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

Serological tests for Q fever abortion

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

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

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1. Material and methods

1.1. Study design

1.1.1. Selection of goat herds

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

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

1.1.2. Selection of goats and sera collection

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

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

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

1.2. Indirect diagnostic tests

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

1.2.1. ELISA

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

1.2.2. IFA

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

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IgG (Jackson ImmunoResearch Lab. Inc., West Grove, PA, USA). The *C. burnetii* Spot IF antigen was a mix of Henzerling and Nine Mile strains in Phase II obtained after culturing the bacteria on Vero cells. Sera were considered negative if the titer was <80, dubious if it was =80 and positive if the titer was >80. When appropriate, the obtained titers were converted 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, 1.9, 2.2, 2.5 and 2.8, respectively.

1.2.3. CFT

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

1.3. Statistical analyses

The ELISA, IFA and CFT test results were first analyzed separately. Wilcoxon rank sum tests were used to search for differences between aborting and non-aborting animals as indicated by the ELISA %OD distributions and the IFA and CFT $\log_{10}(\text{titer})$ distributions. Wilcoxon signed rank tests (paired samples) were used to explore the time trends of the ELISA %ODs and of the IFA and CFT titers within the aborting and non-aborting animal categories. The individual result series (three per animal and per test) were then converted into “serological profiles”, for each serological test, using the positive cut-off: “+++”, animals that had remained above this cut-off throughout the study: “---”, animals that had remained 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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2. Results

2.1. ELISA results

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

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

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

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

2.2. IFA results

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

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

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

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2.3. CFT results

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

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

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

2.4. ELISA–IFA tests comparison

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

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

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

2.5. ELISA–CFT tests comparison

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

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

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

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

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

2.6. Relationships between abortion and serological test results

A strong association was found between abortion and the occurrence of strongly positive ELISA results (Tab. 4). However, the OR decreased rapidly from 10.2 at D15 to 3.7 at D60. A similar association was found between the occurrence of positive IFA results and abortion, the OR decreasing from 5.6 at D15 to 4.6 at D60. At D15, 84 % of the aborting goats gave strongly positive results with ELISA, and 88 % were positive with IFA. No association was found between strongly positive CFT results (titer>40) and abortion. The OR values were not 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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3. Discussion

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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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 (aborting/non-aborting)
	Origin	Size	Multiparous (MP) or primiparous (PP) state	Number (%)	Gestation duration ^b	
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									
Profiles	Non-aborting animals			Total	Aborting animals			Total	
	- - - ^a	+ + + ^b	+ / - ^c		- - -	+ + +	+ / -		
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)