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Serological survey and risk factors for *Coxiella burnetii* infection among Nomadic Cattle in Lanlate, Southwestern Nigeria

O. D. Adelakun[1]*, F. A. Akande[2], O. M. Obisesan[1] and S. I. B. Cadmus[3]

[1]Department of Animal Health Technology, College of Agriculture and Technology, Igboora, Oyo State, Nigeria.
[2]Department of Veterinary Parasitology and Entomology, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.
[3]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria.

*Corresponding author. Email: bukynafaso@gmail.com

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ABSTRACT: Q-fever, an important tick-borne zoonosis caused by *Coxiella burnetii* that affects a broad range of animal hosts, including humans. There is limited data on its prevalence in Nigeria particularly among ruminants in southwestern Nigeria. This study therefore evaluated the seroprevalence of *C. burnetii* antibodies and associated factors among nomadic cattle herds. A cross-sectional study design was adopted and a random selection of cattle was carried out in Lanlate, Oyo State, southwestern Nigeria. Sera were harvested from collected blood of 260 cattle covering 25 cattle herds, these were analyzed using a commercial indirect Enzyme-Linked Immuno-Sorbent Assay (ELISA) kit. Results were analysed by a chi-square ($\chi 2$) test and predictors of *C. burnetii* infections were determined using multivariate logistic regression. Of the cattle screened, 9.2% (24/260) at the individual animal level and 24% (6/25) at the herd level were seropositive to *C. burnetii.* There was an association between seropositivity to *C. burnetii* antibodies, breed of cattle and herd size. However, only the breed of cattle (OR: 3.1; 95% CI: 2.0 – 4.9) was observed to be predictor of *C. burnetii* infection. In conclusion, findings from this study confirm exposure to *C. burnetii* among nomadic cattle herds. It is therefore recommended that awareness of *C. burnetii* infection be created among pastoralists, they should also be educated on ways to improve hygiene. In addition, there is a need to further characterize the circulating strain of *C. burnetti* in the studied area.

Keywords: *Coxiella burnetti,* ELISA, Nomadic cattle, Lanlate, Sero-prevalence.

INTRODUCTION

Q-fever is an important underreported zoonotic disease and ruminants are considered to be the primary source of infection in humans (Tagesu, 2019; Hireche *et al*., 2020). The disease is caused by *Coxiella burnetii,* a rickettsia-like Gram-negative, obligate intracellular, and spore-forming bacterium (Tesfaye *et al*., 2020). The organisms and the diseases caused are distributed worldwide, except in New Zealand and Antarctica (Tagesu, 2019). The World Organisation for Animal Health (OIE) lists Q-fever as a notifiable animal disease and the member countries of this organization are encouraged to report the incidence of the disease in livestock (OIE, 2018). The disease results in direct economic losses for livestock farmers due to increased late-stage abortion rates, stillbirths, delivery of weak offspring and loss of milk production on infected farms (Canevari *et al*., 2018).

C. burnetii can infect a wide range of animals. It has been reported in a wide range of host species including wild animals, zoological collections, domestic ruminants, camels, rabbits, rodents, tortoises, lice, ticks, and humans (Eldin *et al*., 2017; Tagesu, 2019; Theonest *et al*., 2020). It has the ability to withstand environmental stresses and subclinical infections and often contaminates the environment by disseminating highly resistant organisms in birth products, milk, faeces and vaginal discharges (Porter *et al*., 2011). The contaminated environment may facilitate the spread of *C. burnetti* (Eldin *et al*., 2017). Furthermore, animal trade, the porous nature of country borders, and the illegal hunting of wild herbivores for game meat contribute to the spread within livestock (Wardrop *et al*., 2016).

Q-fever is considered to be an occupational hazard for pastoralists, livestock farmers, veterinarians and slaughterhouse workers (Angelakis and Raoult, 2011) due to close contact with livestock and wildlife animals (Kazwala, 2016). It was first described in 1935 in Queensland, Australia during an outbreak of a febrile illness among abattoir workers (Derrick, 1973). Transmission of the disease to humans results from direct contact with infected animals' products as well as inhalation of aerosolized particles from wool, animal placentas, aborted foetuses, environmental dust and rarely via tick bite (Tissot-Dupont *et al*., 2008; Whitney *et al*., 2009; Angelakis and Raoult, 2011). Though infection with *C. burnetii* in humans can be asymptomatic, symptomatic infection, known as Q-fever, may present as an acute undifferentiated febrile illness which can progress to chronic forms typically in individuals predisposed due to valvular heart disease or immunodeficiency (Crump *et al*., 2013).

The nonspecific clinical symptoms of Q-fever make diagnosis very challenging (Angelakis and Raoult, 2011). *C. burnetii* infection can be demonstrated either serologically or molecularly. Incidentally, seropositivity to *C. burnetii* does not confirm the shedding of the bacterium, but it is a valuable tool for the screening of *C. burnetii* infection (Hwang *et al*., 2020). Most importantly, to detect previous exposure to the bacterium (Khademi *et al*., 2020). Serological techniques such as complement fixation test (CFT), immunofluorescence assays (IFAs), and enzyme-linked immunosorbent assays (ELISAs) are used to diagnose Q-fever. ELISA is more sensitive than IFA, hence, it is a more preferred technique (Rousset *et al*., 2007). The disease is associated with poor knowledge and diagnosis among public health professionals, veterinarians, and farmers (Koka *et al*., 2018; Rahaman *et al*., 2019). Despite these perceived risk factors, there is limited data on its prevalence in Nigeria particularly among pastoral cattle that can serve as carriers of the organism. Further, Oyo State constitutes an important hub of pastoralists; hence, a veritable point for disease surveys.

MATERIALS AND METHODS

Study area

This study was carried out in Lanlate, Ibarapa East Local Government Area (Figure 1) situated between latitude 7°43′0.01″N and longitude 3°37′ 0.01″E with an elevation of 192 meters and a total land area of 7 507ha (Olugbemiga and Kehinde, 2021). The administrative headquarters of Ibarapa East LGA is Eruwa and the LGA comprises two major towns namely; Eruwa and Lanlate. The reason for the choice of study area was because of the heavy presence of nomads with their livestock population. The indigenous people of the area are Yorubas and they are mainly farmers and hunters. The town provides good arable land for animal pasture and agricultural production which is the primary occupation of members of the community. The study was conducted on nomadic cattle from the northern part of Nigeria. The nomadic Fulani are a group of people that live in hard-to-reach areas, which makes them inaccessible to health care services despite their vulnerability to zoonotic diseases like Q-fever. They have however settled in large numbers in Oyo State, Nigeria.

Study design

A cross-sectional study was conducted by sampling nomadic herds of cattle in selected pastoral communities in Lanlate, Ibarapa East LGA in Oyo State, southwestern Nigeria between November 2020 and February 2021.

Sample size and sampling

The minimum sample size (n) required was determined according to Thrusfield (2007) using the 95% level of confidence, the prevalence of 6.2% (Adamu *et al*., 2018) and the desired absolute precision of 5%. $n = Z^2 p (1-p)/d^2$ = 250 cattle. A simple random sampling technique was used to select cattle from different cattle herds.

Data and sample collection

Data on the age, sex, breed, body condition score and herd size of cattle were collected. The age was grouped into < 1, 1-3 and > 3 years. Male and female cattle were randomly selected without any preference. The breeds of cattle sampled included Bunaji, Bokolo and Rahaji. The body condition was scored as lean, moderate and good as previously described by Adedipe *et al*. (2014). Three to 5 mls of blood were collected from each cattle with the assistance of a veterinarian using sterile needles and

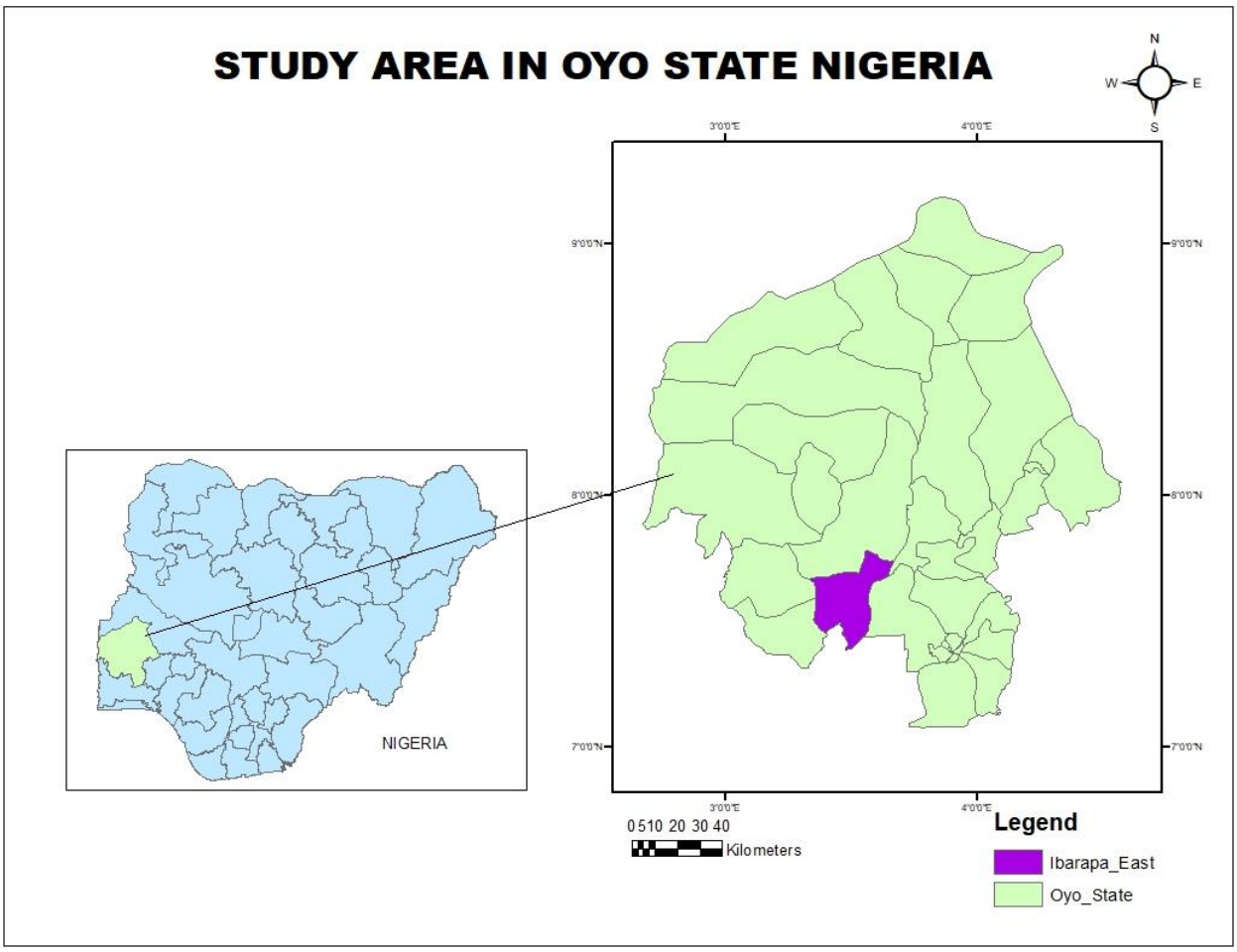

Figure 1. Study area in Oyo State.

syringes into properly labelled plain vacutainers. Samples were transported to the laboratory in flasks containing ice packs and later centrifuged at 3,000 rpm for 10 minutes. Sera were harvested from clotted blood and stored in cryo-tubes at -20°C, before laboratory analysis.

Sample analysis

Sera were tested for antibodies to Phase I and II antigens of *C. burnetti* using commercial Indirect Enzyme-Linked Immunosorbent Assay (IDscreen® Q fever indirect multi-species, IDvet France) Kit. Sample per positive percentages (S/P%) was calculated using optical density (OD) values as 100 × (ODsample − ODnegative control)/ (ODpositive control − ODnegative control). The optical densities (OD) were measured at 450 nm in a microplate ELISA reader and cut-offs for the titres were determined S/P% ≥ 40% was defined as positive, and S/P% < 40% was defined as negative based on the manufacturer's instructions.

Data analyses

Data were analyzed using IBM SPSS software package version 23 and Epi-Info version 3.5.1. Descriptive statistics such as frequencies and percentages were determined. The association between variables was assessed by calculating the odds ratios. A multivariable logistic regression model was used to determine predictors of Q-fever infection while adjusting for other covariates that were significant at $p<0.10$ in the bivariate analysis. The best-fit model was selected based on the Likelihood Ratio. Adjusted odds ratios (AOR) and 95 %Confidence Interval (95%CI) were reported. The level of significance was set at 5 %.

Ethics statement

The study protocol (ref: UI-ACUREC/17/0101) was approved by the Animal Care and Use Research Ethics Committee of the University of Ibadan, Nigeria, before the commencement of the research.

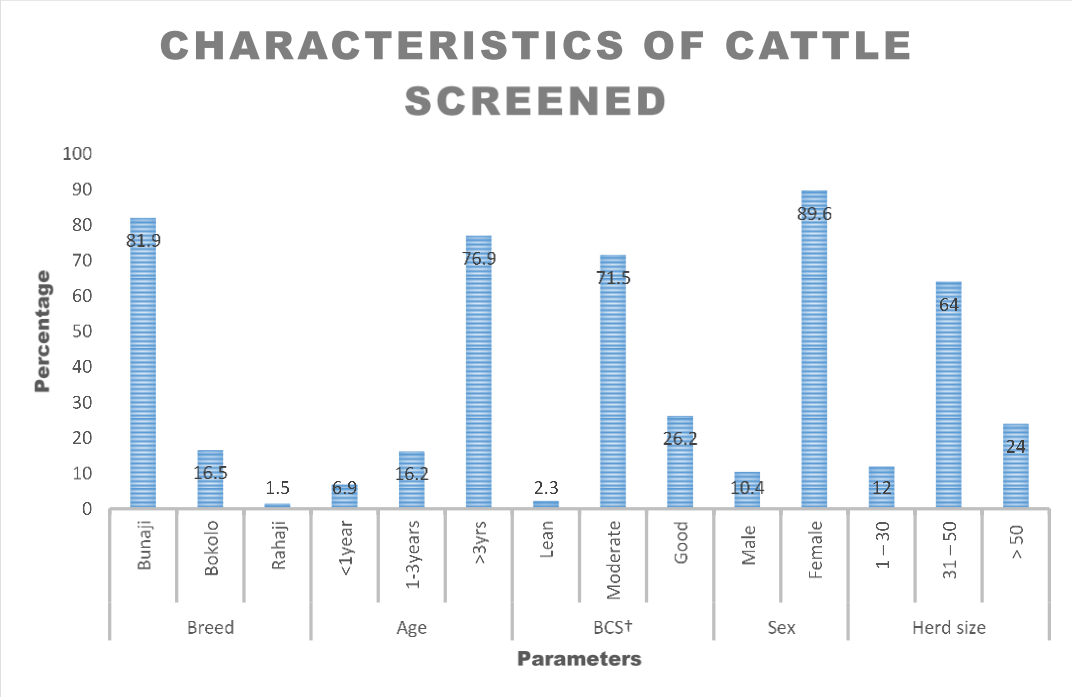

Figure 2. Characteristics of cattle screened at Lanlate, Oyo State.

RESULTS

Demographic characteristics

Two hundred and sixty (260) cattle from 25 nomadic herds were sampled for this study, 213 (81.9%) were of the Bunaji breed and 4 (1.5%) were Rahaji. The majority of the cattle sampled 233 (89.6%) were female. Cattle that were above three years of age 200 (76.9%) were sampled most while cattle below a year 18 (6.9%) were the least sampled. Most 186 (71.5%) of the cattle screened had moderate body condition scores while very few cattle 6 (2.3%) were lean. Most of the herd sizes 16 (64.0%) were in the category of 31-50 heads, while the least number of cattle heads 3 (12.0%) sampled was from the 0-30 group (Figure 2).

Sero-surveillance

A total of 24/260 screened cattle were positive for *Coxiella burnetii* antibodies equivalent to an overall individual animal seroprevalence of 9.2% while six herds (24%) were seropositive for *Coxiella burnetii* antibodies out of the 25 herds sampled. Of the 260 cattle sampled, the Bunaji breed had the highest seropositivity (32.6%) to *C. burnetii* antibodies, while Rahaji breed of cattle screened were all negative. Seropositivity to *C. burnetii* antibodies was higher among male (11.1%) than female (9.0%) cattle. The seropositivity to *C. burnetii* antibodies was highest (76.2%) in the age group < 1 year, with the least (4.8%) in the age group (1-3) years cattle. Cattle with lean BCS had the highest (16.7%) seropositivity to *C. burnetii* antibodies, and the least (7.4%) was in cattle with good condition. Cattle from herds numbering above 50 had the highest (19.4%) seropositivity to *C. burnetii* antibodies, while those from herds between 1 to 30 cattle were negative (Table 1).

The bivariate analysis showed an association between seropositivity to *C. burnetii* antibodies, breed of cattle and herd size. Bokolo cattle show a higher likelihood of being seropositive to *C. burnetii* antibodies when compared to Bunaji (OR=0.1, 95% CI: 0.0-0.3) and Rahaji (OR=0.3, 95% CI: 0.0-10.1). Furthermore, cattle within herds that are >50 in size (OR=3.7, 95% CI: 1.4-9.7) show a higher likelihood of seropositivity to *C. burnetii* antibodies when compared to herds with cattle between (1-30) heads (OR=0.3, 95% CI: 0.0-5.0) and (31-50) heads. On multivariable logistic regression, breed (OR: 3.1; 95% CI: 2.0 – 4.9) remained a predictor of seropositivity to *C. burnetii* infection (Table 2).

DISCUSSION

From the study, there was evidence of exposure to *C. burnetii* and the percentage seropositivity recorded from the present study is consistent with 9.43% reported in Plateau State (Elelu *et al*., 2020). It is however higher than

Table 1. Seroprevalence of Q-fever (*Coxiella burnetii*) in individual cattle screened according to breed, age, BCS, sex and herd size at Lanlate, Oyo State.

Parameters	Variables	No. examined	No. infected (%)	OR† (95%CI)	P-value
Breed	Bunaji	213	10 (4.7)	0.1 (0.0 – 0.3)	0.000*
	Bokolo	43	14 (32.6)	Ref	
	Rahaji	4	0 (0.0)	0.3 (0.0 – 10.1)	1.0
Age	<1year	18	2 (11.1)	2.5 (0.2-36.6)	0.6934
	1-3years	42	2 (4.8)	Ref	
	>3yrs	200	20 (10.0)	2.2 (0.5-20.3)	0.4507
BCS‡	Lean	6	1 (16.7)	2.5 (0.1-30.2)	0.8183
	Moderate	186	18 (9.7)	1.4 (0.5-4.9)	0.7684
	Good	68	5 (7.4)	Ref	
Sex	Male	27	3 (11.1)	0.8 (0.2 – 4.5)	0.9324
	Female	233	21 (9.0)	Ref	
Herd size	1 – 30	26	0 (0.0)	0.3 (0.0 – 5.0)	1.0
	31-50	162	10 (6.2)	Ref	
	> 50	72	14 (19.4)	3.7 (1.4 – 9.7)	0.0059*
Total		**260**	**24 (9.2)**		

*Significant at p≤0.05, † Odds Ratio, ‡Body condition score.

Table 2. Logistic regression analysis of variables associated with individual cattle seropositivity to *C. burnetii* antibodies.

Parameters	OR†	95% CI	p-value
Breed	3.1	2.0 – 4.9	0.000*
Age	1.1	0.5 – 2.6	0.754
BCS	2.3	0.4 – 3.1	0.815
Sex	0.7	0.2 – 2.7	0.562

2.98, 5.56, and 6.2% reported in Sokoto, Bornu and Kaduna States respectively (Adamu *et al*., 2018; Elelu *et al*., 2020; Cadmus *et al*., 2021). Findings from this study are lower than 13% in Taraba State (Nyifi *et al*., 2018), 14.5% reported in Kaduna State (Tukur *et al.,* 2014), 15.6% reported in Kwara State (Elelu *et al*., 2020) and 23.5% in Oyo State (Cadmus *et al*., 2020). The seroprevalence was lower than the one reported from countries in West Africa; In Coˆte d'Ivoire and Togo, 13.9 and 15.7% seroprevalence were reported respectively (Dean *et al*., 2013; Kanoute *et al*., 2017). In Africa; seroprevalence of 10.5 and 19.8% were reported in studies from Kenya and Egypt (Wardrop *et al*., 2016; Selim *et al*., 2023) respectively. Other countries; similar to 9.94% reported in Lebanon (Dabaja *et al*., 2019) but higher than a seroprevalence of 4.18 and 4.6% in Poland and Thailand respectively (Szymańska-Czerwińska *et al*., 2017; Doungngern *et al*., 2017). Findings is however lower than the seroprevalence of 29% reported in in China (El-Mahallawy *et al*., 2016) and 43% in Ecuador (Echeverría *et al*., 2019). The seroprevalence variations between regions and nations may result from a number of variables, including management techniques, research period, and environmental factors that affect *C. burnetii* exposure. Seroprevalence was highest among Bokolo breeds, a finding which agrees with the report of Cadmus *et al*. (2020) but is in contrast to the report of Adamu *et al*. (2018) where Bunaji breeds had higher seropositivity to *C. burnetti.* A review and meta-analysis conducted by Bwatota *et al*. (2022) reports that animal intrinsic factors such as age, sex and breed were risk factors for *C. burnetti* infection, however, age and sex are not statistically significant in this study. This finding gives credence to the importance of cattle as a reservoir of *C. burnetti*, which people can contract by breathing in aerosolized particles from the infected placenta, an aborted animal foetus, or dust contaminated with dried secretion (excrement and urine) of infected animal. This study also shows that cattle from bigger herd sizes are more susceptible to infection, findings which are in agreement with Cadmus *et al*. (2020) and Barlozzari *et al.* 2020. This is very possible because transmission of infection increases in large cattle herds. This could occur from contact with infected animals (Menadi *et al*., 2020) or grazing on contaminated pasture and drinking from the same water source (Porter *et al*., 2011; Adamu *et al*., 2018). Q fever is prevalent but

unrecognized because of poor/lack of surveillance of the disease (Maurin and Raoult, 1999) and diagnosis. This has limited the true prevalence of this disease in animals and consequently mismanagement of cases.

Conclusion

In conclusion, this study confirmed the presence of natural *Coxiella burnetii* antibodies in cattle herds because of ruminants are not vaccinated against coxiellosis in Nigeria. The seroprevalence of 9.2% in Lanlate, Ibarapa East in Oyo State is a cause for concern because of the economic implication on the livestock industry and the public health impact. Hence, subsequent studies should embrace a “one health approach”, to give a better understanding of the epidemiology of the disease. It is also important that enlightenment and awareness be created among the general populace, particularly pastoralists on predisposing factors to the disease in order to improve management practices and hygiene.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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