

Enterically transmitted hepatitis in the third millennium in northeastern Romania

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Abstract. While the incidence of hepatitis A virus (HAV) infections has decreased in the last few years, the incidence of hepatitis E virus (HEV) is increasing in developed countries. Both infections remain a worldwide reality, strongly related to socio-economic conditions. We retrospectively analyzed the medical files of patients with viral hepatitis hospitalized in the Infectious Disease Hospital Iasi, Romania between 2018 and 2019. The serological confirmed cases of HAV and HEV infections were included in the analysis; included in our analysis were 269 HAV-infected patients. The most affected were males (53.9% cases) aged between 8 and 15 years (53.6% cases). Severe infections were recorded at admission in 2.24% cases and evolution was favorable under supportive and symptomatic treatment. Only three adult males, with no recent history of travel, were confirmed with HEV infection. Even though we have identified a small number of cases, recent studies performed in our region found a seroprevalence of 32.5% in the general population, possibly related to zoonotic transmission. While outbreaks of HAV infection are still noted in our region, a lack of hospitalized HEV-infected patients suggest that most cases are asymptomatic or underdiagnosed. HEV infection remains an underreported disease, possibly due to misdiagnosis, subclinical or dual hepatitis infection, but with a significant risk in vulnerable categories, such as pregnant women or immunosuppressed patients.

Introduction

Hepatitis A and E are common acute, self-limited viral infections produced by hepatitis A virus (HAV) and hepatitis E virus (HEV), respectively, generally acquired by the fecal-oral route, via either person-to-person contact or ingestion of contaminated food or water (1,2). A rare modality of transmission is blood transfusion, in the case of contaminated blood or when the donor is in the viremic prodromal phase of the infection at the time of blood donation which is more frequently encountered in the transmission of hepatitis B or C (3). HAV infection affects 120 million people annually worldwide, especially children (4). By contrast, World Health Organization (WHO) estimated that HEV causes 20 million new infections annually and over 55,000 deaths (5). Identified for the first time in 1983, in sporadic cases of infections in low-income countries from Asia and Africa, the number of cases of HEV infections has continuously increased over the last 15 years, especially in developed countries (6). Currently, HEV infection represents a worldwide public health issue (7). Netherlands reported a 5-fold increased incidence of HEV infections in 2014 compared to previous years, while the number of cases in Germany went up 40-fold in the last 10 years (8,9).

In both HAV and HEV infections, the levels of endemicity are strongly correlated with hygienic and sanitary conditions of each geographic area and are divided into areas with high, intermediate or low endemicity as defined by results of age-seroprevalence surveys (10,11). A special interest is given to middle-income countries with varied epidemiological profiles but are an important factor in the spread of fecal-oral transmitted infections through international economic and social globalization (12,13). This process of socio-economic interdependence may lead to significant changes in the epidemiological patterns of infections. Located in southeastern Europe, Romania is part of this globalization process due to a growing number of foreign visitors or international food trade. Romania has an intermediate endemicity of HAV infection (14). On the other hand, the seroprevalence of HEV infection in Romania is still unknown. Based on studies of seroprevalence of IgG HEV antibodies, an incidence of 12% was noted in 2009. Further studies revealed an increased

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number of cases in Romania by 14% among medical staff and up to 28% among the general population (6,14).

In this context, the aim of the present study was to evaluate the most recent epidemiological, clinical, biological and therapeutic data concerning HAV and HEV infections based on clinical practice at 'Sf. Parascheva' Clinic Hospital of Infectious Diseases (Iasi, Romania).

Patients and methods

Patients. This was a retrospective analysis of hospital-based medical records of patients with a diagnosis of hepatitis A and hepatitis E viral infections hospitalized between 2018 and 2019 at 'Sf. Parascheva' Clinic Hospital of Infectious Diseases from Iasi. The inclusion criteria for the study consist of in-patients with a diagnosis of HAV and HEV infections confirmed by blood tests and serological tests. Patients with hepatic disease (other than HAV or HEV) were excluded from the study.

Data collection. The following data were collected: Demographic data, medical and medication history, clinical data, blood and urine tests, serological tests, treatment administered and outcome. All blood and urine tests were performed by the central hospital's laboratory. The District Public Health Directorate, Iasi, Romania, performed the serology tests (IgM HVA and IgM HVE, respectively).

Statistical analysis. Only cases with a complete dataset were included in the statistical analysis. Data were analyzed by descriptive statistics, where applicable. Correlation between demographic parameters, clinical data and outcome was performed using Pearson test in XLSTAT version 2019 software. Kendall's Tau correlation coefficients were calculated (11). Statistical analysis was performed using Statistical Software for Excel (XLSTAT) version 2019.

Results

Our analysis consisted of 272 HAV- and HEV-infected patients with complete dataset, of which 98.9% were HAV infections and only 1.1% were HEV infection cases. There were no recorded cases of HAV and HEV coinfection. Patients were hospitalized at 'Sf. Parascheva' Clinic of Infectious Diseases in Iasi between 2018 and 2019.

Patient characteristics. A wide range of age was noted for the HAV-infected patients (between 11 months and 47 years), but as expected, most patients were aged 8-15 years (53% cases) (Fig. 1).

Most of the patients were male (53.9% cases) with epidemiological context of outbreaks of HAV infections from rural areas (87.73% cases) (Table I).

Comorbidities were recorded in 12 cases (6.3%) of HAV infections, the majority represented by digestive disorders (41.66%, 5 cases). Hypertension (24.99%, 3 cases), chronic peripheral venous insufficiency (8.33%, 1 case), chronic kidney disease (8.33%, 1 case), urinary infection (8.33%, 1 case) and oncological pathology (8.33%, 1 case) were also noted in this group of patients (Fig. 2).

In regards to HEV infection, only 3 male patients with a mean age of 54.3 years were identified in the hospital database (Table I). None of the patients had international travel history within the last 2 months, but only one patient described previous contact with farm animals and possible contaminated water.

Clinical findings. Regarding the onset symptomatology, most of the HAV-infected patients (57.24%, 154 cases) had digestive disturbances (abdominal pain, nausea) and/or jaundice (52.78%, 142 cases) at clinical examination. Flu-like onset was presented in 31 patients (11.52%), fatigue and neurasthenia in 17 patients (6.31%) and pruritus was found in 7 patients (2.6%) (Fig. 3).

Regarding the HEV cases, the patients described a typical onset of infection with digestive symptoms (nausea, vomiting, abdominal pain) in 2 cases or with flu-like associated with somnolence and asthenia in the third case.

Laboratory findings. In the HAV cases, biological investigations revealed cholestasis in 59.65% of the cases. Based on the intensity of cholestasis syndrome, it was found that most patients had the moderate form of the disease, with a slight significant difference between adults (67.19%) (Fig. 4) and children (54.43% cases) (Fig. 5).

During an average hospitalization period of 12 days (maximum period of 36 days), the evolution was favorable with a mean decrease of 83% for alanine transaminase (ALT) and 60% for bilirubin.

In HEV, jaundice with significant cytolysis (transaminases increased up to 100-fold) and moderate cholestatic syndrome were noted in all 3 cases.

HEV infection had a self-limited evolution under hepatoprotective treatment with jaundice remission and significant decrease in cytolysis and cholestasis in up to 16 days of hospitalization (Fig. 6). Extrahepatic manifestations were not identified in all 3 cases and associated disorders (diabetes mellitus, hypertension, pre-renal failure) were not aggravated by viral infection.

Evolution. After hospital isolation, diet, strict hygiene, hepatoprotective treatment and isoprinosine administration (in 2.5% of cases), over 95% of the HAV-infected patients showed a favorable evolution, with only 5 cases of relapse in the first 3 months post-first infection.

There was a weak association between duration of hospitalization and age (Kendall's tau=0.198; Fig. 7) but a stronger correlation with severity of liver dysfunction (Kendall's tau=0.297, Fig. 8).

The cases diagnosed with HEV, found in adult men, had a favorable evolution, with a hospital stay of ~2 weeks.

Discussion

In this retrospective study, we evaluated the epidemiological and clinical-biological evolution of patients with HAV and HEV infections hospitalized at 'Sf. Parascheva' Clinic Hospital of Infectious Diseases in Iasi in northeastern Romania. Only laboratory-confirmed cases with clinical criteria were included. Of the total number of patients hospitalized in our

Table I. Characteristics of the HAV and HEV patients (N=272).

Characteristics	HAV infection	HEV infection
Mean age \pm SD (years)	13.31 \pm 8.56	54.33 \pm 3.06
Male/female ratio	145 (53.9%)/124 (46.1%)	3 (100%)/0 (0%)
Residence area - rural, n (%)	236 (87.73%)	2 (66.67%)
Symptomatology, n (%)	168 (62.45%)	3 (100%)
Comorbidities, n (%)	12 (4.46%)	2 (66.67%)

HAV, hepatitis A virus; HEV, hepatitis E virus.

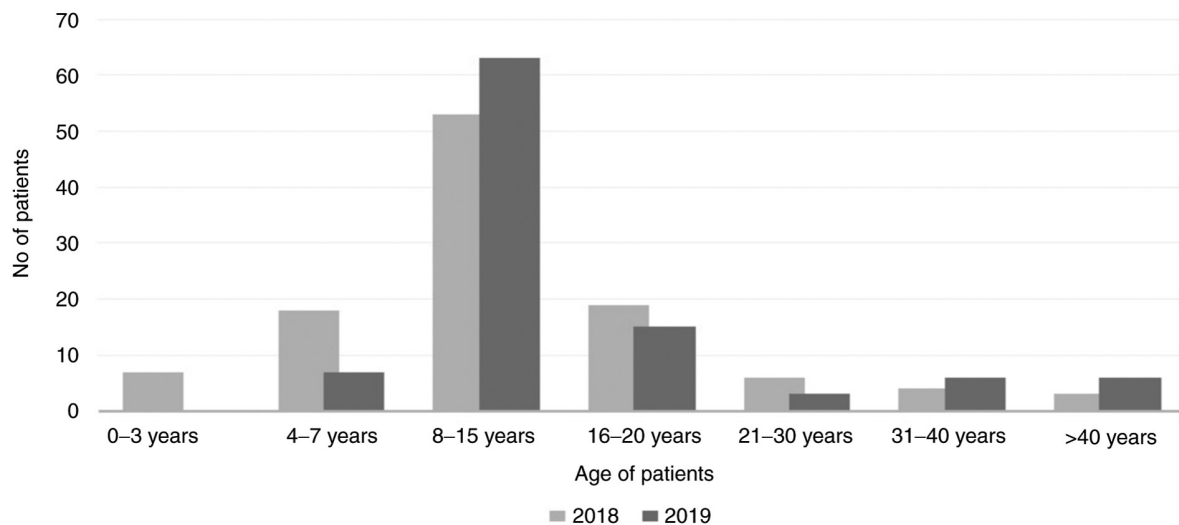


Figure 1. Distribution of HAV infections by age and year. HAV, hepatitis A virus.

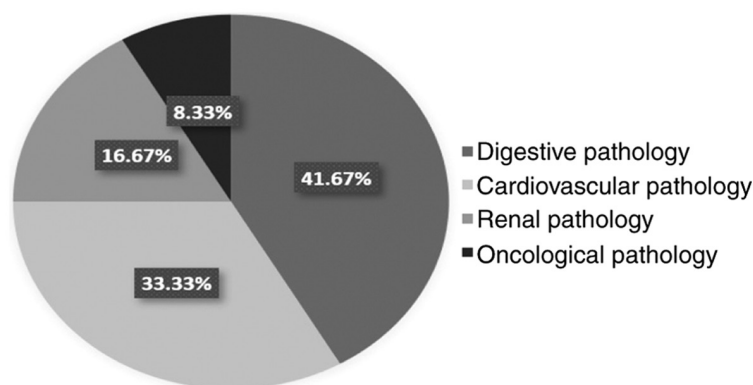


Figure 2. HAV-infected patients distribution by comorbidities (%). HAV, hepatitis A virus.

clinic during the analyzed period, 272 cases were included in our study. The majority of these cases originated from rural areas with poor sanitary conditions and/or bad hygienic habits. Moreover, in some cases with HAV disease, a familial aggregation was noted.

Hepatitis A infection occurs worldwide, sporadically or in an epidemic form. Globally, an estimated 1.4 million cases occur each year (15). The US reported an increase in the incidence of HAV by 294% during 2016-2018 compared

to 2013-2015 and in 2017, more than 650 people in the state of California were infected with hepatitis A (including 417 hospitalizations and 21 deaths) (16).

International outbreaks have occurred via importation of contaminated food from areas where HAV is endemic (17). In some circumstances, seemingly sporadic occurrences may reflect cases from geographically distant outbreaks. In one report, for example, 213 cases of hepatitis A were detected in 23 schools in Michigan and 29 cases in 13 schools in Maine;

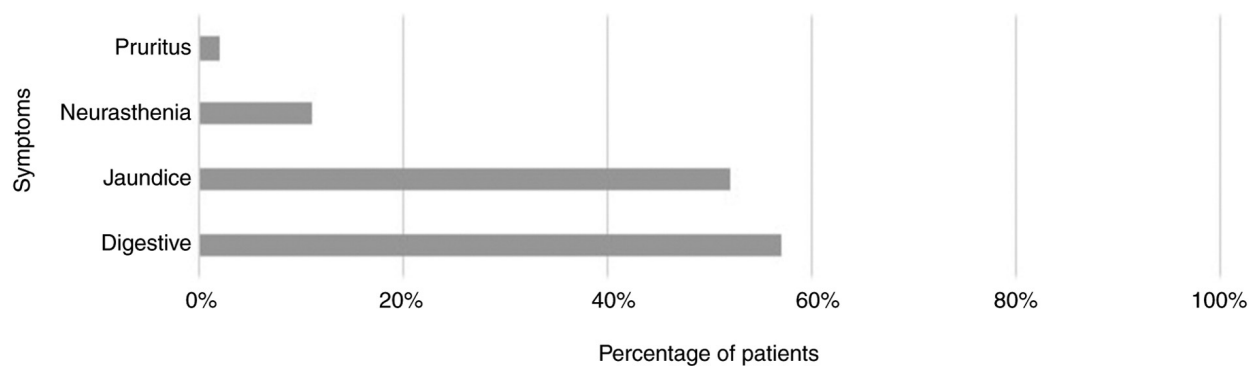


Figure 3. Distribution of symptoms in the HAV study group. HAV, hepatitis A virus.

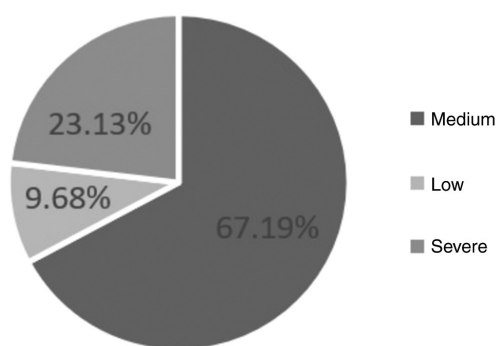


Figure 4. Severity of HAV in the adults. HAV, hepatitis A virus.

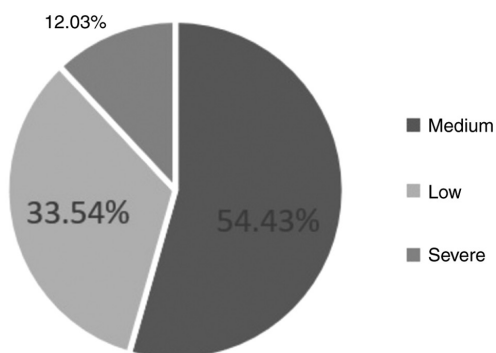


Figure 5. Severity of HAV in the children (0-18 years). HAV, hepatitis A virus.

all were related to contaminated frozen strawberries from a common source (18).

The epidemiological characteristics of HAV disease recorded in our study are in line with other Southeastern European countries (19,20). Tsankova *et al* found in Bulgaria recurrent outbreaks with time and geographic fluctuations. Similar with our data, male gender and children aged less than 9 years are the main risk factors associated with spread of the disease (20).

However, adult patients appear to be more frequently affected in the last few years since we found 9% of cases in 2018 and ~15% of cases aged over 25 years. Even though we have noted this increase in the age of HAV-infected patients

during this period, the incidence of this infection is uncommon in patients over 50 years. When the serological tests for HAV infection in 3 male patients aged over 50 years were negative, we took into consideration HEV infection.

In a recent study performed in northwestern Romania, the mean age of HEV-infected patients was 50.6 years compared to 39.1 years for HAV infection (21). The diagnosis was based on clinical and serological IgM anti-HEV tests. We must mention that only three other cases were confirmed in our clinic in the last 11 years and is not a frequent cause of viral hepatitis in our country.

The cases of HEV previously reported, diagnosed and treated in the infectious diseases clinic in Iași were not numerous, but, nevertheless, recent studies conducted in our region found a seroprevalence of 32.5% in the general population, which indicates the fact that most cases are possibly related to zoonotic transmission (22) similar to the way of transmission of leptospirosis (23,24). Other studies show a seroprevalence rate of IgG HEV antibodies up to 17% in Romania, comparable to other east European countries (e.g. 20.9% in Bulgaria, 15% in Serbia) (25).

In 2014, research on the evidence of HAE infection in pigs and humans in the northeastern part of Romania showed a seroprevalence of 17.14% (12/70) and 12.82% (10/78), in the tested human sera. The authors also reported that HEV infections were found in middle-aged adults exposed to the virus through contact with pig farms (26,27).

The genotype 4 of HEV is the most frequently detected in the European area and is involved especially in cases of zoonotic transmission, including undercooked deer meat, wild boar meat, pig liver sausage, and internal organs of animals (28,29). Information regarding other possible routes of transmission (e.g. blood transfusions, organ donors, vertical transmission) are limited in our region (30).

The risk of infection is increased in travelers in endemic areas (31). However, we could not identify a clear epidemiological context for our HEV-infected patients. Most patients with acute HEV are asymptomatic or mildly symptomatic, but severe cases were noted in middle age male or elderly patients, especially with comorbidities. The HEV infection is often associated with extrahepatic manifestation, including central nervous system (CNS) disorders, acute pancreatitis, glomerulonephritis or hematological abnormalities (32-34) which can

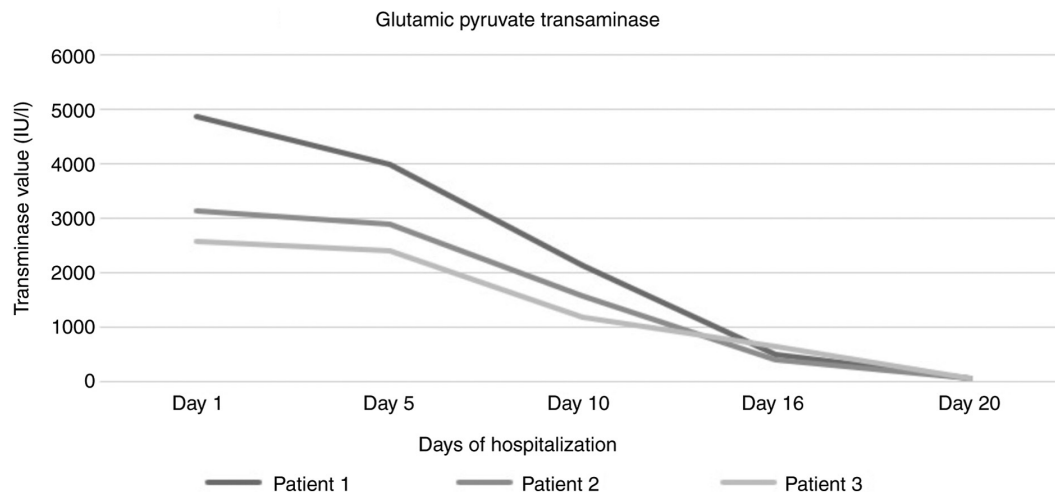


Figure 6. Evolution of cytolysis (TGP, glutamic pyruvate transaminase) in all 3 cases with HEV. HEV, hepatitis E virus.

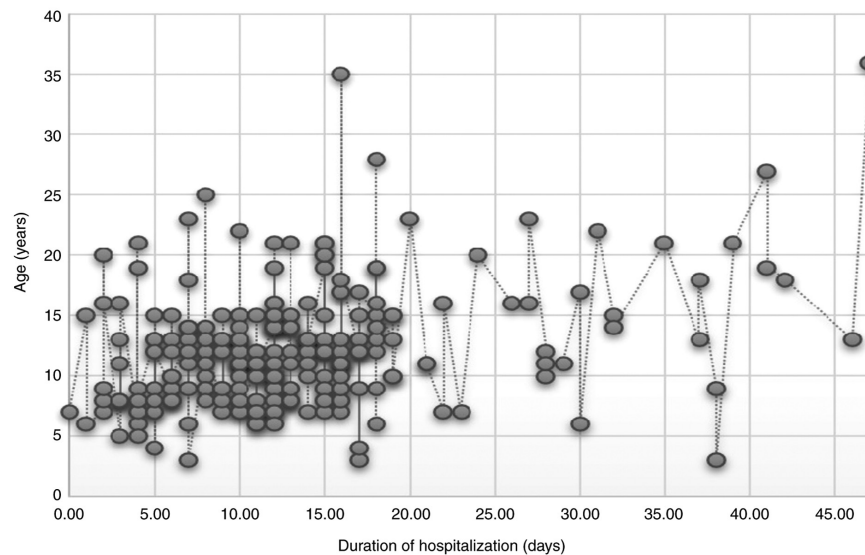


Figure 7. Correlation between age and duration of hospitalization (days).

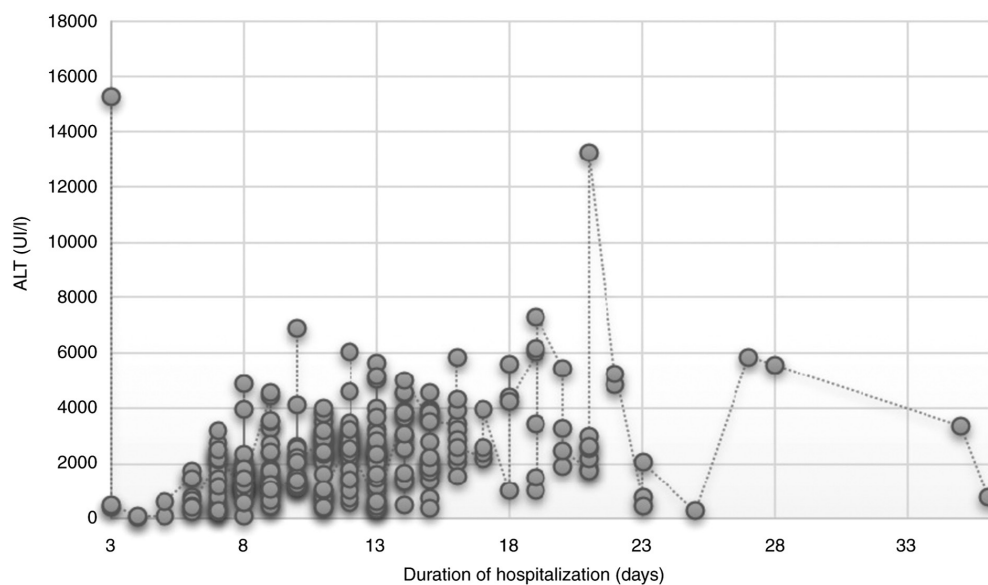


Figure 8. Correlation between severity of cytolysis (ALT) and duration of hospitalization (days). ALT, alanine transaminase.

also cause psychological distress and may require the intervention of a psychologist (35,36).

In conclusion, moderate cases with only hepatic involvement were found in our hospital. The number of cases of HEV infection identified in our hospital was extremely low compared to HAV infections. One reason is that an HAV infection is a nationally notifiable infectious disease that must be reported from all medical specialists to the District Public Health directorates, when detected. By contrast, HEV infections remain underevaluated in humans in our region. In the meantime, studies performed in farmed and wild animals confirm that HEV is a ubiquitously pathogen in swine in northeastern Romania and is a potential reservoir for HEV-associated infection.

HEV infection is a new threat to global public health with fatal outcome in sporadic cases. Even in light of this fact, the evaluation within Europe is still uncertain due to limitations in surveillance systems, differences between diagnosis tests and lack of information.

Although the seroprevalence of HEV infection is increasing in Romania, the use of different methods for the diagnosis of hepatitis and the increased incidence of HEV infection among asymptomatic patients, can allow this pathology to go undiagnosed.

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Availability of data and materials

All data generated or analyzed during this study are included in this published.

Authors' contributions

IFM, SL and MCL designed the study. IFM and GEL retrieved the data concerning the HEV infections. IMH, CM and GAL retrieved the data concerning HAV infections. CM, IAHA and AV were responsible for the data analyses. GEL, IFM and IMH made substantial contributions to discussion of the data. IFM, IMH and GAL drafted the manuscript. MCL, AV, CM and IMH critically revised the manuscript. All authors read and give final approval of the version to be submitted for publication.

Ethics approval and consent to participate

No ethics approval was required as the study was performed based on medical records, retrospectively. The results of this study do not include patient names or any personally identifiable data.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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