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Short communication

Detection of hepatitis E virus (HEV) in goats

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ABSTRACT

Hepatitis E virus (HEV) is a major cause of acute hepatitis worldwide. Genotypes 1 and 2 are restricted to humans, whereas genotypes 3 and 4 also occur in animals and are recognized as zoonotic pathogens. In this study, by screening goat faecal samples collected from six small farms located in the province of Teramo (Abruzzo region, Italy), HEV RNA was found with an overall prevalence of 9.2% (11/119). Upon sequence analysis of a 0.8 kb portion of the ORF2 gene, four strains were grouped with animal and human genotype 3 HEVs, subtype c, with the highest match (94.2–99.4% nt identity) to a wild boar strain, WB/P6-15/ITA, identified in the same geographical area in which the six goat farms were located. Further investigations are needed in order to assess if goat may represent an additional active host for HEV.

Hepatitis E virus (HEV) is the leading cause of enterically-transmitted viral hepatitis. A global burden of disease study in 2005, 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 (Rein et al., 2012).

HEV is a small, non-enveloped, positive-sense, single-strand RNA virus classified in the genus *Orthohepevirus*, family *Hepeviridae*. Based on full-length genome analysis, HEV strains infecting humans are classified into 4 major genotypes (Gt) within the species *Orthohepevirus A* (Smith et al., 2014). Gt1 and Gt2 are endemic in developing countries and restricted to humans, where they are predominantly transmitted through the faecal–oral route, either indirectly through contaminated drinking water or food. Gt3 and Gt4 infect humans and animals and are responsible for sporadic cases of autochthonous human hepatitis E in industrialized countries (Kamar et al., 2012). Also, more recently a Gt7 strain (camelid HEV) has been detected in a liver transplant recipient (Lee et al., 2016).

The accumulating literature indicates that human infections by Gt3 and Gt4 HEVs are due to consumption of raw or undercooked pork or game meat, thus raising public health concerns about the zoonotic transmission of HEV (Meng, 2010). Pigs, wild boars and deer are recognized as the main reservoirs for Gt3 and Gt4 infections, although molecular evidence indicate that several additional animal species may act as HEV hosts, including rabbits, yaks, sheep and cattle (Cossaboom et al., 2011; Xu et al., 2014; Wu et al., 2015; Huang et al., 2016). Also, IgG specific antibodies against HEV have been identified in cats (Peralta et al., 2009), dogs (Arankalle et al., 2001; McElroy et al., 2015) and in farmed animals, such as goats (Peralta et al., 2009; Sanford et al., 2013) emphasizing the need to further investigate the epidemiological relevance of these findings. In this study, by screening stools from clinically healthy goats, we found viruses genetically related to Gt3 HEVs.

A total of 119 faecal samples from adult goats older than 6 months of age were collected between May 2015 and February 2016 from six small farms located in an area of approximately 415 km^2 in the province of Teramo (Abruzzo region, Italy). The flock size ranged from 10 to 50 animals. Goats from all the flocks were bred for both milk and meat and were grazed freely on pastures. Faecal specimens were placed in isothermal boxes using ice bags and transferred in the lab. Samples were kept frozen at −80 °C until tested.

The RNA was extracted from 200 μl of 10% (wt/vol) faecal suspension by using the TRIzol LS (Invitrogen, Ltd, Paisley, UK) procedure. The final RNA pellet was resuspended in 50 μl of RNase free water and used directly in RT-PCR assays. The RNA extracts were screened using the consensus primer sets ConsORF1 and ConsORF2 (Wang et al., 1999) targeting respectively a 287-bp fragment of the methyltransferase region (ORF1) and a 145-bp portion of the ORF2 gene, and the primers Cs/Cas and Csn/Casn (Johne et al.,

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Table 1
Nucleotide identities in the partial RdRp region of the strains detected in goats with HEV strains genetically closest.

Strain	GenBank Accession no.	% identity range
HEV/WB/P6-15/ITA	KU508285	97.4–100
HEV/Hu/NL0085/NL	KC171451	93.3–95.0
HEV/Hu/NL0065/NL	KC171449	91.7–92.5
HEV/Hu/NL0051/NL	KC171446	92.5–93.3
HEV/Hu/NL2008/NL	JQ015412	93.1–94.6
HEV/Hu/NL2009/NL	JQ015422	93.2–94.1
HEV/Sw/43-15/GER (raw sausage)	KR935764	91.6–92.4
HEV/Sw/51-15/GER (raw sausage)	KR935768	92.8–94.3
HEV/Sw/43-15/GER (liver sausage)	KR935761	90.4–91.3

Table 2
List of primers used in this study. Nucleotide position refers to the sequence of the Gt3 subtype c prototype strain wbGER27 (GenBank accession no. FJ705359).

Oligonucleotide	Position	Sequence (5′ to 3′)	Sense	Reference
HEV-ORF1con-s1	54-77	CTGGCATYACTACTGCYATTGAGC	+	Wang et al. (1999)
HEV-ORF1con-a1	449–471	CCATCRARRCAGTAAGTGCGGTC	–	Wang et al. (1999)
HEV-ORF1con-s2	102–122	CTGCCYTKGCGAATGCTGTGG	+	Wang et al. (1999)
HEV-ORF1con-a2	365–388	GGCAGWRTACCARCGCTGAACATC	–	Wang et al. (1999)
HEV-ORF2con-s1	6323–6346	GACAGAATTRATTTCGTCGGCTGG	+	Wang et al. (1999)
HEV-ORF2con-a1	6495–6519	CTTGTTCRTGYTGGTTRTCATAATC	–	Wang et al. (1999)
HEV-ORF2con-s2	6372–6393	GTYGTCTCRGCCAATGGCGAGC	+	Wang et al. (1999)
HEV-ORF2con-a2	6492–6516	GTTCRTGYTGGTTRTCATAATCCTG	–	Wang et al. (1999)
HEV-cs	4181–4203	TCGCGCATCACMTTYTTCCARAA	+	Johne et al. (2010)
HEV-cas	4628–4650	GCCATGTTCCAGACDGTRTTCCA	–	Johne et al. (2010)
HEV-csn	4286–4311	TGTGCTCTGTTTGGCCCNTGGTTYCG	+	Johne et al. (2010)
HEV-casn	4592–4617	CCAGGCTCACCRGARTGYTTCTTCCA	–	Johne et al. (2010)
HEV-5376	5376–5395	CAATCCGGGGCTGGAGCTCG	+	This study
HEV-5469	5469–5488	CGTCGATCTGCCCCAACTGG	+	This study
HEV-6260	6260–6279	AGCAATACCACGGCCCACCT	–	This study

2010), which amplify a 334-bp fragment of the highly conserved region of the RNA-dependent RNA polymerase (RdRp) in the ORF1 gene. RT and PCR were performed in one-step procedure, using the SuperScript III One-Step RT-PCR System kit (Invitrogen, Ltd., Paisley, UK). Thirty-five cycles of 94 °C for 1 min, 50 °C for 45 s and 72 °C for 1 min, with a final extension of 72 °C for 10 min, were used for amplification. Second-round reactions were carried out in a total volume of 50 μl of GoTaq Hot Start Master Mix (Promega, Italy) with 1 μl of first-round product and 25 pmol of each primer. Thermal conditions were the same as the first round except that only 30 cycles of amplification were carried out. By using the primers Cs/Cas and Csn/Casn, HEV RNA was found in a total of 11 specimens with an overall prevalence of 9.2% (11/119) and in 5 out of 6 farms investigated (83.3%). Out of 11 positive samples, two were also positive when screened with the primer sets ConsORF1 and ConsORF2. Partial RdRp sequences were determined from the HEV positive samples and deposited in GenBank under the accession numbers KX470586-KX470596.

Upon sequence analyses, all the strains shared 97.4-100% nucleotide (nt) identities to each other and displayed the highest identity to a wild boar Gt3 HEV strain, WB/P6-15/ITA (KU508285), detected recently in Italy (97.0–100% nt identity) (Di Profio et al., 2016) and to other Gt3 strains identified in humans and in pig sausages (Table 1).

A genome region of ~800-nt, encompassing the 5′ end of the ORF2 gene, was determined for four strains (GenBank accessions KX470597-KX470600) by using newly consensus primers (Table 2) designed on the bases of the sequences obtained in this study and the corresponding conservative regions of HEVs available on NCBI website. Tree was generated using the Neighbor-joining method and the *p*-distance model with Geneious software package vers. 9 (Biomatters, New Zealand, http://www.geneious.com), supplying a statistical support with bootstrapping of 1000 replicates. Phylogenetic analysis was inferred with a selection of strains representative of the Gt3 HEV, including the reference sequences of each subtype group (http://talk.ictvonline.org) (Smith et al., 2016). In the partial capsid-based tree, the four strains segregated within the clade 3abchij, with the highest identity (89.6–99.4% nt) to wild boar and human HEVs assigned to subtype c (Fig. 1).

Altogether, these findings provide firm evidence for the circulation of Gt3 HEVs in goats in Abruzzo region (Italy), although it remains to be clarified if these viruses are commonly harbored in this animal species or incidentally transmitted from other mammals acting as HEV reservoirs. Susceptibility of goats to HEV has been assessed in several serological investigations. HEV antibodies have been detected in goats in Spain (Peralta et al., 2009), Egipt (El-Tras et al., 2013), eastern China (Zhang et al., 2008) and USA (Sanford et al., 2013) with prevalence rates ranging from 0.6% to 24.0%, while they were not found in goats from India (Arankalle et al., 2001) and from the northeast of China (Wang et al., 2002).In a study conducted in Virginia (Sanford et al., 2013) IgG antibodies against HEV were detected in 16% (13/80) of goat sera screened by using an antigen-based immune-enzyme assays. Of interest, the majority of the seropositive animals had neutralizing antibodies to the human HEV Gt1 Sar-55, suggesting that the strain infecting goats was antigenically related to human HEVs. However, attempts to infect goats experimentally with HEV strains Sar-55 (Gt1), Meng (Gt3) and TW6196E (Gt4) were unsuccessful (Sanford et al., 2013). Intriguingly, in the 0.8 kb portion of the ORF2 gene the caprine HEV strains exhibited the closest nt identity (94.2–99.4%) to a wild boar strain, WB/P6-15/ITA, identified (Di Profio et al., 2016) in the same geographical area in which the six goat farms were located and where the density of free-ranging wild boar population is high. The circulation of similar HEVs in different animal hosts in the same geographical areas have been previously observed. The high sequence identity (93.8%–99.1% nt identity) among yak (KF736234), swine (JU119961) and human (JQ740781) HEV strains detected in Northwestern China (Xu et al., 2014) demonstrated a cross-species transmission cycle for HEV genotype 4 in the regions investigated. Also, in a molecular survey in Hokkaido (Japan), HEVs genetically similar to swine Gt3 strains (95.2-100%) have been identified in Norway rats (Rattus norvegicus) caught near the