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Original article

Estimation of the hepatitis E assay-dependent seroprevalence among Croatian blood donors

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ABSTRACT

Background. – Seroprevalence of hepatitis E virus (HEV) in blood donors presenting to the Croatian Institute of Transfusion Medicine was assessed with 4 available tests (3 ELISA tests and 1 immunoblot (IB) test).
Materials and Methods. – In October and November 2014, a total of 1,036 serum samples of blood donors were collected for the study. Samples were primarily tested for total HEV antibodies by Dia.Pro HEV Ab test (a). All reactive samples were tested by ELISA tests: Dia.Pro HEV IgG (b) and IgM (c), Mikrogen *recom*Well HEV IgG_old (d) and IgM_old (e), *recom*Well HEV IgG_new (f) and IgM_new (g), and IB Mikrogen *recom*Line HEV IgG (h) and IgM (i). HEV IgM reactive samples also positive by the IB were further tested for HEV RNA.
Results. – There were 21.5% of samples reactive for total HEV antibodies (a). Seroprevalence of HEV IgG according to the b, d, f and h tests was 20.2%, 9.6%, 18.1% and 17.8%, respectively. Seroprevalence of HEV IgM according to the c, e, g and i tests was 4.4%, 1.5%, 2.0% and 1.7%, respectively. Out of 46 HEV IgM (Dia.Pro HEV IgM) positive samples, 18 (39.1%) were also positive by IB. HEV RNA was not detected in any of those samples. There was a significant association between age and HEV seroprevalence ($P < 0.001$).
Conclusion. – Different HEV antibody detection assays showed a high HEV IgG seroprevalence in Croatian blood donors. Among HEV IgG and HEV IgM positive samples HEV RNA was not detected.

Mots clés :
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RÉSUMÉ

Objectif La séroprévalence du virus de l'hépatite E (VHE) a été évaluée à l'aide de 4 tests disponibles (3 ELISAs et 1 test immunoblot [IB]) chez les donneurs de sang se présentant à l'Institut croate de médecine transfusionnelle.
Matériels et méthodes. – En octobre et novembre 2014, 1036 échantillons de sérum de donneurs de sang ont été prélevés pour cette étude. La présence d'anticorps anti-VHE totaux a été initialement testée dans ces échantillons à l'aide du test Dia.Pro HEV Ab (a). Tous les échantillons réactifs ont ensuite été testés avec les tests ELISAs IgG Dia.Pro HEV (b) et IgM (c), Mikrogen recomWell HEV IgG_old (d) et IgM_old (e), recomWell HEV IgG_new (f) et IgM_new (g), et le test IB Mikrogen recomLine HEV IgG (h) et IgM (i). L'ARN VHE a également été recherchés dans les échantillons montrant une réactivité IgM anti-VHE et trouvés positifs en IB.
Résultats. – Un total de 21,5 % des échantillons étaient réactifs pour les anticorps anti-VHE totaux (a). La séroprévalence IgG anti-VHE était respectivement de 20,2 %, 9,6 %, 18,1 % et 17,8 % avec les tests b, d, f et h. La séroprévalence IgM anti-VHE était respectivement de 4,4 %, 1,5 %, 2,0 % et 1,7 % avec les tests c, e, g et i. Parmi 46 échantillons IgM VHE positifs (Dia.Pro HEV IgM), 18 (39,1 %) étaient également positifs en IB. L'ARN du VHE n'a été détecté dans aucun de ces échantillons. Une association significative a été observée entre l'âge des donneurs et la présence d'anticorps anti-VHE ($p < 0{,}001$).

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Conclusion. – Différents tests de détection des anticorps anti-VHE ont montré une forte séroprévalence des IgG anti-VHE chez les donneurs de sang croates. L'ARN VHE n'a pas été détecté dans les échantillons IgG et IgM VHE positifs.

1. Introduction

Hepatitis E virus (HEV) is a long-standing public health problem in poorly developed or high endemic countries in Asia, Middle Africa and the Middle East, where HEV genotypes 1 and 2, with fecal-oral transmission, result in thousands of patients. The annual morbidity of hepatitis E in these countries in 2005 has been estimated to 3.4 million cases and 70,000 deaths, most commonly associated with pregnancy or comorbidity with other chronic liver diseases [1]. Epidemic hepatitis E in developed countries indicates an increase not only in "imported" (acquired during stay in high endemic countries) but also in autochthonous hepatitis E, genotype 3 (and genotype 4 in Japan and China), which refers to transmission of infection mostly by inadequately heat-treated food, pork meat and processed products.

Hepatitis E virus infection is the most common asymptomatic, self-limited infection, and for a long time it was considered an acute disease without progression to chronic infection. However, HEV infections with chronic and high mortality characteristics are described in immunocompromised patients and organ recipients [2,3]; that is why HEV prevalence studies have been initiated in blood and organ donors [4] or HIV/AIDS patients [5].

Data on HEV seroprevalence in the population of blood donors and in the general population vary, resulting from different sensitivity and specificity of the tests used, but also from differences in the seroprevalence per se among countries and regions within countries, as well as among particular age groups. HEV seroprevalence in blood donors in eastern Japan is 5.6% and 1.8% in western Japan [6], 9.7% (Wantai test) and 8.1% (MP Diagnostic test) in New Zealand [7], 32.6% in China [8], 52.5% in southwestern France [9], 16% in Southwest England [10], 6.8% in Germany [11], 10% to 20% among Danish blood donors, depending on the assay used [12], 2.9% in Greece [13], 49% in Italian region Abruzzo (0.6% also anti-HEV IgM positive) [14] and among Swiss blood donors in Vaud 4.9% (MP Diagnostics), 4.2% (Dia.Pro) and 21.8% (Fortress) [15]. Several studies showed increased prevalence with age [12–14]. The risk of HEV transmission by organ transplantation exists [16], but testing for HEV is still not mandatory for tissue and organ donors.

In the past few years, HEV has become a topic in transfusion medicine because its occurrence is increasing in developed countries, thus implying the risk of viral transmission *via* blood transfusion. In Croatia, blood donors are not routinely screened for HEV and there are no data on HEV seroprevalence in the general population. Seroprevalence of hepatitis E virus in blood donors presenting to the Croatian Institute of Transfusion Medicine was assessed with 4 available tests (3 ELISA tests and 1 immunoblot test).

2. Material and Methods

2.1. Collection of blood donor samples

In October and November 2014, a total of 1,036 serum samples of blood donors were collected by the Croatian Institute of Transfusion Medicine (CITM), from the following counties: Bjelovar-Bilogora ($n = 175$), Koprivnica-Križevci ($n = 103$), Krapina-Zagorje ($n = 186$), Požega-Slavonia ($n = 199$), Sisak-Moslavina ($n = 79$) and Zagreb ($n = 294$). All donors had previously completed a medical questionnaire to verify that they fulfilled the criteria for blood donation and had given their informed consent. The study was approved by the CITM Ethics Committee.

2.2. Testing

All tests were performed at the CITM Department for Blood Borne Diseases Diagnosis, according to manufacturers' instructions. Samples were first tested for total HEV antibodies using a commercial enzyme immunoassay, HEV Ab (Dia.Pro Srl, Milan, Italy) on a Gemini analyzer. All weakly positive ($S/CO \leq 2$) samples were retested. All reactive samples were tested for HEV IgG and IgM antibodies by three commercial ELISA assays: HEV IgG and IgM (Dia.Pro Srl, Milan, Italy) on Gemini analyzer; *recom*Well HEV IgG_old (sensitivity 1.4 IU/mL) and IgM_old, *recom*Well HEV IgG_new (sensitivity 0,29 IU/mL) and IgM_new (improved specificity, 98.6%) (Mikrogen GmbH, Neuried, Germany) on Evolis analyzer; and one immunoblot test: *recom*Line HEV IgG and IgM (Mikrogen GmbH, Neuried, Germany) on Dynablot Plus analyzer. All HEV IgM reactive samples confirmed by *recom*Line HEV IgM were further tested for the presence of HEV RNA at the Croatian Veterinary Institute. Viral RNA extraction (QIAamp viral RNA Mini Kit®, Qiagen, Hilden, Germany) was performed on 140 μL of each serum sample. A real time RT-PCR protocol for detecting a highly conserved fragment within ORF3 was employed [17]. Amplification was done in a Rotor-Gene Q machine (Qiagen, Hilden, Germany) by use of commercially available kits (Rotor-Gene Probe RT-PCR kit, Qiagen, Hilden, Germany) according to the manufacturer's instructions.

2.3. Statistical analysis

On data processing, descriptive and inferential statistics were used. The number of participants according to sex is expressed by absolute frequency, and according to age by median and range. Comparison of proportions was used on statistical analysis between male and female, and Mann Whitney test for comparison of results according to age. Kruskal–Wallis test was used for statistical analysis of results among counties. For interrelationship of individual tests, Cohen's kappa coefficient was applied. The level of statistical significance was set at $P < 0.05$.

3. Results

3.1. Overall HEV seroprevalence

The study included 1,036 blood donors, 913 (88.1%) male and 123 (11.9%) female, median age 47 years (95% confidence interval [CI], 45 to 48 years), range 18–69 years. Out of 1,036 collected blood donor samples, 223 samples were reactive for total HEV antibodies (21.5%, 95%CI 19.1%-24.1%). These 223 reactive samples were further submitted to four different IgG and IgM tests, three ELISA tests: Dia.Pro HEV IgG and IgM, Mikrogen *recom*Well HEV IgG_old and IgM_old, *recom*Well HEV IgG_new and IgM_new, and