

ORIGINAL ARTICLE

A systematic review and meta-analysis of predictors of human hepatitis E virus exposure in non-endemic countries

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Abstract

The reported incidence of clinical hepatitis E cases is rising in some non-endemic countries, with concurrent concerns regarding potential hepatitis E virus (HEV) contamination of the blood supply. Therefore, the characterization of major potential sources of human HEV exposure is important to inform risk assessment and public health policy. A systematic review was conducted, including a comprehensive search in six electronic bibliographic databases, verified by hand-searching reference lists of HEV reviews, and a grey literature search, of the broad research question 'what is the evidence of the association between predictors of human HEV exposure, and HEV IgG seropositivity, in non-endemic countries?' Using forms designed a priori, captured studies were appraised at first-level screening, second-level characterization, and third-level data extraction and risk of bias assessment. Meta-analysis yielded summary estimates of association between potential predictors and odds of HEV seropositivity. Meta-analysis and meta-regression of the odds of HEV seroprevalence in specific groups characterized potential sources of HEV exposure. From 4,163 captured citations, 245 relevant studies underwent data extraction, investigating HEV seroprevalence or predictors in both healthy subjects and targeted patient groups. Across these groups, increasing age was a predictor of HEV IgG seropositivity. Both human immunodeficiency virus patients and haemodialysis patients had significantly increased odds of HEV seropositivity relative to the general population. Working with pigs, in forestry, or in hospitals, was significantly associated with increased odds of HEV seropositivity, as were consumption of meat, pork or game meat, or hunting. Chronological time was not associated with HEV seropositivity within our data sets. Further study of the distribution of potential dietary or behavioural predictors between high and lower prevalence areas within non-endemic countries could improve our understanding of the relative importance of specific HEV transmission pathways.

KEYWORDS

exposure, hepatitis E virus, systematic review

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1 | BACKGROUND

Hepatitis E virus (HEV) has been recognized for decades as a potential cause of waterborne outbreaks of human jaundice, spread by the faecal–oral route, in HEV-endemic countries with limited sanitary infrastructure (Capai, Charrel, & Falchi, 2018). However, sporadic locally acquired clinical cases of hepatitis E have been increasingly reported in patients from countries considered non-endemic for HEV, that is industrialized countries in which public health supports help to prevent the occurrence of waterborne disease outbreaks (European Centre for Disease Prevention and Control (ECDC), 2017).

While only one HEV serotype is reported, currently eight genotypes are recognized; genotypes 1 and 2, involved in human HEV outbreaks in endemic areas, have only been detected in humans (Capai et al., 2018). In contrast, genotypes 3 and 4 have been detected in humans, but also swine, wild boar and deer, and a variety of rodents including rabbits and rats, with genotypes 5 and 6 currently reported only in wild boar (Sridhar, Teng, Chiu, Lau, & Woo, 2017). More recently, genotypes 7 and 8 have been detected in dromedary and Bactrian camels, respectively, but have not to date been isolated from humans (Sridhar et al., 2017).

Current HEV IgG assays do not discriminate between HEV genotypes. It is possible that a very small proportion of HEV IgG-positive subjects in non-endemic countries may reflect prior exposure to HEV genotype 1 acquired via travel. Human exposure to HEV as evidenced by the presence of HEV IgG antibodies seems to vary across non-endemic countries, broadly ranging from less than 10% of the population in countries such as Canada and Australia, to more than 20% in France and the Netherlands (Petrik et al., 2016; Wilhelm, Waddell, Greig, Young, & of., 2019). Seroprevalence in endemic countries tends to be even higher (e.g., 60.5% of blood donors, as reported by Katiyar et al. (2018)). While differences in laboratory methods and surveillance systems across non-endemic countries preclude direct comparisons of the incidence hepatitis E, consensus is that the proportion of those exposed developing clinical disease is very small (Adlhoch et al., 2016; Petrik et al., 2016).

In non-endemic countries, several different HEV exposure sources have been implicated as the cause of sporadic locally acquired hepatitis E cases, including consumption of raw pork sausage (Colson et al., 2010) and receipt of contaminated blood transfusions (Hewitt et al., 2014). However, the exposure source remains unknown in many cases of hepatitis E (ECDC, 2017). Case isolates recovered from these sporadic cases tend to be either genotype 3 or genotype 4 (in contrast with human cases in endemic areas, where the case isolates are either genotype 1 or genotype 2), and in a small number of sporadic cases, both epidemiological evidence and genomic evidence have identified pork as the source of human infection (Sooryanarain & Meng, 2019). HEV is widely prevalent in swine and pork, globally, with meta-analysis summary estimates of HEV shedding in swine four to six months of age ranging from 5% (95% confidence intervals [CI]: 2%, 13%) in Asia to 23% (95% CI: 13%, 36%) in North America (Wilhelm et al., 2015). HEV has also been detected in retail pork in non-endemic countries; therefore, swine and pork are widely hypothesized to be an

Impacts

- Increasing age is consistently a significant predictor of hepatitis E virus (HEV) IgG seropositivity.
- Some medical conditions, as well as some behaviours, occupations and dietary choices, were significantly associated with increased odds of HEV seropositivity in non-endemic countries.
- Chronological time was not a significant predictor within our data set.

important source of human exposure to HEV in non-endemic countries (European Food Safety Authority (EFSA), 2017; Wilhelm et al., 2014, 2015). Other behaviours, and specific population subgroups, have been studied to identify potential predictors of HEV exposure as demonstrated by HEV IgG antibodies (ECDC, 2017). More complete characterization of these predictors and the magnitude of their association with HEV exposure would be helpful in mitigating the incidence of hepatitis E in non-endemic countries, and to understand the varying level of HEV exposure in the general population across non-endemic countries (Wilhelm et al., 2019).

Systematic review methodology is a standardized and reproducible process used to describe and synthesize a specific body of research (Higgins & Green, 2011). Consequently, systematic review and meta-analysis are important methods for informing health policy (Munn, Moola, Lisy, Riitano, & Tufanaru, 2015). Following systematic review, meta-analysis may allow the pooling of results to compute a summary estimate of effect; if data regarding potential predictors have been captured, meta-regression, that is the regression of one or more study-level covariates on the dependent variable, allows computation of measures of association between multiple predictors and outcome, across studies (Borenstein, Hedges, Higgins, & Rothstein, 2009), as well as an understanding of how well predictors account for between-study heterogeneity.

Therefore, a systematic review of predictors of HEV exposure in non-endemic countries was undertaken with the broad research questions: What risk factors (predictors) for human HEV exposure have been identified among human populations from non-endemic HEV countries? Does the measure of association for a given predictor vary across non-endemic countries and/or sub-populations?

2 | METHODS

2.1 | Scope

This systematic review was conducted as part of a larger project studying reported HEV seroprevalence across non-endemic countries. The protocol was prepared a priori (available as section S1, Appendix S1), and the manuscript is reported according to the

Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines (Moher, Liberati, Tetzlaff, & Altman, 2009). Inclusion/exclusion criteria were defined using the CoCoPop acronym (Munn et al., 2015):

2.1.1 | Condition (outcome of interest)

Measurement of HEV IgG antibodies was deemed the relevant outcome. Total HEV antibodies, IgM antibodies or detection of HEV RNA (e.g., using RT-PCR) was deemed not relevant for this review. Included studies were required to report a defined, reproducible HEV IgG assay.

2.1.2 | Context

Some demographic descriptors (age, sex) have frequently been significantly associated with odds of HEV seropositivity (ECDC, 2017), with many others also investigated, including socio-economic status (Unzueta et al., 2016), occupation (Meng et al., 2002), recreational activities (Mansuy et al., 2011), dietary preferences (Kuniholm et al., 2009) and rural, relative to urban, residence (Mansuy et al., 2008). Therefore, these parameters were captured when reported by investigators. A complete list of contextual parameters captured is listed in the data extraction tool available in the Appendix S1 (section S1).

2.1.3 | Population

Inclusion

People living in countries was categorized as 'very high' human development index by the United Nations, and therefore likely to have access to public health infrastructure precluding outbreaks of genotype 1 HEV transmission by the faecal-oral route (United Nations, 2016). A list of the countries categorized as 'very high' by the UN human development index is presented in the study protocol (S1). Descriptors of population, age and sex structure were captured when reported. Specific populations as described by the investigators (e.g., farmers, haemodialysis patients, recreational hunters) were captured. Exclusion: travellers, recent immigrants and travelling members of armed forces were excluded due to the difficulty in establishing the country of origin of infection in these groups; liver patients were also excluded from this review as we deemed the probability of selection bias to be high in this group. Surveys of only blood donors or the general population, not reporting analysis of potential predictors such as age or sex structure, were excluded from meta-analysis in this manuscript; such studies do not support investigation of our current research question, but have been analysed in a systematic review of HEV seroprevalence in non-endemic countries (Wilhelm et al., 2019). However, comparisons reported within studies of blood donors or the general population (e.g., comparing the odds of seropositivity between blood donors of different ages or dietary habits) were deemed relevant.

2.1.4 | Epidemiological outcomes

Hepatitis E virus seroprevalence in defined groups hypothesized to be at increased risk of HEV exposure, or targeted patient groups, or comparisons of HEV IgG seropositivity (e.g., between specific groups, relative to a comparison group, preferably expressed as an odds ratio [OR]) was deemed relevant.

Inclusion criteria for meta-analysis were as follows: two or more studies, investigating similar comparisons, employing an assay used in at least five studies in this review (assays employed in four or less studies were categorized as 'other' in analysis).

2.2 | Search strategy

The search strategy for the overall review of HEV seroprevalence across non-endemic countries has been previously described (Wilhelm et al., 2019) and is available in Appendix S1 (section S1). Briefly, a broad electronic search was conducted in six electronic bibliographic databases on 29 November 2016: EMBASE, PubMed, Scopus, Global Health, Epub Ahead of Print, In-Process & Other Non-Indexed Citations in Ovid MEDLINE(R) Daily and Ovid MEDLINE(R). A pre-tested search algorithm was employed:

("hepatitis E virus" OR "Hepatitis E virus" OR HEV) AND (blood OR serum OR serology OR sero-prevalence OR plasma OR "plasma products").

The electronic database search was verified by hand-searching the reference lists of nine randomly selected literature reviews (Appendix S1, section S1). Hand searching of selected grey literature websites (The International Liver Congress of the European Association for the Study of the Liver; European Congress of Clinical Microbiology and Infectious Diseases; and IDWeek) was conducted for the previous three years; additionally, a search was conducted using the Google search engine, employing the same terms as the electronic bibliographic search (Appendix S1, section S1).

2.3 | Review management

Citations were saved to RefWorks (ProQuest LLC), de-duplicated and then uploaded to the Distiller electronic platform (Evidence Partners). All reviewing forms (i.e., used for first- and second-level relevance screening, risk of bias assessment and data extraction) were pre-tested on a selected subset of citations (first-level screening) and full papers (second-level screening, risk of bias and data extraction).

First-level relevance screening excluded irrelevant citations; second-level screening excluded studies reporting irrelevant study locations and outcomes; then, data extraction and risk of bias assessment were completed on all relevant papers. Each phase of the systematic review was conducted on each citation or paper independently by two epidemiologists (Figure 1). Parameters captured in data extraction included location and year of sampling, assay(s) employed, storage and handling of samples, and demographics of

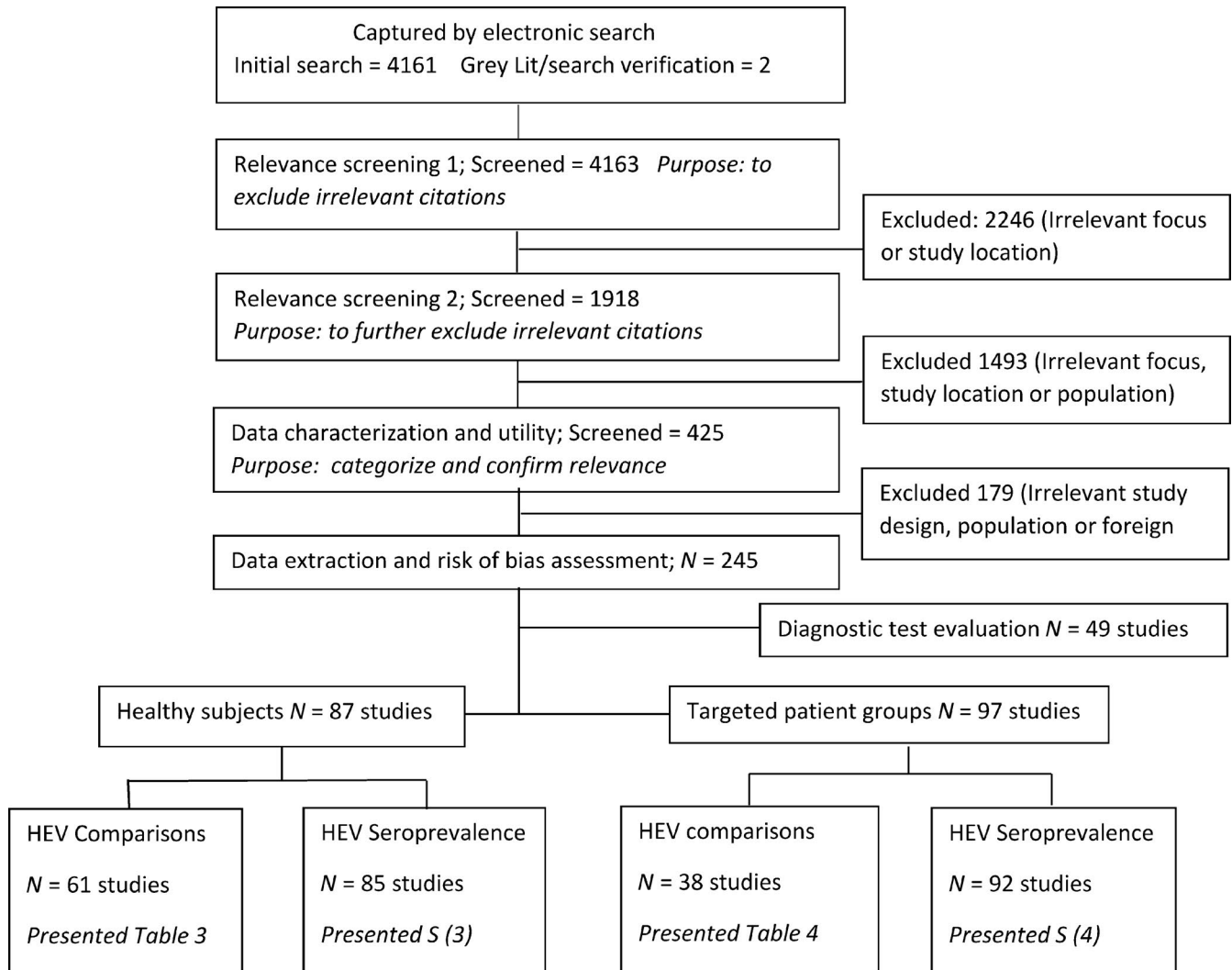


FIGURE 1 Review process

the population sampled and all risk factors examined for HEV exposure. All forms used in this review are available in the Appendix S1 (section S1).

Risk of bias of individual studies was assessed using several selected criteria appropriate for both seroprevalence and comparison studies, adapted from widely endorsed criteria for assessment of non-randomized intervention studies (Sterne et al., 2016). These included reporting of all intended study outcomes relevant to this review, consideration of the presence of potential confounders and reporting of appropriate adjusted outcomes. From individual risk of bias criteria assessed for each study, overall assessment of the risk of bias for the population(s) studied was determined. Additionally, the method of sample collection (e.g., random/convenience) and the procedures for sample handling and processing were captured, for each study.

2.4 | Analysis

Two types of study designs were captured: analytical studies and seroprevalence surveys. Outcome measures from analytical

cross-sectional studies reporting comparisons of HEV IgG seropositivity between groups were measures of association between a defined exposure (e.g., working with animals) and HEV IgG seropositivity, relative to a baseline unexposed group (e.g., the general population), either captured as reported, or estimated from raw data.

For the seroprevalence surveys, the Freeman–Tukey double arc-sine transformation was applied to data sets in which the median prevalence was less than or equal to 10%, given the relatively low HEV seroprevalence reported in many studies (Freeman & Tukey, 1950; Munn et al., 2015). For those data sets in which the majority of studies reported prevalence >10% and/or the Shapiro–Wilk statistic was significant ($p < .05$), the logit transformation was applied (Barendregt, Doi, Lee, Norman, & Vos, 2013).

Comparisons of HEV IgG seroprevalence between groups were computed as summary estimates of the ORs of seropositivity between individuals in the exposed and referent groups, using the ‘metagen’ command in the R package ‘meta’ (Schwarzer, 2018). Odds ratios and confidence intervals (CIs) were captured from relevant studies. Data from studies reporting only measures of association other than ORs were extracted, and converted, if possible, to ORs

using the 'OR2RR' calculator included in the EpiGear group of tools (EpiGear, 2017). If conversion to ORs was not possible, the other measures were reported separately, and meta-analysis was conducted if two or more studies within the same data set reported the same outcome measure (e.g., within the consumption of pork data set, 8 studies reported ORs and 3 reported risk ratios, meta-analysis was performed across the respective sets of studies reporting the same outcome measures).

Most of the data sets were hierarchical, with multiple relevant comparisons reported within the same study. To appropriately adjust the estimated variance for these data sets, robust standard errors were fitted for each data set having more than four data clusters (Fisher, Tipton, & Zhipeng, 2017).

In some analytical studies, age was a potential predictor of HEV seropositivity, reported as a continuous or categorical variable. Additionally, some categorical variables (e.g., socio-economic status) were reported in ordinal categories (e.g., occupation = professional, technical, labourer). These were dichotomized for analysis with selection of the referent group chosen to have least probable exposure time (e.g., least contact with animals or faecal material), based on known HEV transmission routes. Chronological time (i.e., year) of sampling was captured, when reported by authors, or imputed as previously described (Wilhelm et al., 2019).

Meta-analysis was conducted in the R Studio platform (R Studio, 250 Northern Ave, Boston, MA, USA) using the R software environment (Venables, Smith, & R Core Team, 2019) computing summary estimates of the two broad outcomes (seroprevalence or measures of association) described above (Figure 1). Random effects meta-analysis was selected, based on the assumption of true variation across studies. Heterogeneity was quantified by calculation of Higgins' I^2 (Higgins, Thompson, Deeks, & Altman, 2003) and T^2 , an estimate of τ^2 , which represents the true variance across studies (Borenstein et al., 2009). The restricted maximum-likelihood (REML) method was selected to compute T^2 (Viechtbauer, 2005).

Heterogeneity of effect estimates within a data set was categorized as 'low' if $I^2 \leq 60\%$ and T (the computed estimate of τ , or the true standard deviation) was less than the meta-analysis summary estimate. For data sets not categorized with 'low' heterogeneity, the median and range of individual study summary estimates are presented in lieu of meta-analysis summary estimates and 95% confidence intervals (CIs). Assessment that the data met the meta-analysis model assumptions of normality, the Shapiro-Wilk normality test (de Vries & Meys, 2015) and a visual examination of the quantile-quantile normal plots (Dohoo, Martin, & Stryhn, 2009) were used.

Random effects meta-regression in the R package 'meta', using the 'metareg' command, was employed to assess the association between study-level predictor variables and HEV IgG seroprevalence, or measure of association between two groups (Schwarzer, 2018). For inclusion in the multivariable model, all variables for which $p < .20$ in univariable analysis were considered for inclusion in the multivariable model.

Potential publication bias, a form of small study bias, was assessed by Egger's regression test (Egger, Davey Smith, Schneider, & Minder,

1997), the rank correlation test (Begg & Mazumdar, 1994) and the trim-and-fill method of Duval and Tweedie (Duval & Tweedie, 2000). These tests were applied to all data sets meeting the 'lenient' criteria outlined by Ionnidis and Trikalinos (2007): a minimum of five surveys within the data set, and Higgins' $I^2 \leq 50\%$.

3 | RESULTS

The search captured 245 studies examining IgG HEV seropositivity in high developed countries from 4,163 citations screened for relevance (Figure 1). Within these 245 studies, 163 studies examined predictors of HEV exposure and are summarized in this systematic review. Characteristics of 87 studies on healthy individuals and 97 studies on targeted patient groups are summarized in Table 1; characteristics for each study are listed in Appendix S1 (section S2).

The most frequently reported countries of sampling were France ($n = 24$ studies), Italy ($n = 16$), Germany ($n = 14$) and Japan ($n = 12$). The time span during which the greatest number of relevant papers was published was the interval from 2010 to 2014 ($n = 63$ papers published). The most frequently employed assays included the Wantai ($n = 45$ studies), Abbott ($n = 32$), MP Biomedical ($n = 19$) and 'in-house' or non-commercial assays ($n = 19$). Some assays were relatively more frequently employed in specific countries (Appendix S1, section S2). A summary of the specific populations sampled within included sampling frames of healthy subjects and targeted patients is presented in Table 2.

The association between potential predictors or risk factors for HEV IgG seropositivity in each of these broad groups are presented separately in Tables 3 and 4, respectively. For brevity, references for each data set in Tables 3 and 4 are presented in Appendix S1 (sections S3 and S4).

Our analysis focused on synthesizing the findings of studies comparing odds of HEV seropositivity between 'exposed' groups (either healthy subjects, or targeted patient groups) with baseline comparator groups (mainly general population or blood donors). However, the search also captured HEV seroprevalence surveys in both broad groups of interest, without a baseline or comparator group (Figure 1). Where meta-regression on study-level predictors was possible on these HEV seroprevalence surveys, we have reported findings in Appendix S1 (sections S3c and S4c) to provide additional information regarding predictors of HEV exposure.

3.1 | Healthy populations

3.1.1 | Comparisons of HEV IgG seropositivity in 'exposed' and baseline groups of healthy subjects

The estimated association between potential predictors and odds of HEV seropositivity between 'exposed' and baseline groups of healthy subjects are presented in Table 3. Age, measured as both a categorical and continuous variable, was consistently

TABLE 1 Major characteristics of 163 hepatitis E virus studies from which data were extracted

Parameter	Parameter categories	Number of studies
Total number of studies		163
Publication date	2015 on	39 (24%)
	2010–2014	63 (39%)
	2005–2009	21 (13%)
	2000–2004	15 (9%)
	Before 20,000	25 (15%)
Sampling date ^a	2015 on	4 (2%)
	2010–2014	61 (37%)
	2005–2009	44 (27%)
	2000–2004	30 (18%)
	Before 20,000	40 (25%)
	Unclear	29 (18%)
Study design	Prevalence survey	27 (17%)
	Cross-sectional study	123 (75%)
	Cohort study	13 (8%)
	Case–control study	3 (2%)
Assays	Abbott	32 (20%)
	Bioelisa	5 (3%)
	EIAgen	19 (12%)
	Dia.Pro	15 (9%)
	In-house	19 (12%)
	Mikrogen	15 (12%)
	MP Biomedical	19 (12%)
	Wantai	45 (28%)
	Other ^b	9 (6%)
Countries	Argentina	5 (3%)
	Austria	3 (2%)
	Australia	3 (2%)
	Canada	3 (2%)
	Chile	2 (1%)
	Croatia	2 (1%)
	Czech Republic	1 (1%)
	Denmark	3 (2%)
	Estonia	1 (1%)
	France	24 (15%)
	Germany	14 (9%)
	Greece	6 (4%)
	Hong Kong	1 (1%)
	Iceland	1 (1%)
	Ireland	1 (1%)
	Israel	2 (1%)
Italy	16 (10%)	
Japan	12 (7%)	

TABLE 1 (Continued)

Parameter	Parameter categories	Number of studies
	Korea	3 (2%)
	Netherlands	10 (6%)
	Norway	1 (1%)
	Poland	3 (2%)
	Portugal	3 (2%)
	Saudi Arabia	4 (2%)
	Spain	18 (11%)
	Sweden	3 (2%)
	Switzerland	3 (2%)
	United Kingdom	6 (4%)
	United States	13 (8%)

^aSome studies reported findings from samples collected over more than one time period, study design, assay or country of sample collection.

^b'Other' assays = Axiom, Euroimmun, Immunlon, Institute of Immunology Co., Viragent.

reported to be a significant predictor of HEV IgG seropositivity in healthy subjects, employing a sampling frame of blood donors or the general population. Males also had higher odds of HEV seropositivity compared with females (OR = 1.34, 95% confidence interval [CI] [1.16, 1.55]) across 18 studies and 25 comparisons (Table 3). Across two studies reporting four general population comparisons, increasing level of education was significantly associated with reduced odds of HEV seropositivity (OR = 0.49, 95% CI [0.30, 0.80]). Using North American or European countries of origin or Caucasian race as referents, sampling blood donors, the general population or prisoners, five studies reported individuals from other ethnic background had increased odds of HEV IgG seropositivity and one found no association. Selected locations within several European countries, and Japan, were identified as significantly associated with increased odds of HEV seropositivity relative to other locations within the same country. Rural residence (relative to urban) was an inconsistent predictor across seven studies, with three studies reporting a protective association and four reporting a harmful one (median OR = 1.35, range [0.40, 1.91]) (Table 3).

Several occupations hypothesized to have greater probability of HEV exposure were studied. Occupational contact with pigs was associated with increased odds of HEV seropositivity relative to the general population, across five studies sampling farmers, as well as veterinarians (median OR = 1.95; 95% CI [1.06, 3.60]).

Employment in forestry was associated with significantly increased odds of HEV seropositivity relative to the general population across three studies conducted in France and the United States, with high heterogeneity but consistent direction of association (median OR = 2.49, range [1.62, 6.76]). Across two studies, hospital workers had significantly greater odds of HEV seropositivity relative to the general population (Ding et al., 2003), or subjects presenting for HIV testing (Lanini et al., 2015).

(Continues)

Hunting, described as recreational in three of the four underpinning studies, was a non-significant predictor in meta-analysis (Table 3). Animal contact, variably defined by individual investigators, was investigated as an HEV IgG predictor across six studies and 29 comparisons sampling blood donors or the general population. Overall, there was a borderline significant association between animal contact and odds of HEV IgG seropositivity (OR = 1.25, 95% CI [0.97, 1.62]). Contact with pigs (not categorized as occupational), cats or horses was non-significantly associated with HEV IgG seropositivity (Table 3). In contrast, contact with dogs was significantly associated with increased odds of HEV IgG seropositivity.

The association between diet and HEV IgG seropositivity was investigated in five studies, four of which reported data which could be expressed as an odds ratio (Kuniholm et al., 2009; Mansuy et al., 2015, 2016; Verhoef et al., 2012) and included in meta-analysis of risk factors for HEV IgG seropositivity in blood donors or the general population. Prevalence ratio outcomes reported by Lucarelli et al. (2016) are presented in Appendix S1 (section S3). The consumption of meat was consistently a significant ($p < .05$) predictor of HEV IgG seropositivity, with high heterogeneity across four studies reporting 14 comparisons studying the consumption of uncooked liver sausage, rabbit meat, game meat, liver or organ meats, bacon or ham, and pork (median OR = 1.44, range [1.12, 2.77]). Underpinning this association were significant positive associations between consumption of pork or game meat, and HEV IgG seropositivity (Table 3). Seafood (mussel or oyster consumption) consumption was significantly associated with seropositivity; in contrast, there was no association with vegetable consumption across two studies (Table 3). Similarly, consumption of 'treated' tap water was inconsistently associated with HEV IgG seropositivity, across five comparisons with high heterogeneity (median OR = 1.07, range [0.64, 7.44]).

An association with exposure of HEV through intravenous drug use (IVDU) was investigated in three studies and yielded a significant positive association with IgG seropositivity (OR = 1.98, 95% CI [1.45, 2.68]).

3.1.2 | Meta-regression of studies of HEV seroprevalence in healthy subjects

The prevalence of HEV IgG seropositivity was investigated among several groups of healthy subjects, including animal workers, forestry workers, hospital workers, sewage workers and intravenous drug users (Table 2); blood donor and general population seroprevalence surveys have been analysed and reported previously (Wilhelm et al., 2019). Meta-regression data sets were stratified by assay due to the correlation between assay and country (Wilhelm et al., 2019). Only the Abbott and Wantai data sets had sufficient studies to meet our meta-regression criteria. Only certain specific groups (e.g., those with occupational contact with animals) were significant ($p < .05$) predictors of HEV seroprevalence; country

and chronological time (year) were non-significant predictors (Appendix S1, section S3c).

3.2 | Targeted patient groups

3.2.1 | Comparisons of HEV IgG seropositivity in 'exposed' and baseline groups of targeted patients

The estimated association between potential predictors and odds of HEV seropositivity between 'exposed' and comparison groups, across the broad group of targeted patients captured, are presented in Table 4. Age was a borderline significant predictor of HEV IgG seropositivity across four studies of patient groups including both transplant patients (Legrand-Abravanel et al., 2011) and human immunodeficiency virus (HIV) patients (Payne et al., 2013; Pineda et al., 2014; Riveiro-Barciela et al., 2014) with low heterogeneity (OR = 1.32, 95% CI [0.97, 1.79]). Males had greater odds of HEV IgG seropositivity across 10 studies of patients containing 11 comparisons (OR = 1.29, 95% CI [1.17, 5.16]). In meta-regression, country of sampling and assay were significant in explaining heterogeneity across the studies of sex as a predictor of HEV seropositivity in patients (Table 4). No significant association was reported between HEV seropositivity and occupational exposure to swine among HIV patients (Pineda et al., 2014) environmental manual workers relative to professionals, or among transplant patients (Unzueta et al., 2016). Ethnicity was not significantly associated with HEV seropositivity across two studies containing five comparisons, sampling patients, with low heterogeneity (OR = 0.68, 95% CI [0.20, 2.28]).

Several specific groups of patients were sampled. HIV patients had increased odds of HEV IgG seropositivity relative to the general population, across five studies, with low heterogeneity (OR = 2.13, 95% CI [1.47, 3.09]). In contrast, three studies reported inconsistent findings for the association between transplant patients relative to the general population, and HEV IgG seropositivity (Table 4) (Harrison et al., 2013; Riveiro-Barciela et al., 2014; Unzueta et al., 2016).

Haemodialysis patients were consistently associated with increased odds of HEV seropositivity relative to the general population, or other patient groups, with high heterogeneity across six studies (median OR = 3.33, range [1.02, 20.20]). In univariable meta-regression, country was a significant predictor of seropositivity, with Japanese haemodialysis patients having increased odds relative to patients from other countries (Greece, Italy, Saudi Arabia, United Kingdom) in our data set.

A medical history of receiving blood or blood products was not associated with HEV IgG seropositivity across two studies (OR = 1.12, 95% CI [0.50, 2.53], $I^2 = 0$, $\tau^2 = 0$). In contrast, Mallet et al. (2013) reported that receiving therapeutic plasma exchange (TPE) was significantly associated with greater risk of HEV seropositivity relative to those not receiving TPE (RR = 2.62, 95% CI [1.09, 6.31]) (Appendix S1, section S4).

TABLE 2 Populations sampled in 163 hepatitis E virus seroprevalence and comparison studies

Broad population studied	Specific population studied	Number of seroprevalence studies ^a	Number of comparison studies ^a
Healthy subjects (<i>n</i> = 87 studies)	Total: all healthy subjects studied	85	61
	Blood donors	N/A ^b	36 (59%)
	General population	N/A	31 (51%)
	Occupational contact with animals ^c	24 (28%)	6 (10%)
	Abattoir workers	6 (7%)	
	Farmworkers	13 (15%)	6 (10%)
	Foresters	3 (4%)	3 (5%)
	Veterinarians		
	All veterinarians studied	11 (13%)	1 (2%)
	Swine veterinarians	6 (7%)	1 (2%)
	Non-swine veterinarians	2 (2%)	
	Hunters	4 (5%)	4 (7%)
	Hospital workers	1 (1%)	
	Sewage workers	2 (2%)	
	Rural residents	1 (1%)	7 (11%)
	Consume pork		5 (8%)
	Consume raw or undercooked pork	1 (1%)	5 (8%)
	Consume other raw or undercooked meat	2 (2%)	1 (2%)
	Intravenous drug use	8 (9%)	4 (7%)
	Subjects reporting high-risk sexual behaviour	1 (1%)	
Prisoners	2 (2%)	2 (3%)	
Homeless	3 (4%)	1 (2%)	
Targeted patient groups (<i>n</i> = 97 studies)	Total: all targeted patients studied	92	38
	Transplant patients	22 (24%)	3 (8%)
	Haemodialysis patients	15 (16%)	6 (16%)
	Frequent blood or blood product recipients	10 (11%)	4 (11%)
	Human immunodeficiency virus (HIV) patients	29 (32%)	8 (21%)
	Hospital outpatients/clinic attendees	9 (10%)	1 (3%)
	Hospital ward patients	9 (10%)	2 (5%)
	Sexual health or illness patients	3 (3%)	
	Patients triggering submission to diagnostic laboratory	2 (2%)	
	Other immune-suppressed groups, for example CVID patients	3 (3%)	
	Guillain-Barré syndrome patients	2 (2%)	
	Other ^d	5 (5%)	

^aSome studies sampled more than one specific group. Thus, totals may sum to more than 100% or more than 163 studies.

^bN/A = HEV IgG seroprevalence of blood donors and the general population across non-endemic countries was previously reported by Wilhelm et al. (2019) and outside the scope of this systematic review.

^cOccupations captured include farmworkers, veterinarians, slaughterhouse workers and foresters.

^dOther = peritoneal dialysis patients, infectious disease patients, psychiatric patients, inflammatory bowel disease patients, thalassaemia patients.

An inconsistent association was found between previous exposure to hepatitis A virus (HAV) and HEV IgG seropositivity (Table 4). One Argentinian study reported significantly increased odds of HEV IgG seropositivity in hospital ward patients relative to blood donors (Rey et al., 1997).

3.2.2 | Meta-regression of studies of HEV seroprevalence in targeted patient groups

HEV IgG seroprevalence was reported in 88 studies of targeted patient groups (Appendix S1, section S4). In meta-regression of

TABLE 3 Predictors of odds of hepatitis E virus (HEV) seropositivity in 67 studies of healthy subjects relative to baseline groups

Predictor	Number of studies (Number of comparisons)	Meta-analysis summary estimate ^a (95% CI)	I^2 (τ^2)	Comparisons reporting/ adjusting for age	Comments
1. Demographic predictors					
Age ^b					
Analysed as dichotomous variable	8 (8)	Med = 2.84 (1.46, 5.07)	95.9% (0.18)	N/A	Baseline or referent group = younger group as defined by individual studies. All subjects were 18 years or older. Dichotomization cut point ranged from 21 to 45 years of age, across studies Meta-regression models were non-significant or did not converge
Analysed as continuous variable	1 (1)	OR = 1.04 (1.03, 1.04)	N/A		Odds ratio estimate from extracted from single study
Sex	18 (25)	OR = 1.34 (1.16, 1.55)	34% (0)	6/25	Referent = female Non-significant publication bias across this data set. Meta-regression models did not converge.
Education	2 (4)	OR = 0.49 (0.30, 0.80)	60.3% (0.15)	0/4	Higher education associated with lower odds of HEV seropositivity
Ethnicity	6 (11)	Med = 1.8 (0.42, 3.54)	75.2% (0.26)	2/11	Referent = Caucasian/European or North American country of origin; comparison group defined by individual studies
Location within one country	7 (14)	Med = 1.40 (1.13, 2.85)	75.2% (0.09)	5/14	Referent = area in a given country with lower HEV IgG seroprevalence
Rural versus urban residence	7 (10)	Med = 1.35 (0.40, 1.91)	86.1% (0.23)	2/10	Referent = not living in rural area as defined by authors; comparison group = subjects living in a rural area
Occupation ^b					
Occupational contact with swine ^b	5 (11)	OR = 1.95 (1.06, 3.60)	53.4% (0.04)	4/11	Referent = general population; comparison group = farmworkers, veterinarians, abattoir workers Meta-regression models were non-significant or did not converge
Occupation in forestry ^b	3 (3)	Med = 2.49 (1.62, 6.76)	95.9% (0.51)	0/3	Referent = general population or blood donors; comparison group = woodcutter, forester or employee of Department of Natural Resources
Hospital worker	2 (2)	OR = 1.56 (1.16, 2.10)	0 (0)	0/2	Referent = general population or subjects presenting for HIV testing; comparison group = healthcare workers as defined by authors
2. Voluntary exposures					
All animals ^b	6 (29)	OR = 1.25 (0.97, 1.62)	35.4% (0.05)	0/29	Referent = no animal contact
Meta-regression					
Country		Significant (France)			Assay and time were non-significant predictors; model for population did not converge
Swine ^b	2 (2)	OR = 0.98 (0.37, 2.61)	0 (0)	0/2	Referent = no swine contact
Cats	4 (4)	Med = 0.87 (0.3, 1.49)	68.7% (0.16)	0/4	Referent = no cat contact

(Continues)

TABLE 3 (Continued)

Predictor	Number of studies (Number of comparisons)	Meta-analysis summary estimate ^a (95% CI)	<i>I</i> ² (τ^2)	Comparisons reporting/ adjusting for age	Comments
Dogs	3 (3)	OR = 1.22 (1.07, 1.40)	0 (0)	0/3	Referent = no dog contact
Horses	3 (3)	OR = 1.37 (0.66, 2.83)	0 (0)	0/3	Referent = no horse contact
Diet					
Consume meat ^b	4 (14)	Med = 1.44 (1.12, 2.77)	73.5% (0.06)	6/16	Referent = no meat consumption
Consume pork ^b	4 (5)	Med = 2.36 (1.38, 3.0)	67.7% (0.05)	3/5	Referent = no pork consumption
Consume game	2 (4)	OR = 1.38 (1.29, 1.48)	0 (0)	2/4	Referent = no game consumption
Consume seafood ^b	2 (2)	OR = 1.45 (1.23, 1.71)	0 (0)	0/2	Referent = no mussel consumption
	2 (2)	OR = 1.81 (1.63, 2.0)	0 (0)	0/2	Referent = no oyster consumption
Consume vegetables ^b	2 (2)	Range = (1.10, 1.35)	90.7% (0.02)	0/2	Referent = no vegetable consumption
Consume offal	2 (2)	OR = 1.98 (1.81, 2.16)	0 (0)	0/2	Referent = no offal consumption
Consume treated water	4 (5)	Med = 1.07 (0.64, 7.44)	73.8% (0.10)	2/5	Referent = no treated water consumption
Hunting ^b	4 (6)	Med = 1.31 (0.51, 4.11)	72.1% (0.28)	0/6	Referent = no hunting
High-risk behaviours					
Intravenous drug use (IVDU)	3 (3)	OR = 1.98 (1.45, 2.68)	0 (0)	1/3	Referent = no IVDU

Abbreviations: CI, confidence intervals; HEV, hepatitis E virus; IVDU, intravenous drug use; MA, meta-analysis; Med, median; MR, meta-regression; MV, multivariable; OR, odds ratio; UV, univariable.

^aSummary estimates include odds ratio and 95% confidence interval or median and range are presented where data sets are categorized as 'High' heterogeneity.

^bReported outcomes other than odds ratios are summarized for this data set in Appendix S1 (section S3b).

this data set stratified by assay used, the only significant ($p < .05$) predictor of HEV seropositivity was the specific group of patients (e.g., HIV patients, blood product recipients) sampled (Appendix S1, section S4); country and chronological time were non-significant. For example, patients identified as blood or blood product recipients were at significantly increased odds of HEV seropositivity relative to other patient groups assayed, in the Mikrogen and Wantai assay data sets (Appendix S1, section S4).

3.3 | Risk of bias assessment

Risk of bias assessments for individual included studies are presented in the Appendix S1 (section S5); a summary of the findings for risk of bias assessment across studies is presented in Table 5. Most ($n = 102/163$) included studies were categorized overall as 'low' risk of bias using a small number of design-appropriate criteria. However, across the probing questions selected for additional risk of bias assessment for this systematic review, less than half of the included studies reported appropriate sample storage and processing, or validation of the representativeness of the study population to the target population, with convenience sampling the most frequent sampling strategy employed.

4 | DISCUSSION

Since HEV was first detected in swine, contact with swine or pork has been hypothesized to be an exposure source for humans (Sooryanarain & Meng, 2019). More recently, clinical hepatitis E has been reported in some specific groups of patients, particularly the immune-compromised, such as cancer patients and transplant patients, sometimes presenting with chronic disease (Halac et al., 2012; Harrison et al., 2013). The potential for hepatitis E to cause complications such as chronic hepatitis and cirrhosis in some patient groups has intensified interest in identifying potential predictors of HEV IgG seropositivity (Legrand-Abraham et al., 2011).

In our data set, increasing age was associated with increased odds of HEV seropositivity across populations studied (Tables 3 and 4). This is consistent with many primary research studies as well as other reviews and is also consistent with the epidemiology of other hepatitis viruses such as HAV (Lin et al., 2017). Additionally, the increased odds of HEV seropositivity among males are consistent with the greater proportion of males relative to females reported in studies of clinical hepatitis E (Faber, Askar, & Stark, 2018). The significance of age and sex as predictors provides clues to further defining important specific HEV exposure sources, and these variables should be controlled for in future analytical studies.

TABLE 4 Predictors of odds of hepatitis E virus seropositivity in 30 studies comparing targeted patient groups relative to baseline groups

Predictor	Number of studies (Number of comparisons)	Meta-analysis summary estimate ^a (95% CIs)	<i>I</i> ² (τ^2)	Comparisons reporting/ adjusting for age	Comments
1. Demographics					
Age					
Analysed as dichotomous variable	4 (4)	OR = 1.32 (0.97, 1.79)	24.3% (0.03)	N/A	Referent = younger group as defined by individual studies. All subjects were 18 years or older. Dichotomization cut point ranged from 35 to 55 years of age, across studies
Continuous variable	1 (1)	OR = 1.04 (1.01, 1.07)			Odds increase with each additional year of age
Sex ^b	10 (11)	OR = 1.29 (1.17, 5.16)	0.5% (0.05)	0	Referent = female Publication bias was non-significant across this data set.
Meta-regression					
Country		Significant UV (Japan)			MV model did not converge
Assay		Significant UV (Bioelisa, Dia.Pro, In-house, MP Bioi)			
Time		Non-significant			
Occupation					
Swine exposure	1 (1)	OR = 1.21 (0.64, 1.78)		Not reported	Referent = not occupationally exposed to swine; comparison = occupationally exposed
Environmental worker	1 (1)	OR = 1.78 (0.15, 3.40)		Not reported	Referent = environmental worker; comparison = professional environmental worker
Ethnicity	2 (5)	OR = 0.68 (0.20, 2.28)	39.9% (0.80)	0/5	Referent = Caucasian or European; comparison = Hispanic/Black/ Native American/Asian/African
Location	1 (1)	OR = 3.69 (2.40, 4.99)	N/A	Reported	Referent = area of lower seroprevalence; comparison = area of higher seroprevalence
Socio-economic status	1 (1)	OR = 2.13 (1.14, 3.11)		Not reported	Referent = 'Middle-High' income; comparison = 'Low' income
2. Specific patient groups					
Immune-compromised					
HIV patients ^b	5 (5)	OR = 2.13 (1.47, 3.09)	0 (0)	1/5	Referent = not HIV-positive patients
Transplant patients ^b	1 (1)	OR = 2.22 (1.43, 3.02)	N/A	Not reported	Referent = healthy controls; Comparison = HIV-positive patients + transplant patients
	1 (1)	OR = 1.18 (0.61, 1.75)	N/A	Not reported	Referent = healthy controls; Comparison = transplant patients
	1 (1)	OR = 1.44 (0.69, 3.02)	N/A	Reported	Referent = kidney transplant patients; comparison = heart transplant patients
Blood-borne exposures					
Haemodialysis patients ^b	6 (8)	Med ^a = 3.33 (1.02, 20.20)	93.1% (0.40)	4/8	Referent = no haemodialysis
Meta-regression					

(Continues)

TABLE 4 (Continued)

Predictor	Number of studies (Number of comparisons)	Meta-analysis summary estimate ^a (95% CIs)	I^2 (τ^2)	Comparisons reporting/ adjusting for age	Comments
Country		Significant UV, MV (Japan)			
Assay		Significant UV			
Time		Non-significant			
Blood recipients ^b	2 (2)	OR = 1.12 (0.50, 2.53)	0 (0)	2/2	Referent = no history of transfusion
Other exposures					
HAV exposure	4 (5)	Med = 1.28 (0.80, 2.27)	91.3% (0.16)	0/5	Referent = HAV IgG-negative
Hospital patients	1 (1)	OR = 1.74 (1.33, 2.16)		1/2	Referent = blood donors; comparison group = hospital patients

Abbreviations: CI, confidence intervals; HAV, Hepatitis A virus; HEV, hepatitis E virus; HIV, Human immunodeficiency virus; IVDU, intravenous drug use; MA, meta-analysis; Med, median; MR, meta-regression; MV, multivariable; OR, odds ratio; UV, univariable.

^aSummary estimates include odds ratio and 95% confidence interval or median and range are presented where data sets are categorized as 'High' heterogeneity.

^bReported outcomes other than odds ratios are summarized for this data set in Appendix S1 (section S4b).

The increased odds of HEV seropositivity frequently reported in healthy Asian, African or non-Caucasian populations could reflect exposure to genotype 1 or 2 HEV acquired in areas traditionally considered endemic (Sooryanarain & Meng, 2019). In contrast, ethnicity was not a predictor of HEV IgG in the targeted patient groups, possibly due to a stronger association with other exposures unique to these patient groups, masking the potentially weaker association with ethnicity.

Interestingly, in both broad groups, residence in some locations within-country was associated with increased odds of HEV IgG seropositivity (Tables 3 and 4), suggesting areas of relatively higher or lower probability of HEV exposure, which could be promising candidates for further research into known and unknown HEV transmission routes. For example, in France, both healthy subjects and HIV patients living in the south were associated with increased odds of HEV exposure relative to residents of the north, possibly reflecting a local source of HEV that is specific to the south. Further research is required to explore this phenomenon.

Swine have historically been considered a reservoir for HEV; therefore, epidemiological research has studied contact with pigs and consumption of pork products as potential exposure routes (Sooryanarain & Meng, 2019). The significant association across studies within this systematic review with dietary consumption of pork, and HEV IgG seropositivity in the general population (Table 3) is consistent with previously published literature. Likely because of the logistical difficulties involved in estimating the measures of association of dietary exposures within specific patient groups, we did not capture similar data pertaining to patient groups.

The origin of the inconsistent association between hunting and HEV exposure is less clear and could reflect variable exposure to wild mammals known to be potential hosts for HEV infection such as rats, rabbits or wild boar (Sooryanarain & Meng, 2019), or exposure

to HEV-contaminated surface waters (Pisano, Balderramo, et al., 2018). The potential role of drinking water as an exposure source of HEV remains unclear, as demonstrated by the opposing findings of individual primary investigations, and the non-significant and heterogeneous meta-analysis summary estimate. One French study reported a significant protective association between consumption of bottled water, originating from deep under impervious rock, relative to tap water, suggesting that in some settings tap water could possibly be an exposure source for HEV (Mansuy et al., 2016).

The significant positive association between IVDU and HEV IgG seropositivity is consistent with the relationship between IVDU and blood-borne diseases generally. In Canada and Australia, among other countries, the association between IVDU and blood-borne infections is considered so important that potential blood donors reporting any history of IVDU receive indefinite deferral from blood donation (Canadian Blood Services, 2019; Quinn et al., 2017).

We identified two patient groups with significantly increased odds of HEV exposure within our data set: haemodialysis patients and HIV patients. Haemodialysis patients have been reported to have significantly increased odds of HEV exposure relative to the general population in primary studies as well as one systematic review (Haffar et al., 2018). The specific HEV exposure source in haemodialysis patients remains unclear, although several have been hypothesized, including the widespread use of swine-derived heparin for maintaining indwelling intravenous catheter patency (Crossan et al., 2013; Haffar et al., 2018) or potentially increased probability of HEV exposure in renal patients generally (Zhang et al., 2017). The high heterogeneity across the haemodialysis data set in this review could reflect differences in comparison groups across studies (Table 4), differences in mean time receiving dialysis across study populations or variation in distribution of currently unknown predictors.

TABLE 5 Summary of risk of bias assessment across 163 hepatitis E virus seroprevalence surveys and comparison studies

Parameter	Increased risk Number of studies (%)	Targeted patients Number of studies (%)	Overall Number of studies (%)
Were samples stored appropriately and processed/tested within a reasonable period of time after collection?	Yes = 35 (40%) No = 3 (4%) Not reported = 49 (56%)	Yes = 32 (33%) No = 2 (2%) Not reported = 63 (65%)	Yes = 56 (34%) No = 8 (5%) Not reported = 96 (61%)
Does the study report validation of the representativeness of the sample population with the target population?	Yes = 13 (15%) No = 74 (85%)	Yes = 8 (8%) No = 79 (92%)	Yes = 21 (13%) No = 142 (87%)
How were individual subjects selected to participate in this study?	Whole registry = 4 (5%) Random = 0 Reported random = 11 (12%) Systematic = 4 (5%) Convenience = 68 (78%)	Whole registry = 24 (25%) Random = 0 Reported random = 9 (9%) Systematic = 3 (3%) Convenience = 61 (63%)	Whole registry = 28 (17%) Random = 2 (1%) Reported random = 14 (9%) Systematic = 8 (5%) Convenience = 111 (68%)
What is the probability of bias from selective reporting?	Low = 71 (82%) Unclear = 11 (12%) High = 5 (6%)	Low = 85 (88%) Unclear = 7 (7%) High = 5 (5%)	Low = 144 (88%) Unclear = 13 (8%) High = 6 (4%)
What is the risk of bias from potential confounding factors?	Low = 19 (22%) Unclear = 65 (75%) High = 3 (3%)	Low = 21 (22%) Unclear = 66 (67%) High = 10 (10%)	Low = 33 (20%) Unclear = 117 (72%) High = 13 (8%)
Overall risk of bias	Low = 59 (68%) Unclear = 20 (23%) High = 8 (9%)	Low = 60 (62%) Unclear = 23 (24%) High = 14 (14%)	Low = 102 (63%) Unclear = 38 (23%) High = 23 (14%)

Across the studies captured within this review, HIV patients had significantly increased odds of HEV IgG seropositivity with negligible heterogeneity across studies, relative to comparator groups (Table 4). This is especially noteworthy given the reduced humoral immune response reported in HIV patients to viral vaccines such as HAV (Lin et al., 2017). The exposure source for HEV in HIV patients is unclear, and several potential routes could be involved in the increased probability of HEV exposure in this group. Men who have sex with men are estimated to comprise more than half of the HIV patients currently diagnosed in Canada and the United States, and this group is deemed at greater risk of exposure to blood-borne pathogens in general (Community AIDS Treatment Information Exchange (CATIE) 2018; Centers for Disease Control and Prevention (CDC), 2019). In contrast with HIV patients, organ transplant patients were not associated with HEV IgG seropositivity, which is consistent with other published findings (Pisano, Lugo, et al., 2018).

Receiving blood or blood products, within our small data set, was not associated with odds of HEV seropositivity, despite international concern regarding the potential for asymptomatic HEV-infected blood donors to contribute contaminated blood (Petrik et al., 2016; Westhölter et al., 2018). This could reflect the small number of comparable studies captured yielding inadequate power to detect a significant difference between blood product recipients and the general population. In contrast, in HEV seroprevalence (as opposed to comparison) studies, blood recipients as a group had significantly increased odds of exposure relative to other patient groups in the meta-regression (Appendix S1, section S4). Additional primary studies of HEV seroprevalence in blood or blood product recipients, and comparisons with the general population, would be useful to further

characterize the potential probability of HEV exposure via contaminated blood (ECDC, 2017; Westhölter et al., 2018). Concurrently, studies of HEV RNA detection and load in blood donors, and critical evaluation of the currently used methods (e.g., individual samples or mini-pools) could help to clarify the frequency of HEV viraemic blood donation falling above or below a reported infectious dose (Vollmer, Diekmann, Knabbe, & Dreier, 2019).

In contrast with reported HEV seroprevalence in blood donors or the general population of non-endemic countries (Wilhelm et al., 2019), HEV seroprevalence in the healthy subjects studied in the meta-regression data set (e.g., swine workers) did not vary significantly across countries. This may reflect the relatively small data sets having insufficient power to detect a difference, or a truly non-significant association between country and seroprevalence for the specific healthy groups studied.

The relationship between country and the measures of association studied was difficult to examine within our data set of comparisons between specific exposed and baseline groups, since the relatively small data sets for many individual predictors precluded meta-regression. The significance of country as a predictor in specific comparison data sets (e.g., animal contact, or haemodialysis patients, relative to the general population) suggests currently unknown covariates potentially present in those countries deemed statistically significant predictors.

Risk of bias for individual studies was assessed across several domains and computed across studies separately for the healthy subjects and targeted patient data sets but did not differ significantly between these two broad groups (Table 5). Convenience sampling was the most frequent sampling strategy employed, reflecting the affiliation of many investigators with a university or

hospital which was used as the sampling frame. The direction of this potential bias is difficult to predict and could vary across subgroups (e.g., some investigators might see more severely ill patients). Except for the dietary data sets underpinned by several large blood donor studies, and several patient group data sets (transplant patients, haemodialysis patients, patients receiving blood products), most studies in each data set in this review did not consider age as a potential confounder (Tables 3 and 4). The direction of potential bias could vary with the specific exposure investigated. For example, studies investigating occupational exposure, and not adjusting for the occupation in question possibly being performed by people significantly older than those in the comparator group, could over-estimate the true measure of association.

Additionally, given the hierarchical nature of our data set (surveys or comparisons within studies), those data sets in which the number of clusters (four or less) precluded adjustment by estimating robust standard errors likely have yielded under-estimates of the true data set variance (Dohoo et al., 2009).

Our study has several limitations. Lacking resources for translation of papers in foreign languages, 25 potentially relevant papers published in 10 different languages were excluded from data extraction, including studies in Spanish ($n = 7$), Italian ($n = 4$) and German or Japanese ($n = 3$ each). While ideally all relevant research would be included, we feel that exclusion of a relatively small proportion of the total number of relevant papers captured is unlikely to have significantly influenced our findings. Additionally, some data sets investigating a specific exposure contained one or more relevant studies reporting outcomes other than ORs in a way that precluded conversion, thereby excluding some potentially relevant studies from meta-analysis. Selection of a preferred measure of outcome would be helpful in maximally leveraging the potential contribution of existing research within research synthesis.

A related challenge arises from the variation in specific details of exposure definitions across studies. Given the intrinsically greater risk of bias in observational studies relative to controlled trials, it would be especially useful to draft definitions for commonly investigated exposures such as diet and occupation, to be generally adopted for observational research. A similar initiative has been undertaken to standardize the definition and measurement of clinical outcomes in some areas of experimental research (Wuytack et al., 2018), and guidelines have been drafted by the Core Outcome Measures in Effectiveness Trials (COMET) project to expand this work to other areas of investigation (Williamson et al., 2017).

The variation in performance of HEV IgG assays is widely recognized, and a review of HEV seroprevalence across non-endemic countries reported that assay was a significant predictor of HEV seroprevalence in meta-regression (Wilhelm et al., 2019). All the individual estimates of association captured by this review employed the same assay in baseline and exposed groups. Additionally, assay was a not a significant predictor in multivariable meta-regression (Tables 3 and 4), and for these reasons, we do not consider variation in assay performance to have had a notable influence on our findings.

5 | CONCLUSIONS

Some demographic and other predictors are associated with significantly increased odds of HEV seropositivity in non-endemic countries. Heterogeneity of the measure of association varies across specific exposures. The most commonly occurring significant predictors captured in this review were age and sex, and these predictors provide clues to further defining important HEV exposures, as opposed to defining the exposures themselves.

This systematic review summarizes evidence suggesting that humans are exposed to HEV via contact with swine and pork. However, the distribution of other less strongly associated and perhaps also less consistently distributed predictors, such as contact with shedding domestic animals, or contaminated water, may also contribute to the existence of regions of higher or lower human exposure, as indicated by HEV seroprevalence studies. Further study of the distribution of potential dietary or behavioural predictors between high and lower prevalence areas within countries/regions could help to identify specific predictors and transmission pathways. Coordination in defining common exposures of broad research interest such as dietary choices or occupation could help to expand the utility of each individual primary study in further synthesis work.

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CONFLICTS OF INTEREST

The authors have no conflicts of interest to declare.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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