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

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

2

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

4

5

6 **Highlights**

7 • *Meta-analysis of HEV sporadic cases confirms fecal-oral and zoonotic transmissions*

8 • *Consumption of pork products and processed meat are highly at risk of HEV infection.*

9 • *Consumption of produce or shellfish is associated with HEV exposure.*

10 • *Blood transfusion, dialysis and other contact with needles are risk factors for HEV*

11

12 **Keywords**

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

14

15

16 **Abstract**

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

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

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

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

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

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

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

## 511. **Introduction**

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

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

76 In most parts of Asia and Africa, the prevalence rate of anti-HEV antibodies in the general  
77 population ranges between 10 and 40%, with the highest levels in older age groups (>50 years  
78 of age) (WHO, 2010). Anti-HEV seroprevalence estimates in Europe range from 0.6% to  
79 52.5% (Hartl et al., 2016), increased with age, but unrelated to gender. Available  
80 epidemiological data showed an increase in the number of HEV cases reported in  
81 industrialized countries. In Europe, the number of reported cases has increased from 514 cases  
82 per year in 2005 to 5617 in 2015, with most infections being locally acquired (Aspinall et al.,

83 2017). In developed countries, the seroprevalence estimate level in the population is high, but  
84 the number of detected symptomatic cases is low.

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

107

108

## 1092. **Material and methods**

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

112

### 1132.1 **Systematic review**

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

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

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

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

138

$$139 \quad OR = \frac{RR(1-p_0)}{1-p_0RR}$$

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

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

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

151  
152 Specific partitions were made to investigate more deeply risk factors of acquiring hepatitis E.  
153 such as blood transfusion, transplant recipients, dialysis, chronic diseases, and other medical  
154 conditions. Personal hygiene (e.g « not washing hands after toilets ») was considered  
155 separately. Person-to-person transmission was stratified by the type of contact; namely:  
156 contact with an ill person in the family or relatives (“contact”), venereal contact (“venereal  
157 contact”), healthcare worker (“occupational contact”), and other blood contact without blood  
158 transfusion, such as history of injective drug or tattoo (“other contact”). Some other  
159 exceptional sub-partitions were created for HEV as the risk factor of consuming pig liver.

160

## 161 **2.2 Data synthesis**

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

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

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



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

191  
192 All analyses were produced in the R software (R Development Core Team, 2008)  
193 implemented with the metafor package (Viechtbauer, 2010).

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

197

### 198 **3. Results**

#### 199 **3.1 Descriptive statistics**

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

214

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

219 The 77 publications selected concern studies carried out between 1986 and 2016. Around  
220 80% of studies are post-2000. The majority of publications concern, in descending order,  
221 Europe (n=31), Asia (n=20), Africa (n=12), South America (n=9) and North America (n=5).  
222 In 68 publications, mostly transversal studies with healthy individuals, cases were defined by  
223 positive anti-HEV antibodies (IgG or IgM). Ten studies used a case definition based on  
224 symptoms with confirmation by serology or HEV detection by RT-PCR (Appendix 2) (107  
225 ORs). One publication investigated separately both populations (Houcine et al., 2012).  
226 Seventy-two studies employed an unmatched experimental design. During the methodological  
227 quality assessment, a potential for selection bias was assigned to two case-control studies.  
228 While in Delarocque-Astagneau et al. (2012), the controls were hepatitis A positive, in  
229 Mellgren et al. (2017), the associations between host-specific factors and hepatitis E were  
230 measured in patients with chronic hepatitis C. The ten ORs extracted from the studies above  
231 were marked as having potential for bias, and their influence on the meta-analyzed OR  
232 estimates was appraised by means of the Cook's distance.

233

234 All publications were the source of 578 ORs. The risk factors studied include food  
235 transmission pathways (145 ORs) (including hygiene before meal), environmental pathways  
236 (142 ORs), contact with animals (135 ORs), human-to-human transmission (37 ORs) and  
237 personal hygiene practices (5 ORs). Host factors (90 ORs) and travel (24 ORs) were also  
238 studied. The genotype was rarely described, as a consequence of the main way of recruitment  
239 of (non-symptomatic) cases by serology only. Studies on pregnant women population are  
240 limited to serological surveys. Moreover, only one publication refers to symptomatic hepatitis  
241 in the other susceptible populations. Therefore, risk factors associated with symptomatic  
242 hepatitis E can only be investigated in the mixed population, the other populations were  
243 considered with a serological definition (or infection status) only.

244

### 245 **3.2 Meta-analysis**

246

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

### 252 **Meta-analysis for travel**

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

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

260

### 261 **Meta-analysis for host-specific risk factors**

262 In the mixed population (with infected case definition), blood transfusion (pooled OR=2.005;  
263 95% CI [1.468- 2.738]) and dialysis (pooled OR=2.699; 95% CI [1.391- 5.236]) were found  
264 significant (Table 1). Other explored factors were not found significant: other medical  
265 conditions (like HAV, HBV or HCV antibodies), chronic diseases, HIV seropositivity,  
266 surgery, and to be a recipient transplant (Figure 2). However the forest plot in Figure 2  
267 shows that ORs associated with kidney transplant are lower than those associated with liver  
268 transplant. For the susceptible population, chronic disease were found significantly associated  
269 with HEV infection (pooled OR=2.454; 95% CI [1.827- 3.298]; Table 1). The susceptible  
270 individuals investigated were dialysis patients (Alavian et al., 2015) or patients on dialysis  
271 and solid organ transplant recipients in Argentina (Pisano et al., 2017).

272

### 273 **Meta-analysis for person-to-person transmission factors**

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

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

283

#### 284 **Meta-analysis for animal contacts**

285 Contact with a pet (cat/dog) or farm animal or livestock as occupational exposure (mainly  
286 swine) is associated with HEV positive serology in the mixed population (pooled OR=1.426,  
287 2.071 and 2.077 respectively). Occupational activities such as hunting or farming display a  
288 significant relationship with symptomatic hepatitis (pooled OR=3.354; 95% CI: [1.092-  
289 10.302]) (Table 1). Contact with pets or farm animals was not associated with symptomatic  
290 cases of Hepatitis E (Appendix 3). For pregnant women, contact with pets or occupational  
291 exposure was also found to be associated with HEV infection (pooled OR=2.061 and=2.549,  
292 respectively). Contact with farm animals was not investigated in pregnant women.

293

#### 294 **Meta-analysis for environmental factors**

295 For the environmental factors, contact with soil (“playground”, including gardening) is  
296 associated with HEV for the mixed population (HEV positive serology) and pregnant women  
297 (pooled OR=1.253 and 1.683, respectively). Other significant risk factors for the mixed  
298 population were: contact with wastewater (unsanitary toilets or exposure to sewage) (pooled  
299 OR=2.068; 95% [1.497 - 2.857]); consumption of insufficiently treated water (pooled  
300 OR=1.692; 95% CI [1.434 - 1.996]); living in a farm environment (pooled OR=2.187; 95% CI  
301 [1.654 - 2.893]) or forestry activity (pooled OR=1.614; 95% CI [1.357 - 1.919]) (Table 1).  
302 For symptomatic cases in the mixed population consumption of insufficiently treated water  
303 (pooled OR=5.105; 95% CI [1.327 - 19.633]) and contact with wastewater (“unsanitary toilet  
304 mainly” pooled OR= 5.205; 95% CI[2.305 - 11.754]) were significant risk factors, (Table 1 ).  
305 Consumption of insufficiently treated water and living in a farm environment were not found  
306 significantly associated with HEV infection in pregnant women (Appendix 3).

307

#### 308 **Meta-analysis for food consumption**

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

313 meats (mainly “game meat”) (pooled OR=1.850 and 1.799) (Table2; Figure 3), and processed  
314 meat products (mainly pork sausages including liver made *figatelles*, and some other deli  
315 products) (OR =1.613 and 3.987) (Table 2 and Figure 4). Further analysis of products labeled  
316 “pork liver” showed an association with HEV infection in the mixed population (pooled OR =  
317 1.992; 95% CI [1.618 - 2.452]) (Table 2).

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

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

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

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

341

#### 342 **4. Discussion**

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

346 (Khuroo et al., 2016). The genotypes HEV-3 and -4 are zoonotic (with animal reservoir  
347 mostly) and circulate in humans and several animal species including domestic and wild  
348 swine, and to lesser extent red and roe deers (Anheyer-Behmenburg et al., 2017). HEV-3 and  
349 -4 are associated with sporadic outbreaks in Europe, Asia, and North America (Kamar et al.,  
350 2017). Then, exploring risk factors at a global scale, ignoring the genotype, due to the lack of  
351 available data can be seen as a limitation of the study. However, different genotypes can  
352 cohabit in the same countries, in particular for HEV-3, which seems to be widespread in pig  
353 populations worldwide (Khuroo et al., 2016). Even in industrialized countries, where HEV-1  
354 and -2 are not endemic, HEV-3 can be excreted in human stools (Fenaux et al., 2018) and  
355 then can be seen as a potential fecal-oral risk. We also have chosen to keep all publications  
356 using different detection methods for anti- HEV antibodies, regardless of sensitivity or  
357 specificity, as there were not so many studies and we opted for being as conservative as  
358 possible.

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

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

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

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

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

403 With regards to the role of food, pork, pig sausages, and game meat were identified as  
404 significant risk factors for HEV infection, in particular undercooked pig meat, or meat  
405 preparations containing pig liver (virus multiplication organ). As the liver is part of the offal,  
406 it is also expected to play a significant role in the risk of exposure to HEV. The presence of  
407 HEV contamination of fresh or ready-to-eat pork product or meat (e.g. pork pies, liver pate,  
408 liver sausages, pork sausages, salami) is demonstrated in several European countries with  
409 HEV prevalence of up 70% of the product tested (Boxman et al., 2019; Mykytczuk et al.,  
410 2017; Pavio et al., 2014; Szabo et al., 2015).

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

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

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

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



444 In general, symptomatic cases of hepatitis E could be better explored, in particular for  
445 populations at risk of developing severe or chronic forms (Aspinall et al., 2017). Sporadic  
446 cases should be better investigated with precise definitions of exposure specifying by example  
447 the mode of preparation (washing of vegetables, cooking of meat). Whenever possible,  
448 whatever the country of origin, common most potential risk factors should be studied. Finally,  
449 an harmonization of the case definition of HEV infection (clinical criteria, detection HEV  
450 IgG/IgM, HEV RNA) is still a challenge at a global scale and European level (Adlhoch et al.,  
451 2019; Bohm et al., 2020). It could allow better comparison between studies and a better  
452 understanding of the epidemiological situation.

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

458

## 459 **5. Conclusion**

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

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

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

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

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#### 486 **Appendices: Supplementary material**

487 Appendix 1: Complete bibliographic references.

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

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

491

#### 492 **Data statement**

#### 493 **Figures**

494 • Figure 1: Flow chart of literature search for case-control or cohort studies of human  
495 hepatitis E

496 • Figure 2: Forest plot of the association of hepatitis E infection with transplant organ  
497 recipient in the mixed population:

498 (in separate file) (**legend \* adjusted OR as described in Gonzales-Barron et al. (2019)**)

499 • Figure 3: Forest plot of the association of hepatitis E infection with other red meat (game  
500 meat) in the mixed population

501 (in separate file) (**legend \* adjusted OR as described in Gonzales-Barron et al. (2019)**)

502 • Figure 4: Forest plot of the association of hepatitis E infection with processed meat (pork  
503 sausages) in the mixed population

504 (in separate file) (**legend \* adjusted OR as described in Gonzales-Barron et al. (2019)**)

505 • Figure 5: Forest plot of the association of hepatitis E infection with dairy products in all  
506 populations

507 (in separate file) (**legend \* adjusted OR as described in Gonzales-Barron et al. (2019)**)

508

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

511

512 **Legend**

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

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521 • **Tables**

- 522 • Table 1. Results of the meta-analysis on the main risk factors  
523 • Table 2. Results of the meta-analysis on disaggregated risk factors  
524 • Table 3. Effect of handling on the pooled OR for food products

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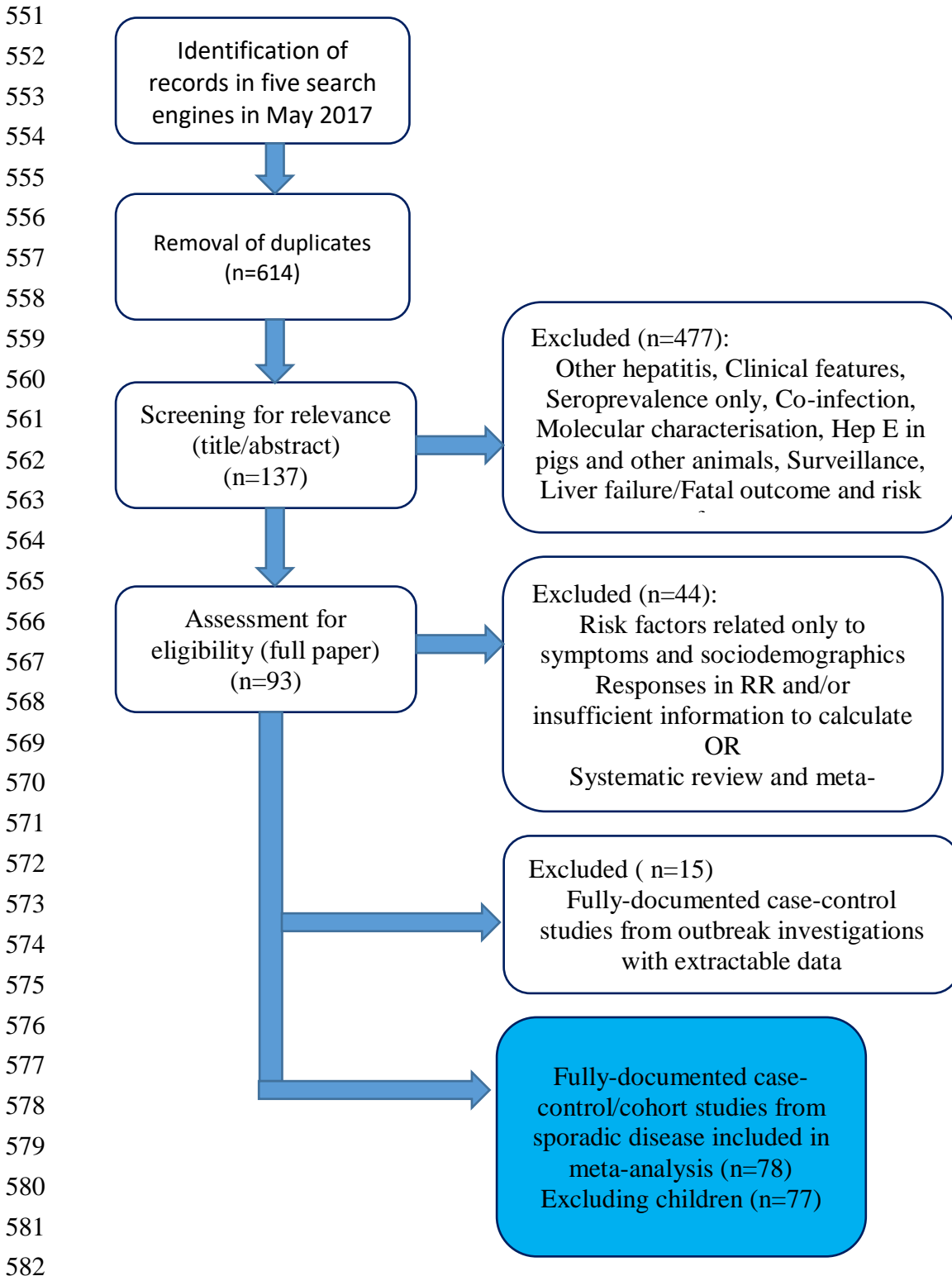
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550 **Figure 1: PRISMA Flow chart of included studies**



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

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***
<b>Travel</b>									
Mixed	Asia, South America, Europa	Infection	Abroad	1.043[1.012-1.075]	9/15	0.006	0.15	0	$\tau^2=0$ ; 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	$\tau^2=0.918$ Q(df = 4) = 14.855, p-val = 0.005 $s^2=1.626$ ; $I^2= 36.079$
<b>Host specific</b>									
Mixed	Africa, Asia, Europa, North and South America	infection	Blood Transfusion	2.005[1.468 - 2.738]	10/14	<.0001	0.156	0	$\tau^2=0.137$ 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	$\tau^2=0.204$ QE(df = 8) = 11.520, p-val = 0.174 $s^2=0.379$ ; $I^2= 34.908$
<b>Person to person by type of contact</b>									
Mixed	Africa, Asia, Europe, North america	infection	Occupational (healthcare worker)	1.570 [1.092 - 2.258]	2/3	0.0148	0.028	0	$\tau^2=0.144$ QE(df = 21) = 23.922, p-val = 0.297 $s^2= 0.152$ ; $I^2=487$
			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	$\tau^2=1.043$ 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			
<b>Personal Hygiene</b>									
All	Africa, Asia	infection		1.613 [1.324 - 1.965]	3/5	<.0001	0.750	0	$\tau^2=0$ ; Q(df = 4) = 9.164, p-val = 0.057 ; $s^2=0.081$ ; $I^2=0$
<b>Animals</b>									
Mixed	Africa,	Infection	Farm animals	2.071 [1.506 - 2.848]	10/17	<.0001	0.602	0	$\tau^2=0.456$

	Asia, Europe, North and South America		Occupational exposure	2.077 [1.795 - 2.403]	23/59	<.0001			QE(df = 96) = 192.480, p-val < .0001; s <sup>2</sup> =0.325 I <sup>2</sup> = 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	r <sup>2</sup> = 0.823 QE(df = 10) = 17.509, p-val = 0.064 s <sup>2</sup> = 0.533 I <sup>2</sup> = 60.69
Pregnant	Africa, Asia, South America	infection	Pets	2.061 [1.563 - 2.718]	3/6	<.0001	0.815	0	r <sup>2</sup> =0 ; QE(df = 15) = 16.039, p-val = 0.379; S <sup>2</sup> =0.231 I <sup>2</sup> =0
			Occupational exposure	2.549 [1.989 - 3.267]	3/11	<.0001			
<b>Environment</b>									
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	r <sup>2</sup> =0.775 QE(df = 100) = 337.2926, p-val < .0001 S <sup>2</sup> = 0.371 I <sup>2</sup> =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	r <sup>2</sup> =1.425 QE(df = 8) = 18.016, p-val = 0.021 S <sup>2</sup> = 0.845 I <sup>2</sup> =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	r <sup>2</sup> =0.111 QE(df = 8) = 10.740, p-val = 0.217 S <sup>2</sup> =0.149 I <sup>2</sup> =42.635
<b>Food</b>									
Mixed (y)	Africa, Asia, Europa, North and South America	infection	Dairy	1.711 [1.101 - 2.660]	3/3	0.0170	0.623	0	r <sup>2</sup> =0.406 QE(df = 73) = 282.648, p-val < .0001 S <sup>2</sup> =0.232 ; I <sup>2</sup> =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	r <sup>2</sup> =0.024 QE(df = 26) = 31.355, p-val = 0.215 ;

	<b>Europa</b>								S <sup>2</sup> =0.483 ; I <sup>2</sup> =4.69
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585 \*N/n Number of studies/number of OR; \*\* points removed by sensitivity analysis, all results are given after removing data concerned; \*\*\*Between-study variability ( $\tau^2$ ), test for  
586 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  
587 account

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

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 ****
<b>Meat (y)</b>	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	$\tau^2=0.319$ 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			
<b>Meat</b>	Europa	symptomatic cases	Other red meats	1.799 [1.186 - 2.728]	2/5	0.0057	0.557	0	$\tau^2=0.425$ 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			
<b>Seafood</b>	South America, Europa, South Africa	infection	Molluscs (shellfish)	1.396 [1.256 - 1.552]	2/7	<.0001	0.201	0	$\tau^2=0$ QE(df = 8) = 21.298, p-val = 0.006 $s^2=0.419$ ; $I^2=0$
<b>Dairy</b>	Asia, South America	infection	Dairy(raw milk)	1.932 [1.279 - 2.918]	4/5	0.0018	0.369	0	$\tau^2=0.057$ Q(df = 4) = 6.242, p-val = 0.182; $s^2=0.358$ $I^2=13.67$
<b>Produce</b>	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; $s^2=0.207$ $I^2=0$
<b>Pig products</b>	Africa, Asia, Europa, North and South America	infection	Liver presence	1.992 [1.618 - 2.452]	4/16	<.0001	2.10 <sup>-16</sup>	0	$\tau^2=0.2282$ 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			
<b>Poor handling : poor handwashing before meal</b>	China	infection	Poor handwashing	1.295 [1.028 - 1.632]	2/4	0.028	0.385	0	$\tau^2=0$ Q(df = 2) = 0.051, p-val = 0.975 $s^2=0.027$ ; $I^2=0$

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

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

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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 <sup>-06</sup>	τ <sup>2</sup> =0.118 QE(df = 28) = 193.568, p-val < .0001 S <sup>2</sup> =0.336 I <sup>2</sup> =25.91
	Base	1.973 [1.407- 2.767]	8/25	0.1028	–			

596 \*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<sup>2</sup>), test for  
597 residual heterogeneity (QE), variance of residuals (s<sup>2</sup>), intra-class correlation (I<sup>2</sup>).

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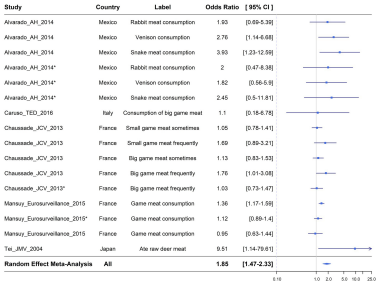
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