Hepatitis E Virus in Pork Food Chain, United Kingdom, 2009–2010

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We investigated contamination by hepatitis E virus (HEV) in the pork production chain in the United Kingdom. We detected HEV in pig liver samples in a slaughterhouse, in surface samples from a processing plant, and in pork sausages and surface samples at point of sale. Our findings provide evidence for possible foodborne transmission of HEV during pork production.

During the past 10 years, hepatitis E virus (HEV) infection acquired in industrialized regions worldwide in the apparent absence of contaminated drinking water for fecaloral transmission has been reported (1). In Japan, foodborne transmission of HEV through ingestion of contaminated Sika deer, wild boar, and pig meat has been demonstrated (2) and, in France, after ingestion of pig liver sausage (3). Detection of HEV in pig liver sold in retail locations has been reported from Japan, the United States, and the Netherlands in 1.9%, 14.0%, and 6.5%, respectively (4,5). PCR indicated that 1 (1.3%) of 76 pig livers collected at retail outlets in southwestern England was positive for HEV (6).

The European Commission Framework Program 7 project, Integrated Monitoring and Control of Foodborne Viruses in European Food Supply Chains, aimed to gather data on virus contamination of food and environmental sources for quantitative viral risk assessment and development of virus-specific guidance for food supply chain operators. The UK Animal Health and Veterinary Laboratories Agency investigated the pork food chain for HEV from slaughterhouse to point of sale. We investigated fecal contamination of pork and work surfaces during this study.

The Study

During September 2009–October 2010, we collected samples from slaughtered pigs, human hands, and the environment at perceived critical points for virus contamination in a pig slaughterhouse, a processing plant, and 3 points of retail sale. Food safety fact-finding visits were made to the premises during which, through direct

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observations of conditions and practices, more points were identified where contamination with viruses possibly could occur and from where ad hoc surface samples were collected by using sterile gauze swabs and placed in in phosphate-buffered saline plus antimicrobial drugs.

We tested all samples collected by using real-time reverse transcription PCR (rRT-PCR) (7) for HEV. In addition, samples were tested for porcine adenovirus (PAdV) and human adenovirus by PCR as indicators of porcine and human fecal contamination, respectively. Nucleic acid extraction and rRT-PCR were performed according to standardized Integrated Monitoring and Control of Foodborne Viruses in European Food Supply Chains protocols. An extraction control virus, murine norovirus (MNoV), was placed in all samples before the lysis step of the extraction (8) to demonstrate the extraction of amplifiable nucleic acid. We performed all rRT-PCRs with an internal amplification control (8).

At the slaughterhouse, 40 carcasses were selected. Ten carcasses were randomly selected from each of 4 groups of pigs slaughtered that day; each group corresponded to a different farm of origin. From each carcass, the visceral pack was removed and 2–3 g of liver and 8–10 g of feces were collected. Also, 10 swab samples were collected from the handlers and environment (Table 1).

At the processing plant, 10, 18, and 14 carcasses from 3 slaughterhouses, representing 12 farms of origin, were randomly selected. The set of 14 carcasses came from the slaughterhouse we had visited, but for logistical reasons the carcasses were not the same as those that we sampled at the slaughterhouse. We collected 5 g of muscle from the ventral abdomen of each carcass. The extraction control MNoV was not detected in 2 of the samples, which we therefore excluded from the study, leaving 40 samples. Also, 10 surface swab samples were collected (Table 1).

At points of retail sale, 75 sausages were collected in 11 batches from 3 locations representing 2 types of retail outlet (2 UK supermarket chains and 1 butcher). Sausages were collected on different days to ensure that they were from different batches of pigs. MNoV was not detected in 12 of the samples, leaving 63 sausages for investigation. Eight surface swab samples were collected at point of sale (Table 1).

HEV RNA was detected at each of 3 sites in the pork food supply chain (Table 2). At the slaughterhouse, 5 (13%) of 40 fecal samples, 1 (3%) of 40 livers, and 1 (25%) of 4 swabs of workers' hands were HEV positive. At the processing plant, each of 40 pig muscle samples was negative for HEV, and a surface swab from a metal point used to hook the carcasses was HEV positive. At points of sale, 6 (10%) of 63 sausages and 2 (25%) of 8 surface samples (knife and slicer swabs) were HEV positive. Five of the 6 positive sausages were in 1 of the 11 batches

Slaughterhouse	Processing/cutting plant	Point of sale	
Surface swab			
Bar under operator inspecting livers	Bench on which meat is sold Chopping boa		
Floor under carcasses in clean area	Box in which cuts are collected	Slicer	
Floor under which livers are hung	Door handle	Cold-room door handle	
Boxes in which livers are collected before freezing and sale	Hook	Sausage maker	
Knife used immediately after scraping	General purpose knife	General purpose knife	
Knife used on livers	Point	NT	
	Saw	Toilet	
	Scale	Sink	
Swab, handlers' skin			
Hand 1	Hand 1	Hand 1	
Hand 2	Hand 2	Hand 2	
Hand 3	NT	NT	
Hand 4	NT	NT	

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collected. Control results showed no evidence of crosscontamination in the laboratory.

The indicator of pig fecal contamination, PAdV, was detected at 2 of 3 sites (Table 2). Of 40 fecal samples, 39 (98%) from the slaughterhouse were PAdV positive, as were 6 (15%) of 40 livers and 4 (40%) of 10 swab samples (knife swab immediately after evisceration, 2 hand swabs, and floor swab from area under which pigs were hung). At the processing plant, PAdV was not detected in any of the 40 pig muscle samples or the 10 swab samples tested. At points of sale, PAdV was not detected in any of the 63 sausages tested, but 1 (13%) of the 8 swab samples from the door handle of the cold room was PAdV positive. This finding could have resulted from transfer from a contaminated pig carcass, but the in-test controls and method of sampling exclude this contamination as a source of the HEV in the sausage meat. Human adenovirus was not detected in any samples from any of the locations.

Conclusions

In industrialized regions, although the incidence of clinical hepatitis E in humans is low, the seroprevalence of antibodies against HEV is relatively high (9), indicating a high proportion of subclinical disease and/or underdiagnosis (10). A small proportion of this exposure to HEV is likely to result from travel to regions to which the virus is endemic or migration from such regions (11). However, a substantial level of exposure to HEV seems to have an indigenous source.

Pork products are eaten in several industrialized regions, including the United Kingdom (2,12,13). A recent cluster of illnesses in southern France was associated with ingestion of *figatelli*, a pig liver sausage mainly eaten raw (3). Foodborne illness has been associated with ingestion of pig liver and pig muscle tissue (14,15). Our study showed that in the United Kingdom, pork products with highvolume nationwide consumption might be contaminated with HEV. We chose sausages as the type of point-ofsale pork product investigated for HEV because they are widely eaten throughout the United Kingdom (>212,746 tons in Great Britain during February 2011-February 2012, BPEX, Agriculture and Horticulture Development Board, UK), unlike pig liver, and a 10% HEV detection rate in pork sausages at point of sale could be a cause for concern.

In terms of the potential for virus transmission, the surface swabs provided evidence that PAdV and HEV contamination occurs in the slaughterhouse and at point of sale. From the processing plant, HEV was detected on 1 surface swab. The 98% positive rate recorded for pig feces with the PAdV indicator validates this approach to detecting fecal contamination of porcine origin. No evidence of human fecal contamination was detected at any point in the chain, indicating that personal hygiene standards were high and that the HEV detected was unlikely to have come from human contamination of the samples.

Because the numbers of samples tested for viral contamination in this study were relatively small, these results should be taken as indicators only. For greater

Table 2. Prevalence of PAdV, H	EV, and HAdV in the pork food c	hain, 2009–2010*		
		No. PAdV positive/	No. HEV positive/	No. HAdV positive/
Point in chain	Sample type	no. collected (%)	no. collected (%)	no. collected
Slaughterhouse	Feces	39/40 (98)	5/40 (13)	NT
	Liver	6/40 (15)	1/40 (3)	NT
	Surface swab	4/10 (40)	1/10 (10)	0/10
Processing plant	Muscle	0/40	0/40	NT
	Surface swab	0/10	1/10 (10)	0/10
Point of sale	Sausage	0/63	6/63 (10)	NT
	Surface swab	1/8 (13)	2/8 (25)	0/8

*PAdV, porcine adenovirus; HEV, hepatitis E virus; HAdV, human adenovirus; NT, sample not taken.

DISPATCHES

confidence in the results, we recommend a larger study. In addition, we will assess the viability and genotype of HEV by further work of in vitro culture and nucleotide sequencing, respectively.

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References

- Dalton HR, Stableforth W, Thurairajah P, Hazeldine S, Remnarace R, Usama W, et al. Autochthonous hepatitis E in southwest England: natural history, complications and seasonal variation, and hepatitis E virus IgG seroprevalence in blood donors, the elderly and patients with chronic liver disease. Eur J Gastroenterol Hepatol. 2008;20:784– 90. http://dx.doi.org/10.1097/MEG.0b013e3282f5195a
- Tei S, Kitajima N, Takahashi K, Mishiro S. Zoonotic transmission of hepatitis E virus from deer to human beings. Lancet. 2003;362:371– 3. http://dx.doi.org/10.1016/S0140-6736(03)14025-1
- Colson P, Borentain P, Queyriaux B, Kaba M, Moal V, Gallian P, et al. Pig liver sausage as a source of hepatitis E virus transmission to humans. J Infect Dis. 2010;202:825–34. http://dx.doi. org/10.1086/655898
- Bouwknegt M, Lodder-Verschoor F, van der Poel WH, Rutjes SA, de Roda Husman AM. Hepatitis E virus RNA in commercial porcine livers in the Netherlands. J Food Prot. 2007;70:2889–95.

- Feagins AR, Opriessnig T, Guenette DK, Halbur PG, Meng XJ. Detection and characterization of infectious hepatitis E virus from commercial pig livers sold in local grocery stores in the USA. J Gen Virol. 2007;88:912–7. http://dx.doi.org/10.1099/vir.0.82613-0
- Banks M, Martelli F, Grierson S, Fellows HJ, Stableforth W, Bendall R, et al. Hepatitis E virus in retail pig livers. Vet Rec. 2010;166:29. http://dx.doi.org/10.1136/vr.b5602
- Jothikumar N, Cromeans TL, Robertson BH, Meng XJ, Hill VR. A broadly reactive one-step real-time RT-PCR assay for rapid and sensitive detection of hepatitis E virus. J Virol Methods. 2006;131:65– 71. http://dx.doi.org/10.1016/j.jviromet.2005.07.004
- Diez-Valcarce M, Cook N, Hernández M, Rodríguez-Lázaro D. Analytical application of a sample process control in detection of foodborne viruses. Food Analytical Methods. 2011;4:614–8. http:// dx.doi.org/10.1007/s12161-011-9262-9
- Bouwknegt M, Frankena K, Rutjes SA, Wellenberg GJ, de Roda Husman AM, van der Poel WH, et al. Estimation of hepatitis E virus transmission among pigs due to contact-exposure. Vet Res. 2008;39:40–51. http://dx.doi.org/10.1051/vetres:2008017
- Meng XJ, Wiseman B, Elvinger F, Guenette DK, Toth TE, Engle RE, et al. Prevalence of antibodies to hepatitis E virus in veterinarians working with swine and in normal blood donors in the United States and other countries. J Clin Microbiol. 2002;40:117–22. http://dx.doi. org/10.1128/JCM.40.1.117-122.2002
- Péron JM, Mansuy JM, Poirson H, Bureau C, Dupuis E, Alric L, et al. Hepatitis E is an autochthonous disease in industrialized countries. Analysis of 23 patients in south-west France over a 13-month period and comparison with hepatitis A. Gastroenterol Clin Biol. 2006;30:757–62.
- 12. Péron JM, Mansuy JM, Izopet J, Vinel JP. Hepatitis E virus: an emerging disease [in French]. Sante. 2006;16:239–43.
- Yazaki Y, Mizuo H, Takahashi M, Nishizawa T, Sasaki N, Gotanda Y, et al. Sporadic acute or fulminant hepatitis E in Hokkaido, Japan, may be food-borne, as suggested by the presence of hepatitis E virus in pig liver as food. J Gen Virol. 2003;84:2351–7. http://dx.doi. org/10.1099/vir.0.19242-0
- Deest G, Zehner L, Nicand E, Gaudy-Graffin C, Goudeau A, Bacq Y. Autochthonous hepatitis E in France and consumption of raw pig meat. Gastroenterol Clin Biol. 2007;31:1095–7. http://dx.doi. org/10.1016/S0399-8320(07)78342-2
- de Deus N, Seminati C, Pina S, Mateu E, Martin M, Segales J. Detection of hepatitis E virus in liver, mesenteric lymph node, serum, bile and faeces of naturally infected pigs affected by different pathological conditions. Vet Microbiol. 2007;119:105–14. http://dx.doi. org/10.1016/j.vetmic.2006.08.027

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