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Hepatitis E virus (HEV), an enterically transmitted pathogen, is one of the major causes of acute hepatitis in humans, being an important public health concern responsible for outbreaks and epidemics in regions with sub-optimal sanitary conditions, in many of which it is endemic. In contrast, hepatitis E is unusually reported in industrialized countries, but recent studies have revealed quite variable seroprevalence rates among European’s populations and a possible porcine zoonotic transmission has been postulated. Accumulating evidences indicates that hepatitis E is a zoonotic disease, and that domestic pigs, wild boars and maybe other animal species are reservoirs for HEV. Recent studies have also indicated that intensive contact with pigs and consumption of some pork products, such as raw liver and sausage, as well as deer or wild boar uncooked meat and liver were major sources of exposure for autochthonous HEV infection and routes for HEV transmission in non-endemic countries. In this regard, a few studies on HEV presence in human and animal population have been conducted in Serbia in the last few years. The aim of this paper is to summarize the results of those studies.

In Serbia, the prevalence of anti-HEV antibodies in the general human population has never been studied. During 2010, presence of anti-HEV IgG was examined in serum samples of 200 blood donors from Novi Sad and surrounding settlements by a previously validated ELISA based on a recombinant ORF 2 protein of HEV genotype 3. Obtained results showed that 15% (30/200) of the analyzed blood donors tested positive in the ELISA test. No significant differences in terms of anti-HEV IgG seropositivity were found between different gender, but HEV seroprevalence increase with age, as a higher one was recorded in individuals older than 51 years (21.5%) than in those between 31 and 50 years of age, or younger than 30 years of age (14.2% and 5.4%, respectively, p<0.027). No relationship between seroprevalence and blood donors occupation was found. Also, none of the anti
HEV IgG positive sera gave positive results on the presence of HEV RNA in nested RT-PCR test.

The first study on HEV presence in swine population in Serbia started during 2007 when 30 pooled stool samples from 3 pig farms, 10 pooled stool samples from wild boars, 20 pooled tissues samples (spleen, mesenteric lymph nodes and liver) of pigs from 2 pig farms and 15 pooled tissue samples from backyard pigs from 3 different municipalities were collected and examined for the presence of HEV RNA by the nested RT-PCR. Nine (30%) out of 30 examined pooled stool samples and 9 (45%) out of 20 examined pooled tissues samples from pig’s farms tested positive for swine HEV RNA. HEV RNA was detected in 4 out of 5 examined pig farms. The presence of HEV was not detected in stool samples from wild boars and tissues samples from the backyard pigs at that time.

The first HEV serology testing in pigs in Serbia was done on 315 blood serum samples collected from 3 to 4 months-old healthy backyard pigs, during 2006 and 2007 in 63 herds from 28 towns and villages of 4 different districts of the Vojvodina province of Serbia. The presence of anti-HEV IgG antibodies in swine sera samples was tested by in house ELISA based on a recombinant ORF 2 protein of HEV genotype 3. Of the tested sera 34.6% (109/315) were found positive. The prevalence of anti-HEV antibodies varied widely between municipalities (range 16.7–75.0%) and herds (range 0–100%).

During 2010, 5 pooled stool samples were collected from 6 big industrial pig farms and tested on the presence of HEV by nested RT-PCR amplification of the ORF1 part of virus genome. HEV presence has been detected in 70% (21/30) of examined samples, and the virus was present in all examined farms (from 20% to 100% virus positive pooled stool samples). Six HEV isolates from different farms was sequenced and molecularly characterized. All isolates have been typed as HEV genotype 3 strains and grouped in 2 clusters, 4 isolates in HEV genotype 3a and 2 isolates in HEV genotype 3g.

Recently, the presence of specific IgG antibodies against HEV and HEV RNA was examined by ELISA and TagMan based one step RT-qPCR in 201 blood and 298 liver samples from wild boars culled during hunting seasons from January 2010 until February 2011 in Serbia. The samples were collected from 27 hunting grounds located on the territory of 7 counties/regions of the country. The overall seroprevalence rate found was 34.33% (69/201), but ranges greatly between different hunting grounds (0 - 93.33%) and different counties/regions analyzed (4.55% - 48.65%). RT-qPCR analysis revealed a relatively high prevalence of 9.40 % (28/298) of HEV positive animals with regional differences. Notably,
a high proportion of adult wild sows and wild boars have been found positive for the presence of HEV RNA. In addition, samples of faeces, liver, bile and meat from a total of 145 animals (95 fatteners and 50 eight weeks old piglets) were collected on the line of slaughter and tested on HEV presence by RT-qPCR. Among fatteners, HEV have been detected just in faecal samples (7.37%; 7/95), but in piglets HEV have been detected in 54%, 26%, 16% and 10% samples of faeces, bile, liver and meat respectively. Study conducted among blood donors in Serbia indicates that HEV infection is not just occasionally present in human population in the area. The high prevalence of HEV found in pigs and wild boars population in Serbia may represent a risk for human health. Further research on the presence of HEV in humans, wild boars and domestic pigs as well as molecular characterization of isolates from all these species in Serbia, but also in all other Balkan counties, is necessary to obtain the real prevalence of this zoonotic virus, to study the possible connection and transmission between different species and to access the risk for human health in the area.

**Keywords:** HEV, seroprevalence, RT-PCR, blood donors, domestic pigs, wild boars