Role of wildlife and domestic pigs as reservoirs for hepatitis E virus (HEV): study of the infection in suids and cervids and of the susceptibility of pigs to HEV originating from wild boar

Auteurs

Thiry, D. [3]

Trefwoorden

2. Pigs [5]
4. wild boar [7]

Samenvatting:

The zoonotic transmission of hepatitis E virus (HEV) is of special concern, particularly in high income countries were waterborne infections are less frequent than in
developing countries. High HEV seroprevalences can be found in European pig and wild boar populations. The aims of the first study of this thesis were to obtain prevalence data on HEV infection in swine in Belgium and to phylogenetically compare Belgian human HEV sequences with those obtained from swine. Sampling was carried out in the pig serum banks made by the regional animal health laboratories in Belgium between September...

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The zoonotic transmission of hepatitis E virus (HEV) is of special concern, particularly in high income countries were waterborne infections are less frequent than in developing countries. High HEV seroprevalences can be found in European pig and wild boar populations. The aims of the first study of this thesis were to obtain prevalence data on HEV infection in swine in Belgium and to phylogenetically compare Belgian human HEV sequences with those obtained from swine. Sampling was carried out in the pig serum banks made by the regional animal health laboratories in Belgium between September 2010 and October 2011. In total, 420 serum samples of fattening pigs aged less than 6 months were used for virological studies and 420 serum samples of lactating sows for serological testing. The presence of HEV-specific antibodies was demonstrated by ELISA. An individual seroprevalence of 73 % (95 % CI 68.8-77.5) was found in Belgium. The individual seroprevalence was significantly different between the two regions (Chi2 = 4.83; 1 degree of freedom (df); P = 0.03): 66 % (95 % CI 56.6-74.2) in the Walloon Region and 76 % (95 % CI 71.1-81) in the Flemish Region. Moreover, 93 % (95 % CI 89-100) of the tested herds were found to contain at least one seropositive pig. A collection of 98 pig sera already tested by ELISA was further analysed by a Western blot (WB) against IgG or IgM. These data were obtained in order to use WB as a reference test in a ROC curve analysis. Therefore the ELISA cut-off value was re-evaluated by the ROC curve analysis and different scenarios were analysed. Whatever the scenario, the seroprevalence remained high, from 58 to 77 % for the ELISA seroprevalence and from 69 to 81 % after confirmation by WB. In addition to the investigation of the high HEV seroprevalence in pigs, the risk of zoonotic transmission of the infection was approached by comparing viral sequences identified during this study in pigs and humans in Belgium. Nine human positive serum samples, from the bank of serum of hepatitis E cases of the Belgian National Center for viral hepatitis, obtained between 2009 and 2011, were available in sufficient amount and used for genetic comparison with swine samples. Four out of 420 pig sera were detected positive for HEV RNA. All sequences from the 4 positive pig sera belonged to genotype 3, subtype f. Eight sequenced human HEV fragments belonged to genotype 3 (7 subtypes f and 1 e) and one to genotype 1. These results indicate the possible zoonotic potential of HEV in Belgium. In addition, the existence of other HEV genotypes or subtypes circulating in pigs in Belgium cannot be excluded, as highlighted by the detection of genotype 4 subtype...
b in a previous survey (Hakze-van de Honing et al., 2009). In Europe and more accurately in Belgium, the wild boar population is in constant increase with a population size estimated to be more than 25000 heads in 2012 in the Walloon Region (16903 km²). The red and roe deer bag statistics were respectively 5300 and 14 400 in 2012 in the same area. In Belgium, four-fifths of the forests are in the Walloon Region and this zone has a wooded area of 4952 km², representing one third of its total area. Moreover, this region contains also a high density of human population (210 people/km²). The aims of the second study of this work were to obtain prevalence data on HEV infection in wild fauna in Belgium and to phylogenetically compare Belgian human and swine HEV sequences with those obtained from wild boars and cervids. Sampling of sera and livers of wild boars was made during the hunting season from September 2010 to February 2011. A total of 383 sera from wild boars over 6 months of age were selected for serology. For the virological study, all the samples available from young wild boars (less than 6 months of age) were used: 69 sera and 61 livers. Sampling of sera and livers from cervids was also made by the Walloon wildlife surveillance network during the hunting season from October 2012 to December 2012; 189 and 235 sera of respectively red deer and roe deer were collected for serological analysis. For the virological analyses, 84 and 68 sera as well as 29 and 27 livers from respectively red and roe deer were sampled. An overall apparent seroprevalence of 34 % (95 % CI 29.71-39.46) was found in wild boars, of 1 % (95 % CI 0-2.4) in red deer and 3 % (95 % CI 0.8-4.2) in roe deer. In order to assess the ELISA screening prevalence, WB analyses were performed. The data obtained by the WB analysis in wild boars have been used for the conception of a ROC curve analysis with WB as reference test and different scenarios were analysed. Seroprevalence remained high whatever the scenarios in the wild boar population. Indeed, it was from 27 to 64 % for the ELISA screening prevalence and from 34 to 42 % after confirmation by WB. In wild boars, 4 out of 69 sera and 5 out of 61 livers were detected as positive for HEV RNA. All sequences obtained from sera belonged to HEV genotype HEV-3, three to subtype 3f and one to 3c according to the classification of Lu et al. (2006). Comparison with the human and swine strains belonging to genotype HEV-3 showed that most of these sequenced fragments also belonged to subtype 3f. HEV RNA was detected in one out of 29 livers from red deer, it belonged to genotype 3f. No HEV RNA was detected in red and roe deer sera. Using a multivariate logistic regression, a significant effect of age was observed: young animals were less seropositive in comparison with adult animals as reference. A significant effect of density was also observed. Wild boar can be considered as a host reservoir of the virus in Belgium. However, the low prevalence in deer disregards these species as reservoir contrarily to the epidemiological role played by them in other countries. This evidence needs further investigation in order to determine in which situation deer can serve as a reservoir. These results also raise the question of the dynamics of HEV infection between wild fauna, domestic pigs and humans. An increasing number of animal species have been recognized as susceptible to HEV. Direct zoonotic transmission to human beings, followed by symptomatic infection, has been documented several times as in the case of uncooked meats from wild boar, deer and pigs infected with HEV. The particular epidemiological role of wild boars in the HEV transmission route has
recently been investigated. The aim of the third study was first to investigate the early consequences of pig infection with a wild boar HEV strain (WbHEV) inoculated by intravenous route and second to observe the infection pattern of a WbHEV strain, a WbHEV strain previously passed in swine, and a SwHEV strain after oral inoculation. A HEV-3 subtype f isolated from the livers of two young wild boars hunted in the same Belgian forest during the same hunting and phylogenetically similar for the analysed sequences was used for the production of inocula for the intravenous and the oral infection experiments, respectively. In the intravenous infection experiment, the piglets were divided in two groups. Group 1 included three piglets (A, B and C) inoculated with a wild boar HEV strain (WbHEV). Group 2, representing a negative control, was composed of two piglets (D and E) inoculated with the HEV-free liver homogenate. In the oral infection experiment, 12 piglets were divided in four groups. Group 3 included three piglets inoculated with a swine HEV strain (SwHEV); group 4 included three piglets inoculated with a wild boar HEV strain previously passed in swine; group 5 included three piglets inoculated with a wild boar HEV strain (WbHEV); and group 6 was composed of three piglets inoculated with HEV-free liver homogenate representing a negative control. After intravenous inoculation, HEV RNA was detected in serum, bile, liver, spleen, duodenum, jejunum, colon, lung, gastro-hepatic lymph nodes and faeces in all group 1 piglets. Pigs from both groups remained seronegative until the end of the experiment and hepatic enzymes remained within the normal range. Furthermore, no clinical signs were observed. After oral inoculation, HEV RNA was detected in serum, bile, liver, gastro-hepatic lymph nodes and faeces in groups 3, 4 and 5. Most of HEV inoculated pigs became seropositive at day 15 post-inoculation and one Sw-WbHEV inoculated pig became seropositive at day 12. Hepatic enzymes remained within the normal range. Furthermore, no clinical signs were observed. In both oral and intravenous infection experiments, HEV inoculated pigs showed infiltration of lymphocytes, plasmocytes, eosinophils in the portal areas, interlobular septa and sinusoids. Additionally parenchymal aggregates of lymphocytes, plasmocytes and macrophages could be noticed in parenchyma of the livers. No histopathological differences were observed between the pigs of both groups in duodenum, jejunum and colon samples. In conclusion, domestic pigs and wild boars can be considered as reservoir hosts for HEV in Belgium. However, contrarily to the apparent epidemiological role of cervids in other countries, the low seroprevalence data obtained in Belgium suggest they are accidental hosts. The results of experimental infections reinforce the putative role of wild boars in the transmission of HEV in pigs. The oral inoculation model better mimics the natural route of infection than the intravenous model and shows a limited spread of the virus in the organism, a low viremia and low levels of the virus in the organs. These points should be considered when assessing the risk of human infection with HEV in the context of the consumption of pork products.

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