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First Detection of Hepatitis E Virus (*Rocahepevirus ratti* Genotype C1) in Synanthropic Norway Rats (*Rattus norvegicus*) in Romania

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Introduction

Hepatitis E virus (HEV) is an emerging zoonotic pathogen with different viral genera and species reported in a wide range of animals. Rodents, particularly rats, carry the specific genus rat HEV (*Rocahepevirus* genus, genotype C1) and are exposed occasionally to HEV-3 (*Paslahepevirus* genus, genotype 3), a zoonotic genotype identified in humans and widely distributed in domestic and feral pigs.

The detection of rat HEV in humans with acute or chronic hepatitis and the worldwide distribution of rat HEV in rodents suggest that rat HEV infection may be an emerging disease. The role of rats in the transmission of the zoonotic HEV-3 genotype, the most commonly detected genotype in humans worldwide, the main animal reservoir of which is domestic pigs, also needs to be clarified.

In this study, the presence of HEV was investigated in synanthropic Norway rats (*Rattus Norvegicus*) from Eastern Romania, in areas where the presence of HEV-3 was previously reported in pigs, wild boars and humans.

Materials & Methods

Using methods capable of detecting different HEV species, the presence of HEV RNA was investigated in 69 liver samples collected from 52 rats and other animal species, originated in different counties in Eastern Romania (Figure 1).

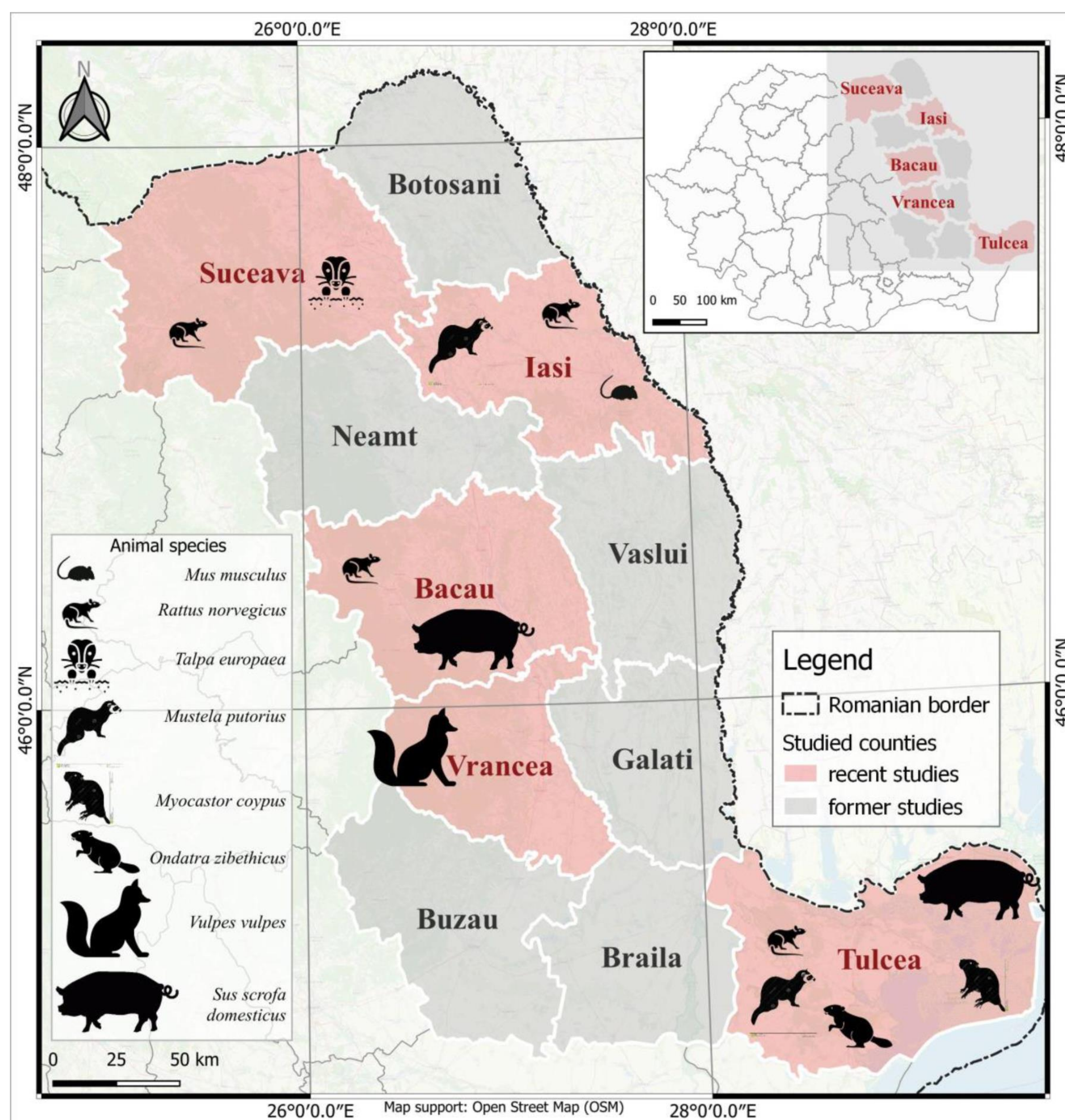


Figure 1. Geographical distribution of the counties included in the study, with the representation of the analyzed species.

Rats were trapped at various locations near human settlements and animal farms (Table 1). In 2017, 10 pool fecal samples were collected from two indoor pig farms (*Sus scrofa domestica*) where rats were trapped in Tulcea and Bacau counties.

Table 1. Data on the samples from rats

No.	Location/Site	Number of Rats/Location/Counties			
		Suceava	Iasi	Bacau	Tulcea
1	Intensive system pig farm	-	-	-	12
2	Semi-intensive system pig farm	5	-	11	-
3	Extensive system pig farm	1	-	5	-
4	Farm with different animal species, forestry site	10	-	-	-
5	Cattle farm, metropolitan area	-	1	-	-
6	Urban settlements, near garbage cans	-	5	-	-
7	Rural settlements, population's household	-	-	-	2
Total		16	6	16	14

For HEV RNA detection, three different assays were used:

- . A TaqMan real-time RT-PCR assay (Jothikumar et al., 2006; Barnaud et al., 2012), and a RT-nested PCR specific for HEV genotypes 1-4 (Cooper et al, 2005; Bouquet et al., 2011),
- . A nested broad-spectrum RT-PCR (NBS-RT-PCR) for hepeviruses, including rat HEV (Johne et al., 2010).

Results

Nine rat liver samples were identified as being positive for rat HEV RNA (17.3%). Positive rats originated from each analyzed county and from four of the seven sites studied, indicating a broad geographical distribution of rat HEV in this species in the study area. All samples tested from other animal species, within the same environment, were negative for HEV RNA (Table 2).

Table 2. Molecular detection of HEV genome.

Species	Type of Samples	County				
		Suceava	Iasi	Bacau	Tulcea	Vrancea
Wild						
<i>Rattus norvegicus</i>		3/16 (3/10 ⁴)	1/6 (1/1 ⁵)	2/16 (2/11 ²)	3/14 (3/12 ¹)	-
<i>Vulpes vulpes</i>	Liver	-	-	-	-	0/9
<i>Mustela putorius</i>		-	0/1	-	0/3	-
<i>Mus musculus</i>		-	0/1	-	-	-
<i>Talpa europaea</i>		0/1	-	-	-	-
<i>Myocastor coypus</i>		-	-	-	0/1	-
<i>Ondatra zibethicus</i>		-	-	-	0/1	-
Domestic						
<i>Sus scrofa domestica</i>	Pool faecal	-	-	-	0/10	-

1, 2, 4, 5 Numbers corresponding to the different settings indicated in Table 1 (column 1).

High sequence identity (85–89% nt) was found with other European *Rocahepevirus*. The nucleotide identity between our isolates ranged from 85% to 91%, and phylogenetic analysis showed that they were clustered by county. These sequences clustered within the proposed subtype GI, but are more distant from those of Southern Europe such as Spain and may constitute a new European clade or subtype. The analysis of complete genomes would improve this comparison. As rat populations may have followed trade routes, the identification of other sequences from different countries would contribute to drawing a possible map of rat HEV virus evolution and spread.

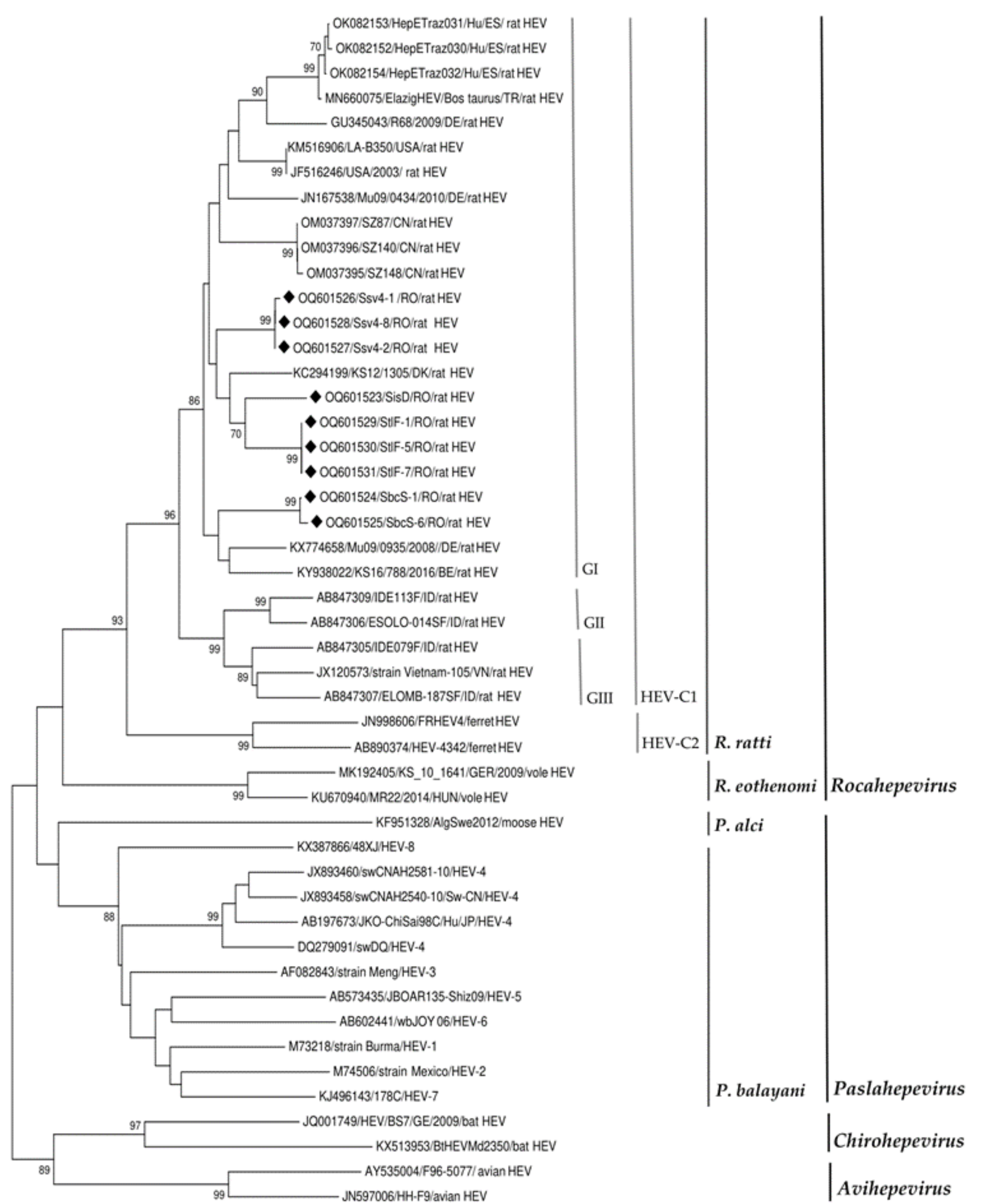


Figure 2. Phylogenetic tree based on the alignment of the generated sequences with the best hits to our sequences in GenBank and with reference sequences for the members of the subfamily *Othohepevirinae* (the rat HEV strains obtained in our study are highlighted by black diamonds).

Conclusions

The first detection and molecular characterization of rat HEV in Romania represent the novelty of the present study.

The results of the molecular investigations regarding the presence of hepatitis E virus in rats allowed the classification of the isolated strains into the rat HEV genotype and indicated the presence of a new source of human infections in the study area.

To better assess the role of rats in the epidemiology of HEV infection in humans, it is recommended to screen for rat HEV in humans with a suspicion of hepatitis. (Porea et al 2023, Viruses)