PEER REVIEW HISTORY

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ARTICLE DETAILS

<table>
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<tr>
<th>TITLE (PROVISIONAL)</th>
<th>Epidemiology of HEV in the Mediterranean basin: 10-year prevalence in Italy</th>
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<tr>
<td>AUTHORS</td>
<td>Lanini, Simone; Garbuglia, Anna; Lapa, Daniele; Puro, Vincenzo; Navarra, Assunta; Pergola, Catia; Ippolito, Giuseppe; Capobianchi, Maria</td>
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VERSION 1 - REVIEW

| REVIEWER                  | Prof. Dr. Andy I.M. Hoepelman Medisch afdelingshoofd Interne Geneeskunde & Infectieziekten | Divisie Interne Geneeskunde en Dermatologie, MS Interne Geneesk. en Infectieziekten |
|---------------------------|------------------------------------------------------------------------------------------|
| REVIEW RETURNED           | 09-Jan-2015                                                                               |

GENERAL COMMENTS

major:

This is an interesting study, however, there is a varying diagnostic value between the available assays. The type of assay was not given.

Moreover, there is among experts a general consensus that serological screening alone may be insufficient to diagnose HEV and should be complemented with detection of HEV by PCR. This was not discussed in the text nor tested.

A study which shows this in MSM was not referenced:


Minor: typo on page 9 line 15/16 apparently no reference was found by the reference manager.

<table>
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<tr>
<th>REVIEWER</th>
<th>Jacques Izopet Toulouse University - France</th>
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<td>REVIEW RETURNED</td>
<td>20-Jan-2015</td>
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GENERAL COMMENTS

This study by Lanini and colleagues is a ten-year anti-HEV IgG seroprevalence study carried out in Italian individuals from Roma area who underwent anti-HIV screening (2002-2011). The main findings are a seroprevalence of 5.38% and an increased risk of HEV infection in men who have sex with men (p=0.04) and in subjects born outside Italy (p=0.027).

This study has several limitations:

1. The limit of detection of the serologic assay used in this study is not provided. It has been reported that the assay sensitivity is a major factor influencing anti-HEV IgG seroprevalence data.
2. The 5.38% seroprevalence found in Roma area must be compared to seroprevalence data found in different European countries based on sensitive assays.
3. The population composed mainly of young adults is quite homogeneous for age, a key parameter associated to HEV seroprevalence.
4. Food habits (major risk for HEV infection) were not assessed in the studied population.

**VERSION 1 – AUTHOR RESPONSE**

**Reviewer Name** Hoepelman

**Institution and Country** Prof. Dr. Andy I.M. Hoepelman

Universitair Medisch Centrum Utrecht

Please state any competing interests or state ‘None declared’: none

**Major Remark 1**

This is an interesting study, however, there is a varying diagnostic value between the available assays. HEV type of assay was not given.

Moreover, there is among experts a general consensus that serological screening alone may be insufficient to diagnose HEV and should be complemented with detection of HEV by PCR. This was not discussed in the text nor tested.

**Answer**

We thanks the reviewer for appreciating our study and for the opportunity he provided us to better define the aim of the paper.

We agree that serology alone is insufficient to yield a reliable diagnosis of HEV infection. However the diagnosis of either acute or persistent HEV infection is beyond the scope of our study (in that case a PCR assay would have been crucial). In fact the study was mainly aimed to assess whether the prevalence of anti-HEV IgG (as proxy of past, not current, infection with HEV) significantly changed between different groups (e.g. MSM and HIV positive subjects) according to exposure to certain risk factors.

According to you suggestion we revised the text in order to eliminate any potential ambiguity *(see tack change manuscript lines: 45; 61; 83; 107-108; 163; 183)*

**Major Remark 2**

A study which shows this in MSM was not referenced, Van Welzen J Acquir Immune Defic Syndr. 2012 Jun 1;60(2):e65-7. doi:10.1097/QAI.0b013e318251b01f

**Answer**

The reference has been now quoted *(see ref 14)*

**Minor Remark**

Typo on page 9 line 15/16 apparently no reference was found by the reference manager
Answer

Thanks for noticing the mistake. It has been currently corrected (see ref 12)

Reviewer 2

Reviewer Name Jacques Izopet

Institution and Country Toulouse University France

Please state any competing interests or state 'None declared': None declared

This study by Lanini and colleagues is a ten-year anti-HEV IgG seroprevalence study carried out in Italian individuals from Roma area who underwent anti-HIV screening (2002-2011). The main findings are a seroprevalence of 5.38% and an increased risk of HEV infection in men who have sex with men (p=0.04) and in subjects born outside Italy (p=0.027).

This study has several limitations:

Remark 1

The limit of detection of the serologic assay used in this study is not provided. It has been reported that the assay sensitivity is a major factor influencing anti-HEV IgG sero-prevalence data (Bendall J Med Virol 2000; Mansuy Emerg Infect Dis 2011).

Answer:

We agree that assay sensitivity is a major factor in influencing HEV seroprevalence; high variability in the performances has been observed between currently available commercial tests detecting anti-HEV IgG, both in the acute setting and in the measurement of seroprevalence (the references indicated by the reviewer have been quoted in the revised version); moreover no gold standard has been defined for HEV antibody measurement. (see tack change manuscript lines 236-245 and ref 27; 28; 29; 30; 31)

In a recent study [Pas SD J Clin Virol 2013; 58:629-634] 8 different HEV-IgG and IgM assays were compared. In this study, the performances of the test used in our study (DIA.PRO) were similar to those of other assays (i.e MP diagnostics, DSI, DRG). The authors conclude that “current commercial HEV ELISAs could be used to diagnose HEV genotype 3” (the most diffused HEV genotype in Europe) adequately in a clinical setting. (see ref 31)

Clinical sensitivity of the serological assay used in our study was based on our experience gained in the acute HEV infection setting; with our test, HEV-specific IgG development was observed in 40 over 41 patients with PCR-positive or IgM-positive HEV diagnosis (97.6% sensitivity). The only case who did not develop IgG response was immuno-suppressed; therefore we concluded that our test has suitable clinical sensitivity for detecting HEV sero-positivity. However, the duration of HEV IgG has not been clearly established so far, hence it is difficult to infer the test sensitivity for past infection (seroprevalence). (see tack change manuscript lines 119-120)

Remark 2

The 5.38% seroprevalence found in Roma area must be compared to seroprevalence data found in different European countries based on sensitive assays.

Answer
Available data suggest high variability of seroprevalence rate in European countries, ranging between 0.26% (Greece, Stefanidis et al. 0.26% to 31%, 2004) and 52% (Mansuy et al. EID 2011) in blood donors. However, assay-specific differences in sensitivity are at least in part responsible for the observed differences; in fact, discrepancies between anti IgG prevalence assayed with different serological test have been described [Rossi-Tamisier M. J Clin Virol 2013; 56:62-64; Bendall J Med Virol 2010; 82:796-805]. For these reason comparative analysis on seroprevalence data based on different HEV assays should be carefully interpreted [Khudyakov Y, Kamili S Virus research 2011, 161:84-92]. (see tack change manuscript lines 236-240 and ref 27; 28; 29; 30)

Remark 3
The population composed mainly of young adults is quite homogeneous for age, a key parameter associated to HEV seroprevalence.

Answer
This is reported and potential limitation discussed (see tack change manuscript lines 226-235)

Remark 4
Food habits (major risk for HEV infection) were not assessed in the studied population.

Answer
We agree that food habit is a major risk for HEV infection. Unfortunately, this factor could not be taken into account in our study, since the appropriate information was not available in the database, that was established for a purpose not correlated to HEV and other food-borne infections, as stated in the methods. In fact, as stated in the background, we specify that “the aim of this study is to explore potential risk factors (other than food and occupational exposure during animal handling)”. This limitation is acknowledged (see tack change manuscript line 247)
Epidemiology of HEV in the Mediterranean basin: 10-year prevalence in Italy

Simone Lanini, Anna Rosa Garbuglia, Daniele Lapa, Vincenzo Puro, Assunta Navarra, Catia Pergola, Giuseppe Ippolito and Maria Rosaria Capobianchi

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