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1 **Molecular prevalence of *Coxiella burnetii* in bulk-tank milk from bovine dairy**

2 **herds: systematic review and meta-analysis**

3

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15

16 **Abstract**

17 *Coxiella burnetii* is an obligate intracellular zoonotic bacterium that causes Q

18 fever. Ruminants, including cattle, are broadly known to be reservoirs for this

19 bacterium. Since 2006, many research groups have evaluated the herd-level

20 prevalence of *C. burnetii* in cattle by molecular techniques on composite milk

21 samples. This study explored the global *C. burnetii* herd-level prevalence from

22 studies done on bovine bulk-tank milk (BTM) samples using PCR-based

23 analysis. Also, moderators were investigated to identify sources of

24 heterogeneity. Databases (CAB Abstracts, Medline via Ovid, PubMed, Web of

25 Science and Google Scholar) were searched for index articles on *C. burnetii*

26 prevalence in BTM samples by PCR published between January-1973 and

November-2018. Numerous studies (1,054) were initially identified, from which

seventeen original publications were included in the meta-analysis based on the

pre-defined selection criteria. These studies comprised 4,031 BTM samples

from twelve countries. A random-effects model was used because of

considerable heterogeneity (I^2=98%) to estimate the herd-level prevalence of

C. burnetii as 37.0%(CI$_{95\%}$25.2-49.5%). The average herd size appeared to

account for a high level of the heterogeneity. No other moderators (geographic

location, gross national income or notification criteria for Q fever) seemed to be

determinant. This systematic evaluation demonstrated a high molecular

prevalence of *C. burnetii* in BTM samples both in European and non-European

countries, evidencing a widespread herd-level circulation of this agent in bovine

dairy farms around the world. Meta-regression showed herd size as the most

relevant moderator with the odds of a BTM sample testing positive doubling with

every unit increase..

Keywords

Q fever*; Coxiella burnetii*; coxiellosis, meta-prevalence; PCR; *IS1111*

1. Introduction

Coxiella burnetii the intracellular Gram-negative bacterium responsible for the zoonotic

disease Q fever [1] has many reservoirs, including ruminants, that represent the

primary source of environmental contamination and of infection in people [2]. This

agent causes fertility disorders and metritis in cattle and is implicated in bovine abortion

[3,4,5]. It often leads to abortion in small ruminants when a pregnant dam is infected,

as *C. burnetii* exhibits a specific tropism for the trophoblast cells in placental cotyledons

[6].

Coxiella burnetii has a complex epidemiological pattern and characteristics that make its control challenging. It is widely disseminated in nature and infects a large number of species, including mammals, birds, reptiles and fish [7]. There are two maintenance cycles in nature, one involving domestic species, and another including wild animal species and their ectoparasites. Ticks may be involved in the transmission of *C. burnetii* between wildlife and domestic species [8]. Additionally, the agent is extremely resistant remaining viable in the environment over extended periods [8]. *Coxiella burnetii* can also undergo air-borne transmission by contaminated dust particles, which can be facilitated by hot and dry weather conditions [9,10].

A large human outbreak of Q fever reported in the Netherlands (2007-2010), comprising more than 4000 cases, emphasised the need for robust surveillance campaigns and highlighted its importance as a threat to public health [9,11]. Transmission to people is principally by the inhalation of aerosolised contaminated animal placenta and birth fluids during abortions or the birth of normal offspring [12]. Practices such as the assistance of calving, handling of birth products, and manure spreading may present a high risk for *C. burnetii* transmission to humans [13,14,15]. There is no consensus about the importance or effectiveness of the digestive route of infection by the consumption of raw milk and dairy product [6,16-18]. Nevertheless, respiratory exposure to aerosols produced during milking of animals should not be underestimated [19].

The level of bacterial load by the different routes differs among ruminants [6]. While parturition products are the primary source of shedding in small ruminants, milk seems to play a central role as a shedding route of *C. burnetii* in dairy cattle [20,21]. Even asymptomatic animals [20] or seronegative cattle [22] have been identified as *C. burnetii* milk shedders. *Coxiella burnetii* can be excreted in milk for up-to 13 months [9,23], although this may be intermittent [6]. Two patterns of shedding have been

77   identified in dairy cows which can be persistent heavy shedders or sporadic shedders

78   [20].

79   Based on these heterogeneous shedding patterns, composite samples such as bulk-

80   tank milk (BTM) constitute useful and easily accessible specimens for large scale

81   epidemiological investigation. A positive result provides robust evidence for the

82   identification of infected herds. Bulk-tank milk testing is the preferred diagnostic

83   approach for disease notification in many countries [24] and has epidemiological value

84   for the monitoring of infection status over time in follow-up evaluations [25].

85

86   Recent large human Q fever outbreaks in the Netherlands, Spain, France and

87   Germany have increasingly focussed attention on coxiellosis in many European

88   countries where strategies including mandatory notification of the disease have been

89   implemented. We systematically review studies of the herd prevalence of *C. burnetii* in

90   dairy cattle using PCR on BTM samples, conduct a meta-analysis to determine the

91   overall European and global prevalences and assess geographic region, average herd

92   size, local legislation for coxiellosis and per capita income in each country where

93   studies were conducted as potential moderators.

94

95   **2. Material and methods**

96   *2.1 Literature search and study selection*

97   The systematic review and meta-analysis followed the Preferred Reporting Items for

98   Systematic Reviews and Meta-Analyses (PRISMA) guidelines [26] (Figure 1). The

99   search strategy identified publications reporting the prevalence of *C. burnetii* on BTM

100   samples analysed by molecular studies. The following electronic databases were used

101   to identify studies published from January 1973 up to November 2018 (week 43 of

102   2018): CAB Abstracts, Medline, PubMed, Web of Science, Scopus, Science Direct and

4

103   Google Scholar. The literature search comprised the terms: "*Coxiella burnetii"* or "Q

104   fever" or "coxiellosis" and "PCR" or "qPCR" or "real-time PCR" or "molecular diagnosis"

105   and "BTM" or "milk", with no language restriction. No constraint in study designs was

106   applied at this phase. Additional publications were identified by cross checking

107   references included in the articles. Duplicates were identified by reference

108   management software (Mendeley) and manually removed.

109   *2.2 Eligibility- Inclusion criteria*

110   Publications on studies fulfilling all the following criteria were eligible for inclusion: (i)

111   molecular investigation of *C. burnetii* by PCR, (ii) random sampling, (iii) composite

112   single test-day samples obtained from the bulk storage tank located on a dairy cattle

113   farm, (iv) primary studies, but not reviews, (v) cross-sectional studies reporting

114   prevalence. Authors of articles not stating the total number of dairy cattle herds from

115   which the sample was drawn were contacted to provide this missing data. Publications

116   were examined by two independent reviewers (AR and MF) to ensure they matched

117   the inclusion criteria. Discrepancies between the two reviewers on eligibility were

118   discussed with the rest of authors until reaching agreement.

119   *2.3 Data extraction and Meta-analysis*

120   Studies were screened by title, and abstract and irrelevant publications were excluded.

121   The remaining studies were full-text checked against the inclusion criteria described

122   above. Articles that did not fulfil all these criteria were excluded. The number of

123   publications excluded are shown in Figure 1. Data were systematically extracted from

124   all the studies that satisfied the inclusion criteria, including: the first author identity, year

125   of publication, study title, journal title, country, study methodology (duration of

126   sampling, herd size, sample size, the number of positives herds and/or prevalence,

127   randomisation), molecular technique and target gene used. When available,

128   information about the factors associated with the *C. burnetii* infection was also

129   reported.

130   The *C. burnetii* herd prevalence determined in BTM samples (dependant variable) was

131   considered as the effect size for the studies included in the meta-analysis. This meta-

132   analysis of proportions was performed as outlined by Wang [27]. The heterogeneity

133   among studies was first investigated by Cochran's Q (X^2) that tests the null hypothesis

134   of homogeneity, and then quantified by the Higgins' I^2 statistic [28]. The heterogeneity

135   was measured to select the model for the overall weighted *C. burnetii* herd prevalence

136   estimation. As the level of heterogeneity was high, a random-effects model was first

137   used to address both within-study variance (the sampling error) and the between-

138   studies variance (τ^2). Possible sources of heterogeneity were investigated through the

139   analysis of moderators. The evaluated moderators included: i) geographic region:

140   Europe vs non-Europe; ii) average herd size; iii) local legislation for Q fever: mandatory

141   notification vs non-mandatory notification [29-37], and iv) gross national income (GNI)

142   per capita classification from the year the study was conducted, based on the Atlas

143   method [38]. A subgroup analysis was performed for the categorical moderators.

144   Categorical moderators were analysed using a mixed-effects model. The statistical

145   significance of the moderators was evaluated by an omnibus test (QM) within the

146   mixed-effects model [39]. The proportion of heterogeneity accounted for by each

147   moderator was explored by the R^2 index. Meta-regression was also utilised to explore

148   heterogeneity among the studies. All the moderators and their interactions were

149   entered in the initial model and non-significant terms were then dropped stepwise (from

150   lowest R^2 to highest R^2) [40]. The odds ratio (OR) for $\log_e$ average herd size was

151   additionally investigated. Association among moderators was assessed by the Pearson

152   correlation coefficient (r). Results from the meta-analysis with the corresponding 95%

153   confidence intervals were summarized using forest plots. Egger's test was used to test

154   for the possibility of a publication bias for studies with low or high effect sizes [41]. All

155   the assessments were conducted using open RStudio software (Boston, MA) with

156   metafor package, mvmeta package and metaprop commands [39,42].

157   **3. Results**

158   *Description of the studies*

159   After removal of duplicates, a total of 179 studies were identified initially (Figure 1).

160   Seventeen studies from twelve different countries (Belgium, Colombia, Hungary, Iran [2

161   studies], Italy [3 studies], Latvia, Netherlands [2 studies], Portugal, Spain, South Korea,

162   UK and USA [2 studies]) were eligible for the meta-analysis based on the inclusion

163   criteria. Six of those studies were conducted in non-European countries and 11 in

164   European countries; 10 were conducted in countries where Q fever is a notifiable

165   disease, while 7 were from countries where it is not. The study conducted in the

166   Basque Country was included in the subgroup with mandatory notification, although

167   this is the only Spanish province where the notification for Q fever is compulsory.

168   Finally, 3 studies were conducted in upper-middle-income countries and 14 studies

169   were in high income countries. The seventeen selected articles are summarised in

170   Table 1 and included test results for a total of 4,031 BTM samples collected over 9

171   years (2006 to 2015). Studies employed either conventional PCR (n=5), quantitative

172   PCR (n=9) or nested PCR (n=3). The transposon-like repetitive region of the bacterial

173   genome (*IS1111*) was the gene most frequently used as the target in these PCRs

174   (n=14), followed by *com1* (n=2), *icd* (n=1) and 16S rRNA genes (n=1) (Table 1).

175

Table 1: Characteristics and main results of the eligible studies ordered by molecular prevalence of *Coxiella burnetii* in bulk-tank milk samples

Author	Year	Country	Study area	Average herd size	Period of study	Risk factor analysis	Gross national income per capita [38]	Is Q fever a mandatory notifiable disease?	Molecular approach	Target gene	N herds in study area	Percentage of herds sampled	BTM[i] samples tested	Positive BTM samples	Prevalence	95% CI	
Boroduske *et al.* [43]	2017	Latvia	Nationwide	8.6	2015	Yes	High-income	Yes	qPCR	*IS1111*	5,040	5	252	27	10.7	7.2	14.9
Kargar *et al.* [23]	2013	Iran	Johrom	3.7	-	Yes	Upper-middle-income	Yes	nPCR	*com1*	3,000	3.3	100	11	11	5.5	18.0
Seo *et al.* [44]	2018	South Korea	Gyeongsang	74	2015	No	High-income	Yes	nPCR	16S rRNA	869	69.9	607	108	17.8	14.8	20.9
Rahimi *et al.* [45]	2010	Iran	Chaharmahal and Bakhtiari	48	2008	No	Upper-middle-income	Yes	nPCR	*com1*	95	29.5	28	5	17.9	5.5	34.5
van Engelen *et al.* [46]	2014	Netherlands	Nationwide	71.7	2009-2011	Yes	High-income	Yes	qPCR	*IS1111*	20,746	1.5	309	58	18.8	14.6	23.3
Anastácio *et al.* [47]	2016	Portugal	Nationwide	21.7	2009-2013	Yes	High-income	No	PCR	*IS1111*	1,712	2.6	45	9	20	10.9	33.8
Velasova *et al.* [48]	2017	UK	Nationwide	133	2014-2015	No	High-income	No	qPCR	*icd / IS1111*	10,491	2.1	220	57	25.9	20.3	31.9
Czaplicki *et al.* [49]	2012	Belgium	Wallonia	28.5	2006	Yes	High-income	No	qPCR	*IS1111*	5,086	1	50	15	30	8.7	51.3
Magnino et al. [50]	2009	Italy	Cremona, Montova and Pavia	180	2007-2008	No	High-income	No	PCR	*IS1111*	3,550	11.2	400	161	40.2	35.5	45.1
Valla *et al.* [51]	2014	Italy	Nationwide	42.5	2011-2013	No	High-income	No	PCR	*IS1111*	30,000	1.1	344	138	40.1	35.0	45.4
Contreras *et al.* [37]	2015	Colombia	Monteria	150-600	2012	No	Upper-middle-income	No	PCR	*IS1111*	3,341	0.3	11	5	45.5	16.7	75.8
Astobiza *et al.* [52]	2012	Spain	Bizkaia	46.1	2009-2010	No	High-income	No / Yes[ii]	qPCR	*IS1111*	178	100	178	92	51.7	44.4	59
Muskens *et al.* [25]	2011	Netherlands	Nationwide	65.7	2007	No	High-income	Yes	qPCR	*IS1111*	21,313	1.6	341	193	56.6	50.7	61.9
Vicari et al. [34]	2013	Italy	Lombardy	182	2011	No	High-income	No	PCR	*IS1111*	5,750	5	287	173	60.3	54.5	65.9
Bauer *et al.* [53]	2015	USA	Indiana	145.3	2011	No	High-income	Yes	qPCR	*IS1111*	1,225	25.8	316	193	61.1	55.6	66.4
Gyuranecz *et al.* [54]	2012	Hungary	Nationwide	14.5	2010-2011	No	High-income	Yes	qPCR	*IS1111*	17,172	0.1	15	10	66.7	40.5	88.7
APHIS [55]	2007	USA	18 states[iii]	162.6	2007	No	High-income	Yes	qPCR	*IS1111*	54,100	1	528	406	76.9	73.2	80.4

(i): BTM: bulk-tank milk samples, one per herd; PCR: conventional PCR; qPCR: real-time PCR; nPCR: nested PCR. (ii) mandatory notification in Basque Country. (iii) California, Idaho, Indiana, Iowa, Kentucky, Michigan, Minnesota, Missouri, New Mexico, New York, Ohio, Pennsylvania, Texas, Vermont, Virginia, Washington, Wisconsin.

Table 1: Characteristics and main results of the eligible studies ordered by molecular prevalence of *Coxiella burnetii* in composite milk samples.

The estimated overall meta-prevalence of Coxiella burnetii *in BTM samples*

The median size of the eligible studies was 252 BTM samples. Of the total 4,031 BTM samples, 1,661 were diagnosed positive by molecular techniques. The percentages of positive BTM samples among the studies ranged from 10.7 to 76.9%. The overall weighted prevalence of *C. burnetii* in the random-effects meta-analysis was estimated at 37.0% ($CI_{95\%}$25.2-49.5%). The I^2 value of 98.0% ($CI_{95\%}$95.9–99.0) suggested high heterogeneity, with a τ^2 of 0.0654 ($CI_{95\%}$0.3296-1.4997), and an X^2 statistic of 892.97 (P<0.0001). The overall meta-analysis is shown in a forest plot (Figure 2a). No obvious evidence of publication bias was detected in the meta-analysis on the basis of Egger's test (P=0.599).

The meta-prevalence of Coxiella burnetii *and moderator analyses*

The weighted average prevalence was similar within each of the two geographic subgroups (36.9% in European countries and 37.1% in non-European countries; (I^2=98%; X^2=870.29, P<0.01; QM (df=1)=0.002, P=0.98), albeit with differing 95% confidence intervals of 22.8%– 52.2% in the former and 18.0%–58.5% in the latter group of countries (Figure 2b). Similarly, countries with mandatory and non-mandatory notification of Q fever had a prevalence around 37.0% ($CI_{95\%}$22.3–52.9% and $CI_{95\%}$19.4–56.4%, respectively; (I^2=98%; X^2=892.61, P<0.01; QM (df=1)=0.010, P=1.00) (Figure 2c). In the subgroup analysis based on the GNI per capita (Figure 2d), the prevalence was 40.1% ($CI_{95\%}$27.9–52.9%) in high-income countries and 21.2% ($CI_{95\%}$2.2–50.2%) in upper-middle-income countries (I^2=98%; R^2=3.10%; X^2=844.20, P<0.01; QM (df=1)=1.39, P=0.24). None of the three factors above appeared to contribute meaningfully to the observed level of heterogeneity based on the subgroup analysis. The meta-regression revealed that average herd size accounted for a significant proportion of the heterogeneity (I^2=97%; R^2=33.01%; X^2=552.23, P<0.01; QM =4.55, P=0.03). As a significant moderator, high-size herds presented a higher

202 herd-level *C. burnetii* BTM prevalence (Figure 3). The odds ratio for the $\log_e$ of herd

203 size was 2.00 ($CI_{95\%}$1.24-3.52; *P*=0.02). A strong positive correlation was found

204 between countries being located in Europe and high GNI per capita income (r=0.633,

205 *P*<0.05), but between location in Europe and compulsory disease notification (r=-0.239,

206 *P*=0.24), and between high GNI per capita and notification (r=-0.076, *P*=0.82)

207 correlations were weak and negative. Herd size was not meaningfully correlated with

208 the origin of the studies (r=-0.468, *P*=0.12), notification (r=-0.428, *P*=0.16), or with GNI

209 per capita (r=-0.444, *P*=0.14).

210

211 **4. Discussion**

212 Global serological or molecular prevalences from pathogens as diverse as *Toxoplasma*

213 *gondii* and *Helicobacter pylori* have been estimated by meta-analyses following a

214 systematic review of the published body of studies [56,57]. We conducted a

215 comprehensive keyword-based systematic review of the literature on the global

216 molecular prevalence of *C. burnetii* in bovine BTM samples and data from those

217 studies matching the inclusion criteria was extracted and included in a meta-analysis.

218 For the purpose of this review, only adequately randomised studies with a cross-

219 sectional design were included.

220

221 Heterogeneity among studies was first investigated by Higgins' I^2 statistic which

222 indicates the proportion of heterogeneity not due to chance. A high level of

223 heterogeneity (≥ 75%) indicates another source of variability besides the random

224 error. The high I^2 value (98%) led to the choice of a random-effects model for

225 estimating the overall weighted *C. burnetii* herd-level prevalence among eligible

226 articles, which makes no assumption that the prevalence is constant across the

227 studies. The meta-analysis shows that *C. burnetii* is widely distributed in dairy farms

228 around twelve countries from 3 continents (America, Europe, and Asia). The best

229 estimate of global *C. burnetii* herd-level prevalence, based on the studies matching the

230 current inclusion criteria, was 37.0%. While there was no obvious evidence of

231 publication bias based on Egger's test, this test has limited power and the possibility of

232 bias cannot be altogether excluded [58].

233

234 Bulk tank milk samples are a widely used approach for studying infectious diseases of

235 dairy livestock at the population level, despite that dry cows and unhealthy animals are

236 not included and hence BTM only provides a partial representation of the herd sanitary

237 status. The analysis of BTM samples represents a suitable and convenient approach

238 for the investigation of *C. burnetii*, not only for initial farm-level screening in situations

239 where their disease status is unknown, but also for repeated analyses during

240 monitoring programmes or after sanitary interventions such as antibiotic administration

241 [59] or vaccination [60,61]. A positive BTM result confirms herd exposure to *C. burnetii*.

242

243 The molecular diagnostic methods of studies included in this meta-analysis targeted

244 different regions of the bacterial *C. burnetii* genome. The repetitive element

245 *IS1111* was selected in most of the published studies as this multiple copy gene is

246 presumed to increase the sensitivity of the test [62]. Other studies used PCRs targeting

247 *com1*, *icd* and 16S rRNA genes. The *com1* element is frequently used for accurate

248 quantification, as this is a single-copy gene [63]. Additionally, the analysis of 16S RNAs

249 may reveal the prevalence of *Coxiella* as a genus, by the identification of both

250 *C. burnetii* and *Coxiella*-like organisms [44].

251

252 The overall weighted *C. burnetii* prevalence found in bovine dairy herds was higher

253 than the 5.1% to 22.1% range reported for BTM samples from sheep dairy flocks

254 [47,64,65]. This difference could be explained by the primary route of bacterial

255 transmission in each species. A higher *C. burnetii* prevalence might be expected in

256   bovine milk, which is the predominant route of shedding for cows (and with a longer

257   duration), whereas milk is less important for transmission from goats and sheep [9,23].

258   Two nationwide studies in Dutch dairy herds revealed markedly different prevalence

259   levels in 2011 (56.6%) and 2014 (18.8%) [25,46], when using the same molecular

260   approach in a similar number of herds. The lower prevalence in 2014 might be related

261   to compulsory control measures applied in dairy goat farms after the large human Q

262   fever outbreak in 2007-2010 [11,66]. There is some albeit limited evidence that the

263   same outbreak strain may affect both cattle and goats in the Netherlands [67], and

264   measures applied to goat farms might have indirectly helped to reduce prevalence in

265   bovine herds. Similarly, three studies conducted in Italian herds in 2013 and 2014 also

266   reported differences in *C. burnetii* prevalence. Valla *et al.* (2014) [51] revealed a

267   nationwide prevalence of 40.0%, while Vicari *et al.* (2013) [34] found a higher

268   prevalence of 60.0% in the northwest region of Lombardy, where almost half of Italian

269   cows' milk is produced [68]. The molecular prevalence of *C. burnetii* found in Lombardy

270   represented a marked increase compared to a previous two-year study (2007-2008)

271   conducted in the same region (40.0%) [50].

272

273   Differences in the bacterial shedding patterns among ruminants and uncertainty about

274   the importance of milk-borne infection may result in emphasis on different control

275   measures depending on the species. In small ruminants, the identification of high-risk

276   dams before parturition is important in avoiding zoonotic risk [69]. In cattle where milk

277   is the primary shedding route, pre-partum monitoring may not be as appropriate [69].

278   Identification of chronic *C. burnetii* milk shedding cattle may be more effective in

279   preventing environmental contamination, decreasing the risks of transmission among

280   animals and preventing the spread of the bacterium.

281

282   Only five of the seventeen selected articles included analysis of factors associated with

283   *C. burnetii* infection. Herd size, cattle density and purchasing replacement animals from

284   external sources were all linked with *C. burnetii* infection [43,46]. Additionally, the

285   presence of ticks on cattle was associated with BTM PCR positivity [46].

286

287   For both cattle and small ruminants, a positive correlation between herd size and herd

288   prevalence of *C. burnetii* has been reported [70,71]. The association between herd

289   size, density of animals and an enhanced risk of *C. burnetii* infection has been well

290   demonstrated [10,72]. Close contact between cows is an intrinsic characteristic of dairy

291   herd management systems, and larger herds offer even greater chances for contact

292   and transmission. Densely populated farms are prone to a higher risk of transmission

293   of the pathogen within the herd after *C. burnetii* is introduced into the farm. Additionally,

294   high animal density leads to greater bacterial load and thus higher environmental

295   contamination [73], which may represent an increased risk of transmission to either

296   cattle or people. This meta-analysis showed that elevated prevalence of *C. burnetii* is

297   associated with large-sized herds, where the odds of a BTM sample testing positive

298   double with every unit increase in $\log_e$ herd size (odds ratio $CI_{95\%}$1.24-3.52).

299   Accordingly, of the moderators analysed, average herd size had the largest effect,

300   accounting 33.0% of the observed level of heterogeneity among studies.

301

302   While Q fever has been studied in both European and non-European countries, these

303   two contexts have not previously been contrasted. The overall prevalence of *C. burnetii*

304   infection was remarkably similar in European and non-European studies (both 37%).

305   The greater variability among non-European studies ($CI_{95\%}$ 18.0%–58.5%) than among

306   European studies ($CI_{95\%}$ 22.8%–52.2%) could be accounted for by the differences in

307   the numbers of studies and herds investigated.

308

309   The mandatory notification of a disease should be helpful not only for early

310   identification of outbreaks but also to enable evaluation of the effectiveness of control

311   strategies. For instance, legislation implemented by the Dutch government in the face

312 of the largest Q fever outbreak ever recorded included compulsory notification of

313 coxiellosis [66]. In the current meta-analysis, a remarkable similarity was noted

314 between overall weighted prevalence of *C. burnetii* in BTM samples from countries with

315 mandatory (37.0%, $CI_{95\%}$22.3–52.9%) and non-mandatory (36.9%, $CI_{95\%}$19.4–56.4%)

316 notification legislation.

317

318 In our meta-analysis, the GNI per capita seems to have a minor effect as a moderator

319 of the prevalence of *C. burnetii* in BTM samples. When the studies were stratified

320 according to this indicator of economic development, high-income countries had twice

321 the overall weighted prevalence of upper-middle income countries, albeit that this

322 difference was not statistically significant (*P*=0.24). All publications matching the

323 inclusion criteria were conducted in high and upper-middle income countries. None of

324 the studies conducted in low-middle and low-income countries that were identified in

325 the initial search fulfilled the inclusion criteria and were rejected from the meta-analysis.

326 For instance, an ineligible study carried out in Egypt reported a 22% molecular

327 prevalence of *C. burnetii* in individual milk samples [74] and one carried out in

328 Bangladesh reported 15.6% seroprevalence in herd milk specimens [75]. These

329 findings suggest that further field studies could prove rewarding. The overall

330 prevalence in low-middle and low-income countries remains unknown. There is

331 evidence of extensive ruminant infection with *C. burnetii* throughout African countries

332 where the threat of human exposure and significant economic impact are possibly

333 underestimated [76].

334

335 Some heterogeneity might have resulted from methodological variation among nine of

336 the 17 studies that used qPCR to detect the *IS1111* target. Four of these [25,46,49,52]

337 used the TaqVet *Coxiella burnetii* LSI kit and followed the same manufacturer's

338 instructions for the amplification reaction and for the interpretation of the results. These

339 four studies considered samples as positive with a cycle threshold (Ct) < 40. Two

340 further studies used threshold Ct values of 36.5 [53] and 36.95 [54], while the

341 remaining three studies using qPCR to detect *IS1111* did not report threshold Ct

342 values.

343

344 Moreover, whereas the *IS1111* transposon-like element is a multi-copy gene [77], the

345 16S rRNA target used in South Korean study [44] and the *com1* target used in the two

346 studies in Iran [23,45] are both single copy genes. The assays used in these studies

347 might have had lower sensitivity and indeed, the studies using the single copy assays

348 had three of the four lowest prevalence values. All three of these studies were in non-

349 European countries where the disease is notifiable, and the two Iranian studies were in

350 an upper-middle income country, which may have introduced a degree of bias in the

351 analysis.

352 Although the moderator analysis identified average herd size as one source, most of

353 the heterogeneity remained unexplained (residual heterogeneity I^2=97.0%; *P*<0.01). It

354 is quite possible that other factors, not currently addressed, influence the *C. burnetii*

355 herd-level prevalence. Unsurprisingly, two of the moderators were highly correlated;

356 studies in European and in high-income countries showed a significant and positive

357 correlation (r=0.627, *P*<0.01). Awareness of the relationships between moderators that

358 may potentially induce bias in the analysis should be considered when drawing

359 conclusions [78].

360 **5. Conclusion**

361 This meta-analysis reports a high overall global prevalence of *C. burnetii* in BTM

362 samples of 37.0% ($CI_{95\%}$25.2-49.5%), showing widespread herd-level circulation of this

363 agent in bovine dairy farms. These results should be of interest not only for European

364 countries where *C. burnetii* is a well-known health threat, but also in countries where

365 epidemiological investigations have been limited, its importance as a zoonosis may be

366 underestimated and prevention strategies may need to be implemented. Information on

367 local biosecurity practices and environmental conditions would be valuable for a full

368 understanding of *C. burnetii* prevalence globally, but such descriptions were lacking in

369 most of the publications considered in the meta-analysis. While this study has shown

370 the global herd prevalence of *C. burnetii* in dairy cattle to be high, in many countries,

371 including high-income countries such as Belgium, Italy, Portugal and UK, the disease is

372 not currently notifiable, and control is not mandatory. To make it so might represent an

373 additional burden on dairy farmers and would require justification on economic or public

374 health grounds for which further study might be required. The high herd-level

375 circulation of *C. burnetii* in bovine dairy farms in several countries showed by this study

376 reinforces the need for further investigations on this globally important zoonosis.

377

378 **Author contributions**

379 AR and MCE conceptualised the study. AR and MF performed the systematic review,

380 including data collection and screening the retrieved records. AR, MCE and LGC

381 conducted the data-analysis. All authors (AR, MF, LGC, KT, FRC and MCE) made

382 contributions to the interpretation of results. All authors participated in the manuscript

383 drafting and reviewing.

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388

389 **Declaration of Competing Interest**

390 None declared.

391

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Figure 1

(a)

Study and Year	Events	Samples	Proportion	95%-IC	Weight
APHIS, 2007	406	528	76.9	[73.2; 80.4]	6.2%
Rahimi et al., 2010	5	28	17.9	[5.5; 34.5]	5.5%
Muskens et al., 2011	193	341	56.6	[51.3; 61.8]	6.1%
Czaplicki et al., 2012	15	50	30.0	[18.0; 43.5]	5.8%
Astobiza et al., 2012	92	178	51.7	[44.3; 59.0]	6.1%
Gyuranecz et al., 2012	10	15	66.7	[40.5; 88.7]	5.0%
Kargar et al., 2013	11	100	11.0	[5.5; 18.0]	6.0%
Vicari et al., 2013	173	287	60.3	[54.5; 65.9]	6.1%
van Engelen et al., 2014	58	309	18.8	[14.6; 23.3]	6.1%
Magnino et al., 2014	161	400	40.2	[35.5; 45.1]	6.1%
Valla et al., 2014	138	344	40.1	[35.0; 45.4]	6.1%
Contreras et al., 2015	5	11	45.5	[16.7; 75.8]	4.7%
Bauer et al., 2015	193	316	61.1	[55.6; 66.4]	6.1%
Anastácio et al., 2016	9	45	20.0	[9.4; 33.1]	5.7%
Boroduske et al., 2017	27	252	10.7	[7.2; 14.9]	6.1%
Velasova et al., 2017	57	220	25.9	[20.3; 31.9]	6.1%
Seo et al., 2018	108	607	17.8	[14.8; 20.9]	6.2%
Combined prevalence	4031		37.0	[25.2; 49.5]	100.0%

Heterogeneity: $I^2 = 98\%$, $\tau^2 = 0.0654$, $\chi^2_{16} = 892.97$

Prevalence (95% CI)

(b)

Study and Year	Events	Samples	Proportion	95%-IC	Weight
Origin = Non-Europe					
APHIS, 2007	406	528	76.9	[73.2; 80.4]	6.2%
Rahimi et al., 2010	5	28	17.9	[5.5; 34.5]	5.5%
Kargar et al., 2013	11	100	11.0	[5.5; 18.0]	6.0%
Contreras et al., 2015	5	11	45.5	[16.7; 75.8]	4.6%
Bauer et al., 2015	193	316	61.1	[55.6; 66.4]	6.1%
Seo et al., 2018	108	607	17.8	[14.8; 20.9]	6.2%
Combined prevalence	1590		37.1	[18.0; 58.5]	34.6%
Heterogeneity: $I^2 = 99\%$, $\tau^2 = 0.0641$, $\chi^2_{10} = 556.32$ ($p < 0.01$)					
Origin = Europe					
Muskens et al., 2011	193	341	56.6	[51.3; 61.8]	6.1%
Czaplicki et al., 2012	15	50	30.0	[18.0; 43.5]	5.8%
Astobiza et al., 2012	92	178	51.7	[44.3; 59.0]	6.1%
Gyuranecz et al., 2012	10	15	66.7	[40.5; 88.7]	5.0%
Vicari et al., 2013	173	287	60.3	[54.5; 65.9]	6.1%
van Engelen et al., 2014	58	309	18.8	[14.6; 23.3]	6.1%
Magnino et al., 2014	161	400	40.2	[35.5; 45.1]	6.1%
Valla et al., 2014	138	344	40.1	[35.0; 45.4]	6.1%
Anastácio et al., 2016	9	45	20.0	[9.4; 33.1]	5.7%
Boroduske et al., 2017	27	252	10.7	[7.2; 14.9]	6.1%
Velasova et al., 2017	57	220	25.9	[20.3; 31.9]	6.1%
Combined prevalence	2441		36.9	[22.8; 52.2]	65.4%
Heterogeneity: $I^2 = 97\%$, $\tau^2 = 0.0641$, $\chi^2_{10} = 313.98$ ($p < 0.01$)					
Combined prevalence	4031		37.0	[25.3; 49.4]	100.0%

Heterogeneity: $I^2 = 98\%$, $\tau^2 = 0.0641$, $\chi^2_{16} = 892.97$ ($p < 0.01$)
Residual heterogeneity: $I^2 = 98\%$, $\chi^2_{15} = 870.30$ ($p < 0.01$)
Test for subgroup differences: $\chi^2_1 = 0.00$, df = 1 ($p = 0.98$)

Prevalence (95% CI)

(c)

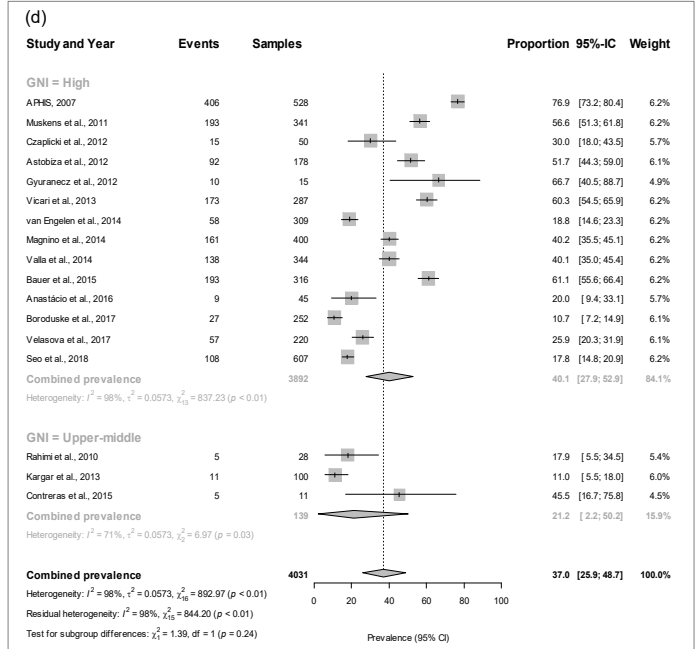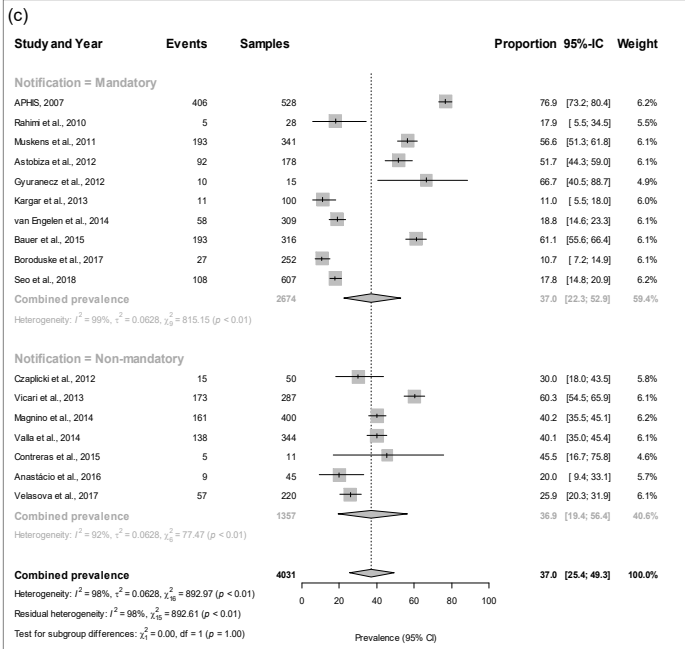| Study and Year	Events	Samples	Proportion	95%-IC	Weight
Notification = Mandatory					
APHIS, 2007	406	528	76.9	[73.2; 80.4]	6.2%
Rahimi et al., 2010	5	28	17.9	[5.5; 34.5]	5.5%
Muskens et al., 2011	193	341	56.6	[51.3; 61.8]	6.1%
Astobiza et al., 2012	92	178	51.7	[44.3; 59.0]	6.1%
Gyuranecz et al., 2012	10	15	66.7	[40.5; 88.7]	4.9%
Kargar et al., 2013	11	100	11.0	[5.5; 18.0]	6.0%
van Engelen et al., 2014	58	309	18.8	[14.6; 23.3]	6.1%
Bauer et al., 2015	193	316	61.1	[55.6; 66.4]	6.1%
Boroduske et al., 2017	27	252	10.7	[7.2; 14.9]	6.1%
Seo et al., 2018	108	607	17.8	[14.8; 20.9]	6.2%
Combined prevalence	2674		37.0	[22.3; 52.9]	59.4%
Heterogeneity: $I^2 = 99\%$, $\tau^2 = 0.0628$, $\chi^2_9 = 815.15$ ($p < 0.01$)					
Notification = Non-mandatory					
Czaplicki et al., 2012	15	50	30.0	[18.0; 43.5]	5.8%
Vicari et al., 2013	173	287	60.3	[54.5; 65.9]	6.1%
Magnino et al., 2014	161	400	40.2	[35.5; 45.1]	6.2%
Valla et al., 2014	138	344	40.1	[35.0; 45.4]	6.1%
Contreras et al., 2015	5	11	45.5	[16.7; 75.8]	4.6%
Anastácio et al., 2016	9	45	20.0	[9.4; 33.1]	5.7%
Velasova et al., 2017	57	220	25.9	[20.3; 31.9]	6.1%
Combined prevalence	1357		36.9	[19.4; 56.4]	40.6%
Heterogeneity: $I^2 = 92\%$, $\tau^2 = 0.0628$, $\chi^2_6 = 77.47$ ($p < 0.01$)					
Combined prevalence	4031		37.0	[25.4; 49.3]	100.0%

Heterogeneity: $I^2 = 98\%$, $\tau^2 = 0.0628$, $\chi^2_{16} = 892.97$ ($p < 0.01$)
Residual heterogeneity: $I^2 = 98\%$, $\chi^2_{15} = 892.61$ ($p < 0.01$)
Test for subgroup differences: $\chi^2_1 = 0.00$, df = 1 ($p = 1.00$)

Prevalence (95% CI)

(d)

Study and Year	Events	Samples	Proportion	95%-IC	Weight
GNI = High					
APHIS, 2007	406	528	76.9	[73.2; 80.4]	6.2%
Muskens et al., 2011	193	341	56.6	[51.3; 61.8]	6.2%
Czaplicki et al., 2012	15	50	30.0	[18.0; 43.5]	5.7%
Astobiza et al., 2012	92	178	51.7	[44.3; 59.0]	6.1%
Gyuranecz et al., 2012	10	15	66.7	[40.5; 88.7]	4.9%
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Bauer et al., 2015	193	316	61.1	[55.6; 66.4]	6.2%
Anastácio et al., 2016	9	45	20.0	[9.4; 33.1]	5.7%
Boroduske et al., 2017	27	252	10.7	[7.2; 14.9]	6.1%
Velasova et al., 2017	57	220	25.9	[20.3; 31.9]	6.2%
Seo et al., 2018	108	607	17.8	[14.8; 20.9]	6.2%
Combined prevalence	3892		40.1	[27.9; 52.9]	84.1%
Heterogeneity: $I^2 = 98\%$, $\tau^2 = 0.0573$, $\chi^2_{13} = 837.23$ ($p < 0.01$)					
GNI = Upper-middle					
Rahimi et al., 2010	5	28	17.9	[5.5; 34.5]	5.4%
Kargar et al., 2013	11	100	11.0	[5.5; 18.0]	6.0%
Contreras et al., 2015	5	11	45.5	[16.7; 75.8]	4.5%
Combined prevalence	139		21.2	[2.2; 50.2]	15.9%
Heterogeneity: $I^2 = 71\%$, $\tau^2 = 0.0573$, $\chi^2_2 = 6.97$ ($p = 0.03$)					
Combined prevalence	4031		37.0	[25.9; 48.7]	100.0%

Heterogeneity: $I^2 = 98\%$, $\tau^2 = 0.0573$, $\chi^2_{16} = 892.97$ ($p < 0.01$)
Residual heterogeneity: $I^2 = 98\%$, $\chi^2_{15} = 844.20$ ($p < 0.01$)
Test for subgroup differences: $\chi^2_1 = 1.39$, df = 1 ($p = 0.24$)
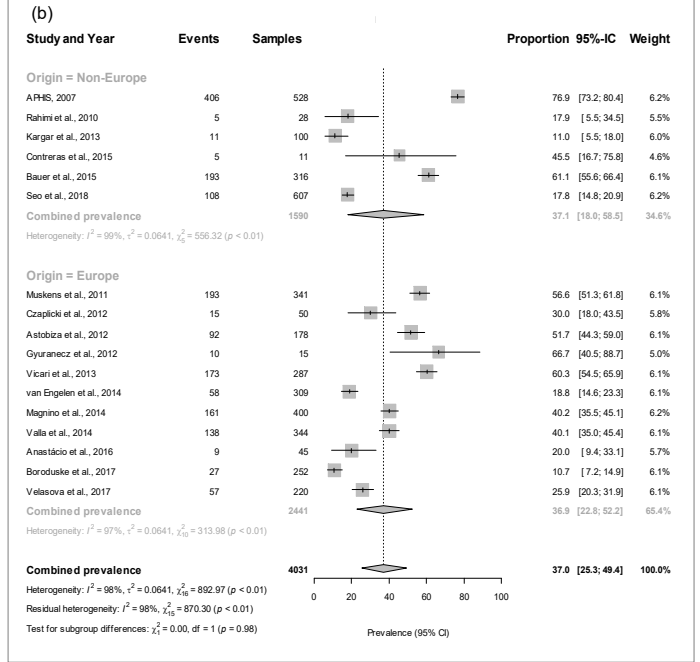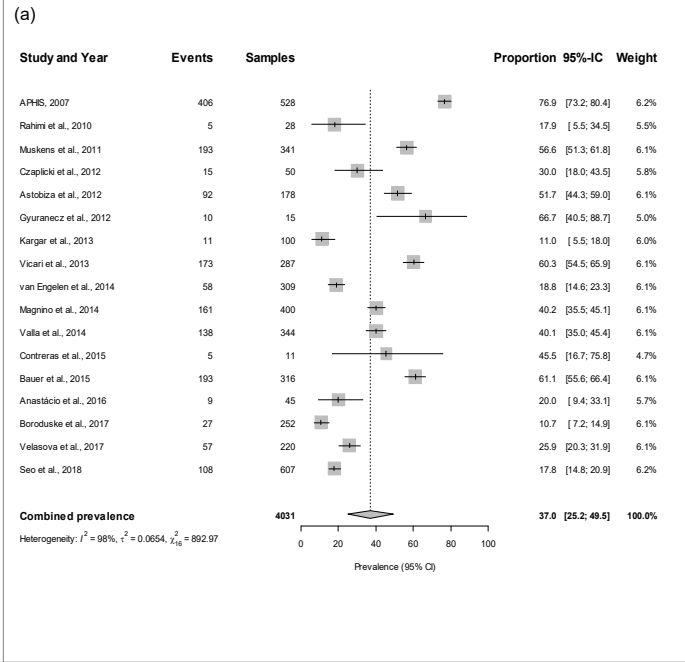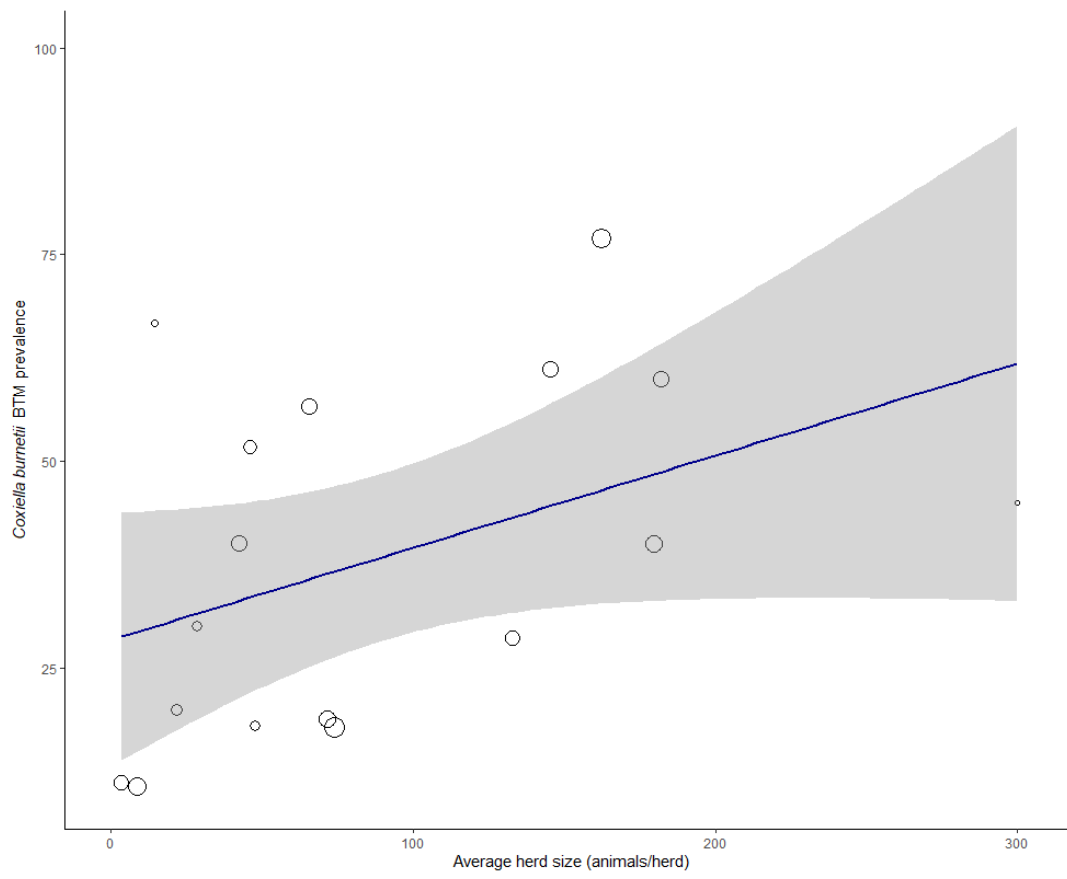
Prevalence (95% CI)

Figure 3

Figure Captions

Figure 1: PRISMA flow diagram for the systematic review describing the study design process. Articles reporting the herd-level *Coxiella burnetii* prevalence based on bulk-tank milk samples by molecular investigation were systematically reviewed and further evaluated by a meta-analysis.

Figure 2: Forest plot for the meta-analysis of herd-level *Coxiella burnetii* prevalence based on bulk-tank milk samples from the seventeen studies that matched the inclusion criteria in the systematic review. (a) All studies. (b) European and non-European country subgroups. (c) Grouped by mandatory and non-mandatory notification. (d) Grouped by the per capita Gross National Income (GNI) level.

Figure 3: Bubble plot for meta-regression of herd-level *Coxiella burnetii* prevalence based on bulk-tank milk with average herd size as continuous covariate. Points represent the seventeen studies that matched the inclusion criteria in the systematic review. Bubble size is in relation to the weight of each primary study.