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Case report

Circulation of genotype 4 hepatitis E virus in Europe: First autochthonous hepatitis E infection in France

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ABSTRACT

Background: Human HEV infections reported in Europe without previous travel to endemic regions are linked to exposure to genotype 3 Hepatitis E virus (HEV).Genotype 3 is widely distributed through human cases and zoonotic reservoir. The geographical distribution of genotype 4 is limited to Asian countries.
Objectives: The first human case of autochthonous genotype 4 hepatitis E infectionwasreported in France.
Study design: The HEV infection was described in an immunosuppressed patient, presenting an acute myeloblastic leukemia. Investigation of the case was performed on detection of HEV markers in the patient and in the environment.
Results: Hepatitis E infection was diagnosed on the basis of HEV RNA viremia, and detection of anti-HEV IgM. The prognostic of leukemia was favorable and HEV was cleared without relapsing. HEV isolate was classified into genotype 4.
Conclusions: The recent characterization of genotype 4 HEV through swine surveillance in Europe and the description of the first human case in France open interesting questions about the circulation of this genotype: health risks in human population, transmission patterns, and zoonotic reservoir.

1. Why this case is important?

Hepatitis E virus (HEV) causes sporadic and epidemic viral hepatitis. According to their genetic variability and reservoir, human HEV isolates have been classified into four major genotypes.[1]

The four genotypes are associated with different epidemiological profiles. While genotype 1 and 2 are reported in epidemics in developing countries, genotypes 3 and 4 are associated with sporadic cases and found in both industrialized and developing countries. Genotypes 3 and 4 share a zoonotic transmission with the description of human cases after consumption of raw or undercooked meat.[2–4] Moreover, several differences exist between genotypes 3 and 4. Hepatitis E, due to a virus of genotype 3 is mostly reported in middle-aged and elderly men. In contrast, genotype 4 strains are isolated from young adults, without discrimination of gender, and with a high mortality among pregnant women.[5]

In France, like in other European countries, autochthonous human infections by HEV are due to genotype 3 viruses. Additional cases of HEV infection have been documented in immunosuppressed patients, after organ transplantation or leukaemia disease.[6,7] A recent phylogenetic analysis[8] showed the same proportion of subtypes in both human and swine origins and over 99% identity was found between HEV sequences isolated from human and swine.

Genotype 4 has been isolated from Asian[1] countries and India[9] and recently through swine[10] surveillance in Europe. Moreover, a sporadic human case due to a genotype 4 virus was described in Germany in 2008.[11]

This report describes the first human case of genotype 4 hepatitis E infection, in France, in absence of previous travel of the patient to endemic regions.

2. Case description

A 30-year-old Caucasian female, living in East of France, was hospitalised for acute hepatitis with common clinical features: jaundice and anorexia. Physical examination revealed moderate abdominal pain and dark urines. This patient presented a pre-existing disease with an acute myeloblastic leukaemia diagnosed seven months before the onset of acute hepatitis. Two cures of induction therapy according to protocol LAM 2006 IR associated with ozogamicin led to complete remission. Three months after

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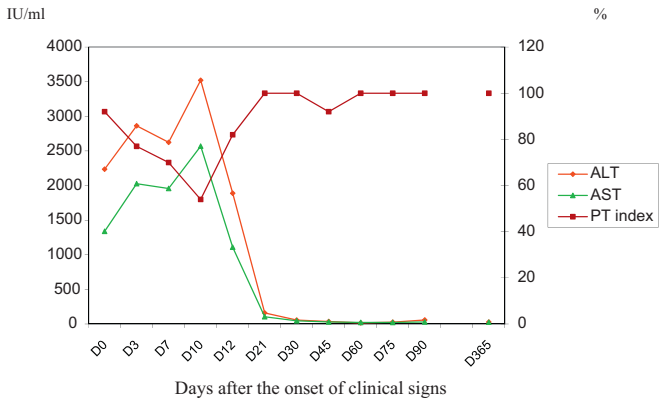

Fig. 1. Evolution of liver functions (biochemical markers and prothrombin time index) on sera collected the day of the onset of clinical signs (D0) to one year after the clinical signs. The patient clinically completed recovered at D30. ALT: alanine aminotransferase; AST: aspartate aminotransferase; PT index: prothrombin time index.

the initial diagnosis of leukemia, 2 cures of consolidation therapy were achieved before the conditioning regimen for allogenic stem transplantation. But the bone marrow transplantation was not performed because of the development of acute hepatitis.

At onset of clinical signs, biochemical markers confirmed hepatitis with alanine aminotransferase (ALT): 2236 U/L ($N < 45$), total bilirubin: 44 mol/L ($N < 20$), gamma glutamyl transferase: 362 IU/L ($N < 50$) (Fig. 1). Initial investigation performed at admission on acute and chronic liver disease showed negative results for hepatitis A (Abbott Architect HAVAb-IgM), hepatitis C (Abbott RealTime HCV), human cytomegalovirus (Argene, CMV R-gene), human immunodeficiency virus infection (Abbott Architect HIV Ag/Ab Combo, Abbott RealTime HIV) and autoimmune hepatitis (negativity for antinuclear, anti-Liver Kidney Microsomal type 1, anti-smooth muscle antibodies). She was immunised against hepatitis B (anti-HBs >1000 IU/ml, negative Ag HBs, negative Ag HBe, negative anti-HBc (Abbott Architect), herpes simplex virus (DiaSorin Liaison HSV), Epstein–Barr virus (DiaSorin ETI-VAC-G) and varicella zona virus (DiaSorin Liaison VZV).

Hepatitis E infection was investigated using sera collected four weeks before and six weeks after initial clinical investigation. The patient recovered from this hepatitis and a third cure of consolidation therapy could be achieved within one month after the recovery. After one year of supervision, the prognostic is favourable for acute myeloblastic leukaemia and HEV was cleared without relapsing.

2.1. Diagnosis of hepatitis E infection

Hepatitis E infection was diagnosed on both HEV RNA and serological data. Six sera were tested within one month between the onset of clinical signs and the time of hepatitis recovery (Fig. 2). Serological tests for anti-HEV IgG and IgM (EIAgen HEV IgG® and EIAgen HEV IgM® kits, Adaltis, Bologna, Italy), were performed according to the manufacturer's instructions. HEV RNA was amplified by real time RT-PCR[12] used for quantitative detection (Fig. 2). HEV RNA viremia was found to be positive within two weeks, from one week before the onset of clinical signs to one week after. Sequential serum collected at D21 and D30 were negative for HEV RNA.

Anti-HEV IgM and anti-HEV IgG were detectable from D7 after the onset of clinical signs and the anti-HEV IgG index (optical density/reactivity index > 1 as positive) increased in following specimens. The IgG avidity index value for the sera collected at D7 was 20%.[6,13] Serological results and amplification of HEV RNA by PCR showed a recent infection.

Chronic and prolonged HEV infections were described in patients after solid organ transplantation[7] or stem cell transplantation.[14] In the present case, no episode of hepatitis E was observed after cure of consolidation therapy.

2.2. Investigation of the case

During the two cures of consolidation therapy, administered before the onset of acute hepatitis (three months and one month, respectively) the patient received 36 blood units. Only five aliquots units transfused one month before the hepatitis were available for HEV marker tests. No HEV RNA, HEV IgG or IgM antibodies were detected in these five samples. The suggestion that HEV could have been acquired parentally could thus not be excluded with only 15% of blood products tested.

Contamination from a zoonotic source was investigated. No consumption of uncooked or poorly cooked pork, wild boar, or shellfish, no contact with animals were reported.

A patient-to-patient transmission of HEV was previously demonstrated in France, in 2009.[15] In the present case, no hepatitis E infection was reported at the same ward. Nevertheless, a person-to-person contamination could not be excluded.

Therefore, none of the investigations allowed deciphering the source of hepatitis E contamination of the patient.

2.3. Phylogenetic analysis

The amplified 343-bp product of the partial ORF2 capsid region was sequenced. HEV genotype 4 strain was identified in 6/6 HEV RNA samples of the patient (Genbank access number GU982294) and the sequences were 99.5% identical between them. They shared 94% identities with strains isolated from human in China (AJ344192, AJ344188) and 93–94% with strains isolated from swine in China (EU375332, EU375330) (Fig. 3). No phylogenetic analysis on genotype 4 in Europe was carried out by lack of sequences available in the database.[11]

3. Other similar and contrasting cases in the literature

In non-endemic areas, genotypes 3 and 4 are isolated from both human and animals (particularly swine) and implicated in sporadic human cases. Sporadic cases due to genotype 4 are mainly reported in Asia.

In France, until now, only genotype 3 viruses have been implicated in sporadic cases without travel in endemic areas. Nevertheless, the first sporadic human case due to genotype 4 was described in Germany in 2008[10] without travel in Asia. Source of contamination was not clearly defined but contaminated meat

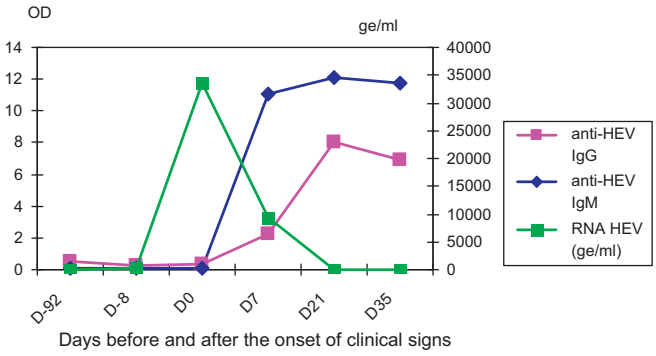

Fig. 2. Hepatitis E serological markers and quantitative HEV RNA (genomes copies/ml) on sequential sera samples collected within 5 months. DO: onset of clinical signs. OD: optical density of anti-HEV IgG and IgM markers.