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## Seroprevalence of *Coxiella burnetii* in Sheep and Goats and associated Risk Factors

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### ABSTRACT

A cross-sectional seroprevalence study was conducted among flocks of sheep and goats in three agricultural zones of Borno State. Seven hundred sixty-eight small ruminants (384 sheep and goats each) of both sexes (282 males and 486 females) of different age groups from 90 flocks were randomly selected for blood collection and related epidemiological data. A commercial indirect enzyme-linked immunosorbent assay (iELISA; I.D. Vet) was used to test the sera samples for the presence of both phases I and II antibodies against *Coxiella burnetii* infections. The overall seroprevalence in sheep and goats was 10.9% (44/384) and 12.0% (46/384). There was no statistically significant association between the two species of the animals tested and the infection rates of coxiellosis ( $P = 0.734$ , OR = 0.924, 95% CI = 0.578–1.408). Bivariate analysis showed that female animals of all species tested were more seropositive for antibodies to *Coxiella burnetii* than males. There was a statistically significant association between the sex of both sheep and goats tested ( $P = 0.032$ , OR = 0.439, 95% CI = 0.204–0.9470) and ( $P = 0.022$ , OR = 0.434, 95% CI = 0.208–0.903). Age of sheep and goats were not statistically significant ( $P = 0.199$ , OR = 0.577, 95% CI = 0.247–1.346), ( $P = 0.304$ , OR = 0.670, 95% CI = 0.311–1.444). There was no statistically significant association between the breeds of sheep and goats tested ( $P = 0.861$ , OR = 0.787, 95% CI = 0.315–1.964), and ( $P = 0.742$ , OR = 0.736, 95% CI = 0.264–2.050). The study indicates that seroprevalence of coxiellosis was high in the studied small ruminant population, particularly among female sheep and goats, and can be considered a potential risk for both susceptible animals and humans in the study area.

**Keywords:** *Coxiella burnetii*; iELISA; Serosurvey; Sheep; Goats

### INTRODUCTION

Query fever (Q fever) is a zoonotic disease caused by the intracellular bacterium *Coxiella burnetii* (CFSPH, 2017). *Coxiella burnetii* is a small coccobacillus, an obligate intracellular pathogen in the family Coxiellaceae, order Legionellales and gamma subdivision of the Proteobacteria (CFSPH, 2017). Coxiellosis is one of the essential zoonotic diseases of livestock that has remained linked with chronic fatigue syndrome (Angelakis and Raoult, 2010). The organism was well-known in all species of animal and human beings; however, sheep and goats are an essential reservoir and common source of infection in humans (OIE, 2016). *Coxiella burnetii* existed in most countries except a few countries such as New Zealand, Norway, Iceland and French Polynesia (CFSPH, 2017). *Coxiella burnetii* is highly resistant to environmental conditions and can resist high

temperature, drying, and several disinfectants (Cekani *et al.*, 2008). Animals become infected either by direct contact with infected animals and contaminated environments or via aerosolized bacteria, which is considered the primary route of infection for both animals and humans (Angelakis and Raoult, 2010). Q fever is typically asymptomatic with a sub-clinical presentation in affected animals. It is usually considered not a problem for animal health apart from ruminants, where the organism causes abortions, stillbirths, and the birth of small or weak offspring (EFSA, 2010; Carbonero *et al.*, 2015; CFSPH, 2017). Reproductive losses may occur as outbreaks in sheep and goats, but they seem to be irregular in cattle. In pregnant women, there is placentitis which leads to premature birth, growth restriction, spontaneous abortion or fetal death (EFSA, 2010). Only a few studies on *C. burnetii* infection have been demonstrated in Nigeria. Prevalence of 14.5% (Tukur *et al.*, 2014; Kaduna

Metropolis), 6.8% (Adamu *et al.*, 2018; Kaduna State) and 11.7% (Adamu *et al.*, 2019; Yobe State) were reported in dairy cows, cattle and sheep respectively.

Recently, in another study in Kaduna State, seroprevalence rates of 8.8% and 8.0% were documented in goats and sheep respectively (Adamu *et al.*, 2020; Adamu *et al.*, 2021). Because of the paucity of information on the infection of small ruminants with *C. burnetii*, this study was to determine the seroepidemiology of *Coxiella burnetii* infection in sheep and goats in three agricultural zones of Borno State, Nigeria. This study may determine the actual status of small ruminants regarding exposure to this bacterial agent in small ruminants in Borno State, Northeastern Nigeria.

## MATERIALS AND METHODS

### Ethical Statement

The experiment was performed in accordance with the care and use of experimental animals' protocol (Ochei and Kolhatkar, 2000) and was approved by the Faculty of Veterinary Medicine Ethics and Research Committee, Ahmadu Bello University Zaria, Nigeria.

### Study Area

This study was conducted in Borno State, which lies between Latitudes 10° 2' N and 13° 4' N and Longitude 11° 4' E and 14° 4' E and covers an area of 69,436 km<sup>2</sup> with an elevation of 35 meters above sea level. The State has a landmass area of 75,540 square kilometres and is located in the Northeastern part of Nigeria (Fig. 1). (BOSG, 2009). Farming and livestock rearing is the main occupation of the people of Borno State, the farming is mainly for food and cash crops and rearing of livestock. Most part of the State generally consists of semi-arid Savannah or sub-desert. The arid zone has rather austere climate conditions with a hot, dry season from late January to late June (Fig. 1). The average daily peak temperature, especially in April and May, is 34.4°C to 37.8°C.

### Study Design

A cross-sectional study was conducted from October 2019 to February 2020. The target population was sheep and goats from farms, households, and settled and semi-sedentary nomadic Fulani flocks. The sampling frame was from a list of sheep and goat farms compiled data from the Veterinary Teaching Hospital (VTH), University of Maiduguri, Borno State, Animal Health Workers and private veterinary practitioners.

### Sample Size Determination

Sample size for the study was determined using the following formula by Thrusfield (2005) with an expected disease prevalence of 11.7 % (Adamu *et al.*, 2019), and accepted absolute error of 5%, and a confidence interval of 95%:

$$N = \frac{Z^2 pq}{d^2}$$

where;

N was the sample size,

Z was the standard for the 95% confidence interval (1.96),

P was the prevalence (11.7%) (Adamu *et al.*, 2019),

d was the desired precision (0.05), and

q was 1-P,

Therefore,

$$n = \frac{1.96^2 \times 0.117 (1 - 0.117)}{(0.05)^2}$$

$$n = 158$$

Using the prevalence of Q-fever in sheep in Yobe State as 11.7% (Adamu *et al.*, 2019), a minimum of 158 samples was required for the study. However, 768 samples from both sheep and goats were randomly collected to increase the precision of the seroprevalence estimate.

### Blood Sample Collection and Handling

Five (5 mL) of blood sample was collected from the animal's jugular vein into plain vacutainer tubes (Becton Dickson, UK). Each sample was labelled using codes describing the specific animal and flock, with a unique identification number. Information about the species, age, breeds, location and sex of the animal was recorded for data analysis. Any missing information was recorded as "unknown". The ages of the animals were verified using the method of Pace and Wakeman (2003). The breed of the animals sampled was recorded based on the physical characteristics of each animal (Bourn *et al.*, 1994; Felius *et al.*, 2011). The samples were then transported in ice-packed coolers to the laboratory. Then, the plain vacutainer tubes were tilted on a table at room temperature for clotting and the clotted blood samples were centrifuged (at 3000 G for 5 min) to obtain clear serum. The harvested sera were stored at -20°C pending testing for the detection of *C. burnetii* infection. Samples that showed haemolysis were discarded and replaced.

### Inclusion Criteria

Only flocks/households whose owners consented were included, flocks/households with a minimum of 5 sheep and goats were included, and sheep and goat flocks/households within Borno State were included.

### Exclusion criteria

Flocks / households outside the location area were excluded, flocks/ households with a maximum of 4 sheep and goats were excluded, sheep and goats that were less than 5 months were excluded from the study.

### Serological Test

The indirect enzyme-linked immunosorbent assay (iELISA) was used for determining the seroprevalence of Q fever in sera samples collected from the animals. The serology test was conducted in the Department of Veterinary Public Health and Preventive Medicine Bacterial Research Laboratory, Ahmadu Bello University Zaria, Nigeria. The iELISA ID Screen® Q Fever Indirect Multi-species kits IDvet, (France). The sera were tested for antibodies against *Coxiella burnetii* using iELISA following the manufacturer's instructions. The samples, reagents and plate(s) were brought to room temperature, and all reagents were homogenized by vortexing before starting the test. The reagents were reconstituted as directed by the manufacturer's guide.

In a 96 well pre-dilution microplate, five microlitres (5 µL) of the negative control was added to wells A1 and B1, and five microlitres (5 µL) of the positive control was added to wells C1 and D1. Five microlitres (5 µL) of each sample were put in the remaining wells, and two hundred and forty-five microlitres (245 µL) of the dilution buffer 2 added to each

well. The plate was then covered and incubated at 21°C for 45 minutes. After incubation, all the contents of the wells were emptied and washed three times with 300 µL of the wash solution. The microtitre plates were tapped against a clean absorbent tissue paper to remove all the contents of the plates. The drying of the wells was avoided between washings. After washing, an anti-multi-species horseradish peroxidase (HRP) conjugate was added to the wells and incubated for 30 min at room temperature. Then substrate solution tetramethylbenzidine (TMB) was added to each well to eliminate conjugate. The plates were incubated for 15 min at room temperature. Subsequently, the stop solution was added to the wells to stop reaction. Then the optical density (O.D) of the microtiter plate was read at an absorbance of 450 manometers using an ELISA reader machine.

**Interpretation of test results**

For each sample, the S/P percentage (S/P%) was calculated following the manufacturer's formula:

$$S/P = \frac{O.D_{\text{sample}} - O.D_{\text{NC}}}{O.D_{\text{P.C.}} - O.D_{\text{NC}}} \times 100$$

After competition, samples S/P ≤ 40% are considered as negative, while from 40 % < S/P ≤ 50 % were considered doubtful and 50 % < S/P ≤ 80 % were considered positive and S/P > 80 % were strong positive.

**Statistical Analysis**

All data generated were analysed using SPSS version 21.0 statistical software (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to determine frequencies and percentages. The prevalence of the disease was then determined using the number of positive samples divided by the total number of samples examined. Association between demographic, management and other variables with infection was determined using the Chi-square (χ<sup>2</sup>) test and Fishers' Exact test where applicable to test for the association. The strength of association was calculated using Odds Ratio (OR) at a 95% Confidence Interval (CI).

**RESULTS**

**Table 1:** Seroprevalence of Q fever in sheep and goats in Borno State based on sex

| Species | Sex    | Number Examined | Number Positive (%) | Number Negative (%) | χ <sup>2</sup> | OR    | 95% CI lower upper | P value |
|---------|--------|-----------------|---------------------|---------------------|----------------|-------|--------------------|---------|
| Sheep   | Male   | 140             | 9 (6.4)             | 131 (93.6)          | 4.598          | 0.439 | 0.204–0.947        | 0.032** |
|         | Female | 244             | 33 (13.5)           | 211 (86.5)          |                |       |                    |         |
|         | Total  | 384             | 42 (10.9)           | 342 (89.1)          |                |       |                    |         |
| Goats   | Male   | 142             | 10 (7.0)            | 132 (93.0)          | 5.208          | 0.434 | 0.208–0.903        | 0.022** |
|         | Female | 242             | 36 (14.9)           | 206 (85.1)          |                |       |                    |         |
|         | Total  | 384             | 46 (12.0)           | 338 (88.0)          |                |       |                    |         |
| Overall |        | 768             | 88 (11.5)           | 680 (88.5)          | 0.1155         | 0.924 | 0.578–1.408        | 0.734   |
| Total   |        |                 |                     |                     |                |       |                    |         |

1 (Ref.) = Reference \*\* = statistically significant

**Table 2:** Seroprevalence of Q fever in sheep and goats in Borno State based on age

| Species | Age (Years) | Number Examined | Number Positive (%) | Number Negative (%) | χ <sup>2</sup> | OR    | 95% CI lower upper | P-value |
|---------|-------------|-----------------|---------------------|---------------------|----------------|-------|--------------------|---------|
| Sheep   | ≤ 2 Years   | 95              | 7 (7.4)             | 88 (92.6)           | 1.651          | 0.577 | 0.247–1.346        | 0.199   |
|         | > 2 Years   | 289             | 35 (12.1)           | 254 (87.9)          |                |       |                    |         |
| Goats   | ≤ 2 Years   | 99              | 9 (9.1)             | 90 (90.9)           | 1.055          | 0.670 | 0.311–1.444        | 0.304   |
|         | > 2 Years   | 285             | 37 (13.0)           | 248 (87.0)          |                |       |                    |         |

1 (Ref.) = Reference

Out of the 768 sera samples from sheep and goats tested for *Coxiella burnetii* antibodies, overall seroprevalence was 11.5%. The seroprevalence recorded for goats and sheep were 46/384 (12.0%) and 42/384 (10.9%) respectively. There was no statistically significant association in the seroprevalence rates between the two species tested ( $P = 0.734$ , OR= 0.924, 95% CI= 0.578–1.408). For sheep, the higher seroprevalence was observed in females (33/244; 13.5%) than males (9/140; 6.4%). There was statistically significant difference in the seroprevalence rates between male and female sheep tested ( $P = 0.032$ , OR= 0.439, 95% CI= 0.204 – 0.947). Similarly, in goats, seroprevalence rate was higher in females (36/242; 14.9%) than the male goats tested (10/142; 7.0%). There was statistically significant difference in the seroprevalence rates between the female and male goats ( $P = 0.022$ , OR= 0.434, 95% CI= 0.208 – 0.903) (Table 1).

Based on age distribution, seroprevalence recorded was higher among sheep older than two years 35 (12.1%) than in sheep less than two years 7 (7.4%). There was no statistically significant association in the seroprevalence rates between the age of sheep tested ( $P = 0.199$ , OR = 0.577, 95% CI= 0.247–1.346). The seroprevalence recorded was higher among goats older than two years 37 (13.0%) than in goats less than two years 9 (9.1%). There was no statistically significant association in the seroprevalence rates between the age of goats tested ( $P = 0.304$ , OR = 0.670, 95% CI= 0.311–1.444) (Table 2).

Based on breeds distribution in sheep, seroprevalence recorded was higher in the Balami breed (11.9%) than in the other two breeds, Yankasa (10.6%) and Uda (9.6%). There was no statistically significant association in the seroprevalence rates between the different breeds of sheep tested ( $P = 0.861$ , OR = 0.787, 95% CI = 0.315–1.964). In goats, seroprevalence recorded was higher in the Sahel breed (13.4%) than in the Red Sokoto breed (11.0%) and the West African Dwarf breed (10.2%). There was no statistically significant association in the seroprevalence rates between different breeds of goats tested ( $P = 0.742$ , OR = 0.736, 95% CI = 0.264–2.050) (Table 3).

**Table 3:** Seroprevalence of Q fever in sheep and goats in Borno State based on breed

| Species | Breed      | Number Examined | Number Positive (%) | Number Negative (%) | $\chi^2$ | OR    | 95% CI      |       | P value |
|---------|------------|-----------------|---------------------|---------------------|----------|-------|-------------|-------|---------|
|         |            |                 |                     |                     |          |       | lower       | upper |         |
| Sheep   | Balami     | 160             | 19 (11.9)           | 141 (88.1)          | 0.301    | 0.787 | 0.315–1.964 | 0.861 |         |
|         | Yankasa    | 151             | 16 (10.6)           | 135 (89.4)          |          |       |             |       |         |
|         | Uda        | 73              | 7 (9.6)             | 66 (90.4)           |          |       |             |       |         |
| Goats   | Sahel      | 172             | 23 (13.4)           | 149 (86.6)          | 0.598    | 0.736 | 0.264–2.050 | 0.742 |         |
|         | Red Sokoto | 163             | 18 (11.0)           | 145 (89.0)          |          |       |             |       |         |
|         | WAD        | 49              | 5 (10.2)            | 44 (89.8)           |          |       |             |       |         |

WAD = West African Dwarf, l(Ref.) = Reference

## DISCUSSION

The seroprevalence of *Coxiella burnetii* obtained in sheep in this study was lower than the 11.7% reported by Adamu *et al.* (2019) in sheep from Yobe State, Nigeria. The prevalence recorded in this study was also lower than the 14.19% reported by Karagul *et al.* (2019) from the Marmara region of Turkey and the 16.57% reported by Raphael *et al.* (2020) from Kumasi, Ghana. The seroprevalence obtained in sheep was higher than the 8.0% reported by Adamu *et al.* (2021) from Kaduna State, Nigeria, the 9.5% reported by Rahman *et al.* (2016) from Bangladesh.

Furthermore, the seroprevalence of *Coxiella burnetii* obtained in goats in this study was lower than the 28.57% reported by Raphael *et al.* (2020) from Ghana, the 28.20% reported by Abushahba *et al.* (2017) from El Minya Governorate Egypt and the 14.1% reported by Khaled *et al.* (2016) from Algeria, the 31.9% reported by Mohabbati *et al.* (2017) from Iran. The seroprevalence obtained in this study in goats was higher than the 10.0% reported by Nyifi *et al.* (2018) from Jalingo abattoir in Nigeria, the 8.8% reported by Adamu *et al.* (2020) from Kaduna State, Nigeria and the 10.24% reported by Karagul *et al.* (2019) from the Marmara region of Turkey.

The seroprevalence of *Coxiella burnetii* was higher in female sheep than in male sheep, though there was no statistically significant association between the sex of sheep and positive serological reactions. The report agreed with the study in Nigeria by Adamu *et al.* (2019) in sheep, Adamu *et al.* (2020) in goats and Adamu *et al.* (2021) in sheep. It also agreed with the reports from Iran by Edalati-Shokat *et al.* (2015) in sheep and goats. Our report also agreed with the findings of Sakhaee and Khalili (2010), who reported higher seroprevalence in females. The high seroprevalence in female animals could be because the organism has a high affinity for foetal membranes, mammary glands and the placenta. The organism appears in large numbers in these tissues. However, the seroprevalence was in contrast to Rahman *et al.* (2016) report in Bangladesh, which reported high seroprevalence of coxiellosis in male animals than in females. The hormonal changes between males and females play an essential role in determining susceptibility to infection (Cantas *et al.*, 2011). Estrogen enhances antibody production, and androgen suppresses both T-cell and B-cell immune responses, but immunity in females can be depressed due to various factors such as age, pregnancy and environmental factors (Cantas *et al.*, 2011; Porter *et al.*, 2011).

Seroprevalence was higher in sheep and goats older than two years than in sheep and goats less than two years. Though, there was no statistically significant association in seroprevalence of coxiellosis and the age of sheep and goats.

This finding agreed with those of the previous reports from Spain by Ruiz-Fons *et al.* (2010), the Gambia by Klaasen *et al.* (2014), Iran by Ezatkhaha *et al.* (2015) and Nigeria by Adamu *et al.* (2020) who variously reported high seroprevalence in the age greater than two years old. However, this contrasts with Esmaeili *et al.* (2013) report in Spain, who reported high seroprevalence in animals less than two years old. (Ruiz-Fons *et al.*, 2010, Astobiza *et al.*, 2011). Though there was no statistically significant association between the seroprevalence of coxiellosis and the breeds of sheep and goats studied. But the seroprevalence was higher in Balami than in Yankasa and Uda breeds of sheep, the seroprevalence was also higher in the Sahel breed than in Red Sokoto and the West African Dwarf breeds of goats

In conclusion, the present study revealed that *Coxiella burnetii* infection is present in sheep and goats in Borno State, Northeastern Nigeria.

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## Conflict of Interest

The authors declare that they have no conflict of interest.

## Authors' Contribution

SGA, GSNK and SNAS: designed and planned the research. SGA: performed the field work, SGA and AOT financed the research. SGA, AOT and SMJ: wrote the paper and editing. MAS, HIM and FEE provided support assistance to the study. GSNK and SNAS supervised the work. All authors read and approved the final manuscript

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