Risk factors for sporadic hepatitis E infection: a systematic review and meta-analysis

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Short Title : A meta-analysis to characterize risk factors associated to hepatitis E

Highlights

- *Meta-analysis of HEV sporadic cases confirms fecal-oral and zoonotic transmissions*
- *Consumption of pork products and processed meat are highly at risk of HEV infection.*
- *Consumption of produce or shellfish is associated with HEV exposure.*
- *Blood transfusion, dialysis and other contact with needles are risk factors for HEV*

Keywords

Research synthesis; case-control studies; cohort studies; systematic review; Hepatitis E

Abstract

Hepatitis E virus (HEV) is one of the main causes of viral hepatitis infection worldwide. Sources of contamination can vary greatly according to geographical regions and HEV genotypes. HEV is endemic and responsible for large waterborne epidemics involving human HEV-1 or HEV-2 genotypes in regions with limited sanitation, in contrast to industrialized countries, where HEV is mainly a foodborne zoonosis involving HEV-3 and HEV-4 zoonotic genotypes. Limited data on HEV source attribution are available, and all possible sources and transmission pathways of HEV are not fully identified.

A systematic review and a meta-analysis of case-control and cohort studies (including transversal studies) were performed to determine the main risk factors associated with sporadic hepatitis E infection. Suitable scientific articles were identified through a systematic literature search and subjected to a methodological quality assessment. From each study, odds-ratio (OR) measures were extracted/calculated, as well as study characteristics such as population type, design, and risk factor hierarchy. Mixed-effects meta-analyses models were adjusted by population type to appropriate data partitions.

Seventy-seven cohort and case-control studies conducted between 1986 and 2016 and investigating risk factors in mixed population, susceptible population, and pregnant women, were included in this meta-analysis. Hepatitis E cases were defined with serological exams and differentiated whenever the serological exam is associated or not with symptoms.

This meta-analysis identified the parenteral pathway (blood transfusion, tattooing or IV injection, dialysis or hemodialysis), and routes of infection related to contaminated water, animal contact (occupational exposure) and consumption of foods as relevant risk factors for hepatitis E infection.

With regards to the role of food, as suspected and sometimes proven in several studies, pig meat, pork sausages, and game meat are identified as significant risk factors for HEV, in particular undercooked pig meat, or meat preparations containing pig liver. In addition, consumption of shellfish (oysters and mussels), in which HEV can accumulate when water is environmentally contaminated (from animal or human origin), is also associated with the detection of anti-HEV antibodies.

The results of this meta-analysis show that symptomatic and infected cases share the most explainable risk factors, and are in agreement with recent studies conducted in Europe. This meta-analysis reveals that some sources such as consumption of insufficiently treated water, shellfish, or vegetables are under-investigated. Future case-control studies should include

population at risk but under-investigated, such as transplant recipients, pregnant women and children, and investigate other potential sources of HEV.

1. Introduction

Hepatitis E virus (HEV) is one of the main causes of viral hepatitis infection worldwide (EFSA BIOHAZ Panel, 2017). HEV is a quasi-enveloped virus, similar to hepatitis A virus (HAV), with a fecal-oral route of transmission. HEV strains infecting humans belong to genotypes 1 to 4 (HEV-1 to HEV-4) within the Orthohepevirus A genus of the Hepeviridae family (Smith et al., 2014). In a typical HEV infection, anti-HEV IgM are produced with a maximum level at 6 or 8 weeks after infection and last till 5-to 6 months (Hakim et al., 2018). IgG gradually increase and would persist at least 14 years after infection (Hakim et al., 2018). The diagnosis of acute hepatitis is usually made by the demonstration of increased serum bilirubin and liver enzymes. The differential diagnosis includes IgM (as a marker of recent infection), IgG anti-HEV as a marker of past infection (WHO, 2014). Detection of HEV-RNA by RT-PCR is also feasible: HEV RNA can be detected in the blood after 3 weeks till the beginning of symptoms (Kamar et al., 2017).

In most cases, the infection by HEV is asymptomatic and benign, yet it can turn into self-limited acute hepatitis in humans (Kamar et al., 2017). Jaundice usually persists one to six weeks. Furthermore, fulminant hepatic failure can occur in patients with underlying liver chronic diseases, ~~in the elderly~~, immunosuppressive conditions, and pregnant women (EFSA BIOHAZ Panel, 2017). Excess mortality during pregnancy, estimated at 21%, and premature delivery are associated with HEV-1 and -2 infections and have not been yet reported for HEV-3 and HEV-4 (Kamar et al., 2017). Chronic cases of HEV infections are also reported in immunocompromised patients such as solid organ transplant recipients (Kamar et al., 2017) and in patients with pre-existing liver disease (EFSA BIOHAZ Panel, 2017). Chronic hepatitis E with HEV-3 can lead to steatosis, fibrosis, and even cirrhosis (Kamar et al., 2017). Extrahepatic manifestations, including kidney dysfunctions or neurological syndromes, have also been described during acute or chronic HEV infections (Kamar et al., 2017).

In most parts of Asia and Africa, the prevalence rate of anti-HEV antibodies in the general population ranges between 10 and 40%, with the highest levels in older age groups (>50 years of age) (WHO, 2010). Anti-HEV seroprevalence estimates in Europe range from 0.6% to 52.5% (Hartl et al., 2016), increased with age, but unrelated to gender. Available epidemiological data showed an increase in the number of HEV cases reported in industrialized countries. In Europe, the number of reported cases has increased from 514 cases per year in 2005 to 5617 in 2015, with most infections being locally acquired (Aspinall et al.,

2017). In developed countries, the seroprevalence estimate level in the population is high, but the number of detected symptomatic cases is low.

Sources of contamination are different according to the genotype of the virus. In tropical and sub-tropical areas, HEV is endemic and responsible for large waterborne epidemics due to HEV-1 or HEV-2 contamination of drinking water (Aggarwal and Goel, 2018). In industrialized countries, HEV sporadic infections can be diagnosed after travelling to endemic regions, with HEV-1 or HEV-2 being involved, but the majority of sporadic or grouped cases observed are locally-acquired and due to HEV-3 and HEV-4 (Kamar et al., 2017). In these cases, zoonotic transmission through the consumption of contaminated foods (EFSA BIOHAZ Panel, 2017) or by direct contact with infected animals (mainly swine) is the major transmission route to humans. Several cases were related to the consumption of raw or undercooked infected pork meat or pork liver (Doceul et al., 2016). Apart from the consumption of pork products, there is growing evidence of other routes of infection related to other animal species (wild boar, deer, rabbits, etc.), food products (crops, shellfish), the environment, and blood transfusion (Kamar et al., 2017). Since HEV exposure to humans may have multiple origins, there is an increasing number of published epidemiological studies in recent years investigating the main sources and transmission pathways of sporadic hepatitis E infection, with more than 25 publications after 2010. In the present study, a systematic review and a meta-analysis of case-control and cohort studies (including transversal studies) were performed to determine the main risk factors associated with sporadic hepatitis E infection. As far as possible, we compared risk factors between different type of susceptible populations, for symptomatic and infected cases (with or without symptoms). Characterization of risk factors to HEV exposure will contribute to identifying measures to reduce the burden of hepatitis E.

2. Material and methods

The protocol of the systematic review and the meta-analysis model are described in depth in the methodological paper of this issue (Gonzales-Barron et al., 2019).

2.1 Systematic review

The Literature search was conducted between March 2017 and December 2017 using a combination of keywords related to (1)“ hepatitis E*”*, (2) “case-control” “OR” “risk factor”

"OR" "cohort" (3) *"*infection" "*OR" "*disease*'",* joined by the connector "AND". Relevant studies were identified from five bibliographic search engines, Science Direct, PubMed, Scielo, ISI Web of Science and Scopus. No restrictions were defined for the year of the study or type of publication. The search was limited to the languages English, French, Portuguese and Spanish.

Each reference record was screened manually for relevance for inclusion in the meta-analysis study, and subsequently, the methodological quality of the "candidate" studies were assessed using pre-set quality criteria comprising (1) appropriate selection of the controls; (2) adjustment to correct for confounders, (3) comparability between cases and controls, (4) acceptable responses rates for the exposed and control groups; (5) Data analysis appropriate to the study design; (6) provision of Odd ratio (OR) with confidence interval or p-value; or provision of sufficient data to calculate ORs; overall quality of the study (Gonzales-Barron et al., 2019). Primary studies that passed the screening for relevance were marked as having potential for bias if they failed to meet at least one of the methodological quality assessment criteria.

Data from primary studies were then extracted using a standardized spreadsheet. Data extracted included the relevant study characteristics (location, time period, population, genotype, case definition, design, sample size of the groups, type of model, etc.), the categorized risk factors, the setting, the handling practices and the outcome of the study OR (Odds-Ratio in case control-studies) or RR (Relative Risk in cohort or transversal studies). When data were extracted from cohort studies, OR could be either computed from raw data or RR (Relative. OR was computed from RR, using the equation:

$$OR = \frac{RR(1-p_0)}{1-p_0 RR}$$

A data categorization scheme was established to hierarchically group the risk factors into travel, host-specific factors and pathways of exposure (i.e., person-to-person, animal, environment, and food routes (Gonzales-Barron et al., 2019).

The variable "Population" was stratified into mixed (adults and no specific age), pregnant women, and other vulnerable populations ("susceptible"). Indeed, pregnant women and other susceptible populations such as immunosuppressed people or persons with pre-existing liver disease are considered at higher risk of severe disease following infection. Two "cases" definitions were considered in publications : seropositivity (in general not associated with

symptoms at time of sampling), defined here as “infection cases”, and symptomatic cases (associated with positive serology). In order to better investigate risk factors associated with possible severity, we keep separate results from those two definitions.

Specific partitions were made to investigate more deeply risk factors of acquiring hepatitis E. such as blood transfusion, transplant recipients, dialysis, chronic diseases, and other medical conditions. Personal hygiene (e.g « not washing hands after toilets ») was considered separately. Person-to-person transmission was stratified by the type of contact; namely: contact with an ill person in the family or relatives (“contact”), venereal contact (“venereal contact”), healthcare worker (“occupational contact”), and other blood contact without blood transfusion, such as history of injective drug or tattoo (“other contact”). Some other exceptional sub-partitions were created for HEV as the risk factor of consuming pig liver.

2.2 Data synthesis

The meta-analysis procedures are described in depth in the methodological paper of this issue (Gonzales-Barron et al., 2019).

The joint meta-analytical data was first described using basic statistics. Next, data was partitioned into subsets of categories of risk factors. Meta-analysis models were then fitted to each of the data partitions or subsets in order to estimate the overall OR due to- travel, host-specific factors and transmission pathways related to person-to-person contagion, animal contact, environmental exposures, and food vehicles. The meta-analytical models were fitted separately by population type. For some food classes, the effects of handling (i.e., eating raw, undercooked) and setting (i.e., eating out) on the overall OR were assessed by the calculation of the ratio of the mean OR when food is mishandled (or, when food is prepared outside the home) to the base OR.

The statistical analysis was designed to assess the effect of the geographical region and is taking into account the study period (before/after 2000) and the analysis type (univariate/multivariate) on the final result. The objective of the region-specific meta-analysis was to inform the decision on the geographical regions that should be maintained for the subsequent pooling of ORs. A Geographical region (Asia, North America, South America, Africa, Europe, Oceania) was removed from a particular meta-analysis partition only if its pooled ORs were different from those associated with the other regions or if less than 3 ORs represented the region (Gonzales-Barron et al., 2019).

All meta-analysis models were essentially weighted random-effects linear regression models. Once a meta-analysis model was fitted, influential diagnostics statistics were applied in order to remove any influential observation originating from studies marked as having potential-for-bias. Publication bias was assessed by funnel plots and a statistical test investigating the effect of the study sample size on the ORs (Tables 1, 2 and 3) (Gonzales-Barron et al., 2019). Heterogeneity between studies was assessed by different indicators such as the between-study variability (τ^2), the QE test investigating residual heterogeneity, the variance of residuals and the intra-class correlation I^2 (Gonzales-Barron et al., 2019). Publication bias and remaining heterogeneity were not further corrected for, but were taken into account for the interpretation of the results.

All analyses were produced in the R software (R Development Core Team, 2008) implemented with the metafor package (Viechtbauer, 2010).

The meta-analyzed risk factors are presented in summary tables only when significant. Pooled ORs were considered as significant when the lower bound of the 95% CI was equal or greater than 1.

3. Results

3.1 **Descriptive statistics**

In the systematic review of risk factors for human infection with Hepatitis E, a total of 614 clean bibliographic sources were identified using appropriate keywords in five bibliographic search engines, from which 93 case-control and cohort studies passed the full assessment for eligibility (Figure 1). From these, fifteen fully-documented case-control studies investigated the source(s) of outbreaks and were kept in the JabRef file as their data can be readily extracted. Meta-analysis was undertaken on data either extracted or calculated from 78 primary studies – cohort and case-control studies – focusing on sporadic disease (Figure 1). These published studies were conducted in years spanning from 1986 and 2016. Appendix 2 compiles a list of the primary studies along with their main features. Primary studies investigated risk factors in different types of population, namely children (1 study), mixed population (69 studies), susceptible population (7 studies) and pregnant women (6 studies) (five studies investigate different populations, refer to Appendix 2 for details). All publications concerning pregnant women were coming from Mexico, China, India, Turkey, Egypt, and Tunisia.

For children, all ORs came from only one study (Meng et al., 2015) which was finally removed from the analysis. Risk factors for children can be seen as having their specificity (Verghese and Robinson, 2014) and it was not pertinent to join them to the general (mainly adult) population.

The 77 publications selected concern studies carried out between 1986 and 2016. Around 80% of studies are post-2000. The majority of publications concern, in descending order, Europe (n=31), Asia (n=20), Africa (n=12), South America (n=9) and North America (n=5). In 68 publications, mostly transversal studies with healthy individuals, cases were defined by positive anti–HEV antibodies (IgG or IgM). Ten studies used a case definition based on symptoms with confirmation by serology or HEV detection by RT-PCR (Appendix 2) (107 ORs). One publication investigated separately both populations (Houcine et al., 2012). Seventy-two studies employed an unmatched experimental design. During the methodological quality assessment, a potential for selection bias was assigned to two case-control studies. While in Delarocque-Astagneau et al. (2012), the controls were hepatitis A positive, in Mellgren et al. (2017), the associations between host-specific factors and hepatitis E were measured in patients with chronic hepatitis C. The ten ORs extracted from the studies above were marked as having potential for bias, and their influence on the meta-analyzed OR estimates was appraised by means of the Cook's distance.

All publications were the source of 578 ORs. The risk factors studied include food transmission pathways (145 ORs) (including hygiene before meal), environmental pathways (142 ORs), contact with animals (135 ORs), human-to-human transmission (37 ORs) and personal hygiene practices (5 ORs). Host factors (90 ORs) and travel (24 ORs) were also studied. The genotype was rarely described, as a consequence of the main way of recruitment of (non-symptomatic) cases by serology only. Studies on pregnant women population are limited to serological surveys. Moreover, only one publication refers to symptomatic hepatitis in the other susceptible populations. Therefore, risk factors associated with symptomatic hepatitis E can only be investigated in the mixed population, the other populations were considered with a serological definition (or infection status) only.

3.2 Meta-analysis

The meta-analyzed risk factors are presented in summary tables only when significant. Pooled ORs were considered as significant when the lower bound of the 95% CI was equal or greater than 1. All results are given in tables 1, 2 and 3. Whenever a category is significant but is only described in one publication, the result is not given in the main tables but in Appendix 3. Non-significant results of the main risk factors are also reported in Appendix 3.

Meta-analysis for travel

Travel factors can be analyzed for mixed population with infected or symptomatic cases definition. The pooled OR for travel abroad is significant for the mixed population (infected definition; pooled OR=1.043; 95% CI [1.012-1.075]; Table1). Except for two publications (Alvarado-Esquivel et al., 2014, 2015), all countries exploring traveling abroad as a risk factor, are localized in developed areas. Most of the time, the destination is not mentioned.

For symptomatic cases, traveling in different areas (Egypt, Bangladesh, or endemic area is also a significant risk factor (pooled OR=3.547; 95%CI [1.1.159-10.859]; Table 1)

Meta-analysis for host-specific risk factors

In the mixed population (with infected case definition), blood transfusion (pooled OR=2.005; 95% CI [1.468- 2.738]) and dialysis (pooled OR=2.699; 95% CI [1.391- 5.236)) were found significant (Table 1). Other explored factors were not found significant: other medical conditions (like HAV, HBV or HCV antibodies), chronic diseases, HIV seropositivity, surgery, and to be a recipient transplant (Figure 2). However the forest plot in Figure 2 shows that ORs associated with kidney transplant are lower than those associated with liver transplant. For the susceptible population, chronic disease were found significantly associated with HEV infection (pooled OR=2.454; 95% CI [1.827- 3.298]; Table 1). The susceptible individuals investigated were dialysis patients (Alavian et al., 2015) or patients on dialysis and solid organ transplant recipients in Argentina (Pisano et al., 2017).

Meta-analysis for person-to-person transmission factors

In the mixed population, contact with needles (tattoo, injective drug user) and occupational exposure (being a healthcare worker) were found associated with HEV infection (Table1). Venereal and family contact with an ill person at home were not found associated with Hepatitis E (Appendix 3). Contact with jaundice or hepatitis patient, was found associated with symptomatic hepatitis cases (pooled OR= 3.399; 95 % CI [1.121-10.308]. ~~However, ORs in this category coming from 3 publications were highly different each other~~ .History of

injection or tattoo was also found significant for symptomatic cases (Table 1). Hygiene factors (e.g. lack of handwashing after toilet) are also associated with HEV infection (pooled OR=1.613; 95% CI: [1.324- 1.965]).

Meta-analysis for animal contacts

Contact with a pet (cat/dog) or farm animal or livestock as occupational exposure (mainly swine) is associated with HEV positive serology in the mixed population (pooled OR=1.426, 2.071 and 2.077 respectively). Occupational activities such as hunting or farming display a significant relationship with symptomatic hepatitis (pooled OR=3.354; 95% CI: [1.092-10.302]) (Table 1). Contact with pets or farm animals was not associated with symptomatic cases of Hepatitis E (Appendix 3). For pregnant women, contact with pets or occupational exposure was also found to be associated with HEV infection (pooled OR=2.061 and=2.549, respectively). Contact with farm animals was not investigated in pregnant women.

Meta-analysis for environmental factors

For the environmental factors, contact with soil ("playground", including gardening) is associated with HEV for the mixed population (HEV positive serology) and pregnant women (pooled OR=1.253 and 1.683, respectively). Other significant risk factors for the mixed population were: contact with wastewater (unsanitary toilets or exposure to sewage) (pooled OR=2.068; 95% [1.497 - 2.857]); consumption of insufficiently treated water (pooled OR=1.692; 95% CI [1.434 - 1.996]); living in a farm environment (pooled OR=2.187; 95% CI [1.654 - 2.893]) or forestry activity (pooled OR=1.614; 95% CI [1.357 - 1.919]) (Table 1). For symptomatic cases in the mixed population consumption of insufficiently treated water (pooled OR=5.105; 95% CI [1.327 - 19.633]) and contact with wastewater ("unsanitary toilet mainly" pooled OR= 5.205; 95% CI[2.305 - 11.754]) were significant risk factors, (Table 1). Consumption of insufficiently treated water and living in a farm environment were not found significantly associated with HEV infection in pregnant women (Appendix 3).

Meta-analysis for food consumption

With respect to the role of food, meat consumption is associated with HEV infection in the mixed population, whatever the case definition (infected or symptomatic: pooled OR=1.597 and 2.887, respectively) (Table 1). Among meats, the following categories are associated with HEV infected and symptomatic cases: pork (pooled OR=2.267 and 2.730; Table 2), other red

meats (mainly “game meat”) (pooled OR=1.850 and 1.799) (Table2; Figure 3), and processed meat products (mainly pork sausages including liver made *figatelles*, and some other deli products) (OR =1.613 and 3.987) (Table 2 and Figure 4). Further analysis of products labeled "pork liver" showed an association with HEV infection in the mixed population (pooled OR = 1.992; 95% CI [1.618 - 2.452]) (Table 2).

Raw milk consumption (mixed and pregnant population) is associated with HEV infection (pooled OR=1.932; 95% CI [1.279 - 2.918]); Table 2 and Figure 5).

Produce consumption is associated with positive serology in the mixed population (including pregnant women and susceptible persons) (pooled OR=1.094; 95% CI: 1.046- 1.144] (Table 2). Consumption of fishery products, in particular shellfish, is associated with positive serology in the mixed population (pooled OR=1.396; 95%CI [1.256 - 1.552]) (Table 2).

Overall, the consumption of undercooked pork and processed meat products (pork sausages) is a significant risk factor (pooled OR= 2.604; 95% CI: [1.33064 - 5.094512]) and multiplies the basic OR, comparing with unknown or well-cooked pork products, by a factor of 1.319 (Table 3). Finally, the lack of handwashing prior to meal preparation is a significant risk factor (pooled OR=1.295; 95% CI: [1.028 - 1.6321] (Table 2).

For all the meta-analytical models reported in Tables 1 and 2, the statistical tests indicated the absence of potential significant publication bias at 5% significance. Exceptions are observed in partitions related to travel in mixed population (symptomatic cases), “person-to-person” in mixed population (both definition of cases), food for symptomatic cases, pig products (with or without liver) and handling for undercooked pork and pork sausages (Tables 1, 2 and 3). For better assessing the publication bias, the funnel plots for those models are provided in Figure 6. For “travel”, “person-to-person” in mixed population (both definition of cases), “food”, “pig products” and “handling pork products” in symptomatic cases, some asymmetry was appreciated due to the lack of small studies with low ORs. Moreover, the intra-class correlation I^2 (Table 1) is always below high heterogeneity (<75%). Sometimes, remaining between-study heterogeneity (significant p-values below 0.05 for Q or QE) was observed for the data partitions.

4. Discussion

All HEVs are transmitted orally but may have different origins depending on the genotypes considered. HEV-1 and -2 are from human origin and are responsible for major outbreaks in developing countries where drinking water is contaminated with unsanitized effluents

(Khuroo et al., 2016). The genotypes HEV-3 and -4 are zoonotic (with animal reservoir mostly) and circulate in humans and several animal species including domestic and wild swine, and to lesser extent red and roe deers (Anheyer-Behmenburg et al., 2017). HEV-3 and -4 are associated with sporadic outbreaks in Europe, Asia, and North America (Kamar et al., 2017). Then, exploring risk factors at a global scale, ignoring the genotype, due to the lack of available data can be seen as a limitation of the study. However, different genotypes can cohabit in the same countries, in particular for HEV-3, which seems to be widespread in pig populations worldwide (Khuroo et al., 2016). Even in industrialized countries, where HEV-1 and -2 are not endemic, HEV-3 can be excreted in human stools (Fenaux et al., 2018) and then can be seen as a potential fecal-oral risk. We also have chosen to keep all publications using different detection methods for anti- HEV antibodies, regardless of sensitivity or specificity, as there were not so many studies and we opted for being as conservative as possible.

The fecal-oral waterborne route is in agreement with factors found significant such as the consumption of untreated drinking water for the mixed population (symptomatic and infection case definition). Concerning the fecal-oral route, the lack of toilets ("personal hygiene"), lack of handwashing before meals, contact with wastewater, or playground are plausible ways of transmission. However, consumption of untreated drinking water was not found significantly associated with seroconversion for pregnant women in developing countries; although the corresponding OR was close to being significant (Appendix 3), and the contamination of water heterogeneously dispersed. HEV is commonly found in wastewater treatment plants and may persist in the environment, and it has been detected in rivers, even in European countries (Rutjes et al., 2009). Traveling in endemic countries can be a risk of exposure from food or the environment and the finding of this factor as significant is not surprising.

Person-to-person transmission, even considering the fecal-oral pathway, needs further confirmation in both sporadic and epidemic settings, since the occurrence of several cases in one family can be attributed to person-to-person transmission but also to high disease attack rates resulting from high levels of environmental contamination (Kamar et al., 2017). In this meta-analysis, a significant association was only found for contact with an ill person for symptomatic cases. This factor should deserve further investigation. The question was also raised and questioned in a systematic review of outbreaks (Hakim et al., 2017).

This meta-analysis confirms the role of the parenteral pathway (tattooing/injection/contact with blood) that could be further investigated for healthcare workers (significant with two

publications). Blood transfusion is significant for the mixed population, and it is consistent with descriptions of proven transmissions of hepatitis E after a blood transfusion (Kamar et al., 2017). Cases of hepatitis E after blood transfusion are described and human blood supply can be frequently contaminated in some European countries (Domanovic et al., 2017). HEV testing of blood products is implemented in some European countries as well (Domanovic et al., 2017). Dialysis can also be seen as a confirmed risk factor, hemodialysis was also found associated with HEV seroprevalence in a recent meta-analysis (Haffar et al., 2017). Being a transplant recipient was not found significant, but including recent and new studies, like Mallet et al. (2018) could change the significance of this factor in the future. Then it seems plausible that Hepatitis E can share routes of transmission with Hepatitis A, B, or C, explaining the significance of the risk factor linked to other medical conditions.

Other risk factors found in the meta-analysis can be seen as a consequence of the zoonotic transmission. Occupational exposure, contact with farm animals, was found significant for mixed and pregnant populations in this study. Several seroprevalence studies have suggested that contacts with infected animal reservoirs are risk factors for HEV infection. Professional exposure is higher than in the general population or control cohorts in butchers (contact with pork meat), pig farmers (contact with pigs), slaughterhouse personnel (contact with pigs and pork meat), hunters (contact with wildlife) or forestry worker (contact with wildlife) (Pavio et al., 2017). Forestry activities can also be linked to hygiene or closer contact with wild animals in relationship to HEV-3 or -4. On rare occasions, HEV transmission from a pet pig was reported (Renou et al., 2014). However, in this meta-analysis, contact with pets (dogs and cats) was found significant for pregnant women in developed countries, and the mixed population This result should be confirmed by further studies, perhaps investigating rabbits as a zoonotic pathway (Geng et al., 2019a).

With regards to the role of food, pork, pig sausages, and game meat were identified as significant risk factors for HEV infection, in particular undercooked pig meat, or meat preparations containing pig liver (virus multiplication organ). As the liver is part of the offal, it is also expected to play a significant role in the risk of exposure to HEV. The presence of HEV contamination of fresh or ready-to-eat pork product or meat (e.g. pork pies, liver pate, liver sausages, pork sausages, salami) is demonstrated in several European countries with HEV prevalence of up 70% of the product tested (Boxman et al., 2019; Mykytczuk et al., 2017; Pavio et al., 2014; Szabo et al., 2015).

The consumption of seafood, particularly shellfish (oysters and mussels), which can accumulate HEV when water is environmentally contaminated (from animal or human origin), is also associated with infection. The shellfish were recently shown contaminated in Italy, Spain, and Scotland (La Rosa et al., 2018; Mesquita et al., 2016; O'Hara et al., 2018). Vegetable products (mostly unwashed or raw) were found significantly associated with positive serology in this meta-analysis. As a possible consequence of environmental contamination by infected animals or human effluents, HEV RNA was detected on vegetables (leafy salads), fruits, and spices (Loisy-Hamon and Leturnier, 2015; Maunula et al., 2013; Santarelli et al., 2018).

As said by another author, factors exploring indirect contamination such as insufficient sanitization of water or shellfish or vegetable consumption should be better investigated in developed countries (King et al., 2018).

To a lesser extent, raw milk was identified as a risk factor. ~~This result is based on three publications, two from Mexico and one from India (figure 5).~~ To date, zoonotic transmission by domestic ruminants has not been established. Excretion of HEV-4 is described in cow milk in China (Huang et al., 2016), but was not confirmed by other studies (Geng et al., 2019b; Vercouter et al., 2018). This result could also be related to a lack of hygiene (human or environmental contamination).

It is important to differentiate between infected cases (with positive serology definition), the large proportion of which corresponds to asymptomatic forms, and symptomatic cases of hepatitis. In France, where HEV seroprevalence is high (22.4%) (Mansuy et al., 2016), it is estimated that 70% of cases are asymptomatic (Guillois et al., 2016). Such difference can be explained, generally, by the virulence of the strain, or human susceptibility or higher dose associated with higher severity. Most of the explanation could be attributed to human susceptibility, but this meta-analysis was an opportunity to compare, roughly, exposures between symptomatic and infected cases (healthy at the time of the study, and in some studies, clearly asymptomatic, without hepatitis history). From the systematic review, it was evident that few studies investigated symptomatic cases of Hepatitis E. However, when it was possible to make a comparison, we could see that symptomatic and infected cases share the same most explainable risk factors, such as occupational exposure (contact with animals or hunting), untreated drinking water, contact with wastewater, game meat or pork products. This comparison was not feasible for the susceptible population as only one study investigated symptomatic hepatitis E cases.

In general, symptomatic cases of hepatitis E could be better explored, in particular for populations at risk of developing severe or chronic forms (Aspinall et al., 2017). Sporadic cases should be better investigated with precise definitions of exposure specifying by example the mode of preparation (washing of vegetables, cooking of meat). Whenever possible, whatever the country of origin, common most potential risk factors should be studied. Finally, an harmonization of the case definition of HEV infection (clinical criteria, detection HEV IgG/IgM, HEV RNA) is still a challenge at a global scale and European level (Adlhoch et al., 2019; Bohm et al., 2020). It could allow better comparison between studies and a better understanding of the epidemiological situation.

~~This is in agreement with recent studies conducted in Europe: consumption of pork meat, pork liver, wild boar, produce or contact with waste water have been identified as significant risk factors in a German study (Faber et al., 2018), whereas contact with farm or wild animals, and contact with cat have been pointed out as risk factors in a Polish study (Baumann-Popczyk et al., 2017).~~

5. Conclusion

~~In summary, this meta-analysis identified parenteral pathways (blood transfusion, dialysis) and routes of infection related to contaminated water, animal contact and consumption of foods (mainly pork products) as relevant risk factors for hepatitis E infection. This is in agreement with recent studies conducted in Europe: consumption of pork meat, pork liver, wild boar, produce or contact with waste water have been identified as significant risk factors in a German study (Faber et al., 2018), whereas contact with farm or wild animals, and contact with cat have been pointed out as risk factors in a Polish study (Baumann-Popczyk et al., 2017). Future case-control studies should focus on susceptible individuals, at risk of developing severe or chronic forms, and showing recent seroconversion. In general, symptomatic cases of hepatitis E could also be better explored (Aspinall et al, 2015). Lack of information was also identified for pregnant women in developing countries, and to a lesser extent for children. As suggested in another publication, factors exploring indirect contamination such as insufficient sanitization of water or shellfish or vegetables consumption should be better investigated in developed countries (King et al., 2018).~~

This meta-analysis provides an original complete view of identified risk factors for HEV infection from published literature till December 2017. In summary, this meta-analysis confirms parenteral pathways (blood transfusion, dialysis) and other routes of infection

related to contaminated water, animal contact and, consumption of foods (mainly insufficiently cooked pork or pig liver products) as relevant risk factors for hepatitis E infection. This meta-analysis also identifies less-known risk factors, such as shellfish, and raw milk, which need confirmation in concerned areas.

More precise and harmonized definition of exposure and cases in epidemiological studies may help for better understanding the pathway of transmission.

Appendices: Supplementary material

Appendix 1: Complete bibliographic references.

Appendix 2: Characteristics of the 78 primary studies investigating risk factors for acquiring sporadic hepatitis E included in the meta-analysis.

Appendix 3: Non-significant results on the main risk factors

Data statement

Figures

- Figure 1: Flow chart of literature search for case-control or cohort studies of human hepatitis E
- Figure 2: Forest plot of the association of hepatitis E infection with transplant organ recipient in the mixed population:
  (in separate file) **(legend * adjusted OR as described in Gonzales-Barron et al. (2019)**
- Figure 3: Forest plot of the association of hepatitis E infection with other red meat (game meat) in the mixed population
  (in separate file) **(legend * adjusted OR as described in Gonzales-Barron et al. (2019)**
- Figure 4: Forest plot of the association of hepatitis E infection with processed meat (pork sausages) in the mixed population
  (in separate file) **(legend * adjusted OR as described in Gonzales-Barron et al. (2019)**
- Figure 5: Forest plot of the association of hepatitis E infection with dairy products in all populations
  (in separate file) **(legend * adjusted OR as described in Gonzales-Barron et al. (2019)**

- Figure 6: (in separate file) **Funnel plots of studies investigating categorized risk factors with significant p bias (Table 1, 2, 3):**

Legend

  - A: Host-specific factors in mixed population (infection case definition)
  - B: Person-to-Person factors in mixed population (infection case definition)
  - C: Person-to-person factors in mixed population (symptomatic case definition)
  - D: Food in mixed population (symptomatic case definition)
  - E: Pig products in mixed population (infection case definition)
  - F: Handling pig products in mixed population (infection case definition)

- ***Tables***
- Table 1. Results of the meta-analysis on the main risk factors
- Table 2. Results of the meta-analysis on disaggregated risk factors
- Table 3. Effect of handling on the pooled OR for food products

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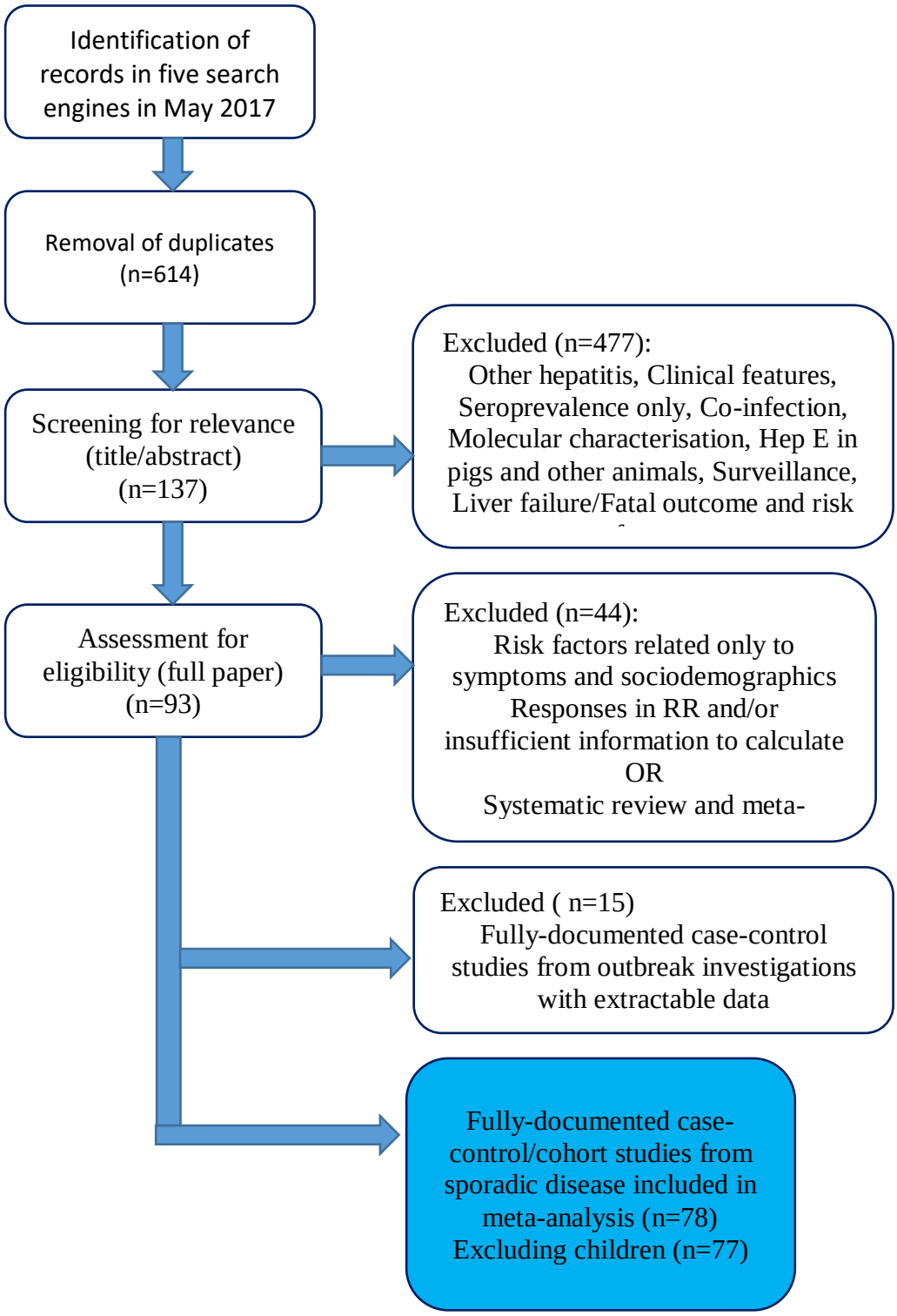
Figure 1: PRISMA Flow chart of included studies



583 • **Table 1: Results of the meta-analysis on main risk factors**
584

Population	Included study area	Case definition	Risk factor	Pooled OR [95% CI]	N/n*	p-value of risk factor	Publication bias p-value	Points removed **	Heterogeneity analysis***
Travel									
Mixed	**Asia, South America, Europa**	Infection	Abroad	1.043[1.012-1.075]	9/15	0.006	0.15	**0**	τ^2=0 ; Q(df = 15) = 21.045, p-val = 0.135 ; s^2=0.241; I^2=0
Mixed	**Asia, Africa, Europa**	Symptomatic	Any Travel	3.547 [1.1.159-10.859]	4/5	0.027	0.004	**0**	τ^2=0.918 Q(df = 4) = 14.855, p-val = 0.005 s^2=1.626;I^2= 36.079
Host specific									
Mixed	**Africa, Asia, Europa, North and South America**	infection	Blood Transfusion	2.005[1.468 - 2.738]	10/14	<.0001	0.156	**0**	τ^2=0.137 QE(df = 50) = 223.494, p-val < .0001 s^2= 0.469; I^2=22.643
			Dialysis	2.699 [1391 - 5.236	2/3	0.0033			
Susceptible (y)	**Asia, Europa,South America**	Infection	Chronic	2.454 [1.827 - 3.298]	3/8	<.0001	0.464	**0**	τ^2=0.204 QE(df = 8) = 11.520, p-val = 0.174 s^2=0.379;I^2= 34.908
Person to person by type of contact									
Mixed	**Africa, Asia, Europe, North america**	infection	Occupational (healthcare worker)	1.570 [1.092 - 2.258]	2/3	0.0148	0.028	0	τ^2=0.144 QE(df = 21) = 23.922, p-val = 0.297 s^2= 0.152I^2=487
			Tattoo/injective drug user	1.579 [1.027 - 2.427]	5/7	0.0373			
Mixed	**Africa, Asia, Europa**	symptomatic	Contact (jaundice exposure)	3.399[1.121-10.308]	5/6	0.0306	0.0143	0	τ^2=1.043 QE(df = 9) = 23.707, p-val = 0.005 s^2= 1.694; I^2=38.112
			Tattoo/injection	2.125[1.190- 3.793]	3/5	0.0108			
Personal Hygiene									
All	**Africa, Asia**	infection		1.613 [1.324 - 1.965]	3/5	<.0001	0.750	0	τ^2=0 ; Q(df = 4) = 9.164, p-val = 0.057 ; s^2=0.081; I^2=0
Animals									
Mixed	**Africa,**	Infection	Farm animals	2.071 [1.506 - 2.848]	10/17	<.0001	0.602	0	τ^2=0.456

	Asia, Europa, North and South America		Occupational exposure	2.077 [1.795 - 2.403]	23/59	<.0001			QE(df = 96) = 192.480, p-val < .0001; s2=0.325 I2= 58.381
			Pets	1.426 [1.121 - 1.814]	9/23	0.004			
Mixed	**Africa, Asia, Europa**	symptomatic cases	Occupational exposure	3.354 [1.092 - 10.302]	2/4	0.035	0.158	0	τ^2= 0.823 QE(df = 10) = 17.509, p-val = 0.064 s^2= 0.533 I^2= 60.69
Pregnant	**Africa, Asia, South America**	infection	Pets	2.061 [1.563 - 2.718]	3/6	<.0001	0.815	0	τ^2=0 ; QE(df = 15) = 16.039, p-val = 0.379; S^2=0.231 I^2=0
			Occupational exposure	2.549 [1.989 - 3.267]	3/11	<.0001			
Environment									
Mixed (y)	**Africa, Asia, Europa, North and South America**	infection	Untreated drinking water	1.692 [1.434 - 1.996]	22/46	<.0001	0.105	0	τ^2=0.775 QE(df = 100) = 337.2926, p-val < .0001 S^2= 0.371 I^2=67.615%
			Farm environment	2.187 [1.654 - 2.893]	19/27	<.0001			
			Forestry	1.614 [1.357 - 1.919]	2/9	<.0001			
			Playground	1.253 [1.034 - 1.520]	5/7	0.022			
			Waste water	2.068 [1.497 - 2.857]	8/17	<.0001			
Mixed	**Africa, Asia, Europa**	symptomatic cases	Untreated drinking water	5.105[1.327 - 19.633]	4/4	0.018	0.941	1	τ^2=1.425 QE(df = 8) = 18.016, p-val = 0.021 S^2= 0.845 I^2=62.771%
			Waste water	5.205 [2.305 - 11.754]	3/4	<0.001			
Pregnant	**Africa, Asia,**	infection	Playground	1.683 [1.233 - 2.297]	2/2	0.001	0.312	**0**	τ^2=0.111 QE(df = 8) = 10.740, p-val = 0.217 S^2=0.149 I^2=42.635
Food									
Mixed (y)	**Africa, Asia, Europa, North and South America**	infection	Dairy	1.711 [1.101 - 2.660]	3/3	0.0170	0.623	**0**	τ^2=0.406 QE(df = 73) = 282.648, p-val < .0001 S^2=0.232 ; I^2=63.71
			Meat	1.597 [1.401 - 1.821]	14/55	<.0001			
			Seafood	1.451 [1.262 - 1.669]	3/9	<.0001			
			Produce	1.092 [1.043 - 1.143]	6/9	0.0002			
Mixed	**Africa, Asia,**	symptomatic cases	Meat	2.887 [2.108 - 3.954]	2/24	<.0001	0.023	**0**	τ^2=0.024 QE(df = 26) = 31.355, p-val = 0.215 ;

	Europa								S^2=0.483 ; I^2=4.69

*N/n Number of studies/number of OR;** points removed by sensitivity analysis, all results are given after removing data concerned; ***Between-study variability (τ^2), test for residual heterogeneity (QE), variance of residuals (s^2), intra-class correlation (I^2). **(**γ): year is significant (before/after 2000) in this model and the estimates are taking this effect into account

589 • **Table 2: Results of the meta-analysis on disaggregated risk factors**
590

Risk Factor	Included Area	Case definition	Risk factor precise	Pooled OR [95% CI]	N/n*	p-value of risk factor	Publication bias p-value	Points removed **	Heterogeneity analysis ****
Meat (y)	Africa, Asia, Europa, North and South America	infection	Other red meats (game meat)	1.850 [1.469 - 2.330]	5/16	<.0001	0.870	0	τ^2=0.319 QE(df = 59) = 222.731, p-val < .0001 s^2=0.243 ; I^2=56.699
			Others	1.414 [1.171 - 1.707]	7/17	0.0003			
			Pork	2.267 [1.675 - 3.068]	9/17	<.0001			
			Processed meat	1.613 [1.339 - 1.942]	6/14	<.0001			
Meat	Europa	symptomatic cases	Other red meats	1.799 [1.186 - 2.728]	2/5	0.0057	0.557	0	τ^2=0.425 QE(df = 24) = 29.227, p-val = 0.212; s^2=0.369 I^2=53.447
			Pork	2.730 [1.381- 5.398]	2/4	0.0039			
			Processed meat	3.987 [2.745 - 5.792]	2/12	<.0001			
Seafood	South America, Europa, South Africa	infection	Molluscs (shellfish)	1.396 [1.256 - 1.552]	2/7	<.0001	0.201	0	τ^2=0 QE(df = 8) = 21.298, p-val = 0.006s^2=0.419; I^2=0
Dairy	Asia, South America	infection	Dairy(raw milk)	1.932 [1.279 - 2.918]	4/5	0.0018	0.369	0	τ^2=0.057 Q(df = 4) = 6.242, p-val = 0.182; s^2=0.358 I^2=13.67
Produce	Africa, Asia, South America, Europa	infection	Produce	1.094 [1.046 - 1.144]	8/13	<.0001	0.647	0	T^2=0 Q(df = 12) = 7.603, p-val = 0.815; s^2=0.207 I^2=0
Pig products	Africa, Asia, Europa, North and South America	infection	Liver presence	1.992 [1.618 - 2.452]	4/16	<.0001	2.10^{-16}	0	τ^2=0.2282 QE(df = 29) = 215.671, p-val < .0001 s^2=0.361; I^2=0
			No liver	1.737 [1.104 - 2.733]	7/15	0.017			
Poor handling : poor handwashing before meal	China	infection	Poor handwashing	1.295 [1.028 - 1.632]	2/4	0.028	0.385	0	τ^2=0 Q(df = 2) = 0.051, p-val = 0.975 s^2=0.027; I^2=0

*N/n Number of studies/number of OR;** points removed by sensitivity analysis, all results are given after removing data concerned; ***Between-study variability (τ^2), test for residual heterogeneity (QE), variance of residuals (s^2), intra-class correlation (I^2). y): year is significant (before/after 2000) in this model and the estimates are taking this effect into account

- **Table 3: Effect of handling on the pooled OR for pork consumption**

Risk Factor	Risk factor precise	Pooled OR [IC95%]	N/n*	p-val risk factor	OR ratios and 95% CI	Points removed**	Publication bias p-value	Heterogeneity analysis***
Pork (only sero)	Undercooked	2.604 [1.331- 5.095]	3/6	<.0001	1.319 [0.946 - 1.841]	0	$5.43.10^{-06}$	τ^2=0.118 QE(df = 28) = 193.568, p-val < .0001 S^2=0.336 I^2=25.91
	Base	1.973 [1.407- 2.767]	8/25	0.1028	_			

*N/n Number of studies/number of OR;** points removed by sensitivity analysis, all results are given after removing data concerned; ***Between-study variability (τ^2), test for residual heterogeneity (QE), variance of residuals (s^2), intra-class correlation (I^2).

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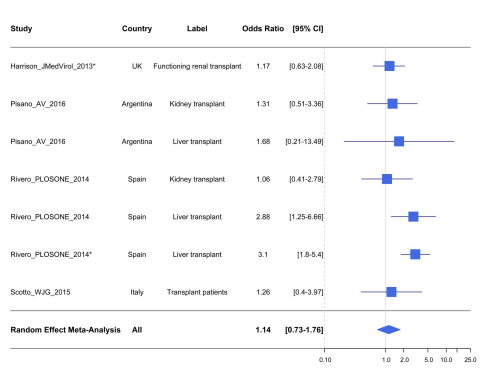
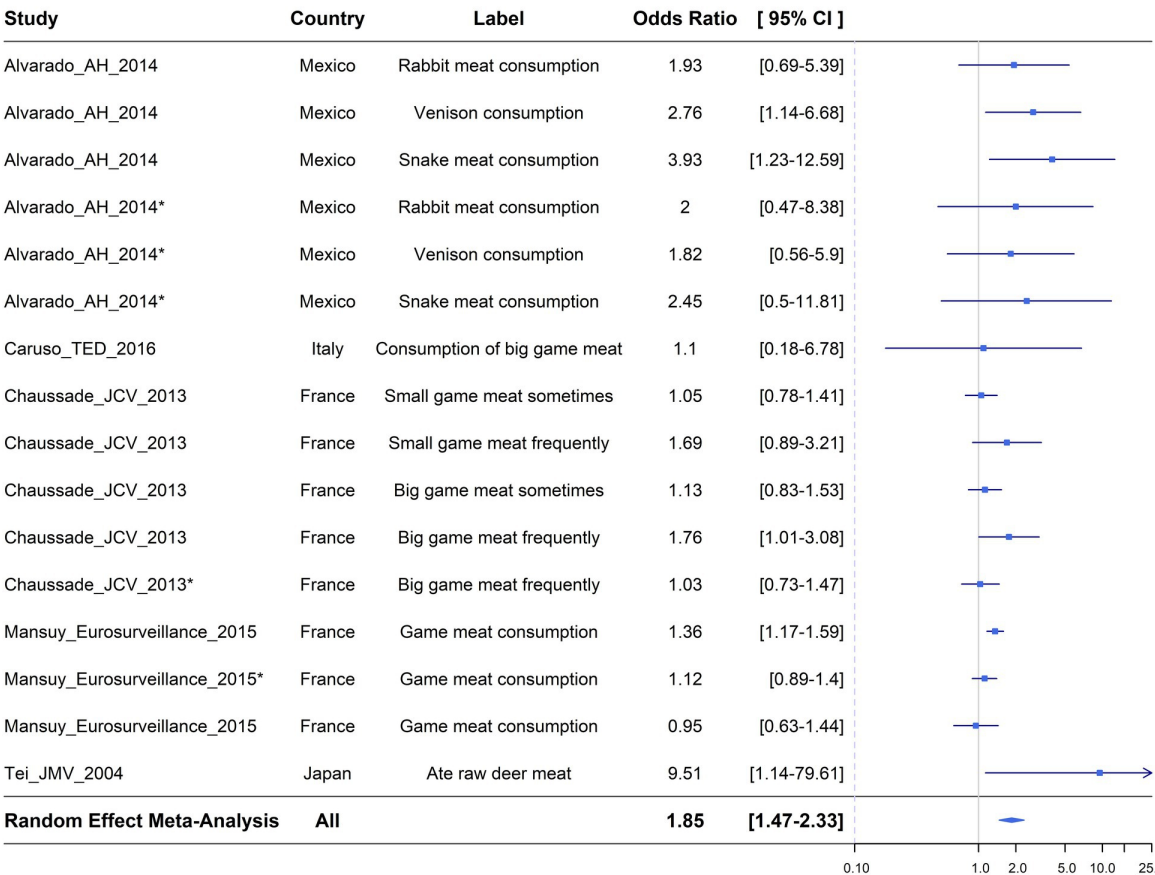
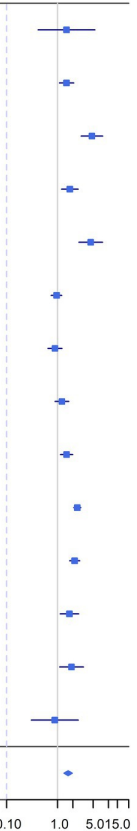
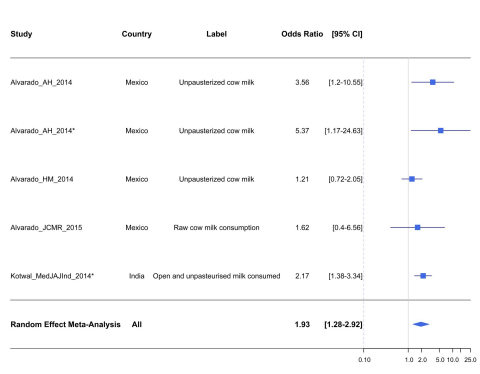
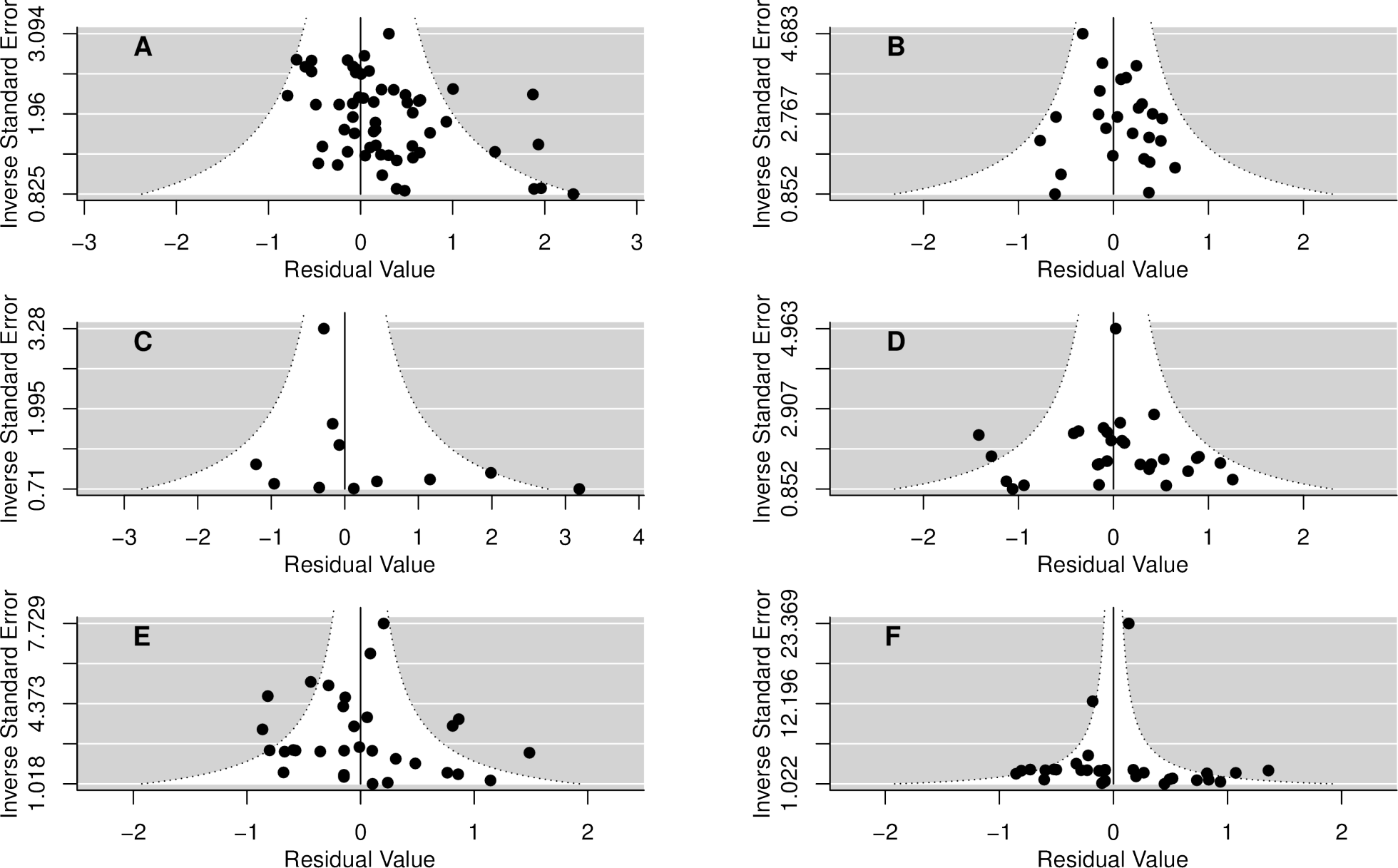

Study
Country
Label
Odds Ratio
[95% CI]
Harrison_JMedVirol_2013*
UK
Functioning renal transplant
1.17
[0.63-2.08]
Pisano_AV_2016
Argentina
Kidney transplant
1.31
[0.51-3.36]
Pisano_AV_2016
Argentina
Liver transplant
1.68
[0.21-13.49]
Rivero_PLOSONE_2014
Spain
Kidney transplant
1.06
[0.41-2.79]
Rivero_PLOSONE_2014
Spain
Liver transplant
2.88
[1.25-6.66]
Rivero_PLOSONE_2014*
Spain
Liver transplant
3.1
[1.8-5.4]
Scotto_WJG_2015
Italy
Transplant patients
1.26
[0.4-3.97]
Random Effect Meta-Analysis
All
1.14
[0.73-1.76]
0.10
1.0
2.0
5.0
10.0
25.0


Study
Country
Label
Odds Ratio
[95% CI]
Alvarado_AH_2014 Mexico Rabbit meat consumption 1.93 [0.69-5.39]
Alvarado_AH_2014 Mexico Venison consumption 2.76 [1.14-6.68]
Alvarado_AH_2014 Mexico Snake meat consumption 3.93 [1.23-12.59]
Alvarado_AH_2014* Mexico Rabbit meat consumption 2 [0.47-8.38]
Alvarado_AH_2014* Mexico Venison consumption 1.82 [0.56-5.9]
Alvarado_AH_2014* Mexico Snake meat consumption 2.45 [0.5-11.81]
Caruso_TED_2016 Italy Consumption of big game meat 1.1 [0.18-6.78]
Chaussade_JCV_2013 France Small game meat sometimes 1.05 [0.78-1.41]
Chaussade_JCV_2013 France Small game meat frequently 1.69 [0.89-3.21]
Chaussade_JCV_2013 France Big game meat sometimes 1.13 [0.83-1.53]
Chaussade_JCV_2013 France Big game meat frequently 1.76 [1.01-3.08]
Chaussade_JCV_2013* France Big game meat frequently 1.03 [0.73-1.47]
Mansuy_Eurosurveillance_2015 France Game meat consumption 1.36 [1.17-1.59]
Mansuy_Eurosurveillance_2015* France Game meat consumption 1.12 [0.89-1.4]
Mansuy_Eurosurveillance_2015 France Game meat consumption 0.95 [0.63-1.44]
Tei_JMV_2004 Japan Ate raw deer meat 9.51 [1.14-79.61]
Random Effect Meta-Analysis All 1.85 [1.47-2.33]
0.10
1.0
2.0
5.0
10.0
25.0

Study	Country	Label	Odds Ratio	[95% CI]
Alvarado_JCMR_2015	Mexico	Salami consumption	1.5	[0.41-5.43]
Chaussade_JCV_2013	France	Pork liver sausages sometimes	1.5	[1.09-2.08]
Chaussade_JCV_2013	France	Pork liver sausages frequently	4.73	[2.91-7.69]
Chaussade_JCV_2013*	France	Pork liver sausages sometimes	1.74	[1.2-2.53]
Chaussade_JCV_2013*	France	Pork liver sausages frequently	4.48	[2.63-7.64]
Kuniholm_JID_2009*	USA	11-20 times/mo bacon, sausage or processed meat	0.96	[0.75-1.21]
Kuniholm_JID_2009*	USA	>20 times/mo bacon, sausage or processed meat	0.89	[0.65-1.22]
Madden_WJG_2016	SouthAfrica	Sausage	1.22	[0.89-1.66]
Madden_WJG_2016	SouthAfrica	Bacon/ham	1.5	[1.14-1.98]
Mansuy_Eurosurveillance_2015	France	Ate uncooked pork liver sausage	2.44	[2.09-2.86]
Mansuy_Eurosurveillance_2015*	France	Ate uncooked pork liver sausage	2.17	[1.73-2.72]
Mansuy_Eurosurveillance_2015	France	Ate uncooked pork liver sausage	1.71	[1.13-2.59]
Mansuy_Eurosurveillance_2015*	France	Ate uncooked pork liver sausage	1.88	[1.1-3.23]
Pisano_AV_2016	Argentina	Charcuterie consumption	0.88	[0.3-2.55]
Random Effect Meta-Analysis	**All**		**1.61**	**[1.34-1.94]**




Study
Country
Label
Odds Ratio
[95% CI]
Alvarado_AH_2014
Mexico
Unpausterized cow milk
3.56
[1.2-10.55]
Alvarado_AH_2014*
Mexico
Unpausterized cow milk
5.37
[1.17-24.63]
Alvarado_HM_2014
Mexico
Unpausterized cow milk
1.21
[0.72-2.05]
Alvarado_JCMR_2015
Mexico
Raw cow milk consumption
1.62
[0.4-6.56]
Kotwal_MedJAInd_2014*
India
Open and unpasteurised milk consumed
2.17
[1.38-3.34]
Random Effect Meta-Analysis
All
1.93
[1.28-2.92]
0.10
1.0
2.0
5.0
10.0
25.0


A
Inverse Standard Error
0.825
1.96
3.094
−3
−2
−1
0
1
2
3
Residual Value
B
Inverse Standard Error
0.852
2.767
4.683
−2
−1
0
1
2
Residual Value
C
Inverse Standard Error
0.71
1.995
3.28
−3
−2
−1
0
1
2
3
4
Residual Value
D
Inverse Standard Error
0.852
2.907
4.963
−2
−1
0
1
2
Residual Value
E
Inverse Standard Error
1.018
4.373
7.729
−2
−1
0
1
2
Residual Value
F
Inverse Standard Error
1.022
12.196
23.369
−2
−1
0
1
2
Residual Value