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Serological screening for Schmallenberg virus in exotic and indigenous cattle in Nigeria

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Abstract

Schmallenberg virus (SBV), a recently emerged Orthobunyavirus, is associated with abortions, stillbirths and congenital malformations in ruminants. Considering that *Culicoides* species which transmit this disease have previously been identified in Nigeria as vectors of bluetongue, another livestock disease that causes abortions, it is speculated that SBV also circulates in the Nigerian ruminant population. We therefore conducted a pilot study to investigate the occurrence of anti-SBV antibodies in a limited population of cross-breed, exotic and indigenous cattle in Nigeria. Serum samples obtained from 60 Friesian-White Fulani (FWF), 7 Jersey and 53 indigenous cattle were screened for SBV antibodies using a commercial indirect ELISA kit that detects antibodies against recombinant SBV nucleoprotein in ruminant sera. An overall seropositivity of 29.2% (35/120) was obtained with antibodies being detectable in 23.3% FWF (14/60), 42.9% Jerseys (3/7) and 34.0% (18/53) indigenous cattle. All indigenous breeds of cattle tested had seropositive animals: White Fulani (13/38, 34.2%), Sokoto Gudali (2/8, 25.0%), Red Bororo (1/5, 20.0%) and Bunaji (2/2, 100.0%). The detection of antibody-positive animals among unvaccinated cattle provides evidence of possible SBV infection in Nigeria. However, there is also the probability of cross-reactivity with other Simbu serogroup viruses, especially considering that some of these viruses have previously been reported in Nigeria. Further studies to confirm these preliminary findings using serum neutralisation assay, viral isolation or detection of SBV RNA from ruminants or *Culicoides* are necessary.

Keywords: Abortions, Antibodies, Cattle, Congenital malformations, Nigeria, Schmallenberg virus

Introduction

Schmallenberg virus (SBV), a novel member of the family Bunyaviridae, genus *Orthobunyavirus* was first reported in cattle from Germany and The Netherlands in 2011 (Hoffmann et al., 2012). It is an enveloped, negative-sense, segmented, single-stranded RNA virus that is closely related to Shamonda virus (SHAV), which belongs to the Simbu serogroup of viruses that includes Sango, Sabo and Shuni viruses (Causey et al., 1972; Goller et al., 2012). The virus is associated with abortions, stillbirths and congenital malformations in cattle, sheep and, much less frequently, goats (Beer et al., 2013). Since the first cases from Germany and The Netherlands, SBV has caused a large epidemic in ruminants with several reports of clinical manifestation and serologic evidence of the disease mostly from European countries (Doceul et al., 2013).

Apart from a retrospective report of suspected SBV infection of lambs in South Africa and the recent detection in Mozambique of SBV antibodies in ruminants (Leask et al., 2013; Blomstrom et al., 2014), there is limited information on the disease in Africa. In Nigeria, although there are no reports of SBV infection in ruminants hitherto, congenital malformations associated with SBV such as arthrogryposis, kyphosis, scoliosis and brachygnathia inferior have been reported among Nigerian ruminants (Ibrahim et al., 1987; Ate & Allam, 2002; Ibrahim et al., 2006). Moreover, several *Culicoides* species identified as vectors of SBV have previously been identified in Nigeria (Dipeolu & Ogunrinade, 1977). Since these Culicoides vectors also transmit bluetongue virus (BTV) which causes abortions in ruminants and has been reported in Nigeria (Herniman et al., 1983), it is hypothesized that SBV infection is prevalent in

Nigeria but its diagnosis might have been missed because it produces clinical signs and pathology similar to those caused by other known disease agents such as BTV. Thus, in this pilot study, we investigated the presence of anti-SBV antibodies in a limited number of cross-breed, exotic and indigenous cattle in Nigeria.

Materials and methods

Serum collection was carried out from 120 cattle comprising 60 adult Friesian-White Fulani (FWF), seven Jersey (5 adults and 2 calves) and 53 adult indigenous cattle. The FWF, which are cross-breeds between Friesians imported from The Netherlands over three decades ago and White Fulani cattle, were managed intensively on a private dairy farm in Jos, Plateau state while the adult Jersey cattle, whose country of origin could not be ascertained, were reared privately with their calves in Ibadan, Oyo state. The indigenous cattle belonged to four breeds: White Fulani (n=38), Sokoto Gudali (n=8), Red Bororo (n=5), and Bunaji (n=2) and were located in Abeokuta (Ogun state), Ibadan (Oyo state), Ejigbo (Osun state) and Badagry (Lagos state) (Figure 1). Most of them were managed intensively on privately-owned farms while a few were transported from northern Nigeria for slaughter in major cattle markets in the southwest region. Sera from the FWF were archived samples collected in January 2011 while those from the indigenous and Jersey cattle were collected between March and July 2012, and in January 2013, respectively. The sera were screened for SBV antibodies using the commercial ID Screen® Schmallenberg virus indirect ELISA kit (IDvet, Montpellier, France) that detects antibodies against recombinant SBV nucleoprotein in ruminant sera and was reported by the manufacturer to have excellent (98.9%) correlation with the virus neutralization test. The ELISA was performed according to the instructions of the manufacturer and assay characteristics as reported in the manufacturer's validation data were a relative sensitivity of 96.47% (95% CI: 93.43-98.13%) and specificity of 99.75% (95% CI: 99.26-99.92% (IDvet, 2012). Results were expressed as *S/P* percentage (*S/P* %) using the optical densities (OD) from the ELISA reader (*S/P*% = OD_{sample} / $OD_{positive\ control}$ x 100). Samples that presented *S/P*% lower or equal to 60%, between 60% and 70%, and > 70% were considered negative, doubtful and positive respectively. Data obtained were analysed with Graph Pad prism version 5.0 (Graph Pad software, San Diego, USA) and p-values < 0.05 were considered significant. Using two-tailed Fisher's exact test, prevalence rates were compared based on region of sample collection and breed of cattle.
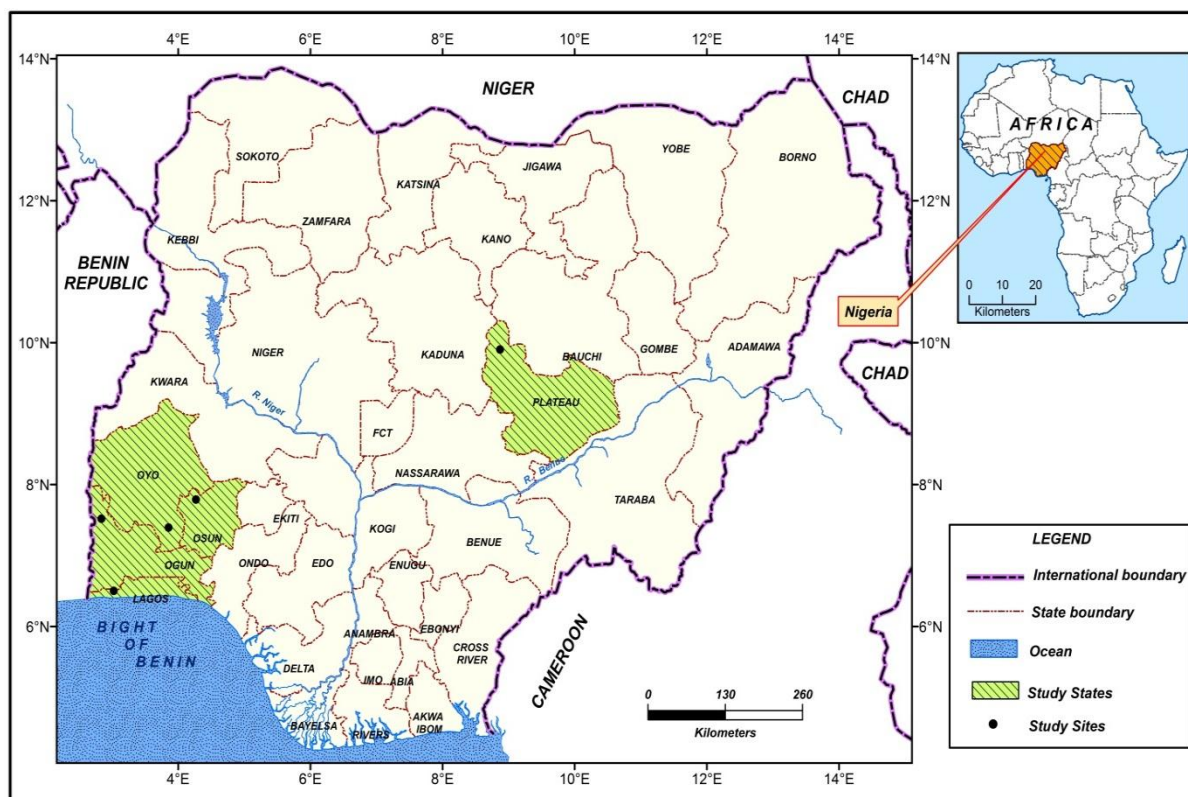

Figure 1: Map of Nigeria showing sample collection sites

Table 1: Seroprevalence of Schmallenberg virus in tested cattle samples

Breed of cattle	No. sampled	Positive (%)	Doubtful (%)	Negative (%)
Friesian-White Fulani	60	14 (23.3)	4 (6.7)	42 (70.0)
Jersey	7	3 (42.9)	1 (14.3)	3 (42.9)
Indigenous	53	18 (34.0)	7 (13.2)	28 (52.8)
Total	120	35 (29.2)	12 (10.0)	73 (60.8)

Results

Thirty-five (29.2%) of the 120 sera tested reacted against recombinant SBV nucleoprotein antigen with 12 (10.0%) and 73 (60.8%) being doubtful and negative, respectively (Table 1). Specifically, antibody-positive animals were 14 FWF (23.3%), 3 Jersey (42.9%) and 18 indigenous cattle (34.0%). For the indigenous cattle, seropositivity was 34.2% (13/38), 25.0% (2/8), 20.0% (1/5) and 100.0% (2/2) among White Fulani, Sokoto Gudali, Red Bororo and Bunaji breeds, respectively. Although a higher SBV seroprevalence (35.0%) was obtained for cattle from the Southwest region compared to those from the North-Central (23.3%), the difference was not statistically significant.

Discussion

The findings of this study provide a first indication of the possible circulation of SBV infection in cattle in Nigeria. As vaccination against SBV is not currently practised in the country, the detection of seropositive animals among the tested cattle suggests natural exposure of these animals to the virus. Considering that the FWF cattle sera were collected in January 2011, this finding of antibody-positive animals presupposes that the virus may have existed in Nigeria, at least, as far back as 2011 when the first outbreak of the disease was recorded in Europe. Our detection of positive reactors among cattle in this study is consistent with the report of Azkur *et al.* (2013) who also found SBV-positive animals in Turkey using the same ELISA kit and concluded, based on personal communication with the manufacturer, that antibodies detected in the screened animals were actually due to SBV and not a related virus like Akabane virus (AKAV).

However, the possibility that the antibodies could be due to cross-reactivity with other Simbu serogroup viruses cannot be completely excluded. This possibility is particularly relevant to the present study because SHAV and Sathuperi virus (SATV) were originally isolated in Nigeria (Causey *et al*., 1972), and it is likely that other currently unidentified Simbu viruses might be present in the country. Moreover, SBV is reported to be a reassortant having the S and L RNA segments from SHAV and the M segment from SATV (Yanase *et al*., 2012). The prospect of cross-reactivity as it relates to the Nigerian (or African) origin of some of these Simbu viruses is underscored by the report of Kupferschmidt (2012) in which it was proposed that SBV could have gained entry into Europe through insects imported on aircraft, or with an infected animal or cut flowers from sub-Saharan Africa. Furthermore, in a study to validate the commercial ELISA used for the current investigation, Bréard *et al*. (2013) reported that despite limited cross-reactions observed with sera from ruminants experimentally infected with AKAV, cross-reactivity with other members of the Simbu serogroup such as SHAV and SATV cannot be ruled out using this ELISA.

Although 23.3% and 42.9% SBV seropositivity were detected in FWF and Jersey cattle sampled respectively, our study could not ascertain whether the exotic cattle (Friesian component of FWF and Jersey) acquired the infection locally or were already incubating the disease at the time of their importation into Nigeria. Also, since there is illegal transborder movement of ruminants from neighbouring West African countries, it is possible that some of the seropositive indigenous cattle could have imported the disease into the country. This highlights the need for screening of all imported animals for both endemic and emerging infectious disease agents. Moreover, the detection of seropositive animals among exotic and indigenous cattle located in different geographical regions of Nigeria suggests that the disease is not breed-restricted and that the virus is present over a large area of the country, being likely sustained by the activity of *Culicoides* vectors.

Except for the report of suspected SBV infection in South Africa (Leask *et al*., 2013) and recent serologic evidence of the disease in Mozambique (Blomstrom *et al*., 2014), the infection has so far been reported only from Europe and Asia (Doceul *et al*., 2013; Azkur *et al.*, 2013). Therefore, our detection of seropositive cattle in Nigeria indicates that SBV infection may actually be more widespread in distribution than previously believed. Coupled with previous reports of congenitally malformed sheep in Nigeria (Ibrahim *et al*., 1987; Ate & Allam, 2002), the results of this study suggest that Nigerian sheep may also be infected with SBV or other related Simbu serogroup viruses since the latter are usually reared with cattle. Furthermore, it is possible that SBV infection may have been present for some time in Nigeria but cases were wrongly attributed to BTV as the latter also causes abortions, stillbirths and congenital malformations.

Although the findings of this study provide an indication that SBV is present in Nigeria, further investigations based on serum neutralisation test, viral isolation and/or detection and sequencing of SBV RNA from ruminants that have stayed only in Nigeria or from *Culicoides* would be critical to conclude that SBV has emerged in the country. Additionally, even though SBV is not yet a notifiable disease, we advocate that veterinarians and farmers be encouraged to report cases of abortions, stillbirths and congenital malformations in ruminants to appropriate authorities.

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Observations on placentome diameters in gestating West African dwarf does experimentally infected with *Trypanosoma brucei*

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Abstract

This study investigated the effect of experimental *Trypanosoma brucei* infection on the placentome diameter (PD) of twenty four gestating West African dwarf does. The does were randomized into 4 equal groups with 'G1' as control while 'G2', 'G3' and 'G4' were intravenously inoculated with 5 x 10^8 *trypanosomes* on days 25, 51 and 101 post breeding (PB), respectively. Real-time trans-abdominal scan was carried out with 3.5-5MHz convex transducer. The differences between the mean PD readings on days 60, 75 and 132 for 'G1' (1.18 ± 0.32, 1.63 ± 0.83 and 2.43 ± 0.69) cm, 'G3' (1.20 ± 0.82, 0.49 ± 3.13) cm and 'G4' (1.19 ± 0.26, 1.65 ± 0.05 and 2.39 ± 1.16) cm, respectively were statistically significant (P ≤ 0.05). At day 60, the mean value for does in 'G1' (1.18 ± 0.32) cm was significantly (P ≤ 0.05) higher than for 'G2' (0.88 ± 1.53) cm. At day 75, the mean value for does in 'G1' (1.63 ± 0.83) cm was significantly (P ≤ 0.05) higher than for 'G3' (0.49 ± 3.13) cm. At day 132, the difference between the mean values of PD for does in 'G1' (2.43 ± 0.69) cm and 'G4' (2.39 ± 1.16) cm was not significant (P ≥ 0.05). The placenta tissue loss following infection for 'G2' and 'G3' were 25.4% and 69.9% at 36 DPI and 25 DPI, respectively. No values were obtained at days 75 and 132 for does in 'G2' and at day 132 for does in 'G3' either due to abortion or death. These findings indicate that experimental *T. brucei* infection led to reduced placentome diameter during critical periods of increased foetal development.

Keywords: Gestation, Placentome diameter, *Trypanosoma brucei,* Ultrasonography, West African dwarf does

Introduction

Trypanosomosis has not only been described as the greatest constraint to agricultural development and growth of livestock industry in sub-Saharan Africa (Guy, 1994), it is perhaps the largest single aetiology responsible for the acutely short supply of animal protein to humans in Tropical Africa (Swallow, 2000). Its association with the reproductive system had been reported (Silva *et al.,* 2013). Some of these symptoms/clinical signs in female mammalian livestock include intrauterine infection, abortion and fetal death *in-utero* (Ogwu *et al.,* 1986; El-Hassan *et al*., 1995; Bawa *et al*., 2000; Faye *et al*., 2004).

The activities of the placenta in pregnancy are pivotal for successful gestation. The mammalian placenta is the major determinant of intrauterine growth through its supply of nutrients to the fetus (Fowden *et al*., 2006). The efficiency of the placenta has often been evaluated as fetal grams produced per gram placenta (Wilson & Ford, 2001). Baur (1977) also reported that fetal and placental weight near term were positively correlated. Reductions in determinants of placental efficiency (e.g. size) affects its nutrient transfer capacity (Jones *et al*., 2007) which therefore causes restrictions in intrauterine growth (Fowden *et al*., 2006).

Based on the above, the integrity of the organ placenta during trypanosome challenge raises issues to ponder over. Older literatures observed trans-placental infection, neo-natal deaths, with other