8%; 4/41 wild ungulates), one red deer from Fratel (2.9%; 1/35 wild ungulates), two wild boar from Lousa (4.5%; 2/44 wild ungulates), four red deer from Marvão (12.9%; 4/31 wild ungulates), two wild boar from Niza (7.7%; 2/26 wild ungulates), four red deer and two wild boar from Rosmaninhal (15.4%; 6/39 wild ungulates), eight red deer from Vila Velha de Ródão (12.5%; 8/64 wild ungulates), and three red deer from Vale Pousadas (13.6%; 3/22 wild ungulates).

Three variables were associated ($p < 0.05$) with seropositivity in wild ungulates. Seropositivity significantly correlated with the following factors (Table 1): age, species, and animals with clinical signs. These variables were included in the multiple model. A backward stepwise conditional logistic regression was employed using all the above statistically significant variables. Multiple logistic regression analysis of the OR for being seropositive to the potential risk factors listed above is presented in Table 2. At the individual level, the odds of HEV seropositivity were found to be higher for adult animals, OR = 3.66 (95% CI: 1.72–18.11%), and also for in red deer, OR = 4.2 (95% CI: 1.64–10.69%).

Table 2. Risk factors associated with HEV infection of wild ungulates in the Centre of Portugal.

Risk factor	**β[a]**	**S.E. β[b]**	***p***	**OR[c]**	**95% CI[d] (OR)**
Age	1.298	0.641	0.043		
Juvenil				1	

Adult				3.662	1.042–12.867
Species	1.431	0.479	0.003		
Wild boar				1	
Red deer				4.184	1.637–10.692

[a]β: logistic regression coefficient; [b]S.E. β: standard error; [c]OR: odds ratio; [d]CI: confidence interval.

4. Discussion

Each year, foodborne pathogens, including the HEV, result in numerous infections across various continents. Ingestion of contaminated food of animal origin exposes the final consumers to the risk of infection. HEV has been identified as an emergent public health risk in several European countries [45].

Annually, HEV leads to a considerable number of infections through its zoonotic transmission. Moreover, it has gained recognition as an occupational disease due to the substantial prevalence of antibodies found in the blood samples of professionals like veterinary workers, slaughterhouse employees, hunters, and similar occupations [12]. Individuals such as veterinary foods inspectors, pig breeders, hunters, pork product sellers, and meat processing workers are frequently exposed to the risk of HEV infection [46]. Even in developed countries where the origins of human hepatitis E cases were previously thought to be rare, detection of HEV in sewage, water sources, coastal, surface waterways, drinking water, and produce raises environmental safety concerns [36,47].

Epidemiological studies utilize molecular biology techniques like real-time PCR assays, along with seroprevalence screening methods involving the detection of immunoglobulin G (IgG) and/or immunoglobulin M (IgM), as the established and highly reliable approaches for diagnosing and assessing the epidemiological situation [48].

This study shows that approximately 5.1% of wild game that are hunted for human consumption have antibodies for hepatitis E virus. As far as we know, the present study is the first serological study conducted on red deer in Portugal, and the largest seroprevalence study on wild boar from Portugal to date.

HEV human infections caused around 21,000 clinical cases in Europe between 2005 and 2015. Most were limited to specific countries (e.g., Germany, France, Italy, and Spain [4]. Serological studies have shown that people are at danger of infection, as reported by Raji et al. [49], who found substantial seroprevalence titres in hunters and in blood donors [31]. Consumption of infected (raw or undercooked) pork liver or wild boar meat products is the mode of HEV infection in humans, and traditional home-cooked foodstuffs, in particular, allow HEV persistence in specific geographical locations [18,31,50,51]. Occupational exposure of workers to swine and sheep represents other risk factors associated with seropositivity [52,53].

In the Portuguese human and animal population, there are reports of evidence of infection detected by molecular and serological methods [54-60]. In Portugal, HEV has previously been detected by molecular methods in wild ungulates. Namely, in two red deer with a prevalence of 2.1% [54], 20 wild boar livers (25.0%), and four wild boar stools in two different studies (10% and 2.8%) [55,56].

In the Iberian Peninsula, serological reports of HEV in wild ungulates have ranged between 8.2−57.4% in wild boar [11,39,61,62,63,64,65] and 4%−13.9% in red deer [62-67]. The seroprevalence in wild ungulates found in the present study is lower than in previous studies from the Iberian Peninsula.

Moreover, the values found in this serological study are lower than those found in other similar European studies. In Italy, a study found seroprevalences between 87.3% and 100% in pigs [68], and similar results have been found in white-tailed deer (*Odocoileus virginianus*) in Finland (1.4%) [69]. Interestingly, other studies in Italy also detected HEV RNA in 14.3−83.7% of liver samples [11,40,41,42,70,71,72], and in farmed ruminants the majority of HEV genotypes discovered until to date are from the zoonotic HEV3 and HEV4 [73].

Although the seroprevalence shown in this study appears to be low, the etiological agent seems to infect the wild ungulates under evaluation. Seropositive variance between red deer and wild boars indicates the presence of current infections and previous exposures in the examined animals.

For the statistical analysis, only two factors were associated in the final model: age and species. The high seropositivity level reported in adult wild ungulates supports prior research and could be explained by longer-term exposure to the HEV in the environment with similar studies on wild ungulates from other countries of Europe also finding the same pattern [74,75]. Furthermore, this tendency has been observed in humans as well, with anti-HEV seroprevalence increasing with age [76,77] and seropositivity associated with the female gender, probably due to the potential role of sexual hormones peaks which may increase host receptivity to infection [78,79]. Interestingly, in the present study, the test chosen to detect HEV infection proved to be practical and rapid in comparison to the expensive and time-consuming molecular testing.

Domestic pigs play a prominent role as a reservoir for the HEV among wildlife and domestic animal species. HEV surveillance is critical over the world to close the knowledge gap about its transmission and reservoirs, especially given its zoonotic potential [80]. They are closely linked, from an epidemiological standpoint, with other important wild species, such as *S. scrofa* (wild boars) and wild ruminants, which serve as additional sources for the environmental spread of the virus [18,28,81,82,83]. Results of this study should be interpreted from a One Health perspective. The interaction between different species, particularly wild ungulates, and domestic ruminants, in their shared habitats, can facilitate the transmission of infectious agents such as HEV. Previous studies reveal a similar HEV RNA detection rate in red deer and wild boars inhabiting the same regions, implying that the same HEV-3 variant is frequently transmitted among various ungulate species [64,84] and, ungulate species like deer serve as true host for HEV. However, wild and domestic pigs are still the principal cause of infection for ruminants living in the same regions [84]. Additionally, the proximity of small ruminants and wild animals to humans increases the risk of pathogen transmission to people [85].

However, the results, it is important to interpret these findings cautiously as the present study has some limitations. Specifically, this study involved a cross-sectional design with self-selection animals and the designs constraints prevent the establishment of a definitive cause-and-effect relationship.

The results of this ELISA-based survey indicate that HEV infection is widely distributed among red deer and wild boar from the central region of Portugal. Considering the lack of similar studies in the country, particularly in red deer, our results could contribute to the effective control of HEV in wildlife. These results also confirm that the seroprevalence of HEV infection in red deer and wild boar has been underestimated in Portugal.

The present study aims to contribute to the detection and identification of potential future threats related to HEV infections in wild ungulates. Wildlife monitoring plays a crucial role as it enables the identification of changes in disease occurrence among wild populations [86-88]. Regularly monitoring and studying the health status of wild ungulates can detect any shifts or patterns in transmission dynamics in the wildlife population [88]. This information is essential for understanding the potential risks and impacts associated with HEV infections.

5. Conclusions

In conclusion, the present study emphasizes the importance of a One Health, multidisciplinary approach for assessing wild boar and red deer exposure to HEV in the Centre of Portugal and also for controlling HEV disease. Further research about the role of wildlife in the epidemiology of HEV infection should be conducted. By addressing the concerns associated with wildlife reservoirs and integrating them into disease control, better efforts may be conducted to safeguard animal and human health in the face of HEV.

Author Contributions: Conceptualization, H.P., A.C.C., A.C.M., and J.R.M.; methodology, H.P., A.C.M., A.C.C., C.P., and J.R.M; software, H.P. and A.C.C.; validation, A.C.M., A.C.C., and J.R.M; formal analysis, H.P., L.C., and A.C.C.; investigation, H.P.; resources, L.C., M.d.C.F., and A.P.L.; data curation, A.C.C. and L.C.; writing—

original draft preparation, H.P. and A.C.C.; writing—review and editing, H.P., A.C.C., L.C., A.P.L., L.F., M.d.C.F., J.R.M., C.P., S.S, A.C.M., and M.M; visualization, H.P., C.P., M.d.C.F., A.P.L., S.S. and M.M.; supervision, A.C.C., J.R.M., and A.C.M. project administration, A.C.C., A.C.M., and J.R.M.; funding acquisition, A.C.C., L.C., M.d.C.F., and A.P.L. All authors read and agreed to the published version of the manuscript.

Funding: This research was funded by the projects UIDP/00772/2020 and LA/P/0059/2020 funded by the Portuguese Foundation for Science and Technology (FCT). Sérgio Santos-Silva thanks Fundação para a Ciência e a Tecnologia (FCT) for the financial support of his Ph.D. work under the scholarship 2021.09461.BD contract through the Maria de Sousa-2021 program.

Institutional Review Board Statement: Ethical review and approval were waived for this study, because the blood samples were obtained from carcasses sourced through a regional passive surveillance system during hunting season. It is important to stress that the carcasses were not obtained through experimental experiments, but rather in partnership with hunters and assisting veterinarians for the express purpose of monitoring hunting activities. Throughout the investigation, there was no intervention with the usual health management of wildlife.

Informed Consent Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. World Health Organization. Hepatitis E. WHO. 2023. Available online: https://www.who.int/news-room/fact-sheets/detail/hepatitis-e (accessed on 19 July 2023).
2. Melgaço, J.G.; Gardinali, N.R.; de Mello, V.; Leal, M.; Lewis-Ximenez, L.L.; Pinto, M.A. Hepatitis E: Update on Prevention and Control. *Bio. Med. Res. Int*. **2018**, *5769201*. doi: 10.1155/2018/5769201
3. Shirazi, R.; Pozzi, P.; Wax, M.; Bar-Or, I.; Asulin, E.; Lustig, Y., Mendelson, E., Ben-Ari, Z.; Schwartz, E.; Mor, O. Hepatitis E in Pigs in Israel: Seroprevalence, Molecular Characterization and Potential Impact on Humans. *Euro Surveill.* **2018,** *23*, 1800067. doi: 10.2807/1560-7917.ES.2018.23.49.1800067
4. Aspinall, E.J.; Couturier, E.; Faber, M.; Said, B.; Ijaz, S.; Tavoschi, L.; Takkinen, J.; Adlhoch, C. The Country Experts Hepatitis E Virus Infection in Europe: Surveillance and Descriptive Epidemiology of Confirmed Cases, 2005 to 2015. *Euro Surveill.* **2017**, *22*, 30561. doi: 10.2807/1560-7917.ES.2017.22.26.30561.
5. Wang, B.; Yang, X.-L. *Chirohepevirus* from Bats: Insights into Hepatitis E Virus Diversity and Evolution. *Viruses* **2022**, *14*, 905. https://doi.org/10.3390/v14050905
6. Purdy, M.A.; Drexler, J.F.; Meng, X.J.; Norder H, Okamoto H, Van der Poel WHM, Reuter G, de Souza WM, Ulrich RG, Smith DB. ICTV Virus Taxonomy Profile: Hepeviridae 2022. *J. Gen. Virol.* **2022**, *103*. doi: 10.1099/jgv.0.001778.
7. Nagashima, S.; Takahashi, M.; Kobayashi, T.; Tanggis; Nishizawa, T.; Nishiyama, T.; Primadharsini, P.P.; Okamoto, H. Characterization of the Quasi-Enveloped Hepatitis E Virus Particles Released by the Cellular Exosomal Pathway. *J. Virol.* **2017**, *91*, e00822-17. doi: 10.1128/JVI.00822-17.
8. Kamar, N.; Bendall, R.; Legrand-Abravanel, F.; Xia, N.S.; Ijaz, S.; Izopet, J.; Dalton, H.R. Hepatitis E. *Lancet.* **2012**, *379*, 2477-2488. doi: 10.1016/S0140-6736(11)61849-7.
9. Debing, Y.; Moradpour, D.; Neyts. J.; Gouttenoire, J. Update on hepatitis E virology: Implications for Clinical Practice. *J. Hepatol.* **2016**, *65*, 200-212. doi: 10.1016/j.jhep.2016.02.045.
10. Smith, D.B.; Izopet, J.; Nicot, F.; Simmonds, P.; Jameel, S.; Meng, X.J.; Norder, H.; Okamoto, H.; van der Poel, W.H.M.; Reuter, G.; Purdy, M.A. Update: Proposed Reference Sequences for Subtypes of Hepatitis E Virus (species *Orthohepevirus A*). *J. Gen. Virol.* **2020**, *101*, 692-698. doi: 10.1099/jgv.0.001435.
11. Martinelli, N.; Pavoni, E.; Filogari, D.; Ferrari, N.; Chiari, M.; Canelli, E.; Lombardi, G. Hepatitis E Virus in Wild Boar in the Central Northern Part of Italy. *Transbound. Emerg. Dis.* **2015**, *62*, 217–222.
12. EFSA Panel on Biological Hazards (BIOHAZ) Ricci, A.; Allende, A.; Bolton, D.; Chemaly, M.; Davies, R.; Fernandez Escamez, P.S.; Herman, L.; Koutsoumanis, K.; Lindqvist, R.; Nørrung, B.; Robertson, L; Ru, G.; Sanaa, M.; Simmons, M.; Skandamis, P.; Snary, E.; Speybroeck, N.; Ter Kuile, B.; Threlfall, J.; Wahlström, H.; Di Bartolo, I.; Johne, R.; Pavio, N.; Rutjes, S.; van der Poel, W.; Vasickova, P.; Hempen, M.; Messens, W.; Rizzi, V.; Latronico, F.; Girones, R. Public Health Risks Associated with Hepatitis E Virus (HEV) As a Food-Borne Pathogen. *EFSA J.* **2017**,*15*, e04886. doi: 10.2903/j.efsa.2017.4886.
13. Velavan, T.P.; Pallerla, S.R.; Johne, R.; Todt, D.; Steinmann, E.; Schemmerer, M.; Wenzel, J.J.; Hofmann, J.; Shih, J.W.K.; Wedemeyer, H.; Bock, C.T. Hepatitis E: An Update on One Health and Clinical Medicine. *Liver Int.* **2021**, *41*, 1462-1473. doi: 10.1111/liv.14912.
14. Behrendt, P.; Steinmann, E.; Manns, M.P.; Wedemeyer, H. The Impact of Hepatitis E in the Liver Transplant Setting. *J. Hepatol.* **2014**, *61*, 1418–1429. 10.1016/j.jhep.2014.08.047
15. Takakusagi, S.; Kakizaki, S.; Takagi, H. The Diagnosis, Pathophysiology, and Treatment of Chronic Hepatitis E Virus Infection—A Condition Affecting Immunocompromised Patients. *Microorganisms* **2023**, *11*, 1303. https://doi.org/10.3390/microorganisms11051303

16. Meng, X.J.; Purcell, R.; Halbur, P.; Lehman, J.; Webb, D.; Tsareva, T.; Haynes, J.; Thacker, B.; Emerson, S. A Novel Virus in Swine is Closely Related to the Human Hepatitis E Virus. *Proc. Natl. Acad. Sci. USA.* **1997**, *94*, 9860–9865. doi: 10.1073/pnas.94.18.9860
17. Colson, P.; Borentain, P.; Queyriaux, B.; Kaba, M.; Moal, V.; Gallian, P.; Heyries, L.; Raoult, D.; Gerolami, R. Pig Liver Sausage as a Source of Hepatitis E Virus Transmission to Humans. *J. Infect. Dis.* **2010**, *202*, 825–834. doi: 10.1086/655898.
18. Matsuda, H.; Okada, K.; Takahashi, K.; Mishiro, S. Severe Hepatitis E Virus Infection After Ingestion of Uncooked Liver from a Wild Boar. *J. Infect. Dis.* **2003**, *188*, 944. doi: 10.1086/378074
19. Serracca, L.; Battistini, R.; Rossini, I.; Mignone, W.; Peletto, S.; Boin, C.; Pistone, G.; Ercolini, R.; Ercolini, C. Molecular Investigation on the Presence of Hepatitis E Virus (HEV) in Wild Game in North-Western Italy. *Food Environ. Virol.* **2015**, *7*, 206–212. doi: 10.1007/s12560-015-9201-9
20. Dai, X.; Dong, C.; Zhou, Z.; Liang, J.; Dong, M.; Yang, Y.; Fu, J.; Tian, H.; Wang, S.; Fan, J.; Meng, J.; Purdy, M.A. Hepatitis E Virus Genotype 4, Nanjing, China, 2001-2011. *Emerg. Infect. Dis.* **2013**, *19*,1528-30. doi: 10.3201/eid1909.130013.
21. Pallerla, S.R.; Harms, D.; Johne, R.; Todt, D.; Steinmann, E.; Schemmerer, M.; Wenzel, J.J.; Hofmann, J.; Shih, J.W.K.; Wedemeyer, H.; Bock, C.T.; Velavan, T.P. Hepatitis E Virus Infection: Circulation, Molecular Epidemiology, and Impact on Global Health. *Pathogens* **2020**, *9*, *856*. https://doi.org/10.3390/pathogens9100856
22. Takahashi, M.; Nishizawa, T.; Sato, Y.; Miyazaki, S.; Aikawa, T.; Ashida, K.; Tamaru, T.; Oguro, K.; Hayakawa, F.; Matsuoka, H.; Ozaki, H.; Kodera, Y.; Irokawa, M.; Hirose, H.; Nagashima, S.; Kawakami, M.; Mizuo, H.; Okamoto, H.; Murata K. Prevalence and Genotype/Subtype Distribution of Hepatitis E Virus (HEV) among Wild Boars in Japan: Identification of a Genotype 5 HEV Strain. *Virus Res*. **2020**, *287*, 198106. doi: 10.1016/j.virusres.2020.198106.
23. King, N.J.; Hewitt, J.; Perchec-Merien, AM. Hiding in Plain Sight? It's Time to Investigate Other Possible Transmission Routes for Hepatitis E Virus (HEV) in Developed Countries. *Food Environ. Virol.* **2018**, *10*, 225–252 (2018). https://doi.org/10.1007/s12560-018-9342-8
24. Treagus, S.; Wright, C.; Baker-Austin, C.; Longdon, B.; Lowther, J. The Foodborne Transmission of Hepatitis E Virus to Humans. *Food Environ. Virol*. **2021**, *13*,127-145. doi: 10.1007/s12560-021-09461-5.
25. Woo, P.C.; Lau, S.K.; Teng, J.L.; Tsang, A.K.; Joseph, M.; Wong, E.Y.; Tang, Y.; Sivakumar, S.; Xie, J.; Bai, R.; Wernery, R.; Wernery, U.; Yuen, K.Y. New hepatitis E virus genotype in camels, the Middle East. *Emerg. Infect. Dis*. **2014**, *20*, 1044-1048. doi: 10.3201/eid2006.140140.
26. Woo, P.C.; Lau, S.K.; Teng, J.L.; Cao, K.Y.; Wernery, U.; Schountz, T.; Chiu, T.H.; Tsang, A.K.; Wong, P.C.; Wong, E.Y.; Yuen, K.Y. New Hepatitis E Virus Genotype in Bactrian Camels, Xinjiang, China, 2013. *Emerg. Infect. Dis*. **2016**, *22*(12), 2219-2221. doi: 10.3201/eid2212.160979.
27. Kukielka, D.; Rodriguez-Prieto, V.; Vicente, J.; Sánchez-Vizcaíno, J.M. Constant Hepatitis E Virus (HEV) Circulation in Wild Boar and Red Deer in Spain: An Increasing Concern Source of HEV Zoonotic Transmission. *Transbound Emerg. Dis*. **2016,** *63*, e360-8. doi: 10.1111/tbed.12311.
28. Pavio, N.; Doceul, V.; Bagdassarian, E.; Johne R. Recent Knowledge on Hepatitis E Virus in Suidae Reservoirs and Transmission Routes to Human. *Vet. Res.* **2017**, *48*, 78. https://doi.org/10.1186/s13567-017-0483-9
29. Tei, S.; Kitajima, N.; Takahashi, K.; Mishiro S. Zoonotic Transmission of Hepatitis E Virus from Deer to Human Beings. Lancet. 2003, 362, 371–373. doi: 10.1016/S0140-6736(03)14025-1
30. Dalton, H.R.; Kamar, N.; Izopet, J. Hepatitis E in Developed Countries: Current Status and Future Perspectives. *Future Microbiol.* **2014**, *9*, 1361–1372. doi: 10.2217/fmb.14.89.
31. Spada, E.; Simeoni, M.; Martina, A.; Pati, I.; Villano, U.; Adriani, D.; D'Angiò, A.; Tritarelli, E.; Taffon, S.; Bellino, S.; Boros, S.; Urciuoli, R.; Masiello, F.; Marano, G.; Bruni, R.; Pezzotti, P.; Ciccaglione, A. R.; Pupella, S.; De Angelis, V.; Pisani, G. Prevalence and Risk Factors for Hepatitis E Virus Infection in Blood Donors: A Nationwide Survey in Italy, 2017 to 2019. *Euro Surveill.* **2022**, *27*, 2100516. https://doi.org/10.2807/1560-7917.ES.2022.27.22.2100516
32. Dalton, H.R.; Kamar, N.; van Eijk, J.J.; McLean, B.N.; Cintas, P.; Bendall, R.P.; Jacobs, B.C. Hepatitis E Virus and Neurological Injury. *Nat. Rev. Neurol.* **2016**, *12*, 77–85. doi: 10.1038/nrneurol.2015.234.
33. Boccia, D.; Guthmann, J. P.; Klovstad, H.; Hamid, N.; Tatay, M.; Cigelnecki, I.; Nizou, J.; Nicand, E.; Guerin, P.J. High Mortality Associated with an Outbreak of Hepatitis E Among Displaced Persons in Darfur, Sudan. *Clin. Infect. Dis.* **2006**, *42*, 1679-84. doi: 10.1086/504322
34. Meng X.J.; Lindsay, D.S.; Sriranganathan, N. Wild Boars as Sources for Infectious Diseases in Livestock and Humans. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **2009**, *364*, 2697–2707.http://doi.org/10.1098/rstb.2009.0086
35. Salines, M.; Andraud, M.; Rose, N. From the Epidemiology of Hepatitis E Virus (HEV) Within the Swine Reservoir to Public Health Risk Mitigation Strategies: A Comprehensive Review. *Vet. Res.* **2017**, *48*, 31. https://doi.org/10.1186/s13567-017-0436-3
36. Yugo, D.M.; Meng, X.-J. Hepatitis E Virus: Foodborne, Waterborne and Zoonotic Transmission. *Int. J. Environ. Res. Public Health* **2013**, *10*, 4507-4533. https://doi.org/10.3390/ijerph10104507

37. Wang, B.; Meng, X.J. Hepatitis E Virus: Host Tropism and Zoonotic Infection. *Curr. Opin. Microbiol*. **2021**, 59, 8-15. doi: 10.1016/j.mib.2020.07.004.
38. Ferri, G.; Lauteri, C.; Festino, A.R.; Piccinini, A.; Olivastri, A.; Vergara, A. Hepatitis E Virus Detection in Hunted Wild Boar Liver and Muscle Tissues in Central Italy. *Microorganisms* **2022**, *10*, 1628. doi: 10.3390/microorganisms10081628.
39. Adlhoch, C.; Wolf, A.; Meisel, H.; Kaiser, M.; Ellerbrok, H.; Pauli, G. High HEV presence in four different wild boar populations in East and West Germany. *Vet. Microbiol.* **2009**, *18*, 270–278.
40. Caruso, C.; Modesto, P.; Bertolini, S.; Peletto, S.; Acutis, P.L.; Dondo, A.; Robetto, S.; Mignone, W.; Orusa, R.; Ru, G.; Masoero, L. Serological and Virological Survey of Hepatitis E Virus in Wild Boar Populations in Northwestern Italy: Detection of HEV subtypes 3e and 3f. *Arch. Virol.* **2015**, *160*, 153–160. https://doi.org/10.1007/s00705-014-2246-5
41. Aprea, G.; Amoroso, M.G.; Di Bartolo, I.; D'Alessio, N.; Di Sabatino, D.; Boni, A.; Cioffi, B.; D'Angelantonio, D.; Scattolini, S.; De Sabato, L.; Cotturone, G.; Pomilio, F.; Migliorati, G.; Galiero, G.; Fusco, G. Molecular Detection and Phylogenetic Analysis of Hepatitis E Virus Strains Circulating in Wild Boars in South-Central Italy. *Transbound. Emerg. Dis.* **2018**, *65*, e25–e31. https://doi.org/10.1111/tbed.12661
42. Montone, A.; De Sabato, L.; Suffredini, E.; Alise, M.; Zaccherini, A.; Volzone, P.; Di Maro, O.; Neola, B.; Capuano, F.; Di Bartolo, I. Occurrence of HEV-RNA in Italian Regional Pork and Wild Boar Food Products. *Food Environ. Virol.* **2019**, *11*, 420–426. https://doi.org/10.1007/s12560-019-09403-2
43. Bonardi, S.; Filipello, V.; Pavoni, E.; Carta, V.; Bolzoni, L., Corradi, M.; Gilioli, S.; Losio, M.N. Geographical Restriction of Hepatitis E Virus Circulation in Wild Boars (Sus Scrofa) In Emilia-Romagna Region, Northern Italy. *Ital. J. Food Saf.* **2020**, *9*, 8463. doi: 10.4081/ijfs.2020.8463.
44. Hosmer, D.W.; Lemeshow, S. Applied Logistic Regression. 2000, Second edition. John Wiley and Sons, New York, NY.
45. Ferri, G.; Giantomassi, G.; Piccinini, A.; Olivastri, A.; Vergara, A. Hepatitis E Virus RNA Detection from Hunted Wild Boars in Central Italy: an Epidemiological Investigation. *Food Environ. Virol.* **2023**, *15*,158-166. doi: 10.1007/s12560-023-09554-3
46. Montagnaro, S.; De Martinis, C.; Sasso, S.; Ciarcia, R.; Damiano, S.; Auletta, L.; Iovane, V.; Zottola, T.; Pagnini U. Viral and Antibody Prevalence of Hepatitis E in European Wild Boars (*Sus scrofa*) and Hunters at Zoonotic Risk in the Latium Region. *J. Comp. Pathol.* **2015**, *153*, 1–8. doi: 10.1016/j.jcpa.2015.04.006
47. Salvador, D.; Neto, C.; Benoliel, M.J.; Caeiro, M.F. Assessment of the Presence of Hepatitis E Virus in Surface Water and Drinking Water in Portugal. *Microorganisms* **2020**, *8*, 761. https://doi.org/10.3390/microorganisms8050761
48. De Schryver, A.; De Schrijver, K.; François, G.; Hambach, R.; van Sprundel, M.; Tabibi, R.; Colosio, C. Hepatitis E virus infection: An emerging occupational risk? *Occup. Med. (Lond.)* **2015**, *65*, 667–672
49. Raji, Y.E.; Toung, O.P.; Taib, N.M.; Sekawi, Z.B. Hepatitis E Virus: An Emerging Enigmatic and Underestimated Pathogen. *Saudi J. Biol. Sci.* **2022**, *29*, 499–512. https://doi.org/10.1016/j.sjbs.2021.09.003
50. Masuda, J.; Yano, K.; Tamada, Y.; Takii, Y.; Masahiro, I.; Omagari, K.; Kohno, S. Acute Hepatitis E of a Man Who Consumed Wild Boar Meat Prior to the Onset of Illness in Nagasaki, Japan. *Hepatol. Res.* **2005**, *31*, 178–183. doi: 10.1016/j.hepres.2005.01.008
51. Yazaki, Y.; Mizuo, H.; Takahashi, M.; Nishizawa, T.; Sasaki, N.; Gotanda, Y.; Okamoto, H. Sporadic Acute or Fulminant Hepatitis E in Hokkaido, Japan, May Be Food-Borne, As Suggested by the Presence of Hepatitis E Virus in Pig Liver as Food. *J. Gen. Virol.* **2003**, *84*, 2351–2357. doi: 10.1099/vir.0.19242-0.
52. Teixeira, J.; Mesquita, J.R.; Pereira, S.S.; Oliveira, R.M.; Abreu-Silva, J.; Rodrigues, A.; Myrmel, M.; Stene-Johansen, K.; Øverbø, J.; Gonçalves, G.; Nascimento, M.S. Prevalence of Hepatitis E Virus Antibodies in Workers Occupationally Exposed to Swine in Portugal. *Med. Microbiol. Immunol.* **2017**, *206*, 77-81. doi: 10.1007/s00430-016-0484-8.
53. Mesquita, J.R.; Santos-Ferreira, N.; Ferreira, A.S.; Albuquerque, C.; Nóbrega, C.; Esteves, F.; Cruz, R.; Vala, H.; Nascimento, M.S.J. Increased Risk of Hepatitis E Virus Infection in Workers Occupationally Exposed to Sheep. *Transbound. Emerg. Dis*. **2020**. doi: 10.1111/tbed.13524.
54. Moraes, D.; Lopez-Lopez, P.; Palmeira, J.D.; Torres, R.T.; Rivero-Juarez, A.; Dutra, V.; Nascimento, M.; Mesquita J.R. Screening for Hepatitis E Virus Genotype 3 in Red Deer (*Cervus elaphus*) and Fallow Deer (*Dama dama*), Portugal, 2018-2020. *Transbound. Emerg. Dis.* **2022**, *69*, 2764-2768. doi: 10.1111/tbed.14427.
55. Mesquita, J.R.; Oliveira, R.M.; Coelho, C.; Vieira-Pinto, M.; Nascimento, M.S. Hepatitis E Virus in Sylvatic and Captive Wild Boar from Portugal. *Transbound. Emerg. Dis.* **2016**, *63*, 574-578. doi: 10.1111/tbed.12297.
56. Santos-Silva, S.; Moraes, D.F.d.S.D.; López-López, P.; Palmeira, J.D.; Torres, R.T.; Nascimento, M.; Dashti, A.; Carmena, D.; Rivero-Juarez, A.; Mesquita, J.R. Survey of Zoonotic Diarrheagenic Protist and Hepatitis E Virus in wild boar (*Sus scrofa*) of Portugal. *Animals* **2023**, *13*, 256. https://doi.org/10.3390/ani13020256
57. Mesquita, J.R.; Abreu-Silva, J.; Sousa, J.C.; Aguiar, A.; Nascimento, M.S. Evidence of Autochthonous hepatitis E in a Portuguese Pediatric Cohort, 1992-1995. *J. Med. Virol.* **2016**, *88*, 919-921. doi: 10.1002/jmv.24414.

58. Oliveira, R.; Mesquita, J.R.; Pereira, S.; Abreu-Silva, J.; Teixeira, J.; Nascimento, M.S.J. Seroprevalence of Hepatitis E Virus Antibodies in Portuguese Children. *Pediatr. Infect. Dis J.* **2017**, *36*, 623-626. doi: 10.1097/INF.0000000000001525.
59. Gonçalves, D.; Pereira-Vaz, J.; Duque, V.; Bandeira, V.; Fonseca, C.; Donato, A.; Luxo, C.; Matos, A.M. First Serological Evidence on Endemicity of HEV Infection In Wild Boar (*Sus scrofa*) Populations from Portugal. *Virol. Sin.* **2018**, *33*, 197–200. https://doi.org/10.1007/s12250-018-0008-3.
60. Abrantes, A.; Santos-Silva, S.; Mesquita, J. R.Vieira-Pinto, M. Meat Juice as an Alternative Sample to Hepatitis E Virus ELISA: A Pilot Study in Wild Boar. *SafePork* **2023**, *14*. doi: https://doi.org/10.31274/safepork.16342
61. de Deus, N.; Peralta, B.; Pina, S.; Allepuz, A.; Mateu, E.; Vidal, D.; Ruiz-Fons, F.; Martin, M.; Gortazar, C.; Segales, J. Epidemiological Study Of Hepatitis E Virus Infection In European Wild Boars (Sus scrofa) in Spain. *Vet. Microbiol.* **2008**, *129*, 163–170. doi: 10.1016/j.vetmic.2007.11.002
62. Rutjes S.A.; Lodder-Verschoor, F.; Lodder, W.J.; van der Giessen, J.; Reesink, H.; Bouwknegt, M.; de Roda Husman, A.M. Seroprevalence and Molecular Detection of Hepatitis E Virus in Wild Boar and Red Deer in The Netherlands. *J. Virol. Methods.* **2010**, *168*, 197–206. doi: 10.1016/j.jviromet.2010.05.014.165,167,169
63. Boadella, M., Ruiz-Fons, J.F.; Vicente, J.; Martın, M.; Segales, J.; Gortazar, C. Seroprevalence Evolution of Selected Pathogens in Iberian Wild Boar. *Transbound. Emerg. Dis.* **2011**, *59*, 395–404. doi: 10.1111/j.1865-1682.2011.01285.x
64. Kukielka, D.; Rodriguez-Prieto, V.; Vicente, J.; Sánchez-Vizcaíno, J.M. Constant Hepatitis E Virus (HEV) Circulation in Wild Boar and Red Deer in Spain: An Increasing Concern Source of HEV Zoonotic Transmission. *Transbound. Emerg. Dis.* **2016**, *63*, e360–e368. doi: 10.1111/tbed.12311.
65. Roth, A.; Lin, J.; Magnius, L.; Karlsson, M.; Belák, S.; Widén, F.; Norder, H. Markers for Ongoing or Previous Hepatitis E Virus Infection Are as Common in Wild Ungulates as in Humans in Sweden. *Viruses.* **2016**, *8*, 259. doi: 10.3390/v8090259.
66. Di Bartolo, I.; Ponterio, E.; Angeloni, G.; Morandi, F.; Ostanello, F.; Nicoloso, S.; Ruggeri, F.M. Presence of Hepatitis E Virus in a Red Deer (Cervus elaphus) Population in Central Italy. *Transbound. Emerg. Dis.* **2017**, *64*, 137–143. https://doi.org/10.1111/tbed.12353
67. Sacristán, C.; Madslien, K.; Sacristán, I.; Klevar, S.; das Neves C.G. Seroprevalence of Hepatitis E Virus in Moose (*Alces alces*), Reindeer (*Rangifer tarandus*), Red Deer (*Cervus elaphus*), Roe Deer (*Capreolus capreolus*), and Muskoxen (*Ovibos moschatus*) from Norway. *Viruses,* **2021**, *13*, 224. doi: 10.3390/v13020224.
68. Martino, C.; Rampacci, E.; Pierini, I.; Giammarioli, M.; Stefanetti, V.; Hyatt, D.R.; Ianni, A.; Di Paolo, G.; Coletti, M.; Passamonti, F. Detection of Anti-HEV Antibodies and RNA of HEV in Pigs From A Hyperendemic Italian Region with high Human Seroprevalence. *Eur. J. Public Health*. **2021**, *31*, 68-72. doi: 10.1093/eurpub/ckaa112.
69. Loikkanen, E.; Oristo, S.; Hämäläinen, N.; Jokelainen, P.; Kantala, T.; Sukura, A., Maunula, L. Antibodies Against Hepatitis E Virus (HEV) in European Moose and White-Tailed Deer in Finland. *Food Environ. Virol.* **2020**, *12*, 333-341. doi: 10.1007/s12560-020-09442-0.
70. Di Pasquale, S.; De Santis, P.; La Rosa, G.; Di Domenico, K.; Iaconelli, M.; Micarelli, G.; Martini, E.; Bilei, S.; De Medici, D.; Suffredini, E. Quantification and Genetic Diversity of Hepatitis E Virus in Wild Boar (*Sus Scrofa*) Hunted for Domestic Consumption in Central Italy. *J. Food Microbiol.* **2019**, *82*, 194–201. https://doi.org/10.1016/j.fm.2019.02.005
71. De Sabato, L.; Amoroso, M.G.; Ianiro, G.; Esposito, C.; De Grossi, L.; Fusco, G.; Barone, A.; Martini, E.; Ostanello, F.; Di Bartolo, I. Detection of Hepatitis E Virus in Livers and Muscle Tissues of Wild Boars in Italy. *Food Environ. Virol.* **2020**, *12*, 1–8. https://doi.org/10.1007/s12560-019-09405-0
72. De Sabato, L.; Di Bartolo, I.; Lapa, D.; Capobianchi, M.R.; Garbuglia, A.R. Molecular Characterization of HEV Genotype 3 in Italy at Human/Animal Interface. *Front. Microbiol.* **2020**, *11*, 137. https://doi.org/10.3389/fmicb.2020.00137
73. Santos-Silva, S.; López-López, P.; Gonçalves, H.M.R.; Rivero-Juarez, A.; Van der Poel, W.H.M.; Nascimento, M.S.J.; Mesquita, J.R. A Systematic Review and Meta-Analysis on Hepatitis E Virus Detection in Farmed Ruminants. *Pathogens.* **2023**, *12*, 550. doi: 10.3390/pathogens12040550.
74. Barroso, P.; Risalde, M.A.; García-Bocanegra, I.; Acevedo, P.; Barasona, J.Á.; Caballero-Gómez, J.; Jiménez-Ruiz, S.; Rivero-Juárez, A.; Montoro, V.; Vicente, J. Long-Term Determinants of the Seroprevalence of the Hepatitis E Virus in Wild Boar (*Sus scrofa*). *Animals (Basel).* **2021**, *11*, 1805. doi: 10.3390/ani11061805.
75. Rinaldo, C.H.; Nymo, I.H.; Sánchez Romano, J.; Breines, E.M.; Murguzur, F.J.A.; Tryland, M. Serological Evidence of Hepatitis E Virus Infection in Semi-Domesticated Eurasian Tundra Reindeer (*Rangifer tarandus tarandus*) in Norway. *Pathogens* **2021**, *10*, 1542. https://doi.org/10.3390/ pathogens10121542
76. Hartl, J.; Otto, B.; Madden, R.G.; Webb, G.; Woolson, K.L.; Kriston, L.; Vettorazzi, E.; Lohse, A.W.; Dalton, H.R.; Pischke, S. Hepatitis E Seroprevalence in Europe: A Meta-Analysis. *Viruses* **2016**, *8*, 211. doi: 10.3390/v8080211.
77. Olsøy, I.B.; Henriksen, S.; Weissbach, F.H.; Larsen, M.; Borgen, K.; Abravanel, F.; Kamar, N.; Paulssen, E.J.; Hirsch, H.H.; Rinaldo, C.H. Seroprevalence of Hepatitis E Virus (HEV) in a General Adult Population in

Northern Norway: The Tromsø Study. *Med. Microbiol. Immunol.* **2019**, *208*, 715–725. doi: 10.1007/s00430-019-00599-5

78. Arnaboldi, S.; Righi, F.; Carta, V.; Bonardi, S.; Pavoni, E.; Bianchi, A.; Losio, M.N.; Filipello, V. Hepatitis E Virus (HEV) Spread and Genetic Diversity in Game Animals in Northern Italy. *Food Environment. Virol.* **2021**, *13*, 146–153. https://doi.org/10.1007/s12560-021-09467-z
79. Oechslin, N.; Moradpour, D.; Gouttenoire, J. (2020). On the Host Side of the Hepatitis E Virus Life Cycle. *Cells*, **2020**, *9*, 1294. https://doi.org/10.3390/cells9051294
80. Prpić, J.; Kunić, A.; Keros, T.; Lojkić, I.; Brnić, D.; Jemeršić, L. Absence of Hepatitis E Virus (HEV) Circulation in the Most Widespread Wild Croatian Canine Species, the Red Fox (Vulpes vulpes) and Jackal (*Canis aureus moreoticus*). *Microorganisms* **2023**, *11*, 834. https://doi.org/10.3390/microorganisms11040834
81. Pineda, J.A.; Cifuentes, C.; Parra, M.; Merchante, N.; Pérez-Navarro, E.; Rivero-Juárez, A.; Monje, P.; Rivero, A.; Macías, J.; Real, L.M. Incidence and Natural History of Hepatitis E Virus Coinfection Among HIV-Infected Patients. *AIDS* **2014**, *28*, 1931–1937. doi: 10.1097/QAD.0000000000000378.
82. Denner, J. Hepatitis E virus (HEV) - The Future. *Viruses* **2019**, *11*, 251. doi: 10.3390/v11030251.
83. Fredriksson-Ahomaa, M. Wild Boar: A Reservoir of Foodborne Zoonoses. *Foodborne Pathog. Dis.* **2019**, *16*, 153-165. doi: 10.1089/fpd.2018.2512.
84. Karlsen, A.A.; Kichatova, V.S.; Kyuregyan, K.K.; Mikhailov, M.I. Phylodynamic Analysis Suggests That Deer Species May Be a True Reservoir for Hepatitis E Virus Genotypes 3 and 4. *Microorganisms* **2023**, *11*, 375. https://doi.org/10.3390/microorganisms11020375
85. Pires, H.; Cardoso, L.; Lopes, A.P.; Fontes, M.d.C.; Matos, M.; Pintado, C.; Figueira, L.; Mesquita, J.R.; Matos, A.C.; Coelho, A.C. Seropositivity for *Coxiella burnetii* in Wild Boar (*Sus scrofa*) and Red Deer (*Cervus elaphus*) in Portugal. *Pathogens* **2023**, *12*, 421. https://doi.org/10.3390/pathogens12030421
86. Matos, A.C.; Figueira, L.; Martins, M.H.; Pinto, M.L.; Matos, M.; Coelho, A.C. New Insights into *Mycobacterium bovis* Prevalence in Wild Mammals in Portugal. *Transbound. Emerg. Dis.* **2014**, *63*, e313–e322, https://doi.org/10.1111/tbed.12306.
87. Matos, A.C.; Figueira, L.; Martins, M.H.; Loureiro, F.; Pinto, M.L.; Matos, M.; Coelho A.C. Survey of *Mycobacterium avium* subspecies *paratuberculosis* in Road-Killed Wild Carnivores in Portugal. *J. Zoo Wildl. Med*. **2014**, *45*, 775-781. doi: 10.1638/2014-0010.1.
88. Matos, A.C.; Dias, A.P.; Morais, M.; Figueira, L.; Martins, M.H.; Matos, M.; Pinto, M.L.; Coelho, A.C. Granuloma Coinfection with *Mycobacterium bovis*, *Mycobacterium avium* subsp. *paratuberculosis*, and *Corynebacterium pseudotuberculosis* in Five Hunted Red Deer (*Cervus elaphus*) in Portugal. *J. Wildl. Dis.* **2015**, *51*, 793–794.