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Seroepidemiological Investigation of Human and Swine Hepatitis in Botoşani County

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Abstract. The swine hepatitis E virus (HEV) is considered to be a new zoonotic agent due to its close genomic resemblance to the human HEV and its ability to infect nonhuman primates. Hepatitis caused by HEV infection has been a serious public health problem in developing countries. However, recent seroprevalence studies indicate that the HEV also circulates in industrialized countries. Swine HEV is prevalent in pig populations and does not cause abnormal clinical symptoms in infected pigs, further implicating a likelihood of a risk of transmission to humans by normal contact

In this study were collected 85 swine blood samples from seven localities of Botoşani county. All de swine samples are from household system. From the 75 samples tested, seventeen were found positive for IgG anti-HEV, representing a prevalence of 22,66%. Also we tested 61 human serums for the presence of antibodies against hepatitis E virus and we detected 8 positive samples.

Keywords: hepatitis E virus antibodies, human, swine.

INTRODUCTION

Hepatitis E virus (HEV) is a non-enveloped, single stranded, positive sense RNA virus of 27–34 nm in diameter (Emerson and Purcell, 2003). HEV is the sole member of the Hepeviridae family, genus Hepevirus, which includes all HEV strains detected in human and animals (Emerson et al., 2004). Based on genetic variability, HEV present in mammals has been classified into four genotypes and 24 subtypes (Lu L. et al., 2006). A fifth type has been detected in birds. HEV has been found in humans and animals: genotype 1 and 2 is found only in humans whereas genotypes 3 and 4 have been found in humans and animals (pigs, boar, and deer). Genotypes 1 and 2 are mainly found in the subtropical and tropical areas of Asia, Africa, and the Americas. Genotype 3 is found worldwide and genotype 4 is confined mostly to Asia.

Genotype distribution is indicative of transmission modes. For instance, genotypes 1 and 2 are mainly from contaminated water, whereas genotypes 3 and 4 can be transmitted from pigs or other animals to humans.

Europe is considered a non-endemic region for human hepatitis E, with few clinical cases, although seroprevalences among healthy population can vary from 1% to 16% (Mansuy et al., 2008). In contrast, recent studies showed that seropositive animals are present in up to 97% of the pig herds (Rutjes et al., 2007; Seminati et al., 2008). In both cases, humans and pigs, European autochthonous isolates belong to genotype 3 (Clemente-Casares et al., 2003; De Deus et al., 2008). Like in humans, HEV in pigs is mainly transmitted by faecal-oral route or by direct contact with infected animals (Kasorndorkbua et al., 2004).

Therefore, the objective of the present work was to determine the presence of hepatitis E infection in Botoşani County at humans and swine using serology.

MATERIAL AND METHODS

Blood samples were collected from swine aging 5 to 18 months in household system. A total of 75 swine serums were tested, pigs sampled appeared to be clinically healthy.

Sixty-one human serums used in this study were collected from three different categories of patients. 20 samples were collected from patients hospitalized in Infectious diseases clinic with suspicion of viral hepatitis, 25 serums were obtained from patients diagnosed with hepatitis type B or C being under serological surveillance and 16 serums were obtained from patients undergoing blood tests.

Serum samples were stored until testing at -20^{0}C. Swine and human serums were tested for IgG antibodies against hepatitis E virus by enzyme immunoassay. For the detection of antibodies anti-HEV was used the HEV ELISA kit produced by MP Biomedicals, according to the manufacturer's recommendations. Blank solution, non-reactive and reactive controls were included for each plate. Briefly, 10 µl of the serum sample was added to each well containing 200 µl of the diluent, and the microplate was incubated for 30 min at 37°C. The microplate was washed six times with 300 µl of a wash solution. Horseradish peroxidase-labeled goat anti-human IgG (MP Biomedicals) and rabbit anti-pig IgG (P.A.R.I.S. anticorps) were used as the conjugates for the human and pig sera, respectively. One hundred microliters conjugate was added to each well, and the microplate was incubated for 30 min at 37°C. The microplate was washed six times with 300 µl of the wash solution. Subsequently, 100 µl of the substrate solution was added to each well. The microplate was incubated for 15 min in the dark at room temperature. The color-developing reaction was stopped by adding 100 µl of the stop solution to each well. The absorbance was determined at 450 nm with reference at 620 nm.

RESULTS AND DISCUSSION

Eighty-four swine samples were collected from seven different localities of Botoşani county and 75 were analyzed for the presence of IgG anti-hepatitis E virus. Investigations have been extended in places where is practiced growing pigs in household system, where private owners grow 3-5 pigs for family consumption. The contact with these animals, in most cases accompanied by poor hygiene increase the risk of zoonotic transmission of infection.

In five localities were detected a total of 17 HEV-seropositive pigs. The highest prevalence of anti-HEV IgG was observed in locality Vorniceni, showing a 70% seroprevalence, followed by Romăneşti with 43,75%. In two localities: Băluşeni and Cristeşti, were not detected positive swine.

Table no. 1.

The result of serologic test on swine serums

Locality	Tested samples	Positive serums	Negative serums
Albeşti	10	1	9
Mihai Eminescu	10	1	9
Băluşeni	10	0	10
Cristeşti	9	0	9
Gorbăneşti	10	1	9
Vorniceni	10	7	3
Romăneşti	16	7	9
Total	75	17	58

The presence of seropositive animals in five localities from the study demonstrates the presence and movement of hepatitis E virus in pigs in the household system, drawing attention to the risk of interspecific transmission. A study made in Moldova Republic (Drobenciuc J. et al., 2001) using a serological surveys carried out by groups of people estimated that the risk of infection with HEV of pig owners who participate in the shelter and assist in cleaning the births are 2.46 times more prone to infection than anyone else.

Serological investigations of human hepatitis E infection were undertaken on 61 samples collected from three different groups of patients. Group 1 consists of persons hospitalized in Botoşani Infectious Diseases Hospital with suspected viral hepatitis. The persons included in the first group showed classic symptoms of hepatitis: jaundice, anorexia, nausea, abdominal pain, fever and increased liver enzymes. Group 2 consists of patients who have diagnosed hepatitis type B or C and are under serological surveillance. Group 3 consists in patients undergoing blood tests without heaving clinical signs of hepatitis.

Out of 61 human samples tested, 8 samples were HEV-positive representing 13.11% of total samples tested. A positive serum corresponds to group 1 (suspected of viral hepatitis), three positive samples correspond to the second group, and four positive samples were detected in group 3.

Table no. 3.

Results obtained from HEV ELISA on human serums

Group	Samples tested	Positive serums	Negative serums
Group 1	20	1	19
Group 2	25	3	22
Group 3	16	4	12
Total	**61**	**8**	**53**

Identifying a single person positive for IgG anti-HEV in Group 1, in patients with signs of acute hepatitis admitted in the Infectious Diseases Hospital Botoşani is explained by the fact that IgG is detectable after at least four weeks of infection. The presence of IgG indicates an old infection.

Detection of anti-HEV antibodies in three people diagnosed with hepatitis B or C under serological surveillance shows us the possibility of overlapping human infection with different viruses with liver tropism.

Identification of four people positive for IgG anti-HEV in group 3, indicate that the human populations in Botoşani is subclinically infected by the hepatitis E virus.

In non-endemic regions, the number of hepatitis E cases in humans seems to be on the rise largely due to an increasing interest in this disease. One retrospective study has reported that the incidence of hepatitis E in France was stable over a period of 5 years, suggesting that hepatitis E is generally under-diagnosed (Mansuy J.M. et al., 2009).

Our findings are compatible with previous speculation that the presence of anti-HEV in healthy adults without history of jaundice may reflect subclinical infection of humans, even in developed areas where clinical HEV is rare or nonexistent.

CONCLUSIONS

- Of the 61 human samples from Botoşani County were detected 8 positive samples representing 13,11%. Moreover prevalence of HEV infection in pigs from household system in Botoşani County is 22,66%.
- Anti-HEV antibodies detected at three people diagnosed with hepatitis B or C, under serological surveillance, shows us the possibility of overlapping human infection with different viruses with liver tropism.
- Correlation of positive serological results to hepatitis E in the same area both at human and swine draws our attention to the emergence and potential zoonotic infection.

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