The added value of hepatitis E diagnostics in determining causes of hepatitis in routine diagnostic settings in the Netherlands
Doting, M. H. E.; Weel, J.; Niesters, H. G. M.; Riezebos-Brilman, A.; Brandenburg, A.

Published in:
Clinical Microbiology and Infection

DOI:
10.1016/j.cmi.2017.02.026

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2017

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license. More information can be found on the University of Groningen website: https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment.

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.
The added value of hepatitis E diagnostics in determining causes of hepatitis in routine diagnostic settings in the Netherlands

M.H.E. Doting 1,*, J. Weel 2, H.G.M. Niesters 1, A. Riezebos-Brilman 1, A. Brandenburg 2

1) The University of Groningen, University Medical Center Groningen, Department of Medical Microbiology, Groningen, The Netherlands
2) Izore Centre for Infectious Diseases Friesland, Medical Microbiology, Leeuwarden, The Netherlands

ABSTRACT

Objectives: Hepatitis E virus (HEV) genotype 3 is endemic in Europe and an underdiagnosed and emerging (public) health issue. In recent years commercial enzyme immunoassays (EIAs) that detect antibodies to HEV more adequately, became available. We investigated the added value of this HEV serology in the diagnostic work flow to detect viral causes of recent hepatitis.

Methods: During a 2-year period (May 2013 to May 2015), HEV serology was added to the hepatitis work flow, consisting of serological detection of hepatitis viruses A, B and C (HAV, HBV, HCV), Epstein–Barr virus (EBV) and cytomegalovirus (CMV). Samples positive for HEV IgM were also analysed using PCR to detect HEV RNA. If positive, HEV sequencing was performed for genotyping purposes.

Results: In 235 out of 2521 patients (9.3%), a viral cause for hepatitis was found. Recent HAV, HBV, HCV, EBV or CMV infections were serologically diagnosed in 3, 34, 10, 69 and 42 patients, respectively. Seventy-eight patients (3.1%) had a recent HEV infection. In 49 of them, sufficient HEV RNA was present for genotyping. All patients were infected with HEV genotype 3.

Conclusions: In our region, an HEV infection is the most frequently diagnosed viral cause for recent hepatitis. These results indicate that, in a country where HEV is endemic, serological HEV diagnostics should be added to the standard work-up for viral hepatitis.

© 2017 European Society of Clinical Microbiology and Infectious Diseases. Published by Elsevier Ltd. All rights reserved.

Introduction

For decades, hepatitis E virus (HEV) has been known as a causative agent of human viral hepatitis. The HEVs are recently described as members of the genus Orthohepevirus in the family Hepeviridae. This genus comprises in total four major human genotypes. Genotypes 1 and 2 are strictly human viruses and are identified as a cause of epidemic hepatitis in developing countries, associated with waterborne and faecal–oral transmission. Genotypes 3 and 4 are swine viruses (common in domestic and wild pigs, boar and deer) and infect humans as an accidental host [1]. In industrialized countries, non-travel-related HEV infections are caused mainly by genotype 3 [2,3].

Usually HEV infections cause a mild self-limiting hepatitis. Incidentally, a fulminating hepatitis may develop. In immunocompromised patients, however, HEV genotype 3 viral infections may develop into a chronic infection with rapidly progressing cirrhosis [4–7].

Global immunization programmes have changed the epidemiology of viral hepatitis. In the Netherlands, viral hepatitis caused by hepatitis A virus (HAV) and hepatitis B virus (HBV) has decreased considerably. Recently, HEV has been recognized as an increasingly important cause of viral hepatitis [8,9]. Often, a recent HEV infection may be mistaken for drug-induced hepatitis, especially in the elderly where poly-pharmacy is more common. In the Netherlands, the majority of patients with an HEV infection are immunocompetent with no travel history [10]. HEV testing in healthy blood donors showed an overall IgG seroprevalence of 27% in 2011 [11]. The prevalence appears to increase with time and with age [12]. Commercially available HEV IgM enzyme immunoassay (EIA) kits now detect HEV infections more accurately, with a described sensitivity of 96.7% in immunocompetent
individuals and 83.3% in immunocompromised patients, and a specificity >99% [13,14]. We therefore investigated the value of adding HEV serology to our routine workflow of viral causes of hepatitis.

Methods

Between May 2013 and May 2015, we prospectively added HEV serology (IgM and IgG) for all samples submitted to our laboratory for detecting viral causes of hepatitis. The samples were obtained from patients seen by general practitioners (GPs) and medical specialists working in the province of Friesland. Indication for testing was at the discretion of the attending physician. This could be anything from mildly elevated liver enzymes only (alanine transaminase >45 IU/mL) to severe hepatitis or during regular monitoring, for example in potential hepatotoxic drug use.

Samples were tested for HAV, HBV, hepatitis C virus (HCV), cytomegalovirus (CMV), Epstein–Barr virus (EBV) and HEV. Work-up is illustrated in Fig. 1. In accordance with the manufacturer’s instructions, HAV, HBV and HCV serological testing was performed using an i2000 Architect analyser (Abbott, Wiesbaden, Germany). For EBV (VCA-IgM, VCA-IgG and EBNA-IgG) testing an indirect chemiluminescence-assay (CLIA) Liaison (DiaSorin, Saluggia, Italy) was used. For CMV (IgM and IgG, and if necessary IgG avidity) a VIDAS (bioMérieux, Marcy l’Etoile, France) was used during the first year and a CLIA Liaison was used during the second year.

A recent HAV infection was diagnosed by a positive anti-HAV IgM. A recent HBV infection was diagnosed by HBsAg, HBeAg and anti-HBc IgM positivity and first detection of chronic HBV infection was by positive HBsAg, negative anti-HBcore IgM and positive anti-HBc IgG, and 6 months later an unchanged serological profile. First detection of HCV infection was diagnosed by positive anti-HCV IgG confirmed by HCV-RNA positivity. A recent EBV infection was diagnosed by high positive VCA-IgM and low VCA-IgG titre and negative EBNA-IgG. A recent CMV was diagnosed by positive IgM titres and low IgG titres with a low avidity index of the IgG.

HEV IgM and IgG testing was performed using a Wantai ELISA kit (Biological Pharmacy Enterprise, Beijing, China). The Wantai HEV IgM kit is a capture EIA using a HEV ORF2 recombinant antigen conjugate. The Wantai HEV IgG ELISA kit is a binding EIA using microwell strips pre-coated with HEV ORF2 recombinant antigen.

Hepatitis E serology was interpreted as follows: if both IgM and IgG were positive the patient was considered to have had a recent HEV infection. If IgM was borderline (optical density/cut-off (OD/CO) ratios >0.9 and <1.1) and IgG was positive (OD/CO ≥1), an HEV RNA PCR was performed [15]. If PCR was positive, these patients were regarded as having had a recent HEV. If HEV IgM was found positive or borderline and IgG was negative, an HEV RNA PCR was performed and a follow-up sample was requested for serology. If HEV RNA PCR was found positive and/or subsequent HEV IgG seroconversion was detected in the follow-up sample, the patient was considered as suffering from a recent HEV infection.

Sera from all other HEV IgM-positive patients were analysed for HEV RNA as well. If HEV RNA was detected and the viral load was high enough for sequencing, a phylogenetic analysis was performed as described before [11,16].

This study was reviewed by the ethics commission of the University Medical Centre Groningen, who concluded that it did not fall within the scope of the rules on medical research involving human subjects. Therefore ethical approval was not applicable to our study.

Results

From May 2013 until May 2015, we received 2567 serum samples from 2521 patients for the diagnosis of viral hepatitis. The age

![Fig. 1](https://example.com/fig1.png) Flow diagram for the diagnosis of recent viral hepatitis.
of the patients varied from 6 months to 93 years (median 42 years). In 235 patients (9.3%), a viral cause of their hepatitis was found. A recent HAV was diagnosed in three patients. A recent HBV or first detection of chronic HBV was found in 34 patients. First detection of HCV occurred in ten patients. A recent EBV was found in 69 patients and a recent CMV was found in 42 patients (see Fig. 1).

**Recent HEV infections**

In 94 patients, a positive or borderline HEV IgM was detected. In 74 patients, both HEV IgM and IgG was found. In 45 of these 74 patients, HEV RNA was also detected. In eight patients, HEV IgM, but no HEV IgG, was found. In four of these eight patients HEV RNA was found, whereas in the remaining four patients no RNA was found and no HEV IgG seroconversion was observed in the follow-up sample. In 12 patients, a borderline IgM and positive IgG was detected but none of them were positive for HEV RNA. In summary, 78 patients (3.1% of all patients tested) were regarded as having a recent HEV infection. Among the 78 patients with a recent HEV infection, men were more often affected than women (ratio 2 : 1), whereas in our total study population men did not outnumber women. The median age of those recently affected by HEV was 56 years for women and 59 years for men (range 11–85 years) (see Fig. 2). Of the patients with a recent HEV, 68% were seen by medical specialists and 32% by GPs.

No seasonal pattern in HEV infections was observed and no multiple infections were diagnosed.

Seven patients with a recent HEV infection turned out to be immunocompromised. In four of them, HEV RNA was found. HEV infection resolved in all four of them, in two by discontinuing the immune-suppression, whereas in the other two patients this had to be combined with an oral ribavirin treatment.

**HEV IgG seroprevalence**

For the HEV seroprevalence rate calculation, patients with recent HEV were excluded. HEV IgG was found in 475 of 2443 patients (18.8%). IgG was detected in 20.4% of male patients, and in 17.6% of female patients. The seroprevalence steadily increased from 4.9% in those younger than 10 years, to 40.2% in those over 70 years of age.

**Phylogenetic analysis of HEV**

Forty-nine of 78 patients with a recent HEV infection tested positive for HEV RNA. In 30 patients HEV could also be genotyped (Fig. 1). All 30 appeared to be genotype 3 and 25 patients had subtype c (Fig. 3). No epidemiological link was observed.
Discussion

In our region, HEV infection appeared to be the most frequently diagnosed viral cause of hepatitis in patients seen by medical specialists and GPs. Physicians sent in serum samples for viral hepatitis testing if abnormal liver function (alanine transaminase >45 IU/mL) was found, either in patients with complaints of hepatitis or during regular monitoring for, for example, potential hepatotoxic drug use. This open approach, reflecting ‘routine diagnostics’, probably contributed to our finding that a viral cause of hepatitis was only discovered in a relatively low percentage of patients (9.3%). To our surprise, HEV was most prevalent. It outnumbered EBV and CMV, which are regularly observed causes of abnormal liver function and hepatitis in our population. In particular the GPs were quite often surprised by the outcome, as they frequently suspected alcohol consumption as the cause of the diseased liver.

In several other European studies looking for recent HEV infections, percentages are reported in the same range as in our study [17–20] or higher (up to 11%) [21–23]. Differences in regional epidemiology may cause this variability, although differences in test selection and study population may also play an important role. Despite the described high specificity and sensitivity of the Wantai ELISA, positive cases during early infection could have been missed. Borderline IgM with positive IgG was found in 12 patients. No HEV RNA was detected in these 12 patients, suggesting just missed. Borderline IgM with positive IgG was found in 12 patients. Wantai ELISA, positive cases during early infection could have been test selection and study population may also play an important role.

Despite the described high specificity and sensitivity of the Wantai ELISA, positive cases during early infection could have been missed. Borderline IgM with positive IgG was found in 12 patients. No HEV RNA was detected in these 12 patients, suggesting just missed. Borderline IgM with positive IgG was found in 12 patients. Wantai ELISA, positive cases during early infection could have been test selection and study population may also play an important role.

Of eight patients with a positive IgM and negative IgG, in four patients the recent HEV infection could be confirmed by detection of HEV RNA and a seroconversion to IgG positivity in the follow-up samples.

A limitation in our study is that detailed clinical information was not available for every patient. Although HEV serology alone is considered to be sufficient to diagnose a recent HEV infection in immunocompetent patients, it lacks sensitivity in the patient group most at risk of a complicated course of disease, the immunocompromised [24–26]. The results could have been more complete if PCR had been performed in all immunocompromised patients, allowing us to report in more detail on sensitivity levels in this group of patients. This is a risk in our test algorithm. However, in our open population only a minority is expected to be immunocompromised. These findings underline a need to keep educating physicians in our region to adequately inform the laboratory about the immune status of patients for whom tests are requested. For the ‘high-risk’ immunocompromised patient group testing primarily with PCR is advised, similar to HCV diagnostics.

In accordance with other findings in the Netherlands, HEV IgG seroprevalence in our total study population was 18.8%, suggesting that asymptomatic HEV infections do occur frequently [25,27]. HEV seroprevalence varies both between and within countries and is higher in individuals exposed to swine and wild animals. The predominance of HEV that we found might be related to the importance of the pig industry in the Netherlands compared with surrounding countries such as Belgium and northern France [28]. We also observed that older people were significantly more likely to test positive for HEV IgG, suggesting a lifelong cumulative exposure of HEV.

The notion of autochthonous zoonotic transmission is supported by phylogenetic studies that have shown that HEV strains circulating in human beings and pigs are closely related [10,29]. To clarify farm-to-table risk assessments, more studies on HEV circulation are necessary.

Although most zoonotic HEV cases are sporadic, a point-source foodborne HEV outbreak has been documented [30]. In our study, virus RNA sequences were not identical, and were found over a longer time period of 2 years, making a common source unlikely. If typeable, all HEV infections found in our patients were genotype 3. HEV genotype 3 is the endemic genotype causing viral hepatitis in our population. The majority of HEV–RNA-positive samples were genotype 3c. This is in accordance with the study of Rutjes et al. [29], who found that cluster 3c comprises 35% of animal and environmental HEV sequences and 75% of human HEV sequences.

In conclusion, HEV genotype 3 is more frequently diagnosed as a cause of viral hepatitis than HAV, HBV, HCV, EBV and CMV in our patients. Our results warrant the addition of HEV diagnostics in the standard diagnostic work up for recent viral hepatitis, both in a hospital and in a GP setting.

Funding

The authors have no support or funding to report.

Transparency declaration

The authors declare no conflict of interest related to this article. Ethical approval did not apply to this work.

Authors’ contributions

MHED, AB and AR-B drafted the manuscript, AB contributed to the design of the study, JW and HGMN provided a detailed critical review of the manuscript. All authors approved the final manuscript.

Acknowledgements

We would like to thank Gatske Kraak and Yvonne Roelofs for their help with GLIMS database searches. We are also grateful to Mieke Vogelzang for setting up the database, and Randy Poelman and Renze Borger for the phylogenetic analysis. Advice in English grammar and style given by Fiona Nijkamp-Lyell has been of great help.

References


