# Serosurvey and Risk Factors of Coxiella burnetii in Sheep and Goats in three agricultural zones of Borno State, Nigeria

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**Abstract**

Coxiellosis (Q-fever) caused by *Coxiella burnetii* is a cosmopolitan zoonosis that causes significant losses through abortions and stillbirths in small ruminants. A cross-sectional seroprevalence study was conducted in three agricultural zones of Borno State in the flocks of sheep and goats. 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 to collect sera and related epidemiological data information. A commercial indirect enzyme-linked immunosorbent assay (iELISA; I.D. Vet) was used to test the samples for the presence of both phases I and II antibodies to *Coxiella burnetii* infections. The seroprevalence in sheep and goats was 10.9% (44/384) and 12.0% (46/384). There was no statistically significant association in the two species of the animals tested and the infection rates of coxiellosis. 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 and the infection rates of coxiellosis. Breed and age of sheep and goats were not statistically significant for seropositivity to *Coxiella burnetii.* The study indicates that seroprevalence of coxiellosis was high in the studied small ruminants' population, particularly among female sheep and goats and can be considered a potential risk for both susceptible animals and humans in the study area.

## Introduction

Query 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 well-being 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. While 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. Tukur *et al* . (2014) reported a prevalence of 14.5% in dairy cows in Kaduna Metropolis, Adamu *et al* . (2018) reported

a prevalence of 6.8% in cattle herds in Kaduna State, Adamu *et al* . (2019) reported a prevalence of 11.7% in sheep from Yobe State, Nigeria. Recently, in another study in Kaduna State, seroprevalence rates of 8.8% and 8.0% were reported in goats and sheep (Adamu *et al.,* 2020; Adamu *et al* ., 2021). Because of the scanty information concerning the report of 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 bacterium causing agent in small ruminants in Borno State, Northeastern Nigeria.

## MATERIALS AND METHODS

### 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$^2$ 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. The State shares boundaries with the Republic of Niger to the North, the Cameroon Republic to the East, and the Northeast with the Chad Republic (BOSG, 2009). Borno State has 27 Local Government Areas (LGAs) spread over six Agricultural Zones (Fig. 1). 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. The average daily peak temperature, especially in April and May, is 34.4°C to 37.8°C (BOSG, 2009).

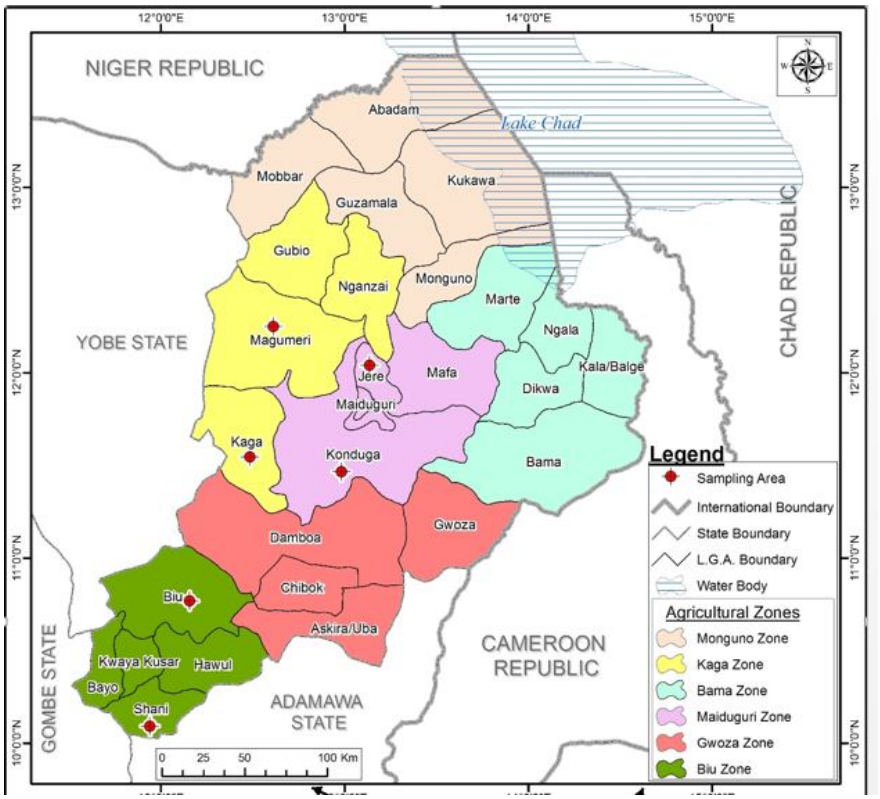


**Figure 1:** Map of Borno State showing the sampled area

### Study design

A cross-sectional study was used to conduct this study, which lasted six months, 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 goats 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 with an expected disease prevalence of 11.7 % (Adamu *et al* ., 2019), and accepted absolute error of 5%, and a confidence interval of 95% (Thrusfield, 2005):

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

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,

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

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 samples 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. The plain vacutainer tubes were then tilted on a table at room temperature for clotting, the clotted blood samples were centrifuged (at 3000 G for 5 min) to obtain clear serum. The harvested sera were stored at -20$^0$C pending testing for the evidence of *C. burnetii* infection. Samples that showed haemolysis were discarded and replaced. A total of 768 blood/sera samples from sheep and goats were collected for this study.

**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.

**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 were from IDvet, Innovative Diagnostics, Montpellier, France. The sera were tested for antibodies against *Coxiella burnetii* using commercial Multi-species iELISA following the manufacturer's instructions. The reagents for the test were reconstituted as directed by the manufacturer's guide. The samples, reagents and plate (s) were brought to room temperature, and all reagents were homogenized by vortexing before starting the test. Each plate had 96 wells comprised of 8 rows and 12 columns each.

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 tested in the remaining wells, and two hundred and forty-five microlitres (245 µL) of the dilution buffer two added to each well. In the ELISA microplate, one hundred microlitres (100 µL) of the pre-diluted negative control was transferred to wells A1 and B1. The remaining wells tested one hundred microlitres (100 µL) of each pre-diluted sample. The pre-diluted positive control was transferred to wells C1 and D1; the remaining wells tested one hundred microliters (100 µL) of each pre-diluted sample. The plate was then covered and incubated at temp 21°C for 45 min. 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. After washing the plates with a wash solution to eliminate conjugate, the substrate solution tetramethylbenzidine (TMB) was added to each well. The plates were incubated for 15 min at room temperature. The stop solution was added to stop the reaction. Then the optical density (O.D) of the microtitre plate was read at an absorbance of 450 nanometers 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:

$$S/P = \frac{\text{O.D}_{\text{sample}} - \text{O.D}_{\text{NC}}}{\text{O.D}_{\text{P.C.}} - \text{O.D}_{\text{NC}}} \; X \; 100$$

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. All the doubtful results were classified as negative for analysis.

**Statistical analysis**

All data generated were analyzed using Statistical Package for Social Sciences (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 ($\chi 2$) test and Fishers' Exact test to test for the association. The strength of association was calculated using Odds Ratio (OR) at a 95% Confidence Interval (CI).

## RESULTS

Out of the 768 sera samples from sheep and goats tested for *Coxiella burnetii* antibodies, overall seroprevalence was 11.5%. The highest seroprevalence was recorded in goats with 46 (12.0%) out of the 384 samples tested, and the least seroprevalence was recorded in sheep with 42 (10.9%) out of 384 samples tested. There was no statistically significant association in the seroprevalence rates between the two species tested ($P$ >0.05), (OR= 0.902, 95% CI= 0.578–1.408). In sheep, the highest seroprevalence was recorded in females with 33

(13.5%) out of 244 sheep sampled, while the seroprevalence of 9 (6.4%) was recorded out of 140 males sheep sampled. There was statistically significant difference in the seroprevalence rates between male and female sheep tested ($P < 0.05$), (OR= 0.439, 95% CI= 0.204 – 0.947). In goats, the highest seroprevalence rate was recorded in females, with 36 (14.9%) out of 242 females goats' tested, while a seroprevalence of 10 (7.0%) was recorded out of 142 males goats tested. There was statistically significant difference in the seroprevalence rates between the female and male goats tested ($P < 0.05$), (OR= 0.434, 95% CI= 0.208 – 0.903) (Table 1).

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

| Species | Sex | Number Examined | Number Positive (%) | Number Negative (%) | ?² | OR | 95% CI lower upper | P valu |
|---|---|---|---|---|---|---|---|---|
| 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) | | 1* | | |
| | **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) | | 1* | | |
| | **Total** | **384** | **46 (12.0)** | **338 (88.0)** | | | | |
| | **Overall Total** | **768** | **88 (11.5)** | **680 (88.5)** | **0.1155** | **0.924** 1* | **0.578–1.408** | **0.734** |

1* = Reference

** = statistically significant

Table 2 shows the age distribution of *Coxiella burnetii*antibodies in sheep and goats samples. In sheep, the highest seroprevalence was recorded in the age group older than two years with 35 (12.1%) out of the 289 samples tested, while the least was recorded in the age group less than or equal to 2 years with the seroprevalence of 7 (7.4%) out of 95 samples tested. There was no statistically significant association in the seroprevalence rates between the age of sheep tested ($P > 0.05$), (OR = 0.577, 95% CI= 0.247–1.346). In goats, the highest seroprevalence was recorded in the age group older than 2 years with 37 (13.0%) out of the 285 samples tested, while the least seroprevalence was recorded in the age less than or equal to two years with 9 (9.1%) out of 99 samples tested. There was no statistically significant association in the seroprevalence rates between the age of goats tested ($P > 0.05$), (OR = 0.670, 95% CI= 0.311–1.444).

**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 (%) | ?² | OR | 95% CI lower upper | P-valu |
|---|---|---|---|---|---|---|---|---|
| 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) | | 1* | | |
| Goats | [?] 2 Years | 99 | 9 (9.1) | 90 (90.9) | 1.055 | 0.670 | 0.311–1.444 | 0.304 |

| Species | Age (Years) | Number Examined | Number Positive (%) | Number Negative (%) | $?^2$ | OR | 95% CI lower upper | P-valu |
|---|---|---|---|---|---|---|---|---|
| | > 2 Years | 285 | 37 (13.0) | 248 (87.0) | | 1* | | |

1* = Reference

Based on breed, the highest seroprevalence was recorded in the Balami breed of sheep, with 19 (11.9%) out of 160 samples tested, followed by seroprevalence of 10.6% recorded from the Yankasa breed of sheep tested. The least seroprevalence was recorded in the Uda breed of sheep, with a seroprevalence of 9.6%. There was no statistically significant association in the seroprevalence rates between the different breeds of sheep tested ($P > 0.05$), (OR = 0.787, 95% CI = 0.315–1.964). In goats, the highest seroprevalence was recorded among the Sahel breed, with a seroprevalence of 13.4% out of 172 samples tested. The Red Sokoto (Maradi) breed recorded a seroprevalence of 11.0% out of 163 samples tested. Of the 49 samples tested from West African Dwarf (WAD) goats, a seroprevalence of 10.2% was recorded. There was no statistically significant association in the seroprevalence rates between different breeds of goats tested ($P$>0.05), (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 (%) | $?^2$ | OR | 95% CI Lower upper | P valu |
|---|---|---|---|---|---|---|---|---|
| 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) | | 0.895 | 0.351–2.281 | |
| | Uda | 73 | 7 (9.6) | 66 (90.4) | | 1* | | |
| 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) | | 0.915 | 0.321–2.607 | |
| | WAD goats | 49 | 5 (10.2) | 44 (89.8) | | | | |

WAD = West African Dwarf, 1* = Reference

## DISCUSSION

The overall seroprevalence of Q fever recorded in this study was 11.5% in sheep and goats. In sheep, the seroprevalence of Q fever obtained in this study was 10.9%, which was found to be higher than the 9.5% reported in Bangladesh by Rahman *et al* . (2016), 9.0% reported in Jalingo, Taraba State, Nigeria by Nyifi *et al* . (2018) and the 8.0% reported from Kaduna State, Northwestern Nigeria by Adamu *et al* . (2021). Though the seroprevalence obtained was lower than 25.68% reported from El Minya Governorate, Egypt by Abushahba *et al* . (2017), the 24.7% reported from Iran by Mohabbati *et al* . (2017). The seroprevalence was also lower than the 11.7% reported from Yobe State, Northeastern Nigeria by Adamu *et al* . (2019), 14.19% reported by Karagul *et al* . (2019) from the Marmara region, Turkey and 16.57% reported by Raphael *et al* . (2020) from Kumasi, Ghana. In goats, the seroprevalence of Q fever obtained in this study was 12.0%, which

was higher than the 7.6% reported in Bangladesh by Chakrabartty *et al* . (2016), the 10.0% was reported by Nyifi*et al* . (2018) from Jalingo Taraba State, Nigeria. The seroprevalence was also higher than 10.24% reported by Karagul *et al* . (2019) from the Marmara region, Turkey and the 8.8% reported by Adamu *et al* . (2020) from Birnin Gwari and Maigana agro-ecological zones of Kaduna State, Nigeria. However, the seroprevalence was lower than 14.1% reported by Khaled *et al* . (2016) from Algeria and 19.5% reported by Rizzo *et al* . (2016) from Italy. The seroprevalence was also lower than 28.20% reported by Abushahba*et al* . (2017) from El Minya Governorate Egypt, the 31.9% reported by Mohabbati *et al* . (2017) from Iran and 28.57% reported by Raphael *et al* . (2020) from Ghana.

The infection rates were higher in females than in males in both sheep and goats; there was a statistically significant association between seroprevalence of infection and the sex of sheep and goats studied. 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) on sheep and goats. Our report also agreed with the findings of Sakhaee and Khalili (2010), who reported seroprevalence of 18.8% in females and 10.6% in male animals from Iran, a statistically significant difference ($P<0.05$). 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).

Animals greater than two years old exhibited a greater rate of seropositivity for *Coxiella burnetii* than did the animals less than or equal to 2 years old in both sheep and goats studied. There was no statistically significant association in seroprevalence of coxiellosis and the sheep and goats' studied ages. 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. This finding also suggests horizontal transmission among animals and the maintenance of infection within adult populations (Ruiz-Fons *et al* ., 2010, Astobiza *et al* ., 2011). There was no statistically significant association between the seroprevalence of coxiellosis and the breeds of sheep and goats studied. While in sheep, the seroprevalence was higher in Balami, followed by Yankasa and the least was in the Uda breed. In goats, the seroprevalence was higher in the Sahel breed, followed by Red Sokoto or Maradi breed, and the least was in West African Dwarf breed.

In conclusion, the present study revealed that *Coxiella burnetii*infection is present in sheep and goats in Borno State, Northeastern Nigeria, at seroprevalence rates of 10.9% and 12.0%. *Coxiella burnetii* antibodies were demonstrated at a higher prevalence in female sheep (13.5%) and female goats (14.9%) and in older animals (12.1% in sheep and 13.0% in goats) than in younger ones (7.4% in sheep and 9.1% in goats). The high seroprevalence rates recorded in this study are of public health concern knowing how pastoralists mingle with their animals and consume unpasteurized milk from these animals. The possibility of the spread of infection due to *C burnetii* beyond Borno State is because of the lack of livestock movement control and poor disease surveillance program. There is, therefore, a need to determine the status of *C. burnetii* infection among small ruminants in Borno State with a view to advice on husbandry management practices.

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## REFERENCES

Abushahba, M.F.N., Abdelbaset, A.E., Rawy, M.S. *et al.* (2017). Cross-sectional study for determining the prevalence of Q fever in small ruminants and humans at El Minya Governorate, Egypt. *BMC Res Notes* **10,** 538 (2017). https://doi.org/10.1186/s13104-017-2868-2v

Adamu, S. G., Kabir, J., Umoh, J. U. and Raji, M. A. (2021). Seroprevalence of Coxiella burnetii in sheep flocks in Kaduna State, Northwestern Nigeria. *Acta Veterinaria Hungarica,* 69(3), 234–238. http://doi:10.1556/004.2021.00034

Adamu, S.G., Kabir, J., Umoh, J. U. and Raji, M.A (2020). Seroprevalence of Coxiellosis (Q fever) in Flocks of Goat in Birnin Gwari and Maigana Agro-Ecological Zones of Kaduna State, Nigeria. *Sahel Journal of Veterinary Science*, 17(1), 12-16, https://doi.org/10.54058/saheljvs.v17i1.75

Adamu, S.G., Tijani, A.O., Adamu, N. B., Atsanda, N.N., Dauda, J. and Lawan, F.A. (2019). Epidemiology of Q fever in flocks of sheep in Yobe State, Nigeria.*Journal of Veterinary and Biomedical Sciences*, 2, 70–76. http://10.36108/jvbs/9102.20.0180

Adamu, S. G, Kabir, J., Umoh, J.U and Raji, M. A. (2018). Seroprevalence of brucellosis and Q fever (coxiellosis) in cattle herds in Maigana and Birnin Gwari agro-ecological zone of Kaduna State, Nigeria. *Tropical Animal Health and Production*, 50, 1583–1589. https://doi.org/10.1007/s11250-018-1598-3

Angelakis, E. and Raoult, D. (2010). Q fever. *Veterinary Microbiology* , 140(3-4), 297–309.

Astobiza, I., Barral, M., Ruiz-Fons, F., Barandika, J.F., Gerrikagoitia, X., Hurtado, A. and Gardia-Perez, A. L. (2011). Molecular investigation of *Coxiella burnetii*in wildlife and ticks in an endemic area. *Veterinary Microbiology*, 147, 190–194.

Borno State Government (BOSG) (2009). "Borno State Government Diary, 19–29

Bourn, D., Wint, W., Blend, R. and Wolley, E. (1994). Nigerian livestock Resource survey. FAO *World Animal Review,* 78 (1), 49–58.

Cantas, H., Muwonge, A., Sareyyupoglu, B., Yardimci, H. and Skjerve, E. (2011). Q fever abortions in ruminants & associated on farm risk factors in northern Cyprus.*BMC Veterinary Research,* 7, 13. https://doi.org/10.1186/1746-6148-7-13

Carbonero, A., Guzman, L.T., Montano, K., Torralbo, A., Arenas-Montes, A., Saa, L.R. (2015). *Coxiella burnetii* seroprevalence and associated risk factors in dairy and mixed cattle farms from Ecuador. *Preventive Veterinary Medicine*, 118(4), 427-435. https://doi.10.1016/j.prevetmed.2015.01.007

Cekani, M. M., Papa, A., Kota, M., Velo, E. and Berxholi, K. (2008). Report of a serological study of *Coxiella burnetii* in domestic animals in Albania, *The Veterinary Journal*, 175, 276–278. https://doi.org/10.1016/j.tvjl.2007.01.005

Center for Food Security and Public Health (CFSPH) (2017). Q fever. Institute for International Cooperation in Animal Biologics an OIE Collaborating Center College of Veterinary Medicine Iowa State University Ames, Iowa 50011. Q Fever. Retrieved from http://www.cfsph.iastate.edu/DiseaseInfo/ fact-sheets.php. Pp1 – 12.

Chakrabartty, A., Bhattacharjee, P.K., Sarker, R.R., Rahman, A.K.M.A., Henning, K., Neubauer, H.

and Rahman, M.S. (2016). Prevalence of *Coxiella burnetii*Infection in Cattle, Black Bengal Goats and Ticks in Bangladesh.*Bangladesh Journal of Veterinary Medicine*, 14 (1), 65-68.

Edalati-Shokat, H., Abbasi-Doulatshahi, E., Hajian-Bidar, H., Gharekhani, J. and Ali-Akbar Rezaei,

A.A. (2015). Q fever in domestic ruminants: A Seroepidemiological survey in Hamedan, Iran. *International Journal of Current Microbiology and Applied Sciences*, 4(1): 589–596.

European Food Safety Authority (EFSA) (2010). Panel on Animal Health and Welfare (AHAW);

Scientific Opinion on Q Fever. *EFSA Journal*, **8** (5), 1595, 114 pp. https://doi:10.2903/j.efsa.2010.1595

Esmaeili, S., Mostafavi, E., Shahdordizadeh, M., Mahmoudi, H. (2013). A seroepidemiological

survey of Q fever among sheep in Mazandaran province, northern Iran.*Annals of Agricultural and Environmental Medicine*, 20(4), 708–710.

7Ezatkhah, M., Alimolaei, M., Khalili, M and Hamid Sharifi, H. (2015). Seroepidemiological study of Q fever in small ruminants from southeast Iran. *Journal of Infection and Public Health* , 8 (2), 170–176.

Felius, M., Koolmees, P. A., Theunissen, B. (2011). European Cattle Genetic Diversity Consortium

and Johannes A. Lenstra on the Breeds of Cattle Historic and Current Classifications. *Diversity*,3, 660–692. doi: 10.3390/d3040660.

Heydel, C. and Willems, H. (2011). Pathogens in Milk – *Coxiella burnetii* . In: Fuquay, J. W., Fox,

P. F. and McSweeney, P. L. H (eds.) Encyclopedia of dairy sciences (Second edition); **4:** 54–59. *Academic Press*, London.

Karagul, M.S., Malal, M.E. and Akar, K. (2019). Seroprevalence of Q fever in sheep and goats from

the Marmara region, Turkey. *Journal of Veterinary Research* , 63(4), 527 – 532.

https://doi.org/10.2478/jvetres-2019-0070

Keyvanirad, N., Azizzadeh, M., Taghavi Razavizadeh, A.R., Mehrzad, J and Rashtibaf, M. (2014).

Seroepidemiology of coxiellosis (Q fever) in sheep and goat populations in the Northeast of Iran. *Iranian Journal of Veterinary Research*, 15(1), 1–16.

Khaled, H, Sidi-Boumedine, K., Merdja, S., Dufour, P., Dahmani, A., Thiéry, R., Rousset, E. and

Bouyoucef, A. (2016). Serological and molecular evidence of Q fever among small ruminant flocks in Algeria. *Comparative Immunology, Microbiology and Infectious Diseases*, 47,19-25. https://doi.org/10.1016/j.cimid.2016.05.002

Klaasen, M., Roest, H.J., van der Hoek, W., Goossens, B., Secka A and Stegeman, A. (2014). .

*Coxiella burnetii* Seroprevalence in Small Ruminants in The Gambia. *PLoS ONE,* 9(1), 1–6.

Mohabbati, M.A., Bagheri, A.F. and Esmaeili, S. (2017). Seroprevalence of Q fever among human

and animal in Iran; A systematic review and meta-analysis. *PLoS Neglected Tropical Diseases,* 11(4), 1–15. https://doi.org/10.1371/journal

Nyifi, A. S., Ardo, M. B., Ja'afar J. N. and Mbaya, P. Y. (2018). Detection of *C. burnetii* antibodies among humans and slaughtered ruminants in Jalingo abattoir, Nigeria. *Savannah Veterinary Journal,* 1(1), 14 – 18. https://doi.10.36759/svj.2017.005

OIE. 2016. Manual of the Diagnostic Tests and Vaccines for Terrestrial Animals, Office

International des Epizootics: Paris, France, pp. 1–15.

Raphael, D. F, Opoku-Agyemang, T., Amemor, E., Opoku, E. D., Bentum, K. E. and Emikpe, B. O.

(2020). Serological evidence of Coxiella burnetii infection in slaughtered sheep and goats at Kumasi Abattoir, Ghana. *Journal of Immunoassay and Immunochemistry*, 41, (2) 152 – 157. http://doi.org/10.1080/15321819.2019.1701012

Pace, J. E. and Wakeman, D. L. (2003). Determining the age of cattle by their teeth. CIR253. Florida

Cooperative Extension Service, Institute of Food and Agricultural Sciences. University of Florida, Gainesville, FL., 23, 345–357.

Porter, S.R., Czaplicki, G., Mainil, J., Guatteo, R. and Saegerman, C. (2011). Q Fever: Current

State of Knowledge and Perspectives of Research of a Neglected Zoonosis.*International Journal of Microbiology*. doi:10.1155/2011/248418, Pp1-2

Rahman, M.A., Alam, M.M., Islam, M.A., Bhuiyan, A.K.F.H. and Rahman, A.K.M.A. (2016).

Serological and Molecular Evidence of Q Fever in Domestic Ruminants in Bangladesh. *Veterinary Medicine International*, 1–8. doi.org/10.1155/2016/098416.

Ruiz-Fons, F., Astobiza, I., Barandika, J. F., Hurtado, A., Atxaerandio, R., Juste, R. A. and García-

Pérez, A. L. (2010). Seroepidemiological study of Q fever in domestic ruminants in semi-extensive grazing systems. *BMC Veterinary Research*, 6(3), 1746 – 6148.

Rizzo, F., Vitale, N., Ballardini, M., Borromeo, V., Luzzago, C., Chiavacci, L. and Mandola, M. L.

(2016). Q fever seroprevalence and risk factors in sheep and goats in northwest Italy. Medicine. *Preventive Veterinary Medicine*, 130, 10–17.

Sakhaee, E. and Khalili, M. (2010). The first serologic study of Q fever in sheep in Iran. *Tropical*

*Animal Health and Production* , 42, 1561–1564.

Thrusfield, M. (2005). Veterinary epidemiology, 3rd edition. Wiley-Blackwell, Oxford. pp.

228–330.

Tukur, H.B., Ajogi, I., Kabir, J. and Umoh, J.U., 2014. Seroprevalence of *Coxiella burnetii* in Cattle

and Its Risk Factors in Kaduna Metropolis, Kaduna State, Nigeria.*IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)*, 7(2), 01–05.