

Brief Original Article

Seroprevalence of hepatitis E in pigs and wild boars in the region of the city Belgrade

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Abstract

Introduction: Hepatitis E is considered an emerging human viral disease with many evidences of zoonotic nature of disease, and swine are the main reservoir of HEV. The aim of this study was to determine HEV seroprevalence in commercial pig farms, backyard pigs, slaughtered pigs and wild boars in the region of the city Belgrade.

Methodology: A total of 405 sera samples: 150 samples from 3 commercial pig farms, 70 samples from backyard pigs, 119 samples from slaughtered pigs and 66 samples from wild boars of the region of the city Belgrade, Serbia were analysed by commercial ELISA test.

Results: The overall HEV seroprevalence in 3 commercial pig farms was 55.33% (83/150). All tested farms (farm A, B and C) were positive on the presence of anti-HEV antibodies, respectively 58% (29/50), 54% (27/50) and 54% (27/50). From 70 tested backyard pigs, 75.71% (53/70) were tested seropositive. In total, 26 backyard pig holdings were confirmed as positive to anti-HEV antibodies (81.25%). At slaughterhouse, 25% (8/32) weaned piglets and 20.69% (18/87) fattening pigs were tested positive on anti-HEV antibodies. Overall HEV seroprevalence in tested wild boar population was 52.25% (36/66).

Conclusions: Detected very high seroprevalence of anti-HEV antibodies indicated an active circulation of HEV, being enzootic in the swine population, and wild boars, as well, in the region of the city Belgrade.

Key words: hepatitis E; pig, ELISA; prevalence; farm; slaughterhouse.

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Introduction

Hepatitis E virus (HEV), the pathogen causing acute hepatitis E, has become a worldwide public health concern. HEV infection is self limited disease in immunocompetent patients, with a low mortality rate [1]. The infection is considered an emerging human viral disease with many evidences of zoonotic nature of disease [2]. In developing countries of Asia, Africa and Central America hepatitis E is an enterically transmitted, waterborne acute viral infection. It is an important infection in humans of EU/EEA countries, and over the last 10 years, more than 21,000 acute clinical cases with 28 fatalities have been notified with an overall 10-fold increase in reported HEV cases [2,3]. Autochthonous, sporadic cases of hepatitis E have been reported in the United Kingdom, France, Italy, Spain, the Netherlands, Greece, Hungary, Germany, Austria,

Poland, and in various regions from the USA [4,5]. Most of these human cases are caused by HEV genotype 3 which is common in swine and is considered a zoonosis of porcine origin. Contact with pigs or consumption of undercooked or raw pork meat are recognized as risk factors for transmission of infection, given the high seroprevalence observed in pig veterinarians, pig farmers, and in the populations that usually consume uncooked pork or pork raw liver products [3,4,6,7]. Although HEV infection in pigs is subclinical, swine are the main reservoir of the virus [8].

Besides pigs, HEV infection was proved serologically in many animal species including sheep, goats, cattle, horses, dogs, rabbits chickens, rodents, deer and wild boar [9,10].

HEV infection is highly prevalent among the domestic pigs in Europe. Jemeršić *et al.* [11] reported

that the overall seroprevalence in Croatia was 32.94%. In Bulgaria, the total seroprevalence of HEV in pigs 1 to 6 months of age was 40%, being in piglets 50% and in fattening pigs 29.2% [12]. The individual seroprevalence in pigs over 6 months of age varied between countries being: 92.8% in the UK [13], 80% in Italy [14] and 68.6% in Germany [15]. Recent studies from Serbia indicated HEV infection seroprevalence of 34.6% in backyard pigs [16] and presence of the virus in commercial pig herds and slaughtered pigs, as well [17-19]. In a human population in Serbia, the prevalence of anti-HEV IgG among blood donors as representatives of the general population is 15% [20], which is higher than compared to data from some other European countries [21,22].

The aim of this study was to determine HEV seroprevalence in commercial pig farms, backyard pigs, slaughtered pigs and wild boars in the region of the city Belgrade. It is currently unknown whether HEV seroprevalences differ between pigs raised in different farming systems in Serbia. Because of the zoonotic nature of HEV, in terms of food safety, slaughtered pigs were included in this investigation, as well. Additionally, to prove the potential role of the wild boars in the epidemiology of the HEV, samples from wild boars were also analyzed.

Methodology

Blood samples were collected from 3 commercial pig farms (with 1000 - 1500 sows), backyard pigs, slaughtered pigs from one slaughterhouse and from hunted wild boars from the region of the city Belgrade, Serbia, from 2016 - 2018. According to official data of Statistical Office of the Republic of Serbia total number of swine population in Serbia is 2,792,286, out of which 146,397 (5.24%) were kept in sampling area at the time of sampling (https://publikacije.stat.gov.rs/G2019/Pdf/G20191032.pdf). Blood samples were collected into vacutainers without anticoagulant but with clot activator. From commercial pig farms (farm A, B and C), a total of 150 blood samples were taken by puncture of the jugular vein. From each farm, 50 animals, showing no clinical symptoms, were randomly selected for sampling considering the expected high seroprevalence. From each category suckling piglets, weaned piglets, fattening pigs, sows and boars, 10 samples were taken.

From 70 backyard pigs, blood samples were taken by puncture of the jugular vein. Examined backyard pigs were originated from 32 herds (2 to 15 pigs per herd).

Sampling at the slaughterhouse, was done in December 2018, in one slaughterhouse with capacity 100 animals per hour. According to official data of Statistical Office of the Republic of Serbia total number of slaughtered pigs in Belgrade region is 6669 in sampling area at the time of sampling (https://publikacije.stat.gov.rs/G2019/Pdf/G20193003.pdf). Blood from 32 weaned piglets (aged between 70 and 80 days) and 87 fattening pigs (aged between 150 and 160 days) was taken from the bleeding wound at exsanguinations point. Slaughtered pigs were originated from the one commercial farm, which was not included in the previously tested commercial pig farms (farm A, B and C).

From 66 wild boars, blood samples were taken after hunting, by puncture of heart, in accordance with an ongoing classical swine fever monitoring program of the Serbian Veterinary Directorate.

After centrifugation at 1000 g for 15 minutes, sera were decanted and stored at -20 °C until analyzed. Serum samples were analysed by commercial ELISA test (PrioCHECK® HEV Ab porcine ELISA, Prionics AG, Schlieren-Zurich Switzerland) for the presence of antibodies directed against HEV. ELISA plate was coated with recombinant HEV antigen of the ORF 2 and ORF 3 of the genotypes 1 and 3.

All results above or equal to the cut-off value (the mean optical density calculated at 450 nm of the cut off control multiplied by 1.2) were considered positive, as recommended by the manufacturer. The optical densities were measured by an ELISA reader, with 7.2 Magellan software (Tecan Sunrise, Vienna, Austria). Beside kit controls, previously tested sera were used as negative and positive internal controls [16].

Results

Overall HEV seroprevalence in 3 commercial pig farms was 55.33% (83/150). All tested farms (farm A, B and C) were positive on the presence of anti-HEV antibodies, respectively 58% (29/50), 54% (27/50) and 54% (27/50). Seroprevalences in different age categories at commercial pig farms are shown in Table 1.

From 70 tested backyard pigs, 75.71% (53/70) were tested seropositive. In total, 26 backyard pig holdings were confirmed as positive to anti-HEV antibodies (81.25%). Seroprevalence ranged from 50% to 100% at holding level.

At slaughterhouse, 25% (8/32) weaned piglets and 20.69% (18/87) fattening pigs were tested positive on anti-HEV antibodies.

Table 1. Number of anti-HEV antibodies positive samples in different age categories in commercial pig farms.

Farm	Age category					
	Suckling piglets (%)	Weaned pigs (%)	Fattening pigs (%)	Sows (%)	Boar (%)	Total (%)
A	3/10 (30)	4/10 (40)	8/10 (80)	9/10 (90)	5/10 (50)	29/50 (58)
B	2/10 (20)	5/10 (50)	8/10 (80)	7/10 (70)	5/10 (50)	27/50 (54)
C	0/10 (0)	7/10 (70)	9/10 (90)	7/10 (70)	4/10 (40)	27/50 (54)
Total	5/30 (16.67)	16/30 (53.33)	25/30 (83.33)	23/30 (76.67)	14/30 (46.67)	83/150 (55.33)

Overall HEV seroprevalence in tested wild boar population was 52.25% (36/66).

Discussion

Recently, a number of zoonoses have emerged and hepatitis E has become a global health concern. Therefore, investigation of zoonotic pathogens aiming at safeguarding of animal health and ensuring safe food for human kind.

On the tested commercial pig farms, the detected seroprevalence was pretty high (55.33%). This finding goes in favour of the fact that HEV is spreading very quickly throughout the dense pig population at farm via the faecal-oral route [2]. On the tested commercial pig farms, biosafety measures were at a high level as well as pig health status, according to the national legislation which refers to biosafety measures. Those measures are very important to stop spreading of different pathogens [23]. Despite those applied measures, faecal-oral route of HEV transmission, very high density of pig population at the farms, as well as close contact between pigs contributed to the seroprevalence being high. Significantly higher seroprevalence was found in backyard pigs (75.71%) in this investigation. Very poor biosafety measures in backyard holdings are of importance in the faster spreading of HEV in the tested pig population. In such conditions, repetitive exposure to HEV via greater contact frequency between different age categories of pigs and more exposure to pig manure, increasing the transmission rate. In one investigation HEV seroprevalences in pigs reared on conventional farms were statistically significantly lower than the prevalences in pigs reared on organic pig farms [24], confirming the importance of biosecurity measures in pig production. On the other hand, backyard pigs are quite often in close contact with the environment, water sources and wild boars which might serve as a source for HEV transmission.

A lower seroprevalence of 32.94% was detected in swine in Croatia [11] and 40% in Bulgaria [12] but very similar seroprevalence (58.1%), as in our investigation, was demonstrated in Switzerland [25]. On the other hand, higher seroprevalence of 80% was established in

fattening pigs in northern Greece [26]. However, data from different studies should be compared with caution because prevalence can be influenced by the type of farming, sampling strategy and performance of ELISA method used. In one investigation conducted in Japan seroprevalence in 2 months, 3 months, 4 months and 5-6 months old pigs were as follows: 7%, 40%, 87% and 90%, respectively [27]. Detected seroprevalence in suckling piglets in the present study can be a consequence of passive maternal immunity. In one investigation, the efficiency of HEV transmission was 13 times lower in piglets with maternally derived antibodies than in susceptible piglets [28]. An increase in seroprevalence occurs with ageing, which was confirmed also in the present investigation. However, in another study, all tested pigs 6 months old, were negative on the presence of HEV RNA in their sera [27]. It can be assumed that early infection in suckling piglets and weaned pigs, lead to the production of protective humoral immunity, which can protect older pig from reinfection.

In the present study, at slaughterhouse 25% weaned piglets and 20,69% fattening pigs were tested positive on anti-HEV antibodies. This prevalence is lower than the seroprevalence detected in other studies. Usually, in slaughtered pigs, seroprevalence is very high and can be ranged from 4% [29] to 90% [27]. Slaughtered pigs in this investigation, originated from one farm with implemented high-level biosafety measures, batching of piglets after weaning, and good farm and hygiene practice, which may be the reasons for the detected lower seroprevalence.

It is known that active HEV infection occurs naturally in most farm pigs around 2 months of age [30]. Therefore, most market-weight pigs >6 months of age at the time of slaughter are no longer actively infected by HEV. However, some studies have shown that 5.7% slaughterhouse market-weight pigs in the UK [13], and 44.4% in Scotland [31] have been proven to be viremic.

HEV transmission from animals to humans is documented through direct and indirect evidence in many countries [2]. The relatively high seroprevalence of 15% of anti-HEV IgG-positive individuals found

among Serbian blood donors in one recent study [20] is similar to that found previously in Serbia (16.9%) [32]. Those data can be connected to the results obtained in this study, keeping in mind the fact that HEV infection has zoonotic nature and widely traditional consumption of pork meat and products in human population in Serbia.

More than half tested samples originated from wild boars revealed the presence of anti-HEV antibodies. There are many studies throughout Europe about HEV seroprevalence in wild boar population. In one investigation in Italy, HEV seroprevalence in wild boars was 40.7% [33]. The finding in the present investigation indicated an active circulation of HEV in the Serbian wild boar population suggests that wild boars can be source of HEV infection for domestic pigs, as well as for humans since infection with the genotype 3 has been proved in wild boars and humans [33]. This finding is very important in some regions as is an area near the Sava River, where extensively reared pigs can be in direct contact with wild boar population during the summer season.

Conclusion

Detected very high seroprevalence of anti-HEV antibodies indicated an active circulation of HEV, being enzootic in the swine population, and wild boars, as well, in the region of the city Belgrade. This investigation suggesting that both types of husbandries could be of importance in spreading of HEV in swine, as well as to be a potential source of HEV infection for humans. Sows are most affected by HEV, while piglets are at least.

Lower seroprevalence detected in slaughtered pigs could not be overlooked, bearing in mind health risk for slaughter workers and also risk for entering in food chain of HEV contaminated pork.

Hence, further investigation on the presence of HEV in pork and pork products are needed to evaluate potential risks for acquired human infection.

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