SHORT COMMUNICATION

Prevalence of hepatitis E virus antibodies in pigs in Northern Italy

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The prevalence of the hepatitis E virus (HEV) infection in pigs in Northern Italy was serologically examined. The survey was carried out on 39 farms: 17 farrow-to-feeder, 10 farrow-to-finish, and 12 fattening enterprises. There were 1,422 sera that were tested using commercial indirect ELISA. This method originally developed for testing human sera was adapted for the analysis of pig sera. All farms except one (97.43%) and 714 sera samples (50.21%) resulted positive for anti-HEV IgG antibodies. This study confirms that HEV is widespread in pigs in Italy and might be endemic on most farms.

Keywords: *Hepatitis E virus*; *pig*; *antibodies*; *prevalence*; *ELISA*

Received: 30 May 2011; Revised: 16 June 2011; Accepted: 17 June 2011; Published: 12 July 2011

Hepatitis type E (HE) is considered an emerging zoonotic disease in developed countries (1). The hepatitis E virus (HEV) is a small, non-enveloped, single-stranded RNA virus classified in the Hepeviridae family as Hepevirus genus. Up to now, four major genotypes (2) and only one serotype have been identified (3). Genotypes 1 and 2 are restricted to humans and often associated with large outbreaks and epidemics in developing countries with poor sanitation. Genotypes 3 and 4 infect humans, pigs, and other animal species and have been responsible for sporadic cases of hepatitis E in both developing and industrialised countries. In humans, HEV is responsible for acute hepatitis that rarely leads to death, except in pregnant women where the fatality rate is up to 25%. In developed countries, autochthonous HEV infections are suspected due to contact with infected animals, in particular pig and wild boar, and certainly due to ingestion of contaminated raw meat and seafood (4). Nucleotide sequence analysis has shown that swine and human HEV isolates from the same geographic area are more similar than swine HEV isolates from different regions (5). Furthermore, it has been reported that there are more anti-HEV antibodies among swine handlers than in a control population (6). Anti-HEV antibodies have been found in several animal species: swine, bovine, dog, horse, wild boar, deer, and rodents. In swine, several studies on anti-HEV prevalence show high levels of seroprevalence proving that it is endemic in developed countries.

Hepatitis E virus has been identified by RT-PCR (reverse transcriptase-polymerase chain reaction) on pig farms both in Northern and Central Italy (7), but data on seroprevalence are not yet available. In this report we describe the results of a study to define HEV seroprevalence in Northern Italian pig herds.

Materials and methods

From January to June 2008, 1,422 pig blood samples were collected on 39 pig farms in Northern Italy. Ten farms were farrow-to-finish, 17 farrow-to-weaning, and 12 were fattening operations. On average, 10% of animals per farm were sampled and the sera were analysed for anti-HEV IgGs using an indirect enzyme-linked immunosorbent assay (ELISA).

The ELISA test was a human commercial kit (HEV-Ab, Diagnostic Bioprobes, Milan, Italy), modified with a specific tracer; that is, goat anti-swine instead of goat anti-human IgG. This test is based on the use of plates coated with a recombinant antigen containing immunodominat epitopes from the ORF2 and ORF3 regions of Mexican (genotype 2) and Burmese (genotype 1) viral human strains. The ELISA method was performed following the kit instructions. Each pig serum (50 µl/well) was examined at a fixed dilution (1:100 in PBS containing 1% yeast). The peroxidase-conjugated goat

Citation: Infection Ecology and Epidemiology 2011, **1**: 7331 - DOI: 10.3402/iee.v1i0.7331

anti-swine IgG (Goat anti-pig IgG, Serotec, Oxford, UK) were used at 1:3000 dilution. The absorbance value was measured at 492 nm wavelength and the results expressed as optical density (OD). The pre- and post-infection serum from pigs experimentally infected with HEV were included as positive and negative controls, respectively. The cut-off value used was 0.274 and was calculated as the mean OD value plus three standard deviation (sd), of 80 antibody-negative pig sera. The Chi-squared test was performed on contingency tables to find *P*-values.

Results and discussion

The OD values of pig sera for anti-HEV IgG values ranged from 0.045 to 3.369 with an average OD of 0.52 (sd 0.62) and a median value of 0.352. Using the cut-off value of 0.274, 38 out of 39 farms had at least one seropositive sample (97.43%, 95% CI: 92.5–100%) and 714/1422 serum samples (50.21%, 95% CI: 47.7–52.8%) were positive for anti-HEV IgG. The mean OD of positive samples was 0.914 (sd 0.67). The mean anti-HEV IgG seroprevalence on farms was 52.8%.

The sows presented the highest seroprevalence (70.6%, 95% CI: 67–74.1%) and the risk of developing seroconversion was about four times higher than all the other groups put together (OR = 4.7; IC = 3.7–5.9; Table 1).

Considering the different type of farms, the mean seroprevalence value was 70.5% (ranged 21.6–100%) in farrow-to-weaning farms, 61.2% (0–94.1%) in farrow-to-finish farms, and 30.3% (3.6–81.3%) in fattening farms (Table 2). A direct correlation between farm size and seroprevalence was also evident (Table 3).

The data indicate a high seroprevalence for anti-HEV antibodies in an Italian pig population, even if the percentage of seropositive animals varied widely among herds, being around 0% on some farms and almost 100% on others. In particular, the seroprevalence was higher in larger herds and it varied greatly among different age classes. In fact, we found the highest and the lowest seroprevalence in sows and in weaners, respectively; in piglets (8–16 weeks of age) we got a lower seroprevalence than in fattenings (up to 24 weeks). Unexpectedly, seroprevalence decreased in finishers (30.8%), perhaps due to the mixing of pigs from different farms and to the absence of sows in these herds. Such differences are probably due to the dynamics of the infection in swine, which is influenced by maternal immunity. Passive immunity protects piglets up to 2 months old and, after the infection, seroconversion occurs with IgG increase mainly at 15 weeks old. This infection dynamic is supported by studies based on detected viral RNA in faeces, with the highest values in pigs around 6 months old and also at slaughter time, suggesting that HEV can infect at any age (8). Seminati and collaborators (9) had similar results in Spain: the total anti-HEV IgG seroprevalence was 41.9% in sera collected from 1998 to 2000 and 60.8% in sera of gilts and sows collected from 1998 to 1999. Other studies conducted on small-sized pig sera samplings showed seroprevalences in developed countries: United Kingdom (85%), Sweden (58%), Germany

Table 1. Number of positive serum samples displayed by productive age

Productive age	Positive samples	Seroprevalence 95% CI	OR 95% CI	*P*
Sow	447/633	70.6% (67–74.1%)	4.7 3.7–5.9	<0.00001
Weaner (up to 2 months)	7/58	12.1% (3.7–20.4%)	0.1 0.05–0.3	<0.00001
Slips (2–3 months)	41/133	30.8% (23–38.7%)	0.4 0.3–0.6	<0.00001
Fattening (4–6 months)	135/325	41.5% (36.2–46.9%)	0.6 0.5–0.8	0.0005
Finisher (over 6 months)	84/273	30.8% (25.3–36.2%)	0.4 0.3–0.5	<0.00001
Total	714/1422	50.2% (47.6–52.8%)		

Table 2. Seroprevalence in different type of herd, displayed by productive age

Farm type	Farrow-finish (10 herds)			Farrow-weaning (17 herds)			Fattening (12 herds)		
Prevalence	61.2% 95% IC 55.8–66.6			70.5% 95% IC 66.3–74.6			30.3% 95% IC 26.8–33.9		
Productive age	Sow	Fattening	Finisher	Sow	Slips	Weaner	Slips	Fattening	Finisher
Tested sera	182	91	32	451	23	58	110	234	241
Positive sera	129	44	12	318	16	7	25	91	72
Prevalence	70.9%	48.3%	37.5%	70.5%	69.6%	12.1%	22.7%	38.9%	29.9%
IC 95%	64.3–77.5	38–58.6	20.7–54.3	66.3–74.7	50.8–88.4	3.7–20.4	14.9–30.5	32.6–45.1	24.1–35.6

Citation: Infection Ecology and Epidemiology 2011, **1**: 7331 - DOI: 10.3402/iee.v1i0.7331

Table 3. Seroprevalence in farms according to the herd size

Herd size	Seroprevalence (95% IC)	OR (95% IC)	*P*
<1000	51.2% (45.8–56.8)	1.1 (0.8–1.4)	0.70
1000–4000	40.7% (37.4–44.1)	0.4 (0.3–0.5)	<0.0001
>4000	75.1% (70.2–80)	3.9 (2.9–5.2)	<0.0001

(23%), and the United States (34.5%) (6). In Canada, 594 out of 998 (59.5%) pig sera were seropositive with significant variations between geographic regions (10).

Human genotype 1 and 2 antigens have previously been used to test human samples with good specificity and sensitivity (11). Due to the existence of common immunodominant epitopes in human and swine HEV (3), these antigens are efficient in capturing pig antibodies produced against the HEV swine strain circulating in Northern Italy (7).

In conclusion, although there are a few cases recognising human infection, HEV is widespread on pig farms in Northern Italy. The high seroprevalence in pigs should raise concern as it has been described previously that seropositive animals, despite developing an immunological response, could still contain HEV at slaughter age (12), representing a risk for food security and for persons in contact with pig or pork products.

Acknowledgements

For technical support we must thank Mrs Daniela Bresciani and Dr Paolo Bonilauri for their precious collaboration. We also thank Michael John of the Vita-Salute San Raffaele University, Milan for the English language editing of this manuscript.

Conflict of interest and funding

The authors declare no conflict of interest and they have not received any funding or benefits that could have affected this study.

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