Hepatitis E virus antibodies in workers occupationally exposed to ovine, Portugal

Favoured occupational exposure to hepatitis E virus in sheep Shepherds and Cheesemakers

Occupational exposure to hepatitis E virus in sheep Shepherds and Cheesemakers

Occupational exposure to hepatitis E virus in sheep workers

Exposure to hepatitis E virus in sheep workers

Running head: Hepatitis E virus in ovine workers

João R Mesquita[1,2], Nanci Santos-Ferreira[3], Ana S Ferreira[3], Carlos Albuquerque[4,5], Cármen Nóbrega[5,6], Fernando Esteves[5,6], Rita Cruz[5,6], Helena Vala[5,6], Maria S J Nascimento[2,3]

[1]Instituto de Ciências Biomédicas Abel Salazar, Universidade do Porto, Porto, Portugal.

[2]Epidemiology Research Unit, Instituto de Saúde Pública, Universidade do Porto, Porto, Portugal.

[3]Faculdade de Farmácia, Universidade do Porto, Porto, Portugal.

[4]Escola Superior de Saúde, Instituto Politécnico de Viseu, UNICISA-E, CIEC, CI&DEI Viseu, Viseu, Portugal.

[5]Centre for Studies in Education, Technologies and Health, Instituto Politécnico de Viseu, Viseu, Portugal.

[6]Escola Superior Agrária, Instituto Politécnico de Viseu, Viseu, Portugal.

Address for correspondence: JR Mesquita. Instituto de Ciências Biomédicas Abel Salazar. Rua de Jorge Viterbo Ferreira, 228. 4050-313 Porto. Portugal jrmesquita@icbas.up.pt; +351220428000

Summary

Hepatitis E virus (HEV) is an enteric RNA virus from the family Hepeviridae with five genotypes (genotypes 1-4 and 7) known to infect humans. HEV infection is known to have a zoonotic swine origin in industrialized countries. The role of pigs and wild boars as major reservoirs for human infection is today well-established; however, the list of new animal reservoirs is ever-expanding as new HEV strains are continuously being found in a broad host range. The recent detection of HEV in sheep stools brings concerns on the possibility of HEV transmission from these animals to humans, particularly in those occupationally exposed. The present work investigated the potential occupational risk of HEV infection in shepherds and sheep milk cheesemakers-workers occupationally exposed to ovine (WOEOs; N = 96)-from a region of the Centre of Portugal ('Serra da Estrela') based on the differences of anti-HEV IgG seroprevalence rates between these professionals and the general population (N = 192). The presence of HEV-specific antibodies in sheep (N = 90) from the same region was also evaluated. The HEV seroprevalence in WOEOs (29.3%) was found to be significantly higher (p = .0198) when compared with population controls (16.1%) which suggests an increased risk for HEV infection in these workers. HEV-specific antibodies were also found in 16.6% of the studied sheep showing that HEV circulates in these animals. Further studies are needed to confirm the zoonotic potential of sheep HEV.

Keywords: hepatitis E virus; occupational exposure; ovine; sheep; zoonoses.

800 words text

50 words abstract

10 references

Introduction

Hepatitis E virus (HEV) is an enteric, small non-enveloped, positive-sense, single-stranded RNA virus from the genus *Orthohepevirus A*, family *Hepeviridae* (1). Originally thought to be a human specific virus, the concept of hepatitis E in industrialized countries has radically changed with the discovery of zoonotic swine strains of HEV genotype 3 (HEV3), closely related to human HEV (2). The role of swine as major reservoirs for human infection by HEV3 is today well established (3), however the list of new animal reservoirs is ever-expanding as new HEV strains are continuously being found in a broad host range (4). A very recent study has described for the first time the presence of HEV3 in sheep stools from Italy, sharing high identity with human HEV3 identified in hepatitis patients and blood donors from France and The Netherlands, as well as to strains found in porcine blood products (5). In light of the circulation of genetically similar HEV3 strains amongst sheep and humans, the possibility of transmission between these domestic animals and humans is present and needs to be addressed. Considering the importance of sheep as livestock animals in many regions of the world, occupational exposure to HEV excreted from ovine might be favoured in professions such as Shepherds and sheep milk Cheesemakers. Hence, serologic surveillance for HEV exposure in possible risk groups is important in understanding if ovine are also reservoirs for HEV. For this, we investigated the potential occupational risk of HEV infection in Shepherds and sheep milk Cheesemakers – workers occupationally exposed to ovine (WOEOs) - in Portugal based on the differences of anti-HEV IgG seroprevalence rates between these professionals and the general population.

The study focused on the sub-region of Estrela Mountain ("Serra da Estrela"), the Portuguese mainland point with highest altitude, where the National Association of "Serra da Estrela" sheep breed (ANCOSE) manages the "Serra da Estrela" autochthonous sheep breed. Flocks are continuously confined to farm sites and produce milk that is solely used for the globally recognized "Serra da Estrela" cheese. A list of "Serra da Estrela" WOEOs ~~and~~ was retrieved from ANCOSE and each was individually contacted and asked to provide a blood sample. In total 96 WOEOs blood samples of Shepherds (N=22; 18 male and 3 female; median age = 58.5) and Cheesemakers (N=75; 44 male and 31 female median age = 56.2) were obtained between November and December 2017. Sera from anonymous volunteers (n = 192) matched with the WOEOs by age (5 year age group), sex and region (all from NUTSII centre region) were also retrieved from blood donations and used as controls. Survey procedures were conducted in accordance with the recommendations outlined in the Declaration of Helsinki and were approved by a national ethics board (Ethics commission of HSJ; reference: 99/2015). Serum samples were tested for anti-HEV IgG using a commercial enzyme immunoassay (*Recom*Well HEV-IgG, Mikrogen, Neuried, Germany). Prevalence estimates and 95% confidence intervals (CIs) were calculated for WOEOs and control groups. P values were calculated from chi-squared tests and were considered significant at <0.05 (SPSS version 15.0 statistical software).

We have detected anti-HEV IgG in 29.3% (95% CI: 20.2-38.5) of the WOEOs compared with 16.1% (95% CI: 10.9-21.3) in the control group. The seroprevalence rate of anti-HEV IgG was significantly higher in WOEOs then in controls (P = 0.0198). Higher anti-HEV IgG prevalence has also been widely reported in individuals with occupational exposure to pigs (pig farmers, slaughterers, meat inspectors~~,~~ and veterinarians) linking the frequent and close contact with swine with human HEV infection (6-10). Like in swine we can speculate that contact to ovine

93 can also be a source for HEV infection in WOEOs. The precise route of transmission to WOEOs
94 still remains to be demonstrated, but it is plausible that there is an increased risk of becoming
95 infected during regular animal husbandry practices. For workers that are at continued risk of
96 exposure, strict hygiene measures should be enforced, especially in middle-aged, elderly men
97 or patients with an underlying liver disease, where complications by HEV3 are more frequent
98 (1).
99 Sheep products are frequently and widely consumed throughout the world, thus further
100 studies are needed to clarify the risk of sheep products to HEV infection.
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104 **References**
105 1. Kamar N, Abravanel F, Lhomme S, Rostaing L, Izopet J (2015) Hepatitis E virus: chronic
106 infection, extra-hepatic manifestations, and treatment. *Clin. Res. Hepatol. Gastroenterol.* 39,
107 20-27.

108 2. Meng XJ, Purcell RH, Halbur PG, Lehman JR, Webb DM, Tsareva TS, Haynes JS, Thacker BJ,
109 Emerson SU (1997) A novel virus in swine is closely related to the human hepatitis E virus.
110 *Proc. Natl. Acad. Sci. USA* 94, 9860-9865.

111 3. Meng XJ. From barnyard to food table: the omnipresence of hepatitis E virus and risk for
112 zoonotic infection and food safety. Virus Res. 2011 Oct;161(1):23-30. doi:
113 10.1016/j.virusres.2011.01.016.

114 4. Kenney S. P. (2019). The Current Host Range of Hepatitis E Viruses. Viruses, 11(5), 452.
115 doi:10.3390/v11050452

116 5. Sarchese V, Di Profio F, Melegari I, Palombieri A, Sanchez SB, Arbuatti A, Ciuffetelli M,
117 Marsilio F, Martella V, Di Martino B. Hepatitis E virus in sheep in Italy. Transbound Emerg Dis.
118 2019 May;66(3):1120-1125. doi: 10.1111/tbed.13157.

119 6. Bouwknegt M, Engel B, Herremans MM, Widdowson MA, Worm HC, Koopmans MP,
120 Frankena K, de Roda Husman AM, De Jong MC, Van Der Poel WH (2008) Bayesian estimation
121 of hepatitis E virus seroprevalence for populations with different exposure levels to swine in
122 The Netherlands. *Epidemiol. Infect.* 136, 567-576.

123 7. Krumbholz A, Mohn U, Lange J, Motz M, Wenzel JJ, Jilg W, Walther M, Straube E, Wutzler
124 P, Zell R (2012) Prevalence of hepatitis E virus-specific antibodies in humans with occupational
125 exposure to pigs. *Med. Microbiol. Immunol*, 239-244.

126 8. Chaussade H, Rigaud E, Allix A, Carpentier A, Touzé A, Delzescaux D, Choutet P, Garcia-
127 Bonnet N, Coursaget P (2013) Hepatitis E virus seroprevalence and risk factors for individuals
128 in working contact with animals. *J Clin Virol*. 58, 504-508.

129 9. Galiana C, Fernández-Barredo S, García A, Gómez MT, Pérez-Gracia MT (2008) Occupational
130 Exposure to Hepatitis E Virus (HEV) in Swine Workers. *Am. J. Trop. Med. Hyg*. 78, 1012-1015.

131 10. Siochu A, Froesner G, Tassis PD, Kyriakis CS, Alexopoulos C, Kyriakis CS (2004) First report
132 of the prevalence of anti-hepatitis E (anti-HEV) lgG in blood serum of blood donors,
133 slaughtermen and swine farmers in Greece. *Acta Veterinaria* 59, 205-211.