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1 **Comparative diagnostic potential of three serological tests for abortive Q fever in goat**
2 **herds**

3 *Serological tests for Q fever abortion*

4

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22 **Abstract**

23 Performances of an ELISA, an immunofluorescence assay (IFA) and a complement fixation
24 test (CFT) were assessed for detecting antibodies against *Coxiella burnetii* after Q fever
25 abortions in naturally infected goats. The goal of the study was to provide information useful
26 for veterinary serodiagnosis in regard to categories of goats either experiencing Q fever
27 abortion or not, blood sampling times and recommended cut-offs. The study was conducted
28 on 8 goat herds with evidence of *C. burnetii* abortions. In each herd, at least 5 goats that had
29 aborted and 10 goats prior to parturition or at term were monitored 15, 30 and 60 days (D15,
30 D30, D60) after the onset of Q fever abortion. The overall CFT results distribution did not
31 differ between the two groups of goats and showed poor agreement with the ELISA results. In
32 contrast, the ELISA and IFA results revealed comparable significant differences, but overall
33 the ELISA test was slightly more sensitive than the IFA test. Seroprevalence, according to
34 ELISA and IFA respectively, was higher in the aborting (88% and 82%) than in the non-
35 aborting group (60% and 50%). High levels of serum antibodies were detected in goats post-
36 abortion with an average of 114 %OD using ELISA and a $\log_{10}(\text{titer})$ of 2.4 using IFA.
37 Strongly positive ELISA (%OD>80) and positive IFA results ($\log_{10}(\text{titers})>1.9$) were
38 significantly associated with abortion. Sampling on D15 gave the best association with ORs
39 of 10 for ELISA and 6 for IFA. The practical interest of these results is discussed.

40

41 Keywords: Q fever, *Coxiella burnetii*, goat, abortion, serological test

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43 Introduction

44 Q fever is a worrisome zoonosis caused by *Coxiella burnetii*, an obligate intracellular
45 bacterium that can also survive in spore-like form with high resistance to environmental
46 conditions. Human infection is asymptomatic or apparent as a flu-like syndrome. Recovery is
47 spontaneous but may lead to a broad spectrum of manifestations, that may be severe when
48 chronic (Raoult et al., 2005; Parker et al., 2006). Most human cases have been related to
49 airborne infection from cattle, sheep and goat, followed by pets and occasionally birds.
50 Virtually all species can be asymptomatic carriers and shed bacteria in various secretions and
51 excreta (Lang, 1990; Arricau-Bouvery et al., 2005).

52 In goat herds, Q fever has been associated with variable frequencies of abortion ranging
53 from a few cases to more than 90% (Palmer et al., 1983; Arricau-Bouvery and Rodolakis,
54 2005). *C. burnetii* infection is still considered a common cause of caprine abortion in several
55 countries (Hatchette et al., 2001; Moeller, 2001; Chanton-Greutmann et al., 2002; Masala et
56 al., 2004; Parisi et al., 2006). However, diagnosis is currently hindered by considerable
57 ambiguity in results interpretations that does not encourage systematic research and
58 complicates sanitary surveillance and active measures. Ideally, diagnosis of the etiological
59 agent requires taking samples from aborting animals (placenta, vaginal discharges) and from
60 aborted fetuses (liver, lung or abomasum contents) and sera during the actual abortion event.
61 Differential diagnosis involves microbiological and serological examination for the major
62 infectious causes of abortion (Palmer et al., 1983; Schopf et al., 1991; Moeller, 2001).

63 Direct diagnosis usually consists of modified Ziehl-Neelsen staining of placental or fetal
64 sample smears (i.e., Stamp or Gimenez). The samples analysed only permit a presumptive
65 diagnosis due to lack of specificity with *Chlamydomphila* and *Brucella* species (Rousset et al.,
66 2004). The sera are usually tested by complement fixation test (CFT), indirect
67 immunofluorescence assay (IFA) or enzyme-linked immunosorbent assay (ELISA) (Rousset

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68 et al., 2004). Evaluation of coxiellosis serodiagnosis in goats has usually been for serologic
69 surveys rather than for clinical diagnosis (Behymer et al., 1985; Lang, 1988; Soliman et al.,
70 1992). High seroprevalence or prevalence of strong serological levels were observed in goat
71 herds affected by Q fever abortions (Palmer et al., 1983; Schopf et al., 1991; Sanford et al.,
72 1994; Hatchette et al., 2003; Berri et al., 2005; Berri et al., 2006). However, a relationship
73 between abortion and response level of *C. burnetii* antibodies could only be suggested. In
74 contrast, diagnostic test cut-offs have been defined in human Q fever serology (Tissot-Dupont
75 et al., 1994; Fournier et al., 1998). The signs and symptoms of the disease are not specific and
76 the need for serology in the routine primary clinical diagnosis of Q fever in humans has been
77 clearly established (Fournier et al., 1998).

78 The goal of the present study was to assess the response of *C. burnetii* antibodies,
79 obtained by three different serological tests, used in veterinary serodiagnosis, after Q fever
80 abortions in several naturally infected goat herds. As ELISA methods supplement other
81 methods, an ELISA test was compared with two other commercial tests, an IFA and a CFT.
82 The serological results, obtained for goats that aborted and goats that did not abort, were
83 compared according to cut-offs recommended by the manufacturers and blood sampling times
84 after the Q fever abortive episode.

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85 **1. Material and methods**86 *1.1. Study design*87 *1.1.1. Selection of goat herds*

88 Each herd was selected after a differential diagnostic investigation following the
89 occurrence of at least five abortions. Stamp staining was used to detect *Coxiella burnetii*,
90 *Chlamydophila abortus* and *Brucella melitensis* on at least one placenta and/or aborted foetal
91 tissues as already described (Rousset et al., 2004). The five aborted goats were sampled for
92 serological analysis for chlamydiosis, brucellosis and Q fever. The CHEKIT Q-fever ELISA
93 kit (Idexx laboratories, Broomfield CO., USA), according to the manufacturer's instructions,
94 was used by the county laboratories. The inclusion criteria for herds with Q fever abortions
95 were therefore based on positive *C. burnetii* Stamp staining, seropositive results with
96 CHEKIT Q-fever ELISA and absence of reliable evidence for the other infectious agents
97 tested. Herds showing multi-infections evidence of abortions were not investigated.

98 The study was then conducted on 8 dairy goat herds with abortion cases attributed only to
99 Q fever (Tab. 1). These herds originated from 6 French departments and varied in size.
100 Abortions had always occurred at the end of gestation and both primiparous and multiparous
101 goats were concerned. About 20% or more of the reproductive females had aborted in five
102 herds, indicating a clinical enzootic Q fever episode. Neither Q fever events nor vaccination
103 against Q fever have been notified in these herds during the three previous years, according to
104 historical records of veterinarians.

105 *1.1.2. Selection of goats and sera collection*

106 The goats selected in each herd included the five Q fever aborting goats, five goats at full
107 term gestation and five that were in the final month of gestation (Tab. 1). The ratio of 1
108 aborting to 2 non-aborting goats per flock was not strictly respected because of difficulties in

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109 systematically finding pre- or post-partum goats around the same date as the abortions.
110 Moreover, 5 of 35 animals that were still pregnant at D15 aborted less than one week later.

111 A veterinarian visited each farm three times (15, 30 and 60 days post-abortion i.e.D15,
112 D30 and D60), based on the first period of Q fever abortions. Selected goats were bled from
113 the jugular vein into vacutainers. In total, 354 blood samples were collected: 145 from the 50
114 aborting goats and 209 from 70 goats that delivered normally.

115 *1.2. Indirect diagnostic tests*

116 Sera were obtained by centrifuging the blood samples at 1500 g for 20 minutes and were
117 stored at -20 °C before testing for *C. burnetii* antibodies. Three different assays were used as
118 already described in detail (Rousset et al., 2004). Internal positive and negative laboratory
119 control sera were used to control each test run.

120 *1.2.1. ELISA*

121 The ELISA CHEKIT Q-fever test was performed according to the manufacturer's
122 instructions. Briefly, the ELISA antigens consist of Phases I and II from the Nine Mile strain
123 of *C. burnetii*. Sera were prepared at 1:400 dilution and specific antibodies were measured
124 using a peroxidase-labeled anti-ruminant immunoglobulin G (IgG) conjugate. Results were
125 expressed as a percentage of the optical density reading of the test sample (%OD) calculated
126 as $\%OD = 100 \times (S - N) / (P - N)$ where S, N and P are the OD values of the test sample, the
127 negative and positive controls, respectively. Sera were considered to be ELISA negative if
128 $\%OD \leq 40$, dubious if $40 < \%OD \leq 50$, positive if $50 < \%OD \leq 80$ and strongly positive if
129 $\%OD > 80$.

130 *1.2.2. IFA*

131 An indirect immunofluorescence assay (IFA) (*Coxiella burnetii* Spot IF, Bio-Merieux SA,
132 Marcy-l'Etoile, France) was carried out as recommended by the manufacturer but was adapted
133 to goat sera by using a fluorescein isothiocyanate-conjugated rabbit affinity-purified anti-goat

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134 IgG (Jackson Immunoresearch Lab. Inc., West Grove, PA, USA). The *C. burnetii* Spot IF
135 antigen was a mix of Henzerling and Nine Mile strains in Phase II obtained after culturing the
136 bacteria on Vero cells. Sera were considered negative if the titer was <80 , dubious if it was
137 $=80$ and positive if the titer was >80 . When appropriate, the obtained titers were converted
138 into $\log_{10}(\text{titers})$. The IFA titers of 40, 80, 160, 320 and 640 were ranged in $\log_{10}(\text{titers})$ of 1.6,
139 1.9, 2.2, 2.5 and 2.8, respectively.

140 1.2.3. CFT

141 The serum samples were also tested by complement fixation test (CFT). Briefly, the *C.*
142 *burnetii* antigen provided by Symbiotics Europ (Lyon, France) was a mixture of Henzerling
143 and Nine Mile strains in Phase II, produced on embryonated eggs. Any sample with CFT
144 titer <10 was considered negative, titer $=10$ was dubious, titer $=20$ or 40 was positive, and
145 titer >40 was strongly positive (Rousset et al., 2004). When appropriate, the obtained titers
146 were converted into $\log_{10}(\text{titers})$. The CFT titers of 10, 20, 40, 80, 160, 320 were ranged in
147 $\log_{10}(\text{titers})$ of 1, 1.3, 1.6, 1.9, 2.2 and 2.5, respectively.

148 1.3. Statistical analyses

149 The ELISA, IFA and CFT test results were first analyzed separately. Wilcoxon rank sum
150 tests were used to search for differences between aborting and non-aborting animals as
151 indicated by the ELISA %OD distributions and the IFA and CFT $\log_{10}(\text{titer})$ distributions.
152 Wilcoxon signed rank tests (paired samples) were used to explore the time trends of the
153 ELISA %ODs and of the IFA and CFT titers within the aborting and non-aborting animal
154 categories. The individual result series (three per animal and per test) were then converted
155 into “serological profiles”, for each serological test, using the positive cut-off: “+++”, animals
156 that had remained above this cut-off throughout the study: “---”, animals that had remained
157 below this cut-off throughout the study; and “+/-”: other dynamics comprising sequences such

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158 as “-++”, “--+”, “+-+”, “++-”, “-+-” and “+-+”. The distributions of these serological profiles in
159 aborting and non-aborting animals were computed and compared.

160 The ELISA results were then compared with the IFA and CFT results. The qualitative
161 agreement was assessed by computing the kappa coefficient. The kappa values were
162 interpreted according to the usual scale (Landis and Koch, 1977). The quantitative agreement
163 was evaluated using linear regressions, the independent variable being the ELISA %OD and
164 the dependent variable the IFA (or CFT) titer.

165 Finally, the association between abortion and serological results was explored by
166 computing the proportions of strongly positive (ELISA and CFT) or positive (IFA)
167 serological test results in aborting- and non-aborting goats and the associated odds-ratio (OR).

168 The statistical analyses were done with R (Ihaka and Gentleman, 1996).

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169 **2. Results**170 *2.1. ELISA results*

171 The average ELISA value was 68 %OD (95% confidence interval [CI]: 60–77) for
172 samples taken from non-aborting goats. In comparison, the average ELISA value for sera
173 from aborting animals was 114 %OD (95% CI: 104–125). The negative–dubious cut-off (40
174 %OD) was clearly apparent on the density plot of the ELISA results for non-aborting goats,
175 giving the curve a bimodal shape (Fig. 1). The strongly positive cut-off (80 %OD) was also
176 apparent on this curve, but less markedly. Conversely, in samples taken from aborting goats,
177 these cut-offs were apparent on the density plot as local maxima instead of local minima. The
178 two curves overlapped to give large areas of equivocal results on either side of the cut-off
179 line. Nevertheless, a significant difference between the two %OD distributions was found,
180 both globally (Fig.1) and when the distributions obtained for each of the three sampling series
181 at D15, D30 and D60 post-abortion episode were compared separately (Fig. 2). A significant
182 decrease in %OD with time, from 126 %OD (95 % CI: 106–147) at the second week to 103
183 %OD (95 % CI: 87–119) at the second month, was observed in the aborting goats. The
184 decrease in %OD level for the non-aborting goats was only significant for the D30–D60
185 transition (Fig. 2).

186 Sixty percent of the samples from non-aborting goats and 88 % from aborting goats were
187 positive (Tab. 2). These percentages were significantly different (Fisher's exact test:
188 $p < 0.0001$). Moreover, strongly positive sera were obtained for 78 % of the aborting goats and
189 two times less for non-aborting goats (35 %). The serological profiles of the aborting and non-
190 aborting animals, considered individually, were significantly different (Fisher's exact test:
191 $p = 0.0003$) (Tab. 3). In particular, 30 % (21/70) of the non-aborting goats remained negative
192 or dubious throughout the study compared to 8 % (4/50) of the aborting goats. These four
193 aborting goats came from three different herds; two of them were primiparous and two were

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194 in their second parturition. Seronegativity was observed in 9 % (6/70) of non-aborting goats
195 and 2 % (1/50) of aborting goats. Seroconversion profiles were obtained for 11 % (8/70) of
196 non-aborting goats and 4 % (2/50) of aborting goats.

197 The overall ELISA results showed significant differences between the two goat
198 categories.

199 2.2. IFA results

200 The average \log_{10} (titer) was 1.9 (95 % CI: 1.7–2.0) for the samples from non-aborting
201 goats and 2.4 (95 % CI: 2.3–2.5) for those from aborting goats. A two titer difference is
202 commonly considered significant when using IFA. The difference between the two
203 distributions, as with the ELISA, was significant for total samples (Fig. 1) and also for each of
204 the three sample series at D15, D30 and D60 (Fig. 2). The time trends obtained for IFA were
205 similar to those observed for ELISA, with a global decrease in titer for the aborting animals.
206 This decrease was only significant between D30 and D60 for the non-aborting animals
207 (Fig.2).

208 Fifty percent of the samples from non-aborting goats and 82 % from aborting goats were
209 positive (Tab. 2). These percentages were significantly different (Fisher's exact test:
210 $p < 0.0001$). The serological profiles of the aborting and non-aborting animals, taken
211 individually throughout the study, were also significantly different (Fisher's exact test:
212 $p = 0.0001$), as 38 % (27/70) of the non-aborting goats and 12 % (6/50) of the aborting goats
213 remained negative throughout the study (Tab. 3). Seronegativity occurred in 17 % (12/70)
214 of non-aborting goats and 12 % (6/50) of aborting goats. Three percents (2/70) of non-
215 aborting goats seroconverted. No seroconversion profile was observed in aborting goats.

216 Thus, the IFA results showed significant differences between the two goat categories.

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217 *2.3. CFT results*

218 Although the average $\log_{10}(\text{titer})$ of samples taken from both non-aborting animals (0.9,
219 95 % CI: 0.8–1.0) and aborting animals (1.0, 95 % CI: 0.9–1.1) was low and the
220 corresponding distributions apparently similar, the difference between these two distributions
221 was significant (Fig. 1). However, no significant difference was found when the distributions
222 obtained for each sample series were compared separately (Fig. 2). Also, no significant time
223 trend was observed in the CFT titers of non-aborting goats. Although the average titers for the
224 aborting goats decreased, the variance was too high for this trend to be significant (Fig. 2).

225 Forty percent (83/209) of the samples obtained from non-aborting goats were positive or
226 strongly positive as well as 50 % (72/145) from aborting animals (Tab. 2). High titers were
227 evenly distributed in 7 aborting (5 %) and 4 non-aborting goats (1 %). The results were
228 dubious for approximately one third (126/354) of the samples. The serological profiles of the
229 aborting and non-aborting animals were not significantly different (Tab. 3). The proportions
230 of animals that remained negative or dubious throughout the study were similar (36 % for the
231 non-aborting goats and 32 % for the aborting goats). Seronegativity profiles were observed
232 in 16 % (11/70) of non-aborting goats and 24 % (12/50) of aborting goats. Seroconversion
233 profiles were observed in 19 % (13/70) of the non-aborting goats and 10 % (5/50) of the
234 aborting goats.

235 No significant difference of interest between the two goat categories was observed.

236 *2.4. ELISA–IFA tests comparison*

237 The proportion of positive samples obtained with IFA was lower than with ELISA, but the
238 difference was not significant for either non-aborting or aborting goats. Similarly, no
239 significant difference was found between the IFA and ELISA serological follow-up profiles
240 for either goat category (Mc Nemar's tests, $p \geq 0.05$, Tab. 3).

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241 The linear regression results showed a parallel increase in ELISA %ODs with IFA titers
242 (Fig. 3). The slope values obtained for aborting- and non-aborting goats, (24.0 and 21.2,
243 respectively), were similar. An overall “good” agreement was obtained between ELISA and
244 IFA results ($\kappa=0.63$, 95 % CI: 0.55–0.72). This agreement was 0.62 for non-aborting
245 goats (95 % CI: 0.51–0.72) and 0.54 for aborting goats (95 % CI: 0.33–0.75) (Tab. 2). Most
246 of the discordant test results were ELISA-positive and IFA-negative or dubious (43/120), and
247 few ELISA-negative or dubious and IFA-positive sera were observed (14/120) (Tab. 2). Ten
248 of these latter sera were obtained from non-aborting animals. Six obtained from 6 goats
249 originating from 4 different herds were ELISA-dubious whereas the 4 other sera were all
250 below 40 %OD and obtained on D15 from pregnant goats in the same herd. No ELISA-
251 negative and IFA-positive sera were obtained from aborting animals. Four ELISA-dubious
252 and IFA-positive sera were obtained from aborting animals, all from 3 goats in 2 different
253 herds. This trend was confirmed in the serological profiles of each goat (Tab. 3). All goats
254 that gave a continuous ELISA-negative or dubious individual response throughout the study
255 also remained IFA-negative or dubious. In contrast, approximately one quarter of the goats
256 (8/33) in which the IFA profile was continuously negative or dubious were ELISA-positive at
257 least once.

258 Both tests gave similar results, but overall the ELISA test appeared slightly more sensitive
259 than the IFA test.

260 2.5. ELISA–CFT tests comparison

261 The proportions of CFT-positive samples obtained for aborting and non-aborting animals
262 were significantly lower than those obtained with ELISA (Fisher’s exact tests: $p<0.0001$, Tab.
263 2). Similarly, significant differences were observed between the CFT and ELISA serological
264 profiles, both in aborting goats (Mc Nemar’s test, $p<0.0001$) and non-aborting goats (Mc
265 Nemar’s test, $p=0.0004$, Tab. 3).

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266 The trends in the linear regression of ELISA %ODs against CFT titers for non-aborting
267 and aborting goats were divergent (Fig. 3). The slope was significantly greater than zero in
268 the former (although the lower bound of the 95% confidence interval was close to zero),
269 whereas it did not differ significantly from zero in the latter, despite the higher average CFT
270 titer.

271 The overall ELISA–CFT agreement was “poor” ($\kappa=0.17$, 95 % CI: 0.08–0.27), and
272 significantly lower than the ELISA–IFA agreement. A κ value of 0.21 (95 % CI: 0.08–
273 0.34) was obtained for the non-aborting animals, with 41 % of discordant sera, most of which
274 were ELISA-positive and CFT-negative. The κ value obtained for aborting animals (0.07,
275 95 % CI: -0.09–0.23) was not significantly greater than zero, with 47 % of discordant sera.
276 Again, most of the discordant sera were ELISA-positive and CFT-negative.

277 However, comparison of the serological profiles showed that a significant proportion of
278 goats that were ELISA-negative or ELISA-dubious during the study were CFT-positive at
279 least once: 7 of the 21 non-aborting animals, and 2 of the 4 aborting animals (Tab. 3).

280 Thus, most of the CFT results differed from those obtained with ELISA, the titers
281 measured were frequently very low and the sensitivity of CFT was apparently weaker than
282 that of ELISA.

283 *2.6. Relationships between abortion and serological test results*

284 A strong association was found between abortion and the occurrence of strongly positive
285 ELISA results (Tab. 4). However, the OR decreased rapidly from 10.2 at D15 to 3.7 at D60.
286 A similar association was found between the occurrence of positive IFA results and abortion,
287 the OR decreasing from 5.6 at D15 to 4.6 at D60. At D15, 84 % of the aborting goats gave
288 strongly positive results with ELISA, and 88 % were positive with IFA. No association was
289 found between strongly positive CFT results ($\text{titer}>40$) and abortion. The OR values were not
290 significantly higher than 1 whatever the time interval.

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291 Only one of the non-aborting goats remained negative with ELISA, IFA and CFT at D15,
292 D30 and D60. All aborting goats yielded at least one dubious or positive result in one of the
293 three tests. The individual serological follow-up CFT results indicated a large number of
294 seronegativation and seropositivation profiles. In ELISA and IFA, seronegativation and
295 seroconversion were observed mostly in the non-aborting goats.

296 Four aborting goats remained ELISA-negative or dubious throughout the study. All four
297 animals also remained IFA-negative or dubious, but each was CFT-positive at least once
298 (Tab. 4). Two were CFT-positive (titer: 20) at D15 and dubious at D30, the other two gave a
299 single dubious CFT result (one at D15, the other at D30).

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300 **3. Discussion**

301 Q fever serology is used for different veterinary research objectives throughout the world
302 but is far from being standardized. In this study, three different serological methods were
303 assessed for Q fever diagnosis in naturally-infected goat herds using the recommended cut-
304 offs values. Sampling was based on a strategy often used to characterize an abortion problem.
305 Statistical comparison of the serological results obtained from aborting and non-aborting
306 goats, originating from eight different flocks, revealed significant differences between the
307 three tests.

308

309 The results clearly showed that CFT was less performant than ELISA and IFA. The
310 percentage of seropositive animals was higher with the ELISA and IFA tests. A large
311 proportion of dubious CFT results were observed, i.e. 71 % (89/126), whereas these same sera
312 gave positive results with ELISA. Moreover, no association was found between positive (or
313 strongly positive) CFT results and Q fever abortion. Finally, no trend was apparent in the
314 serological response over time to distinguish between aborting and non-aborting goats.

315 The antigen might be responsible for defects in antibody-binding capacity and thus for the
316 poor test performance. However, the poor sensitivity of several CFT methods had already
317 been demonstrated in serologic evaluations of human Q fever (Peter et al., 1987; Peter et al.,
318 1988; Cowley et al., 1992). Firstly, CFT failed to detect some cases when anti-complementary
319 substances were present in the tested sera. Secondly, some antibodies were not revealed by
320 CFT because of differences in ability of the IgG subclasses to activate the complement. In
321 ruminants, only IgG1 antibodies are known to fix the complement in CFT (Micusan and
322 Borduas, 1977; Schmeer, 1985). Moreover, CFT titers may be reduced because the presence
323 of IgG2 and IgM antibodies can suppress complement fixation by IgG1 antibodies (Schmeer,
324 1985). Our results demonstrated that the CFT technique used gave incorrect negative results,

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325 too many uninterpretable results and would not be suitable for the serological investigation of
326 Q fever dynamics.

327

328 There was good agreement between ELISA and IFA, both qualitatively, as indicated by
329 kappa coefficient, and quantitatively, as shown by the linear relation between IFA titers and
330 ELISA %ODs. In addition, monitoring of the serological profiles showed that the same 25
331 goats were seronegative with both tests. It should be noted that a 100% specificity for this
332 ELISA kit was showed with sera collected from goats, that were not infected by *C. burnetii*
333 and monitored in a controlled environment (Arricau-Bouvery et al., 2005). Moreover,
334 specificity as well as serological cross-reactions between antibodies against *C. burnetii* and
335 against *Chlamyphila* were checked before commercialisation of this ELISA test by the
336 manufacturer (personal communication).

337 Nevertheless, ELISA seems to show slightly higher sensitivity than IFA. Firstly, all
338 animals that were IFA-positive at least once were also ELISA-positive at least once.
339 Secondly, 22 % (6/27) of the animals that remained IFA-negative or dubious throughout the
340 study were ELISA-positive at least once.

341

342 The ELISA %ODs and IFA titers in the aborting group decreased over time whereas this
343 trend was less marked in the non-aborting group. ELISA was best able to detect the
344 persistence of high level specific antibodies response in aborting goats during the test period.
345 Interestingly, this ELISA kit was used to monitor Q fever humoral response in sequential
346 serum samples taken over 11 weeks post-inoculation from pregnant goats experimentally-
347 infected with *C. burnetii* (Arricau-Bouvery et al., 2005). The mean antibody %OD increased
348 to around 100 %OD about 2 weeks after the abortions. The ELISA level obtained under our
349 field conditions was in agreement with data obtained under experimental conditions.

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350 Although technical differences between the ELISA and IFA methods must be taken into
351 account, the major difference between these two commercial tests is the *C. burnetii* antigen.
352 The importance of the choice of antigen in Q fever serology has already been reported
353 (Kovacova et al., 1996; Kovacova et al., 1998). The ELISA detects anti-Phases I and II
354 antibodies whereas IFA detects only anti-Phase II antibodies. In human Q fever serodiagnosis
355 and therapeutic follow-up, the gold standard method is a household IFA (Tissot-Dupont et al.,
356 1994; Fournier et al., 1998). Serum samples are measured for both specific immunoglobulin
357 M (IgM) and G (IgG) antibodies against either Phase I or Phase II antigens. Anti-Phase II
358 antibodies predominate after the primary infection and persist throughout the course of
359 infection, whereas higher titers of antibodies to the Phase I antigen are present only in the
360 chronic form of the illness. As Phase II antibodies are present in all stages of infection,
361 screening for epidemiological purposes is based on the IFA detection of anti-Phase II
362 antibodies. The typical serological profiles of Q fever in ruminants are largely unknown. The
363 tests available are based on the Phase II antigen or a mix of antigen Phases but are unable to
364 discriminate between the different antigen antibodies.

365 Interestingly, the IFA used for human diagnosis was applied to twenty-four goats from the
366 same farm during the next two kidding seasons following a Q fever outbreak (Hatchette et al.,
367 2003). Phase I antibody levels were maintained at high levels whereas the Phase II antibody
368 levels declined. Thus, anti-Phase I antibodies could be serological indicators after an outbreak
369 of Q fever abortions. Taken together, the differences between ELISA and IFA results
370 observed in our study could be mainly explained by the level of anti-Phase I antibodies.

371

372 This study clearly demonstrated the existence of a relationship between abortion and the
373 occurrence of strongly positive ELISA or positive IFA serological test results. Thus, these
374 cut-offs should be used for interpretation for diagnosis respecting the analysis of several sera

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375 sampled from aborting goats. Importantly, this association is strongest when sera are obtained
376 from goats 15 days after the abortions. It is thus recommended to take and test serum samples
377 15 days after abortions rather than later.

378

379 However, no IFA- or ELISA-positive results were observed in four aborting animals. Two
380 of these gave a single transiently CFT-positive result suggesting that the role of *C. burnetii* in
381 these four abortions was questionable. In addition, high response levels were obtained in both
382 ELISA and IFA, for approximately one third of non-aborting goats. These data suggest that
383 pathological states other than abortion, such as respiratory affections, might be associated
384 with *C. burnetii* (Arricau-Bouvery et al., 2005; Sanchez et al., 2006). Also, as reported long
385 ago, high colonization of the placenta does not always induce abortion (Welsh et al., 1951).
386 Welsh demonstrated the presence of 10^9 bacteria per gram of placenta from a ewe after
387 normal parturition, but no similar data are available in goats. Evaluation of serological tests
388 for Q fever should be improved by a better understanding of *C. burnetii* pathogenesis.

389

390 In this study, the serological analysis of goats that delivered normally indicated
391 considerable circulation of *C. burnetii* within the herds, as demonstrated by the high
392 seroprevalence and the serological profiles. Relatively few non-aborting goats remained
393 negative or dubious throughout the study, and most of them tested positive at least once. The
394 single goat that constantly remained seronegative in all tests was a goat that delivered healthy
395 kids. Our study clearly showed that CFT should not be used for serological screening, because
396 of its low sensitivity. Performances of the ELISA and IFA commercial tests were comparable.
397 However ELISA is apparently more sensitive than IFA, requires a single dilution of sera and
398 can be automated. Thus, ELISA should be preferable to IFA for practical reasons and because
399 of its slightly higher sensitivity. The negative–dubious cut-off was clearly apparent on the

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400 ELISA %ODs distribution for the non-aborting animals. The bimodal shape of the curve
401 around 40 %OD suggests that the corresponding biological state was unstable, as antibody
402 rates were rapidly rising (or decreasing) around this threshold. Thus when ELISA is used as a
403 serological test for screening, the negative–dubious cut-off should be preferred to the
404 dubious–positive cut-off. However, because our study was conducted in herds in which
405 enzootic abortion had been reported, the results cannot be transposed to other epidemiological
406 contexts, in particular to assign Q fever-free status to a herd. Work is needed to evaluate the
407 sensitivity and accuracy of the tests on sera from herds with unapparent Q fever infection.

408

409 In conclusion, none of the three tests can be used to accurately discriminate an abortion
410 case from a normal delivery at the individual level. Occurrence of strongly positive ELISA
411 results or positive IFA results in several aborting goats can constitute a strong presumption for
412 the etiological diagnosis of abortions at the herd level. However, it remains imperative to
413 perform bacteriological analyses for confirmation of this presumption. The descriptive and
414 epidemiological study reported here showed an extremely wide circulation of *C. burnetii* in
415 herds experiencing serial abortions resulting in numerous strongly positive serological results
416 in non-aborting goats. Complementary studies should thus be carried out to assess the
417 specificity of serological tests for etiological diagnosis of abortion. In particular, anti-*C.*
418 *burnetii* serological responses should be analysed in herds where abortions appear enzootic
419 but are caused by other pathogens than *C. burnetii*. Similar studies are also needed in herds
420 where abortions occur sporadically.

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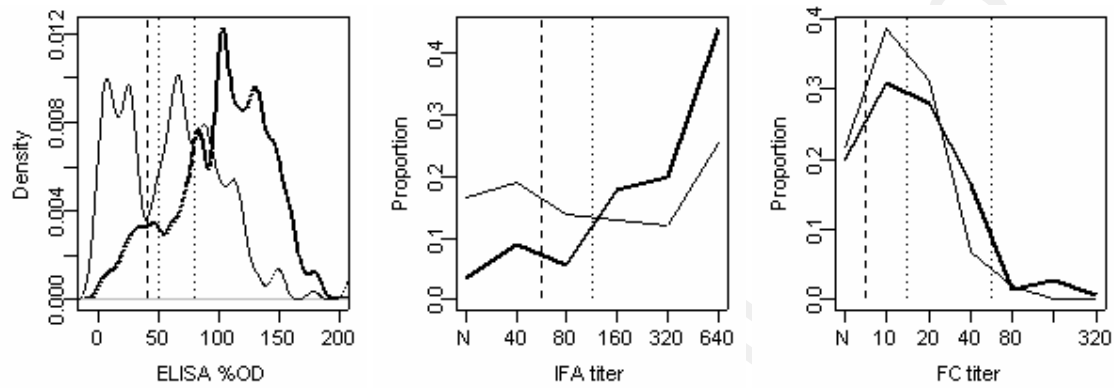
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Figure 1

Distributions of the Q fever ELISA %ODs (left), IFA titers (middle) and CFT titers (right) for non-aborting (thin line) and aborting (thick line) goats tested for antibodies against *C. burnetii*. Dashed lines: negative–dubious cut-off; dotted lines: dubious–positive and positive–strongly positive (ELISA and CFT only) cut-offs.



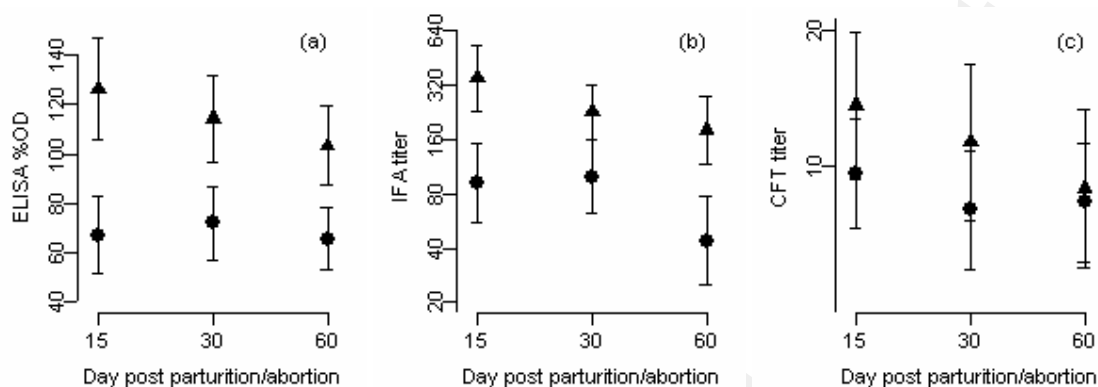
Wilcoxon rank sum tests: ELISA: $p < 0.0001$; IFA: $p < 0.0001$; CFT: $p = 0.02$

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Figure 2

Evolution of the average Q fever ELISA %OD (a), IFA titer (b) and CFT titer (c) for non-aborting (circles) and aborting goats (triangles) tested for antibodies against *C. burnetii*.

Vertical segments: 95% confidence interval of the mean.

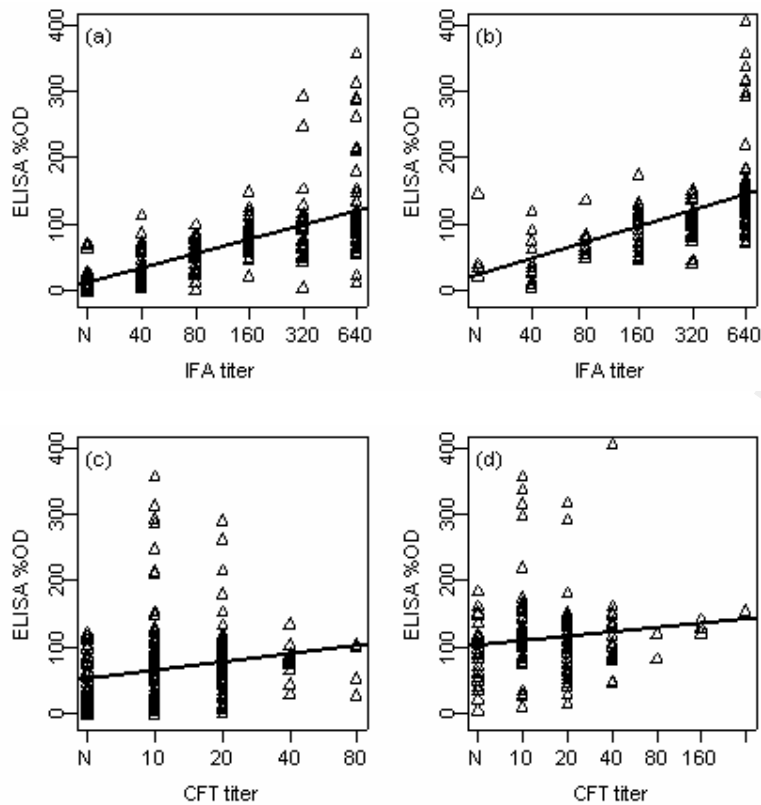


- (a) Wilcoxon signed rank (paired samples) tests: (i) aborting animals: D15–D30: $p=0.005$, D30–D60: $p=0.004$; (ii) non-aborting animals: D15–D30: $p=0.45$, D30–D60: $p=0.03$
- (b) Wilcoxon signed rank (paired samples) tests: (i) aborting animals: D15–D30: $p=0.0005$, D30–D60: $p=0.02$; (ii) non-aborting animals: D15–D30: $p=0.82$, D30–D60: $p=0.0004$
- (c) Wilcoxon signed rank (paired samples) tests: (i) aborting animals: D15–D30: $p=0.34$, D30–D60: $p=0.33$; (ii) non-aborting animals: D15–D30: $p=0.24$, D30–D60: $p=0.97$

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Figure 3

Comparison of ELISA %ODs with IFA titers (a, b) and with CFT titers (c, d) for non-aborting (a, c) and aborting (b, d) goats tested for antibodies against *C. burnetii*.



(a) Slope: 21.2, 95% confidence interval (CI): 17.8–24.6

(b) Slope: 24.0, 95% CI: 18.1–30.0

(c) Slope: 12.4, 95% CI: 3.8–21.0

(d) Slope: 6.4, 95% CI: -2.0–14.8

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Table 1

Characteristics of selected dairy goat herds affected by a Q fever abortion episode and number of selected goats.

Identification	Herds		Aborting goats			Number of selected goats
	Origin	Size	Multiparous (MP) or primiparous (PP) state	Number (%)	Gestation duration ^b	(aborting/non-aborting)
1801	Cher	N ^a	MP and PP	N	N	10/5
2601	Drôme	90	MP	N	4.7 to 5	5/10
4201	Loire	135	MP	40 (30%)	4 to 4.5	7/8
4901	Maine et Loire	110	MP and PP	24 (22%)	3.3 to 5	5/10
6401	Pyrénées Atlantique	39	PP	9 (23%)	4 to 5	5/10
7901	Deux Sèvres	132	PP	25 (19%)	3.7 to 4.7	7/8
7902	Deux Sèvres	200	MP and PP	71 (36%)	3.5 to 4.7	5/10
7903	Deux Sèvres	390	PP	30 (8%)	4 to 5	6/9

^a N: information not provided.

^b Gestation duration of aborting goats expressed in months.

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Table 2

Comparison of ELISA results to IFA or CFT results obtained in non-aborting and aborting goats tested for antibodies against *C. burnetii*.

	Titers	ELISA %OD ^a				Total
		≤40	40–50	50–80	>80	
Non-aborting goats						
Total		75 (36%)	9 (4%)	51 (25%)	74 (35%)	209 (100%)
IFA titer ^b	<80	60	2	11	2	75 (36%)
	80	11	1	12	5	29 (14%)
	>80	4	6	28	67	105 (50%)
CFT titer ^c	<10	28	1	7	9	45 (22%)
	10	31	2	22	26	81 (39%)
	20–40	15	6	21	37	79 (38%)
	>40	1	0	1	2	4 (1%)
Aborting Goats						
Total		12 (8%)	5 (2%)	15 (10%)	113 (78%)	145 (100%)
IFA titer ^b	<80	12		3	3	18 (12%)
	80	0	1	4	3	8 (6%)
	>80	0	4	8	107	119 (82%)
CFT titer ^c	<10	6	1	5	16	28 (19%)
	10	4	0	3	38	45 (31%)
	20–40	2	4	7	52	65 (45%)
	>40	0	0	0	7	7 (5%)

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^a %OD, percentage of the optical density. ELISA cut-offs: %OD \leq 40: negative, 40<%OD \leq 50:

dubious, 50<%OD \leq 80: positive, >80: strongly positive

^b IFA cut-offs: titer<80: negative, titer=80: dubious, titer>80: positive

^c CFT cut-offs: titer<10: negative, titer=10: dubious, titer=20 or 40: positive, titer>40:

strongly positive.

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Table 3

Q fever serological profiles obtained using ELISA, IFA and CFT in each non-aborting and in aborting goats tested for antibodies against *C. burnetii* at D15, D30 and D60 post-abortion episode.

	ELISA								
	Non-aborting animals				Aborting animals				
	Profiles	- - - ^a	+++ ^b	+/- ^c	Total	- - -	+++	+/-	Total
Total		21 (30%)	34 (49%)	15 (21%)	70	4 (8%)	42 (84%)	4 (8%)	50
IFA	- - -	21	4	2	27 (38%)	4	1	1	6 (12%)
	+++	0	21	4	25 (36%)	0	34	3	37 (74%)
	+/-	0	9	9	18 (26%)	0	7	0	7 (14%)
CFT	- - -	14	10	1	25 (36%)	2	13	1	16 (32%)
	+++	1	6	5	12 (17%)	0	13	2	15 (30%)
	+/-	6	18	9	33 (47%)	2	16	1	19 (38%)

^a - - - = Negative or dubious test result at D15, D30 and D60

^b +++ = Positive (or strongly positive) test result at D15, D30 and D60

^c +/- = Other serological profiles, i.e. -+ +, --+, +- -, ++-, +- - and +-+ at D15, D30 and D60

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Table 4

Evolution of the association between abortion and positive (IFA) or strongly positive (ELISA, CFT) test results in goats tested for antibodies against *C. burnetii*.

		Day post-abortion episode		
		15	30	60
ELISA %OD>80	Aborting goats	84%	78%	70%
	Non-aborting goats	33%	36%	38%
	OR (95% CI) ^a	10.2 (3.9–29.6)	6.3 (2.6–16.1)	3.7 (1.6–9.1)
IFA titer>80	Aborting goats	88%	82%	76%
	Non-aborting goats	56%	54%	41%
	OR (95% CI) ^a	5.6 (2.0–18.3)	3.8 (1.5–10.3)	4.6 (1.9–11.8)
CFT titer>40	Aborting goats	6%	6%	2%
	Non-aborting goats	3%	0%	3%
	OR (95% CI) ^a	2.2 (0.2–27.3)	NA ^b	0.7 (0–14.7)

^aOdds-ratio (OR) and 95% confidence interval (CI)

^bNot analysed (NA)