

## SHORT REPORT

# Prevalence of hepatitis E virus in slaughter-age pigs in Scotland

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### SUMMARY

The prevalence of anti-HEV isotype-specific antibodies and viraemia were investigated in serum samples collected from slaughter-age pigs (aged 22–24 weeks) from 23 farms in Scotland. Of 176 serum samples tested, 29·0% ( $n = 51$ ) were anti-HEV IgG positive, 36·9% ( $n = 65$ ) anti-HEV IgA positive and 29·0% ( $n = 51$ ) anti-HEV IgM positive. Overall seroprevalence (anti-HEV IgG+ and/or IgA+ and/or IgM+) was 61·4% ( $n = 108$ ). HEV RNA was detected in 72/162 serum samples (44·4%). Partial sequence of ORF2 (98 nt) was obtained from eight HEV RNA-positive samples and phylogenetic analysis confirmed that they were all of genotype 3. This is the first report on the prevalence of HEV in pigs in Scotland. Given the increasing incidence of locally acquired HEV infection in the UK, evidence that HEV is a foodborne zoonosis emphasizes the need for surveillance in pigs.

**Key words:** Hepatitis E, zoonoses, zoonotic foodborne diseases.

There is growing evidence for zoonotic transmission of hepatitis E virus (HEV) as a cause of autochthonous (locally acquired) hepatitis E in developed countries, where it causes an acute cholestatic hepatitis, varying in severity from sub-clinical to fulminant [1]. In the UK there has been an increase in the number of autochthonous HEV infections in England and Wales in the last few years [2] and in a case-control study the consumption of processed pork products was found to be a risk factor for autochthonous HEV infection [3]. HEV has also been detected in

the pork food chain in England, with an incidence of 9·5% in retail sausages [4]. Similar findings are also being reported in other developed countries; however, it is worth noting that HEV can be inactivated by adequate cooking [1].

Swine are considered a natural reservoir for the virus, where infection is asymptomatic. The virus is ubiquitous in the pig population worldwide, and serological studies have reported herd prevalence of up to 100% [5]. In the UK, around 85% seropositivity (at individual level) was reported based on the analysis of 256 pig serum samples from 1991, 1994 and 2001 [6]. However, the majority of samples (95%) originated in England, and only 13 samples were from Scotland. In the absence of existing data, the aim of this study was to investigate the prevalence in pigs in Scotland.

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The sample set for the study comprised 176 serum samples that had been collected from pigs at slaughter age within a 12 week time-frame during 2006. The samples were part of a larger collection representing all major commercial pig units in Scotland that were obtained to provide a disease surveillance and research resource for the Scottish pig industry. For this study, 23 farms were selected from locations representing different regional areas of Scotland, with testing of between 6 and 15 samples per farm. All farms were breeder/finisher herds with sizes ranging from 48 to 1200 breeding sows plus progeny. The samples were analysed to investigate the prevalence of anti-HEV isotype-specific antibodies and HEV viraemia.

A commercial ELISA was used to test for anti-HEV IgG, performed according to the manufacturers' instructions [HEV-IgG ELISA (Swine); Beijing Wantai Biological Pharmacy Enterprise, China]. The commercial IgG assay was modified to test for anti-HEV IgA and anti-HEV IgM, through the use of horseradish peroxidase (HRP)-conjugated goat anti-porcine IgA and goat anti-porcine IgM (Bethyl Laboratories Inc., USA), respectively. For each isotype-specific assay all samples were tested across two 96-well plates. Following testing, review of the frequency distribution of absorbance value densities did not demonstrate clear bimodal distributions for the data. However, a clear peak in the frequency distribution of values of absorbance (that was assumed to represent the negative population) was observed. From this, the most frequent absorbance value (mode value) for each ELISA plate was delineated (0.131, 0.139 for anti-HEV IgG; 0.047, 0.075 for anti-HEV IgA; 0.166, 0.147 for anti-HEV IgM). Based on this, and on the assumption that the frequency distribution was a composition of a density function of both negative and positive samples, all data with a value less than the mode value was used to simulate a Gaussian distribution of negative samples and to calculate the standard deviation (s.d.). Cut-off values for the ELISA assays (including the commercial anti-HEV IgG ELISA) were based on the mode value plus 3 s.d. and were 0.312, 0.387 for anti-HEV IgG, 0.080, 0.132 for anti-HEV IgA and 0.318, 0.258 for anti-HEV IgM. Absorbance values for positives ranged from 0.312–0.901, 0.399–0.854 for anti-HEV IgG, 0.082–1.581, 0.133–1.381 for anti-HEV IgA and 0.333–1.204, 0.261–1.293 for anti-HEV IgM.

For detection of HEV RNA in serum, nucleic acid was extracted using the QIAamp viral RNA mini kit

(Qiagen, UK), and screened for the presence of HEV RNA by nested reverse transcription–polymerase chain reaction (RT–PCR) using degenerate primers corresponding to a 145 bp region in ORF2, capable of detecting all HEV genotypes in both human and porcine samples [7]. The positive control used was the WHO RNA standard ([http://whqlibdoc.who.int/hq/2011/WHO\\_BS\\_2011.2175\\_eng.pdf](http://whqlibdoc.who.int/hq/2011/WHO_BS_2011.2175_eng.pdf)). Appropriate negative controls were included at all stages of the reaction. PCR for HEV was performed on 162 samples only due to an insufficient amount of sample remaining for 14 samples within the study sample set. Positive samples were identified by gel electrophoresis, and a representative sample from each region cloned using the Promega TA cloning kit (Promega, UK) and sequenced by GATC Biotech (Konstanz, Germany).

Of 176 serum samples tested, 29.0% ( $n = 51$ ) were anti-HEV IgG positive, 36.9% ( $n = 65$ ) anti-HEV IgA positive and 29.0% ( $n = 51$ ) anti-HEV IgM positive, with some samples positive for more than one isotype. The overall seroprevalence was 61.4% ( $n = 108$ ). Anti-HEV antibodies were detected in at least one sample from each of the 23 farms sampled.

HEV RNA was detected in 72/162 serum samples (44.4%). A partial sequence of ORF2 (98 nt) was obtained from eight HEV RNA+ serum samples, and five unique sequences were shown to cluster within genotype 3 (Supplementary Fig. S1).

Sixty-eight of 176 pigs (38.6%) did not have detectable antibody. Of these seronegatives, 63 were also tested by HEV PCR and 32 (50.8%) were viraemic, suggesting recent exposure and infection. Anti-HEV IgA has been reported to be a useful indicator of viraemia in humans [8] and pigs in Japan [9]. This was a finding based on the analysis of serum sampled from pigs aged 1–6 months (55% and 10% anti-HEV IgA positivity in viraemic and non-viraemic pigs, respectively). When comparing seroprevalence for viraemic and non-viraemic pigs, the prevalence of anti-HEV IgG was 34.7% and 27.8%, anti-HEV IgA was 25.0% and 46.7% and anti-HEV IgM was 19.4% and 35.6%, respectively. Fisher's exact test gives  $2P = 0.005$  for IgA+,  $2P = 0.03$  for IgM+ and  $2P = 0.4$  for IgG+, suggesting that IgA correlates better with viraemia than IgM. However, given the high IgA and IgM positive rates in HEV RNA-negative animals, it seems impractical to use either as an indicator of viraemia.

Heterogeneity between the geographical regions in Scotland was significant for IgA, IgM and HEV

Table 1. Prevalence of isotype-specific anti-HEV antibody and viraemia by region. All pigs were aged ~5 months. Regional differences were observed to be significant with regard to IgA, IgM and HEV RNA ( $P < 0.05$ )

Region	No. of farms tested	Total no. of animals tested	IgG + (%)	IgA + (%)	IgM + (%)	HEV RNA + (%)
Highlands	3	25	6 (24)	5 (20)	8 (32)	16 (67)
Grampian	6	42	13 (31)	16 (38)	11 (26)	17 (41)
Central Region	1	7	4 (57)	3 (43)	3 (43)	5 (71)
Tayside & Fife	7	51	16 (31)	24 (47)	16 (31)	21 (48)
Lothian & Borders	4	29	5 (17)	5 (17)	2 (7)	9 (36)
Dumfries & Galloway	2	22	7 (32)	12 (55)	11 (50)	4 (19)
Total	23	176	51 (29)	65 (36.9)	51 (29)	72 (44.4)

RNA ( $P < 0.05$ ). However, regional comparisons should be viewed with caution in view of the small number of herds in some of the regional subsets (Table 1). Anti-HEV IgG seroprevalence was significantly lower than that reported for pigs in a previous study in England (29.0% vs. 85.5%, respectively) [6]. This may reflect differences in the assays used. The strategy for setting cut-off values for the ELISAs used in this study prioritized specificity over sensitivity to provide confidence in true positives identified. As a consequence some low antibody positives may have been missed. Using the cut-off value recommended by the manufacturer for IgG (mean of negative of kit +0.16) the seroprevalence for anti-HEV IgG would be 49%. Differences in anti-HEV IgG seroprevalence reported here and in the previous study could also reflect temporal changes in the seroprevalence in UK pigs since the time period of sampling for the previous study (1991–2001). Interestingly, a lower seroprevalence was observed in the healthy blood donor population in Scotland relative to other parts of the UK, with a seroprevalence of 4.7% [10] relative to 16% and 10% in South West England and Wales respectively [1].

It is generally assumed that the natural course of infection in pigs involves infection at around age 8–12 weeks coinciding with declining maternal antibody, with viraemia lasting from 1–2 weeks followed by a more prolonged period of virus shedding in the faeces (from 3–7 weeks). Data from this study suggests that a significant number of the slaughter-age pigs had only recently been infected ( $n = 32$ ). However, understanding of the dynamics of infection in pigs is also limited. In an experimental infection study, Sanford *et al.* [11] observed more prolonged periods of viraemia in some pigs, and one pig was viraemic continuously for 12 weeks post-infection.

In this study 44.4% of pigs tested were viraemic at slaughter age. The literature currently presents limited information on prevalence of viraemia in slaughter-age pigs; however, it is clear that regional variations occur [1, 4, 5]. In a longitudinal on-farm study in North East Spain, De Deus *et al.* [12] reported that 12.5% of pigs (2/16) were viraemic at a similar age. Both the assay used in this study and the one used by De Deus *et al.*, amplified a region at the start of ORF2. Unfortunately it is not possible to compare the sensitivity of the assays as the Spanish group did not use the WHO standard as a control. However, we compared the sensitivity of our assay to the study published by Mokhtari *et al.* [13], where one commercial and four published real-time RT-PCR assays were tested against serial dilutions of the WHO HEV nucleic acid standard. The most sensitive assay tested could detect 100% of replicates at 250 IU/ml and 25% of replicates at 25 IU/ml. The assay we employed could detect 100% of replicates at 250 IU/ml and 50% of replicates at 25 IU/ml making it at least as sensitive as the current leading real-time assay [13].

Recent reports have cited concerns over HEV presence in foodstuffs in the UK (<http://www.defra.gov.uk/ahvla-en/files/pub-survrep-p0312.pdf>). With the increasing evidence for zoonotic transmission of HEV there is a need for a better understanding of the natural course and dynamics of HEV infection within the commercial pig population, with a view to better understanding the risk and potential for control of foodborne transmission of HEV.

## SUPPLEMENTARY MATERIAL

For supplementary material accompanying this paper visit <http://dx.doi.org/10.1017/S0950268814003100>.

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## DECLARATION OF INTEREST

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