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Rabbit HEV in immunosuppressed patients with hepatitis E acquired in Switzerland

To the Editor:

Hepatitis E virus (HEV) infection is one of the most common causes of acute hepatitis and jaundice in the world.[1,2] HEV is a positive-strand RNA virus classified in the *Hepeviridae* family. Human pathogenic strains belong to species *Orthohepevirus A* which comprises 8 genotypes (gt) infecting a broad spectrum of animals as well as humans.[3,4] Human-restricted gt 1 and 2 are transmitted by the fecal-oral route and can cause large, primarily waterborne outbreaks in resource-limited settings.[5] HEV gt 3 and 4 infection is caused by zoonosis in high-income regions. Zoonotic transmission of HEV is believed to result from the consumption of raw or undercooked pork or game meat, with certain regional specialties such as figatellu in the South of France[6] or mortadella di fegato in the Ticino region of Switzerland[7] representing high-risk alimentary sources. Congruent HEV sequences identified in a Swiss patient with hepatitis E and a suspected food source recently led Wist *et al.* to propose a new gt 3 subtype, provisionally named 3s, as being present in Switzerland.[7–9]

We recently described 93 patients with PCR-proven acute hepatitis E acquired in Switzerland.[10] While most HEV isolates clustered phylogenetically to a distinct gt 3 subtype, the limited length of the genotyping target and the lack of (sub)genotypes from Switzerland recorded in GenBank precluded robust subtyping. Recently, 4 full-length gt 3s sequences were deposited in GenBank (accession numbers MF346772, MF346773, MG573193 and KY780957), allowing for more accurate subtyping. The gt 3s sequences share greater than 95% similarity but only 73 to 88% similarity when compared to other gt 3 subtypes. We have thus been able to reassess subtype assignment of the gt 3 isolates described in our previous report as well as of all other independent isolates for a total of 114 patients (according to protocol 478/15 approved by our ethics committee [CER-VD]), comprising 104 with acute and 10 with chronic hepatitis E, collected in Switzerland and diagnosed in our center over a 5.5-year period of time (November 2011 until April 2017).

Plasma samples from all 114 patients were subjected to HEV genotyping following amplification of open reading frame (ORF) 2/3 (191–195 base pair) and ORF2 (592 base pair) sequences, as described in Fraga *et al.*[10] Subtype assignment and phylogenetic analyses were performed using the HEVnet genotyping tool (https://www.rivm.nl/en/Topics/H/HEVNet) and confirmed by BLAST[11] within the Geneious software (Geneious 11.1.5, https://www.geneious.com) using a local database containing 360 non-recombinant full-length HEV sequences available in GenBank in August 2018, each being annotated with the subgenotype provided by HEVnet (support values ≥94%). All sequences from this study have been deposited in GenBank (accession numbers MK342954-MK343086).

Of 114 cases of hepatitis E acquired in Switzerland, genotyping information based on the ORF2/3 region could be obtained for 98 samples (87%) (Table 1). Sixty-two ORF2/3 positive PCR-samples were also subjected to the ORF2 genotyping assay. Positivity of genotyping PCR assays was associated with viral load (89,250 *vs.* 2,550 IU/ml for ORF2/3 and 456,750 *vs.* 34,500 IU/ml for ORF2; Mann-Whitney *U*, $p < 0.0001$).

HEV gt 3 was identified in 95 of 98 (96%) samples; the remaining 3 were of gt 4 (Table 1). Sixty-eight of the 95 gt 3 isolates could be assigned to a subtype, including 64 from patients with acute and 4 from patients with chronic infection. Fifty-two

Table 1. Distribution of HEV genotypes and subtypes in 114 independent samples subjected to ORF2/3- and/or ORF2-based genotyping.

HEV genotype	3						4	n.i.	Total
HEV subtype	3a	3f	3o*	3ra	3s*	n.a.			
Acute hepatitis E	2	6	4	1	51	22	3	15	104
Chronic hepatitis E		1		2	1	5		1	10
Total	2	7	4	3	52	27	3	16	114

n.a., non assigned to a subtype; n.i., non informative, i.e. the genotyping PCR was negative.
* Provisional subtype assignment according to HEVnet.

(77%) of these 68 isolates were assigned to the newly proposed subtype 3s. There was no noticeable difference in the clinical presentation of patients infected with HEV gt 3s *vs.* other gt 3 subtypes. Together with the recent reports identifying HEV gt 3s in fattening pigs, pork sausage and a few patients,[7–9] our results indicate that this particular subtype represents the main HEV gt 3 circulating in Switzerland. To our knowledge, this subtype has not been identified in other regions of Europe so far. Beyond topological features of Switzerland, with mountains and lakes defining its natural borders, gt 3s geographical restriction likely reflects the economic traits specific to Switzerland. Indeed, as non-member of the European Union, Switzerland applies its own economic rules and taxes, especially concerning the exchange of agriculture-derived products. Thus, about 96% of the pork meat consumed in Switzerland is from local production while at the same time only 1.5% is exported (Annual Report 2017 of the 'Schweizer Fleisch-Fachverband'; Ref.[12] Swiss agricultural policy, which guarantees national food supply, may therefore play an important role in the restriction of HEV gt 3s to Switzerland.

Interestingly, 3 solid organ transplant recipients were found to be infected with rabbit HEV (gt 3ra), as confirmed by reverse transcription PCR amplification of a 298-base pair region encompassing the rabbit-specific 93-nucleotide insertion between the macro (also known as X) and helicase domains of ORF1 using forward primer 5′-GCTTGGTTTGAGGCCAA-3′ and reverse primer 5′-ACCACAACATCAACATC-3′. Phylogenetic analysis of the ORF1 sequence of these 3 isolates together with 22 non-redundant gt 3ra sequences deposited in GenBank (November 2018) confirmed that all 3 patient isolates cluster with the rabbit HEV isolates recently identified in 5 humans in France[13] as well as with those identified in rabbits in France and Germany (Fig. 1). Interestingly, the sequence of 2 of our cases, *i.e.* CH_HEV3ra-1 and -2, are very closely related while the patients were infected at a 1.5-year time interval and were living in distinct regions of Switzerland. Although only a limited number of rabbit HEV sequences are available in public databases so far, the observed phylogenetic clustering indicates that gt 3ra circulating in Europe can infect and cause hepatitis E in humans.

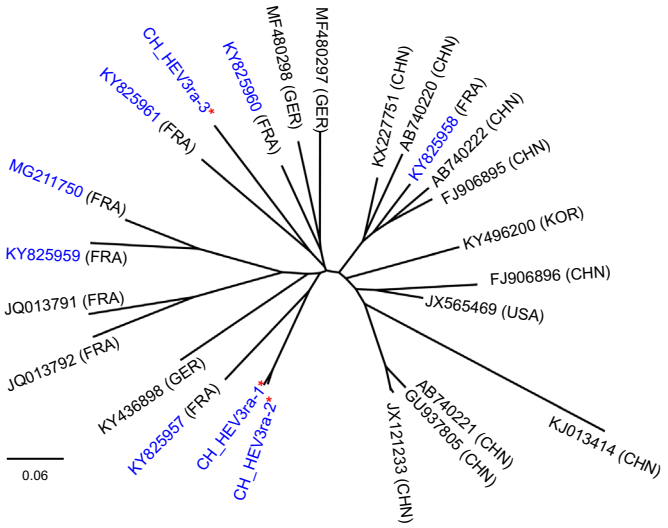

Fig. 1. Phylogenetic relationship of the rabbit HEV isolates identified in our study. The 298-bp ORF1 sequences from the 3 HEV genotype 3ra isolates identified in our study (red asterisks) and of 22 gt 3ra isolates retrieved from GenBank were aligned with ClustalW, followed by phylogenetic tree building using the neighbor-joining method (Geneious 11.1.5 software, Biomatters). The accession numbers of sequences identified in rabbits or humans are indicated in black or blue, respectively. The geographical origin of the samples is indicated by country code. The scale bar indicates the number of nucleotide substitutions per site. HEV, hepatitis E virus; ORF1, open reading frame 1. (This figure appears in colour on the web.)

Of note, our 3 patients infected with rabbit HEV were immunosuppressed and 2 developed chronic hepatitis E. Ribavirin treatment was started early in the third patient, allowing viral elimination before month 3. Restricting the analyses to successfully subtyped HEV gt 3 samples (n = 68), the proportions of gt 3ra among chronic *vs.* acute infections were 50% (2/4) *vs.* 1.6% (1/64), respectively. Very similarly, 4 of the 5 HEV gt 3ra infections recently identified by Abravanel *et al.* in a cohort of 919 patients from France were found in immunosuppressed patients. Despite the still limited number of cases, one may speculate that immunosuppression may have facilitated human infection and possibly persistence by this particular HEV strain, as recently suggested for the first human case of rat HEV infection.[14]

Remarkably, and in line with the observations of Abravanel *et al.*[13] none of our patients with rabbit HEV infection had consumed rabbit meat or been in contact with rabbits. Thus, HEV gt 3ra infection has likely been acquired either *via* another animal or *via* contaminated vegetables, fruits or water supplies.

In summary, our observations lend support to the existence of a dominant HEV subtype in Switzerland, provisionally designated as 3s. Importantly, we confirm that rabbit HEV can infect and cause hepatitis E in humans, especially in a setting of immunosuppression.

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Conflict of interest

The authors declare no conflict of interest related to this article.

Please refer to the accompanying ICMJE disclosure forms for further details.

Authors' contributions

RS, MF, DS, DM and JG contributed to collection and analysis of the data; RS, DM and JG wrote the manuscript.

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Supplementary data

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Splenic artery aneurysms, portal hypertension and pregnancy

To the Editor:

I thank Andrade and the VALDIG investigators for their study of pregnancy outcomes in women with idiopathic non-cirrhotic portal hypertension, noting liver-related events in one-third of patients.[1] I would be interested to know whether imaging was performed with regard to splenic artery aneurysms (SAAs).

SAAs have been reported at autopsy in 7.1% of individuals with cirrhotic portal hypertension.[2] The incidence of SAA in non-cirrhotic portal hypertension is not known. Women are affected approximately 4 times as often as men, and there is an association with cumulative parity.[3]

The risk of rupture of an existing SAA during pregnancy is estimated to be 25%.[4] SAA rupture during pregnancy is associated with maternal and foetal mortality rates of 70% and 90%, respectively.[5] Of the reported cases of SAA rupture, 30% occurred during pregnancy.[6]

Imaging modalities for detection of SAA include colour flow doppler ultrasound, computerised axial tomography angiography (CTA), digital subtraction angiography and magnetic resonance angiography. Ultrasound avoids radiation to the foetus but may be dependent on the operator and body habitus of the patient. CTA is associated with a foetal radiation dose of approximately 30 milligray, below the level associated with foetal harm.[7] Gadolinium is usually avoided in pregnancy as studies have demonstrated adverse foetal and neonatal effects.[8]

While current recommendations advise intervention with SAA greater than 20 mm in diameter, more than half of SAAs which rupture during pregnancy are smaller in size. Some authors therefore recommend treatment of all SAAs in women of childbearing age, and that pregnancy is an absolute indication for proactive management.[9] Minimally invasive techniques include transcatheter or percutaneous angiographic embolization, and laparoscopic ligation.[10] Surgery should ideally be performed in second trimester, though laparoscopic aneurysm resection and splenectomy have been successfully performed in third trimester.

Given the extremely high mortality rates for both mother and foetus with rupture, regular surveillance for SAA should be performed in all women of childbearing age with portal hypertension, as well as during pregnancy.

Keywords: Splenic artery aneurysm; Portal hypertension; Pregnancy.

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