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Seroprevalence and risk factors of brucellosis in goats in selected states in Nigeria and the public health implications

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Abstract

Available reports on brucellosis in Nigeria are largely confined to cattle while it is believed that other ruminants like sheep and goats are equally exposed to the disease. To have an insight into the role of goats in the epidemiology of brucellosis in Nigeria, we conducted a cross-sectional study between June 2011 and May 2013 to determine the seroprevalence of brucellosis in goats in some selected states in Nigeria. Serum samples were collected from goats at different locations and tested for antibodies to *Brucella* spp using the Rose Bengal Test (RBT), samples positive by RBT were further subjected to Competitive Enzyme Linked Immunosorbent Assay (cELISA). Data collected to determine risk factors were also analysed using chi-square and logistics regression statistics.

Out of a total of 2827 samples tested from the different states (Benue = 331; Borno =195; Oyo = 2155; Sokoto = 146), we recorded an overall seroprevalence of 2.83% (Benue = 17.30%; Borno = 2.05%; Oyo = 0.60% and Sokoto = 0.00%) by RBT. The cELISA further supported 9.45% (7/74) of the total RBT positive samples. Logistic regression analysis showed that the location ($p = 0.004$) and source ($p < 0.0001$); are probable risk factors to be considered in the epidemiology of brucellosis with sex ($p = 0.179$); age ($p = 0.791$) and breed ($p = 0.369$) not playing any major role.

Our findings reveal a relatively low seroprevalence of brucellosis among goats screened except for Benue State. Since most of the goats sampled in the present study were from the abattoirs, further farm level investigations are required to determine the role of goats in the epidemiology of brucellosis in Nigeria since they share common environment with sheep and cattle that are natural hosts of *Brucella* species which are of major public health threat.

INTRODUCTION

Brucellosis is caused by the bacteria of the genus *Brucella*, a Gram-negative intracellular coccobacilli that occurs in a wide variety of animals including cattle, sheep, goats, pigs and other livestock as well as humans. It is a contagious systemic disease characterized by the inflammation of the genital organs and foetal membranes, abortion, sterility and formation

of localized lesions in the lymphatic system and joints (CDC, 2005). Brucellosis is a zoonotic disease noted for its public health importance and threat to food security globally. Though controlled in many developed countries (Corbel, 1997), brucellosis is endemic in Africa (Cadmus *et al*., 2013), South America (Lucero, 2005) and many Asian countries (Sofian *et al*., 2008).

In Nigeria, several serological studies have shown that brucellosis is endemic in the livestock population (Ocholi, 1993; Cadmus *et al*., 2006, Ibironke *et al*., 2008, Mai *et al*., 2012). Recently, the prevalence of 8.6% was recorded in Lagos State (Cadmus *et al*., 2009), 37% in three Northern States of Nigeria (Kaduna, Kano and Adamawa) (Mai *et al*., 2012) as well as 16.1% in Plateau State (Bertu *et al*., 2010) in north-central Nigeria. Incidentally, most of these studies have been concentrated on cattle; while few documented evidence have shown that the disease also exists in goats in Nigeria; with prevalence of 0.86%, 14.00% and 25.80% reported in south-western, north-eastern and north-central Nigeria respectively (Cadmus *et al* 2006; Tijjani *et al*., 2009; Kaltungo *et al*., 2013).

In many rural and nomadic communities in Nigeria, goats are continuously in close contact with humans. Thus, there is an increased likelihood of zoonotic transmission of brucellosis to individuals in such settings. In addition, abattoir workers involved in the slaughter and processing of goats are at high risk of being infected, especially from infected uterine and udder contents (European Commission., 2001) since *Brucella* can also be excreted through these routes. Among the *Brucella* species, *Brucella melitensis* which is the major cause of brucellosis in caprine, is noted to be the most pathogenic in humans (OIE, 2009). Hence, serologic studies focusing on brucellosis in goats is not only needful but useful. This study therefore sets out to investigate the sero-prevalence of brucellosis in farms/households as well as goats slaughtered at major abattoirs in some selected states in Nigeria.

MATERIALS AND METHODS

Study site

The study was conducted in selected states in Nigeria including Oyo, Benue, Borno and Sokoto States.

Oyo State—The state has a total land mass of 27,036km.^{2}. The dry season lasts from November to March while the wet season starts from April and ends in October. Average daily temperature ranges between 25 °C (77.0 °F) and 35 °C (95.0 °F), almost throughout the year. Though Oyo State is situated in the forest belt in Nigeria, there are the derived savannah areas in the state that favour livestock rearing. Traditionally, the West African dwarf (WAD) goats are kept but the Sokoto Red goats and their crosses are common (Blench, 1999). However a good number of goats slaughtered in the state come from northern Nigeria. For this study, samples from goats were collected from the households and abattoirs.

Benue State—Benue has a land area of 30,755km^2 and is situated in north-central Nigeria. Though the Sokoto Red goats are generally common in northern Nigeria, Benue State is one of the northern states of the country that still harbours a good population of the West African

dwarf goats (Blench, 1999). In Benue State, blood samples were collected from goats in the market and goats slaughtered in the abattoirs.

Borno State—Borno State has a land mass of 72,767 km^2. The state is located in the semi – arid zone of north-eastern Nigeria. It is noted for the presence of large numbers of the Sahel breed of goats (Blench, 1999). Blood samples were collected from goat herds from various farms.

Sokoto State—Sokoto State is in the north-western part of Nigeria and has a land area of 32,146 km^2. Sokoto is the home of the Sokoto Red goats in Nigeria (Ngere *et al*., 1984) and samples were collected from the abattoirs.

Animal sampling, sample collection and handling

Goats from the selected states were sampled. Blood samples were collected from goats available at the various sites. However, due to varied level of support and cooperation received during the course of the study, sample collection did not follow a systematic approach. Thus, varying numbers of animals were screened across the four states. Animals' parameters such as breed, sex and age, source and location were recorded. For each animal, about 10mls of blood was collected into 15ml sterile tubes. The blood samples were allowed to clot and centrifuged at 3000rpm for 15minutes. Serum samples were then decanted and stored at –20 °C until they were assayed. The serum samples were examined by Rose Bengal test (RBT) (Alton *et al*., 1988) and the positives were further examined with competitive enzyme-linked immunosorbent assay (cELISA) (MacMillan *et al*., 1990).

The Rose Bengal test (RBT)—The RBT antigen consisting of standardized *B. melitensis* antigen was sourced from the Animal Health and Veterinary Laboratories Agency, Weybridge U.K. and used to carry out the test (Alton *et al.,* 1988). Briefly, equal volumes (30µl) of antigen and test serum were mixed thoroughly on a plate using a stick applicator and the plate was rocked for 4 minutes. The appearance of agglutination within 2 minutes was scored 2+ (++), while the agglutination after 2 minutes was scored 1+ (+). The absence of agglutination after 4 minutes was scored negative (-). However, all RBT positive samples were further subjected to cELISA test.

Competitive Enzyme Linked Immnunosorbent Assay (cELISA)

The cELISA kit was sourced from the Animal Health and Veterinary Laboratories Agency, Weybridge U.K. The reagents in the kit were reconstituted as directed by the manufacturers. These included control sera, diluting buffer, conjugate, washing solution, chromogen and stopping solution. The test was performed according to the manufacturer's instructions. The optical density (OD) was measured at 450nm using a microplate ELISA reader (Intertek Multiscan M11®). A positive/negative cut off was calculated as 60% of the mean of the OD of the conjugate control wells. Samples in wells with OD equal to or less than the cut-off point were scored positive, while those above were scored negative.

Data analysis

All data were analysed using the STATA software version 12. Group differences were tested using chi-square statistics for categorical variables. A multi-variable adjusted logistic regression was carried out using all the variables that were statistically significant at the 10% level with the main outcome measure (RBT test) in bivariate analysis. All tests were two-tailed and statistical significance was set at $p<0.05$.

RESULT

The overall sero-prevalence of 2.83% (74/2827) was recorded in all the four States. The highest sero-prevalence of 17.30% was recorded in Benue State, followed by 2.05% in Borno, 0.60% in Oyo State, while a sero-prevalence of 0.00% was recorded in Sokoto using the RBT. Furthermore, the result of the breed prevalence showed the highest infection rate of 2.77% among the Red Sokoto, 2.73% among West African Dwarf and the least sero-prevalence of 1.37% was recorded among the other breeds (Sahel and Kaduna Red; Table 2).

In the same vein, the sex-specific sero-prevalence of 3.47% recorded in female animals was higher than the 1.46% recorded among the male animals. The age specific result showed a sero-prevalence of 7.42% in younger animals which was higher than the 0.65% recorded in older animals. On the basis of sources of samples, the sero-prevalence of 3.00% was recorded in animals sourced from the abattoir while the sero-prevalence of 1.00% was recorded in animals from the households/herds (Table 2).

Our findings as indicated by logistic regression showed that location ($p = 0.004$) and source ($p < 0.0001$) of animals were significantly associated with seropositivity of animals to *Brucella* antibodies. In addition to this, our results showed that animals in Benue State were more likely to be seropositive to *Brucella* infection than those in Oyo State (OR= 34.40; 95% CI: 18.59 – 63.66; Table 3). Also, goat samples sourced from the abattoir were more likely to be seropositive to *Brucella* spp antibodies when compared to those from the household/herds (OR= 1.61; 95% CI: 1.31 – 2.00). Furthermore, the logistic regression analysis revealed that there was no significant association between seropositivity and sex (OR= 1.19; 95% CI: 0.98 – 1.44) as well as age (OR= 1.56; 95% CI: 0.91 – 2.71; Table 3).

DISCUSSION

Our findings reveal an overall seroprevalence of 2.83% (with a range of 0.00% to 17.30%) of brucellosis among goats screened across the four states in Nigeria. This result must be put in the context of the fact that goats are generally not vaccinated against brucellosis, neither are there control programmes for the disease in the country; hence, the low grade persistence of the disease in the absence of any outbreak. Furthermore in Nigeria, cattle and goats are generally reared together on free range in rural communities with the possibility of cross infection from cattle. In this present study however, we did not confirm nor identify the *Brucella* species responsible for infection in the goats in the four states studied. Despite this, we cannot rule out infection of these goats by *Brucella* species like *Brucella abortus* as earlier reported in small ruminants in Nigeria (Ocholi *et al*., 2005). Furthermore, despite the

paucity of data on brucellosis in goats in Nigeria, our current findings reveal that the disease is prevalent among goats and this has significant economic and public health implications. Judging from the different results obtained from earlier studies, the overall seroprevalence recorded in this study is comparable to the prevalence of 2.80% obtained in northern Nigeria (Brisibe *et al*., 1993) and 2.80% in Somalia (Falade and Hussein, 1997); but higher than1.9% in pastoral goats in eastern Ethiopia (Teshale *et al.*, 2007) and 2.00% reported in Uganda (Kabagambe *et al*., 2001). It is however lower than 16.10% in northern Nigeria (Bale and Nuru.,1982) and 45.75% recorded in an outbreak of brucellosis in a goat flock in Abeokuta, south-western Nigeria (Ojo *et al*., 2007) as well as 13.60% in goat herds in north-eastern Ethiopia (Adugna *et al*., 2013). Despite these various findings, we believe that the varying prevalence could be due to geographical differences, sources of animals, sampling techniques, individual differences in interpretation of tests and the number of animals sampled.

Furthermore, our findings recorded a significant association ($p < 0.0001$) between location and seropositivity, with goats sampled in the north having significantly higher sero-prevalence than those sampled in the south of Nigeria. However, it can be observed that the high seroprevalence recorded in the north was mainly due to results from Benue State (17.3%), which is relatively higher when compared to those from other states in this study. The reason for the high prevalence recorded in Benue State may not be unrelated to the fact that animals from this state are sourced from different areas of Nigeria some of which have reported high prevalence of brucellosis in goats (Bertu *et al.*, 2010). For instance, Plateau State, an adjoining state where some of the animals are sourced from, reported a seroprevalence of 16.10% from goats (Bertu *et al.*, 2010). Again, there are similar reports of 29.2%, 23.3% and 26 .7% prevalence reported in Adamawa, Kaduna and Kano (other adjoining states where goats are sourced into Benue State) respectively in cattle (Mai *et al*., 2012). The relevance of the rates of *Brucella* infections recorded in cattle in these states becomes useful because it is usual practice for cattle and small ruminants to share common grazing and watering points in the north. Again, *Brucella abortus* has been isolated from small ruminants kept together with cattle in Bauchi, northern Nigeria (Ocholi *et al.*, 2005).

Intriguing however, is the fact that the 2.05% prevalence recorded in Borno State in this study was lower than the 6.00% earlier reported in Borno and Yobe States (Brisibe *et al*., 1996) and 4.00% in Yobe State (Tijjani *et al*., 2009). The difference recorded may be due to the fact that goats screened from Borno State in this study were sourced from herds/ household animals as against the other studies where slaughter animals were used. It is important to note, that quite a large number of animals in slaughter houses are culled by farmers for poor performance (Mangen *et al*., 2002); which could be an indicator of brucellosis. Furthermore, the prevalence of 0.58% recorded in Oyo State is lower than the 4.75% (Falade, 1981) and 9.0% (Ogundipe *et al.*, 1993) previously reported, but similar to 0.80% recorded in a relatively recent study in the same state (Cadmus *et al*., 2006).

Again, the result of our findings shows that there is no significant association ($p = 0.197$) between seropositivity to *Brucella* antibodies and sex of the animal though with a higher rate of infection in the female than male. This is consistent with the studies of Adugba *et al*. (2013), Teshale *et al*. (2006) and Ashenafi *et al*. (2007) who reported no significant

association between sex and seropositivity, but in contrast to other studies (Junaidu *et al*., 2010 and Tijani *et al*., 2009). Although bucks and does are known to be equally susceptible to *Brucella* infection (E.C., 2001), however male animals are usually sold off at younger age than the females (Kebede *et al*., 2008). The higher infection rate of brucellosis in does than bucks reported by some workers may therefore be due to the fact that bucks are generally more aggressive and with few needed for breeding purposes; hence goat farmers rear fewer males. Generally therefore, female animals may have a possible longer time of exposure to *Brucella* infection than males.

Furthermore, our findings show significantly ($p < 0.0001$) higher seropositive cases among trade animals when compared to goat flocks kept in the farms and households. This is similar to earlier study carried out by Bale and Nuru (1982) in northern Nigeria. The plausible reason for the higher sero-prevalence recorded in slaughter animals could be attributed to the fact that most of the animals slaughtered in the abattoirs are purchased from livestock markets. Farmers are known to sell animals that are mostly underperforming reproductively or sickly (Mangen *et al*., 2002). This partly explains why majority (94.59%) of the seropositive goats in this study were from slaughter houses/markets.

In the same vein, although most goats sampled were adults (70.89%), our study recorded higher sero-positivity in young animals (61%) than adults (13%), however there was no significant association ($p = 0.719$) between *Brucella* infection and age of animals. While young animals may be infected, they generally do not show any clinical sign but only weak and transient serological response. Conversely, susceptibility to brucellosis increases after sexual maturity and especially with pregnancy (E. C., 2001) but most of the positive young animals in this study were in early puberty; hence the possibility of being infected. Although some of them may have been infected as kids through suckling infected dams, however many may have been exposed through mating with infected bucks. Goats are known to reach puberty between 4 to 8 months of age (Delgadillo *et al*., 2007) and because mating in free range is not controlled, they become sexually active during early puberty and are therefore exposed to *Brucella* infection. In addition, these young goats may as well have been exposed to *Brucella* infection at grazing or watering points through contact with contaminated pasture and water. However, in older animals the disease could become chronic resulting in the antibody titre falling to undetectable levels giving rise to false negative results in serological diagnosis of brucellosis (Godfroid *et al*., 2002; Tessaro *et al*., 2004).

It must be noted that although there was no evidence of vaccination of the goats sampled, however the subsequent screening of RBT positive samples with cELISA showed a significantly fewer number of positives ($p<0.001$). This is comparable to findings in Ethiopia (Teshale *et al*., 2006). Specificity has been one of the limitations of the RBT test as it provides more likely false positive results than false negative (Omer *et al.*, 2001). This sometimes may be as a result of an overestimation of agglutination reaction by the individual investigator. Again, cross reaction between smooth *Brucella* antigens and other bacteria species especially *Yersinia enterocolitica* 0:9, which have been recorded in goats (E. C., 2001), may be the likely cause of divergent in result. This can be related to the fact that antibodies to smooth lipopolysaccharide (SLPs) of *Brucella* spp are mainly responsible for hummoral immune responses to *Brucellae* (Cherwonogrodzky *et al*, 1990). However, the

immunodorminant O-side chain of *Yersinia enterocolitica* 0:9 and *Brucella* spp are identical (Caroff *et al*., 1984), resulting in cross reaction between both organisms. On the other hand, while the use of ELISA for the diagnosis of brucellosis in bovines have been well developed, its use in small ruminants still requires more extensive field validation (EC, 2009). Also, while ELISA protocols could be useful in the differentiation of vaccinated and unvaccinated small ruminants (Debbarh *et al.,* 1996), their sensitivity is not very reliable (EC, 2001).

From our findings, the breed specific result revealed no significant association ($p = 0.369$) between seropositivity and breed of animals. Though the highest seroprevalence was recorded among the Red Sokoto breed, this is not significant when compared to that of WAD and others (Sahel and Kaduna Red). This is comparable to reports of Junaidu *et al*. (2010) and Tijjani *et al*, (2009) which also reported highest prevalence in Red Sokoto and Borno white respectively. Junaidu *et al*, (2010) also reported that there was no significant association between seropositivity and breed of animals.

Despite the results of the study, some limitations were observed. Firstly, more than three-quarters of the total animals screened were from Oyo State as more samples could not be collected from the northern parts of Nigeria due to security concerns; this could have introduced bias into the results of the study. Second, majority of the breed of animals screened were of the Red Sokoto breed, thus making the data to be skewed towards this particular breed. Third, cultures which would have helped to confirm the infection status of the serologically positive animals were not carried out.

Conclusion

Despite some of the limitations in the present study, our findings reveal that brucellosis is endemic in the population of goat screened with a significantly higher prevalence in Benue State compared to other states. The source and location of animals are implicated as potential risk factors in the epidemiology of brucellosis in goats. This can therefore constitute potential risks of infection to the human population given the close interactions between goats, livestock farmers and rural dwellers in such settings in Nigeria and other endemic areas of the world. Again, given close human and animal co-habitations, occupational exposure coupled with consumption of unpasteurised milk and other products from goats, zoonotic transmission of the disease could ensue. Finally, since majority of the animals screened in the present study were from markets/ slaughter houses, further studies should be focused on goats under farm settings living alone or co-habiting or sharing common pastures and water points with sheep and goats to shed more light on the role of goats in transmitting of *B. melitensis* and *B. abortus* which are of important zoonotic importance to humans.

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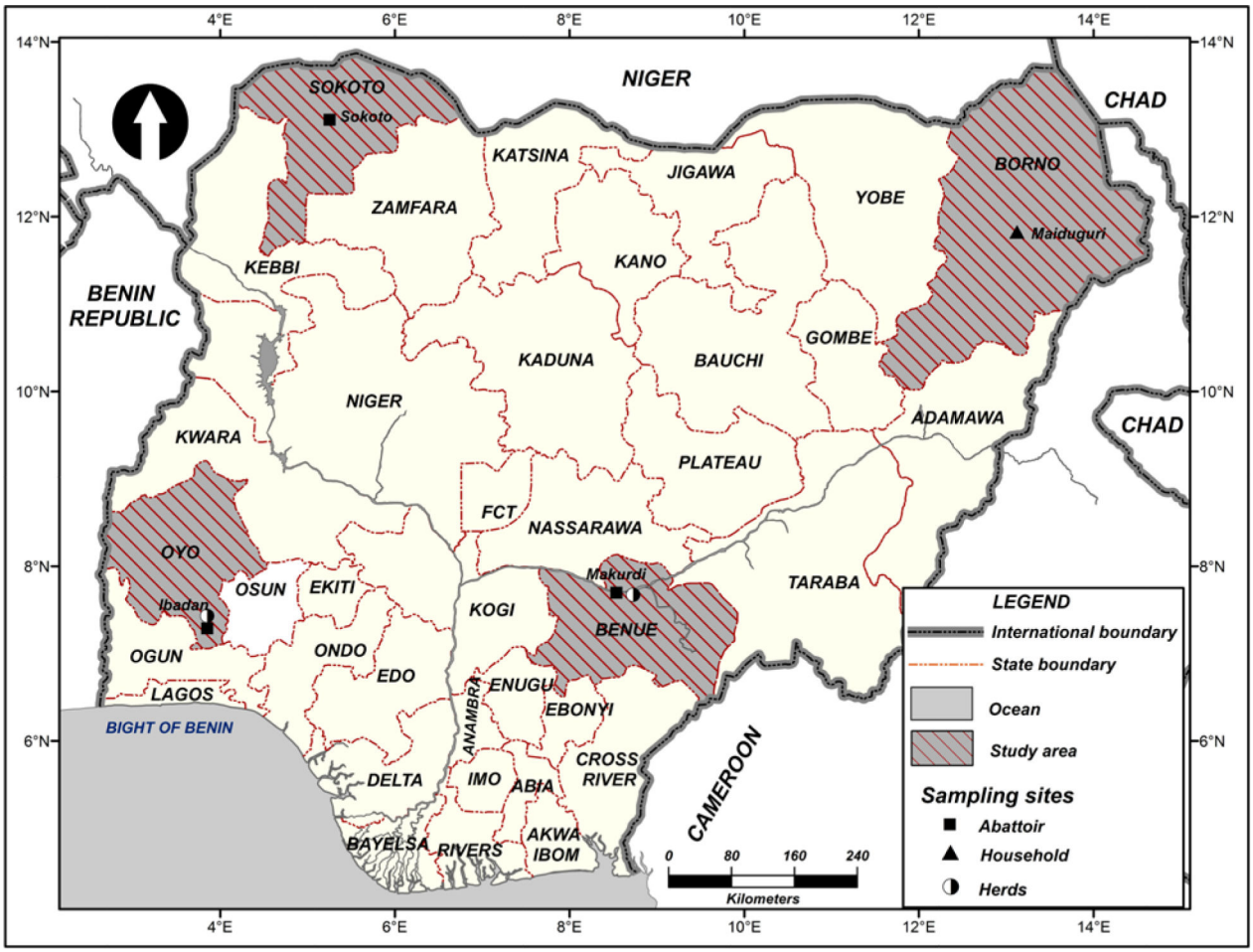

Figure 1.
Map of Nigeria showing the study areas

Table 1

Distribution of goats screened according to sex, age, breed, source and location

Characteristic	Frequency	Percent
Sex		
Male	1098	38.84
Female	1729	61.16
Age		
Adult	2004	70.89
Young adult	823	29.11
Breed		
Red Sokoto	1876	66.36
West African Dwarf	659	23.31
** Others	292	10.33
Source		
Abattoir	2405	85.07
Household/Herds	422	14.93
Location		
Oyo State	2155	76.23
Benue State	331	11.71
Borno State	195	6.90
Sokoto State	146	5.16
Total	**2827**	**100.00**

** Sahel and Kaduna Red

Table 2

Seroprevalence of brucellosis in goats according to sex, age, breed, source and location

	***Brucella* infection**		
Characteristic	**Positive (n)%**	**Negative (n)%**	**p-value**
Sex			
Male	16	1082	0.002
Female	58	1671	
Age			
Adult	13	1991	<0.0001
Young adult	61	762	
Breed			
Red Sokoto	52	1824	0.369
West African Dwarf	18	641	
** Others	4	288	
Source			
Abattoir	70	2335	0.020
Herds	4	418	
Location			
Oyo State	13	2142	<0.0001
Benue State	57	274	
Borno State	4	191	
Sokoto State	0	146	
Total	**74**	**2753**	

** Sahel and Kaduna Red breeds were grouped into others for statistical analysis because they contained cells whose numbers were less than 5

Table 3

Results of logistic regression analysis of variables significant at 10% level with the main outcome measure (RBT) in bivariate analysis

Variable	OR	95%CI	p-value
Sex			
Male	1.0 (referent group)		0.197
Female	1.47	0.82 – 2.65	
Age			
Adult	1.0 (referent group)		0.791
Young adult	1.56	0.91 – 2.72	
Source			
Abattoir	1.0 (referent group)		<0.0001
Herds	0.017	0.004 – 0.059	
Location			
Oyo State	1.0 (referent group)		
Benue State	34.40	18.59 – 63.66	<0.0001
Borno State	3.45	1.11 – 10.68	0.032
Sokoto State	0.016	0.003 – 0.015	<0.0001