sciendo

DOI: 10.2478/fv-2025-0017

FOLIA VETERINARIA, 69, 2: 43—55, 2025

ORIGINAL ARTICLE

SERO-EPIDEMIOLOGY OF *COXIELLA BURNETII* INFECTION IN DOMESTIC RUMINANTS IN THE PREFECTURES OF BEYLA AND MAMOU, GUINEA

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Citation: Tea, M. A., Tialla, D., Soromou, L. W., Salako, A. S., Kaba, L., Tchabo, W., Somda, M. B., 2025: Sero-epidemiology of *Coxiella burnetii* infection in domestic ruminants in the prefectures of Beyla and Mamou, Guinea. Folia Veterinaria, 69, 2, 43–55.

Received: April 28, 2025

Accepted: May 19, 2025

Published: June 16, 2025

Ethical considerations: When reporting experiments on animals Observation of the ARRIVE guidelines 2.0: Updated guidelines for reporting animal research, published on July 14, 2020 (DOI: 10.1371/journal.pbio.3000410), is applied. The authors ensure that all procedures were performed in compliance with the guidelines for animal care of their institutions or with national/international guidelines.

ABSTRACT

This study determined the seroprevalence and identified risk factors for exposure to *Coxiella burnetii* in domestic ruminants in the prefectures of Mamou and Beyla in Guinea. A structured qualitative and quantitative questionnaire was administered by trained interviewers to 48 people, mainly livestock farmers. Besides, 216 serum samples from 75 cattle, 69 sheep, and 72 goats were analysed by indirect enzyme-linked immunosorbent assay (iELISA) for *Coxiella burnetii* IgG and IgM antibodies. The results of the survey showed that farmers had little information about Q fever. Of 216 serum samples analysed from 48 different herds, 101 (46.76 %) animals and 18 (37.5 %) herds were positive for *Coxiella burnetii*. The seroprevalence was significantly higher in Beyla (54.63 %) than in Mamou (38.89 %) (p = 0.029). It was 52.78 % in goats, 40.58 % in sheep and 46.67 % in cattle (p = 0.34). Binary logistic regression analysis showed that tick infestation, age, sex and contact with other animals had a significant impact on the prevalence of Q fever. Given its zoonotic and economic impact, it is important to establish a disease surveillance system in all regions of the country. Further research, particularly in molecular diagnosis, is needed to confirm the involvement of Q fever in cases of reproductive disorders in domestic ruminants in Guinea.

Key words: *Coxiella burnetii*; domestic ruminants; Guinea; risk factors; seroprevalence

INTRODUCTION

Coxiella burnetii is an obligate intracellular Gram-negative bacterium responsible for Q fever [1]. It is a zoonotic disease for which domestic ruminants: cattle, sheep and goats are the reservoirs [2, 3]. Slaughterhouse workers, veterinary officers, and livestock farmers are most exposed to this infection [4]. Animal-to-animal transmissions are important in the sense that they contribute to the persistence and circulation of the bacteria in nature [5, 6]. However, the vast majority of human epidemics of Q fever are epidemiologically related to small ruminants, highlighting their critical role in the zoonotic transmission of *Coxiella burnetii* [7]. Humans mostly contract *Coxiella burnetii* infections mainly through zoonotic route by inhaling contaminated dust with birth debris, abortion, urine, or faeces from infected animals [8]. In addition, infections from contaminated food or unpasteurized milk and transmission by ticks can occur [9, 10]. For this reason, consequently, this disease represents a significant economic burden, by causing, production losses due to reproductive disorders such as infertility, stillbirths, abortions, and the weakness of the offspring, as well as the costs of implementing vaccination and control programmes [11].

Q fever is cosmopolitan with the exception of New Zealand [12]. Information on the epidemiology of this disease has been reported in humans and domestic ruminants in 24 of 54 African countries over the last two decades. Mean seroprevalence estimates were 16 % (95 % CI [11 – 23 %]) in humans, 14 % (95 % CI [10 – 20 %]) in cattle, 13 % (95 % CI [9 – 18%]) in sheep, and 21 % (95 % CI [15 – 29 %]) in goats [13]. In West Africa, several surveys have reported variable prevalences of Q fever in ruminants and humans. Bovine prevalence was estimated at 4 % in Senegal [14], 18 % (30/166) in Ghana [15], 2 % in Togo [16], 10.44 % in northern Nigeria [17], 26.2 % in Burkina Faso [18], and 55.3 % in Mali [19]. In humans, estimated seroprevalences of 10 % and 17 % were recorded in children in Niger [20] and Ghana [21], respectively. As for small ruminants, respective prevalences of 3.16 % and 3.8 % in northern Nigeria [17], 22.6 % and 16.9 % in Mali [19], and 38.2 % and 30.3 % in Burkina Faso [6] were detected in sheep and goats, respectively. Another study reported a prevalence of 21.59 % in small ruminants in Mali [22].

In Guinea, very few data are available on Q fever [23]. While this pathology was previously considered a rare tropical disease restricted to Africa, the findings of some studies show that it circulates in many prefectures of Guinea. In a study carried out in the four regions of Guinea, respective seroprevalences of 0.4 %, 6.9 %, 9,3 %, and 9.2 % were found in Forest Guinea, Upper Guinea, Middle Guinea, and Maritime Guinea, respectively [24]. A few years later, Troupin et al. [25] reported overall prevalence of 20.5 %, 4.4 %, and 2.3 % respectively in cattle, goats and sheep, in the country. This study detected Q fever in cattle in 14 (93 %) out of the 15 prefectures visited, in goats in 11 (69 %), and in sheep in 9 (56 %) out of 16 prefectures [25]. Recently, Naidenova et al. [26] reported the first official case of human Q fever in Mamou (Middle Guinea), admitted to the infectious diseases department of the regional hospital. This suggests that Q fever could be considered a public health problem in Guinea, but due to lack of vigilance on the part of hospital health staff, this infectious disease is underdiagnosed.

Beyla and Mamou are two prefectures located in two different eco-geographical regions of Guinea, where livestock farming represents the second major economic activity after agriculture. However, Q fever prevalences above 20 % were reported between 2017 and 2019 in Dalaba, Dabola, Faranah, and Kouroussa, four sub-prefectures of Mamou. In Belya, located in Forest Guinea, a higher prevalence estimated at 25.9 % was reported by Barry et al. [27]. According to the authors of these studies, climatic conditions with high relative humidity favor the maintenance of the bacteria and a high prevalence of the disease. Indeed, the prefecture of Beyla borders northern Côte d'Ivoire, where seroprevalences of 13.9 %, 9.4 %, and 12.4 % were reported, respectively in cattle, sheep, and goats [28]. Livestock, often transhumant, congregates along this border, sharing watering points and grazing areas.

The aim of this study was to determine the seroprevalence of Q fever in domestic ruminants in the prefectures of Mamou and Beyla and the risk factors associated with transmission among livestock keepers in these localities. The results will provide a better understanding of the epidemiological aspects of this disease, leading to the definition of appropriate prevention and control strategies.

MATERIALS AND METHODS

Study area

Epidemiological data were collected in two prefectures belonging to two different eco-geographical regions: the prefecture of Mamou, located in Middle Guinea, and the prefecture of Beyla, in Forest Guinea (Figure 1). Mamou covers an area of 8,000 km^2 with an estimated population of 318,981 inhabitants, while Beyla has an area of 15,500 km^2 with an estimated population of 379,337 people [29]. The prefectures of Mamou (Middle Guinea) and Beyla (Forest Guinea) are two distinct areas from each other in terms of climate and relief. Mamou is characterized by a tropical climate with the alternation of two seasons: a dry season (from November to May) and a rainy season (from June to October). As for the climate of Beyla, it's tropical wet and dry with two seasons: a rainy season from April to mid-November and a dry season from mid-November to March. Average annual rainfall can be up to 2,400 mm in Mamou but varies between 1,700 mm and 3,000 mm in Beyla. The temperatures vary from 10 ºC to 33 ºC, with an average of 22 ºC in Mamou, and vary between 19 ºC and 29 ºC, with an annual average of 24 ºC in Beyla [30]. The average aridity index is approximately 1.30 in Mamou compared to 1.97 in Beyla. The Mamou region is dominated by tropical savannahs, mountain forests, gallery forests, shrub and grass savannahs, plateaus and plains. As for Beyla, this area is characterised by a transition zone between the savannahs of Upper Guinea and the forest, although the forest is little developed in this region.

Both areas are crossed by numerous rivers and streams that irrigate neighboring countries, making Guinea the water tower of the sub-region. The main economic activity is agriculture, followed by livestock farming. Livestock farming is essentially family-based, with most livestock migrating throughout the year except during the cropping season in both zones in both areas. Common livestock consists of cattle, goats and sheep. However, the grouping of different livestock species, the extensive mode of breeding, and poor breeding management practices are the main factors in the appearance and spread of animal diseases and zoonosis.

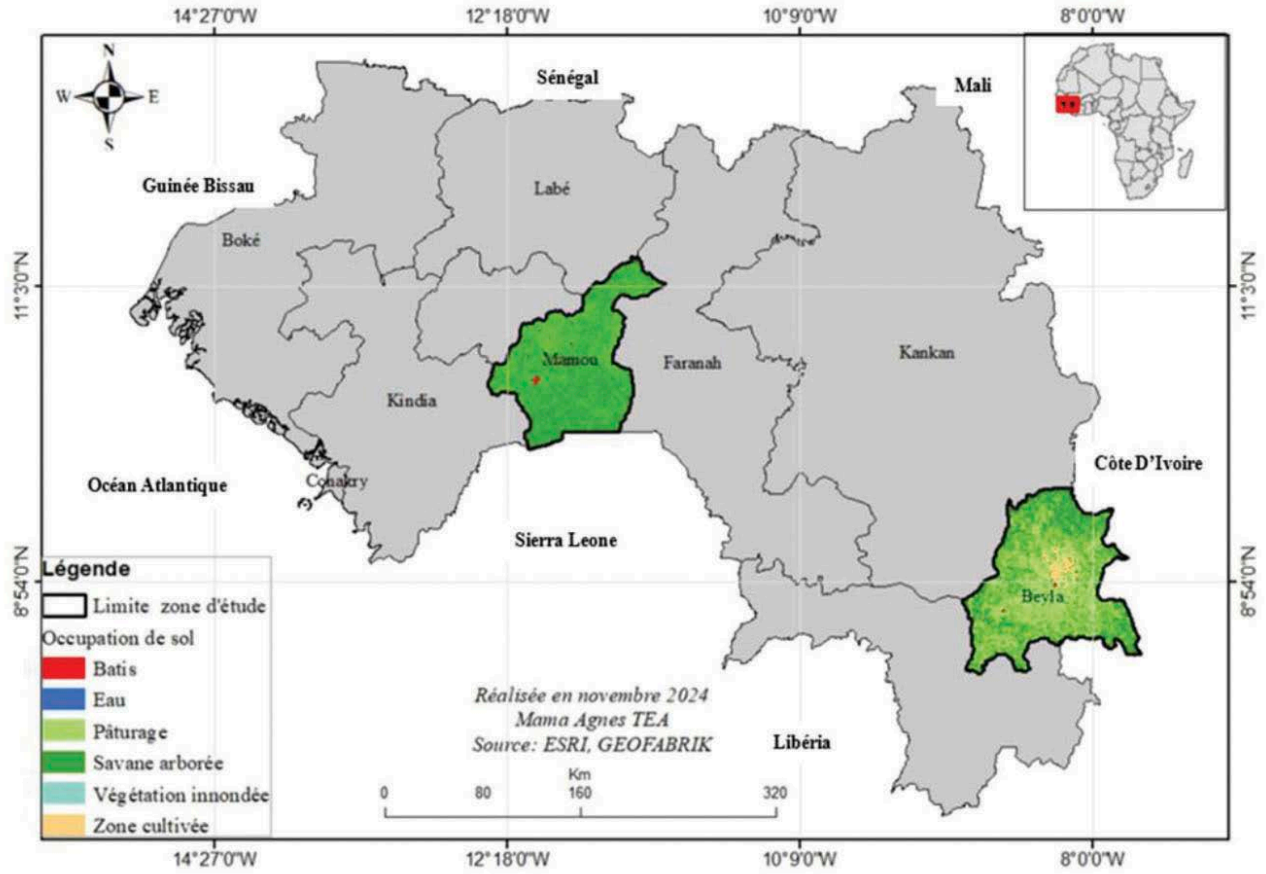

Fig.1. Map of a study area

Epidemiological data collection

The demographic data of the prefectures are presented in Table 1. Before sampling, a predefined data sheet was completed to collect demographic and epidemiological data to assess risk factors for Q fever. Owners/breeders of cattle, goats, and sheep were interviewed in their native language. The geographical coordinates of each sub-prefecture were recorded using GPS. Information on tick infestation was collected by asking owners/breeders whether they had taken precautions against ticks and by inspecting the animals and their habitats. The simple random sampling method was used for the selection of herds, animals, and blood samples from farms scattered throughout the study areas.

Blood sampling and serum separation

A thorough review of the literature revealed that no information was available on the prevalence of Q fever in domestic ruminants (cattle, sheep, and goats) in our targeted study areas. To calculate the sample size, a 95 % confidence interval with an expected prevalence of 15 % and a preferred absolute precision of 5 % was applied using the Thrusfield et al. [31] formula:

$$Nt = \frac{Z^2 . Pexp(1-Pexp)}{d^2}$$

Where *Nt* is the sample size, *Z* is the statistic corresponding to the level of confidence, *Pexp* is the expected prevalence (that can be obtained from the same studies or a pilot study conducted by the researchers), and *d* is precision (corresponding to effect size).

A total of 216 serum samples from all farms were required for this procedure. Blood samples were collected aseptically from the jugular vein of 75 cattle, 72 goats, and 69 sheep. The blood samples were transported to the laboratory and stored at 4 °C. Serum was separated by centrifugation at 3920 rpm for 5 min. Supernatants were pipetted into 1.5 ml Eppendorf tubes and stored at -20 °C.

Serological diagnosis by indirect enzyme-linked immunosorbent assay (iELISA)

The indirect enzyme-linked immunosorbent assay (i-ELISA) was used for *Coxiella burnetii* IgG and IgM antibodies according to the protocol described by Sakamoto et al. [32]. This test was used due to its sensitivity ≥ 95 % (CI 95 %: 89.28 % – 100 %) and specificity ≥ 95 % (CI 95 %: 97.75 % – 100 %).

Each serum sample was tested for the presence of anti-*Coxiella* antibodies using a commercial (indirect) multispecies kit (ID Screen® Q Fever Indirect Multispecies, catalog number FQS-MS-5P). Serum was diluted (1:400) as recommended. Specific antibodies were detected using a peroxidase labeled anti-ruminant conjugate. An ELISA reader was used to measure the optical density (OD) of positive and negative controls, as well as the tested samples, at 450 nm. The results were expressed as a percentage of the optical density (OD) of the tested sample and interpreted according to the manufacturer's recommendations, with S/p values ≤ 30 % being negative and values ≥ 40 % being positive. The test is validated if the mean optical density of the positive control (DOCP) is greater than 0.350 (DOCP > 0.350), and when the ratio between the mean optical density of the positive control (DOCP) and the mean optical density of the negative control (DOCN) is greater than 3 (DOCP / DOCN > 3).

Statistical analysis

Survey data were collected using the KoboCollect mobile application, then transferred to Kobotoolbox and processed. Microsoft Excel spreadsheet software was used for data storage, and R statistical software, version 4.1.2 [33], was used for analysis. The seroprevalence of Q fever was calculated by dividing the number of positive animals by the total number of animal sera tested. The chi-square test for comparison of proportions was used to compare proportions of breeders and seroprevalence in ruminants. Binary logistic regression analysis was applied to identify risk factors associated with seropositive Q fever results, odds ratios, and 95 % confidence intervals. Factors take into account domestic ruminant species, age, sex, contact with other animals, cleaning of premises, rearing method, tick infestation, urbanization of area, metritis and abortion management. A p-value of less than or equal to 0.05 was considered statistically significant.

RESULTS

Extensive livestock farming is the main type of livestock farming practiced in Mamou and Beyla. Indeed, 81.3 % ([66.9 – 90.5]; n = 39) of the 48 farmers surveyed practiced it, with the majority of them (75 % [60.1 – 85.9]; n = 36) being illiterate (Table 1). Overall, a very small

proportion (6.3 % [1.6 – 18.2]; n=3) of livestock farmers were aware of the existence of Q fever in ruminants. The proportion of farmers who were aware of brucellosis (33.3 % [20.8 – 48.5]; n = 16) is significantly higher than that of those who report being aware of Q fever (6.3 % [1.6 – 18.2]) and chlamydia (6.3 % [1.6 – 18.2]). However, this percentage is similar to that of those who are aware of abortive salmonellosis (18.8 % [9.4 – 33.1]), murrain of small ruminants (25.0 % [14.1 – 39.8]) and trichomonas (10.4 % [3.9 – 23.4]) (Table 1).

Regarding the type of livestock farming, out of a total of 48 breeders visited, 22.9 % [12.5 – 37.6] practiced cattle breeding, against 18.8 % [9.4 – 33.1] for sheep breeding, 20.8 % [10.9 – 35.4] for goats and 37.5 % [24.3 – 52.6] for mixed farming (cattle-sheep-goats). For that of hygiene measures on the farm, 83.3 % [69.2 – 92.0] of the breeders did not apply any hygiene measures, 10.4 % [3.9 – 23.4] carried out regular cleaning, 6.3 % [1.6 – 18.2] disinfected the premises. Overall, 87.5 % ([74.0 – 94.8], n = 42) of farmers did not apply any sanitary measures before introducing a new animal to the herd compared to 12.5 % ([5.2 – 25.9], n = 6) who quarantined the animals (Table 1). Furthermore, 64.6 % [49.4 – 77.4] of the farmers surveyed threw the fetus and placenta into the wild, compared to

Table 1. Breeding practices and breeders' knowledge of Q fever

Modality	Designation	Number of breeders surveyed	Percentage (%)	95% CI
Educational level	Literate	12	25.0	[14.1 – 39.8]
	Illiterate	36	75.0	[60.1 – 85.9]
Breeders' knowledge of Q Fever	Knowledgeable	3	6.3	[1.6 – 18.2]
	Unaware	45	93.8	[81.8 – 98.4]
Type of farming	Extensive	39	81.3	[66.9 – 90.5]
	Semi-extensive	9	18.8	[9.4 – 33.1]
	Intensive	0	0.0	[0.0 – 9.2]
Common diseases in livestock	Brucellosis	16	33.3	[20.8 – 48.5]
	Chlamydiosis	3	6.3	[1.6 – 18.2]
	Q-fever	3	6.3	[1.6 – 18.2]
	Abortive Salmonellosis	9	18.8	[9.4 – 33.1]
	Murrain of Small ruminants	12	25.0	[14.1 – 39.8]
	Trichomonas	5	10.4	[3.9 – 23.4]
Livestock species	Cattle	11	22.9	[12.5 – 37.6]
	Sheep	9	18.8	[9.4 – 33.1]
	Goats	10	20.8	[10.9 – 35.4]
	Mixed (Cattle-Sheep-Goats)	18	37.5	[24.3 – 52.6]
Sanitary measures applied before introducing a new animal into the herd	Quarantine	6	12.5	[5.2 – 25.9]
	No measures	42	87.5	[74.0 – 94.8]
Livestock management	Regular cleaning	5	10.4	[3.9 – 23.4]
	Disinfection	3	6.3	[1.6 – 18.2]
	No measures	40	83.3	[69.2 – 92.0]
Management of aborted animals	Burial	7	14.6	[6.5 – 28.4]
	Incineration	2	4.2	[0.7 – 15.4]
	Disposal in the environment	31	64.6	[49.4 – 77.4]
Socio-economic impacts of the disease on farms	No measures	8	16.7	[7.9 – 30.7]
	Abortions	24	50.0	[36.4 – 63.6]
	Treatment costs	6	12.5	[5.2 – 25.9]
	Stillbirths	18	37.5	[24.3 – 52.6]

16.7 % [7.9 – 30.7] who took no measures to manage abortions cases (Table 1). In addition, some minor measures, including burial (14.6 % [6.5 – 28.4]) and incineration (4.2 % [0.7 – 15.4]) of the foetus and placenta, were used in the management of abortion cases.

The socioeconomic impact of Q fever on farms is diverse. According to the farmers interviewed, Q fever abortions and neonatal deaths resulted in economic losses of 50.0 % [36.4 – 63.6] and 37.5 % [24.3 – 52.6], respectively. Other economic losses were due to treatment costs (12.5 % [5.2 – 25.9], n = 6).

Prevalence of anti-*Coxiella burnetii* antibodies in relation to demographic variables of cattle, sheep, and goat populations in Mamou and Beyla

Of 216 serum samples obtained from 48 different herds analyzed, a total of 101 animals and 18 (37.5 %) herds were positive for *C. burnetii*, giving an overall prevalence of Q fever estimated at 46.75 % [39.9 – 53.6]. It was significantly higher in Beyla (54.63 %) compared to Mamou (38.89 %) (p = 0.02912). This prevalence varied significantly from one sub-prefectures to another one in Beyla unlike in Mamou. In the localities of Mamou, the seroprevalence of Q fever was 37.5 % in Dounet, 50 % in Gongoret, and 30.56 % in Poredakar (p = 0.2533). As for Beyla localities, it was 41.18 %, 44.4 % and 76.32 % in Boola, Fouala and Sinko, respectively (p = 0.003). However, the highest seropositivity for *C. burnetii* was observed in the district of Sinko (p = 0.003) (Table 2). The seropositivity of goats for Q fever antibodies (52.78 %) was similar to that of sheep (40.58 %) and cattle (46.67 %) (p = 0.3488). However, it was higher in females (58.02 %) than in males (29.41 %) (p < 0.001). In rural areas, 23.96 % of positive sera were detected compared to 65 % in peri-urban areas. The prevalence was found to be significantly (p < 0.044)

Table 2. Prevalence of anti-*Coxiella burnetii* antibodies in relation to demographic variables in cattle, sheep, and goat populations in Mamou and Beyla

Categories	Variables	Number of samples	Number of positives	Seroprevalence n (%)	Chi-Square	p -Value
Sub-prefectures	Boola	34	14	41.18	11.28	0.003698
	Fouala	36	16	44.44		
	Sinko	38	29	76.32		
	Total Beyla	108	59	54.63	-	-
	Dounet	40	15	37.50	2.7468	0.2533
	Gongoret	32	16	50.00		
	Poredakar	36	11	30.56		
	Total Mamou	108	42	38.89	-	-
Species	Cattle	75	35	46.67	2.1063	0.3488
	Sheep	69	28	40.58		
	Goats	72	38	52.78		
	Total animals	216	101	46.76	-	-
Gender	Female	131	76	58.02	15.812	< 0.001
	Male	85	25	29.41		
	Total Gender	216	101	46.76		
Urbanization	Rural	96	23	23.96	34.45	< 0.001
	Peri-urban	120	78	65		
	Total	216	101	46.76		
Age	Youth [12 – 42 months]	118	63	53.39	4.0248	0.04484
	Adult [43 – 92 months]	98	38	38.78		
	Total	216	101	46.75		

Table 3. Prevalence by age group

Characteristics	0. N =158		1. N = 48		2. N = 10	
Age Group (months)	n (%)	95 % CI	n (%)	95 % CI	n (%)	95 % CI
Youth [12 - 42]	112 (71)	[63.0 – 77.7]	32 (66.6)	[51.4 – 79.1]	10 (100)	[65.5 – 1.00]
Adult [43 - 92]	46 (29,1)	[22.3 – 36.9]	16 (33.3)	[20.8 – 48.5]	0 (0.0)	[0.00 – 34.4]

N = total number; 0 = no farrowing; 1 = primiparous; 2 = multiparous, n = number; % = percentage; CI: Confidence Interval

Table 4. Risk factors associated with the prevalence of anti-*Coxiella burnetii* antibodies in domestic ruminants

Categories	Variable	Nb of positives/ Nb analyzed	Prevalence	Odds ratio	95% CI	p-value
Animal species	Cattle	35/75	46.67	-	-	-
	Sheep	28/69	40.58	1.4	[0.7 – 2.8]	0.29
	Goats	38/72	52.78	0.9	[0.5 – 1.7]	0.17
Gender	Female	76/131	58.02	3.3	[1.9 – 5.9]	0.00004
	Male	25/85	29.41			
Ages	Youth [12 – 42 months]	63/118	53.39	1.8	[1.0 – 3.1]	0.03
	Adult [43 – 92 months]	38/98	38.78			
Introducing a new animal to the herd	Quarantine	18/48	37.50	0.9	[0.4 – 1.95]	0.77
	No measures	23/57	40.35			
Reproductive disorder	Abortion	35/131	26.72	-	-	-
	Placental retention	29/131	22.14	1.3	[0.7 – 2.3]	0.39
	Metritis	12/131	9.16	3.6	[1.8 – 7.3]	0.0002
Breeding method	Extensive	86/185	46.49	0.9	[0.4 – 1.98]	0.84
	Semi-intensive	15/31	48.39			
Urbanicity	Rural	23/96	23.96	0.2	[0.1 – 0.3]	< 0.001
	Peri-urban	78/120	65.00			
Cleaning of the Corrals	Yes	12/25	48.00	1.1	[0.5 – 2.4]	0.89
	No	89/191	46.60			
Contact with other Species	Yes	101/216	46.76	NA	NA	NA
	No	0	0.00			
Ticks Infestation	Yes	44/83	53.01	2.6	[1.5 – 6.4]	0.003
	No	12/133	9.02			

CI: Confidence Interval; Nb: number: p-value of the statistically significant odds ratio; NA: not applicable

impacted by age, with a high prevalence in young animals (53.39 %).

Comparative analysis of prevalence between age groups showed that 71 % [63.0 – 77.7], 66.66 % [51.4 – 79.1], and 100 % [65.5 – 1.00] of young females aged 12 to 42 months who had not given birth, primiparous and multiparous were receptive to *Coxiella burnetii* infection unlike adult females aged 43 to 92 months (Table 3).

Correlation between the prevalence of Q fever and certain risk factors

Table 4 shows the correlation between the prevalence of Q fever and certain risk factors. The seroprevalence of Q fever was higher (53.01 %) in tick-infested animals than in tick-free animals (9.02 %). The presence of ticks was a potentially significant risk factor (p = 0.003). Animals with abortion and retained placenta had high prevalences of 26.72 % and 22.14 %, respectively, compared with animals with metritis (9.16 %). Unlike the history of abortion and retained placenta, which had no significant impact on the prevalence of Q fever, metritis was a significant risk factor (p = 0.0002). Animals that had been in contact with other animals had the highest seroprevalence (46.76 %) compared with zero that had not been exposed. Animals that had been in direct contact with other farm animal species were seropositive at 46.76 %, compared with zero that had not been exposed to other animal species. Higher seropositivity was detected in females (58.02 %) compared

with males (29.41 %) and in young animals aged between 12 and 42 months (53.39 %) compared with adults aged between 43 and 92 months (38.78 %). These two factors, such as sex ($p = 0.00004$) and age ($p = 0.03$), were statistically significant for Q fever seroprevalence.

It is remarkable that animals found in peri-urban areas were significantly more seropositive (65 %) than those in rural areas (23.96 %). Remarkably, animals kept in cleaned corals were significantly less likely to be seropositive (48 %) than others (46.6 %). Furthermore, factors such as animal species (OR = 1.4 [0.7 – 2.8] in sheep vs. cattle; OR = 0.9 [0.5 – 1.7] in goats vs. cattle ($p > 0.05$), the introduction of a new animal into the herd (OR = 0.9 [0.4 – 1.95]; $p = 0.77$), rearing method (OR = 0.9 [0.4 – 1.98]; $p = 0.84$), and cleaning of sheep pens (OR = 1.1 [0.5 – 2.4]; $p = 0.89$) were not significantly associated with the distribution of *C. burnetii* in domestic ruminants in Beyla and Mamou.

DISCUSSION

This cross-sectional study was conducted in the prefectures of Mamou and Beyla in Guinea to determine the prevalence of antibodies to *Coxiella burnetii* in domestic ruminants and to assess associated risk factors to aid in the development of surveillance and control plans. The results obtained in this study confirm that domestic ruminant populations are exposed to *C. burnetii*, which may represent a public health problem. The overall prevalence of antibodies against *Coxiella burnetii* was 46.75 %. It was 54.63 % in Beyla compared to 38.89 % in Mamou. This overall seroprevalence observed in this study is significantly higher than that reported by a previous national study conducted in 2013 in four regions of Guinea, where respective seroprevalences of 0.4 %, 6.9 %, 9.3 % and 9.2 % are recorded in Forest Guinea, Upper Guinea, Middle Guinea, and in Maritime Guinea [24]. Our results are also contrary to those of [25], who found a lower prevalence of 9.1 % in small ruminants seropositive for *C. burnetii* between 2017 and 2019 in Guinea. This increase in *C. burnetii* infectivity in domestic ruminants could be due to a more active circulation of the bacteria in Guinea during the last decade.

Given that extensive livestock farming is extensive in both prefectures, this rapid spread of Q fever in ruminants could be due to the inhalation or ingestion of particles contaminated by farrowing and abortion products, feces, and urine of animals infected with bacteria [34, 35]. This risk factor for *C. burnetii* infection is particularly high in arid areas [36]. Due to a shortage of pasture in arid areas, ruminants are put to pasture on large areas, which indicates an uncontrolled bovine-goat-sheep interaction with a potential risk of transmission. This hypothesis is confirmed with 46.76 % of animals having had contact with other animals detected seropositive in our study. Similarly, the climatic conditions with high relative humidity that prevail in Beyla and Mamou at certain periods of the year could influence the distribution and maintenance of aerosolized bacteria, leading to a higher prevalence of the disease [26, 25]. This hypothesis seems to confirm the results of Troupin et al. [25], who reported a seroprevalence greater than 50 % in cattle in Dalaba, one of the prefectures close to Mamou. In addition to these environmental factors, this potential spread of *C. burnetii* could be favored by the lack of information on the disease and the practices of farmers that would increase the chances of introducing infected ruminants into the herds. As confirmed by the results of our survey, farmers had little knowledge about the pathology (93.8 %), which explains why they did not follow the instructions that would allow them to manage herds free from the disease. It is therefore important to recommend to breeders quarantine and mandatory testing of new animals for *Coxiella* infection before introducing them into the herds. However, it would be interesting to re-evaluate in the coming year the seroprevalence of ruminants in the same areas to verify if this trend of spread remains, in order to recommend appropriate mitigation measures. Besides, the high seroprevalence observed in Beyla compared to Mamou could be due to a higher circulation of the bacteria in this locality, where gatherings of transhumant livestock are observed. Indeed, the prefecture of Beyla borders the west of Côte d'Ivoire, where animals share the same watering points as well as the same grazing areas. Our work corroborates that of [22], who reported higher prevalence rates in the localities of Nioro and Kéniébougouwéré, two localities bordering Beyla in the west of Côte d'Ivoire. Similar observation had already been made in the Volta region of Ghana [37].

Coxiella burnetii seroprevalence rates were similar in sheep (40.58 %), goats (52.78 %) and cattle (46.67 %) in Mamou and Beyla. This result confirms the correlation between infection and risk factors which showed that there is not a significant association between prevalence and ani-

mal species (OR = 1.4 [0.7 – 2.8]; p = 0.29 in sheep versus cattle; OR = 0.9 [0.5 – 1.7]; p = 0.17 in goats versus cattle). These observed seroprevalences are higher than those reported by Troupin et al. [25] in a previous study conducted between 2017 and 2019 in cattle (20.5 %), goats (4.4 %), and sheep (2.3 %) in the Guinea. Prevalence below those found in our study, ranging from 13 % to 32 %, had also been documented in previous studies in Africa [15, 17, 14, 38, 16, 39, 6]. However, our results are lower than those obtained in Mali and Quebec, where seroprevalences of 55.3 % and 68.75 %, respectively, were detected in ruminants [19, 40]. Higher seropositivity rates for *C. burnetii* were reported in areas with higher livestock density (> 100 per 100 animals) in some African countries, including Guinea [41]. In these areas, the probable cause would be the wind-borne distribution of *C. burnetii* spores from agricultural land to more populated areas. Other possible reasons for these variations could be differences in sample size, sampling methods, diagnostic tests used and the geographical locations.

The prevalence was significantly higher in females (58.02 %) than in males (29.41 %). The correlation revealed that gender is a potential statistically significant risk factor for Q fever (p = 0.00004). This result is consistent with previous reports indicating a high affinity of *C. burnetii* with female animals (26.65 %) compared with male animals (17.21 %) [42]. The authors mentioned that males are used for breeding purposes and are therefore transferred from one area to another and may be in contact with many different herds. This method may put them at higher risk of contracting and spreading diseases, which is not the case in our study area. This method may present them with a higher risk of contracting and spreading diseases, which is not the case in our study area. Moreover, this seroprevalence is significantly higher in young non-pregnant females (71 %), primiparous (66.66 %) and multiparous (100) animals aged 12 – 42 months, unlike in adult females aged 43-92 months. This trend could be explained by the fact that young animals (calves, kids, lambs) born to infected mothers are very susceptible to the disease. Similarly, a previous study showed that older animals shedding *C. burnetii* apparently showed no clear signs or symptoms of the disease and had no history of abortion. Our results are in contrast to those of several other studies, which reported that *C. burnetii* seropositivity in domestic ruminants increases with age [13, 43, 44].

In the present study, most of the Q fever-positive samples were collected from animals in peri-urban areas (n = 78; 65 %) where breeding is extensive, without hygiene measures in the facilities, such as cleaning the animals and their premises. However, no correlation was established in this study between infection and lack of cleaning of animal premises. However, these facts may contribute to higher prevalences of the disease. Likewise, contaminated birthing products are thrown into the environment; this justifies a higher risk of Q fever (OR = 0.2 [0.1 – 0.3]; p < 0.001) observed in animals in rural and peri-urban areas.

Ticks are considered an important risk factor and have a significant association with *C. burnetii* seropositivity. In the present study, 53.01 % of seropositive animals were found infested with ticks. These arthropods have a significant link in this infection (OR = 2.6 [1.5 – 6.4]; p = 0.003). This result is consistent with the findings of one of two previous studies conducted in Pakistan that found prevalences of 50 % and 97.9 %, respectively, in animals that harbored ticks [45, 46]. These studies found that ticks can carry the Q fever pathogen and contaminate the environment or the host. Several species of ticks can transfer *Coxiella* spp. by transstadial route as well as transovarial route and can transmit Q fever from a sick animal to a healthy animal via the blood meal [47].

No statistically significant association of infection was associated with abortion product management and retained placenta in this study. This result is contrary to that of Beaudeau et al. [48], who reported that the placentas and aborts of infected females induce a very high bacterial load and are sources of environmental pollution. On the other hand, a significant link was observed with a history of metritis (OR= 3.6 [1.8 – 7.3]; p = 0.0002) in our study and confirms the work of Agag et al. [49], who observed a similar result.

In these different cases, certain preventive measures could be of interest in terms of public health: vector control against ticks, serological tests and vaccination of animals, parturition in buildings, and appropriate disposal of placentas and litter. However, the feasibility of such measures in Guinea, and particularly in the Mamou and Beyla regions, is low since Q fever is not a notifiable disease; tests (in case of abortion) are carried out on request and are the responsibility of the breeder. An effective phase I animal vaccine is not yet available in Guinea. Although indoor parturition is not possible in an extensive livestock

area due to the lack of barns, recommendations have been made regarding the appropriate disposal of placentas. They should not be left on the ground to dry but collected and cremated appropriately, but this is only possible when parturition takes place in the presence of breeders.

Many other personal and behavioral factors could be involved and could be preventable. Further studies are needed to identify and confirm these preventable risk factors.

CONCLUSION

This study demonstrated the presence of Q fever with a higher seroprevalence in cattle, sheep, and goats in the prefectures of Mamou and Beyla in Guinea. These results highlight the need to implement surveillance of this bacterial infection in ruminants in all prefectures of the country. Given the zoonotic nature of the disease, we recommend screening at-risk human populations (veterinarians, breeders, slaughterers) using a One Health approach. Infestation of animals by ticks, metritis, degree of urbanization, management of parturition products, age and sex of animals, as well as climatic variability are risk factors to take into account in the management of this pathology in the study area. Livestock farmers' knowledge of the disease and its effects on animals as well as humans is still limited, as the disease is not considered a priority among zoonoses in Guinea. Therefore, research and awareness are recommended, especially in areas where large numbers of animals and people are at risk. Animals excrete the pathogen during parturition or abortion, which leads to environmental contamination and high exposure of other animals and humans. Hence, separation of animals at parturition and appropriate disposal of placentas and/or aborted fetuses are recommended. We also recommend the inclusion of Q fever in the differential diagnosis of abortion in domestic ruminants using molecular diagnostics to confirm the involvement of Q fever in cases of reproductive disorders in domestic ruminants in Guinea.

Availability of Data and Materials

The datasets that were analyzed in this study are available from the corresponding author and the lead author.

Ethical Approval and Consent to Participate

Prior approval was obtained from the Mamou and Beyla Prefectoral Livestock Directorates. After contacting the farmers, with the agreement of the local chiefs, the objectives and framework of the study were clearly explained. In this way, we were able to obtain the herders' consent to participate in the study without any negative consequences for their activities. Only after their verbal consent was obtained were the questionnaires administered and serum samples taken. The privacy of the participating farmers was respected, as other farmers prefer to hide what is happening in their herds to avoid being judged by their peers. To ensure confidentiality, the questionnaires did not include the farmers' names but rather the names of the villages where they were administered and numbered.

Conflict of Interest

The authors declare that they have no competing interests.

Funding

This study was not financially supported by any grant.

Generative AI Statement

The authors declare that no Gen AI was used in the creation of this manuscript.

Authors' Contributions

The original study was conducted by MAT, DT and LWS who also supplied the data. The idea for the study was conceptualized and generated by MAT, DT and LWS. Data were collected by MAT, DT, MBS, ASS and WT. MAT and ASS drafted the manuscript. Statistical data analysis by LK and ASS. WT, MBS, LK, DT and MAT provided intellectual criticism on the content of the manuscript. All authors have read and approved the final submitted manuscript.

Acknowledgements

This research was made possible thanks to the financial support of the Ministère de l'Enseignement supérieur,

de la Recherche scientifique et de l'Innovation (MESRSI) of Guinea, to whom we express our gratitude. We would also like to thank the General Management of the Institut Supérieur des Sciences et de Médecine Vétérinaire (ISSMV) in Dalaba for their support in carrying out this study.

To all the staff of the Central Veterinary Diagnostic Laboratory Conakry-Guinea of the Serology section, and in particular to Dr. Kouramoudou BERETE for facilitating the work.

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