Seroprevalence of Coxiellosis (Q fever) in Flocks of Goat in Birnin Gwari and Maigana Agro-Ecological Zone of Kaduna State, Nigeria

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Abstract
Coxiellosis is a zoonotic disease caused by the obligate intracellular bacterium Coxiella burnetii which affect the prolific and reproductive competences of animals. A cross sectional study was conducted to determine the seroprevalence of coxiellosis (Q fever) in flocks of goats in Kaduna State, Nigeria. The study aimed to determine the seroprevalence of coxiellosis in goats in Birnin Gwari and Maigana agro-ecological zone of Kaduna State, Nigeria. A total of 400 serum samples from goats of both sexes and of different age groups were collected and screened for Coxiella burnetii antibodies using indirect enzyme-linked immunosorbent assay (iELISA). Out of the 400 sera analysed, 8.8% were seropositive coxiellosis. Of the 253 female goats tested, 9.5% were seropositive, while 7.5% were seropositive out of the 147 male goats tested. There was no statistically significant association between sex of goats and coxiellosis (Q fever). A significant association was detected between age of goats tested and sensitivity of iELISA, non-significant association was found between breed of goats with sensitivity of iELISA. The study indicates that coxiellosis exists with high prevalence predominantly among female goats and is major public health challenge calling for awareness amongst interested party for organized surveillance for the diseases in goats in Nigeria.

Keywords: Goat; Kaduna State; seroprevalence; Coxiella burnetii

Introduction
Q fever is an acute, highly transmissible zoonotic disease that is usually neglected (Njeru et al., 2016). The disease is caused by Coxiella burnetii, an obligate Gram-negative intracellular bacterium (Bielawska-Drozd et al., 2014; Van Leuken et al., 2016). The organism has been classified by Centers for Disease Control and Prevention as a potential bioterrorism agent (El din et al., 2017). Coxiella burnetii can infect a wide variety of animals, humans, birds, and arthropods; though, ruminants act as the main reservoir (Njeru et al., 2016). Q fever infection in animals is mostly clinically in-apparent; however, abortion, stillbirth, decrease in the reproduction efficiency, and infertility are all stated (Guatto et al., 2011). Q fever in humans is considered an endemic disease, mostly occupational, disease with a peculiar epidemiological trend consisting of both sporadic cases and epidemic outbreaks (Van den Brom et al. 2015). The acute C. burnetii infection is characterized by fever, flu-like signs, headache, and pneumonia, whereas hepatitis and endocarditis are serious complications in chronic cases (Hartzell et al., 2008). Infected animals shed C. burnetii in their faeces, milk, urine, aborted fetus, placenta and discharge (Kersh et al., 2013; Salifu et al., 2019). Infection can spread both vertically and horizontally, during contact with body fluids or transmission through arthropod vectors (Raoult et al., 2005).

Transmission of infection to humans occurs mainly through the inhalation of contaminated aerosols, contact with the infected animals and their products (Keyvanirad et al. 2013). In Nigeria, only limited studies on Coxiella burnetii were reported in ruminants (Adesiyan et al. 1984; Adamu et al. 2018; Adamu et al., 2019). The objective of this study was to determine the seroprevalence of Q fever and the associated risk factors influencing the presence of C. burnetii antibodies in goats in Birnin Gwari and Maigana agro-ecological zones of Kaduna State, Nigeria. This may provide greater awareness
among stakeholders and for co-ordinated investigation for the Q fever amongst goats in Nigeria.

Materials and Methods

Study Area
The study was conducted in Birnin Gwari and Maigana agro-ecological zones of Kaduna State. Kaduna State is located in the northwestern part of Nigeria. The state occupies a land area of about 48,473.2 km², and lies between longitude 6° 20' and 9° E and latitude 9° 10’ and 11°30’ N the state is located at an elevation of 704 m above sea level. Kaduna State has a population of 6,113,503 persons (NPC 2006) and an estimated cattle population of 3.1 million, 832,000 sheep, and 988,000 goats (KDSG 2008). The annual rainfall in this area ranges between 750–1100 mm per annum, the area has a typical continental type of climate, with a wide temperature range, sometimes up to 12°C (Jamagani 1998).

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

Study Design
A cross-sectional study was used for detecting C. burnetii infections and probable risk factors influencing the existence of C. burnetii antibodies in goat flocks was carried out between May, 2016 and October, 2016 in Birnin Gwari and Maigana agro-ecological zone of Kaduna State, Nigeria.

Sample Size Estimation and Sample Collection
Sample size for this study was determined using the Thrusfield, 2005 formula, with an expected diseases prevalence of 14.5% (Tukur et al., 2014), accepted absolute error of 5%, and a confidence interval of 95% (Thrusfield 2005);

\[ n = \frac{1.96^2 \cdot P_{\text{exp}} \cdot (1 - P_{\text{exp}})}{d^2} \]

Where: \( n \) = required sample size, 
\( P_{\text{exp}} \) = expected prevalence,  
\( d \) = desired absolute precision.

A minimum of 190 samples was required for the study; however, 400 samples from goats were randomly selected from their flocks to increase precision. Stratified random sampling technique was used to select flocks from each LGA forming the first strata and wards the second strata. In each stratum, simple random sampling was used proportionate to size. Five milliliters of blood sample were collected aseptically from the jugular vein of each animal into clean plain vacutainer tubes. Each sample was labeled with unique identification number and information such as sex, age and breeds of the animals were recorded for data analysis. The samples were transported on ice packs in coolers to the

bacterial research laboratory in the Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, and were centrifuged at 3000g for 5 minutes to obtain sera. The harvested sera were stored at ~20 °C until tested.

Serological Test
Indirect enzyme-linked immunosorbent assay (iELISA) was carried out in the bacterial research laboratory, Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University Zaria, Nigeria. The Indirect Multi-species ELISA kit was supplied by IDvet, Innovative Diagnostics, Montpellier, France. Serum samples were tested for the presence of antibodies against Coxiella burnetii using an iELISA kit following the manufacturer’s instructions.

Statistical Analysis
Data generated were analysed using Statistical Package for Social Sciences (SPSS) version 21.0 statistical software. Prevalence was calculated using number of positive samples divided by the total number of samples tested, expressed as percentage. Chi-square (\( \chi^2 \)) and Fisher’s Exact Test were used to test for association. Strength of association was calculated using Odds Ratio (OR) at 95% Confidence Interval (CI). Values of \( P < 0.05 \) were considered statistical significant.

Results
Out of the 400 goats tested, 35 (8.8%) were seropositive to Coxiella burnetii infection. Of the 147 male goats tested, 11 (7.5%) were seropositive, while 24 (9.5%) were seropositive out of 253 female goats tested. There was no statistically significant association between the sex of goats tested and the presence of Coxiella burnetii antibodies (\( P > 0.05 \)) (Table 1). Based on age distribution, the highest seroprevalence was detected among goats older than 4 years (13.7%), and the least seroprevalence was among goats of 2 to 4 years (8.7%). There was no positive sample detected among the age group less than 2 years old. There was statistically significant association between the age of goats tested and the presence of Coxiella burnetii antibodies (\( P < 0.05 \)) (Table 2). On breeds distribution, the highest seroprevalence was detected among Sokoto Red (10.2%), while the least was detected among Sahelian goats (6.7%). This was followed by West African Dwarf goats (9.2%) while the least was detected among Sahelian goats (6.7%). There was no statistically significant association between the breed of goats tested and the presence of Coxiella burnetii antibodies (\( P > 0.05 \)) (Table 3).

Discussion
The overall seroprevalence of Q fever obtained in this study was 8.8%, which was lower than 24.2% reported by Hussien et al. (2012) from Sudan, 11.0% reported by Johnson et al. (2019) from Ghana. The seroprevalence reported in this study was also lower than 10.24% reported by Karagul et al. (2019)
from Turkey, 29.8% reported by Keyvani Rad et al. (2014) from Iran and 21.4% reported by Schimmer et al. (2011) from the Netherlands.

Table 1: Seroprevalence of Q fever in goats in Birnin Gwari and Maigana agro-ecological zones of Kaduna State based on sex

<table>
<thead>
<tr>
<th>Sex</th>
<th>Number Examined</th>
<th>iELISA +ve No. (%)</th>
<th>OR</th>
<th>95% CI lower</th>
<th>upper</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>147</td>
<td>11 (7.5)</td>
<td>0.772</td>
<td>0.367</td>
<td>1.625</td>
<td>0.468</td>
</tr>
<tr>
<td>Female</td>
<td>253</td>
<td>24 (9.5)</td>
<td>1*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>35 (8.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

However, the seroprevalence obtained was higher than the 5.3% reported by Mohammed et al. (2014) from Saudi Arabia, 6.8% reported by Klemmer et al. (2018) from Egypt.

The seroprevalence obtained in this study was comparable to the works reported by Hussien et al. (2012) in Sudan and Chakrabartty et al. (2016) in Bangladesh.

Table 2: Seroprevalence of Q fever in goats in Birnin Gwari and Maigana agro-ecological zones of Kaduna State based on age

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Number Examined</th>
<th>iELISA +ve No. (%)</th>
<th>OR</th>
<th>95% CI lower</th>
<th>upper</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 2 Years</td>
<td>51</td>
<td>00 (0.0)</td>
<td>&gt; 999</td>
<td>0.000</td>
<td>1.616</td>
<td>0.020</td>
</tr>
<tr>
<td>2–4 Years</td>
<td>254</td>
<td>22 (8.7)</td>
<td>1.672</td>
<td>0.805</td>
<td>3.471</td>
<td></td>
</tr>
<tr>
<td>&gt; 4 Years</td>
<td>95</td>
<td>13 (13.7)</td>
<td>1*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>35 (8.8)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3: Seroprevalence of Q fever in goats in Birnin Gwari and Maigana agro-ecological zones of Kaduna State based on breed

<table>
<thead>
<tr>
<th>Breed</th>
<th>Number Examined</th>
<th>iELISA +ve No. (%)</th>
<th>OR</th>
<th>95% CI lower</th>
<th>upper</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sahel</td>
<td>149</td>
<td>10 (6.7)</td>
<td>1.414</td>
<td>0.491</td>
<td>4.068</td>
<td>0.524</td>
</tr>
<tr>
<td>Sokoto Red</td>
<td>186</td>
<td>19 (10.2)</td>
<td>0.894</td>
<td>0.341</td>
<td>2.345</td>
<td></td>
</tr>
<tr>
<td>WAD</td>
<td>65</td>
<td>6 (9.2)</td>
<td>1*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>400</td>
<td>35 (8.8)</td>
<td></td>
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</table>

The differences in seroprevalence rates could be attributed to climatic variations, density of tick population and type of management practices. The seroprevalence of Q fever obtained in this study was higher among female goats than male goats, and there was no statistically significant difference between the sex of goats tested and positive serological reactions to Coxiella burnetii. This agreed with the work reported in Nigeria by Tukur et al. (2014) and Adamu et al. (2018) in cattle, Adamu et al. (2019) in sheep. In Ghana, Raphael et al. (2020) reported high seroprevalence of Q fever in female goats than in male goats. Similarly, Edalati-Shokat et al. (2015) reported high seroprevalence of Q fever in female goats than in male goats in Iran; likewise, Zahid et al. (2016) reported high seroprevalence of Q fever in female goats than in the male goats in Pakistan. However, this work disagreed with the works reported by Ullah et al. (2019) in Punjab, Pakistan, and Keyvani Rad et al. (2014) in Iran who reported high seroprevalence of Q fever in male goats than in female goats. The high seroprevalence of Q fever in female goats obtained in this study could be probably due to the fact that Coxiella burnetii has a high empathy for placenta, foetal membranes and mammary glands.

Seroprevalence of Q fever obtained in this study was higher in goats greater than 4 years old than the 2-4 and the less than 2 years old groups. There was statistically significant associated between the age of goats studied and positive serological reactions. This study agreed with the reports of Keyvani Rad et al. (2014) in goats in Iran and Filioussis et al. (2017) in goats in Greece. It also agreed with the findings of Adamu et al. (2019) in sheep in Nigeria and Ullah et al. (2019) in goats in Pakistan. But the findings were at variance to previous report from Gambia (Klaassen et al., 2014). This finding also suggests the occurrence of horizontal transmission among animals and the maintenance of
infection within adult populations (Ruiz-Fons et al., 2010, Astobiza et al., 2011). Adult animals are more likely to be breeding and therefore shedding the organism. Though, there was no statistically significant association between the seroprevalence of Q fever and breeds of goats tested, but the seroprevalence was higher in Sokoto Red followed by West African Dwarf and the least was in Sahelian goats.

Conclusions
This study has demonstrated that Q fever exist in the area sampled with a seroprevalence of 8.8% in goat flocks, particularly among female and adult goats. This presents a serious public health concern because people take unpasteurized goat milk which they believe has medicinal effects.

Acknowledgment
The authors are thankful for valuable support of all the technical staff of Department of Veterinary Public Health and Preventive Medicine, Ahmadu Bello University, Zaria Kaduna State, and the livestock owners for their understanding and maximum support during blood samples collection.

Conflict of Interest
The authors declare that there is no conflict of interest.

Author Contribution
SGA and JK: Conceived, designed and planned study. SGA: Did the field work, sample collection, financing the research, wrote the paper. JK, JUU and MAR: Supervised the project and manuscript vetting and editing. All authors read and approved the final manuscript.

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