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ORIGINAL ARTICLE

Unexpected high prevalence of IgG-antibodies to hepatitis E virus in Swedish pig farmers and controls

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Abstract

Hepatitis E virus (HEV) infections are responsible for large waterborne outbreaks in developing countries. Sporadic cases in the developed world are mainly imported via immigrants and travellers from endemic areas. HEV has been suggested to be a zoonotic infection where pigs may be an important reservoir for the disease and specific swine strains of HEV have been identified which can infect also humans. The aim of this study was to analyse if Swedish pig farmers are more exposed to HEV than persons with other occupations. A total of 115 male pig farmers aged 40–60 y and 108 age- and geographically-matched control subjects were tested for IgG anti-HEV antibodies. No statistical difference in anti-HEV prevalence was noted between pig farmers (13.0%) and control subjects (9.3%). The prevalence of anti-HEV antibodies in the pig farmers and controls was higher than that previously reported among other populations in Europe (<1–9%). Further studies are needed to elucidate the routes for infection of indigenous HEV and if sub-clinical infections with pig associated HEV strains occur in Sweden.

Introduction

Hepatitis E was first named enterically transmitted non-A non-B (ETNANB) hepatitis. The disease was first recognized in 1980 when a large waterborne hepatitis epidemic, in India, was analysed with a new and sensitive serological assay for hepatitis A virus (HAV) and found to be caused by another virus than HAV [1,2]. In 1990 HEV was isolated and ETNANB hepatitis virus was denominated hepatitis E virus (HEV) [3]. In 1997 a swine hepatitis E virus strain (swine HEV) was identified [4] and HEV was suggested to be a zoonotic disease. HEV or anti-HEV antibodies have also been found in rats, chickens, cattle and goats [5–7]. Swine HEV has also been detected in piglets both in endemic and non-endemic areas [8]. Reports of human clinical cases in non-endemic areas in patients who lack a history of travelling to endemic areas, and experiments verifying the existence of cross-species infections [8–10] raise the possibility that HEV is a

zoonotic virus which occasionally may be transmitted from animals to humans [11].

HEV is usually transmitted by the faecal-oral route and contaminated drinking water is believed to be the most common source of infection. The spread of HEV is dependent on the overall public health and sanitation levels. Seroprevalence figures from around the world indicate that the seroprevalence is lower (3–26%) than expected in endemic areas, and higher (1–3%) than expected in non-endemic regions [12].

Recent genetic analyses of different HEV strains collected in different geographical regions indicate that HEV strains are distributed in 9 clusters [13]. It also seems that strains from endemic regions group together and that the different swine strains are not clustered in a single separate group but are spread in several groups.

HEV is rarely reported to the health authorities in Sweden although this is mandatory. Hence, less than 10 clinical cases have been reported annually since

1995. In 1998 Sylvan et al. showed that 5.2% of a control population was positive for anti-HEV IgG antibodies and that age was significantly correlated to a positive anti-HEV test [14]. Furthermore, in 1995 nearly all cases in Sweden were imported from endemic countries via tourism or immigration, infections which occurred preferentially in young adults aged 20–30 y [15]. Between 1990 and 1995, in total 17 cases were imported to Sweden and 1 case was secondary transmitted within the family from 1 of these cases [16]. The aim of this study was to analyse the prevalence of anti-HEV antibodies in Swedish farmers and to investigate if pig farmers had been more exposed than age-matched controls from the same geographical location.

Materials and methods

The serum samples in this study had previously been collected in a research project which analysed the health status of Swedish farmers [17]. A total of 1221 active male farmers aged between 40 and 60 y from 9 separated geographical municipalities in Sweden were originally invited to participate along with 1130 matched non-farming controls, all living in rural areas. The project was approved by the Research Ethical Committee of Karolinska Institute and by the National Computer Data Inspection Board in 1990. From this material all pig farmers with their respective controls were selected (124 each) for testing with an anti-HEV IgG immunoassay. A total of 115 pig farmers and 108 controls from 8 municipalities with a mean age of 50 y (both groups) were finally analysed.

A commercially available serological immunoassay, ABBOTT HEV EIA (Abbott GmbH Diagnostica, Weisbaden-Delkenheim, Germany) was used according to the manufacturer's instructions. The serum samples were considered positive when the measured absorbance value of the sample was greater or equal to the cut-off value. Reactive samples were re-tested in duplicate to confirm the initial result. Samples $\pm 10\%$ from the cut-off value were also re-tested. In the statistical comparison of the differences in proportions of anti-HEV positive individuals multiple logistic regression was used. A p -value < 0.05 was considered significant. All statistical analysis was calculated with the computer program Statistica 5.1. [StatSoft, Inc. Tulsa OK 1996]

Results

IgG-antibodies against HEV were found in 13.0% (15/115) of the pig farmers and in 9.2% (10/108) of the controls, $p = 0.4$. The pig farmers were separated

in 2 groups, piglet producers and slaughter pig producers. The prevalence of anti-HEV antibodies among these 2 groups was 13.4% (11/82) and 12.1% (4/33), respectively, a statistically non-significant difference, $p = 0.9$. In a multiple logistic regression analysis, no significant differences could be found due to occupation, geographic location or age.

There was no difference in anti-HEV prevalence according to geographic location. A reason for this might be the uneven distribution and lack of pig farmers in some of the municipalities. To overcome this, the municipalities were divided into 2 geographical areas, north and south. The prevalence of IgG anti-HEV antibodies in the pig farmer population in the northern region was 16.4% and in the southern region 8.3%. The corresponding prevalence in the control populations was 14.4% and 7.1%, respectively (no significant difference).

No statistical difference in anti-HEV prevalence according to age (≤ 50 and > 50 y of age) was detected. Among individuals ≤ 50 y of age the anti-HEV prevalence was 9.3% (11/117) versus 13.2% (14/106) in the older age group (> 51 y), $p = 0.37$, NS. The corresponding figures for pig farmers only were 6/64 (9.4%) and 9/51 (17.6%), respectively, $p = 0.19$.

Discussion

An unexpectedly high seroprevalence of anti-HEV antibodies was found both in the Swedish pig farmers and in the controls. However, no statistical difference in anti-HEV seroprevalence was noted between the pig farmers, expected to have a higher prevalence, and their controls, refuting our hypothesis that pig farmers would have a higher risk of acquiring HEV and in particular HEV caused by swine strains. The lack of statistical difference between pig farmers and controls might, however, have been caused by the small sample size of sera we had available for testing. In a previous study an anti-HEV seroprevalence of 5.2% (18/349) was found in a control group supposed to reflect the general Swedish population. In this study, persons between 18 and 90 y without a risk for contracting swine HEV strains were tested [14]. This prevalence, however, was significantly lower than that found in our study, both in our pig farmers and controls ($p < 0.01$). In the study by Sylvan et al. the anti-HEV prevalence among controls aged between 40 and 60 y was 10% (10/100) (Sylvan, personal communication), a prevalence which did not differ significantly from that noted among our pig farmers in the present study.

The seroprevalence among Swedish pig farmers exceeded the seroprevalence figures reported from

European countries such as Great Britain [18], Italy [19], and the Netherlands [20]. These countries have reported a prevalence of anti-HEV from <1% up to 9.3% in different populations such as blood donors and other possible high-risk groups. In south-west France a 10.7% prevalence of anti-HEV IgG antibodies was found among 431 consecutive patients with acute hepatitis of unknown origin who had not recently visited HEV endemic countries [21]. Furthermore, autochthonous HEV has been shown to circulate in southern Spain, where 9% of more than 300 sera drawn from patients with abnormal ALT levels tested positive for IgM anti-HEV when other hepatitis viruses had been excluded [22].

Our study did not show significantly higher anti-HEV prevalence in farmers than in the controls coming from rural areas, possibly due to our small sample size as discussed above. Another explanation could be that both groups have been exposed to less pathogenic HEV strains causing mainly sub-clinical infections. A further explanation would be that the high seroprevalence is merely a reflection of a cross-reaction in the assay to a yet unidentified but closely related virus or a false positive reaction. Studies which compare the anti-HEV prevalence in urban and rural populations and studies in Swedish pigs are needed to clarify if a reservoir for HEV exists in Swedish pigs which might infect pig farmers and people in the countryside who come into contact with pigs or piglets, as has been shown to be the case in the UK and the Netherlands [23,24].

It is not fully clarified whether the commercial assay we used to test for anti-HEV antibodies includes antigens which detect also swine HEV strains. Possibly a specific ELISA based on antigens produced from swine HEV isolates can clarify this. It has been reported that commercially available anti-HEV ELISA tests vary in their capacity to detect infections caused by swine HEV strains [10]. When our sera were tested with a swine HEV antigen an even higher prevalence of anti-HEV was found (data not shown, personal communication B. Purcell NIH, USA).

If our results show the true anti-HEV prevalence in Sweden an obvious question can be raised, do we have sub-clinical HEV cases caused by an as yet unidentified HEV strain in Sweden, or are the results generated by the commercial serological tests used for detection of anti-HEV unreliable, causing false positive reactions?

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