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Coxiella burnetii excretion in cattle and small ruminants, respectively, during one and two successive calvings following Q fever infection in Guinea

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Abstract

This study examined the excretion of *Coxiella burnetii* (*C. burnetii*) in domestic ruminants and its association with reproductive disorders in Guinea. Blood samples and vaginal swabs were taken from 163 cattles (110 females), 131 goats (99 females), and 142 sheep (110 females) in four ecogeographical regions of Guinea, and then subjected to PCR analysis to determine the prevalence of Q fever. Clinical examinations identified females with reproductive problems. The dynamics of *C. burnetii* shedding were monitored in selected females over two consecutive calving seasons. After the first calving season, PCR tests identified shedding females among those that had calved. During the second season, similar monitoring was only possible in a subgroup of small ruminants. PCR analyses confirmed the shedding of *C. burnetii* in females that aborted or experienced stillbirth during their first pregnancy after infection, with rates of 42.86% in goats, 29.41% in sheep, and 21.87% in cattle, with no statistically significant difference between species (p = 0.348). This shedding was strongly linked to the peripartum period, with an intermittent pattern in cattle (an increase from 4 to 12 positive cases after the first kidding), a transient pattern in goats (no shedding at the second kidding), and a prolonged or recurrent pattern in sheep (an increase from 3 to 8 positive cases). These results confirm species-specific shedding dynamics and the central role of kidding in the dissemination of *C. burnetii*.

Introduction

Q fever is a widespread zoonotic disease caused by the intracellular Gram-negative bacterium *Coxiella burnetii* (*C. burnetii*) (1). It is a significant animal and public health concern, with domestic ruminants (such as sheep,

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goats, and cattle) acting as the primary reservoirs and sources of human infection (2). Following infection, female animals excrete *C. burnetii* into the environment during normal lambing or abortion through the amniotic fluid, placenta and fetal membranes, as well as through urine, milk and faeces (3). While the source of human infection is often unknown, sheep and goats are more frequently associated with human Q fever outbreaks than other animal species (4). Indeed, large quantities of bacteria excreted by infected female animals and those that have given birth normally (5, 6) contaminate humans and other animals through inhalation of contaminated aerosols or dust containing the microorganism excreted by infected animals (4). Therefore, it is necessary to gain a better understanding of the route, duration, and frequency of *C. burnetii* excretion to prevent contamination of humans and animals.

In livestock, *C. burnetii* can cause reproductive disorders, including abortions, stillbirths, premature births, retained placentas, and the birth of weak, non-viable offspring (7). Metritis and infertility due to *C. burnetii* infection are more prevalent in cattle than in other ruminant species (8). Further studies have determined that *C. burnetii* is responsible for abortions with a prevalence of between 1.67% and 11.6%, and other studies have confirmed the presence of postpartum metritis in Q fever infections, with a higher incidence observed in seropositive animals (9). More recently, a study strongly confirmed the role of *C. burnetii* in both clinical and subclinical endometritis, which can lead to low fertility or infertility in cows. The literature also mentions a link between placental retention and Q fever in cows (10).

Currently, there is limited information regarding the clinical signs of Q fever in domestic ruminants. Early 1950s experimental studies showed that Q fever-related epidemics in ruminants were more prevalent in goats and sheep than in cows; however, endemic infection in dairy cows is considered to reduce fertility (8). Pregnant ruminants are highly susceptible to infection and abortions only occur at the first calving after infection. The Subsequent pregnancies proceeded normally, with no reproductive problems. These studies also showed that *Coxiella* was excreted in high quantities in birth products during the first birth following infection. However, the bacteria were no longer present in the placenta or bodily secretions at the time of the second birth. While these earlier studies suggested that *C. burnetii* excretion was mainly limited to the first lambing, recent data showed a more complex dynamic, marked by intermittent excretion (11). Molecular studies conducted on a naturally infected flock of sheep showed that *C. burnetii* was excreted in the vaginal discharge of infected animals long after abortion (12). However, ewes can sometimes excrete the bacterium during subsequent pregnancies after infection with Q fever (13). Nevertheless, few studies followed the same females over several pregnancies to confirm this phenomenon. Studying excretion during two consecutive births therefore appears essential to clarify this contradiction, better understand the actual dynamics of transmission, and re-evaluate Q fever prevention strategies.

In Africa, there is very little data on evidence that *C. burnetii* is regularly excreted during successive births. A study conducted in Algeria found a statistically significant correlation between Q fever seropositivity and a history of placental retention and metritis in cows (14). The presence of *C. burnetii* was detected in 14 (19.1%) placental tissues collected from aborted and calved dairy cows in Algeria using molecular tools (15). Another study, conducted in Egypt, reported a single goat (0.9%) that tested positive for *C. burnetii* in both placental tissue and vaginal discharge out of 108 animals examined, while the prevalence of Q fever in goats is 3.4% (16). In the rural commune of Bama in Burkina Faso, Tialla *et al.* observed prevalence rates of 9.7%, 22.5%, 41.5% and 14.5%, respectively, associated with abortion, retained placenta, metritis, and stillbirth (17).

In Guinea, livestock farming is the second most important rural activity after agriculture. It provides income for 30% of the rural population and is mainly family-based and traditional. Most livestock roam freely all year round, except during the growing season. The livestock population consists almost exclusively of local breeds: N'dama cattle (99.9%), Djallonkés sheep (99.7%) and goats, which are characterised by their hardiness and

ability to adapt to their environment, make the most of natural pastures and are highly resistant to trypanosomiasis (18). According to estimates by the National Institute of Statistics of Guinea, Guinea's livestock population in 2019 amounted to 7, 932,749 cattle, 2, 889,899 sheep and 3, 464,562 goats (18). The number of cattle, goats, and sheep in Boké and Kankan is higher than in other regions. However, factors in the emergence and spread of Q fever could include the grouping of different animal species, the lack of identification of pregnant females, extensive farming practices combined with dry and windy climatic conditions, poor effluent management, and the presence of infected ticks (19).

To date, no studies have assessed the impact of *C. burnetii* on the reproduction of domestic ruminants in Guinea, and the available research has been limited to documenting the seroprevalence or prevalence of Q fever in cattle, goats, sheep, small mammals, and ticks (20-22). While these studies provided valuable information on the circulation of the pathogen, they do not allow us to understand the actual dynamics of its excretion, which is essential for anticipating high-risk periods and implementing prophylactic measures adapted to Guinea's extensive farming systems. In a context where the international literature reports sometimes contradictory patterns of excretion, ranging from excretion limited to the first calving to intermittent excretion, it appears essential to produce robust local data.

It is with this in mind that the present study, entitled « *Coxiella burnetii* excretion in cattle and small ruminants respectively during one and two successive calvings following Q fever infection in Guinea» was conducted. It aimed to examine the presence of *C. burnetii* in vaginal swabs collected during two consecutive pregnancies in sheep/goats and one pregnancy in cattle in order to determine whether the females exhibit a single or recurrent shedding status. This longitudinal approach is an essential step in filling the knowledge gap in Guinea and effectively guiding Q fever surveillance and prevention strategies.

Materials and Methods

Study area and survey of livestock farms

This study was conducted from January to December 2023 in four prefectures of Guinea: Beyla, Boké, Kankan, and Mamou. These prefectures are located in Forest Guinea, Lower Guinea, Upper Guinea, and Middle Guinea, respectively. The choice of study area is justified by its important role in livestock farming. The climatic conditions (temperature, humidity, wind and rainfall) in Lower Guinea, Middle Guinea and Forest Guinea favour the development and multiplication of the highly resistant bacterium *C .burnetii*, which can survive for long periods in the environment.

During the survey, information on livestock management in all towns was collected through direct observations on livestock farms, interviews with farmers, and the collection of biological material from cattles, goats, and sheeps. The questionnaire covered the following main points: the sociodemographic characteristics of the farmers; the zootechnical characteristics, such as assistance with calving and management of animal products (abortuses, placentas, and manure); and the history of reproductive disorders. The geographical coordinates of the surveyed households were recorded using the KoboCollect tool. With regard to animal reproductive health, demographic monitoring was carried out for 12 months on study animals to determine cases of reproductive disorders, such as those associated with Q fever. This survey involved reconstructing the demographics of the herd in the 12 months preceding the survey. The demographic parameters were chosen based on the study's objectives. Parameters such as the abortion rate, normal calving rate, stillbirth rate, placental retention rate, and metritis rate were determined.

Study design and sample size

Simple random sampling was used to collect samples in the study area. The target population consisted of cattle, sheep, and goats. A total of 436 blood samples and 323 vaginal swabs were collected fromcattle, sheep and goats randomly in 10 herds to confirm the presence of Q fever in each prefecture.

Blood samples were aseptically collected from the jugular vein of 163 cattle, 131 sheep, and 142 goats. The samples were then transported to the laboratory and stored at 4 °C.

Vaginal swabs were obtained by inserting a dry, sterile cotton swab into the animals' vaginas. A longitudinal study was conducted to assess the dynamics of *C. burnetii* excretion in female ruminants during two successive calving seasons. The study initially included 114 cows, 99 goats, and 110 ewes, selected based on their reproductive status and availability for prospective monitoring, regardless of whether they actually gave birth. Between January and March 2023, vaginal swab samples were collected from females in the monitored herd, and bacterial shedding was analyzed in those that had never calved but were pregnant. At the end of the first calving season (April–May 2023), the females that had actually calved were sampled. Among them, 48 cows (12 positive, 36 negative), 25 goats (12 positive, 13 negative), and 33 ewes (8 positives, 25 negative) were tested by PCR at first calving. During the second calving season, matched follow-up was possible for a subset of small ruminants only: 17 goats and 11 ewes had comparable results between the first and second calving, allowing for the assessment of the persistence, disappearance, or appearance of bacterial excretion. No matched follow-up could be carried out in cows due to the length of bovine gestation and the total duration of the study.

DNA extraction

DNA was extracted from the samples using the QIAmp DNA Tissue Kit (Qiagen S.A.) according to the manufacturer's instructions. Five microlitres of DNA solution were used in a total volume of 50 µl.

Detection of C. burnetii DNA by real-time PCR

Real-time PCR targeting the *IS1111* gene of *C. burnetii* was performed using the Biobase LEIA-X4 instrument for genomic amplification, according to the following protocol: initial denaturation at 95°C for 10 minutes, followed by 35- 45 cycles at 95°C for 15 seconds and 58°C for 1 minute. Each probe-independent real-time PCR reaction, performed in a final volume of 20 µl, contained 10 µl of Sso Advanced Universal SYBR Green Supermix (Bio-Rad, Italy), 6 µl of an IS1111 primer mixture corresponding to a final concentration of 0.3 µM for each primer (sense primer: 5′-CGGGTTAAGCGTGCTCAGTAT-3′; antisense primer: 5′-TCCACACGCTTCCATCACCAC-3′) (23), and 4 µl of purified DNA template. The data were analysed using Biobase real-time PCR software. We used double-distilled water as a negative control for all reactions, and DNA extracted from a *C. burnetii* strain previously characterised and stored in the laboratory's internal collection as a positive control. A cycle threshold (Ct) ≤ 35 was used for interpreting the results. Due to biosafety and importation constraints, an ATCC reference strain was not available.

Data analysis

Survey data were collected using the KoboCollect mobile application and transferred to KoboToolbox for processing. Microsoft Excel was used to store the data, and the statistical software R (version 4.1.2; R Core Team, 2021) was used to analyse it. The prevalence in cattle, sheep, and goats was calculated by comparing the number of PCR-positive animals with the total number of blood or vaginal samples tested. A chi-squared test was used to compare the prevalence of Q fever and the proportion of animals that excreted the bacteria during their first and second calving. A *p-value* of less than or equal to 0.05 was considered statistically significant.

Results

Detection of C. burnetii DNA in samples

The qPCR amplification profiles showed an expected sigmoid curve for the positive control and no amplification for the negative control, confirming the analytical validity of the method. The samples exhibited heterogeneous kinetics, with threshold crossing only for positive reactions, reflecting variable bacterial loads (Fig. 1).

The results of the PCR analysis of clinical samples are presented in Table 1. Positive amplification was obtained using primers that amplify transposon-like repetitive regions of *C. burnetii* from 221 of the 759 blood and vaginal swab samples (29.11%) analysed in this study (Table 1). Of the 436 animals examined, 132 blood samples (30.28%) were PCR-positive, including 59 of 163 cattles (36.2%), 42 of 131 sheeps (32.1%), and 31 of 142 goats (22.4%) (Table 1). The PCR positivity rate in cattle and sheep was significantly higher than in goats ($X^2 = 6.854$; $p = 0.008$).

Any locality in which a single animal tested positive by PCR was considered infected. All prefectures had at least one animal that tested positive for *C. burnetii* infection by PCR. The prevalence was similar in all localities except in Mamou, where a significant difference was observed (22.94%: 25/109) (p = 0.01276). The highest prevalence was observed in Beyla (39.45%: 43/109), and the lowest in Mamou (Table 1).

After PCR analysis of animal blood, the distribution of prevalence by sex revealed that males were significantly more likely to test positive for infection, with a prevalence of 38.05% [29.22–47.70] (43/113), compared to 27.55% [22.82–32.83] (89/323) for females ($X^2 = 3.888$; $p = 0.04863$).

About the influence of age on the susceptibility of ruminants to the bacterium, the rate of infection did not vary according to age group when the two fluids analysed were considered ($X^2 < 0.001$; $p = 1.00$) (Table 1).

The same trend was observed with vaginal samples, with an overall prevalence estimated at 27.55% (89/323), with significantly higher PCR positivity in cattle and sheep than in goats ($X^2 = 9.978$; $p = 0.001$) (Table 2). However, no significant difference was observed between the prevalence of young people and adults ($X^2 = 0.00049$; $p = 0.9822$).

Table 3 presents the monthly distribution of positive samples recorded in the three animal species. Analysis of the table reveals that almost *C. burnetii* positives were recorded between February and April corresponding to the first parturition period.

Effect of C. burnetii on animal reproductive performance

Analysis of the data in Table 4 (Supplementary file) shows that, of the 114 cows, 99 goats and 110 sheep examined, 42.11% (n = 48), 25.25% (n = 25) and 30% (n = 33), respectively, exhibited various symptoms of reproductive disorders. These symptoms were significantly more prevalent in cows than in the other species tested ($p = 0.0244$). Abortion was the most commonly observed symptom in cows (66.67%), followed by stillbirth (33.33%), with a significant difference between the two ($p = 0.0022$) (Table 4) (Supplementary file). However, the proportion of female goats and sheep that aborted (56% and 51.52%, respectively) was similar to the proportion that gave birth to stillborn offspring (44% and 48.48%, respectively) ($p > 0.05$).

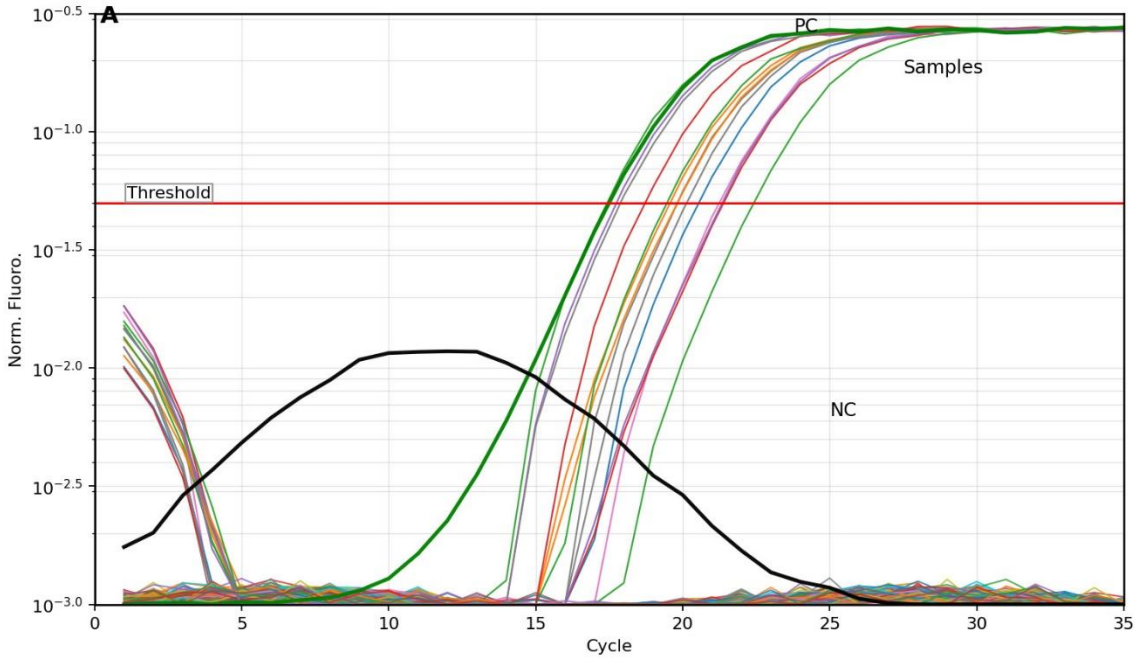

Fig. 1. Results of real-time PCR amplification of *C. burnetii*-positive tick samples. A Ct value < 35 cycles was considered positive for the presence of *C. burnetii*. PC = Positive control. NC = Negative control.

Table 1. Results of PCR tests carried out on blood samples from ruminants

		PCR blood test				
Modality	Variables	Number tested	Positive PCR (%)	95% CI	Chi-Square	*p* -Value
Prefectures	Beyla	109	43 (39.45)	[30.65 – 49.70]	-	-
	Mamou	109	25 (22.94)	[15.65 – 32.16]	6.20	0.0127
	Boke	109	35 (32.11)	[23.67 – 41.82]	0.978	0.3226
	Kankan	109	29 (26.61)	[18.81 – 36.07]	3,504	0.0611
	Total	436	132 (30.28)	[26.04 – 34.86]	N/A	N/A
Species	Cattle	163	59 (36.20)	[28.92 – 44.12]	-	-
	Sheep	131	42 (32.06)	[24.33 – 40.86]	0.382	0.5362
	Goats	142	31 (21.83)	[15.52 – 29.69]	6,854	0.0088
	Total	436	132 (30.28)	[26.04 – 34.86]	N/A	N/A
Gender	Female	323	89 (27.55)	[22.82 – 32.83]	3,888	0.0486
	Male	113	43 (38.05)	[29.22 – 47.70]		
	Total	436	132 (30.28)	[26.04 – 34.86]	N/A	N/A
Age	Young*	305	92 (30.16)	[25.13 – 35.70]	<0.001	1.00
	Adult**	131	40 (30.53)	[22.95 – 39.27]		
	Total	436	132 (30.28)	[26.04 – 34.86]		

NA : not applicable/ * Young: 12 – 42 months/** Adult : 43 – 92 months

Table 2. Results of PCR tests carried out on vaginal swab samples from ruminants

		PCR test of vaginal swabs				
Modality	Variables	Number tested	Positive PCR (%)	95% CI	Chi-Square	*p* -Value
Prefectures	Beyla	84	30 (35.71)	[25.76 – 46.98]	-	-
	Mamou	79	16 (20.25)	[12.36 – 31.09]	4.071	0.0436
	Boke	81	22 (27.16)	[18.14 – 38.36]	1.029	0.310
	Kankan	79	21 (26.58)	[17.55 – 37.91]	1.183	0.2767
	Total	323	89 (27.55)	N/A	N/A	N/A
Species	Cattle	114	40 (35.08)	[26.54– 44.65]	-	-
	Sheep	110	34 (30.90)	[22.63 – 40.53]	0.273	0.6012
	Goats	99	15 (15.15)	[09.00 – 24.07]	9.978	0.001
	Total	323	89 (27.55)	[22.82 – 32.83]	N/A	N/A
Gender	Female	323	89 (27.55)	[22.82 – 32.83]	N/A	N/A
	Male	NA	NA	NA		
	Total	NA	NA	N/A	N/A	N/A
Age	Young*	238	65 (27.31)	[21.85 – 33.51]	0.00049	0.9822
	Adult**	85	24 (28.23)	[19.26 – 39.20]		
	Total	323	89 (27.55)	[22.82 – 32.83]		

NA : not applicable/ * Young: 12 – 42 months/** Adult : 43 – 92

Table 3. Monthly distribution of samples examined during the survey period

Month	Species animals					
	Cattle		Goats		Sheep	
	Number examined	PCR positive	Number examined	PCR positive	Number examined	PCR positive
January	24	8 (33.33)	11	3 (27.27)	9	3 (33,33)
February	32	13 (40.63)	18	5 (27.78)	15	5 (33.33)
March	35	14 (40.00)	29	9 (31.03)	30	12 (40.00)
April	39	16 (41.03)	21	6 (28.57)	17	7 (41.18)
May	6	2 (33,33)	14	3 (21.43)	12	5 (41.67)
June	3	1 (33,33)	5	1 (20.00)	5	1 (20.00)
July	3	0.00	6	1 (16.67)	6	1 (16.67)

August	1	0.00	4	0.00	4	0.00
September	2	0.00	7	1 (14.29)	7	1 (14.29)
October	8	3 (37.50)	6	1 (16.67)	6	1 (16.67)
November	6	1 (16.67)	13	1 (7.69)	14	4 (28.57)
December	4	1 (25.00)	8	0.00	6	2 (33,33)
Total	163	59 (36.20)	142	31 (21.83)	131	42 (32.06)

Excretion of C. burnetii in vaginal secretions

Following the confirmation of an episode of Q fever in all herds, we opted to employ PCR analysis to examine the excretion of *C. burnetii* in vaginal samples post-calving. First, PCR tests were performed on vaginal mucus samples to confirm *C. burnetii* infection. Then, *Coxiella* excretion was studied before and during the following two calving seasons. A total of 323 samples were collected, including 114 from cows, 99 from goats, and 110 from sheep. After the first calving season, PCR tests showed that 21.87% (7/32) of cows, compared to 42.86% (6/14) of goats and 29.41% (5/17) of sheep, had excreted *C. burnetii* in vaginal swabs (*P = 0.3484*) (Table 5) (Supplementary file). In contrast, during the second calving season, none of the female goats that had aborted excreted the bacteria, compared to 27.27% (3/11) of sheep (Table 5). A total of 18 female animals excreted the bacteria vaginally during their first calving, compared to only three after their second calving (Table 5) (Supplementary file). However, approximately seven of the animals that gave birth to healthy calves, kids, and lambs and did not abort also tested positive by PCR. Overall, *C. burnetii* was heavily excreted during the first. Of the females that had stillbirths, 25% (4/16) of cows were still excreting the bacteria compared to 54.54% (6/11) of goats. After their first calving, 18.75% (3/16) of cows were PCR-positive. During the subsequent calving season, only one ewe tested positive for the bacteria using the PCR method (Table 5).

PCR tests revealed that 45% (9/20) of cows, 40% (4/10) of goats, and 27.77% (5/18) of sheep with retained placentae were positive for *C. burnetii*, compared with 20% (3/15) of cows, 34.61% (9/26) of goats, and 23.52% (4/17) of ewes without this history. A similar trend is observed in female animals with or without a history of metritis (Table 5) (Supplementary file).

Table 6 (Supplementary file) shows the transition between positive/negative status during two successive gestations in goats/sheep and one gestation in cattle. Analysis of the table reveals an increase in the number of positive animals after the first calving in all three species. In cows, positive cases increase from 4 before the first calving to 12 after the first calving, while 36 animals remain negative. A cow's gestation period lasts approximately 9 months, while the study lasted 12 months, leaving no data after the second calving.

In goats, positive females increased from 5 to 12 after the first calving, but 0 positive cases were observed after the second calving, with 6 females becoming negative and 11 remaining negative. In ewes, positive cases increased from 3 to 8 after the first calving, of which 4 remained positive after the second calving, 4 became negative, while 1 female that was initially negative became positive, and 2 remained negative (Table 6) (Supplementary file).

Discussion

This study highlights the shedding of *C. burnetii* in domestic ruminants, strongly influenced by the peripartum period and exhibiting distinct dynamics depending on the species. Shedding appeared more pronounced and concentrated in small ruminants, whereas it was more intermittent in cattle, underscoring their differing roles in the dissemination of the agent. These results confirm the value of PCR for identifying shedding animals and better understanding the spread of *C. burnetii* within herds and the associated zoonotic risk.

Although there is no standardised reference test for Q fever, the main diagnostic methods for domestic ruminants are polymerase chain reaction (PCR) and enzyme-linked immunosorbent assays (ELISA). PCR can distinguish between the causes of reproductive disorders (e.g. abortion, metritis, retained placenta, infertility, and stillbirth) associated with Q fever, while ELISA is considered reliable for seroprevalence screening. The latter only detects carriers of antibodies against *C. burnetii*, indicating previous exposure to the pathogen, but not current excretion in the herd (24). In the present study, PCR was considered the reference test for diagnosing these reproductive disorders. A positive PCR result on a vaginal mucus sample indicates the presence of *C. burnetii* in the herd in question. Similarly, as our objective was to evaluate the characteristics of Q fever, it was important to detect *C. burnetii* excretors because they play a key role in controlling the spread of the bacterium between animals and from animals to humans (10).

Out of the 436 whole blood and 323 vaginal swab samples from cattle, sheep, and goats that were analysed, 132 and 89 samples tested positive for *C. burnetii* by PCR, representing respective prevalence rates of 30.28% and 27.55%. PCR detected *C. burnetii* DNA in 35.08% of vaginal swabs from cows, 30.90% from sheep, and 15.15% from goats. Our results contradict those reported by Kiprono *et al.* (25), who found that goats had the highest seropositivity (49.65%), followed by sheep (16.67%) and cows (3.25%), in Kenya. These rates are higher than those recorded in Réunion, France, between March 2011 and August 2012, where *C. burnetii* DNA was detected in 0.81%, 4.4% and 20.1% of vaginal swabs from cows, sheep and goats respectively (26). However, our prevalence rates were similar to those in other tropical countries. In cattle, prevalence was estimated to be between 40% and 59.8% in Nigeria, Sudan, and Zimbabwe, and just 4% in Chad (27). *C. burnetii* DNA detected in vaginal swabs showed a significant association between certain study areas: Belya (35.71% [25.76 – 46.98]), Kankan (26.58% [17.55 – 37.91]), and Boké (27.16% [18.14 – 38.36]) had significantly higher prevalence rates than Mamou. This higher prevalence of *C. burnetii* observed in Beyla, Kankan, and Boké can be explained by the higher concentration of herds with reproductive disorders in these areas, which provide favorable conditions for the circulation and amplification of the bacterium. In these regions, extensive and semi-extensive livestock farming systems promote increased exposure of females during the peripartum period, a key time for *C. burnetii* shedding (28, 29). Conversely, the lower prevalence observed in Mamou could reflect a lesser severity of reproductive disorders or more limited circulation of the agent in the herds studied. These observations are consistent with previous studies showing that the prevalence of *C. burnetii* is closely linked to the frequency of abortions and the intensity of peripartum shedding, rather than to isolated environmental factors, which would explain the similarity of our results with those previously reported (30).

Thus, regional differences primarily reflect the frequency of reproductive disorders and the geographic location of the herds, rather than unmeasured environmental factors or agricultural practices (3).

Analysis of data from animals with a history of reproductive disorders revealed that *C. burnetii* infection has a significant impact on reproductive performance. These disorders were significantly more frequent in cows (42.11%) than in goats (25.25%) and sheep (30%). This study revealed a high but similar incidence of abortions due to *C. burnetii* in cows, goats, and sheep. This finding contradicts other studies that concluded *C. burnetii* was a rare cause of abortion in cattle (3, 31). Since some *C. burnetii*-negative females aborted, this suggests that other infections, such as brucellosis, could be involved in the reproductive disorders in these herds. However, abortion was the most common event observed in cows (66.67%), compared to stillbirth (33.33%). In goats and

sheep, abortion rates were similar to stillbirth rates. These data corroborate those of previous studies that reported abortion rates between 10 and 60% in sheep and higher rates (72–81%) in goats during Q fever outbreaks (32, 33). However, other previous studies conducted in cattle, sheep and goat herds reported contrary results, with lower rates ranging from 1% to 11%, depending on the country compared to our results (34-37).

In addition to these observations, our study showed notable differences in bacterial shedding between species. 42.86% of goats had bacterial shedding in their vaginal mucus, compared to 29.41% of ewes and only 21.87% of cattle after the first abortion. This situation could explain the higher frequency of human epidemics linked to small ruminants (30). The higher shedding rates observed in goats and sheep can be explained by more intense genital shedding of *C. burnetii* at the time of abortion, linked to a strong tropism of the bacterium for the placenta of small ruminants.

C. burnetii shedding is strongly influenced by parturition in our study. In cows, the number of positive cases increased from 4 before the first calving to 12 after the first calving, while 36 females remained negative, indicating intermittent shedding. In goats, the number of positive cases increased from 5 to 12 after the first kidding, but no animals tested positive after the second kidding, suggesting transient shedding (33). In ewes, the number of positive cases increased from 3 to 8, with 4 remaining positive, 4 becoming negative again, and 1 new case, indicating prolonged or recurrent shedding, as confirmed by Angelakis & Raoult (30) and Agerholm (3). The lack of data after the second calving, due to the gestation length (~9 months) and the total follow-up period (12 months), limits the assessment of persistent excretion in cattle (38), which is a limitation of our study. Our data confirm that excretion dynamics differ between species: transient in goats, persistent in sheep, and intermittent in cattle. They highlight the importance of monitoring around parturition and rigorous management of calving products to limit environmental contamination and zoonotic risk. Excretion in the milk of asymptomatic cows also constitutes a potential silent reservoir for transmission to humans and animals that warrants further investigation (33).

These results highlight that postpartum shedding of *C. burnetii* varies by species and can directly influence the risk of transmission within herds. They also suggest that simply detecting the bacterium is insufficient to identify at-risk herds, and that defining clinical thresholds to identify animals or herds with high shedding levels would be beneficial. This would not only improve surveillance but also refine the differential diagnosis of infectious abortions.

However, some limitations must be highlighted. The small number of animals monitored during the second parturition did not allow for definitive conclusions to be drawn regarding the shedding dynamics of *C. burnetti* in goats and sheep, which constituted a limitation to be considered in future studies. Similarly, the fact that samples were taken up to several days after abortion could underestimate postpartum shedding, as several studies indicated a rapid decrease in bacterial levels after abortion in cattle and sheep (33, 38). Furthermore, individual differences among parturient females related to farm management can influence the intensity of shedding and, consequently, the likelihood of exposure for other animals.

These observations highlighted the importance of continuing research on the dynamics of bacterial excretion and the factors influencing its persistence, in order to better understand the role of different species in the transmission of Q fever and to adapt prevention and management strategies for zoonotic risk.

Conclusion

This study highlights the major role of *Coxiella burnetii* in reproductive disorders in cattle, goats, and sheep, with postpartum shedding patterns specific to each species. Cows exhibit intermittent shedding, goat's transient shedding, and ewes prolonged or recurrent shedding, which could explain the higher frequency of human Q fever epidemics linked to small ruminants. These results call for further studies with longer follow-up and larger sample sizes to confirm and expand upon these observations.

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Conflict of Interest

The authors declare no competing interests.

Ethical approval

This study was approved by the Institutional Ethics Committee of ISSMV (IECI) (N°22/2023/ISSMV/D/DG). Prior to conducting the study, we obtained approval from the heads of the Prefectural Livestock Directorates of Beyla, Boké, Kankan, and Mamou. After receiving the agreement of local leaders, we contacted the livestock farmers and clearly explained the objectives and scope of the study to them. Thus, the herders' agreement or refusal to participate was obtained without any negative consequences for their activities. The questionnaires were only administered after obtaining their verbal consent, followed by blood samples and vaginal swabs. We also took care to respect the anonymity of the participating herders, since other herders prefer to hide what is happening in their herds in order to avoid being judged by their peers. To guarantee anonymity, the questionnaires did not include the names of the herders, but rather the names of the villages where the questionnaires were administered, as well as a number for each one.

Artificial Intelligence Statement

Not Applicable.

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