Hepatitis E virus infection in North Italy: high seroprevalence in swine herds and increased risk for swine workers

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Hepatitis E virus infection in North Italy: high seroprevalence in swine herds and increased risk for swine workers

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Running head: Human and swine HEV epidemiology in Italy

Summary

We determined the hepatitis E virus (HEV) seroprevalence and detection rate in commercial swine herds in Italy's utmost pig-rich area, and assessed HEV seropositivity risk in humans as a function of occupational exposure to pigs, diet, foreign travel, medical history, and hunting activities. During 2011-2014, 2700 sera from 300 swine herds were tested for anti-HEV IgG. HEV RNA was searched in 959 faecal pools from HEV-seropositive herds and in liver/bile/muscle samples from 179 pigs from HEV-positive herds. A cohort study of HEV seropositivity in swine workers (*n*=149) was also performed using two comparison groups of people unexposed to swine: omnivores (*n*=121) and vegetarians/vegans (*n*=115). Herd-level seroprevalence was 75.6% and was highest in farrow-to-feeder herds (81.6%). 26/105 (24.8%) herds had HEV-positive faecal samples (25 HEV-3, 1 HEV-4). Only one bile sample tested positive. HEV seropositivity was 12.3% in swine workers, 0.9% in omnivores and 3.0% in vegetarians/vegans. Factors significantly associated with HEV seropositivity were occupational exposure to pigs, travel to Africa, and increased swine workers' age. We concluded that HEV is widespread in Italian swine herds and HEV-4 circulation is alarming given its pathogenicity, with those occupationally exposed to pigs being at increased risk of HEV seropositivity.

Keywords: hepatitis E virus; zoonotic infections; epidemiology; hygiene

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Introduction

Hepatitis E virus (HEV) is a RNA virus belonging to the genus *Orthohepevirus* A, which includes two recognized genotypes infecting only humans (HEV-1and HEV-2) and two genotypes infecting either humans or different animal species (HEV-3 and HEV-4) [1]. In recent years, ~~Hepatitis E virus (~~HEV ~~) is a *Hepevirus* with zoonotic potential that~~ has ~~recently~~ emerged as a threat to public health in developed countries. ~~There are seven recognized HEV genotypes (HEV-1 to HEV-7), but only four occur frequently in humans: w~~While the human-restricted HEV-1 and HEV-2 are ~~restricted to humans and are~~ often associated with outbreaks in developing countries where direct transmission via the fecal-oral route is prominent, HEV-3 and HEV-4 have a zoonotic potential, as they are found in both humans and animals [2]. In Europe, most (sporadic) human HEV infections affect older men and are caused by HEV-3, which is widespread in swine herds [3, 4], whilst HEV-4 is more prevalent in Asia [2]. Yet, autochthonous HEV infections caused by HEV-4 in humans and pigs are being reported in several European countries [5-7], including Italy [8, 9].

Although domestic pigs are the main reservoirs of HEV, viral RNA has also been detected in other animals, particularly wild boar and deer [10, 11]. Accordingly, consumption of (undercooked/raw) meat and offal from these animals has been associated with human HEV infection [12-14], although the public health importance of this transmission route remains unclear [15, 16]. Several studies have highlighted that occupational exposure to animals, particularly swine, may play a role in HEV transmission in developed countries [17-19]. Indeed, HEV infection in pigs is mostly asymptomatic and self-limiting, causing mild liver dysfunction with no macroscopic lesions [20]. Moreover, HEV may persist in manure, posing those in direct contact with infected animals or their living environments at risk of infection [16].

While HEV is a growing public health concern in Europe, epidemiological data in swine and humans in Italy are scattered and heterogeneous with regard to populations, sample types, diagnostic methods, and locations [3, 9, 21-23], making the magnitude of HEV infection difficult to determine. The aim of this study was to determine the seroprevalence of HEV in the domestic swine population of Northern Italy (where over 62% of Italy's swine population is located) and in the corresponding human population, seeking also to detect the circulating HEV strains. Additionally, we aimed to assess

differences in the risk of HEV infection associated with occupational exposure to pigs, foreign travel, medical history, hunting activities, and eating habits.

Methods

Swine sampling

A three-stage sampling design was applied. The first stage determined the HEV seroprevalence in the commercial pig population of the Northern Italian regions of Veneto, Lombardy, and Friuli-Venetia-Giulia (Figure 1). The second stage determined the HEV detection rate in pig faeces at HEV-seropositive herds. The third stage determined the HEV detection rate in tissue samples from slaughtered pigs reared in herds where HEV was detected in faeces. For logistical reasons, these two last stages involved only the herds located in Veneto and Friuli-Venetia-Giulia. All sampling activities were performed during November 2011-April 2014.

Analysis of swine sera

The target pig population consisted of 4184 commercial crossbred pig herds, i.e. breeding herds with ≥5 animals and fattening herds with ≥50 animals registered in the 23 provinces within the aforementioned three regions in 2010, when this study was set up. Sample size calculations based on an expected herd-level seroprevalence of 50%, 95% confidence level, and 5% precision returned a total of 353 herds to be sampled. However, for logistical reasons, only 300 farms could be sampled; these were randomly selected in proportion to their underlying population by province and type of production (farrow-to-finish, farrow-to-feeder, fattening, and weaning herds). ~~Under-sampling of farms had no consequences given the higher observed than expected prevalence (see Results section).~~ Serum samples were collected within the framework of statutory surveillance activities for swine vesicular disease and Aujeszky's disease. From each farm, the sera of nine animals were randomly selected for HEV testing, corresponding to an expected within-farm seroprevalence of 30% [4], 95% confidence level, and 5% precision. In total, 2700 individual serum samples were obtained (Table 1). Sera were tested for the presence of anti-HEV antibodies (IgG) using an in-house non-competitive indirect ELISA (97.5% sensitivity and 87.8% specificity) developed by the Istituto Zooprofilattico

Sperimentale della Lombardia ed Emilia Romagna (IZSLER), according to manufacturer's instructions. Samples with S/P values >10 were considered positive, and negative if S/P values <10.

Analysis of swine faeces

For HEV detection in swine faeces, besides sampling 70 (out of 232) HEV-seropositive herds, 2 (out of 68) HEV-seronegative herds were sampled, as they were epidemiologically linked to the HEV-seropositive ones. Moreover, faeces from a convenience sample of 33 pig herds whose HEV serological situation was unknown were also tested. From each herd, up to 10 pools of faeces from 10 different pens were collected. As the likelihood of detecting HEV in faeces is higher in pigs of 80-120 days of age [3], faecal sampling focused on this age group. In total, 959 faecal pools were collected (Table 1) and analysed by real time RT-PCR targeting a 70 bp fragment of the ORF3 region ~~were processed~~ as previously described [9]; positive samples were also confirmed by nested RT-PCR amplifying a 458 bp fragment of the ORF2 encoding the constitutive protein of the capsid.

Analysis of swine tissues

Presence of viral RNA was investigated in diaphragmatic muscle, liver, and bile samples collected at slaughterhouse from pigs originating from four herds with HEV-positive faeces. In total, 179 animals were tested on at least one of these three tissues (Table 1); 177 of these animals were slaughtered at nine months of age, whereas two animals were slaughtered at five and six months of age for the production of traditional Italian "porchetta" (seasoned and slow-roasted whole pig) to be cooked in smaller pits. All muscle/liver samples were analyzed as described previously [4, 9], whereas a pre-treatment step was applied to bile samples before RNA extraction by diluting them 1:10 in sterile PBS ~~because of the~~to reduce potential inhibitory activity in RT-PCR. All extracted RNAs were further processed as reported elsewhere [9].

Immunohistochemical testing was also performed on a total of 72 liver samples (from 3 different farms) fixed in 10% buffered formalin and embedded in paraffin; slide staining was performed using the automated immunostainer Benchmark Ultra (Ventana, Roche). Tissue sections of 3 µm underwent proteolytic antigen retrieval by incubation with Protease 2 (Roche) at 36°C for 12

minutes, and then were incubated with a casein solution (Antibody Diluent with Casein, Roche) at 36°C for 12 minutes to block non-specific sites. Sections were incubated for 40 minutes at room temperature with 1:50-diluted anti-HEV polyclonal primary antibody (Abbiotec), which recognizes several putative HEV proteins including protein ORF3 (pORF3), the immunogenic protein from the viral capsid and structural proteins~~for 40 minutes at room temperature~~. Finally, the sections were incubated with casein solution at 36°C for 12 minutes and processed with the chromogenic detection kit ultraView Universal DAB Detection Kit (Ventana, Roche) according to manufacturer's instructions. Negative control sections were included in each run by replacing the primary antibody with the buffer to exclude the presence of non-specific reactions with the reagents used.

Human sampling

In parallel with swine faecal sampling, a cohort study of HEV in swine workers was performed. Swine workers in the sampled farms were asked to provide a serum sample for HEV serological testing along with a questionnaire covering basic information on demographics, eating habits, hunting activities, previously experienced hepatitis symptoms, and travel abroad (Table 2). For comparison purposes, two groups of people non-occupationally exposed to swine were sampled from the general population: (i) people following an omnivorous diet, and (ii) people following a vegetarian/vegan diet. The number of subjects to be recruited in these groups was such to guarantee the identification of a statistically significant difference (α=0.05) in the risk of being HEV-seropositive with a confidence level of 95% and a power of 80%; a minimum of 100 subjects per group were then to be sampled.

The omnivores were recruited from the general population of Veneto region via an online recruitment campaign. The same was done to recruit individuals following a vegetarian/vegan diet, with the online recruitment campaign targeting local vegetarian/vegan blogs and websites. Like the swine workers, these participants provided a serum sample and completed the aforementioned questionnaire. Participants were informed about the objective and the methods of the study, which was approved by the Ethical Committee of the Padua's University Hospital, and were enrolled on a

voluntary basis, with no financial incentive being given; informed written consent was obtained from all participants.

The three groups were mutually exclusive. In total, 149 subjects were enrolled in the group of swine workers (median age 43 years, range 16-74; 85% males), 121 in the group of omnivores (median age 43 years, range 20-85; 38% males), and 115 in the group of vegetarians/vegans (median age 39 years, range 19-73 years; 23% males). Serum samples were taken at the Outpatient Service of Microbiology and Virology of Padua's University Hospital or directly on farm upon visit of a specialized nurse. After collection, serum samples were refrigerated at 4°C until arrival at the laboratory and stored in aliquots at –20°C until testing for anti-HEV IgG antibody detection using the commercial Wantai HEV-IgG ELISA kit (Beijing Wantai Biological Pharmacy Enterprise, China), according to manufacturer's recommendations.

Data analysis

A 'design-based' analysis was performed to account for the multilevel serosurvey design for pigs, including the province and type of production as strata, the herds as clusters (principal sampling units), and weighting adjustment for the corresponding population from which the sample was drawn.

For humans, seropositivity rates were calculated for the three groups of participants under study, and their differences were tested for significance using binomial regression including cluster-robust standard errors to account for clustering of swine workers at the farm level; estimates were always adjusted for age and gender. This approach was also used to assess factors associated with HEV seropositivity over the three groups of participants, as well as in each group of participants. Variables were first assessed univariately and those showing a $p<0.20$ for the association with the outcome were included in a multivariate model built in backward stepwise fashion. Non-significant ($p>0.05$) variables were dropped one-by-one from the multivariate models after having evaluated the significance of each partial effect. Associations were expressed as adjusted risk ratios (RR) providing 95% confidence intervals (95%CI). Statistical analysis was performed using STATA 13 (StataCorp, College Station, USA).

Results

HEV seroprevalence in pigs

In total, 232/300 (77.7%) farms had at least one HEV-positive serum sample (Table 1). Adjusting for the serosurvey design resulted in a farm-level seroprevalence of 75.6% (95%CI: 70.3-80.2%) (Figure 1). This was highest in farrow-to-feeder farms (81.6%, 95%CI: 69.1-89.8%, *n*=58 farms), followed by fattening (75.5%, 95%CI: 69.5-80.6%, *n*=223), farrow-to-finish (68.0%, 95%CI: 41.0-86.7%, *n*=18), and weaning farms (0.0%, 95%CI: 0.0-97.5%, *n*=1). Excluding the one weaning farm sampled, farm-level seroprevalence did not differ significantly among the types of farms (χ^2-test, p=0.4806).

With a total of 1217/2700 (45.1%) HEV-positive serum samples, the adjusted pig-level seroprevalence was estimated at 43.1% (95%CI: 39.3-47.0%). Seroprevalence was highest in farrow-to-feeder farms (47.7%, 95%CI: 39.6-56.0%, *n*=522 sera), followed by fattening (44.0%, 95%CI: 39.5-48.6%, *n*=2007), farrow-to-finish (23.1%, 95%CI: 13.8-36.1%, *n*=162), and weaning farms (0.0%, 95%CI: 0.0-33.6%, *n*=9). Excluding the sera from the weaning farm, the pig-level seroprevalence differed significantly among the types of farms (p=0.0109). Specifically, seroprevalence in pigs of farrow-to-feeder farms differed from that of pigs in fattening (p=0.0032) and farrow-to-finish farms (p=0.0028), but the seroprevalence of the pigs housed in these two latter types of farms did not differ significantly with one another (p=0.4405). Under-sampling of farms as mentioned in the methods had no consequences given the higher observed than expected prevalence.

HEV detection in pig faeces and tissues

In total, 26/105 (24.8%) farms had at least one faecal sample positive for HEV (Table 1), of these 25/26 belonged to HEV-3 and one to HEV-4, as reported previously [9]. The latter genotype was detected in a farm in which HEV-3 was detected as well. All liver (*n*=179) and diaphragmatic muscle (*n*=134) samples tested negative, only 1/132 bile sample tested positive. This sample was taken from a 5-month-old animal whose muscle and liver sample tested negative for HEV. All immunohistochemical analyses tested negative.

HEV seropositivity in humans

Anti-HEV IgG antibodies were detected in 14.1% (21/149) of swine workers, 0.8% (1/121) of omnivores, and 2.6% (3/115) of vegetarians/vegans. Seropositivity rates adjusted for age and gender were as follows: swine workers 12.3% (95%CI: 6.4-18.2%), omnivores 0.9% (0.0-2.5%), and vegetarians/vegans 3.0% (0.0-6.6%). While adjusting for age and gender, seropositivity in swine workers was significantly higher than that of the omnivores (p=0.007) and vegetarians/vegans (p=0.041), but these two groups were not significantly different from each other (p=0.291).

In the overall risk factor analysis (Table 2), the only factors significantly associated with HEV seropositivity was occupational exposure to pigs (swine workers *vs*. omnivorous population: RR 15.02, 95%CI 2.17-104.15, p=0.006) and having travelled to Africa (been in Africa once or more times *vs*. never been in Africa: RR 2.20, 95%CI 1.06-4.53, p=0.033). Given the limited number of HEV positivities in the groups of omnivores (#1) and vegetarians/vegans (#3), the group-specific risk factor analysis was performed only for the swine workers. In this group, only age (continuous variable expressed in years) was significantly associated with HEV seropositivity (for every 1-year increase in age: RR 1.03, 95%CI 1.01-1.06, p=0.007).

Discussion

This study was conducted to determine the seroprevalence and detection rate of HEV in commercial swine herds in Italy's utmost pig-rich area and to assess the risk for humans to be HEV-seropositive as a function of several factors, including occupational exposure to pigs. Previous Italian studies were limited by the convenience sampling of only a few swine herds [21, 23, 24]. The present study overcame this issue using a structured sampling scheme representative of the underlying swine population. Moreover, a complete picture was provided by looking at HEV serological evidence in humans as well.

Results indicated that HEV is widespread in Italian swine herds, supporting previous findings in Italy [24-26] and other European countries [24]. For instance, a study in the United Kingdom (UK) reports a pig-level seroprevalence of 93% (n=629) in 6-month-old pigs [27]. Other studies report a herd-level seroprevalence of 80% in Spain (n=85) [28] and 65% (n=186) in France [29], and a pig-

level seroprevalence of 62% (n=380) in Estonia [10] and 61% (n=108) in Scotland [30]. We also found farrow-to-feeder herds to have the highest seroprevalence, followed by fattening, farrow-to-finish, and weaning herds, possibly reflecting the primary productive/age groups represented. For instance, in farrow-to-feeder farms, which are open-cycle herds with sows producing piglets that are sold at 24-28 days for fattening elsewhere, only sows (which usually show the highest HEV seropositivity) were sampled conforming to statutory surveillance activities. In fattening herds, where there are pigs of different ages (usually from 24-28 up to 280 days), some of which would have already seroconverted and some would have not, we found an intermediate seroprevalence. Piglets younger than 60 days are not sampled for swine vesicular disease and more in general they were not included in our study due to maternal immunity. Farrow-to-finish herds, being closed-cycle herds, should introduce new animal less frequently than the others, thereby limiting the introduction of infections; this could explain the lowest HEV seropositivity rates therein. However, a limitation of this study was the lack of information on other factors that may have also played a role in determining the observed seropositivity rates, e.g. type of farm management, infrastructural characteristics of the premises themselves, biosecurity measures implemented, etc. These factors may vary from farm to farm and might not be necessarily associated with the type of farm itself.

Failure to detect HEV in tissues may be due to the age of the pigs slaughtered, as all but two animals were destined to cured ham production and were therefore slaughtered at nine months of age, and the only positive sample (from bile) was collected from a 5-month-old pigs. This is somewhat reassuring with regard to foodborne transmission of HEV from cured pig products, of which Italy is a big producer and consumer, as also evidenced by other studies [4].

Genetic analyses confirmed the wide presence of HEV-3 and the ~~first detection~~co-circulation of HEV-4 among pigs in Italy. For more detailed information on the genetic similarities of the HEV-4 detected here, we refer to the previous publication dedicated to this finding [9]. HEV-4, which is typical of the Asian continent, is believed to have just recently been introduced in Europe [7, 9]. Given the high pathogenicity of this genotype, more focused studies are recommended to better understand how and to which extent this genotype has spread across Europe. We also found that occupational contact with pigs was associated with seropositivity to HEV in humans. HEV-3 and HEV-4 circulating

in Europe have a high level of nucleotide identity between swine and human strains [4], and a recent systematic review and meta-analysis of 12 cross-sectional studies in which HEV seroprevalence (IgG) was compared between people with and without occupational contact with swine has identified a significant association between occupational exposure to swine and seropositivity to HEV [19]. However, the high heterogeneity over the studies (due to, e.g., variations in population susceptibility, test performance, etc.) precluded the calculation of a pooled measure of association. Although this heterogeneity makes also the direct comparison of seropositivity rates among studies rather inappropriate, it is worth reporting that our seropositivity rate of 14.1% among swine workers lays within the range of the (significantly higher) seropositivity rates among people occupationally exposed to pigs reported in the literature, i.e. from 11% to 76% [19]. Our finding therefore adds to the growing body of evidence that direct contact with pigs is a risk factor for human HEV infection. In absence of an effective vaccine against HEV, prevention for swine workers, including farmers, butchers, and veterinarians, can only rely on the implementation of hygiene and individual protection. Yet, more targeted interventions might be planned in the future once an assessment of the working conditions leading to higher risk of HEV infection among swine workers will be performed. As regard to travel to Africa as a risk factor for HEV positivity, a recent comprehensive review has showed that HEV has spread into the human populations of at least 28 of the 56 African countries, with the continent as a whole being among the most severely affected parts in the world [31].

We found no significant effects of diet on HEV seropositivity, as the rate among the omnivores did not differ significantly from that of vegetarians/vegans, even when accounting for how long the vegetarians/vegans did not eat meat. Moreover, consuming specific “risky” food items like pork or shellfish, either raw or cooked, was not significantly associated with HEV seropositivity in this study. Lack of significant differences in HEV seropositivity between meat consumers and vegetarians have been reported previously in the USA [32], but in contrast to hepatitis E in developing countries, sporadic cases in developed countries have mainly been associated with pork consumption, particularly raw/undercooked offal [33]. However, it has been pointed out that it would not be completely fair to attribute the high seropositivity to HEV in developed countries to pork consumption alone, as despite some indications that this might sometimes be relevant [6], raw/undercooked swine

offal consumption remains infrequent and cannot explain the increasing HEV seroprevalence in developed countries [34]. A recent French study [35] involving 10,569 blood donors found an overall IgG prevalence for HEV of 22.4%, with an increased risk of HEV IgG positivity among those eating pork meat, pork liver sausages, game meat, offal, and oysters, whereas drinking bottled water was associated with a lower prevalence of anti-HEV IgGs. Yet, these authors concluded that eating habits alone cannot fully explain the exposure to HEV, and that contaminated water may also play a role in HEV transmission [35].

Available data on HEV seropositivity in Italy are limited to Southern regions and suggest that 1.3-2.9% of people without hepatitis are HEV-seropositive [36], although a retrospective follow-up study (1978–1991) on acute nonA–nonB hepatitis cases at a single referral centre in Northern Italy showed autochthonous cases of acute HEV infections since the 1980s [37]. A recent Italian study on seropositivity to HEV among mainly young adults living in the city of Rome who underwent human immunodeficiency virus (HIV) testing, showed an overall HEV seropositivity of 5.4% and a significant association with male homosexual intercourses, suggesting that besides the oro-faecal and zoonotic transmission, certain sexual practices may also contribute to HEV transmission [22], as well as blood transfusions and solid organ transplants [38].

In conclusion, HEV is widespread in commercial swine herds in Northern Italy, where most of Italy's swine population is located. The circulation of HEV-4, ~~along~~ together with ~~(~~the ~~so far~~ predominant~~)~~ HEV-3, in ~~pigs~~ these swine herds is a cause ~~of~~ for concern, as HEV-4~~it~~ is known to cause more severe illness in humans [7]. Moreover, occupational exposure to pigs stood out as a significant risk factor for HEV seropositivity in humans. Altogether, these findings support current evidence indicating that swine is the most likely source of HEV infection in Italy.

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Conflict of interest: None.

Ethical standards: The part of this study involving human subjects received ethical approval from the Ethical Committee of the Padua's University Hospital (Ethical Approval Number 307/AO/14). Participants were enrolled on a voluntary basis, with no financial incentive being given. Informed written consent was obtained from all participants. Swine data were generated from statutory veterinary public health surveillance activities for swine vesicular disease and Aujeszky's disease in Italy and the EU, so ethical approval was not required.

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Table 1. Total number of farms and sera tested for HEV IgG antibodies and total number of farms and tissues analyzed for HEV RNA presence. Farms were subdivided for pig production categories.

	HEV IgG positive/tested samples (%) and HEV IgG positive/tested farms (%)	HEV RNA positive/tested farms (%)	HEV RNA positive/tested animals (%)		
		Feces	Liver	Bile	Muscle
Farrow-to-feeder	257/522 (49.2); 47/58 (81.0)	3/9 (33.3)	n.t.	n.t.	n.t.
Fattening	917/2007 (45.7); 172/223 (77.1)	21/89 (23.6)	n.t.	n.t.	n.t.
Farrow-to-finish	43/162 (26.5); 13/18 (72.2)	2/7 (28.6)	n.t.	n.t.	n.t.
Weaning	0/9 (0.0); 0/1 (0.0)	n.t.	n.t.	n.t.	n.t.
Total	1217/2700 (45.1); 232/300 (77.3)	26/105 (24.8)			
Slaughtered	-	n.t.	0/179 (0.0)	1/132 (0.75)	0/134 (0.0)

n.t.: not tested

463 **Table 2.** Human HEV seropositivity rates and risk ratios for the variables assessed for association with

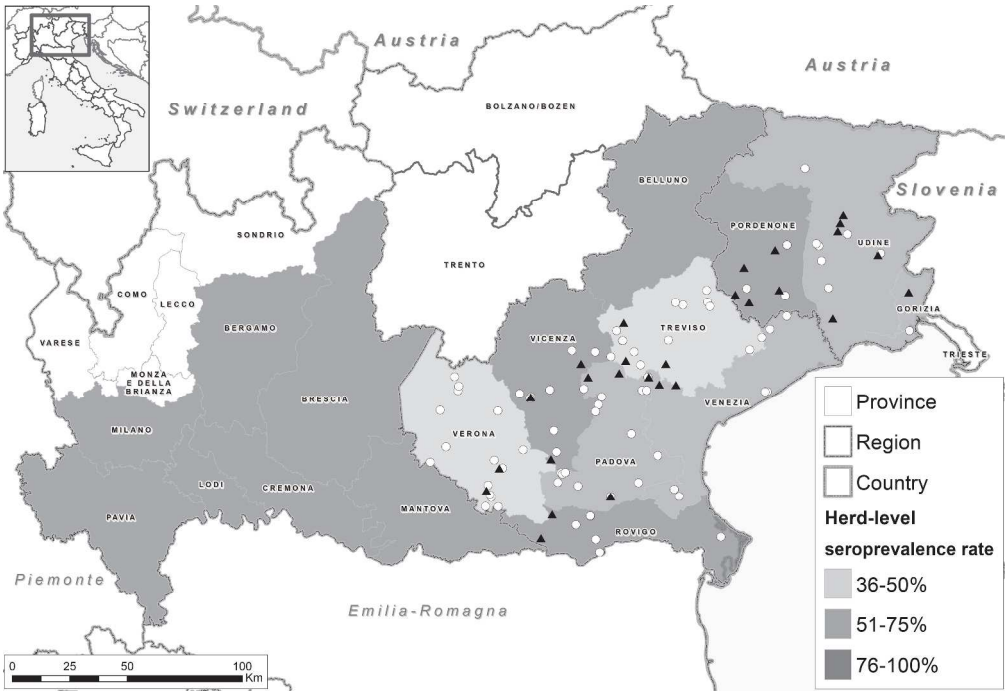
464 HEV seropositivity in the overall binomial regression analysis.

	N	Adjusted % HEV seropositivity (95% CI)†	Adjusted risk ratio (95% CI)† Single-variable analysis	Adjusted risk ratio (95% CI)† Multivariable Analysis
Risk group (occupational exposure to pigs)				
Yes, swine worker	149	12.3 (6.4-18.2)	**14.27 (2.09-97.54)**	**15.02 (2.17-104.15)**
No, omnivore diet	121	0.9 (0.0-2.5)	Reference	Reference
No, vegetarian/vegan diet				
For ≤6 years	59	3.9 (0.0-9.9)	4.54 (0.39-52.61)	3.95 (0.35-44.13)
For >6 years	56	2.1 (0.0-6.0)	2.38 (0.15-38.87)	1.93 (0.12-29.76)
Age (years)	385	6.4 (3.9-8.9)§	1.01 (0.98-1.04)	1.01 (0.98-1.04)
Gender				
Female	187	4.8 (0.7-8.9)	Reference	Reference
Male	198	7.1 (4.1-10.1)	1.48 (0.56-3.88)	1.33 (0.50-3.57)
Hunting				
No	364	6.8 (4.4-9.3)	Reference	
Yes	12	4.0 (0.0-10.9)	0.59 (0.12-2.93)	
Unknown	9	0.0 (0.0-0.0)	0.00 (0.00-0.00)	
Consumption of pork‡				
Never	126	5.8 (0.0-12.0)	Reference	
Sometimes	116	6.8 (2.3-11.3)	1.16 (0.33-4.06)	
Often	143	6.6 (3.2-10.0)	1.13 (0.35-3.70)	
Consumption of cured pork‡				
Never	62	7.3 (0.0-17.7)	Reference	
Sometimes	84	8.7 (3.2-14.2)	1.19 (0.24-5.85)	
Often	166	6.1 (3.1-9.1)	0.84 (0.19-3.69)	
Unknown	73	3.6 (0.0-8.4)	0.49 (0.07-3.38)	
Consumption of raw pork‡				
Never	319	6.0 (3.1-8.9)	Reference	
Sometimes	33	10.0 (2.1-17.9)	1.51 (0.59-3.84)	
Often	24	6.3 (0.0-15.3)	1.10 (0.41-2.93)	
Unknown	9	8.0 (0.0-23.3)	0.77 (0.07-8.18)	
Consumption of shellfish‡				
Never	180	5.7 (2.1-9.3)	Reference	
Sometimes	163	7.7 (3.6-11.7)	1.35 (0.59-3.10)	
Often	36	5.3 (0.0-12.1)	0.93 (0.23-3.85)	
Unknown	6	0.0 (0.0-0.0)	0.00 (0.00-0.00)	
Consumption of raw shellfish‡				
Never	315	5.9 (3.3-8.5)	Reference	
Sometimes	52	10.0 (2.6-17.5)	1.70 (0.77-3.78)	
Often	10	0.0 (0.0-0.0)	0.00 (0.00-0.00)	
Unknown	8	9.8 (0.0-28.1)	1.67 (0.23-12.00)	
Ever had hepatitis symptoms				
No	352	6.4 (3.9-8.8)	Reference	
Yes	18	0.0 (0.0-0.0)	0.00 (0.00-0.00)	
Unknown	15	13.4 (0.0-29.1)	2.10 (0.64-6.86)	
Ever been in Asia				
No	304	6.6 (3.8-9.3)	Reference	
Yes	77	6.6 (2.3-10.9)	1.00 (0.49-2.07)	
Unknown	4	0.0 (0.0-0.0)	0.00 (0.00-0.00)	
Ever been in Central/South America				
No	338	6.6 (4.0-9.1)	Reference	
Yes	43	6.4 (0.0-15.0)	0.98 (0.24-3.93)	
Unknown	4	0.0 (0.0-0.0)	0.00 (0.00-0.00)	
Ever been in Africa				
No	291	5.3 (3.0-7.6)	Reference	Reference
Yes	90	11.6 (4.4-18.8)	**2.20 (1.06-4.53)**	**2.20 (1.06-4.53)**
Unknown	4	0.0 (0.0-0.0)	0.00 (0.00-0.00)	0.00 (0.00-0.00)
Ever been in other European countries than Italy				
No	145	6.6 (3.2-9.9)	Reference	
Yes	236	6.5 (3.0-10.1)	0.99 (0.47-2.08)	
Unknown	4	0.0 (0.0-0.0)	0.00 (0.00-0.00)	

Ever been in North America			
No	333	6.6 (3.9-9.3)	Reference
Yes	48	6.3 (0.0-14.9)	0.96 (0.22-4.16)
Unknown	4	0.0 (0.0-0.0)	0.00 (0.00-0.00)

95% CI = 95% confidence interval. Statistically significant risk ratios are highlighted in bold.
†Adjusted for age (continuous variable expressed in years), gender, risk group (occupational exposure to pigs), except for the eponymous variables, and clustering of swine workers at the farm level.
‡ Risk group (occupational exposure to pigs) excluded from the model because of collinearity with this variable.
§Estimated at the overall average age of participants (42 years)

Figure 1. Map of the study area showing the herd-level seroprevalence rate (anti-HEV IgG antibodies) in pigs per province. Dots indicate farms in which HEV RNA presence was investigated (triangle= positive farms, circles= negative farms).



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