PRESENCE OF BLUETONGUE VIRUS ANTIBODIES IN CATTLE AND SHEEP IN OGUN AND OSUN STATES, NIGERIA

ABSTRACT

Bluetongue is an infectious, arthropod-borne viral disease principally affecting ruminants. The occurrence of bluetongue virus (BTV) antibodies in sheep and cattle from backyard farms, cattle markets and abattoirs in Ogun and Osun states of Nigeria was investigated. Three hundred and forty (340) plasma samples comprising 205 from sheep and 135 from cattle were collected from March to September 2017, noting the sex, breed and age of the animals. The samples were screened with a commercial enzyme-linked immunosurbent assay (ELISA) kit that detects BTV antibodies in ruminant plasma or serum. All cattle tested from both states were positive for BTV antibodies giving a seroprevalence of 100% while 95% seroprevalence was obtained for sheep. In Ogun state, prevalence of 90.5% and 98% were obtained for male and female sheep respectively while 95.6% and 95% prevalence were also obtained for male and female sheep respectively in Osun state. Based on breed, 94%, 95%, 95% and 96% prevalence were obtained for Yankasa, Balami, Ouda and West African Dwarf (WAD) sheep respectively in Ogun state while 93%, 95.5%, 100% and 93% prevalence were obtained for Yankasa, Balami, Ouda and WAD sheep respectively in Osun state. Furthermore, prevalence of 92% and 96.7% were obtained for age groups of ≤ 1 year and > 1 year respectively in Ogun state, while prevalence of 96% and 94.7% were obtained for age groups of ≤ 1 year and > 1 year respectively in Osun state. Since vaccination against bluetongue disease is not practiced in Nigeria, the detection of high prevalence of BTV antibodies observed in apparently healthy animals in this study indicates natural, albeit subclinical, infection with the virus and sustained activity of the *Culicoides* vector. These findings suggest that bluetongue is widespread in southwestern part of Nigeria and highlight the need for continuous surveillance of the disease in the country as well as isolation, identification and characterisation of currently circulating BTV strains in Nigeria.

Keywords: Bluetongue virus, presence, cattle, sheep, Ogun state, Osun state.

1. INTRODUCTION

Bluetongue is an infectious, arthropod-borne viral disease principally affecting ruminants. Other names for this disease include catarrhal fever, sore muzzle, muzzle disease and pseudo-foot-and-mouth disease [1]. It is caused by the pathogenic virus, bluetongue virus (BTV) of the genus *Orbivirus*, family Reoviridae [2]. It is a non-enveloped virus and the genome is made of 10 segments of double-stranded RNA [3][4].

Bluetongue disease was first described in the Cape colony of Southern Africa after Merino sheep were introduced into the region in the late 18th century, and was subsequently recognized in other parts of Africa, Europe, the Middle East, Indian subcontinent, the Americas and Asia [5]. Twenty six serotypes of BTV are recognized globally [6], and the virus has now been isolated on all continents except Antarctica [5].

Orbiviruses are the cause of important and apparently emerging arthropod-borne viral (arboviral) diseases of livestock, including bluetongue virus, African horse sickness virus, equine encephalosis virus and epizootic haemorrhagic disease virus that are all transmitted by haematophagous *Culicoides* insects [5]. The central role of flying insects in bluetongue epidemiology means that the prevalence of the disease is governed by ecological factors that favour insect survival such as high rainfall, temperature, humidity and soil characteristics [7] [8]. It is conceivable that climate change will increase the distribution and severity of arboviral diseases. Recent changes in the global distribution and nature of BTV infection have been especially dramatic, with spread of multiple serotypes of the virus in almost all parts of the world including Europe and USA with previously exotic virus serotypes [5]. Although climate change has been incriminated in the emergence of BTV infection of ungulates, the precise role of anthropogenic factors and the like is less certain [5].

Bluetongue outbreaks generally occur seasonally and in warm climates. In the temperate regions, *Culicoides* vector infects most ruminant species during mid-summer to early fall when it is active [9]. The infection subsides when temperatures drop and hard frosts kill the adult midge vectors [9]. In the tropical and subtropical regions, however, infection occurs throughout the year as the vector is present year-round [10]. In the absence of competent vector populations, animal to animal transmission is not capable of maintaining an endemic state [11].

Bluetongue is not known to be harmful to humans. However, it causes considerable damage to livestock populations. The virulence of BTV varies quite markedly; even strains with matching serotypes have variable virulence [9]. It is a transboundary disease [12][13], and the epidemiological situation in one country can affect neighboring countries while national measures tend not to be sufficient to control its spread. BTV infects many domestic and wild ruminants [14][15]. Sheep are the main hosts and exhibit the most clinical disease [16]. Other ruminants like cattle, goats, camels, buffaloes, and some wild ruminants typically have sub-clinical disease. Cattle are considered amplifiers and maintenance hosts [16].

Bluetongue is characterized by BTV-induced vascular injury that results in haemorrhage and ulceration of the mucous membranes in the upper portion of the gastro-intestinal tract, coronitis and lameness, facial and intramuscular oedema, pleural and pericardial effusions, pulmonary oedema and necrosis of the skeletal and cardiac muscle [17] [18][19]. Swelling of the lips and cyanosis of the tongue give the tongue its typical blue appearance, though this sign is confined to a minority of the animals. Excessive salivation and nasal discharge are also observed. In sheep lameness due to coronitis can lead to knee-walking. In cattle, constant changing of position of the feet gives bluetongue disease the nick-name, the dancing disease [20]. Clinical bluetongue is mainly seen in certain fine wool and mutton breeds of sheep which, seemingly by chance, are found in countries at the limits of the virus distribution (Spain, Portugal, Turkey, Cyprus, USA, South Africa) [21]. Elsewhere, the disease is likely whenever similarly susceptible animals are transported to countries within the bluetongue enzootic areas e.g. Nigeria [22], Cameroon [23] and Indonesia [24]. Since indigenous, unimproved sheep, goat and cattle breeds are usually highly resistant to the clinical effects of the infection, the vast majority of bluetongue episodes throughout the world are completely silent [25]. However, considering the recent alarming global spread of BTV serotypes, it is no longer uncommon to see mortality rates approaching 50 - 100% in susceptible flocks. Other losses are due to morbidity and the need to provide care for the sick animals. Costs associated with morbidity include weight loss, reduced milk yield, abortion and associated veterinary costs. This covert presence of the virus, alternating with occasional outbreaks of severe disease has had a considerable adverse effect upon international trade of bovine and ovine species, and their germ plasms, as countries free from bluetongue attempt to maintain that status [25].

Diagnostic testing for BTV can be very difficult as the virus cross-reacts with many antigenically related viruses including palyam virus and the viruses that cause epizootic haemorrhagic disease of deer and African horse sickness [16][10]. However, the enzyme-linked immunosorbent assay (ELISA) has been successfully used as a serologic test for BTV antibody detection [26][27], and can be used to decrease the chances of cross-reactions [28]. Also, a monoclonal antibody-based competitive ELISA that can distinguish antibodies to virus in the bluetongue serogroup from antibodies to the other related viruses has been developed [28].

Obi *et al*. [29] recorded BTV seroprevalence of 58% and 50% for sheep and goats respectively in southern Nigeria using agar gel precipitin test. Recently, Oluwayelu *et al*. [30] obtained a prevalence of 89.2%, 88.0% and 84.4% for sheep, goats and cattle respectively in Oyo state of Nigeria using a commercial bluetongue ELISA kit.

Considering the fact that bluetongue is a re-emerging disease [9](Purse *et al.*, 2005), this study was carried out to investigate the seroprevalence of the disease in cattle and sheep in Ogun and Osun States, Nigeria, putting into consideration the effect of sex, breed and age of the animals on the prevalence and to show that the indigenous breeds of sheep are resistant to the clinical manifestation of the disease.

2. METHODOLOGY

2.1 Experimental Design and Sample Size Determination

This was a prospective study and the sample size was determined according to the method previously described [31]. The study was aimed at comparing the result obtained from Ogun state to that of Osun state, putting into consideration the age, sex and breed of the animals. The species of animal of interest were cattle and sheep.

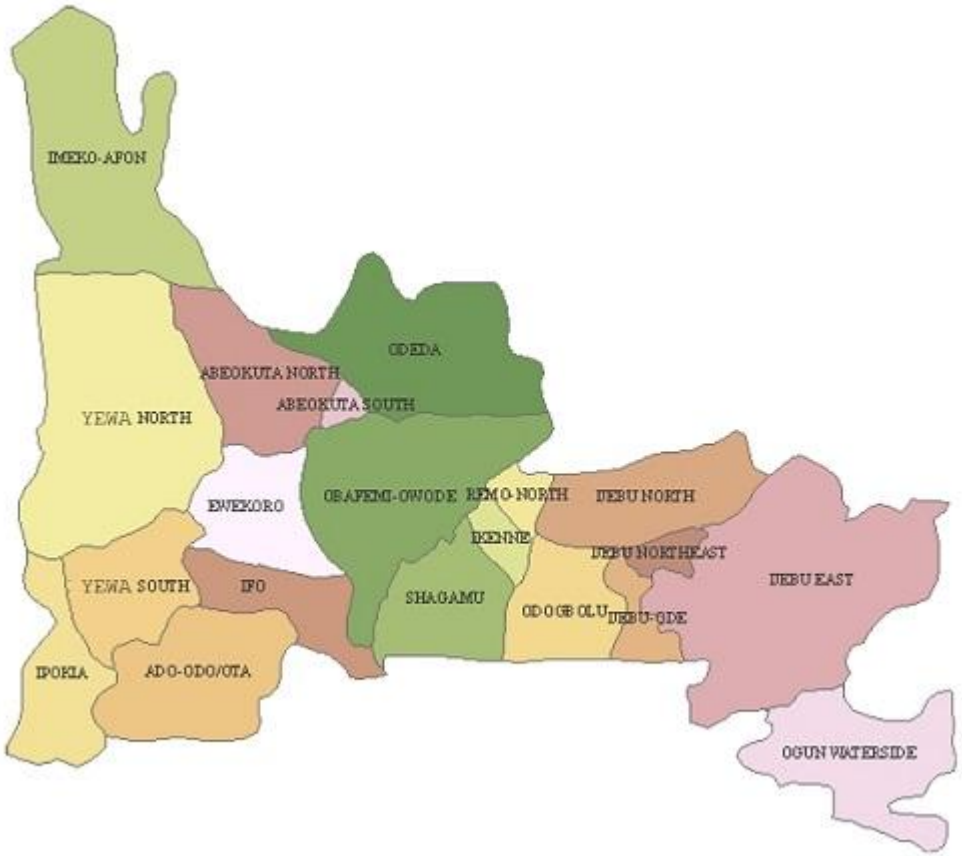
2.2 Study Areas

The areas considered for this study are Ogun and Osun States. In Ogun state, the samples were collected from two different locations which are Abeokuta and Ijebu-Ode while in Osun state samples were collected from Osogbo, Ejigbo and Iwo. For cattle, the sites for sample collection

were ranches, cattle markets and abattoirs while that for sheep were sheep markets and backyard farms in Osun and Ogun states.

Ogun state climate follows a tropical pattern with the rainy season starting from March and ending in November, followed by dry season. The mean annual rainfall varies from 128cm in the southern parts of the state to 105cm in the northern areas [32]. The average monthly temperature ranges from 23^0C in July to 32^0C in February. The northern part of the state is mainly of derived Savannah vegetation, while the central part falls in the rain forest belt [32].

Map of Ogun state (Adopted from NigeriaGalleria,2017).



The climate of Osun state is slightly similar to that of Ogun state, and has a covering of the tropical rain forest. However, Osun state has an annual rainfall of about 60cm [33]. The state climate is less humid when compared to Ogun state although the effects of the harmattan winds are strongly felt in the dry season. The average monthly temperature ranges from 24.5^0C in July to 28^0C in February [33].

Map of Osun state (Adopted from NigeriaGalleria,2017).

The two states are located in the southwestern part of Nigeria and are characterized by a long rainy season, high humidity and temperature favourable for the breeding of the *Culicoides* vectors of bluetongue virus. Since the ambient temperature in Africa allows the survival of the *Culicoides* vectors of BT from January to December, sampling was done without a special interest on a specific season of the year. Several species of *Culicoides* that feed on domestic ruminants have been identified in these areas [34][35].

2.3 Collection of blood samples

Simple random sampling was done. The age, sex and breed of the animals were taken in the process of sampling. The ages of the animals in the farms were determined through oral interview with the farmers, while age estimation was done for the animals in markets and abattoirs. A total number of 340 samples were collected from 205 sheep and 135 cattle from March to September, 2017. About 5ml of blood was collected aseptically from each animal by venipuncture of the jugular vein using sterile Monovette EDTA tubes (Sarstedt, Germany). All samples collected were transported to the laboratory under a cold chain sustained with ice packs.

Antibiotics (Gentamycin and Amphotericin B) were added to the blood samples after which they were centrifuged at 2000 rpm for 5 minutes. The plasma (supernatant) were collected into sterile Eppendorf tubes, labeled and stored in a freezer at -20^{o}.

2.4 Testing the samples

The 340 samples collected (135 cattle and 205 sheep) were screened for the presence of anti-VP7 BTV antibodies using the POURQUIER® BTV cELISA kit. This was done according to the manufacturer's instruction.

2.5 Statistical analysis

The data obtained in this study was analyzed using Chi-square test and the level of significance was set at 95% (0.05). The analysis was done using SPSS version 21.

3. RESULTS

Out of the 340 plasma samples from cattle and sheep screened for the BTV antibodies in Ogun and Osun states, 330 samples were positive which gave an overall seroprevalence of 97%. Furthermore, 195 of the 205 plasma samples from sheep screened were positive which gave a seroprevalence of 95%. All the 135 samples from cattle tested were positive, resulting in 100% seroprevalence irrespective of sex, breeds or age groups. There was a significant difference in BTV antibody prevalence between the cattle and sheep in Ogun and Osun states ($P < 0.05$) (Table 1).

Table 1: Overall prevalence of BTV antibodies in cattle and sheep in Ogun and Osun States

Species	Number of samples	Positive (%)	Negative (%)	X^2
Cattle	135	135 (100)	0 (0.00)	0.04
Sheep	205	195 (95)	10 (5.00)	
Total	340	330 (97)	10	

X^2 represents significant chi square p value

Table 2 shows the seroprevalence of BTV for sheep in Ogun and Osun states to be 95.0% and 95.3% respectively. There was no significant difference. The table also shows the prevalence according to sex. In Ogun state, out of 99 samples from sheep screened for BTV antibodies, 38 (90.5%) and 56 (98.0%) tested positive for males and females respectively, and the difference was not significant (P > 0.05). In Osun state, out of 106 samples from sheep screened, 44 (95.6%) and 57 (95%) tested positive for males and females respectively, and the difference was not significant.

Table 2. BTV antibody prevalence in cattle and sheep based on sex (Ogun and Osun State)

	Ogun		**Osun**	
Sex	Cattle	Sheep	Cattle	Sheep
Male	[a]33/33(100%)	[b]38/42(90.5%)	[a]38/38(100%)	[b]44/46(95.6%)
Female	35/35(100%)	56/57(98.0%)	29/29(100%)	57/60(95.0%)
Total	**68/68(100%)**	**94/99(95.0%)**	**67/67(100%)**	**101/106(95.3%)**

Different superscripts for cattle and sheep for each state represent non-significant p value (p > 0.05)

Table 3 shows results of the BTV antibody prevalence according to the breeds of sheep in Ogun and Osun states. There was no significant difference in the seroprevalence among the breeds in the two states.

Table 3. BTV antibody prevalence in sheep according to breeds in Ogun and Osun States.

	Ogun	Osun
Breed	**Positive (%)**	**Positive (%)**
Yankasa	[a]33/35(94%)	[b]28/30(93%)
Balami	19/20(95%)	21/22(95.5%)
Ouda	20/21(95%)	25/25(100%)
WAD	22/23(96%)	27/29(93%)
Total	**94/99(95%)**	**101/106(95%)**

Different superscripts represent non-significant p value ($p > 0.05$).

Comparison of the BTV seroprevalence based on age groups in Ogun state showed that sheep less than twelve months of age had 92% BTV seroprevalence while those greater than twelve months had a seroprevalence of 96.7%. In Osun state, sheep less than twelve months of age had a seroprevalence of 96% while those greater than twelve months had 94.7% seroprevalence (Table 4). There was no significant difference in BTV antibodies among the age groups.

Table 4. BTV antibody prevalence for sheep in Ogun and Osun States According to age groups.

Age group	Ogun	Osun
1 – 12 months	[a]35/38(92%)	[b]47/49 (96%)
> 12 months	59/61(96.7%)	54/57 (94.7%)
Total	**94/99 (95%)**	**101/106 (95.3%)**

Different superscripts represent non-significant p value ($p > 0.05$)

4. DISCUSSION

The high prevalence of BTV infection observed in cattle and sheep in this study indicates the possible emergence of bluetongue disease in Nigerian cattle and sheep populations. In the present study, 100% and 95% seroprevalence rates were obtained from cattle and sheep respectively in Ogun and Osun states which are located in southern Nigeria that is characterized by high rainfall, high humidity and temperature favourable for the breeding of *Culicoides* vectors of Bluetongue disease [34]. There is constant feeding of the virus vectors on the vertebrates resulting in continual spread of the disease [34]. The prevalence rates obtained in this study for sheep in the two states were slightly different amongst the different parameters such as sex, breed and age group. Our prevalence is higher than 28.9% in sheep reported by Taylor and McCausland [34] in Northern Nigeria. It is also higher than the 58% prevalence obtained by Obi *et al* [29] in Southern Nigeria. Furthermore, the result is also higher than that of Oluwayelu *et al*. [30] which reported BTV seroprevalence rates of 84.4% and 89.2% for cattle and sheep respectively in Oyo state. Other reports on the presence of BTV were made over four decades ago [37][38][39].

The high prevalence obtained in this study together with the stepwise increase in prevalence over time could be attributed to the sustained environmental changes such as global warming and prolonged rainy season as has been proposed [43], and this is constantly facilitating the expansion of the range of *Culicoides* vectors. The insect vectors, biting midges, prefer warm, moist conditions and are in their greatest numbers and most active after rains [8]. Increase in ambient temperature increases the feeding and breeding activities of *Culicoides* midges which results in increased BTV transmission rate [8]. Therefore, the high BTV seroprevalence can be attributed to the sustained activity of *Culicoides* vectors in the study areas. Also, there are so many porous borders in Nigeria, with influx of cattle, sheep and goats from neighbouring West African countries. Preventive measures such as bluetongue disease monitoring and surveillance programme, and quarantine measures are not practiced in Nigeria to ensure that the animals coming into the country are free of the disease. There is no vector control to reduce exposure of the animals to *Culicoides* midges.

Normadism, free-range and backyard systems of farming are the common systems of animal husbandry practices in southwestern Nigeria. Animals are often allowed to roam, almost equally exposed to the vectors of the disease before being taken to the market for sale or slaughter. In addition, even when the animals' movements are restricted, midges easily fly into the pens and ranches through any available space to feed on the confined animals. Moreover, the 100% seroprevalence from cattle in this study could be due to the fact that different breeds of cattle from different parts of the country are usually brought together in the markets before they are slaughtered. The breeds of cattle considered in this study are White Fulani, Red bororo and Sokoto gudali. This convergence could allow for high detection rates of BTV antibodies as the vectors feed on infected animals, become infected themselves and then spread the virus to other ruminants. This could lead to the generation of genetic diversity due to reassortment between BTV strains or serotypes introduced by animals from different parts of the country [9][40].

Cattle are regarded as the maintenance hosts [16]. It has been documented that the disease does not often manifest in cattle but with a prolonged viraemic period which increases the likelihood of feeding *Culicoides* vectors getting infected [16]. Also, the preference for cattle to the other species of ruminants by the *Culicoides* vectors may be a contributory factor [41]. Moreover, it has been reported that African *Culicoides* lay their eggs in deposited dung near the ruminant habitat. Cattle dung, unlike that of sheep, can serve as an efficient environment for breeding of the midges [42]. In this study, the samples from cattle were collected from abattoirs, cattle markets, ranches or farms operating semi-intensive management systems. In most areas, the rearing grounds were covered with animal dungs and moist soils, which served as good habitats for the breeding of *Culicoides* midges.

Reports have shown that the recent spread of several arboviral diseases appears to have resulted from anthropogenic and social factors, rural-urban drift and movement (translocation) of virus infected vectors. *Culicoides* vectors can be transported on wind to long distances of up to several hundred kilometers [44][45]. When the enzootic foci of bluetongue disease are geographically close by, the virus can easily be introduced to the country through the wind-borne insects. Therefore, there is a possibility of occurrence of genetic reassortments between the new strains of the virus introduced by the insects and the existing strains in the locality, resulting in a variant

that may be more compatible with the *Culicoides* species in that region. This variant may be more virulent, and are spread by the vectors to the susceptible hosts.

It is noteworthy that despite the fact that majority of the animals screened in this study were apparently healthy, high seroprevalence rates of the virus were obtained. This shows that clinical manifestation of the disease is not often encountered in Nigeria, even among the sheep population. This is consistent with other reports that bluetongue is endemic in Nigeria and the indigenous breeds of sheep exhibit sub-clinical manifestation of the disease [39][46][21]. Also, Mellor [25] reported that since indigenous, unimproved sheep, goat and cattle breeds are usually resistant to the clinical effects of the disease, the vast majority of bluetongue episodes are completely silent. This could therefore account for the high BTV seroprevalence obtained in this study in the absence of clinical disease.

Since vaccination against bluetongue is not practiced in Nigeria, the detection of high BTV seroprevalence rates indicates natural infection with the virus, as well as sustained activity and possible increased competence of the *Culicoides* vectors in transmission of the disease. Moreover, since field strains of BTV are known to exhibit genetic heterogeneity, it is possible that the genetic profile of circulating Nigerian field BTV strains could have been altered over the past three decades resulting in the emergence of hitherto absent serotypes that could have contributed to the present high antibody prevalence. Therefore, there is a possibility of the indigenous breeds of ruminants becoming susceptible to the emerging and constantly changing strains in Nigeria overtime. It has been reported that BTV serotype 8, which was previously silent is currently causing devastating economic losses in ruminant industries worldwide, and more especially in Northern Europe [18]. BTV-8 is mainly transmitted by *Culicoides imicola* which is the traditional Asian/African species of *Culicoides* [5]. It is possible that this BTV serotype and its principal vector (*Culicoides imicola*) is present in Nigeria without any associated disease outbreak yet.

5. CONCLUSION

The high prevalence of BTV antibodies in cattle and sheep as reported in this study suggests that BTV infection is widespread in Southern Nigeria and stresses the need for continuous

surveillance of the disease in domestic ruminant populations in Nigeria in order to track the possible evolution of the virus. The fact that animals were subclinically infected does not mean that the disease should be over looked. Also, the fact that most of the cattle screened in this study were brought from the northern part of the country suggests that bluetongue may be highly prevalent in the country. Moreover, considering the genetic heterogeneity of field strains of BTV that occurs as a consequence of genetic drift and shift, it is possible that new strains and serotypes of the virus could have emerged in the country.

6. RECOMMENDATIONS

In order to avoid the danger of bluetongue disease outbreak in Nigeria, there is need for continuous national BTV surveillance and further studies to isolate and characterize BTV from Nigerian ruminant populations and *Culicoides* with the aim of identifying currently circulating serotypes and strains which can be used as potential vaccine candidates towards achieving effective control of the disease in Nigeria. Anthropogenic and social factors should be minimized to reduce the rate of climate change. Uncontrolled movement of animals from neighboring West African countries into Nigeria should be checked, and absolute quarantine measures enforced to ensure that animals coming into the country are free of the virus.

ETHICAL APPROVAL

Ethical approval was obtained from the Department of Veterinary Microbiology, Faculty of Veterinary Medicine, University of Ibadan before samples were collected. Data protection act was completely followed in handling the data obtained from the farmers following their informed consent.

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