Food and Environmental Virology
https://doi.org/10.1007/s12560-019-09407-y

BRIEF COMMUNICATION

No Evidence of Hepatitis E Virus Infection in Farmed Deer in Germany

Eva Trojnar[1] · **Bernd Kästner**[2] · **Reimar Johne**[1]

Received: 6 September 2019 / Accepted: 28 September 2019

Abstract
Hepatitis E virus (HEV) is a zoonotic agent, which is mainly transmitted by consumption of undercooked meat products originating from infected animals. Domestic pigs and wild boars are the major animal reservoirs, but HEV infections have been also repeatedly described in wild deer species. However, farmed deer has been only sparsely investigated so far. Here, 108 blood and 106 liver samples from fallow deer, red deer, and sika deer strictly hold in game enclosures from 11 farms in Germany were analyzed for markers of HEV infection. Using a commercial double antigen sandwich ELISA, 3/108 (2.7%) serum samples were scored borderline for HEV-specific antibodies, whereas the remaining samples were negative. No HEV-RNA (0%) was detected in the 106 liver samples. The results suggest a low risk of HEV infection in farmed deer in Germany.

Keywords Hepatitis E virus · Deer · Farmed · Enclosure

The hepatitis E virus (HEV) is the causative agent of acute hepatitis in humans; in addition, chronic disease courses with liver cirrhosis have been described in immunosuppressed transplant patients (Khuroo and Khuroo 2016). The virus has a worldwide distribution. During the last years, the number of notified hepatitis E cases has been steeply increased in many European countries (Aspinall et al. 2017). HEV can be differentiated into four genotypes, which have a distinct geographical distribution. In Europe, genotype 3 is most prevalent. This genotype can also infect animals, enabling zoonotic virus transmission via undercooked meat products. Domestic pigs and wild boars have been identified as the main animal reservoirs of HEV (Pavio et al. 2017). Seroprevalences of approximately 50% were described for HEV-specific antibodies in domestic pigs in Germany (Bächlein et al. 2010) and 33% in wild boars (Denzin and Borgwardt 2013). HEV-RNA was detected in 15–68% of investigated wild boars from Germany (Schielke et al. 2009; Adlhoch et al. 2009). In addition, markers of HEV infection have been described for a large variety of other animal species, although in most cases with lower detection rates (Spahr et al. 2018). Among these, HEV-RNA has been repeatedly detected in several wild deer species (Spahr et al. 2018; Pavio et al. 2017). In addition, case reports from Japan and South Korea identified consumption of raw meat from wild sika deer and wild roe deer as the cause of hepatitis E in patients (Tei et al. 2003; Choi et al. 2013). However, it has been suggested that deer do not represent a true reservoir of HEV but are rather accidentally infected by sharing the same habitat as wild boars (Pavio et al. 2017). Those wild boars, which excrete large amounts of HEV, may therefore serve as a source of infection for wild deer living in the same area (Anheyer-Behmenburg et al. 2017). In contrast to wild deer, only sparse data are available on HEV infection rates of farmed deer, which are not in close contact to wild boars.

The aim of our study was therefore to analyze the HEV infection status of deer, which is strictly held in enclosures in Germany. The data should help to assess the risk of zoonotic transmission of HEV from farmed deer to humans.

Samples from 108 animals (73 fallow deer, 23 red deer, and 12 sika deer) derived from 11 farms located in Thuringia in Germany were included in the study (Fig. 1). This included 19 animals up to 6 months old, 34 females between 7 and 18 months old, 38 males between 7 and 18 months old, 15 females older than 18 months, and 2 males older than 18 months. Serum samples were available from all animals. As two liver samples (from one fallow deer and one red deer) could not be sampled, a total of 106 liver samples were available for analysis. Serum samples

✉ Reimar Johne
Reimar.Johne@bfr.bund.de

[1] German Federal Institute for Risk Assessment, Max-Dohrn-Straße 8-10, 10589 Berlin, Germany

[2] Thuringian State Office for Agriculture and Rural Area, Naumburger Straße 98, 07743 Jena, Germany

Published online: 17 October 2019

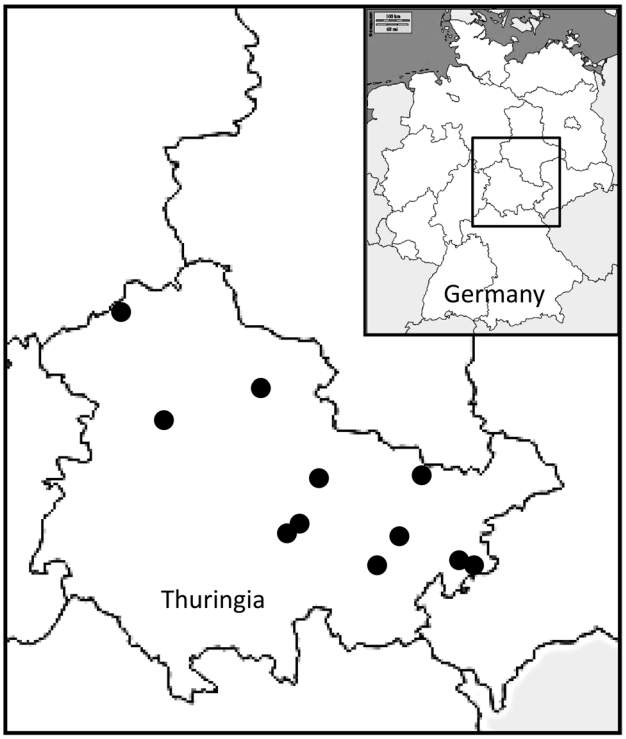

Fig. 1 Location of deer farms included in the study. The maps show the location of the Free State of Thuringia (Thuringia) within Germany (inserted picture, upper right) and the location of deer farms (black circles) within Thuringia

were tested for HEV-specific antibodies using the HEV Ab EIA (Axiom, Bürstadt, Germany) according to the manufacturer's instructions. From the liver samples, 2 g were placed in 50-ml tubes together with 1.6 ml PBS and 3 ceramic beads (1/4″ Ceramic Sphere, MP Biomedicals, CA, USA), and homogenized in a FastPrep®-24 homogenizer (MP Biomedicals) in two cycles of 30 s at speed 5 ms^{-1}. Thereafter, 25 mg of the homogenate was further homogenized with 600 µl RLT buffer (part of the RNeasy Mini Kit, Qiagen, Hilden, Germany) using the sample grinding GK60 Precellys Lysing Kit (Bertin Technologies SAS, Montigny-le-Bretoneux, France) in the FastPrep®-24 homogenizer for one cycle of 30 s at speed 5 ms^{-1}. The bacteriophage MS2 was added to each liver homogenate and used as a process and internal amplification control as described (Althof et al. 2019). After a centrifugation at 13,000×*g* for 3 min at room temperature in a table centrifuge (Eppendorf), the RNA was extracted from the entire supernatant using the RNeasy Mini Kit (Qiagen, Hilden, Germany). HEV-RNA was detected by real-time RT-PCR according to Jothikumar et al. (2006) using the Quantitect Probe RT-PCR Kit (Qiagen) as described (Kreuzer et al. 2012). The bacteriophage MS2 RNA was analyzed by real-time RT-PCR as described (Dreier et al. 2005). By comparing the bacteriophage MS2-specific RT-PCR

Table 1 Results of analysis of farmed deer samples from Germany for HEV-specific antibodies and HEV-RNA

	HEV-specific antibodies (double antigen sandwich ELISA)[a]	HEV-RNA (real-time RT-PCR)[b]
Fallow deer	0/3/73	0/72
Red deer	0/0/23	0/22
Sika deer	0/0/12	0/12
Total	0/3/108	0/106

[a]Number of positive samples/borderline samples/total number of samples

[b]Number of positive samples/total number of samples

Cq value of a sample with that derived from RNA directly isolated from the original bacteriophage preparation, the MS2 recovery rate (in %) was calculated by the formula $2^{-\Delta Cq} \times 100$ (Schmittgen and Livak 2008). Samples showing a recovery rate > 1% were considered as valid as suggested by standardized methods for food-borne virus detection (ISO 2019).

Using the EIA, 3/108 serum samples were scored borderline, and the remaining samples were negative (Table 1). The three borderline samples, originating from fallow deer of three different farms, were re-tested and resulted again in borderline results. According to the EIA manufacturer's instructions, they should therefore be considered as weak positive. All liver samples were negative for HEV-RNA using real-time RT-PCR. A parallel analysis of the RNA preparations for the bacteriophage MS2 added as internal control to each sample revealed recovery rates between 1.8 and 60.7%; therefore, the RT-PCR results were considered as valid for analysis.

Only a few samples of our study showed weakly positive results for HEV-specific antibodies and no sample was scored positive for HEV-RNA. The applied EIA is a double antigen sandwich ELISA, which enables detection of all antibody classes independent of the animal species tested. This kit has been previously shown to be suitable for analysis of sera from a large variety of animal species including those of the family Cervidae (Spahr et al. 2017). By comparison with other assays for HEV antibody detection, this assay turned out to have a high sensitivity, but was considered to exhibit a lower specificity (Krumbholz et al. 2013). Therefore, the weak positive reactions observed in our study may also represent unspecific reactions. The applied real-time RT-PCR was previously shown to have a high sensitivity and specificity and has been successfully used for HEV-RNA detection in a wide range of animal samples including wild deer (Jothikumar et al. 2006; Kreuzer et al. 2012; Anheyer-Behmenburg et al. 2017). Moreover, the applied amplification and inhibition control showed acceptable results in our study. Taken together, the applied methods have to be

considered to generate valid results; therefore, the absence of HEV infection markers in the analyzed farmed deer samples has to be concluded.

Two independently performed recent studies involving wild deer in Germany identified HEV-specific antibodies and HEV-RNA in some of the investigated red deer, roe deer, and fallow deer species indicating the occurrence of HEV infection in wild deer in Germany (Neumann et al. 2016; Anheyer-Behmenburg et al. 2017). In another study, HEV-RNA was detected in 23.8% of the investigated wild boars from Thuringia, suggesting that the virus is present in wild animals of the geographical region investigated in our study (Schielke et al. 2009). The absence of HEV infections in farmed deer in our study may be explained by the different animal holding system. Because the farmed deer is strictly held in enclosure, it has only very limited possibilities of close contact to wild boars, which is known as a source of HEV infection. In recent studies, no HEV-RNA was detected in farmed fallow deer from the Czech Republic (Kubankova et al. 2015) or in farmed sika deer from China (Xia et al. 2015), which is consistent with our results.

Taken together, the results of our study suggest a low risk of HEV infection in farmed deer in Germany. Further studies involving larger animal numbers from more geographical regions and other countries should be performed in order to prove this conclusion.

Acknowledgements We like to thank the deer farmers for cooperation during sampling, PD Dr. Ulrich Methner (Friedich Loeffler Institut, Jena, Germany) for preparation of sera, and Anja Schlosser and Stephanie Prosetzky (BfR, Berlin, Germany) for excellent technical assistance in laboratory analyses. The Free State of Thuringia supported the samplings financially. The laboratory analysis of samples was financially supported by the German Federal Institute for Risk Assessment (BfR)-Center for Land Use Related Evaluation Methods and One Health Approaches.

References

Adlhoch, C., Wolf, A., Meisel, H., Kaiser, M., Ellerbrok, H., & Pauli, G. (2009). High HEV presence in four different wild boar populations in East and West Germany. *Veterinary Microbiology, 139*, 270–278.

Althof, N., Trojnar, E., Böhm, T., Burkhardt, S., Carl, A., Contzen, M., et al. (2019). Interlaboratory validation of a method for hepatitis E virus RNA detection in meat and meat products. *Food and Environmental Virology, 11*, 1–8.

Anheyer-Behmenburg, H. E., Szabo, K., Schotte, U., Binder, A., Klein, G., & Johne, R. (2017). Hepatitis E virus in wild boars and evidence of spillover infection in red and roe deer, Germany, 2013–2015. *Emerging Infectious Diseases, 23*, 130–133.

Aspinall, E. J., Couturier, E., Faber, M., Said, B., Ijaz, S., Tavoschi, L., et al. (2017). Hepatitis E virus infection in Europe: Surveillance and descriptive epidemiology of confirmed cases, 2005 to 2015. *Eurosurveillance, 22*(26), 30561.

Bächlein, C., Schielke, A., Johne, R., Ulrich, R. G., Baumgärtner, W., & Grummer, B. (2010). Prevalence of Hepatitis E virus-specific antibodies in sera of German domestic pigs estimated by using different assays. *Veterinary Microbiology, 144*, 187–191.

Choi, J. Y., Lee, J. M., Jo, Y. W., Min, H. J., Kim, H. J., Jung, W. T., et al. (2013). Genotype-4 hepatitis E in a human after ingesting roe deer meat in South Korea. *Clinical and Molecular Hepatology, 19*, 309–314.

Denzin, N., & Borgwardt, J. (2013). Occurrence and geographical distribution of antibodies to hepatitis E virus in wild boars of Saxony-Anhalt, Germany (2011). *Berliner und Münchner Tierärztliche Wochenschrift, 126*, 230–235.

Dreier, J., Störmer, M., & Kleesiek, K. (2005). Use of bacteriophage MS2 as an internal control in viral reverse transcription-PCR assays. *Journal of Clinical Microbiology, 43*, 4551–4557.

ISO. (2019). Microbiology of the food chain—Horizontal method for determination of hepatitis A virus and norovirus using real-time RT-PCR.ISO15216-2:2019.

Jothikumar, N., Cromeans, T. L., Robertson, B. H., Meng, X. J., & Hill, V. R. (2006). A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. *Journal of Virological Methods, 131*, 65–71.

Khuroo, M. S., & Khuroo, M. S. (2016). Hepatitis E: An emerging global disease—from discovery towards control and cure. *Journal of Viral Hepatitis, 23*, 68–79.

Kreuzer, S., Machnowska, P., Aßmus, J., Sieber, M., Pieper, R., Schmidt, M. F. G., et al. (2012). Feeding of the probiotic bacterium *Enterococcus faecium* NCIMB 10415 differentially affects shedding of enteric viruses in pigs. *Veterinary Research, 43*, 58.

Krumbholz, A., Joel, S., Neubert, A., Dremsek, P., Dürrwald, R., Johne, R., et al. (2013). Age-related and regional differences in the prevalence of hepatitis E virus-specific antibodies in pigs in Germany. *Veterinary Microbiology, 167*, 394–402.

Kubankova, M., Kralik, P., Lamka, J., Zakovcik, V., Dolanský, M., & Vasickova, P. (2015). Prevalence of hepatitis E virus in populations of wild animals in comparison with animals bred in game enclosures. *Food and Environmental Virology, 7*, 159–163.

Neumann, S., Hackl, S.S., Piepenschneider, M., Vina-Rodriguez, A., Dremsek, P., Ulrich, R.G., et al. (2016). Serologic and Molecular Survey of Hepatitis E Virus inGerman Deer Populations. *Journal of Wildlife Diseases, 52*(1), 106–113.

Pavio, N., Doceul, V., Bagdassarian, E., & Johne, R. (2017). Recent knowledge on hepatitis E virus in Suidae reservoirs and transmission routes to human. *Veterinary Research, 48*, 78.

Schielke, A., Sachs, K., Lierz, M., Appel, B., Jansen, A., & Johne, R. (2009). Detection of hepatitis E virus in wild boars of rural and urban regions in Germany and whole genome characterization of an endemic strain. *Virology Journal, 6*, 58.

Schmittgen, T. D., & Livak, K. J. (2008). Analyzing real-time PCR data by the comparative C(T) method. *Nature Protocols, 3*, 1101–1108.

Spahr, C., Ryll, R., Knauf-Witzens, T., Vahlenkamp, T. W., Ulrich, R. G., & Johne, R. (2017). Serological evidence of hepatitis E virus infection in zoo animals and identification of a rodent-borne strain in a Syrian brown bear. *Veterinary Microbiology, 212*, 87–92.

Spahr, C., Knauf-Witzens, T., Vahlenkamp, T., Ulrich, R. G., & Johne, R. (2018). Hepatitis E virus and related viruses in wild, domestic and zoo animals: A review. *Zoonoses and Public Health, 65*, 11–29.

Tei, S., Kitajima, N., Takahashi, K., & Mishiro, S. (2003). Zoonotic transmission of hepatitis E virus from deer to human beings. *The Lancet, 362*, 371–373.

Xia, J., Zeng, H., Liu, L., Zhang, Y., Liu, P., Geng, J., et al. (2015). Swine and rabbits are the main reservoirs of hepatitis E virus in China: Detection of HEV RNA in feces of farmed and wild animals. *Archives of Virology, 160*, 2791–2798.