HEPATITIS E VIRUS IN SHEEP IN ITALY

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BACKGROUND

Hepatitis E virus (HEV) is the leading cause of enterically-transmitted viral hepatitis. A global burden of disease study estimated that HEV accounts for approximately 20.1 million incident HEV infections, 3.4 million cases of symptomatic disease, 70,000 deaths, and 3,000 stillbirths (6). Pigs, wild boars and deer are recognized as the main reservoirs for genotype (Gt) 3 and Gt4 infections, although several additional animal species may act as HEV hosts, including domestic ruminants (1, 7, 8). Considering the importance of sheep as livestock animals in Southern Italy, in this study we investigated serologically and molecularly the epidemiology of HEV in sheep.

MATERIAL AND METHODS

Between January and June 2018, serum and faecal specimens were collected from 192 clinically healthy sheep older than 6 months from seven small farms located in the province of L'Aquila (Abruzzo Region, Italy). Serological investigation for HEV infection was carried out testing all serum samples with a species-independent commercial double-antigen sandwich ELISA kit (Wantai Biological, Beijing, China). Total RNA was extracted individually from each serum and faecal specimen and analysed by HEV-specific real-time reverse transcription PCR (qRT-PCR) (3). Amplification of RNA for sequencing was attempted on all the samples positive by qRT-PCR, using a broadly reactive nested RT-PCR targeting a region of 345 bp of the ORF2 gene (4).

RESULTS

Antibodies against HEV were detected in a total of 41 sera with an overall prevalence of 21.3% (41/192; 95% CI 15.2-26.7%). All 7 farms were seropositive for HEV with rates ranging from 6.6% to 38.3% (**Tab.1**). HEV RNA was detected in 20/192 faecal samples (10.4%) and in six out of seven farms investigated with rates ranging from 5.5% to 26.3%. Upon sequence analyses of eight HEV positive samples (GenBank accession no. MH719221-MH719228), all the ovine strains shared 94.4-100% nucleotide (nt) identity to each other and displayed the highest identity to the goat and wild boar Gt3 HEV strains, previously detected in Abruzzo region (1, 2). Also, high identity (87.7-89.9%) was found to a human HEV strain, Hu/771-2/IT, identified in a blood donor from province of L'Aquila in 2014 (5). After phylogenetic analysis, the Italian sheep strains segregated into the Gt3 subtype c, together with other HEV sequences identified in humans and animals, including those detected in Abruzzo region during 2014-2016 (**Fig.1**).

Positive/Total (%)				
Sheep farm	No. animals tested	HEV antibiodies	Real time RT-PCR	Nested RT-PCR
AQ1	60	23/60 (38.3)	5/60 (8.3)	2/60 (3.3)
AQ2	12	2/12 (16.6)	3/12 (25.0)	2/12 (16.6)
AQ3	18	2/18 (11.1)	1/18 (5.5)	1/18 (5.5)
AQ4	19	3/19 (15.7)	5/19 (26.3)	2/19 (10.5)
AQ5	35	4/35 (11.4)	5/35 (14.3)	1/35 (2.8)
AQ6	30	2/30 (6.6)	0/30 (0)	N.I.
AQ7	18	4/18 (22.2)	1/18 (5.5)	0/18 (0)
Total	192	40/192 (21.3)	20/192 (10.4)	8/192 (4.2)

N.I. not investigated.

Tab.1 Serological and molecular prevalence of HEV in seven sheep farms in province of L'Aquila (Abruzzo, Italy)

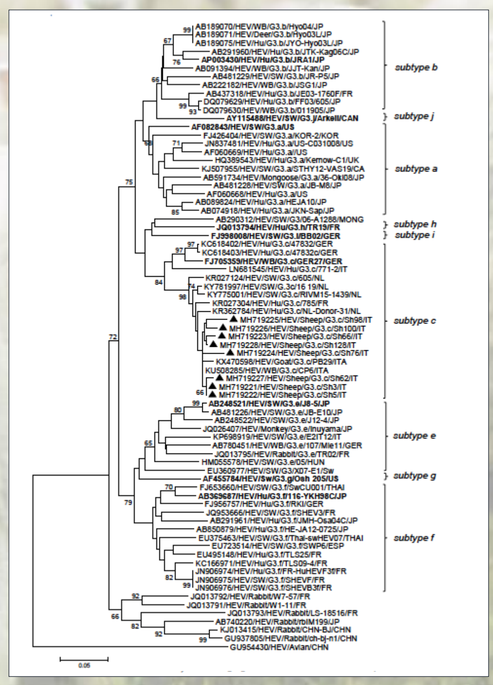

Fig.1 Phylogenetic tree constructed on the 0.3 kb at the 5' end of the ORF2 gene of the sheep strains detected in this study

CONCLUSIONS

The results obtained in this study provide firm evidence for the presence of Gt3 HEVs in sheep in Italy. These findings, along with the previous detection of HEV in goats (1), underline the importance of small domestic ruminants in the epidemiology of HEV. Finally, the circulation of highly genetically similar strains among sheep, goats, wild boars and humans suggests that multiple inter-species transmission of HEV might be occurring in the surveyed area.

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