Molecular detection of hepatitis E virus in wild boars from Botoșani County

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Abstract

Hepatitis E virus (HEV) infections occur in both humans and animals worldwide. The domestic swine and wild boars (Sus scrofa) are known as important reservoirs of HEV, zoonotic hepatitis E infections of foodborne origin being linked to consumption of pig, wild boar and deer raw or undercooked meat or undercooked products. The aim of the study was to highlight the presence of HEV in wild boars using molecular biology methods. During hunting season 2015-2016 were collected blood and faecal samples from 22 wild boars from Suharău hunting fund in Botoșani County. Using degenerate primers, HEV RNA was detected by nested reverse transcriptase PCR in all wild boar faecal samples. The blood (EDTA whole blood) samples tested were negative for hepatitis E virus RNA. Our results indicate that wild boars are a possible source of infection for pigs and human population in Botoșani County.

Keywords*: HEV, wild boar, RNA*

Introduction

Hepatitis E virus (HEV) is a viral pathogen transmitted primarily via fecal-oral route, classified in the genus *Orthohepevirus*, the family *Hepeviridae* (Smith et al., 2014). Human pathogenic HEVs are mainly classified into genotypes 1–4. Although genotypes 1 and 2 infect only humans, genotypes 3 and 4 are zoonotic and infect different animal species and humans. The main animal reservoirs for genotype 3 are domestic pigs and wild boars (Pavio N. et al., 2015).

The zoonotic transmission of hepatitis E virus (HEV) is of special concern. Direct zoonotic transmission to humans has been documented several times from wild boar and pigs infected with HEV. High HEV seroprevalences can be found in European pig and wild boar populations (Van der Poel WH. 2014). To determine wild boar hepatitis E virus (HEV) we analysed molecular markers of HEV infection among feral pigs in Botoșani County.

Materials and methods

Investigations were undertaken during hunting season 2015-2016 on wild boar samples from Suharău hunting fund in Botoșani County. Ten feacal samples and 12 EDTA whole blood samples were collected from 22 animals. Prior to analysis all samples were stored at −80°C.

HEV detection protocol consisted in the following steps: RNA extraction, revers-transcription and nested PCR using specific HEV primers. RNA extraction was achieved using two extraction kits: QIAamp Viral RNA (Qiagen) for faecal samples and RNAeasy Mini kit (Qiagen) for EDTA whole blood samples according to the manufacturer´s instructions.

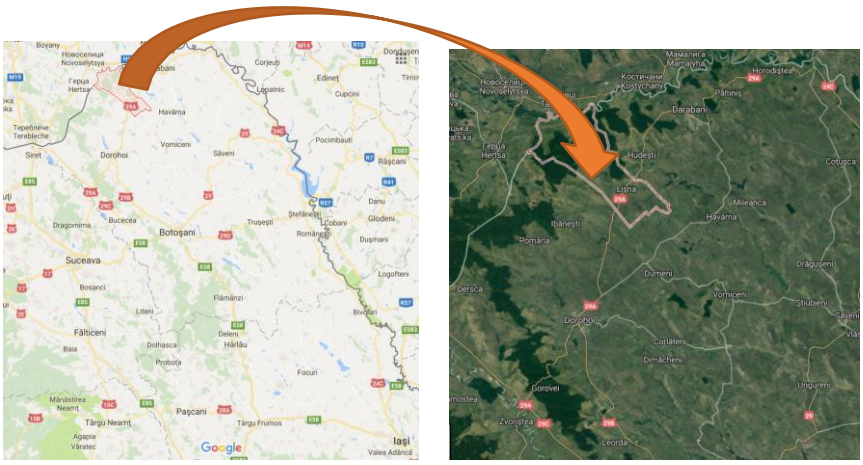

Fig. 1. Sampling area: Suharău hunting fund in Botoşani County

Detection of HEV RNA from faecal and EDTA whole blood samples was performed by nested RT-PCR targeting a fragment of the structural gene of HEV in ORF2 using primers 3156N and 3157N (730 nt product) for the first PCR and primers 3158 N and 3159 N (348 nt product) for the second PCR (nested-PCR) (Table 1). PCR products were separated on a 1% agarose gel and visualized using Gel Doc Documentation System (BioRad).

Table 1. Sets of primers used for nested rt-PCR for HEV RNA detection

Primer	Target gene	PCR method	Sequence	Location	Polarity
3156N	ORF2	PCR	AATTATGCC(T)CAGTAC(T)CGG(A)GTTG	5687–5708	Positive
3157N	ORF2	PCR	CCCTTA(G)TCC(T)TGCTGA(C)GCATTCTC	6395–6417	Negative
3158N	ORF2	nested-PCR	GTT(A)ATGCTT(C)TGCATA(T)CATGGCT	5972–5993	Positive
3159N	ORF2	nested-PCR	AGCCGACGAAATCAATTCTGTC	6298–6319	Negative

Results and discussions

Our previous investigations on hepatitis E in susceptible species from Botoşani County highlighted the existence of a reservoir of infection. Previous results revealed the presence of hepatitis E infection in human population and pigs reared in household system (overall prevalence - 22.66%) in Botoşani County (Aniță A. et al, 2010).

In most cases of autochthonous hepatitis E in developed countries the source and route of infection cannot be identified. However, the evidence suggests that most cases may be due to consumption of undercooked HEV contaminated pork or game meat (Nan Y. et al, 2017). The dynamic of HEV infection in wildlife is still unknown, but recent research highlights the maintenance and circulation of the virus, posing a risk to human health in the case of meat consumption from wild boar and deer (Anheyer-Behmenburg, H.E. et al, 2017).

The aim of this investigation was the detection of molecular markers of hepatitis E infection in wild boars from Botoşani County. Samples (faeces and EDTA whole blood) from 22 animals were tested for the presence of HEV. Using nested RT-PCR the presence of hepatitis E

virus was detected in all faecal samples. Wild boar EDTA whole blood samples were negative for HEV genome (Fig.2).

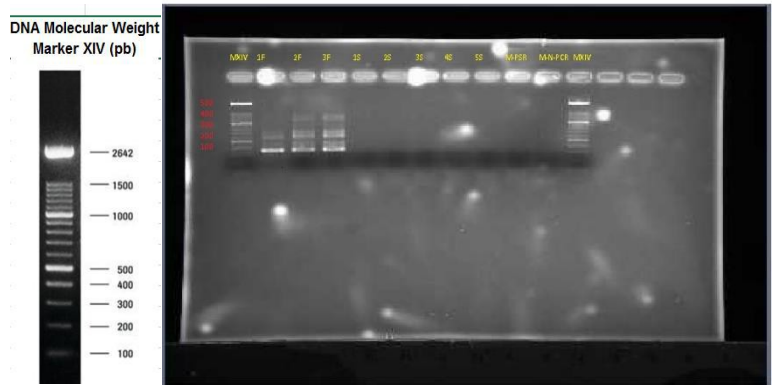

Fig. 2 Migration of the PCR products after nested PCR amplification
1F, 2F, 3F – wild boar faecal samples
1S, 2S, 3S, 4S, 5S – EDTA whole blood samples

HEV has been frequently isolated from wild boars in Europe and in Asia. For the first time, it was detected in wild boars in Japan, and the genotype was the same as most frequently seen in production pigs, HEV-3 (Sonoda H. et al, 2004). Since then, isolation of HEV RNA from wild boars has shown that these animals are important hosts of HEV also in several European countries: Spain (Martelli et al., 2015), Germany (Vina-Rodriguez et al., 2015), Sweden (Roth et al., 2016), Italy (Oliveira-Filho et al., 2014; Di Profio et al., 2016), Slovenia (Zele et al., 2016) and Portugal (Mesquita et al., 2014). Hepatitis E virus was first detected in Romania 2017 by Porea D. et al, highlighting the potential role of wild boars as zoonotic reservoir for HEV.

Conclusions

The results of our investigations revealed the presence of HEV infection in wild boars from Botoşani County. Since large amounts of virus particles are excreted in feces of wild boar, droppings can contaminate the environment and pose a particular risk to susceptible species. Zoonotic hepatitis E infections of foodborne origin have been linked to consumption of pig and wild boar meat, mostly uncooked or undercooked products, raising public health concerns.

References

1. Aniţă A., Aniţă D., Ludu L., Savuţa G., (2010). Seroepidemiological investigation of human and swine hepatitis E in Botoşani County. Bulletin UASVM, Veterinary Medicine 67(2): 19-22.
2. Anheyer-Behmenburg, H. E., Szabo, K., Schotte, U., Binder, A., Klein, G., Johne, R. (2017). Hepatitis E Virus in Wild Boars and Spillover Infection in Red and Roe Deer, Germany, 2013–2015. Emerging Infectious Diseases, 23(1): 130–133.
3. Di Profio, F., Melegari, I., Sarchese, V., Robetto, S., Marruchella, G., Bona, M.C., Orusa, R., Martella, V., Marsilio, F., Di Martino, B., 2016. Detection and genetic characterization of hepatitis E virus (HEV) genotype 3 subtype c in wild boars in Italy. Arch. Virol. 161, 2829–2834.
4. Emerson S.U., Purcell R.H., (2013). Hepatitis e virus. Fields Virology, 2:2242-2258.
5. Martinelli N., Pavoni E., Filogari D., Ferrari N., Chiari M., Canelli E., Lombardi G., (2015). Hepatitis E Virus in Wild Boar in the Central Northern Part of Italy. Transbound Emerg.Dis. 62: 217–222.
6. Nan Y, Wu C, Zhao Q, Zhou EM. (2017). Zoonotic Hepatitis E Virus: An Ignored Risk for Public Health. Front Microbiol, 8:2396.

7. Mesquita J.R., Oliveira R.M., Coelho C., Vieira-Pinto M., Nascimento M.S., 2014. Hepatitis E virus in sylvatic and captive wild boar from Portugal. Transbound Emerg. Dis. 63, 574–578.
8. Oliveira-Filho E.F., Bank-Wolf B.R., Thiel H.J., Konig M., (2014). Phylogenetic analysis of hepatitis E virus in domestic swine and wild boar in Germany. Vet. Microbiol. 174, 233–238.
9. Pavio N., Meng X-J, Doceul V., (2015). Zoonotic origin of hepatitis E. Curr Opin Virol ;10:34-41.
10. Porea D, Anita A, Demange A, Raileanu C, Oslobanu Ludu L, Anita D, Savuta G, Pavio N. Molecular detection of hepatitis E virus in wild boar population in eastern Romania. Transbound Emerg Dis. 2017 Oct 12. doi: 10.1111/tbed.12736.
11. Roth A., Lin J., Magnius L., Karlsson M., Belak S., Widen F., Norder H., (2016). Markers for Ongoing or Previous Hepatitis E Virus Infection Are as Common in Wild Ungulates as in Humans in Sweden. Viruses. 8, 259. doi: 10.3390/v8090259.
12. Sonoda H., Abe M., Sugimoto T., Sato Y., Bando M., Fukui E., Okamoto H. (2004). Prevalence of Hepatitis E Virus (HEV) Infection in Wild Boars and Deer and Genetic Identification of a Genotype 3 HEV from a Boar in Japan. Journal of Clinical Microbiology, 42(11), 5371–5374.
13. Smith, D. B., Simmonds, P., Jameel, S., Emerson, S. U., Harrison, T. J., Meng, X. J., et al., (2014). Consensus proposals for classification of the family Hepeviridae. J. Gen. Virol., 95, 2223–2232.
14. Van der Poel W.H., (2014). Food and environmental routes of hepatitis E virus transmission. Curr Opin Virol, 4:91–96.
15. Vina-Rodriguez A., Schlosser J., Becher D., Kaden V., Groschup M.H., Eiden M., (2015). Hepatitis E virus genotype 3 diversity: phylogenetic analysis and presence of subtype 3b in wild boar in Europe. Viruses. 7, 2704–2726.
16. Zele D., Barry A.F., Hakze-van der Honing R.W., Vengust G., van der Poel W.H., (2016). Prevalence of Anti-Hepatitis E Virus Antibodies and First Detection of Hepatitis E Virus in Wild Boar in Slovenia. Vector Borne Zoonotic Dis. 16, 71–74.