

# Food Safety Risks Associated with Hepatitis E Virus Detection in Wild Boar Liver

Adriana ANITA<sup>1\*</sup>, Daniela POREA<sup>1</sup>, Andreea COZMA<sup>1</sup>, Dragos ANITA<sup>1</sup>, Gheorghe SAVUTA<sup>1</sup>

<sup>1</sup>Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Iași, Romania  
\*corresponding author: aeanita@uaiasi.ro

Bulletin UASVM Food Science and Technology 76(2)/2019  
ISSN-L 2344-2344; Print ISSN 2344-2344; Electronic ISSN 2344-5300  
DOI: 10.15835/buasvmcn-fst: 2019.0004

---

## Abstract

Hepatitis E virus (HEV) has significantly impacted humans due to its potential to cause acute viral hepatitis. Discovery of hepatitis E virus in domestic pigs and wild boars worldwide and the realization that it is highly prevalent, raised concerns of the implications for food-borne transmission of HEV in Europe. Present work focusses on molecular detection of hepatitis E virus in wild boar liver samples, underlining the possible role of wildlife as a source of HEV transmission to humans. During hunting season 2016-2017, liver samples were collected from 37 wild boars in Iași and Suceava County. All tissues samples were submitted for RNA isolation followed by nested RT-PCR. Genetic characterization of wild boar HEV targeted the structural gene in the ORF2 region of hepatitis E virus genome. After specific amplification by nested RT-PCR of a 348 nt fragment from HEV ORF2, five liver samples positive for hepatitis E virus genotype 3 RNA were identified. In the present study HEV detection in Romanian fresh liver from wild boars highlights the importance of swine as a possible source of foodborne transmission. Moreover, our results along with the reviewed literature data emphasize the necessity of efficient food safety control measures implementation.

**Keywords:** hepatitis E virus, food-borne transmission, wild boar

---

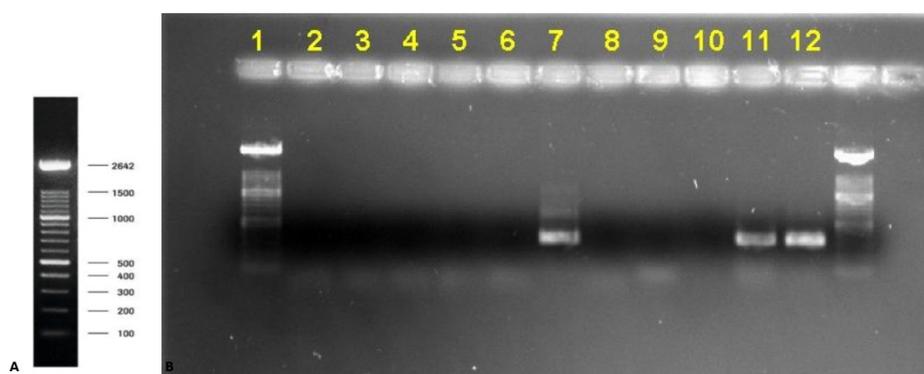
## Introduction

Hepatitis E virus (HEV) is an important public health concern in many industrialized countries. HEV is a positive-stranded RNA virus that belongs to the family *Hepeviridae*, which is divided into two genera: *Orthohepevirus* (all mammalian and avian hepatitis E virus isolates) and *Piscihepevirus* (cutthroat trout virus). Species within the genus *Orthohepevirus* are designated *Orthohepevirus A* (isolates from human, pig, wild boar, deer, mongoose, rabbit and camel), *Orthohepevirus B* (isolates from chicken), *Orthohepevirus C* (isolates from rat, greater bandicoot, Asian musk shrew, ferret and mink) and *Orthohepevirus D* (isolates from bat). To date, *Orthohepevirus A* genus comprises seven genotypes that infect humans (HEV-1, -2, -3, -4 and -7), pigs (HEV-3 and -4), rabbit (HEV-3), wild boar (HEV-3, -4, -5

and -6), mongoose (HEV-3), deer (HEV-3), yak (HEV-4) and camel (HEV-7) (Smith et al., 2016). Hepatitis E virus is a hepatotropic virus endemic in Europe, where it infects humans and animals. Pigs and wild boars are considered to be the main HEV reservoirs (Adlhoch et al., 2016; Wang et al., 2018). Discovery of hepatitis E virus in domestic and feral pigs worldwide and the realization that it is highly prevalent, raised concerns of the implications for food-borne transmission of HEV in Europe (EFSA, 2017; Weigand et al., 2018). Foodborne transmission associated with HEV genotype 3 is and has been linked to ingestion of raw or undercooked meat, liver and liver sausages from infected wild animals (Rivero-Juarez et al., 2017). This calls for an increased public awareness of the zoonotic risk of HEV infection through food

**Table 1.** Sequence characteristics of primers used in this study

Reaction	Primer	Sequence	Target region	PCR product
PCR 1	3156N	5'-AATTATGCC(T)CAG TAC(T) CGG(A)GTTG-3'	5711 5732	730 nt
	3157N	5'-CCCTTA(G)TCC(T)T GCTGA(C) GCATTCTC-3'	6419 6441	
PCR 2	3158N	5'-TT(A)ATGCTT(C)TGC ATA(T)CATGGCT-3'	5996 6017	348 nt
	3159N	5'-AGCCGACGAAATCA ATTCTGTC-3'	6322 6343	



**Figure 1.** Gel image showing nested RT-PCR results . (A) - DNA Molecular Weight Marker XIV (Roche); (B) Position 1 - DNA Molecular Weight Marker; Position 2 to 11 – wild boar samples tested; Position 12 – positive control (348 bp).

consumption or contact with infected animal populations.

Present work focusses on molecular detection of hepatitis E virus in wild boar liver samples, underlining the high importance of this animal species in the epidemiology of HEV and the possible risk of foodborne transmission.

### Materials and Methods

During hunting season 2016-2017 (August 2016- February 2017), liver samples were collected from 37 wild boars in Iași (n=21) and Suceava Counties (n=16). All samples were obtained in accordance with the Directive 2010/63/EU of the European Parliament and of the Council. Samples were transported under chilled condition (4-8°C) and stored at -80°C until testing. All tissues samples were submitted for RNA isolation using RNeasy Mini kit (Qiagen, ref. 52906) according to the manufacturer's protocol. Molecular detection of wild boar HEV consisted in detection of an ORF2 sequence by nested reverse transcription PCR using degenerate primers for genotype 3 hepatitis E virus genome (two sets of primers:

3156-3157 and 3158-3159) described by Cooper *et al.*, (2005) (Table 1.).

### Results and discussions

Animals and humans are the sources of HEV, foodstuffs are vehicles within a transmission pathway. Food-borne transmission may be due to either raw or undercooked products, made of liver and meat from infected domestic animals and game, or food products contaminated with HEV. Consumption of wild boar liver and liver products (pate and sausages) was identified as a potential factor for HEV infection in Europe (Pavio *et al.*, 2010).

In the present study, specific amplification by nested RT-PCR of a 348 nt fragment targeting HEV capsid protein, resulted in identification of five out of thirty-seven wild boar liver samples positive for hepatitis E virus genotype 3 RNA (Figure 1.). Our previous research on hepatitis E infection in wild boars highlighted a seroprevalence of 11,1% (2 out of 18) in Iași County (Porea *et al.*, 2015). Moreover, molecular investigations undertaken during 2013-2015 on wild boars from Suceava County revealed

the presence of HEV genotype 3 in 23.33% (7 out of 30) liver samples (Porea et al., 2018). Previous and present data suggest that HEV genotype 3 is circulating in wildlife in Eastern Romanian Counties. In Europe, hepatitis E virus RNA has been detected in wild boars in Italy (Aprea et al., 2018), Spain (Rivero-Juarez et al., 2017), Germany (Anheyer-Behmenburg et al., 2017), France (Jori et al., 2016) and in several Eastern European countries (Slovenia, Hungary and Czech Republic). Moreover, autochthonous human HEV infections in industrialized countries (due to genotype 3) are increasingly reported and are linked to zoonotic transmission, mainly through the consumption of contaminated meat and offal from swine (Doceul et al., 2016). Hepatitis E virus has been reported in food of animal origin at slaughterhouse and point of sale, respectively products containing raw liver were frequently found HEV-RNA-positive (EFSA, 2017). Cumulative molecular and epidemiological evidence from European countries where wild boars are prevalent suggests that HEV infection is common and poses a zoonotic hazard for hunters and consumers of wild boar meat (Pavio et al., 2017).

There are significant gaps in knowledge regarding HEV survival in foods and environment (including food contact surfaces), and also regarding the effect of elimination procedures used in food supply chain settings (EFSA, 2017). For taste and other sensory reasons, inadequately cooked swine livers are preferred by some consumers. In order to minimize the risk of HEV infection, consumers should thoroughly cook meat and offal, especially wild boar meat products (EFSA, 2017). To prevent hepatitis E, food trade and consumers should cook food thoroughly. For sliced wild boar liver, depending on thickness and quantity, is recommended to boil at 100°C or stir-fry in hot skillet/wok for at least three to five minutes (EFSA, 2017). In addition, food trade personnel and consumers are also advised to implement good personal and food hygiene practices.

### Conclusions

Wild boars (*Sus scrofa*) are probably the main natural reservoir of hepatitis E virus and could therefore represent an important route of transmission in Europe, especially in regions where game meat is widely consumed. High level

of viral shedding may be observed in the faeces, liver and bile of infected animals and a lower level, in meat. This could represent a possible vehicle for cross-contamination during slaughter, evisceration and food processing, including home processing. High virus loads and prolonged viral shedding encountered in infected wild boars constitute an important HEV threat.

In the present study, the detection of hepatitis E virus genotype 3 in fresh liver from Romanian wild boars highlights the importance of swine as HEV reservoir. Moreover, our results along with the reviewed literature data emphasize the necessity of monitoring the presence of hepatitis E virus in pork-derived foods.

### References

1. Adlhoc C, Avellon A, Baylis S A, Ciccaglione A R, Couturier E, De Sousa R, Epstein J, Ethelberg S, Faber M, Feher A, Ijaz S, Lange H, Mandakova Z, Mellou K, Mozalevskis A, Rimhanen-Finne R, Rizzi V, Said B, Sundqvist L, Thornton L, Tosti M E, van Pelt W, Aspinall E, Domanovic D, Severi E, Takkinen J, Dalton H R. (2016). Hepatitis E virus: assessment of the epidemiological situation in humans in Europe, 2014/15. *J Clin Virol* 82, 9–16. doi: 10.1016/j.jcv.2016.06.010
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. *Emerg Infect Dis*, 23(1), 130-133. doi: 10.3201/eid2301.161169
3. 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, (2018). Molecular detection and phylogenetic analysis of hepatitis E virus strains circulating in wild boars in south-central Italy. *Transbound Emerg Dis*, 65(1), e25-e31. doi: 10.1111/tbed.12661
4. Cooper K, Huang F F, Batista L, Rayo C D, Bezanilla J C, Toth T E, Meng X J, (2005). Identification of genotype 3 hepatitis E virus (HEV) in serum and fecal samples from pigs in Thailand and Mexico, where genotype 1 and 2 HEV strains are prevalent in the respective human populations. *J Clin Microbiol*, 43(4), 1684-8. doi: 10.1128/JCM.43.4.1684-1688.2005
5. Doceul V, Bagdassarian E, Demange A, Pavio N, (2016). Zoonotic Hepatitis E Virus: Classification, Animal Reservoirs and Transmission Routes. *Viruses*. 8(10), 270. doi: 10.3390/v8100270
6. EFSA Panel on Biological Hazards (BIOHAZ), Ricci A, Allende A, Bolton D, Chemaly M, (2017). Public health risks associated with hepatitis E virus (HEV) as a foodborne pathogen. In: *EFSA Journal*, 15 (7), 4886 [1-89]. doi: 10.2903/j.efsa.2017.4886
7. Jori F, Laval M, Maestrini O, Casabianca F, Charrier F, Pavio N, (2016). Assessment of domestic pigs, wild boars and feral hybrid pigs as reservoirs of hepatitis E virus in

- Corsica, France. *Viruses*. 8(8) pii: E236. doi: 10.3390/v8080236
8. Pavio N, Meng X J, Renou C, (2010). Zoonotic hepatitis E: Animal reservoirs and emerging risks. *Veterinary Research*, 41(6). 41(6):46. DOI: 10.1051/vetres/2010018
  9. Pavio N, Doceul V, Bagdassarian E, John R. (2017). Recent knowledge on hepatitis E virus in Suidae reservoirs and transmission routes to human. *Vet Res*. 21, 48(1), 78. doi: 10.1186/s13567-017-0483-9
  10. Porea D, Anița A, Paslaru A, Savuta G, (2015). Serological evidence of wild boar hepatitis E infection in three counties from Eastern Romania. *Facultatea de Medicină Veterinară, Timișoara, România, Lucrări Stiintifice - USABT, Medicina Veterinara, XLVIII (3): 174-178.*
  11. Porea D, Anita A, Demange A, Raileanu C, Oslobanu Ludu L, Anita D, Savuta G, Pavio N, (2018). Molecular detection of hepatitis E virus in wild boar population in eastern Romania. *Transbound Emerg Dis*, 65(2), 527-533. doi: 10.1111/tbed.12736
  12. Rivero-Juarez A, Frias M, Martinez-Peinado A, Risalde M A, Rodriguez-Cano D, Camacho A, García-Bocanegra I, Cuenca-Lopez F, Gomez-Villamandos J C, Rivero A, (2017). Familial Hepatitis E Outbreak Linked to Wild Boar Meat Consumption. *Zoonoses Public Health*; 64(7), 561-565. doi: 10.1111/zph.12343.
  13. Smith D B, Simmonds P, Izopet J, Oliveira-Filho E F, Ulrich R G, John R, Koenig M, Jameel S, Harrison T J, Meng X J, Okamoto H, Van der Poel W H, Purdy M A, (2016). Proposed reference sequences for hepatitis E virus subtypes. *J Gen Virol*. 97(3), 537-42. doi: 10.1099/jgv.0.000393.
  14. Wang H, Castillo-Contreras R, Saguti F, López-Olvera J R, Karlsson M, Mentaberre G, Lindh M, Serra-Cobo J, Norder H, (2018). Genetically similar hepatitis E virus strains infect both humans and wild boars in the Barcelona area, Spain, and Sweden. *Transbound Emerg Dis*, 31. doi: 10.1111/tbed.13115.
  15. Weigand K, Weigand K, Schemmerer M, Müller M, Wenzel J J, (2018). Hepatitis E Seroprevalence and Genotyping in a Cohort of Wild Boars in Southern Germany and Eastern Alsace. *Food Environ Virol*;10(2), 167-175. doi: 10.1007/s12560-017-9329-x