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RESEARCH ARTICLE

Seroprevalence of *Coxiella burnetii* in sheep flocks in Kaduna State, Northwestern Nigeria

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ABSTRACT

A cross-sectional study was carried out to determine the seroprevalence and risk factors of Q fever in sheep in the northern part of Kaduna State, Nigeria. This study aimed to determine *Coxiella burnetii* infection and its risk factors in sheep in Kaduna State. A total of 400 blood samples consisting of 259 samples from females and 141 from males were aseptically collected from the jugular vein of sheep from flocks in Kaduna State. The sera obtained were screened for Q fever using an indirect enzyme-linked immunosorbent assay (iELISA). The obtained data were analysed to determine whether there is a relationship between sex, age, and the animals tested. The analysis revealed that 8.0% of the sera was seropositive by iELISA. There was no significant difference in Q fever seropositivity in the study area according to the sex of sheep ($P > 0.05$). There was a statistically significant difference ($P < 0.05$) in Q fever seropositivity according to the age of sheep. This study indicated a high seroprevalence of Q fever mainly among female animals and older sheep. Further studies are required to determine the epizootiology of Q fever in the study area more precisely.

KEYWORDS

Coxiella burnetii, Kaduna state, Nigeria, seroprevalence, sheep

INTRODUCTION

Q fever is a contagious zoonotic disease caused by *Coxiella burnetii*, an obligate intracellular Gram-negative bacterium. This organism has been traditionally placed in the family Rickettsiaceae; however, phylogenetic studies have demonstrated that *C. burnetii* is more closely related to *Legionella*, *Francisella* and *Rickettsiella* (Stein et al., 1993; Asadi et al., 2013). Coxiellosis has remained one of the most under-reported diseases in Africa. The disease has been described worldwide, except in New Zealand (EFSA, 2010). *C. burnetii* can infect a wide range of animals, both aquatic and terrestrial; the organism can survive for an extended period of time in the environment as a highly resistant spore-like form, which favours its spread by wind over long distances (Kersh et al., 2010; Van den Brom et al., 2015). Small ruminants shed the organism in their faeces, milk, urine, aborted fetuses, placenta, and vaginal discharges, which are the most critical infection sources in humans (Guatteo et al., 2007; Nielsen et al., 2013). Transmission among animals can occur through direct contact with infected animals and contaminated environments, as well as by the inhalation of aerosolised *C. burnetii* organisms. Birth products (placenta, fetuses, amniotic and allantoic fluids) and the milk are potential infection sources (Porter et al., 2011). Similarly, infection may also be transmitted by tick bites, and several tick species have been associated with the transmission of coxiellosis (Herrin et al., 2011).

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Humans can contract the disease through inhalation of the pathogen, contaminated aerosols from excreta of infected livestock, especially their birth products and even from excreta of infected parturient domestic pets such as cats and dogs (Cetinkol et al., 2017). Human infections may occur after consuming unpasteurised infected milk and dairy products (Georgiev et al., 2013). The organism can cause reproductive disorders such as abortion, metritis, mastitis, stillbirth, and weak calves at birth in domestic ruminants, and it may also cause infertility in sheep and goats (Marrie, 2007; Muskens et al., 2011; Agerholm, 2013). In Europe, Q fever is a well-known cause of abortion in sheep flocks (Ruiz-Fons et al., 2010). In humans, Q fever is mainly asymptomatic and can be acute, chronic or subclinical. It usually manifests itself as a non-specific febrile disorder that may arise in conjunction with pneumonia or hepatitis (CDC, 2011; Asadi et al., 2013). It is, therefore, a considerably underdiagnosed and underreported disease (Gidding et al., 2009). In Nigeria, only a few studies have been published on *C. burnetii* in small ruminants (Addo and Schnurenberger, 1977; Nyifi et al., 2018; Adamu et al., 2019, 2020). There are only few published data on Q fever of sheep in Kaduna State, one of the main livestock-producing states of Nigeria. The objective of this study was to investigate the occurrence of *C. burnetii* infection and its possible risk factors in sheep flocks of Kaduna State, Nigeria. This study may provide baseline information that could be used in designing control and prevention policies for coxiellosis in Kaduna State as well as throughout Nigeria.

MATERIALS AND METHODS

Ethical guidelines

This experiment was carried out according to the protocol for the care and use of experimental animals and was approved by the Ethics and Research Committee of the Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria.

Study area

This study was conducted in Kaduna State, Nigeria, located in the northwestern part of Nigeria. Kaduna State has a total land area of about 48,473.2 km^2, which lies between latitude 9° 10′ and 11° 30′ N and longitude 6° 20′ and 9° E. Kaduna State has twenty-three Local Government Areas plus a population of 6,113,503 persons (NPC, 2006) and an estimated sheep population 832,000 (KDSG, 2008). Thus, agriculture is the the primary occupation of the state communities, with about 80% of the people engaged actively in livestock and crop farming (KDSG, 2008).

Study design

This study was conducted as a cross-sectional survey to detect *C. burnetii* infections and probable risk factors affecting the presence of *C. burnetii* antibodies in sheep flocks. Blood samples were collected from August to December 2018 in Kaduna State. A simple random sampling technique was used in selecting the animals.

Sample size estimation and sampling

The sample size was calculated by using the formula described by Thrusfield (2005):

$$N = \frac{Z^2 P_{exp} q}{d^2}$$

where:

N was the sample size,
Z was the standard normal deviate for the 95% confidence interval (1.96),
P was the prevalence (14.5%) (Tukur et al., 2014),
d was the desired precision (0.05), and
q was 1–P, i.e. (1–0.145 = 0.855). Thus,

$$N = \frac{1.96^2 \times 0.145(1 - 0.145)}{(0.05)^2}$$

N = 190

Eventually, 400 blood samples were collected randomly from sheep from the selected Local Government Areas (LGAs) of Kaduna State to make full use of the test kit. A stratified random sampling technique was used to select flocks from the selected LGAs, making first levels and wards the second level. In each level a simple random sampling was used proportionately to size.

Five-millilitre (5-mL) blood samples were aseptically collected from the jugular vein of each sheep into clean straight vacutainer tubes. Each sample was numbered using codes describing the sheep flocks, while information about sex, age and breed of the animals was recorded to facilitate data analysis. Blood samples were transported on ice packed in coolers to the laboratory and were centrifuged at 3,000 *g* for 5 min to obtain clear sera. The obtained sera were stored at –20 °C until tested for the evidence of coxiellosis.

Serological test

Serum samples from sheep were tested for the presence of specific phase I and II antibodies to *C. burnetii* using an iELISA kit for coxiellosis (Q Fever Indirect Multi-species, IDvet, France) following the manufacturer's instructions. The test was conducted in the Bacterial Research Laboratory of the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria. Optical density (OD) values were calculated at a wavelength of 450 nm. Sample/positive percentages (S/P %) for each serum sample were determined after adjusting to the negative control using the following formula:

$$S/P = \frac{OD_{sample} - OD_{NC}}{OD_{PC} - OD_{NC}} \times 100$$

The S/P% values thus obtained were divided in different classes, as described by the manufacturer: Negative (S/P ≤

40%), doubtful (40% < S/P ≤ 50%), positive (50% < S/P ≤ 80%) and strong positive (S/P > 80%). All positive and confusing samples were retested.

Statistical analysis

Descriptive statistics were applied to determine the frequencies and percentages of seropositive samples for antibodies to *C. burnetii*. Data produced were analysed using the Statistical Package for Social Sciences (SPSS) version 20.0 statistical software (SPSS Inc., Chicago, IL, USA). Prevalence was calculated using the number of positive samples divided by the total number of tested samples, then expressed as a percentage. Chi-square (χ^2) and Fisher's Exact Test were used to test for association. Strength of the association was calculated using odds ratio (OD) at 95% confidence interval.

RESULTS

Out of the 400 serum samples tested, 8.0% were found to be seropositive to *C. burnetii* infection using iELISA. Out of 141 samples tested from male sheep, 9 (6.4%) were seropositive, whereas 23 (8.9%) were seropositive out of 259 samples tested from female sheep. No statistically significant association could be demonstrated between the sex of the sheep tested and the positive serological reaction ($P > 0.05$) (Table 1). The seroprevalence recorded was higher among sheep older than 2 years (10.6%) than in sheep less than 2 years old (4.3%) based on the age distribution. A significant association was detected between the age of sheep tested and the positive serological reaction ($P < 0.05$) (Table 2). As regards the distribution of seroprevalence by breed, the seroprevalence recorded was higher in the Yankasa breed (11.1%) than in the Uda (7.1%) and the Balami (4.1%) breeds of sheep tested. There was no statistically significant association between the breed of sheep tested and the positive serological reaction ($P > 0.05$) (Table 3).

DISCUSSION

The seroprevalence of Q fever obtained in this study was lower than the 9.0% reported by Nyifi et al. (2018) in ruminants slaughtered at the Jalingo abattoir in Nigeria, the 11.7% reported by Adamu et al. (2019) in sheep from Yobe State, the 26.9% reported by Johnson et al. (2019) in the Volta region of Ghana, and the 12.4% reported by Hireche et al. (2020) from Algeria. The seroprevalence obtained was comparable to the 7.9% value reported in Ethiopia (Tesfaye et al., 2020). The seroprevalence found in this study was higher than the 3.5% reported in the Netherlands and the 8.7% found in Germany (Georgiev et al., 2013). The seroprevalence was higher in female sheep than in male sheep; however, there was no statistically significant association between the sex of sheep and the positive serological results. The findings of this work were consistent with the reports of Nyifi et al. (2018), Adamu et al. (2019), Larson et al. (2019) and Adamu et al. (2020). However, the seroprevalence was in disparity with the findings of Rahman et al. (2016) from Bangladesh, who reported a higher seroprevalence in male than in female animals. The high seroprevalence of Q fever recorded in females could be due to the hormonal variations among males and females, which play an essential role in determining susceptibility to infection. Seroprevalence was higher in sheep older than two years than in sheep under two years of age; there was a statistically significant association between the age of sheep and seropositivity to Q fever. The results of this work agreed with the findings of Adamu et al. (2019) and Adamu et al. (2020) in Nigeria and Lafi et al. (2020) in Jordan. Sheep older than two years are more likely to be kept in the flock for breeding purposes, and thus they more probably shed the *C. burnetii* organisms, particularly during parturition. The seroprevalence was higher in the Yankasa breed than in the Balami and Ouda breeds, although there was no statistically significant association between the breeds of sheep and seropositivity to *C. burnetii*.

Table 1. Seroprevalence of Q fever in the sheep flocks of Kaduna State, Nigeria based on sex

Sex	Number examined	Number positive (%)	Number negative (%)	OR	95% CI		*P* value
					lower	upper	
Male	141	9 (6.4)	132 (93.6)	0.700	0.315	1.556	0.379
Female	259	23 (8.9)	236 (91.1)	1.0 (ref.)			
Total	400	32 (8.0)	368 (92.0)				

OR = odds ratio; CI = confidence interval.

Table 2. Seroprevalence of Q fever in the sheep flocks of Kaduna State based on age

Age (years)	Number examined	Number positive (%)	Number negative (%)	OR	95% CI		*P* value
					lower	upper	
<2 years	164	7 (4.3)	157 (97.3)	0.376	0.159	0.892	0.022
>2 years	236	25 (10.6)	211 (89.4)	1.0 (ref.)			
Total	400	32 (8.0)	368 (92.0)				

OR = odds ratio; CI = confidence interval.

Table 3. Seroprevalence of Q fever in sheep flocks of Kaduna State based on breed

Breeds	Number examined	Number positive (%)	Number negative (%)	OR	95% CI		*P* value
					lower	upper	
Balami	146	6 (4.1)	140 (95.9)	1.795	0.487	6.616	0.059
Yankasa	198	22 (11.1)	176 (88.9)	0.615	0.203	1.866	
Ouda	56	4 (7.1.)	52 (92.9)	1.0 (ref.)			
Total	400	32 (8.0)	368 (92.0)				

The findings of this study clearly show that sheep are the main reservoir of *C. burnetii* and they pose a significant public health risk of transmitting Q fever to humans in areas where they are kept.

In conclusion, the current study demonstrated that Q fever is prevalent in sheep flocks in Kaduna State, Northwestern Nigeria, showing a higher seroprevalence in female sheep (8.9%) and in sheep older than two years (10.6%). The relatively high seroprevalence found in this study is of major public health concern since most pastoralists and their families consume unpasteurised milk.

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