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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 (τ^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):**

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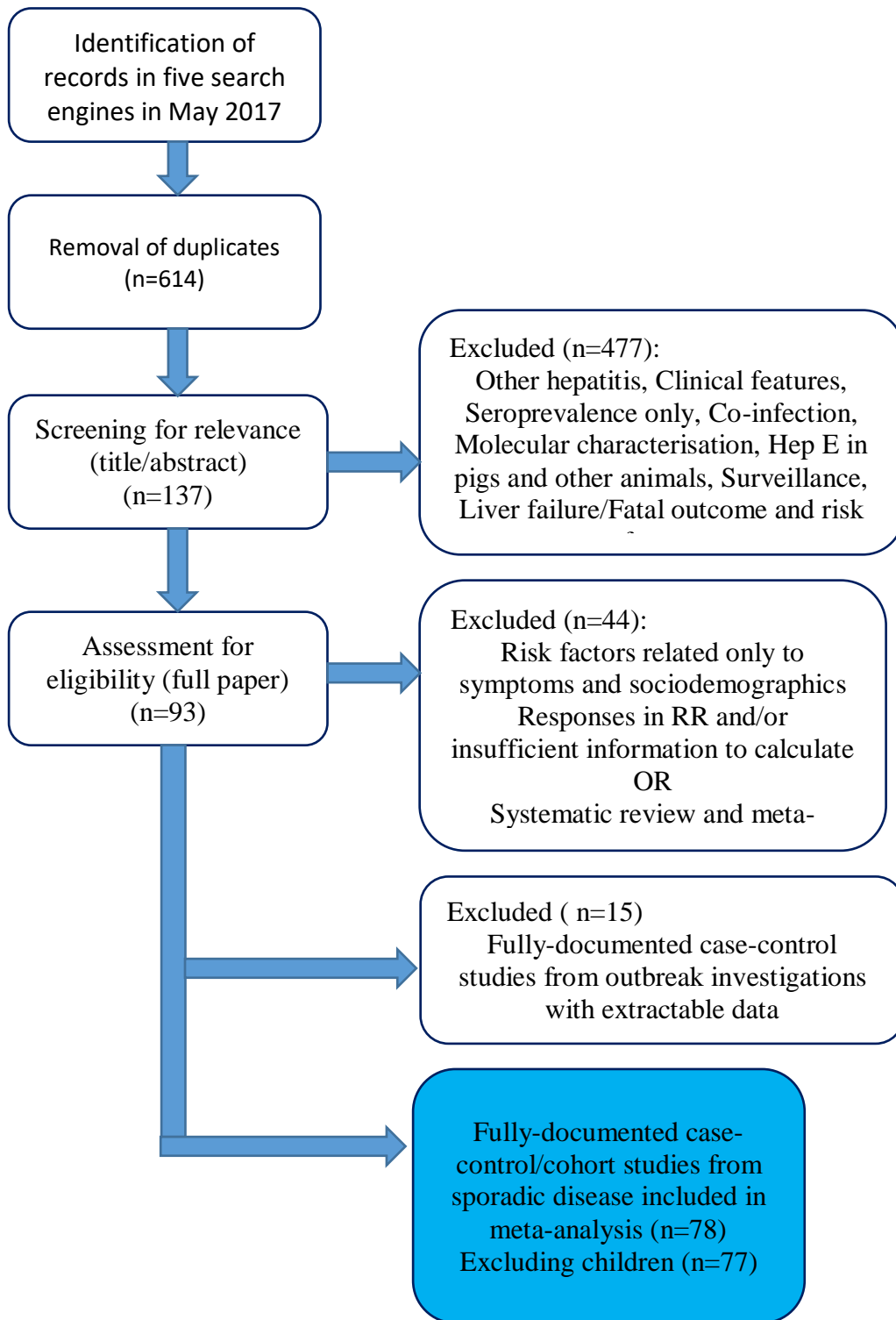
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550 **Figure 1: PRISMA Flow chart of included studies**

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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*** |
|--|---|-----------------|----------------------------------|------------------------|-------|------------------------|--------------------------|-------------------|---|
| Travel | | | | | | | | | |
| 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 $s^2=1.626$; $I^2= 36.079$ |
| 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 | $\tau^2=0.137$ 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 $s^2=0.379$; $I^2= 34.908$ |
| 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 | $\tau^2=0.144$ 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 | | | |
| Personal Hygiene | | | | | | | | | |
| 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 ; $s^2=0.081$; $I^2=0$ |
| Animals | | | | | | | | | |
| Mixed | Africa, | Infection | Farm animals | 2.071 [1.506 - 2.848] | 10/17 | <.0001 | 0.602 | 0 | $\tau^2=0.456$ |

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|--------------------|---|-------------------|--------------------------|------------------------|-------|--------|-------|---|---|
| | 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; 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 | r2= 0.823 QE(df = 10) = 17.509, p-val = 0.064 s2= 0.533 I2= 60.69 |
| Pregnant | Africa, Asia, South America | infection | Pets | 2.061 [1.563 - 2.718] | 3/6 | <.0001 | 0.815 | 0 | r2=0 ; QE(df = 15) = 16.039, p-val = 0.379; S2=0.231 I2=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 | r2=0.775 QE(df = 100) = 337.2926, p-val < .0001 S2= 0.371 I2=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 | r2=1.425 QE(df = 8) = 18.016, p-val = 0.021 S2= 0.845 I2=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 | r2=0.111 QE(df = 8) = 10.740, p-val = 0.217 S2=0.149 I2=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 | r2=0.406 QE(df = 73) = 282.648, p-val < .0001 S2=0.232 ; I2=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 | r2=0.024 QE(df = 26) = 31.355, p-val = 0.215 ; |

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|--|---------------|--|--|--|--|--|--|--|--|---|
| | Europa | | | | | | | | | S ² =0.483 ; I ² =4.69 |
|--|---------------|--|--|--|--|--|--|--|--|---|

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 (τ^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 **** |
|---|---|-------------------|-----------------------------|-----------------------|------|------------------------|--------------------------|-------------------|---|
| 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 | $\tau^2=0.319$ 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 | | | |
| Meat | 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 | | | |
| Seafood | 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 $s^2=0.419$; $I^2=0$ |
| Dairy | 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; $s^2=0.358$ $I^2=13.67$ |
| 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 | $\tau^2=0.2282$ 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 | | | |
| Poor handling : poor handwashing before meal | 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 (τ^2), test for
592 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
593 account

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- **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 ⁻⁰⁶ | $\tau^2=0.118$ 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 | – | | | |

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*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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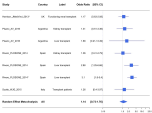
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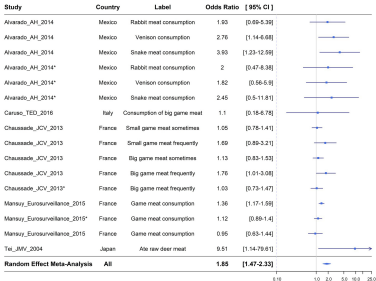
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