

Detection of Hepatitis E Virus Antibodies in Domestic and Wild Animal Species in Central Italy

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Abstract

Hepatitis E virus (HEV) is known for its zoonotic potential. Although several mammalian species have been indicated as possible viral reservoir, the host range of the infection is partially defined. In this work serum samples collected from wild brown hares, red deer, wild rabbits, cattle living in semi-wild state and wild boar-hunting dogs were tested by a multi-species ELISA assay. Only sera from red deer (5.6%), wild rabbit (38.5%) and wild-boar hunting dogs (14.3%) scored positive. The investigation indicated the circulation and the high endemicity of HEV in various animal species in Central Italy, and the importance that these species can play in the epidemiology of infection.

Keywords: ELISA; Hepatitis E virus, Italy; Serology; Zoonosis

Introduction

Hepatitis E virus (HEV) is a well-recognised emerging pathogen distributed worldwide [1]. HEV is a small icosahedral non-enveloped and single-stranded positive-sense RNA virus. It has been designated as the sole member of the genus *Hepevirus* in the family Hepeviridae on account of its unique genomic organization [2].

The HEV genome is about 7.2 kb long and it contains 3 open reading frames (ORFs) as well as 5' and 3' untranslated regions. According to genomic differences HEV has been organized in 4 different genotypes and 1 serotype. Genotype 1 and 2 are isolated in humans while genotype 3 and 4 are zoonotic agents associated with sporadic outbreaks of HEV worldwide [3,4].

Several aspects regarding HEV biology have led to the hypothesis that more than an animal reservoir could be involved in the epidemiology of the virus. Interspecies transmission of HEV has been demonstrated by experimental infection and by genetic analysis of HEV positive samples of human and various animal hosts [5-7]. Although a high seroprevalence is found in pigs and wild-boar, other animal species such as rat, deer, dog, sheep, goat, rabbit, and cattle can also be considered as virus host species [8-11]. Due to the zoonotic potential of HEV it is essential to determine the full host range of the virus and the seroprevalence rate among wildlife animals, in order to control the spreading of the disease to domestic animals and exposed humans.

The purpose of our study was to investigate the presence of anti-HEV antibodies in various wild and domestic animal species in Central Italy, an endemic area for wild-boar HEV infection [12-14].

Materials and Methods

In this study 214 sera belonging to wild brown hares (103), red deer (54), wild-boar hunting-dogs (35), wild rabbits (12) and cattle (10) were tested for the detection of HEV antibodies by multi-species ELISA kit (HEV Ab Version ULTRA, DIA.PRO Milano, Italy). The sera were collected from various sites and all belonging to a large geographical area located in Central Italy (Tuscany), populated by wild-boar with a high seroprevalence rate and with a clear evidence of viral circulation [12-14].

From March to September 2014, serum samples were collected from 54 red deer (40 females, 14 males), captured during the hunting season in the province of Pistoia, in north Tuscany, and from 12 wild rabbits (6 females and 6 males) shot in the province of Pisa in the west Tuscany, during the autumn of 2014.

Sera from 103 wild brown hares (50 females and 53 males) captured by nets from 2011 to 2015 in three different protected areas of the province of Pisa, were also investigated. The animals were stocked in wood cages and blood samples were collected through venipuncture of the saphenous vein prior to their reintroduction.

Blood samples were also collected from ten cattle during 2015 (1 male and 9 females) belonging to a farm where bovine are raised in semi-wild state with the possibility of contact or field sharing with other wild animals. Finally 35 sera from wild boar-hunting-dogs (17 females and 18 males) were collected during 2015 hunting season in the same areas submitted to investigation.

All the sera were classified and stored at -20°C until tested by an ELISA assay following manufacturer's instruction.

The ELISA kit used is designed for the qualitative determination of total antibodies to HEV in serum and plasma samples. Derived by multi-species animals. The principle of the test is based on microplates coated with HEV-specific synthetic antigen. After the capture of anti-HEV antibodies, if present in the sample tested, a second incubation is

performed by the addition of the same HEV highly specific antigen labeled with peroxidase. We considered positive only serum samples having an OD clearly above the assay cut-off value. The kit has a declared diagnostic sensitivity of 100% and an overall value for the diagnostic specificity of >99.5%.

Results and Discussion

A total of 214 sera were examined and seroprevalence from various species was calculated. None of the positive sera resulted equivocal.

The highest seroprevalence rate was detected in wild rabbit samples (38.5%). This result is in agreement with published data recorded in other countries (Table 1) [15-25]. As previously demonstrated by Izopet et al. wild rabbits are one of the hosts for HEV suggesting their zoonotic potential [26]. Considering the high prolific rate of wild rabbits and therefore the high number of susceptible animals that could be constantly infected in a restricted area, their role for the epidemiology of HEV needs to be further investigated.

Animal Species	Seroprevalence from literature*				Seroprevalence in our study	
	Percent	Positive/Total	Country	References	Percent	Positive/Total
Red deer	1.10%	1/84	BE	[15]	5.60%	3/54
	12.85%	9/70	ES	[16]		
	13.90%	35/251	IT	[17]		
Rabbit	57.00%	191/335	CN	[18]	38.50%	5/13
	36%	31/85	US	[19]		
	15.40%	169/1094	CN	[20]		
Hare					0.00%	0/103
Cattle	6%	6/100	CN	[22]	0.00%	0/10
	4.40%	4/91	IN	[11]		
	6.90%	13/188	IN	[11]		
	1.42%	1/70	BR	[22]		
Dog	0.80%	2/247	GB	[23]	14.30%	5/35
	17.80%	18/101	CN	[21]		
	21.12%	139/658	CN	[24]		
	13.50%	26/192	CN	[25]		
	22.70%	10/44	IN	[11]		
	6.97%	3/43	BR	[22]		

*Seroprevalence percentage, number of positive out of sample tested, country and references. (BE: Belgium; ES: Spain; IT: Italy; CN: China; US: United States of America; IN: India; BR: Brazil; GB: United Kingdom)

Table 1: Comparison of seroprevalence rates between data available in literature and the results of our study.

Our study showed a prevalence of 14.3 % in wild-boar hunting-dogs which is also in accordance with similar studies (Table 1). Although the low number of sera examined in our survey does not allow a statistical analysis to determine significant risk of infection studies, the result is an important evidence of canine susceptibility to HEV infection. This aspect could be a veterinary public health problem considering that hunting dogs can easily be infected by direct contact with HEV infected wild boar or by consumption of contaminated material such as wild boar offals. However the zoonotic potential of HEV in dogs is still under discussion.

Finally, our survey shows a HEV seroprevalence of 5.6% in red deer, confirming previous results that indicate deer as one of the main reservoir of the virus in the wildlife [17].

All bovine and wild brown hare's samples resulted negative indicating that so far the virus seems not to circulate in these animals living in the examined area.

In the last decade, an increasing proportion of reported human HEV cases were demonstrated to be autochthonous [27], mainly linked to the ingestion of raw or undercooked meat or viscera of pigs, wild boar and deer [28-30]. Other studies focused the attention about the risk of infection encountered by humans with occupational exposure to pigs [31,32]. Moreover, the presence of seropositive wild animals in the study areas, could constitute a risk of HEV transmission through slaughtering by hunters as demonstrated by other studies [13,33,34]. Particularly intriguing is the high seroprevalence rate recorded in wild-board hunting-dogs, indicating the efficient

transmission of the virus from wild animals to dogs. Although it is rather difficult to understand how exactly this transmission can be achieved, we should consider hunting dogs as a potential zoonotic host for HEV and therefore these animals should be further monitored in order to avoid possible transmission to humans; in particular in owners and veterinarians.

In conclusion our data confirm that HEV infection has a wide distribution with different seroprevalence rates depending on the affected animal species and they reveal that HEV can easily circulate and spread in a Mediterranean ecosystem such as Central Italy.

Acknowledgment

Sera samples were taken for diagnostic purposes from hares captured in protected areas for further restocking of hunting areas and from legally hunted animals (red deer and wild rabbits), and from cattle raised in semi-wild state. Wild boar-hunting dog sera samples were further collected during veterinary cares in accordance with the ethical principles of animal experimentation as approved by the Ethical Committee "Unità etica e tutela animale della ricerca" Pisa University; prot N° 23507. This work was funded by the Provincia of Pisa. We would thank all the hunters and the wildlife rangers of the sampled areas for their precious help in the field's activities and sampling.

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