

Hepatitis E seroprevalence in East and West Flanders, Belgium

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Abstract

Background and study aim : Hepatitis E virus (HEV) infection is increasingly recognized as a cause of hepatitis in developed countries. The goal of this study is to provide an estimate of the seroprevalence of HEV in Belgium, more precisely in East and West Flanders, since data for this country are currently lacking.

Patients and methods : One hundred patients presenting at the gynecological (mainly fertility center) or orthopedic clinics of our hospital were randomly selected to be tested for anti-HEV IgG antibodies using a sensitive indirect ELISA and, in the case of a borderline result, a strip immunoassay.

Results : The anti-HEV IgG seroprevalence was found to be 14%.

Conclusions : The observed seroprevalence rate suggests that HEV infection is not an uncommon occurrence in Belgium. Comparisons with published seroprevalence data of other Western European countries should be made with caution due to differences in the analytical performance of anti-HEV IgG assays. (*Acta gastroenterol. belg.*, 2012, 75, 322-324).

Key words : Hepatitis E, HEV, seroprevalence, IgG, serology, Belgium.

Introduction

Hepatitis E virus (HEV) is a small, non-enveloped and single-stranded RNA virus. It is the sole member of the genus *Hepevirus* in the family of *Hepeviridae*. The virus has four genotypes with one serotype : genotypes 1 and 2 only infect humans, whereas genotypes 3 and 4 also infect animals, particularly pigs (1). HEV typically causes waterborne outbreaks of acute hepatitis in parts of the world with poor sanitation. These cases are usually due to genotype 1 or 2 and are predominantly caused by feco-oral transmission, mainly through contaminated drinking water.

Another variant of the infection, usually due to genotype 3, is increasingly being recognized in industrialized countries. This genotype 3 has been shown to have a high prevalence in pig populations worldwide in which the virus is apathogenic, and is the cause of what is now called autochthonous hepatitis E. This disease mainly affects older people and may be transmitted zoonotically (2). In Western countries, the incidence of HEV infection and the resulting seroprevalence, reflecting previous exposure to HEV, are uncertain because published estimates of seroprevalence in these populations show great variability (3). The goal of this study is to establish the seroprevalence of HEV in East and West Flanders, since no data have hitherto been published for this region nor for other parts of Belgium. Since Belgium is part of

Western Europe, where the occurrence of HEV genotype 3 has been clearly documented (1), a seroprevalence comparable to that in the adjacent countries would be expected.

Patients and methods

A total of 50 male and 50 female patients presenting at the gynecological (mainly fertility center) or orthopedic clinics of our hospital between June 29th and July 2nd 2011 were randomly selected. These individuals were between 17 and 82 years old with a mean age of 45 years. The study was performed on serum leftovers and was approved by the Ethical Committee of Ghent University Hospital under Belgian Registration number B670201110350. Sera were obtained from these patients by drawing 6 mL venous blood into evacuated blood collection tubes without anticoagulant (Venosafe tubes, Terumo, Leuven, Belgium) followed by centrifugation for 8 minutes at 1885 g. Sera were stored at -20°C until analysis.

Anti-HEV IgG antibodies were determined using the HEV-IgG ELISA kit (Biorex, Antrim, United Kingdom). This assay was performed on a BEP III automated microplate processor (Siemens, Munich, Germany). The kit employs a solid-phase, indirect ELISA method for the detection of IgG class antibodies to HEV in a two-step incubation procedure. Polystyrene microwell strips are precoated with recombinant, highly immunoreactive antigens corresponding to the structural regions of HEV (ORF-2). During the first incubation step, anti-HEV specific antibodies, if present, are bound to the solid-phase precoated HEV antigens. Unbound serum proteins are removed by washing and rabbit antibodies against human IgG (anti-IgG) conjugated to horseradish peroxidase (HRP) are added. During the second incubation step, these HRP-conjugated antibodies are bound to any antigen-IgG antibody complexes previously formed. The

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wells are then washed to remove unbound HRP-conjugated antibodies and chromogen solutions containing tetramethylbenzidine and urea peroxide are added to the wells. If antigen-IgG antibody-anti-IgG immunocomplexes are present, the colorless chromogens are hydrolyzed by the bound HRP conjugate to a blue-colored product. The blue color turns into yellow after stopping the reaction with sulfuric acid. The amount of color intensity can be measured and is proportional to the amount of anti-HEV IgG antibody present in the sample. Wells containing samples without anti-HEV IgG antibodies remain colorless. A cut-off is calculated each run based on the mean absorbance value of three negative controls. Results are presented as a ratio between the individual absorbance of the samples (S) and the cut-off absorbance (CO). The decision point to distinguish a positive (> 1.0) from a negative (< 1.0) result is an S/CO ratio of 1.0. However, an S/CO ratio between 0.9 and 1.1 is considered a borderline result.

Further testing of borderline results was done using a strip immunoassay with purified recombinant HEV antigens, the recomLine HEV IgG/IgM assay (Mikrogen, Neuried, Germany). This assay uses separately applied antigens from two different HEV genotypes (the O2N, O2C and O3 antigens of genotypes 1 and 3 as well as the O2M antigen of genotype 1), which enables a high diagnostic sensitivity also for non-endemic regions with a high incidence of autochthonous hepatitis E (genotype 3). A test strip containing the recombinant HEV antigens on a nitrocellulose membrane is incubated with diluted serum from the patient, resulting in the binding of anti-HEV antibodies, if present in the serum sample, to the antigens on the strip. Unbound serum proteins are washed away and the strip is then incubated with anti-human antibodies (IgG specific in this study) coupled to HRP. Specifically bound antibodies are detected by means of a color reaction catalyzed by HRP. If an antigen-antibody reaction has taken place, a dark band appears at the corresponding locus on the strip. The test result is obtained by the sum of the point values of the separate bands that show an intensity equal or greater than a cut-off intensity. Antigens O2N and O2M are assigned two points each, antigen O3 is assigned three points and antigen O2C is assigned 4 points. A sum of points of 2 or less is considered a negative result, whereas 3 points is considered a borderline result and 4 points or more is interpreted as a positive result.

Results

From the 100 serum samples that were evaluated in this study using an indirect ELISA method, 14 were clearly positive (S/CO ratio ranging from 2.15 to 12.89) and 85 were unequivocally negative (S/CO ratio ranging from 0.01 to 0.89) for anti-HEV IgG. One result with an S/CO ratio of 1.09 was considered borderline and therefore evaluated with the recomLine strip immunoassay. This case was found to be negative for anti-HEV IgG.

Taken together, the anti-HEV IgG seroprevalence was 14% in this study. There was no significant difference in anti-HEV IgG seroprevalence according to sex, since cases were equally distributed between the 50 men ($n = 7$) and the 50 women ($n = 7$). Patients positive for anti-HEV IgG were aged between 21 and 77 years old, with a mean age of 54 years.

Discussion

Previous studies in Western Europe have yielded widely differing estimates of seroprevalence of HEV, even within the same countries. For example, reported figures range from 3.2% to 16.6% in France (4,5) and from 2.0% to 15.5% in Germany (6-8). This variability may partly be due to age differences of the population studied, as autochthonous hepatitis E seems to have a predilection for middle-aged and elderly men (2). While for HEV genotypes 1 and 2 in Africa and Asia it was recently modelled that the largest increases in prevalence occur between 5 and 20 years of age, the global modelling data for Europe are lacking (9). Due to the diversity of serological assays used in the available studies, the most important source of variation is probably the analytical performance of the assay used for detection of anti-HEV antibodies (3,10). In this study we have used an anti-HEV IgG ELISA test for which a superior diagnostic sensitivity compared to another commercial assay has been demonstrated (3). The higher sensitivity of this ELISA may be explained by the tendency of the recombinant antigens to associate into dimers that react more strongly with anti-HEV antibodies than linear monomeric antigens (11). In the case of a borderline ELISA result, further anti-HEV IgG testing was performed using a strip immunoassay that provides a high sensitivity for the detection of autochthonous hepatitis E. We observed an overall anti-HEV IgG seroprevalence of 14%. This study does not allow us to determine whether seroconversion has occurred in the context of autochthonous or travel-associated hepatitis E, but based on observations in neighboring countries to Belgium (12-14) we expect a large proportion of these cases to be locally acquired. Published seroprevalence rates of HEV in other Western European countries are as follows: 3.2% to 16.6% in France (4,5), 2.0% to 15.5% in Germany (6-8), 1.1% to 6.0% in the Netherlands (15-18), 5.3% to 16.2% in the UK (3,12,19,20) and 0.8% to 7.3% in Spain (21-23). As pigs may serve as a reservoir for HEV infection in humans, we calculated pig/inhabitant ratios by dividing the number of pigs by the number of inhabitants in these countries (data from the UK government, Department for the Environment, Food and Rural Affairs, 2009 and from the United Nations, Department of Economics and Social Affairs, Population Division, 2009). The pig/inhabitant ratio was 0.59 in Belgium, 0.23 in France, 0.33 in Germany, 0.73 in the Netherlands, 0.08 in the UK and 0.57 in Spain. It is clear from these data that a simple linear correlation between the

seroprevalence of HEV and the pig/inhabitant ratio does not exist. However, it should be kept in mind that HEV seroprevalence rates from different studies are difficult to compare due to large differences in the analytical sensitivity of the available anti-HEV IgG assays (3,10).

In conclusion, we present here the first assessment of the seroprevalence of HEV in Belgium performed in the geographical region representative for East and West Flanders. The observed seroprevalence of 14% seems to be in the upper range of what has been reported for other Western European countries, but a robust comparison is hampered by a lack of standardization of the various anti-HEV IgG assays used in individual studies.

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