

REVIEW ARTICLE

A systematic review/meta-analysis of primary research investigating swine, pork or pork products as a source of zoonotic hepatitis E virus

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SUMMARY

The objectives of our study were to identify and categorize primary research investigating swine/pork as a source of zoonotic hepatitis E virus (HEV) using the relatively new technique of scoping study, and to investigate the potential association between human exposure to swine/pork and HEV infection quantitatively using systematic review/meta-analysis methodology. From 1890 initially identified abstracts, 327 were considered for the review. Five study design types (cross-sectional, prevalence, genotyping, case-report and experimental transmission studies) were identified. A significant association between occupational exposure to swine and human HEV IgG seropositivity was reported in 10/13 cross-sectional studies. The association reported between pork consumption and HEV IgG seropositivity was inconsistent. The quantification of viral load in swine and retail pork, viral load required for infection in primates, cohort and case-control studies in humans, and formal risk assessment are recommended before specific public-health policy actions are taken.

Key words: Hepatitis E virus, public health emerging infections, zoonoses.

INTRODUCTION

Mammalian hepatitis E virus (HEV) is an RNA virus of the genus *Hepevirus*, which includes one serotype with four genotypes [1]. The latter are somewhat distinct with regards to both spatial and host distribution and characteristics. Genotype 1 has been isolated from humans in Asia, genotype 2 from humans in Mexico, genotype 3 from humans and swine in Europe and North America, as well as other animal

species, and genotype 4 from humans and swine in East Asia [2].

Hepatitis E, the clinical disease caused by HEV, occurs frequently as outbreaks of jaundice, primarily in tropical and subtropical regions, where the disease is endemic, and spread by the faecal–oral route [3]. The World Health Organization (WHO) reports that overall mortality due to hepatitis E ranges from 0·5% to 4%, with fulminate hepatitis occurring most frequently in women during pregnancy [4]. Over the past 10 years, sporadic locally acquired cases of hepatitis E have been reported in individuals living in non-endemic areas, and without history of recent travel to endemic regions [5–8]. These cases are typically

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observed in older men [9, 10] in contrast to water-borne outbreaks, which tend to affect younger adults aged between 15 and 40 years. Recent surveys conducted in North America report HEV immunoglobulin G (IgG) seroprevalence in adults ranging from 2.4% to 21% [11, 12] while the diagnosis of locally acquired clinical hepatitis E cases remains rare [5].

The apparently low proportion of HEV infections resulting in clinical disease may be explained by variability of viral load in various geographical areas, as well as differences in virulence among HEV genotypes, host characteristics, and variable sensitivity and specificity of serological assays employed [12, 13].

In non-endemic areas, the source of HEV exposure in asymptomatic seropositive individuals as well as locally acquired hepatitis E cases has been related to various animal reservoirs including swine, wild boar, deer, and rodents [14–16], possibly explaining the geographical clustering of genetically similar human and swine strains of HEV [9, 17].

It is believed that swine are a source of zoonotic hepatitis E, and the supporting evidence is founded upon phylogenetic homology between swine and human strains of HEV, HEV IgG seroprevalence in swine and human populations, and case reports describing patients' specific exposures [1, 2, 18]. This diverse body of evidence, including multiple study designs, is challenging to summarize and critically evaluate, not only in estimating effect of exposure to swine/pork on human outcomes (e.g. HEV IgG seroprevalence), but also with regards to assigning a weight of evidence to the body of studies underpinning the estimates. We therefore chose to sequentially employ two specific methodologies, a scoping study and a systematic review/meta-analysis, to accommodate these challenges. This allowed us to categorize the global evidence, and estimate quantitatively the association between exposures to swine/pork and human HEV infection, which has not been previously reported.

Scoping reviews have been used recently in health-care to investigate broad clinical management questions, such as the role of complementary and alternative medicine in clinical practice, which may be similarly underpinned by a wide range of diverse primary research [19]. The methodological framework varies among studies and is still being validated. However, the end product of a scoping study, regardless of the specific methodology used, is a categorization/cataloguing of the available evidence, by

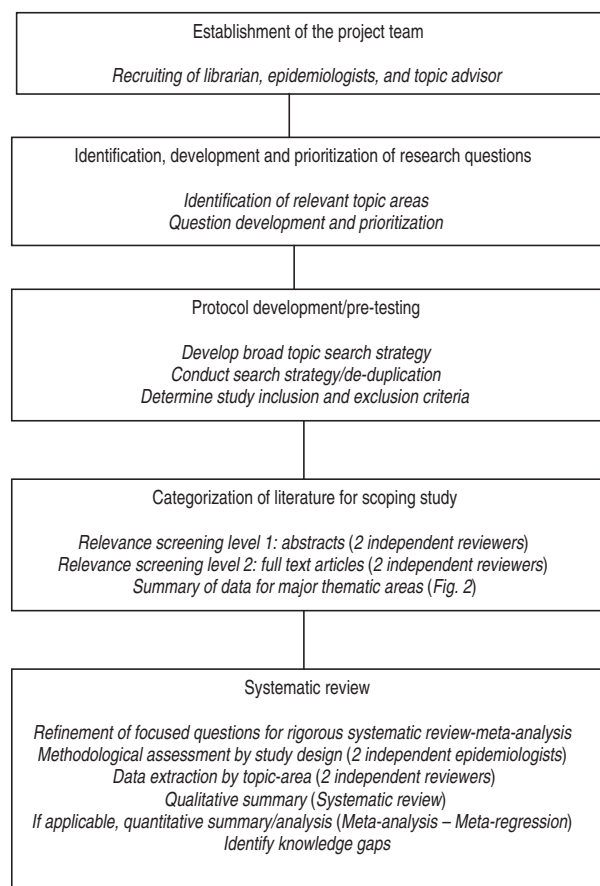


Fig. 1. Framework for scoping study and systematic review methodology used in this study. (Adapted from [120].)

study design, population sampled, outcomes measured, etc. [20]. Systematic review methodology offers a structured, transparent and replicable approach to identifying, critically appraising, and summarizing scientific evidence particularly useful in questions investigating zoonoses, and may allow for the quantification of estimates of association, which traditional narrative reviews do not [21]. Therefore, the study objectives were:

- (1) To identify and categorize the existing global primary research investigating swine or pork as a source of zoonotic HEV using a scoping study.
- (2) To formulate, based on the results of scoping study (i.e. relevance screening and categorization of studies), specific questions related to our broad topic of evidence supporting swine as a source of zoonosis, and conduct a rigorous systematic review, and where appropriate, meta-analysis quantifying the strength of association between exposure and outcome (Fig. 1).

MATERIALS AND METHODS

Definitions used in scoping study and systematic review

The definitions are available as supplementary online material (see Appendix 1).

Review team, questions, and protocol

The scoping study/systematic review process is shown in Figure 1. The review team included a librarian, research assistant, six epidemiologists, and a virologist/topic advisor. The broad research question, i.e. identifying the evidence for swine or pork as a source of zoonotic HEV, was refined into three specific questions that were addressed through subsequent systematic review, after a second level of relevance screening/categorization of full articles was complete:

- (1) Is human HEV IgG seroprevalence as measured by enzyme-linked immunoassay (ELISA) associated with exposure to swine or pork?
- (2) Is detection in humans of HEV RNA measured by reverse transcription–polymerase chain reaction (RT–PCR) associated with exposure to swine or pork?
- (3) Is locally acquired clinical hepatitis E associated with exposure to swine or pork?

A study protocol was developed and pre-tested *a priori* for each step of the review, which commenced when agreement between each pair of reviewers yielded a Cohen's kappa value >0.8 that was not due to chance [22]. Two reviewers independently screened all abstracts or full articles at each stage of the review.

Search terms and search strategy

Multiple broad and specific search terms were developed for population (e.g. pigs OR porcine OR hog) and outcome (e.g. hepatitis E virus OR hepatitis) components of the review question. Human terms were not included, as relevant studies that sampled human populations as well as swine, were captured using the simpler search algorithm containing only swine population terms. For a complete list of search terms and combinations see Appendix 2 (available online). The search was executed in September 2008, and updated in October 2009, in four online electronic databases, with no restrictions on date of publication: Agricola (1970–2009), Current Contents (1999–2009), PubMed (1800s–2009), and Commonwealth

Agricultural Bureau Abstracts (1900–2009). All electronic citations were downloaded and de-duplicated in a bibliographical management program Procite 5.0 (Thomson ResearchSoft, USA), followed by a manual de-duplication.

Using a random number generator, 10 relevant primary research articles captured by the search were selected for a manual search of their reference lists to verify that potentially relevant citations were not missed by the electronic search. Ten topic experts identified by the search were contacted to request any work close to being submitted, or in press; non-responders were contacted once more.

Study inclusion criteria and relevance screening

For inclusion in the scoping study, which comprised the literature search and two levels of reference screening, all abstracts (level 1) and full articles (level 2) reporting primary research in English or French, investigating swine and/or pork as a source of zoonotic HEV, were potentially relevant. Given our broad search strategy, numerous citations not meeting our inclusion criteria were also captured, and these were excluded largely at level 1 or in some cases, level 2 screening, by application of our screening tools. Additionally some studies ($n=57$), while mentioning zoonosis in the abstract, did not, in fact, investigate this topic, and were therefore excluded after appraisal of the full article.

Studies published in languages other than English or French were excluded due to resource constraints. Primary research investigating HEV in other species of animals were categorized, but not considered for methodological assessment or data extraction. The exposure 'swine or pork' was broadly defined as sampling in all production settings, from farm to retail, and within local contextual norms (from agrarian tribes to intensive livestock operations) as described by the authors. Wild boar, although closely related to domestic swine phylogenetically, are usually raised in extensive outdoor settings that have lower population densities than commercial swine production. They are included in a complementary systematic review of the evidence for other animal species (including ruminants and wildlife) as sources of zoonotic HEV, currently underway. Therefore studies investigating HEV IgG seroprevalence or detection of HEV RNA in wild boar populations were categorized, but not considered for further assessment and data extraction. However, case reports investigating locally acquired

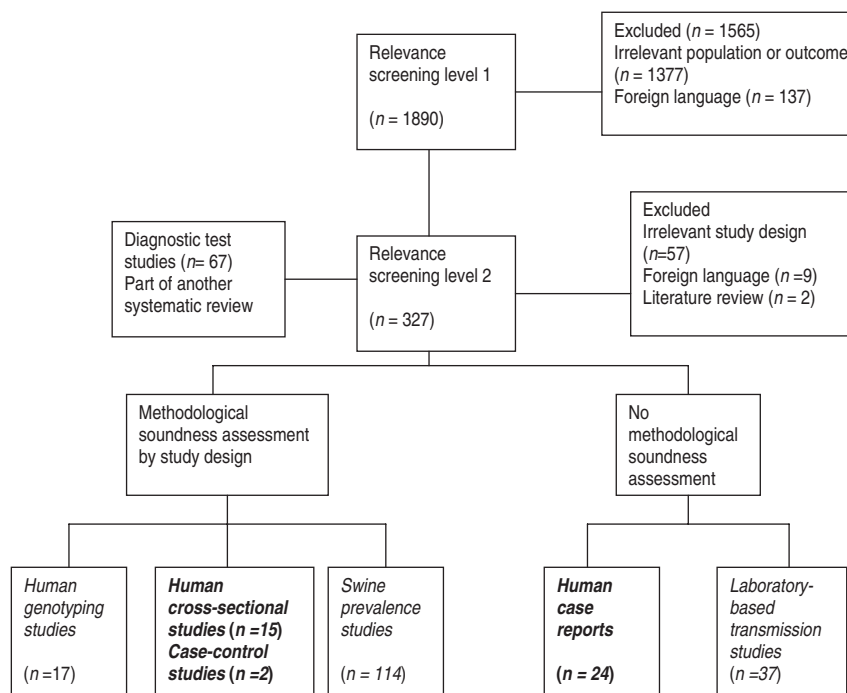


Fig. 2. A summary of scoping study and systematic review results. Papers included in the scoping study are indicated in *italics*. Papers included in the systematic review are indicated in bold.

clinical hepatitis E in individuals who consumed wild boar meat were included in the review, due to the limited volume of information on clinical hepatitis E source attribution. Studies sampling other domestic ($n=16$) and wildlife ($n=42$) species were outside the scope of the current study.

Through first-level relevance screening based on abstracts, studies outside the review scope were excluded. Relevant primary research was categorized at second-level screening, based on the full article, into specific topic-related areas: studies investigating HEV IgG seroprevalence or detection of HEV RNA in humans and/or swine; laboratory-based transmission experiments investigating HEV infection in swine or primates; case reports of locally acquired hepatitis E in humans investigating swine, pork or wild boar as potential sources of infection, and studies developing or evaluating diagnostic test performance (Fig. 2). The first three specific areas are the focus of this review. The studies investigating the performance of diagnostic tests will be analysed and reported separately.

Methodological assessment and data extraction

Studies investigating HEV IgG seroprevalence, or detection of HEV RNA in humans or swine, were assessed for methodological soundness and/or

reporting using a modification of the Grading of Recommendations, Assessment, and Evaluation (GRADE) system developed by the Cochrane Collaboration [23]. Five criteria examined were: potential study design problems (sampling scheme and justification of sample size), inconsistency (studies reported estimates of association less than and/or greater than 1) and imprecision of findings across studies [e.g. 95% confidence interval (CI) includes 'negligible effect'], 'indirectness', or lack of comparability between sample and target population (e.g. measuring seroprevalence when outcome of interest is shedding), and presence/effect of publication bias (e.g. effect estimate, after adjustment for publication bias, was reduced in magnitude) (Appendix 3, online).

The Bradford Hill criteria [24] were applied to examine the evidence for a causal relationship between exposure to swine or pork, and human HEV IgG seroprevalence (Appendix 4, online) particularly with regards to design of the underpinning studies.

The transmission and case-report studies were not assessed for methodological soundness, due to their descriptive nature, but underwent data extraction. The data extraction process included data categorization according to various types of study design and outcome.

We restricted data extraction from the swine survey studies to the two subsets investigating either

HEV IgG by ELISA, or detection of HEV viral RNA using RT-PCR, in either market-age swine, or retail pork, as these two populations are closest to consumers and therefore pose the greatest risk of zoonotic HEV.

Statistical analysis

Data were entered and cleaned in Microsoft Office Excel 2003 (Microsoft Corporation, USA), double-checked for errors, and descriptively summarized. Descriptive statistical analyses were performed in Stata Intercooled 11 (Stata Corporation, USA); meta-analysis was performed in Comprehensive Meta-Analysis 2 (Biostat Inc., USA).

Random-effects meta-analysis was performed on two subsets of data: studies comparing HEV IgG seroprevalence in humans exposed or unexposed to occupational contact with swine, and studies comparing HEV IgG seroprevalence in humans exposed or unexposed to consumption of pork, both based on the *a priori* assumption that heterogeneity existed across studies. Crude odds ratios between exposed and unexposed groups, and 95% CIs were calculated from reported raw data. A pooled estimate of the odds ratio was calculated using the method of DerSimonian & Laird [25]. A forest plot displaying the point estimate and 95% CIs of the effects observed in each study, as well as the summary estimate and percent weights, were generated (Appendix 5, online). Cochran's *Q* statistic (standardized measure of dispersion across studies) and *I*² (the percentage of total variation among studies due to heterogeneity) were used to evaluate heterogeneity [26]. Evidence for the presence of publication bias, in each group of studies was examined using the trim-and-fill method of Duval & Tweedie [27] (Appendix 6, online).

RESULTS

Scoping study

From 1890 de-duplicated citations, 327 were potentially relevant (Fig. 2).

The main characteristics of 15 studies evaluating HEV IgG seroprevalence as measured by ELISA in human populations exposed or unexposed to swine and/or pork are summarized in Table 1. Their design was essentially cross-sectional, although subjects' exposure status was established prior to sampling. The majority of studies (*n* = 10/13 analyses, of 12 unique

sets of samples, i.e. one set of samples received two methods of analysis) investigated occupational contact with swine, and two of three investigated consumption of pork as the exposures of interest, reported a significantly greater odds (*P* < 0.05) of seropositivity in the exposed group.

Clinical disease outcomes were less frequently reported (*n* = 3 studies). In a German case-control study, cases of locally acquired hepatitis E were not associated with consumption of pork, either cooked or under-cooked [10]. However, a small Japanese case-control study found a significant (*P* < 0.001) association between pork liver consumption and locally acquired hepatitis E [28]. In a cross-sectional study conducted in Nepal, the occurrence of human jaundice cases in an agrarian tribe was significantly associated with their close proximity to pigs that were HEV IgG seropositive [29].

A summary of 17 studies assessing relatedness of human HEV isolates recovered from serum samples, and local swine HEV isolates is shown in Table 2. Of 122 HEV genotype 3 and 4 isolates recovered, 23 were reported to be genetically similar to local swine HEV isolates, with the remainder of isolates showing genetic relatedness to local swine isolates ranging from 83–99% based on phylogenetic analysis of the ORF1, ORF2 and full-length genome sequences.

A summary of 24 case reports or case series of locally acquired clinical hepatitis E patients reporting occupational or consumption exposures to swine, pork, or wild boar, as well as a variety of other exposures to putative risk factors, is presented in Table 3. The potential association between clinical outcome and exposure, measured through genotyping of HEV isolates recovered from exposed patients, was confirmed in two studies [30, 31].

Seroprevalence of HEV IgG antibodies as detected by ELISA, and/or detection of viral RNA as identified by RT-PCR, in swine populations of various ages was reported in 114 studies, of which 24 sampled market-age swine (Table 4). All 22 unique studies of HEV IgG seroprevalence, and 15/22 studies investigating HEV RNA as an outcome, reported positive animals. Four of five studies sampling retail pork liver detected HEV RNA in some of the samples tested.

Experimental studies (*n* = 37) of transmission of HEV in swine and/or primates were conducted in controlled research facility settings, and investigators documented the transmission of swine HEV isolates to primates [32] and the transmission of HEV

Table 1. Descriptive summary of 15 studies comparing hepatitis E virus (HEV) immunoglobulin G (IgG) seroprevalence in groups exposed or unexposed to occupational contact with swine, or consumption of pork*

Ref. Country	Populations studied	Prevalence (no. sampled)	Crude OR (95% CI)	Significance level (statistical test)
[16] USA§	SW	10.9% (165)	5.06 (1.46–17.6)	$P=0.006$
	GP	2.4% (127)		(χ^2)
[52] Netherlands‡	SV	11% (49)	5.55 (1.89–16.3)	Significant at 5% level†
	GP	2% (648)		
	SV	8.5% (47)	1.42 (0.34–5.9)	
	GP	6.4% (65)		$P>0.05\#$
[53] Denmark§	F	50% (283)	2.11 (1.42–3.14)	$P=0.046$
	GP	33% (167)		(χ^2)
[54] Moldova§	SW	51% (264)	3.45 (2.37–5.04)	$P<0.0001$
	GP	25% (255)		(χ^2)
[55] Spain‡¶	SW,SV	19% (97)	5.39 (1.76–16.48)	$P=0.03$
	GP	4.1% (101)		(bivariable logistic regression)
[56] Taiwan‡¶	SW	27% (30)	4.18 (1.14–15.4)	$P=0.03$
	GP	8% (50)		(χ^2)
[57] USA§	SV	23% (295)	1.61 (1.12–2.31)	$P<0.05$
	GP	16% (400)		(univariable logistic regression)
[58] China‡¶	SW	75.9% (340)		
	GP			
	(i) upstream from swine production	50.1% (425)	3.10 (2.26–4.24)	$P<0.05$
	(ii) downstream	61.2% (1295)	1.54 (1.23–1.91)	(multivariable logistic regression)
[59] China‡¶	SW	67% (52)	8.09 (4.49–14.5)	$P<0.0001$
	GP	20% (2572)		(χ^2)
[60] China‡¶	SW	31.6% (985)	1.73 (1.48–2.01)	$P<0.01$
	GP	21.1% (3994)		(χ^2)
[61] Sweden‡¶	SW	13% (115)	1.47 (0.63,3.43)	$P>0.05$
	GP	9.3% (108)		
[62] Italy‡¶	SW	3.3% (92)	1.14 (0.35,3.66)	$P>0.05$
	GP	2.9% (3511)		
[12] USA§	Pork/ham eaten			
	0–5 times/month	21.9% (10,600)	0.763 (.641–.911)	$P>0.05$
	> 10 times/month	16.1% (992)		(multivariable logistic regression)
[40] India§¶	Pork-eating tribe	73% (48)	15.7 (6.62–37.4)	$P=0.000$
	Non-pork eating tribe	15% (89)		(χ^2)
[41] Indonesia§¶	Hindus	20% (102)	12.1 (2.74–53.2)	$P=0.012$
	Muslims	2% (101)		(χ^2)

OR, Odds ratio; CI, confidence interval; SV, swine veterinarians; NSV, veterinarians not practising on swine; GP, general population; SW, workers in commercial swine production; F, farmers; animals on-farm were not specified.

* All studies were essentially cross-sectional in design, although exposure status was established prior to measuring outcome measurement (serum HEV IgG antibodies as detected by enzyme-linked immunosorbent assay (ELISA); cut-offs varied and some were not reported).

† Bayesian credible intervals reported for median estimates of seropositivity; informative priors on ELISA specificity selected.

‡ Commercially available ELISA used.

§ Non-commercially available ELISA used.

|| Cut-off values for establishing ELISA seropositivity reported.

¶ Cut-off values for establishing ELISA seropositivity not reported.

Non-Bayesian analysis performed using combined assay results.

genotypes 3 [14] and 4 [33] to swine intravenously, and by contact exposure [34]. Detection of viral particles in the muscle of infected swine was reported in two

studies [34, 35]. In one US study, the infectious nature of the HEV detected in liver sampled at retail was confirmed through the successful transmission of

Table 2. Descriptive summary of 17 studies comparing genotypes of human hepatitis E virus (HEV) isolates with local circulating swine isolates

Ref.	Country	Population (no. of samples)	Laboratory test (sample type)	No. of isolates recovered	Genomic region studied	Genetic relatedness (%) between human and swine HEV isolates
[63]	Korea†	NR (568)	RT-PCR + sequencing (serum)	15 isolates, 7 strains	ORF2	92–99% with swine isolates KOR1, KOR2, and KOR3
[64]		Pooled samples	RT-PCR + sequencing (sewage)		ORF1, ORF2	Spain: swine isolates recovered 87–91% with recovered human isolates
	Spain†	(46)		Barcelona 15		
	USA†	(5)		Washington 1		USA: 98.4% with US swine strain
	France†	(4)		Nancy 1		France: not similar
[65]	New Zealand†	Hepatitis patients (77)	RT-PCR + sequencing (serum)	3	ORF1, ORF2	83–86% with NZ swine; authors conclude not similar
[66]	UK†	Hepatitis patients (333)	RT-PCR + sequencing (serum)	15	ORF2	Similar§
[67]	UK†	Hepatitis E patients (13)	RT-PCR + sequencing (serum)	8	ORF2	Similar
[68]	Spain*†‡	Hepatitis E patients (14)	RT-PCR + sequencing (serum)	14	ORF1, ORF2	4 type 3f, 96% similarity with 3 known swine isolates
[69]	Japan†‡	Blood donors (3185)	RT-PCR + sequencing (serum)	8 type 3, 3 type 4	ORF2	8 type 3 isolates similar to Japanese human, swine and wild boar isolates
[70]	Denmark†, Sweden†	Acute hepatitis patients	RT-PCR + sequencing (serum)	57 type 1, 6 type 3	ORF2	All type 3 isolates acquired in Europe, similar to Swedish and Danish swine isolates
[71]	Spain†	Hepatitis patients (37)	RT-PCR + sequencing (serum)	3	ORF1, ORF2	92–94% similarity between human and swine isolates recovered
[72]	Germany†	Hepatitis case (1)	RT-PCR + sequencing (serum)	1	ORF1, ORF2	95–97% with Dutch swine isolates
[6]	Netherlands†	Confirmed HEV infection (19)	RT-PCR + sequencing (serum)	13	ORF2	12 of 13 strains segregated into two sub-lineages, each containing strains recovered from swine in the Netherlands
[73]	Japan†	Blood donors	RT-PCR + sequencing (serum)	23	ORF2	All ≥92.2% swine isolates
[74]	Japan†	Hepatitis case (1)	RT-PCR + sequencing (serum)	1	Full genome nucleotides, ORF1 amino acids	2 each 99.8% with local swine isolates
[75]	Japan†‡	Hepatitis case (1)	RT-PCR + sequencing (serum)	2	Full genome nucleotides HE J-14	87% with 2 US human, and US swine strains
					ORF1, ORF2 HE-J13	88.6–95.1% with US swine and human strains
[76]	Indonesia‡	Hepatitis patients (57)	RT-PCR + sequencing (serum)	1 human, 5 swine isolates	ORF2	97.3–98.3% similarity with 4 of 5 local swine isolates
[77]	China‡	Non-hepatitis hospital patient (1)	RT-PCR + sequencing (faeces)	1	ORF2	Not similar to local swine isolates
[78]	China‡	General population (13 IgM positive)	RT-PCR + sequencing (serum)	2	ORF2	94–98% with swine isolates recovered

RT-PCR, Reverse transcription–polymerase chain reaction.

Studies sampled various human populations, and compared genetic homology of recovered isolates with known local swine isolates.

* Hepatitis E virus genotype 1.

† Hepatitis E virus genotype 3.

‡ Hepatitis E virus genotype 4.

§ ‘Similar’ is the descriptor used by the authors of each indicated study.

Table 3. Descriptive summary of 24 case reports investigating potential exposures for locally acquired clinical hepatitis E cases*

Physical sampling†

Ref.	Location (no. of cases)	Exposure sampled	Results: Exposures sampled Positive/tested	Genotype/genetic relatedness (%) of isolates recovered from exposures sampled
[6]	Netherlands (19)	Pet pig faeces Garden pig manure Frozen pig liver	0/1 0/1 0/1	Genotype 3, n.a.
[30]	Japan (1)	Wild boar meat consumed	1/1	Genotype 3, 99.5% similarity between remaining boar meat, and case isolate
[79]	UK (1)	Swine faeces from adjacent farm Water downstream from farm	1/3 0/1	Genotype 3, 86.3–86.6% with case; authors conclude not similar
[31]	France (1)	Pet pig faeces	1/1	Genotype 3, 98% similarity with case isolate

Questionnaire‡

Author	Location (no. of cases)	Exposure recorded by questionnaire	Results: Cases sampled/ cases positive	Genotype/genetic relatedness (%) of case isolates with local swine strains
[80]	Germany (1)	Slaughterhouse worker	1/1	Genotype 3f; closest homology Spanish, Dutch, French human and swine strains
[6]	Netherlands (case series of 19)	Eat pork > once/week Eat raw pork/organs Eat processed pork	16/19 0/19 9/19	Genotype 3
[45]	France (1)	Eat undercooked pork Eat wild boar	0/1 0/1	Genotype 3c
[66]	UK (21)	Contact swine Eat pork Contact undercooked pork Contact pigs	0/1 21/21 2/21 1/21	Genotype 3, similar to UK swine isolates
[81]	France (1)	Exposure swine during surgical training	1/1	Genotype 3c only previously reported in swine
[82]	France (2)	Eat undercooked pork	2/2	Genotype 3
[66]	UK (8)	Eat undercooked pork Contact farm animals	0/8 0/8	Genotype 3, similar homology to local swine strains
[83]	Japan (1)	Eat pork	1/1	Genotype 3
[84]	France (62)	Occupational exposure to swine	0/62	Genotype 3, 52 3f isolates recovered, some similar to Dutch swine isolates

Table 3 (cont.)

Author	Location (no. of cases)	Exposure recorded by questionnaire	Results: Cases sampled/ cases positive	Genotype/genetic relatedness (%) of case isolates with local swine strains
[85]	Japan (14)	Grilled pork consumed	8/8	Genotype 4, 99·4% homologous with swJL145 isolated from local retail pig liver
[86]	Japan (1)	Transfusion from index case	1/1	Genotype 3, 1 of 2 other diners HEV IgM positive*
[87]	Japan (2)	Eat grilled wild boar specific meal	1/1	Genotype 4
[88]	Japan (case series of 32)	Eat undercooked pork	2/2	Genotype 3, 7 cases
[89]	France (264)	Eat raw or undercooked pig liver or intestines	25/32	Genotype 4, 25 cases
[89]	France (264)	Eat undercooked pork	42/264	n.r.
[7]	Spain (1)	Occupational exposure swine	5/264	
[90]	Hungary (1)	Work in slaughterhouse	1/1	Genotype 3f homology with local swine strains 87·3–97·3%
[91]	Japan (5)	Eat home-prepared pork sausage	1/1	95–97% with European swine strains
[91]	Japan (5)	Eat wild boar one specific meal	8/12	Genotype 3, isolates from 2 cases 99·4% similar with each other
[92]	Japan (1)	Contact with pigs	0/1	3/10 other diners HEV IgM positive
[93]	UK (1)	Contact with pigs	0/1	Genotype 3 (HE-JA10), 91·6–95·7% with 27 US swine isolates
[94]	Netherlands (case series of 3)	Contact with pigs	0/3	Genotype 3
[28]	Japan (case series of 10)	Eat grilled pig liver	9/10	Genotype 3, 2 isolates Genotype 4, 8 isolates; HE-JA18 (case 9) 100% with swJL145 recovered from local retail liver (Table 4)

n.a., Not applicable; n.r., not reported.

* All cases described were acute sporadic Hepatitis E, non-travel-associated, confirmed by hepatitis E virus (HEV) immunoglobulin M (IgM) antibodies detected by enzyme-linked immunosorbent assay (ELISA) and/or nucleic acids detected by reverse transcription–polymerase chain reaction (RT–PCR).

† Exposures were physically sampled to attempt HEV nucleic acid detection.

‡ Cases' exposures were identified by questionnaire, but were not physically sampled to detect HEV.

Table 4. Descriptive summary of 29 studies investigating hepatitis E virus (HEV) immunoglobulin G (IgG) antibodies, or detection of HEV nucleic acids, in market-age swine and retail pork

Ref.	Location	Laboratory test (sample type)	No. sampled individuals (farms)	Prevalence	Genotype/genetic relatedness (%) of recovered isolates with known human HEV isolates
Studies sampling market-age swine					
Asia					
[95]	India	ELISA (serum)	40 (1)	67.5 %	Genotype 4
		RT-PCR (serum)	40 (1)	2.5 %	
[96]	Thailand	RT-PCR (serum)	10 (1)	0 %	n.a.
[97]	Korea	RT-PCR (faeces)	142 (12)	38 %	Genotype 3a, 89.6–94.8 % with US-1
[98]	China	RT-PCR (liver)	114 (n.r.)	3.50 %	Genotype 4, 2 isolates 96.1–96.4 % local human strains
[99]	Japan	RT-PCR (faeces)	36 (3)	8 %	Genotype 3
[100]	Japan	ELISA (serum)	169 (17)	74.6 %	Genotype 3
		RT-PCR (serum)	169 (17)	1.8 %	
[101]	China	PCR (bile)	603 (n.r.)	1.82 %	Genotype 4
[102]	Thailand	ELISA (serum)	19 (5)	100 %	Genotype 3
		RT-PCR (serum)	19 (5)	0	
[103]	Japan	ELISA (serum)	250 (25)	90 %	Genotype 3, 4 isolated from younger swine
		RT-PCR (serum)	250 (25)	0	
[104]	Japan	ELISA (serum)	6 (1)	100 %	Isolate from younger swine 94 % with HE-JA5 (human case)
		RT-PCR (serum)	6 (1)	0	
[105]	Japan	ELISA (serum)	136 (92)	74 %	n.a.
		RT-PCR (serum)	136 (92)	0	
[106]	Indonesia-Bali	ELISA (serum)	11 (8)	82 %	Genotype 4
[107]	Taiwan	RT-PCR (serum)	112 (4)	1.8 %	Genotypes 3,4
[78]	China	ELISA	193	53.9 %	Genotype 3, 1 isolate
		RT-PCR (serum)	96	5.20 %	Genotype 4, 8 isolates
Europe					
[108]	Spain	RT-PCR (serum)	16 (1)	12.5 %	Genotype 3, 84–100 % other Spanish swine or human isolates
[109]	Spain	RT-PCR (faeces)	27 (17)	7 %	82–99 % other swine
		RT-PCR (serum)	27 (17)	11 %	Genotype 3
[110]	UK	RT-PCR (faeces)	50 (9)	8 %	Genotype 3
[111]	Netherlands	RT-PCR (faeces)	50 (10)	14 %	Genotype 3a, c, e, or f, 1 3c isolate identical to local human isolate
[112]	Spain	ELISA (serum)	9 (1)	100 %	Genotype 3
		RT-PCR (serum)	10 (1)	0 %	
		RT-PCR (faeces)	5 (1)	0 %	
North America					
[113]	Canada	RT-PCR (serum)	51 (1)	11.8 %	Genotype 3
		RT-PCR (feces)	51 (1)	41.2 %	
[114]	USA	ELISA (serum)	54 (6)	95 %	n.a.
[11]	Canada	ELISA (serum)	998 (> 80)	58.2 %	n.a.
South America					
[115]	Brazil	ELISA (serum)	26 (1)	88.4 %	n.a.
[116]	Brazil	ELISA (serum)	37 (10)	92 %	n.a.
Studies sampling retail pork					
[117]	UK	RT-PCR (liver)	80 outlets	0	n.a.
[118]	Netherlands	RT-PCR (liver)	62 livers	6.5 %	Genotype 3, 93 % local human strains; 97 % local swine strains
[36]	USA	RT-PCR (liver)	127 (3)	11 %	Genotype 3, 86–94 % prototype swine; 87–93 % with 2 human US strains
[119]	India	RT-PCR (liver)	240 livers	0.83 %	Genotype 4
[28]	Japan	RT-PCR (liver)	363 livers	1.9 %	Genotype 4, swJL145 100 % with HE-JA18 (human case isolate) Genotype 3, swJL235, 325 98.5–100 % HE-JA4 (human isolate)

ELISA, Enzyme-linked immunosorbent assay; RT-PCR, reverse transcription-polymerase chain reaction; n.a., not applicable; n.r., not reported.

recovered virus to other swine [36]. The infectiousness of HEV recovered from naturally contaminated pork livers, was inactivated through stir-frying or boiling for 5 min [37], but not by heating to a core temperature of 56 °C [38].

A cross-sectional study of wild boar as a source of zoonotic HEV compared HEV IgG seroprevalence in hunters with the general population and reported a significantly higher ($P < 0.0001$) HEV IgG seroprevalence in hunters [39]. One case report described a genetic match between the HEV isolate from partially consumed wild boar and the patient's HEV isolate [30].

Systematic review

(1) *Is human HEV IgG seroprevalence as measured by ELISA associated with exposure to swine or pork?*

The main characteristics of 15 analyses evaluating the potential association between HEV IgG seroprevalence and humans exposed or unexposed to swine or pork via either exposure to swine, or consumption of pork, are shown in Table 1. Twelve studies reported greater odds of seropositivity in the exposed group.

Meta-analysis of 12 cross-sectional studies evaluating potential association between HEV IgG seroprevalence in individuals occupationally exposed to swine, and the general population is shown in Appendix 5. The latter is shown for observation of visual trends only. The pooled estimate effect and corresponding 95% CIs, although statistically significant ($P < 0.05$), are not reported, due to a significant ($P < 0.05$) Q statistic of 47.7, and an I^2 statistic of 77.0% suggesting a high degree of heterogeneity [26]. Analysis for publication bias using the method of Duval & Tweedie yielded an adjusted estimated odds ratio, and imputed three unpublished studies (Appendix 6), suggesting publication bias was present in this set of studies.

Evidence ranking for these studies, based on a modified GRADE system, yields a 'very low' ranking, suggesting the estimate is very uncertain and is likely to change with further research [23] (Appendix 3).

Meta-analysis of three cross-sectional studies investigating the association between consumption of pork, and HEV IgG seropositivity [12, 40, 41] also resulted in a significant Q statistic of 61.2, and I^2 statistic of 96.3% indicating a high level of heterogeneity across studies.

(2) *Is detection of HEV RNA measured by RT-PCR in humans associated with exposure to swine or pork?*

No conclusion can be drawn from the evidence captured as the samples for viral detection were drawn from clinical cases, without a comparison group.

(3) *Is locally acquired hepatitis E associated with exposure to swine or pork?*

Two case-control studies examined locally acquired clinical hepatitis E as the outcome of interest, with conflicting results. German researchers reported consumption of pork, either cooked or undercooked, was not a significant risk factor for clinical disease [10], while a Japanese study reported consumption of pig liver was a significant ($P < 0.001$) risk factor [28]. Twenty-four case reports or case series described locally acquired clinical hepatitis E investigating swine, pork, pork products, or wild boar as possible sources of infection, but these did not allow estimation of an association due to the lack of a comparison group.

DISCUSSION

In HEV non-endemic countries, such as Canada, hepatitis E is not a federally notifiable disease [42]. Thus, a diagnosis of hepatitis E is often not considered in unexplained hepatitis cases in Canada without a history of recent travel abroad (Dr F. Milord, Dr R. Slinger, Dr W. Wong, personal communication). Research from Europe, also a non-endemic region, suggests that HEV may be a cause of both acute and chronic hepatitis in patients with no history of travel [6, 43–45]. Hepatitis E cases may also be under-reported in jurisdictions where there is no domestically licensed test for anti-HEV antibody, such as the USA [12].

The increase in the proportion of relevant papers captured by our first search (220/1650), compared to the updated search (103/238), underlines the current interest in swine or pork as a source of zoonotic HEV. Our search included only 'swine' population terms, and therefore the literature captured might not be representative of the research investigating other populations such as cattle or wildlife. Nevertheless, a wide range of study designs, including transmission experiments, cross-sectional surveys of seroprevalence, genotyping studies, and case reports of clinical disease following exposure to swine or pork, confirm that HEV infection is probably transferable from swine or through pork consumption to humans.

However, the nature of the observational studies precludes appropriate investigations of temporality, namely that exposure to swine results in a health outcome, and for this reason potential exposure to a third, common source of virus cannot be excluded [46, 47]. Laboratory experiments report the transmission of HEV from swine to primates, but under unusual conditions, such as an artificial (intravenous) route of infection, and great caution is required in generalizing these findings to community settings.

Additional questions arise. Given the widespread seroprevalence as well as detection of HEV in market-age swine and retail pork, why are only a few reported cases of hepatitis E directly attributable to pork consumption? Moreover, why do the demographics of locally acquired hepatitis E cases differ from those of waterborne hepatitis E [10, 13]?

Viral load is suggested as an important determinant for developing of HEV infection in humans [12] and primates [48]. Requirement for a certain minimum viral load might explain the relatively low number of reported cases of locally acquired hepatitis E attributable to pork consumption, as well as variations in viral virulence, or host susceptibility.

Overall, our results indicate that swine populations are probably a source of zoonotic HEV. Quantitative summary of evidence for the first systematic review question identified a statistically significant ($P < 0.05$) association between occupational exposure to swine, and human HEV IgG seroprevalence. However, the statistically significant ($P < 0.05$) Q statistic, and the high ($> 75\%$) I^2 indicate heterogeneity across studies [49]. Similarly, for the three studies investigating the association between the consumption of pork and HEV IgG seropositivity, the statistically significant ($P < 0.05$) Q statistic, and the high I^2 statistic indicate heterogeneity, i.e. the effect of exposure was not fixed, but varied across studies. Possible sources of heterogeneity include a variation in susceptibility of different populations, or infectiousness of HEV strains, or intensity of exposure to swine/pork, and variation in test performance.

The performance of different ELISA tests to identify human exposure to HEV has been debated for the past decade [3]. The cross-sectional studies underpinning the first systematic review question employed a variety of ELISA tests, both commercial and in-house, reporting differing cut-off values for identifying seropositivity. The overall impact of the heterogeneity across tests underpinning the association between exposure to swine or pork, and human HEV

seropositivity, is unknown. Additionally, sensitivity of these kit tests may also be variable and/or low, particularly in certain strata of the population, such as remote, i.e. chronologically distant, infections, raising the possibility of differential mis-classification [50] (Dr A. Andonov, personal communication). The combination of these facts suggests that both population estimates of HEV seropositivity in non-endemic regions, and pooled estimates of effect of exposure to swine/pork on seropositivity, may change.

The hierarchical level of evidence associated with this body of studies (Appendix 3) using the GRADE ranking system, is weak. This ranking is a reflection of frequent use of convenience sampling, failure to report justification for sample size, and evidence of publication bias, underlining the need for prospective targeted research in this area, using sound methodological design. This, too, suggests that both the reported and pooled estimates of association between exposure and outcome are uncertain, and may change with time.

Although we have identified a statistically significant association between occupational exposure to swine and seropositivity, the more relevant public health question is: does exposure to swine or pork *cause* increased odds of HEV IgG seropositivity? First described in 1965, the Bradford Hill criteria remain the widely accepted framework for demonstrating a causal relationship [24]. Examination of the primary research supporting the Bradford Hill criteria (Appendix 4) indicates that there is some evidence for each criterion. However, the studies included in our review are observational or laboratory experiments conducted on populations other than the one of interest. Therefore neither is adequate to establish cause-and-effect [47].

More importantly, the public health impacts of an association between exposure to swine and HEV IgG seroprevalence are unknown due to the difference between investigating seroprevalence, vs. clinical disease as an outcome. In all cross-sectional, genotyping and prevalence studies, seroprevalence was the outcome of interest. It is possible that widespread seroconversion in the absence of clinical disease may reflect a desirable public health outcome; however, the potential association between human HEV IgG seroprevalence and occurrence of clinical hepatitis E remains unknown.

The lack of studies investigating association between exposure to swine or pork, and detection of

HEV RNA might be due to the additional cost and logistics that are required to implement large longitudinal field studies using multiple tests in parallel, such as HEV immunoglobulin M (IgM) ELISA and HEV RNA RT-PCR.

Inconclusive evidence for the third review question consists of two case-control studies reporting contradictory findings [10, 28], and 24 case reports that intrinsically do not allow for estimation of an association between exposure and outcome.

We have identified research gaps that merit further research:

- (1) Studies measuring frequency and quantity of HEV viral loads in pork at slaughter and retail levels are needed in order to quantify the magnitude of HEV in pork and potential risk to public health. To accomplish this, it is necessary to develop and validate reasonably sensitive and specific diagnostic tests for HEV detection.
- (2) Laboratory transmission studies conducted on primates with the main aim of establishing a dose-response profile for transmission of HEV infection via the oral route are also needed. These findings would improve understanding of seropositivity to HEV in various exposed and unexposed groups, and the minimum viral load that is necessary for HEV seroconversion as opposed to clinical disease. These studies would still lack direct comparisons to the population of interest (e.g. humans consuming pork, in a community setting).
- (3) Long-term, large cohort studies, investigating the association between exposure to swine/pork, and HEV IgG seroprevalence and clinical disease as outcomes, including genotyping of a larger number of isolates recovered from HEV IgM-positive individuals (i.e. incident cases) are recommended. These would evaluate temporality of exposure as it relates to a more relevant public health outcome (clinical disease), and biological gradient or cumulative exposure. Additionally, a critical review of the performance of ELISA tests, as a first step to developing assays with improved performance in assessing human exposure to HEV is required to establish the specificity of the association between exposure to swine or pork, and human seropositivity.
- (4) Case-control studies are recommended to further define risk factors such as age, gender, occupation, and level of pork consumption and

formulate relevant hypotheses for additional field and experimental research.

- (5) The resulting information (recommendations 1–4) would allow the implementation of a formal risk assessment model with the main aims of quantifying the risk to public health posed by zoonotic HEV via human exposure to swine or pork, as well as to evaluating potential control options.
- (6) Until the information described above becomes available for potential programme or policy actions, we recommend that stakeholders associated with the swine industry develop effective risk communication and knowledge transfer messages for the public, specifically for populations at risk. The key message is to avoid eating raw or inadequately cooked pork, and particularly raw pork liver, which might otherwise occur particularly within certain demographic groups (e.g. older men) [10, 11]. Given the current underpinning evidence, and particularly considering the discrepant results of HEV IgG serology in non-endemic populations [50], we recommend targeted as opposed to general risk communication.

Limitations of review

This review was limited to publications available in French or English, Canada's two official languages. While the effect of language bias in food safety research is unknown, its effects in other areas of medical research are documented [51]. In this review, 128 studies were published in 12 languages other than French or English and excluded at first-relevance screening, including one cross-sectional study. In our updated search, nine foreign-language studies that were published in five languages were also excluded, none of which were observational studies. Bias associated with an exclusion of these potentially relevant studies is possible but unlikely for the questions of our systematic review, as only one cross-sectional study was excluded.

Meta-analysis of the cross-sectional studies suggested the presence of publication bias (Appendix 6). Case reports are particularly prone to publication bias as well as recall bias that could have inflated the reported evidence for swine/pork as a putative risk factor for autochthonous hepatitis E. However, meta-analysis analytical adjustment for publication bias using the method of Duval & Tweedie resulted in a reduced but still significant estimate of association,

suggesting that the original pooled odds ratio was slightly overestimated.

The small number of cases reported in North America, possibly caused by under-diagnosis, may have impacted the findings pertaining to the association between locally acquired hepatitis E cases and exposure to swine and pork.

Overall, the scoping study framework offered a useful and transparent way of mapping evidence for broad and complex questions, with complementary systematic review of specific focused questions allowing a quantitative summary of evidence.

The primary research addressing potential zoonotic questions will inevitably be based on observational studies or laboratory-based studies conducted on primates, which inherently have a lower hierarchical level of evidence compared to randomized controlled trials [47]. We urge policy- and decision-makers to seriously consider funding the priority research areas outlined in this review, and build effective knowledge-transfer and risk-communication capacities in zoonotic public health using an interdisciplinary approach.

CONCLUSION

A diverse body of evidence supports swine as a reservoir of zoonotic HEV infection, and our review finds a significant association between occupational exposure to swine, and HEV IgG seroprevalence. Evidence of an association between consumption of pork, and HEV IgG seroprevalence is inconsistent, as is support for an association between exposure to swine/pork and locally acquired hepatitis E. Further research, including investigation of mechanisms and risk factors for infection, as well as the development of sensitive and specific tests for viral detection, and robust serological tests for identification of infection, are required.

NOTE

Supplementary material accompanies this paper on the Journal's website (<http://journals.cambridge.org/hyg>).

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DECLARATION OF INTEREST

None.

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