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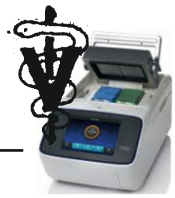

Epidemiology of Q - Fever in Flocks of Sheep in Yobe State, Nigeria

Adamu S. G. [1*], Tijani A. O. [1], Adamu N. B. [1],
Atsanda N. N. [1], Dauda J.[1] and Lawan F. A. [2]
[1]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Maiduguri Borno State, Nigeria
[2]Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Maiduguri Borno State, Nigeria

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ABSTRACT

Q-fever is a zoonotic disease caused by Coxiella burnetii, an intracellular Gram-negative bacterium. A cross sectional epidemiological study was conducted to determine the sero-prevalence of Q-fever in flocks of sheep in Yobe State, Nigeria. Simple random sampling technique was used in selecting the animals. A total of 420 blood samples from sheep of various ages were tested from April, 2018 to July, 2018 for Q-fever using indirect enzyme linked immunosorbent assay (ELISA). Out of the 420 sera tested 49 (11.7%) were seeropositive for Q fever. Of the 315 females sheep tested, 39 (12.4%) were seropositive and out of 105 male sheep tested, 10 (9.5%) were seropositive. There was no significant association (p>0.05) between the sex of sheep tested for Q fever. The seroprevalence was higher in animals greater than 2 years 41 (12.1%) than in animals less than 2 years 8 (9.8%). There were no significant associations between age and infection with Q-fever even though that animal greater than 2 years showed higher prevalence than those less than 2 years. The highest seroprevalence 22 (12.3%) was recorded in Yankasa sheep, and the least seroprevalence 8(9.9%) was recorded in Uda. The seroprevalence of 19 (13.6%), 16 (11.4%) and 14 (10.0%) were recorded in Damaturu, Potiskum and Gashua zones respectively. There was no significant association (p>0.05) between the breed and location of the animals tested for Q fever infection. This study concludes that Q-fever is endemic in sheep in Yobe State. Enlightenment campaign is recommended to educate the livestock farmers, herders and the general public on the dangers of Q-fever infection. There is need for large scale epidemiological investigation of the disease in other livestock farms in the state.

***Keywords*:** Epidemiology, Q-Fever, Sheep, Sero-prevalence, Yobe

Corresponding Author:
email: gidado@unimaid.edu.ng
Tel.: +234 (0)803 538 3010

INTRODUCTION

Coxiellosis (Q Fever) is a highly infectious zoonotic disease that affect both man and animals caused by *Coxiella burnetii*, which is an obligate intracellular gram-negative bacterium [1]. The organism has been described in almost all countries of the world with the exception of New Zealand [2]. A wide variety of animals can be infected with *C. burnetii*. Ticks are considered to be the natural primary reservoir of *C. burnetii* while domestic ruminants are the main reservoirs for the pathogen which can infect a variety of hosts including mammals, reptiles, arthropods and birds [3]. The causative agent of Q fever is classified as a "Category "B" critical biological agent" by the Centre for Disease Control and Prevention and is considered a potential agent of bioterrorism [4]. Q fever can be transmitted by some species of ticks. Animals can be infected by direct contact with infected animals, birth products, excreta, milk and contaminated surroundings and from inhalation of aerosolized bacteria [5]. In humans, transmission of infection is mainly through the consumption of unpasteurized infected milk and milk products, inhalation of contaminated aerosols and direct contacts with birth products and excreta from infected animals [3]. Reproductive disorders such as abortion, metritis, mastitis, stillbirth, weak calves at birth, infertility in cattle, sheep and goats are some of the characteristics of the disease [6]. Only limited studies on *C. burnetii* in cattle, sheep and goats are available in Nigeria. Addo and Schnurenberger [7] reported a prevalence of 11.0% in cattle, 16.5% in sheep and 8.8% in goats in Samaru, Zaria Kaduna State, Adesiyun *et al.,* [8] also reported a prevalence of 59.8% in some dairy cows and their suckling calves in Zaria. Adamu *et al.,* [9] reported a prevalence of 6.8% in Cattle herds in Kaduna State. There is little or no published information on Q-fever in sheep Yobe state, which is one of the leading livestock producers in the Nigeria. The objective of this study was to determine the sero-prevalence of Q-fever in the flocks of sheep in Yobe State, Nigeria.

MATERIALS AND METHODS

Study area

The study was carried out in the three senatorial zones of Yobe State, Nigeria. The state is located in the arid-zone of the North-Eastern part of Nigeria with a total area of 45,502 square kilometers. The state is dry and hot for most part of the year except in the southern part of the state which has a milder climate. The arid zone has a severe climatic condition with a dry season starting from late November to early May with average daily peak temperatures especially in April and May of 34.4–37.80C. The state is one the states in northern Nigeria that shared international border with Niger Republic which enhances Trans boarder movement of livestock between the two countries. Yobe State is one of the leading livestock producers in Nigeria [10].

Study design

A cross-sectional epidemiological method was used in the study. The study was carried out between April, 2018 and March, 2019 in the three senatorial zones of Yobe State. Simple random sampling technique was used in selecting the animals.

A total of 520 samples from sheep were randomly collected to increase precision of the estimate of the seroprevalence and to utilize the kit.

Sample collection and handling

Five milliliters of blood sample were was collected aseptically from the jugular vein of

each animal into clean plain vacutainer sample bottles. Each sample was labeled using codes describing the flocks of animal and information about sex, age, breeds and location were recorded for data analysis. The samples were transported on ice packed in coolers to the laboratory and were centrifuged (at 3000g for 5 min) to obtain clear sera. The harvested sera were stored at -20 °C until tested.

Serological tests and sample analysis

Serological test was carried out using indirect enzyme-linked immunosorbent assay (iELISA) in the bacterial research laboratory, Faculty of Veterinary Medicine, Ahmadu Bello University Zaria, Nigeria. The iELISA ID Screen ® Q Fever Indirect Multi-species kit was supplied by IDvet. Innovative Diagnostics, Montpellier, France Serum samples were checked for the presence of antibodies against *C. burnetii* using commercial iELISA in accordance with the manufacturer's instructions. The reagents in the kit were reconstituted as directed by the manufacturers. Samples, reagents and plate (s) were brought to room temperature (21°C± 5°C) and all reagents were homogenized by vortexing before starting the test. The micro-wells were coated with *C. burnetii* phases I and II strain, isolated in France from an aborted bovine placenta. Each plate had 96 wells of 8 rows and 12 columns each. In order to avoid differences in incubation time between specimens, a 96 well plate containing the test samples and control specimens were prepared before transferring them into an iELISA microtitre plate using multichannel pipette. Ninety microlitre (90µl) of dilution buffer 2 were added into each micro-well, 10µl of the negative control was added into wells A1 and B1. Ten microlitre (10µl) of the positive control was added into wells C1 and D1. Ten microlitre (10µl) of each sample was immediately dispensed into the remaining wells. The plate was then incubated at 21°C for 45 min. After incubation, the contents of the wells (plate) were shaken out and washed 3 times with approximately 300µl of the wash solution. The microtitre plates were tapped against a clean absorbent tissue paper in order to remove all the contents of the plates. The drying of the wells were 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 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 nm using ELISA reader.

Statistical analysis

Data generated were analysed using Statistical Package for Social Sciences (SPSS) version 21.0 statistical software (SPSS Inc., Chicago, IL, USA). Statistical methods used include descriptive statistics to determine frequencies and percentages. Chi square analysis was used for comparison and Odd ratio to test associations between the occurrence of *Coxiella burnetii* infection and sex, age, breed of sheep tested.

RESULTS

Of the 420 sheep samples tested, 49 (11.7%) were seropositive to *Coxiella burnetii* infection by iELISA. Out of the 105 males sheep tested, 10 (9.5%) were seropositive, while out of the 315 females tested, 39 (12.4%) were seropositive. There was no significant association ($p > 0.05$) between sex and the presence of *Coxiella burnetii* antibodies in sheep (Table 1). There was no significant

association ($p > 0.05$) between age and infection with *C. burnetii* in sheep (Table 2). However, sheep of ages > 2 years showed higher prevalence than those of ages < 2 years. There was no significant association ($p > 0.05$) between breed and the presence of *Coxiella burnetii* antibodies ($p > 0.05$) in sheep (Table 3). The prevalence rates in decreasing order were 22 (12.3%), 19 (11.7%) and 8 (9.9%) recorded in Yankasa, Balami and Uda respectively. Table 4 showed distribution of Q fever based on senatorial zone (location), Damaturu zone recorded highest seroprevalence, 19 (13.6%), followed by Potiskum zone, 16 (11.4%) and the least was recorded in Gashua zone, 14 (10.0%). There was no significant association ($p > 0.05$) between location and the presence of *Coxiella burnetii* antibodies in sheep.

Table 1: Sex Distribution of Q fever in sheep in Yobe State

Sex	Number Examined	iELISA +ve No. (%)	iELISA –ve No. (%)	OR	95% CI lower	95% CI upper	P-value
Male	105	10 (9.5)	95 (90.5)	0.745	0.358	1.550	0.430
Female	315	39 (12.4)	276 (87.6)	1*			
Total	**420**	**49 (11.7)**	**371 (88.3)**				

Key: 1* = Reference

Table 2. Seroprevalence of Q fever in sheep in three senatorial zones of Yobe State based on age using iELISA

Age (years)	Number Examined	iELISA +ve No. (%)	iELISA –ve No. (%)	OR	95% CI lower	95% CI upper	P-value
≤ 2 years	82	8 (9.8)	74 (90.2)	0.783	0.352	1.741	0.548
> 2 years	338	41 (12.1)	297 (87.9)	1*			
Total	**420**	**49 (11.7)**	**371 (88.3)**				

Key: 1* = Reference

Table 3: Breed Distribution of Q fever in sheep in of Yobe State

Breeds	Number Examined	iELISA +ve No. (%)	iELISA –ve No. (%)	OR	95% CI lower	95% CI upper	P-value
Balami	160	19 (11.7)	141 (88.1)	0.813	0.340	1.947	0.850
Yankasa	179	22 (12.3)	157 (87.7)	0.782	0.332	1.840	
Uda	81	8 (9.9)	73 (90.1)	1*			
Total	**420**	**49 (11.7)**	**371 (88.3)**				

Key: 1* = Reference

Table 4: Spatial Distribution of Q fever in sheep in of Yobe State

Location Senatorial zone	Number Examined	iELISA +ve No. (%)	iELISA -ve No. (%)	OR	95% CI lower	upper	P-value
Damaturu	140	19 (13.6)	121 (86.4)	0.822	0.404	1.572	0.645
Gashua	140	14 (10.0)	126 (90.0)	1.161	0.544	2.480	
Potiskum	140	16 (11.4)	124 (88.6)	1*			
Total	**420**	**49 (11.7)**	**371 (88.3)**				

Key: 1* = Reference

DISCUSSIONS

The result of this study established the presence of Q-fever in sheep in Yobe State, Northeastern Nigeria. The seroprevalence of 11.9% obtained in this study was comparable to 11.0% reported in Chad [11], but the seroprevalence was lower than the reports of [12,13]. The seroprevalence was higher than 7.0% reported in Tunisia [14] and 1.4% in France [15]. The differences in seroprevalence rates among different areas could be attributed to frequency of tick population, type of management practices, climatic variations and method of sample tests. The seroprevalence was higher in female sheep than in male sheep, though there was no significant association ($p > 0.05$) between the sex and positive serological reactions. Our results agreed with those of Tukur *et al.* [16] and Wardrop *et al*. [17]. The high seroprevalence in female is possibly because; the organism has a high affinity for fetal membranes, mammary glands and the placenta and is found in large numbers in these tissues [18]. This agrees with the study by Cetinkaya *et al.* [19] who reported no significant association between seroprevalence of Q-fever and sex of the animals tested in Turkey. The seroprevalence was higher in sheep greater than 2 years than sheep less than or equal to 2 years. This agreed with the findings of Adamu *et al.,* [9] in Nigeria and in the Netherlands by Schimmer *et al.* [20]. The seroprevalence was higher among Yankasa followed by Balami and the least was among Uda breeds. Although there was no significant association ($p > 0.05$) between the location of sheep and positive serological reactions the seroprevalence was higher in Damaturu zone than in Potiskum and Gashua zones. This study showed that domestic ruminants were very important reservoir of *C. burnetii* and that they pose a significant public health hazard for the transmission of Q fever to humans and animals in areas in which they are raised.

Conclusion and recommendation

This study established that Q fever was present in the three senatorial zones of Yobe State, with the seroprevalence of 12.4% in females' sheep and 9.5% in males sheep, *Coxiella* antibodies was demonstrated at higher prevalence in older sheep 12.1% than in younger sheep 9.8% using iELISA in the study area. Higher *Coxiella burnetii* antibodies in Yankasa breed 12.3% than in Uda breed 9.9% this demonstrated the presence of the organism in the flocks and is of public health concern.

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