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Risk factors for sporadic cryptosporidiosis: A systematic review and meta-analysis

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► To cite this version:

Pauline Kooh, Anne Thébault, Vasco Cadavez, Ursula Gonzales-Barron, Isabelle Villena. Risk factors for sporadic cryptosporidiosis: A systematic review and meta-analysis. *Microbial Risk Analysis*, 2020, pp.100116. 10.1016/j.mran.2020.100116 . hal-03068259

HAL Id: hal-03068259

<https://hal.univ-reims.fr/hal-03068259v1>

Submitted on 9 May 2023

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12 **Abstract**

13 *Cryptosporidium* spp. is an important cause of gastrointestinal disease worldwide, responsible
14 for 69 million cases of illness in 2016. Information on the sources and transmission pathways
15 of human cryptosporidiosis results mainly from outbreak investigations.

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

23 From 1985 identified references, the quality assessment stage was passed by 57 – cohort and
24 case-control studies – focusing on sporadic cryptosporidiosis. The eligible studies were
25 conducted between 1983 and 2016 and provided 568 OR categorized for meta-analysis.

26 This meta-analysis identified travel, immunocompromising conditions, contact with infected
27 humans, waterborne transmission (contact with recreational waters, wastewater, and
28 consumption of untreated drinking water), contact with animals and food consumption as the
29 relevant risk factors for sporadic cryptosporidiosis. With regards to food exposures,
30 consumption of meat, dairy products (raw milk) and dishes consumed outside home were
31 found significantly associated with cryptosporidiosis. The consumption of poorly washed
32 fruits and vegetables significantly increases ORs. This meta-analysis reveals that some
33 potential sources of *Cryptosporidium* such as shellfish or vegetables are under-investigated.

34 Future case-control studies for sporadic cryptosporidiosis should include population at risk,
35 and investigate other potential sources in relation to the genotype and the subtype of
36 *Cryptosporidium* spp.

37 **Keywords:** *Research synthesis; case-control studies; cohort studies; meta-regression;*
38 *Cryptosporidium*

39

40 1. Introduction

41 *Cryptosporidium* spp. is a protozoan parasite that belongs to Apicomplexa phylum.
42 *Cryptosporidium* spp. is a well-known causative agent of gastrointestinal diseases and
43 commonly identified in humans and animals, including livestock and particularly cattle
44 (calves). The main symptom of human cryptosporidiosis is diarrhea that may be responsible
45 for weight loss and dehydration in immunocompetent, but immunocompromised patients are
46 at increased risk of developing a severe disease (Hunter and Nichols, 2002).

47 *Cryptosporidium* spp. are globally distributed, responsible for 69 million cases of illness, and
48 57,203 deaths in 2016 (Troeger et al., 2018). Kirk et al. (2015) estimated that
49 cryptosporidiosis resulted in 2,159,331 DALYs in 2010. A clinical and epidemiological study
50 involving 22,500 children from Africa and Asia revealed that *Cryptosporidium* spp. is one of
51 four pathogens responsible for most of moderate to severe diarrhea in infants and toddlers
52 (Kotloff et al., 2013). In 2016, *Cryptosporidiosis* was estimated to account for 10% of cases
53 of diarrhea mortality among children under 5 years old (Troeger et al., 2018).

54 There are numerous species and genotypes of *Cryptosporidium*, but human infection involves
55 mainly two species: *Cryptosporidium hominis*, whose main host is humans and
56 *Cryptosporidium parvum* which infects animal and ruminants. Transmission can occur
57 through the fecal-oral route, involving direct (person-to-person transmission or contact with
58 animals) and indirect (waterborne or foodborne) pathways.

59 Water is the principal vector of contamination of *Cryptosporidium* and, numerous waterborne
60 outbreaks involving both drinking water and recreational waters have been reported (Moreira
61 and Bondelind, 2017; Ryan et al., 2017). Over the past years, foodborne outbreaks of
62 cryptosporidiosis have been increasingly reported involving a diversity of food products
63 (Ryan et al., 2018). Outbreaks investigations provide useful information about sources and
64 transmission pathways of human cryptosporidiosis. Nevertheless, cryptosporidiosis cases are
65 underreported or underdiagnosed in most countries (ECDC, 2019; Haagsma et al., 2013).

66 Several epidemiological studies of sporadic cryptosporidiosis have been published. A
67 systematic review and a meta-analysis of case-control and cohort studies were performed to
68 determine the main risk factors associated with sporadic cryptosporidiosis. Characterization of
69 risk factors will contribute to identifying measures to reduce the burden of cryptosporidiosis.

70

71 2. Material and methods

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

74

75 **2.1 Systematic review**

76 The literature search was conducted in March 2017 using a combination of keywords related
77 to (1)“*Cryptosporidium*” “OR” “cryptosporidiosis”, (2) “case-control” “OR” “risk factor”
78 “OR” “cohort” (3) “infection” “OR” “disease”, joined by the logical connector “AND”.
79 Relevant studies were identified from five bibliographic search engines, Science Direct,
80 PubMed, Scielo, ISI Web of Science and Scopus. No restrictions were defined for the year of
81 the study or type of publication. The search was limited to the languages English, French,
82 Portuguese and Spanish.

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

92 Data from primary studies were then extracted using a standardized spreadsheet. Data
93 extracted included the relevant study characteristics (location, period, population, case
94 definition, design, sample size of the groups, type of model, etc.), the categorized risk factors,
95 the setting, the handling practices and the outcome of the study (ORs).

96 A data categorization scheme was established to hierarchically group the risk factors into
97 travel, host-specific factors and, pathways of exposure (i.e., person-to-person, animal,
98 environment, and food routes) (see the methodological paper of this issue). In addition to the
99 standard risk factors, the class “Hygiene” (e.g. “no handwashing after toilet”, “poor hygiene
100 habits”) was also used. Person-to-person transmission was stratified in three classes: contact
101 in the household, contact in the community and sexual transmission. The variable
102 “**Population**” was stratified into mixed (adults or undefined), children (under 16 years old)
103 and susceptible (HIV infection, AIDS, elderly population).

104

105 **2.2 Data synthesis**

106 The joint meta-analytical data was first described using basic statistics. Next, data was
107 partitioned into subsets of categories of risk factors. The meta-analytical models were then
108 fitted to each of the data partitions or subsets to estimate pooled OR related to travel, host-
109 specific factors and transmission pathways related to person-to-person contagion, animal
110 contact, environmental exposures, and food vehicles. The meta-analytical models were fitted
111 separately by population type. For some food classes, the effects of food preparation (e.g,
112 eating raw, undercooked) and setting (i.e., eating food prepared outside the home) on the
113 pooled OR were assessed by calculating the ratio of the mean OR when food is mishandled to
114 the base OR.

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

122 All meta-analytical models were essentially weighted random-effects linear regression
123 models. Once a meta-analysis model was fitted, influential diagnostics statistics were applied
124 to remove any influential observation originating from studies marked as having a potential
125 for bias. Publication bias was assessed by funnel plots and a statistical test investigating the
126 effect of the study sample size on the ORs (Tables 2, 3 and 4) (Gonzales-Barron et al., 2019).
127 Heterogeneity between studies was assessed by different indicators such as the between-study
128 variability (τ^2), the QE test investigating residual heterogeneity, the variance of residuals and
129 the intra-class correlation I^2 (Gonzales-Barron et al., 2019). Publication bias and remaining
130 heterogeneity were not further corrected for, but were taken into account for the interpretation
131 of the results.

132 All analyses were carried out in the R software (R Development Core Team, 2008)
133 implemented with the *metafor* package (Viechtbauer, 2010).

134 The meta-analyzed risk factors are presented in summary tables only when significant. Pooled
135 ORs were considered significant when the lower bound of the 95% confidence interval (CI)
136 was equal or greater than 1.0, except for breastfeeding where the upper bound of the
137 confidence interval had to be below 1 for it to be deemed as significant (protective effect).

138

139 **3. Results**

140 3.1 Descriptive statistics

141 From 1985 identified references, the quality assessment stage was passed by 57 primary
142 studies – cohort and case-control studies – focusing on sporadic cryptosporidiosis (Figure 1).
143 These published studies were conducted between 1983 and 2016. Table 1 and Appendix 1
144 compile the list of the primary studies along with their main features. The eligible studies
145 jointly provided 568 odds-ratios categorized for meta-analysis. Meta-analytical data were
146 obtained from primary studies conducted in 31 countries, although studies from only 5
147 countries generated ~70% of the ORs retrieved. These were: USA (9 studies -136 ORs), UK
148 (3 studies - 79 ORs), Australia (3 studies - 66 ORs), the Netherlands (2 studies - 66 ORs) and
149 Canada (2 studies - 47 ORs).

150 Primary studies investigated risk factors in different types of population, namely children (27
151 studies), mixed population (24 studies) and susceptible population, which included
152 immunocompromised individuals (8 studies) and elderly population (1 study). Separate meta-
153 analyses were then adjusted on the mixed population (382 ORs), children (117 ORs) and
154 susceptible (69 ORs). Most studies investigated illness caused by any *Cryptosporidium*
155 species (49) or by *C. parvum* without distinction between *C. parvum* and *C. hominis*. Few
156 studies investigated cases caused by *C. parvum* (3) or *C. hominis* (1). In all studies, the
157 symptomatic cases of cryptosporidiosis were laboratory-confirmed.

158 With regards to the risk factor classes, sporadic illness investigations focused more on
159 multiple pathways of exposure: environment (222 ORs), contact with animals (114 ORs),
160 food (80 ORs), person to person (78 ORs). Host-specific factors (47 ORs), personal hygiene
161 (4 ORs) and travel (23 ORs) were also investigated.

162 During methodological quality assessment, potential for selection bias status was assigned to
163 six case-control studies since, in those, the controls were not healthy individuals but people
164 affected by another enteric disease such as giardiasis (Firdu et al., 2014; Redlinger et al.,
165 2002), salmonellosis (Marder, 2012), amoebiasis (Ravel et al., 2013), campylobacteriosis
166 (Wilson et al., 2008), and one of nine other enteric infections (Pintar et al., 2009). As it is not
167 clear whether these controls shared routes of exposure with the case patients, the ORs
168 extracted from the aforementioned studies were marked as having potential for selection bias.
169 These case-control studies provided 84 potentially-biased ORs whose influence on the meta-
170 analyzed OR estimates was appraised by means of the Cook's distance.

171 Only 13 case-control studies employed a matched experimental design (Table 1). Bringing
172 together the matched and unmatched designs, 379 ORs (67% of the data) were not adjusted by

173 any confounder (crude ORs) (e.g. age, sex, other risk factors), while 189 ORs (33%) were
174 adjusted using either Mantel-Haenzel or logistic regressions.

175

176 3.2 Meta-analysis

177 The meta-analysed significant risk factors are presented in summary tables (Tables 2 and 3).
178 Non-significant results on the main risk factors are presented in Appendix 2. More detailed
179 descriptive results, in particular, funnel plots, forest plots, and OR of non-significant results,
180 are in a complete report available upon request.

181

182 **Meta-analysis for travel**

183 According to this meta-analysis, **foreign travel** is an important risk factor for acquiring
184 cryptosporidiosis. For residents of USA, UK, Switzerland, Netherlands, Australia and New
185 Zealand, traveling abroad increased their odds of acquiring cryptosporidiosis (pooled
186 OR=4.216; 95% CI [2.529 - 7.029]) (Table 2; Figure 2).

187

188 **Meta-analysis for host-specific risk factors**

189 The meta-analysis on **host-specific factors** showed that immunocompromising conditions
190 were associated with cryptosporidiosis for the mixed, children with pooled ORs ranging from
191 2.721 to 4.507. For the mixed and children population, immunocompromising conditions
192 included HIV infection, other immune system illnesses, the use of immunosuppressive
193 medication, etc. Other medical conditions, including chronic disease and HBV infection, were
194 also found to be associated with cryptosporidiosis in the mixed population (pooled OR=2.392;
195 95% CI [1.588 - 3.604]).

196

197 **Meta-analysis for person to person transmission factors**

198 **Person-to-person** transmission was a significant risk factor of acquiring cryptosporidiosis for
199 all the populations (pooled OR ranging from 1.903 to 3.786; Table 2; Figure 3). The same
200 data set related to person-to-person transmission was stratified in three classes according to
201 the type or the location of the contact. Significant associations were found for contact in
202 household (pooled OR=2.191; 95% CI [1.771-2.711]), contact in the community (pooled
203 OR=3.339; 95% CI [2.623- 4.243]) and sexual transmission (pooled OR =2.350; 95% CI
204 [1.439- 3.837]).

205 Poor personal hygiene (e.g “no handwashing after toilet”, “ poor hygiene habits”) could be a
206 risk factor for cryptosporidiosis (pooled OR=1.736; 95% CI [1.286- 2.343]).

207

208 **Meta-analysis for animal contact**

209 Contact with animals was associated with an increased risk of cryptosporidiosis. Significant
210 associations were found for farm animals in the mixed population (pooled OR=2.167; 95% CI
211 [1.703-2.758]; figure 4) and children (pooled OR=1.968; 95% CI [1.284- 3.018]) and pets in
212 children (pooled OR= 1.694; 95% CI [1.297 - 2.212]).

213

214 **Meta-analysis for environmental factors**

215 In both the mixed and children populations, **the environmental pathways** under study were
216 significantly associated with cryptosporidiosis: recreational water (pooled OR = 1.968; 95%
217 CI [1.475- 2.625] for the mixed population (figure 5); pooled OR=4.114; 95% CI [1.579-
218 10.715] for children); farm environment (pooled OR=1.794; 95% CI [1.444- 2.230] for the
219 mixed population and pooled OR=1.802; 95% CI [1.194- 2.719] for children), attendance to
220 daycare (pooled OR=1.539; 95% CI [1.429- 1.659] for the mixed population and pooled
221 OR=1.742; 95% CI [1.031- 2.945] for children), untreated drinking water (pooled OR=1.358;
222 95% CI [1.249- 1.475] for the mixed population and pooled OR=1.367; 95% CI [1.092-
223 1.712] for children) and wastewater (only in the mixed population: pooled OR=1.697; 95% CI
224 [1.127- 2.555]). Data from Oceania (2 ORs) were removed from the children population. This
225 exclusion only affects the significance of the OR related to attendance to daycare.

226

227 **Meta-analysis for food consumption**

228 The meta-analysis on **food consumption** pathways revealed significant associations with mea
229 t (pooled OR=1.934; 95% CI [1.236 - 3.024]; Figure 6) and dairy (pooled OR=1.533; 95% CI
230 [1.009- 2.329]; Figure 7) for the mixed population, and composite foods (pooled OR=1.532; 9
231 5% CI [1.072- 2.189]) for children. Within the food vehicles, associations with cryptosporidio
232 sis were observed for: barbecue foods (pooled OR=2.005; 95% CI [1.624- 2.476]), meat of no
233 n-specified origin (“Others”; pooled OR=1.991; 95% CI [1.288- 3.080]), dishes prepared outs
234 ide the home (pooled OR=1.717; 95% CI [1.220-2.416]) and milk (comprising essentially raw
235 milk in this category) (pooled OR=1.509; 95% CI [1.071- 2.125]). If we restrict the analysis t
236 o raw milk, combining ORs in population mixed and children with 7 OR, the raw milk is still
237 significant at a pooled OR of 1.670 (95% CI [1.035 - 2.695]).

238 Food categories that on meta-analysis had a non-significant association with cryptosporidiosis
239 were produce (comprising raw or fresh vegetables (10 ORs) and unwashed fruits (1 OR) and
240 beverage. The only food data partitions comprising sufficient data that could support the

241 assessment of the effect of handling were those of produce and dairy (Table 4). It was found
242 that people who ate unwashed fruits and vegetables, had their odds of infection significantly
243 increased by a factor of 1.572. Hence, the practice of not washing vegetables before
244 consumption represents on its own a risk factor for cryptosporidiosis.

245
246 For most of the meta-analytical models reported in Tables 2, 3 and 4, the statistical tests
247 indicated the absence of potential significant publication bias at 5% significance. Exception is
248 observed for partitions related to travel, host-specific in the mixed population, person-to-
249 person transmission, animal contact in children, and composite foods. However, for these five
250 partitions, the spread of data points within the funnel plot does not hint any evidence of a
251 strong publication bias problem (Figure 8). Moreover, the intra-class correlation I^2 indicates
252 low (<25%) to moderate (<50%) heterogeneity (Tables 2, 3 and 4). Remaining between-study
253 heterogeneity (significant p-values below 0.05 for Q or QE) was observed for most of the data
254 partitions.

255

256 **4. Discussion**

257 This meta-analysis identified foreign travel (pooled OR = 4,216), immunocompromising
258 conditions (pooled OR ranging from 2.721 to 4.507), person-to-person transmission (pooled
259 OR ranging from 1.903 to 3.786), environmental pathways (pooled OR ranging from 1.358 to
260 1.968 in the mixed population), animal contact (pooled OR ranging from 1.694 to 2.167), and
261 food consumption (pooled OR ranging from 1.533 to 1.934) as risk factors of
262 cryptosporidiosis. For person-to-person, environmental and animal contact pathways, the
263 same risk factors were identified in the mixed population and children. Food exposures were
264 less investigated in children compared to the mixed population. Fewer studies investigated the
265 susceptible population (immunocompromised individuals and elderly) and the pooled OR
266 related to animal, environmental and food exposures were non-significant.

267 Overall, these meta-analytical results are in line with the epidemiology of *Cryptosporidium*
268 (EFSA BIOHAZ Panel, 2018). Few studies investigated cases caused specifically by *C.*
269 *parvum* (11) or *C. hominis* (1). Although the epidemiology of both *C. parvum* and *C. hominis*
270 could involve indirect transmission routes (water, foods), there are some specificities. *C.*
271 *hominis*, which infects mainly humans, is transmitted through the fecal-oral pathway and,
272 hence, person-to-person transmission plays a major role in the transmission. On the other
273 hand, the main reservoir of *C. parvum* is ruminants, and, as such, zoonotic transmission could
274 occur through animal contact.

275 Foreign travel is a known risk factor of cryptosporidiosis (Hagmann et al., 2014). However,
276 due to the lack of information on the countries of travel, it was not possible to identify regions
277 at particular risk (Figure 2).

278 The host susceptibility risk factors (in particular immunosuppression linked to AIDS) have
279 been established in previous studies (Hunter and Nichols, 2002).

280 Person-to-person transmission is a known risk factor of cryptosporidiosis. In this meta-
281 analysis, higher pooled OR were obtained for children compared to adults (Figure 3). This
282 might be related to higher exposure due to the lack of hygiene, greater susceptibility, and less
283 immunity. Regarding the person-to-person pathways, contact with an ill person at home
284 (contact in the household), contact in institutions (child /daycare, schools, etc.) and contact
285 during sexual activity were significantly associated with cryptosporidiosis. The lack of
286 personal hygiene (lack of handwashing), identified as a risk factor, can lead to person-to-
287 person transmission.

288 The meta-analysis confirms the major role of water in the transmission of cryptosporidiosis.
289 Exposure to recreational waters, wastewater (lack of sanitation) and the consumption of
290 untreated drinking water significantly increase the risk of cryptosporidiosis. Many outbreaks
291 of cryptosporidiosis have been associated with the consumption of drinking water (Dalle et
292 al., 2003; Eisenberg et al., 2005; Moreira and Bondelind, 2017), and the ingestion of bathing
293 water in swimming pools or leisure facilities (first cause of outbreak in the United States and
294 the United Kingdom) (Gharpure et al., 2019; Ryan et al., 2017). *Cryptosporidium* is often
295 present in aquatic environments from fecal sources and can be found in a large range of
296 concentrations (1 to several hundred oocysts /L) (Nasser, 2015). *Cryptosporidium* oocysts can
297 bypass common water treatments during occasional failure of the filtration (Lonigro et al.,
298 2006), and are highly resistant to disinfection procedures like chlorination (Erickson and
299 Ortega, 2006).

300 Contact with farm animals and farm attendance are identified as risk factors, which is
301 supported by described outbreaks. In the US, contact with infected cattle is the second cause
302 of cryptosporidiosis outbreaks, responsible for 15 % outbreaks for the period 2009–2017
303 (Gharpure et al., 2019). Several outbreaks have also been reported in Europe (Lange et al.,
304 2014; Utsi et al., 2016; Alsmark et al., 2018). Possession of a pet is only significant in
305 children. The role of pets (dogs and cats) in the transmission of cryptosporidiosis is
306 nevertheless not established in the literature (de Lucio et al., 2017; Lucio-Forster et al., 2010).
307 Among the food-related risk factors, meat was found as a risk factor, which was less
308 expected. Only one outbreak linked to the consumption of raw meat has been reported

309 (Yoshida et al., 2007). Within the meat category, meat of unspecified origin (“others”) is
310 found significant but beef is not a significant risk factor (with only 2 ORs from 2
311 publications). None of the ORs are significant in each study alone (3 studies from Canada and
312 the United Kingdom), but this factor appears significant by the combination of ORs in the
313 meta-analysis (8 ORs) (Figure 6). This association could reflect fecal contamination of beef
314 carcasses during the slaughter process, as observed with other enteric pathogens (e.g
315 *Salmonella*, or Shigatoxin-producing *E. coli*). Data on the contamination of meat by
316 *Cryptosporidium* are however limited. The prevalence of *Cryptosporidium* spp. in feces and
317 meat samples were investigated by Moriarty et al. (2005): *Cryptosporidium* spp. were isolated
318 from fecal samples (7.3%) but not from carcasses samples. To confirm the plausibility of this
319 association, meat should be explored in specific surveys and investigations of outbreaks and
320 sporadic cases of cryptosporidiosis.

321 The consumption of dishes prepared outside home and BBQ foods were also found
322 significantly associated with *Cryptosporidium*. This can be linked to poor hygiene practices
323 (e.g. contamination by an infected handler during the preparation of these products).

324 Unpasteurized milk and dairy products emerged as a risk factor in the meta-analysis. This
325 result is consistent with published outbreaks (Harper et al., 2002; Loury et al., 2019;
326 Rosenthal et al., 2015). *C. parvum* was listed among microbiological hazards potentially
327 transmissible through milk and present in the EU milk-producing animal population (EFSA
328 BIOHAZ Panel, 2015).

329 However, identification and isolation methods of *Cryptosporidium* are not standardized in
330 dairy products and these products are rarely found contaminated during outbreaks
331 investigations (Loury et al., 2019).

332 Produce (washed and not washed in the same category) was not identified as a risk factor, but
333 the consumption of poorly washed fruits and vegetables significantly increases ORs. Fresh
334 produce is the main vehicle of foodborne cryptosporidiosis outbreaks (Aberg et al., 2015;
335 England, 2017; Ethelberg et al., 2009; McKerr et al., 2015). Nevertheless, several case-control
336 studies found that the consumption of vegetables is a protective factor against
337 cryptosporidiosis (Goh et al., 2004; Nic Lochlainn et al., 2019; Roy et al., 2004). Roy et al.
338 (2004) explained this effect by the acquisition of protective immunity following repeated
339 exposure to low doses of oocysts on contaminated vegetables as observed in waterborne
340 outbreaks (Hunter, 2000). Produce (vegetables) should be better studied by taking into
341 account the type of vegetable (more exposed or not to irrigation of contaminated waters, such
342 as lettuce) and the type of preparation (washed or not).

343 Beverages (including cider/bottled water/ice) were not identified as a risk factor in the meta-
344 analysis. Cider was investigated in one study and was found non-significant (Roy et al.,
345 2004). Apple cider/juice has been responsible for two outbreaks in the USA (Blackburn et al.,
346 2006; Millard et al., 1994) and recently in Norway (Robertson et al., 2019).
347 Recommendations have been made on grazing animals in orchards and washing fruits.
348 Shellfish are considered as potential vehicles of *Cryptosporidium* but were not investigated in
349 the included studies. Although shellfish have been found contaminated with *Cryptosporidium*
350 oocysts in several surveys (Giangaspero et al., 2014; Gomez-Bautista et al., 2000; Gomez-
351 Couso et al., 2006; Robertson and Gjerde, 2008), no outbreaks have been reported to date.
352 The role of shellfish in *Cryptosporidium* infections should be investigated in future case-
353 control studies.

354 Our results are comparable to the meta-analysis conducted by Bouzid et al. (2018) who
355 reported diarrhea in the household, animal contact, lack of toilet facility and overcrowded
356 conditions as risk factors for cryptosporidiosis in low and middle-income countries based on
357 11 studies. Food exposures were not investigated in the included studies and poor drinking
358 water was not found significant. These differences may be related to the analysis strategy of
359 Bouzid et al. (2018) as only studies reporting at least four relevant risk factors were included
360 in their meta-analysis.

361

362 **5. Conclusion**

363 In summary, this meta-analysis confirmed known risk factors of cryptosporidiosis linked to
364 anthroponotic and zoonotic pathways of transmission: contact with infected humans,
365 waterborne transmission, contact with animals and food consumption. Except for meat, the
366 identified vehicles are all consistent with described outbreaks.

367 Future case-control studies of sporadic infections should better explore the role of dairy,
368 shellfish, meat, and vegetables, including washing/cooking and hygiene practices. These risk
369 factors should also be included in questionnaires used for outbreak investigations. Moreover,
370 the development of sensitive methods (based on molecular assays) for detection and isolation
371 of *Cryptosporidium* oocysts in these different matrices is necessary to link cases to food items
372 (Rousseau et al., 2018). Susceptible populations, such as children, elderly or
373 immunosuppressed people could be better addressed, due to the severity of cases in those
374 populations. The immunity should be taken into account to reduce misclassification in case-
375 control studies (Hunter, 2000). It may be interesting to consider serology, in addition to
376 criteria related to symptoms, and parasite excretion. In order to improve the detection of

377 cases, biological diagnosis of persistent diarrhea should specify *Cryptosporidium* research
378 (Loury et al, 2019).

379 Lastly, subtyping of human isolates can provide insights into the epidemiology of
380 cryptosporidiosis, allowing the identification of risk factors specific to species or subtypes.

381

382

383 **Appendices: Supplementary material**

384 Appendix 1: References of the 57 primary studies

385 Appendix 2: Non-significant results on the main risk factors

386

387 **Data statement**

388

389 **Acknowledgments**

390 The authors would like to thank ANSES staff and the members of the ANSES Working
391 Group on Source Attribution of Foodborne Diseases: Moez Sanaa, Laurence Watier, Jean
392 Christophe Augustin, Frédéric Carlin, Julie David, Philippe Fravallo, Laurent Guillier,
393 Nathalie Jourdan-Da Silva, Alexandre Leclercq, Lapo Mughini-Gras, Nicole Pavio.

394 Ursula Gonzales-Barron and Vasco Cadavez are grateful to the Foundation for Food Science
395 and Technology (FCT, Portugal) and FEDER under Programme PT2020 for financial support
396 to CIMO (UID/AGR/00690/2019).

397

398

399 **Figures**

- 400 • Figure 1: Flow chart of literature search for case-control or cohort studies of human
401 cryptosporidiosis
- 402 • Figure 2: Forest plot of the association of cryptosporidiosis with travel abroad
- 403 • Figure 3: Forest plot of the association of cryptosporidiosis with person-to-person
404 transmission in children
- 405 • Figure 4: Forest plot of the association of cryptosporidiosis with contact with farm
406 animals in the mixed population
- 407 • Figure 5: Forest plot of the association of cryptosporidiosis with contact with recreational
408 waters in the mixed population

409 • Figure 6: Forest plot of the association of cryptosporidiosis with meat consumption in the
410 mixed population

411 • Figure 7: Forest plot of the association of cryptosporidiosis with dairy consumption in the
412 mixed population

413 • Figure 8: Funnel plots of studies investigating categorized risk factors (travel, host-
414 specific, environment, animal contact, person to person and food)

415 ***Tables***

416 • Table 1. Characteristics of primary studies investigating risk factors for acquiring sporadic
417 cryptosporidiosis included in the meta-analysis

418 • Table 2. Results of the meta-analysis on main risk factors

419 • Table 3. Results of the meta-analysis on disaggregated risk factors

420 • Table 4. Effect of handling on the pooled OR for produce

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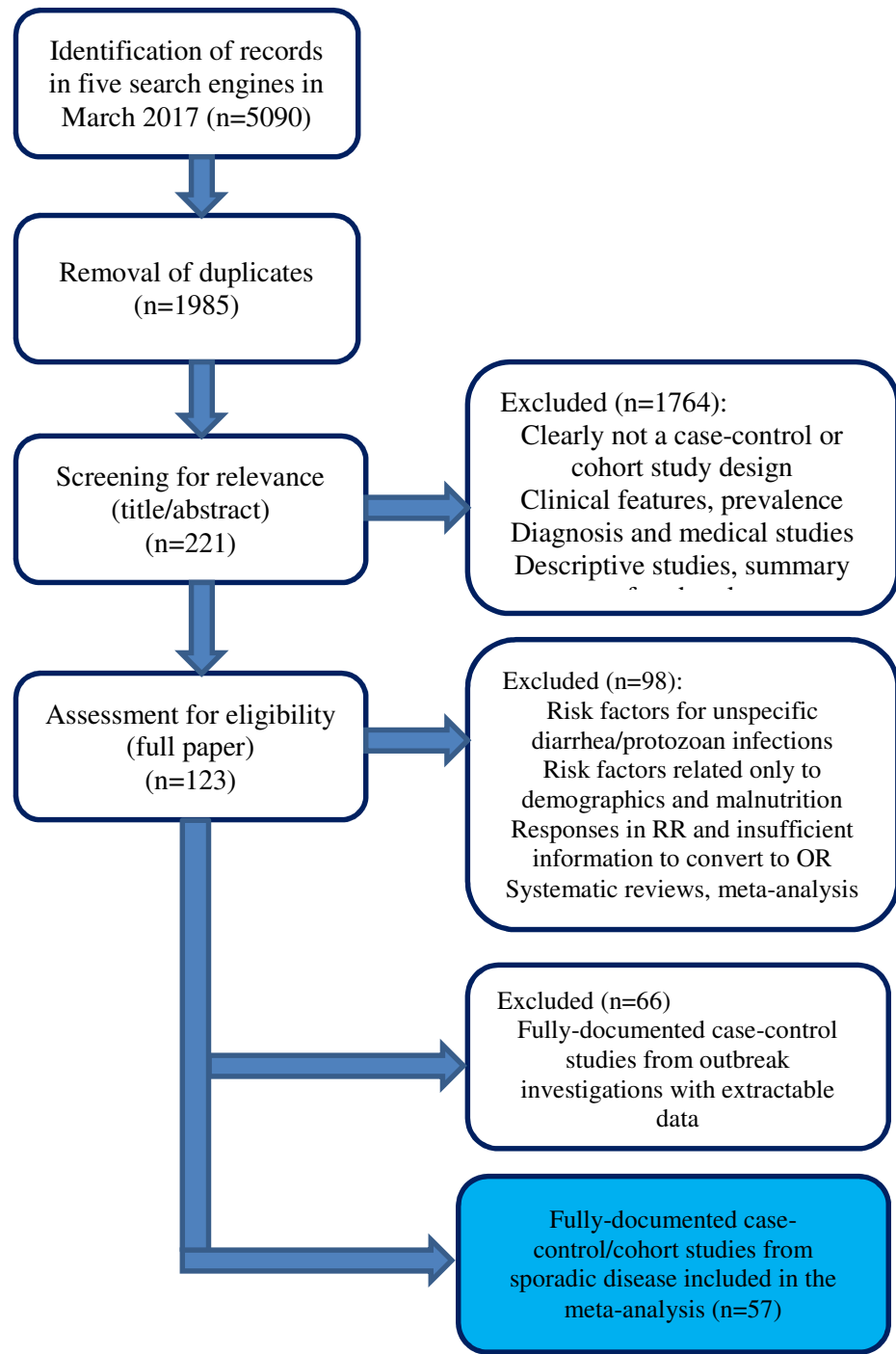


Figure 1. Flow chart of literature search for case-control or cohort studies of human cryptosporidiosis

669

670 **Figure 2. Forest plot of the association of cryptosporidiosis with travel abroad**
671 **transmission in all populations (* adjusted OR as described in Gonzales-Barron et al.**
672 **(2019) n=14**

673

674 **Figure 3. Forest plot of the association of cryptosporidiosis with person-to-person**
675 **transmission in children (* adjusted OR as described in Gonzales-Barron et al. (2019)**
676 **n=7**

677

678 **Figure 4. Forest plot of the association of cryptosporidiosis with contact with farm**
679 **animals in the mixed population (* adjusted OR as described in Gonzales-Barron et al.**
680 **(2019) n=41**

681

682 **Figure 5. Forest plot of the association of cryptosporidiosis with contact with**
683 **recreational waters in the mixed population (* adjusted OR as described in Gonzales-**
684 **Barron et al. (2019) n=65**

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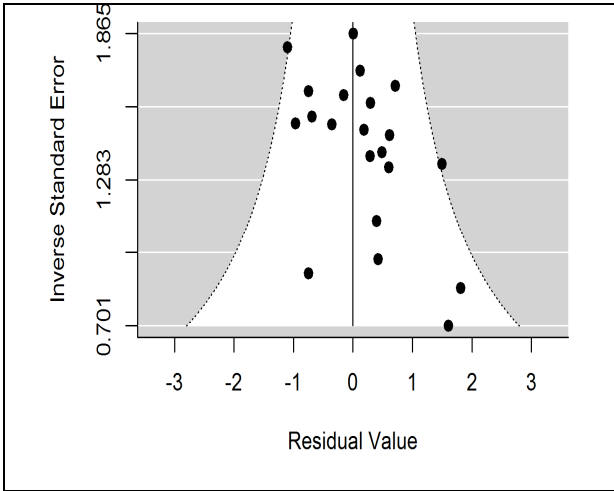
686 **Figure 6. Forest plot of the association of cryptosporidiosis with meat consumption in**
687 **the mixed population (* adjusted OR as described in Gonzales-Barron et al. (2019) n=9**

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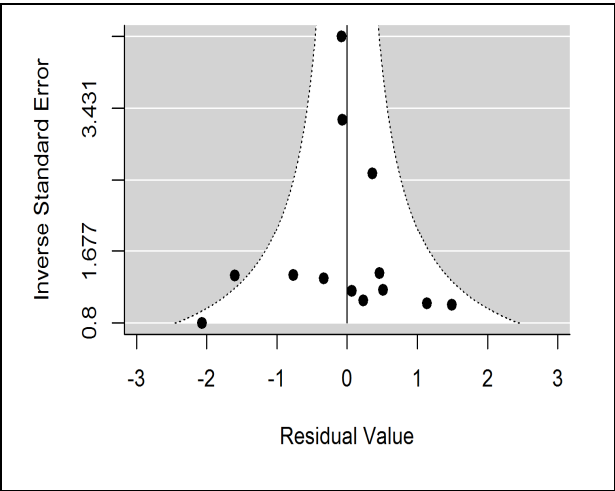
689 **Figure 7. Forest plot of the association of cryptosporidiosis with dairy consumption in**
690 **the mixed population (* adjusted OR as described in Gonzales-Barron et al. (2019) n=10**

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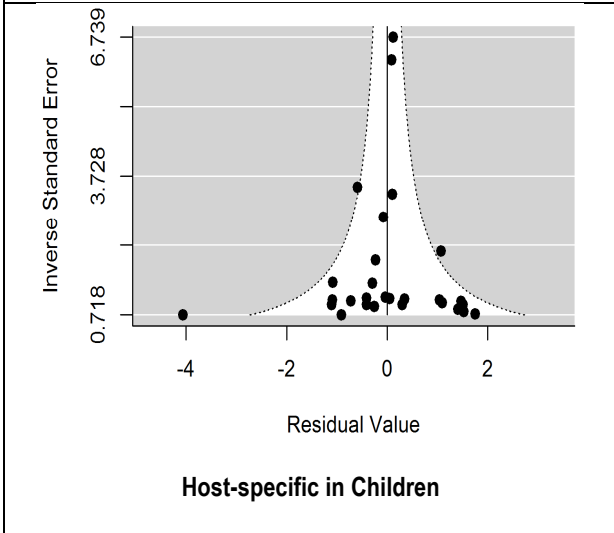
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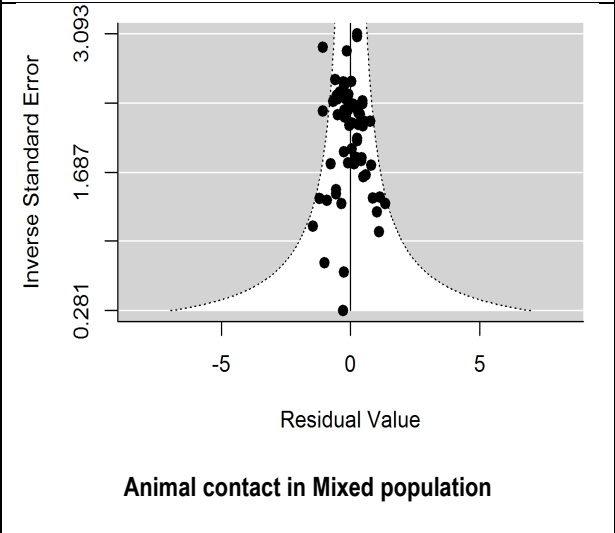
Travel in Mixed population



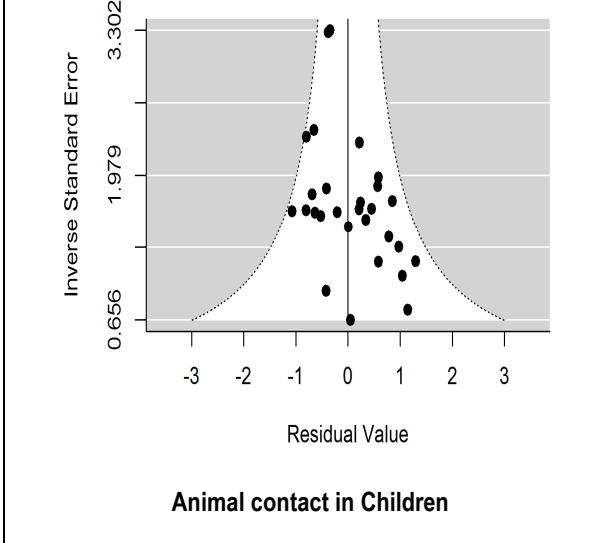
Host-specific in Mixed population



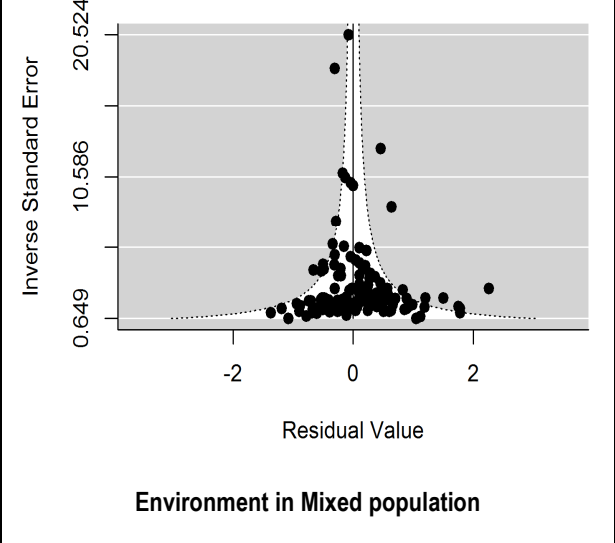
Host-specific in Children



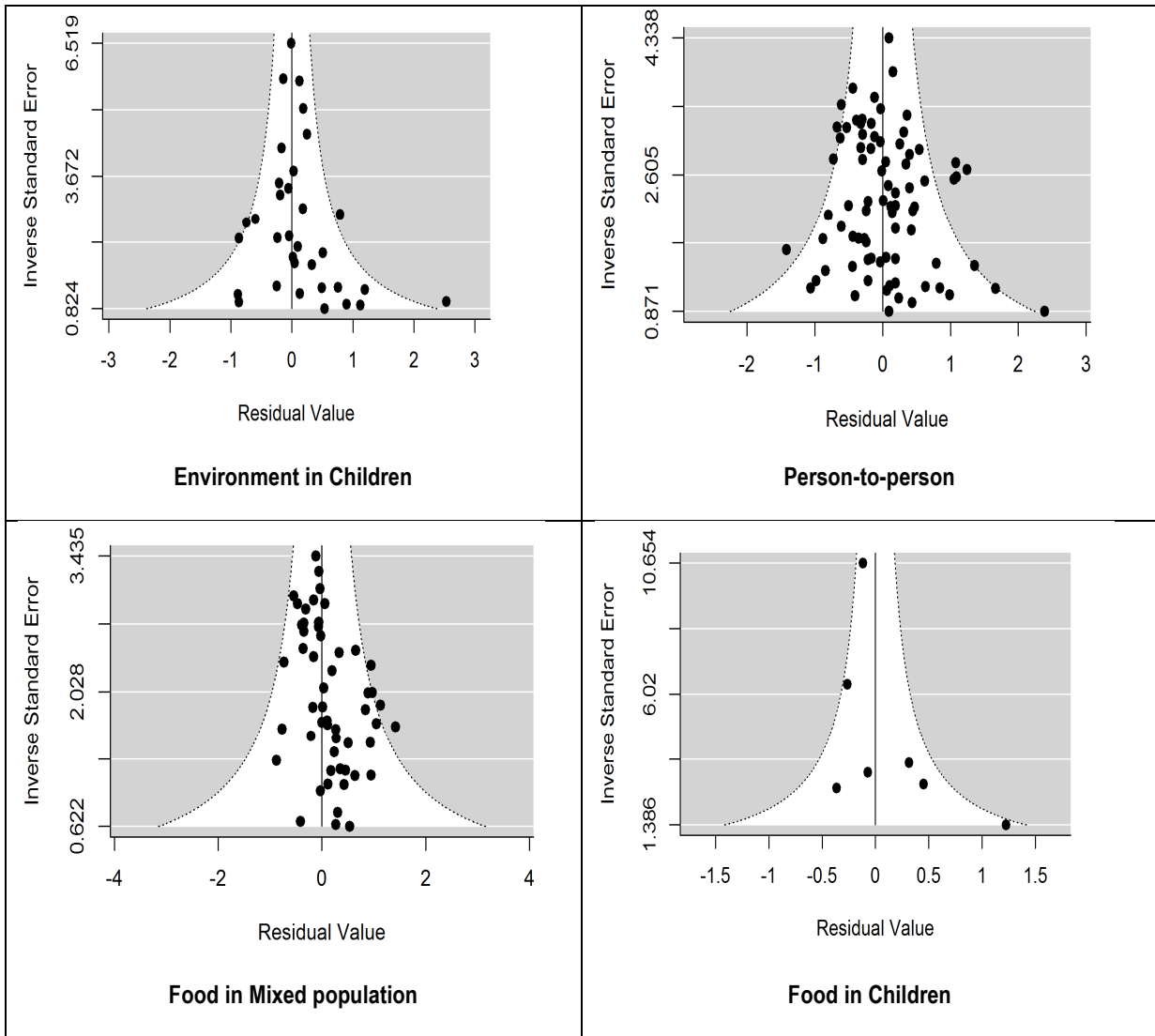
Animal contact in Mixed population



Animal contact in Children



Environment in Mixed population



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694 **Figure 8: Funnel plots of studies investigating categorized risk factors (travel, host-**
 695 **specific, environment, animal contact, person-to-person and food)**

696

Table 1. Characteristics of primary studies investigating risk factors for acquiring sporadic cryptosporidiosis included in the meta-analysis

StudyID	Country	Study period	Population	Design	Analysis & model	# ill/ non-ill	Quality
Abdel-Messih et al., 2000	Egypt	May 2000 - May 2002	Children	Unmatched	Uni -Chi Uni-UL	90 ill 791 non-ill	Good
Al-Dabbagh et al., 2010	Iraq	June 2003– Oct 2003	Children	Matched	Uni –Chi Multi-UL	100 ill 100 non-ill	Good
Al-Shibani et al., 2009	Egypt	2009	Mixed	Unmatched	Uni- Chi	70 ill 222 non-ill	Good
Aragón et al., 2003	USA	May 1996- Sep 1998	Susceptible	Matched	Uni –CL Multi-CL	49 ill 99 non-ill	Good
Bhattacharya et al., 1997	Bangladesh	1991-1994	Children	Unmatched	Uni –Chi Multi-UL	68 ill 204 non-ill	Good
Bouratbine et al., 1998	Tunisia	1997	Children	Unmatched	Uni –Chi	12 ill 120 non-ill	Good
Chacín-Bonilla et al., 2008	Venezuela	2017	Mixed	Unmatched	Multi-UL	67 ill 448 non-ill	Good
Chen et al., 2017	China	2011-2012	Children	Unmatched	Uni –Chi	40 ill 531 non-ill	Good
Cohen et al., 2008	USA	1992-2002	Children Adult Susceptible	Unmatched	Uni-UL Multi-UL	Not stated	Good
Cruz et al., 1988	Guatemala	July 1985- June 1986	Children	Unmatched	Uni –Chi	19 ill 110 non-ill	Good
Egger et al., 1990	Switzerland	June-Sep 1988	Children	Matched	Uni- Chi	19 ill 38 non-ill	Good
El-Shabrawi et al., 2015	Egypt	Sep 2007- Sep 2009	Children	Matched	Uni- Chi	14 ill 236 non-ill	Good
Firdu et al., 2014	Ethiopia	Feb-Aug 2011	Children	Unmatched	Uni –Chi	11 ill 18 non-ill	Poor
Fournet et al., 2013	Netherlands	Aug 2012	Mixed	Unmatched	Uni-Chi Multi-UL	82 ill 125 non-ill	Good
Gallaher et al., 1989	Mexico	July-Oct 1986	Mixed	Matched	Uni-MH	24 ill 46 non-ill	Good
Giroto et al., 2013	Brazil	Dec 2009- Oct 2010	Susceptible	Unmatched	Uni –Chi	3 ill 290 non-ill	Good
Glaser et al., 1998	USA	Apr 1992- Nov 1994	Susceptible	Unmatched	Uni –Chi	48 ill 99 non-ill	Good
Goh et al., 2004	UK	Jan 1998-Feb 2000	Mixed	Unmatched	Uni-Chi Multi-UL	152 ill 466 non-ill	Good
Hellard et al., 2003	Australia	Oct 1998- Aug 2000	Mixed	Unmatched	Uni –Chi	10 ill 24 non-ill	Good
Helmy et al., 2015	Egypt	Apr-June 2011	Children	Unmatched	Uni –Chi	81 ill 84 non-ill	Good
Hunter et al., 2004	UK	Feb 2001- May 2002	Mixed	Unmatched	Uni-Chi Multi-UL	427 ill 400 non-ill 261 ill 351 non-ill	Good
Izadi et al., 2014	Iran	Sep 2009- Mar 2010	Mixed	Unmatched	Uni-Chi Multi-UL	28 ill 394 non-ill	Good
Izadi et al., 2012	Iran	Nov 2008- Mar 2009	Susceptible	Unmatched	Uni-Chi Multi-UL	11 ill 172 non-ill	Good
Khalakdina et al., 2003	USA	July 1999- July 2001	Mixed	Matched	Uni-CL Multi-CL	26 ill 62 non-ill	Good
Khan et al., 2004	Bangladesh	May 2001- Aug 2002	Children	Unmatched	Uni –Chi	46 ill 46 non-ill	Good

Kutima et al., 2015	Kenya	Jan 2011- June 2013	Children	Unmatched	Uni –Chi	36 ill 676 non-ill	Good
Lake et al., 2007	UK	2000-2004	Mixed	Matched	Multi –CL	3368 ill 3368non-ill	Good
Mahdi and Ali, 2002	Iraq	2002	Mixed	Unmatched	Uni –Chi	5 ill 230 non-ill	Good
Manabe et al., 1998	USA	July 1989- 1997	Susceptible	Unmatched	Uni –Chi	68 ill 129 non-ill	Good
Marder, 2012	USA	2003-2010	Mixed	Unmatched	Uni –UL	6534 ill 30890 non-ill	Poor
Mbae et al., 2013	Kenya	Jan 2010- Dec 2011	Children	Unmatched	Uni-Chi Multi-UL	187 ill 1925 non-ill	Good
Mitra et al., 2016	India	2016	Mixed	Unmatched	Uni –Chi	59 ill 233 non-ill	Good
Mølbaek et al., 1994	Guinea- Bissau	1992	Children	Matched	Multi-CL	125 ill 125 non-ill	Good
Mooji et al., 2015	Netherlands	2013-2015	Mixed	Unmatched	Uni-UL Multi-UL	312 ill 587 non-ill	Good
Moore et al., 2016	Cambodia	Apr-June 2012	Children	Unmatched	Uni-Chi Multi-UL	38 ill 460 non-ill	Good
Morse et al., 2008	Malawi	Jan 2001- Dec 2002	Children	Unmatched	Uni-Chi Multi-UL	24 ill 72 non-ill	Good
Nassar et al., 2017	Nigeria	July-Dec 2014	Children	Unmatched	Uni-Chi	88 ill 100 non-ill	Good
Nchito et al., 1998	Zambia	Nov 1995- Mar 1996	Children	Unmatched	Uni-Chi Uni-MH	37 ill 179 non-ill	Good
Ng et al., 2012	Australia	July-Aug 2010	Mixed	Unmatched	Uni-Chi	15 ill 48 non-ill	Good
Nimri and Hijazi, 1994	Jordan	July 1992- Sep 1993	Children	Matched	Uni-Chi	18 ill 18 non-ill	Good
Osman et al., 2016	Lebanon	Jan 2013	Children	Unmatched	Uni-UL	26 ill 223 non-ill	Good
Pereira et al., 2002	Brazil	Aug 1998- May 1999	Children	Unmatched	Uni-UL	64 ill 380 non-ill	Good
Pintar et al., 2009	Canada	Apr 2005- Dec 2007	Mixed	Unmatched	Uni-Chi Multi-UL	36 ill 801 non-ill	Poor
Ravel et al., 2013	Canada	June 2005- May 2009	Mixed	Unmatched	Uni-Chi	51 ill 54 non-ill	Poor
Redlinger et al., 2002	Mexico	Aug 1999- Mar 2000	Mixed	Unmatched	Uni-Chi	298 ill 345 non-ill	Poor
Robertson et al., 2002	Australia	June 1998- May 2001	Children Mixed	Matched	Uni-CL Uni-CL Multi-CL	64 ill 262 non-ill 201 ill 795 non-ill	Good
Roy et al., 2004	USA	1999-2001	Mixed	Matched	Uni-MH Multi-CL	267 ill 464 non-ill 233 ill 467 non-ill	Good
Sarkar et al., 2014	India	2008-2013	Children	Unmatched	Uni-UL Multi-UL	411 ill 180 non-ill 113 ill 51 non-ill	Good
Solorzano-Santos et al., 2000	Mexico	2000	Children	Unmatched	Uni-Chi Multi-UL	10 ill 122 non-ill	Good
Sorvillo et al., 1994	USA	1983-1990	Susceptible	Unmatched	Uni-MH	125 ill 2354 non-ill	Good
Srisuphanunt et al., 2008	Thailand	2007	Susceptible	Unmatched	Uni-Chi	23 ill 120 non-ill	Good
Tellevik et al., 2015	Tanzania	Aug 2010- July 2011	Children	Unmatched	Uni-Chi Multi-UL	23 ill 397 non-ill	Good

Tumwine et al., 2003	Uganda	Nov 1999- Jan 2001	Susceptible	Matched	Uni-Chi	488 ill 1291 non- ill	Good
Valderrama et al., 2009	USA	Aug-Sep 2007	Mixed	Matched	Uni-CL Multi-CL	47 ill 92 non-ill 45 ill 89 non-ill	Good
Velasco et al., 2011	Colombia	Feb-Apr 2009	Susceptible	Unmatched	Uni-Chi Multi-UL	38 ill 93 non-ill	Good
Wilson et al., 2008	NewZealand	2006	Mixed	Unmatched	Uni-Chi	534 ill 5395 non- ill	Poor
Yang et al., 2017	China	Oct-Nov 2014	Mixed	Unmatched	Uni-Chi Multi-UL	73 ill 543 non-ill 73 ill 542 non-ill	Good

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702 **Table 2. Results of the meta-analysis on the main risk factors**

Population	Geographical area	Risk factor	Pooled OR [95% CI]	N/n*	p-value of risk factor	Publication bias p-value	Points removed**	Heterogeneity analysis***
Travel								
All	All	Abroad	4.216 [2.529 - 7.029]	9/14	<.0001	0.0408	0	$\tau^2=0.284$ QE(df = 19) = 73.419, p-val < .0001 S ² =0.656; I ² =30.205%
Host specific								
Mixed(y)	All	Immunocompromising conditions	4.507 [2.168 - 9.367]	6/10	<.0001	0.022	0	$\tau^2=0.5591$ QE(df = 11) = 42.355, p-val < .0001 S ² =0.973 ; I ² = 36.5028
		Other medical conditions	2.392 [1.588 - 3.604]	2/3	<.0001			
Children	All	Immunocompromising conditions	2.721 [2.147 - 3.448]	4/7	<.0001	0.366	0	$\tau^2=0.897$ QE(df = 25) = 102.641, p-val < .0001 S ² =1.39902 I ² = 39.06
Transmission Person to person by population								
Mixed	All		2.489 [2.033 - 3.049]	12/69	<.0001	<.0001	0	$\tau^2=0.2578$ QE(df = 80) = 199.431, p-val < .0001 S ² =0.393 I ² =39.62%
Children			3.786 [1.989 - 7.205]	5/7	<.0001			
Susceptible			1.903 [1.170 - 3.095]	5/7	0.010			
Transmission Person to person by type of contact								
All	All	Contact in the community	3.339 [2.623 - 4.243]	6/14	<.0001	0.304	1	$\tau^2=0.0485$ QE(df = 65) = 167.161, p-val < .0001 S ² =0.315 I ² =13.35%
		Sexual transmission	2.350 [1.439 - 3.837]	3/11	<.0001			
		Contact in the household	2.191 [1.771 - 2.711]	9/43	0.001			
Personal Hygiene								
All	All	All	1.736 [1.286 - 2.343]	4/4	0.0003	0.453	0	$\tau^2=0$ Q(df = 3) = 4.2604, p-val = 0.2347 S ² =0.189 I ² =0
Animal contact								
Mixed	All	Farm animals	2.167 [1.703 - 2.758]	13/41	<.0001	0.698	3	$\tau^2=0.2953$ QE(df = 64) = 224.108, p-val < .0001 ; S ² =0.336 ; I ² =46.772
Children	All	Farm animals	1.968 [1.284 - 3.018]	9/15	0.002	<.0001	0	$\tau^2=0.359$ QE(df = 28) = 59.869, p-val = 0.0004 S ² =0.458 I ² = 43.967
		Pets	1.694 [1.297 - 2.212]	8/15	<.0001			
Environment								
Mixed	All	Farm environment	1.794 [1.444 - 2.230]	5/18	<.0001	0.555	2	$\tau^2=0.601$ QE(df = 143) = 1534.2984, p-val < .0001 ;S ² =0.351 I ² =63.119
		Untreated drinking Water	1.358 [1.249 - 1.475]	14/46	<.0001			
		Recreational water	1.968 [1.475 - 2.625]	14/65	<.0001			
		Wastewater	1.697 [1.127 - 2.555]	5/8	0.011			

		Daycare attendance	1.539 [1.429 - 1.659]	3/5	<.0001			
Children	Oceania removed (2 OR excluded)	Farm environment	1.802 [1.194 - 2.719]	3/3	0.005	0.079	0	$\tau^2=0.0182$ QE(df = 28) = 33.467, p-val = 0.219 S ² =0.464 I ² =3.774
		Daycare attendance	1.742 [1.031 - 2.945]	3/3	0.038			
		Untreated drinking Water	1.367 [1.092 - 1.712]	9/19	0.006			
		Recreational water	4.114 [1.579 - 10.72]	2/2	0.004			
Food								
Mixed	All	Dairy	1.533 [1.009 - 2.329]	4/10	0.045	0.248	0	$\tau^2=0.2602$ QE(df = 50) = 159.0378, p-val < .0001 S ² =0.26 ; I ² =49.99
		Meat	1.934 [1.236 - 3.024]	4/9	0.004			
Children	All	Composite	1.532 [1.072 - 2.189]	2/2	0.019	0.993	0	$\tau^2=0$ QE(df = 4) = 5.9980, p-val = 0.1993 S ² =0.304 ; I ² =0

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

707 **Table 3. Results of the meta-analysis on disaggregated risk factors**

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Risk Factor	Population	Geographical area	Risk factor precise	Pooled OR [95% CI]	N/n*	p-value of risk factor	Publication bias p-value	Points removed **	Heterogeneity analysis***
Meat	Mixed & Susceptible	All	Others****	1.991 [1.288 - 3.080]	3/8	0.002	0.890	0	$\tau^2=0$ QE(df = 8) = 4.5068, p-val = 0.809 S ² =0.243 I ² =0
Dairy	Mixed & Children	All	Milk	1.509 [1.071 - 2.125]	6/8	0.019	0.647	0	$\tau^2=0$ QE(df = 10) = 8.5624, p-val = 0.574 S ² =0.0.205 I ² =0
Composite	Mixed & Children	All	Dishes	1.717 [1.220 - 2.416]	6/17	0.002	0.015	0	$\tau^2=0.142$ QE(df = 18) = 126.1028, p-val < .0001 s ² =0.330 I ² =30.085
BBQ	All	All	BBQ	2.005 [1.624 - 2.476]	2/4	<.0001	0.383	0	$\tau^2=0$ Q(df = 3) = 26.214, p-val < .0001 S ² =0.315 I ² =0

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

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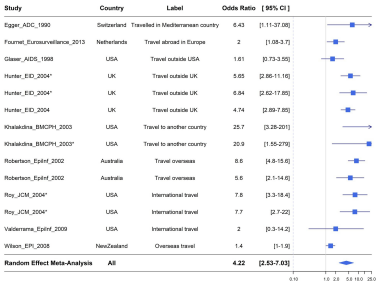
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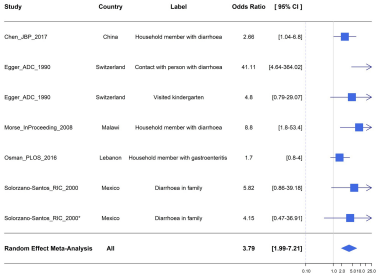
Table 4. Effect of handling on the pooled OR for produce

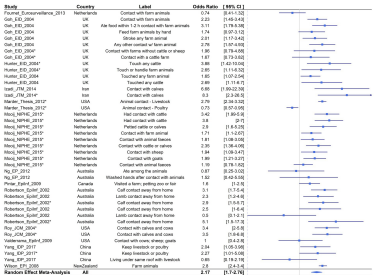
Risk Factor	Risk factor precise	Pooled OR [CI95%]	N/n*	p-value of risk factor	OR ratios [CI95%]	Points removed **	Publication bias p-value	Heterogeneity analysis**
Produce	Unwashed	1.159 [0.615 - 2.185]	3/4	0.039	1.572 [1.021 - 2.419]	0	0.236	$\tau^2=0$ QE(df = 9) = 9.7450, p-val = 0.3715 S ² =0.151 I ² =0
	Base	0.737 [0.602 - 0.903]	6/7	0.003				

719 *N/n Number of studies/number of OR;** points removed by sensitivity analysis, all results are given after removing data
720 concerned; ***Between-study variability (τ^2), test for residual heterogeneity (QE), variance of residuals (s^2), intra-class
721 correlation (I²).

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Random Effect Meta-Analysis

All

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[1.7-2.76]

