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Seroprevalence of *Mycobacterium bovis* in cattle and wildlife in Yankari game reserve, Bauchi State, Nigeria

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Abstract

This study was designed to determine the seroprevalence of *Mycobacterium bovis* (*M. bovis*) in wildlife in Yankari Game Reserve (YGR) and cattle living in settlements surrounding the Game Reserve in Bauchi State, Nigeria. Seven hundred and fifty cattle from 21 herds surrounding the game reserve were conveniently selected and blood samples collected from the animals that were above six months of age in the selected herds. Blood samples were also collected from 250 darted wildlife species during routine examinations and from wild animals captured by hunters with the species, sexes and estimated ages determined at capture. Serum sample was obtained by allowing the blood to coagulate to produce sera. The serum was analyzed using Rapid bovine tuberculosis (TB) antibodies test kits which is specific for *M. bovis*. While 88 (11.7%) of the 750 cattle sera tested were positive for *M. bovis* antibodies, 30 (12.0%) of the 250 wildlife sera were positive for *M. bovis* antibodies. Among the cattle that tested positive to *M. bovis* antibodies, 19 (11.5%) were males, while 69 (11.8%) were females. Of the 250 wildlife species tested 6 (19.35%) zebras, 2 (10.0%) elands, 3 (7.6%), antelopes, 4 (10.0%), baboons, 6 (15.0%), African giant rats, 3 (12.0%) hares, and 6 (30.0%) grass cutters were positive for *M. bovis* antibodies. There was no significant difference ($p < 0.05$) in sero-prevalence of *M. bovis* between cattle living around YGR and the wildlife. The prevalence of *M. bovis* in cattle and wildlife is of public health significance to humans in close proximity to the game reserve and tourists due to the possibility of its transmission to humans. Further studies on the isolation and characterization of *M. bovis* in cattle and wildlife in YGR are recommended.

Keywords: Antibodies, Cattle, *M. bovis*, Wildlife, Yankari Game Reserve

Introduction

Mycobacterium bovis (*M. bovis*) affects many species of animals and is gradually becoming a significant pathogen of free ranging African wildlife (Keet *et al*., 2000; De Vos *et al*., 2001; Keet *et al*., 2001; De Lisle *et al*., 2002; Michel, 2002). The importance of tuberculosis (TB) in the wild has been acknowledged as many of the wildlife have shown possibility of being reservoirs of the infection for both cattle and

other important wildlife species (De Lisle *et al*., 2002). Tuberculosis in wildlife is a potential source of infection for both domestic livestock and humans (Cleaveland *et al*., 2002; Michel, 2002). It also poses threat to valuable wildlife species that are in danger of extinction. For example, there have been reports on death of buffalo (*Syncerus caffer*) (Keet *et al.,* 1996), lion (*Panthera leo*) (Keet *et al*., 2000), and cheetah (*Acinonyx jubatus*) (De Lisle *et al*., 2002) caused by *M. bovis* in the Kruger National Park, South Africa.

Based on the report Livingstone (2000), *M. bovis* has been detected in wildlife such as deer, elk, wild boar, feral goat, buffalo, possum, ferret, mink, hedgehog, lion, cheetah, kudu, baboon, and seal in 22% of countries of the world.

Yankari is one of the biggest game reserves in Nigeria and the most popular destination for tourists in Nigeria. The Yankari game reserve is one of a few remaining areas left in West Africa where wild animals are protected in their natural habitat. The Game Reserve is well-stocked with different species of wildlife including elephants, baboons, waterbucks, bushbucks, crocodiles, hippopotamuses, roan antelopes, buffaloes and various types of monkeys, lions etc. It therefore plays a crucial role in the development and promotion of tourism particularly ecotourism in Nigeria (Yankari National Park, 2000). It is also one of the most popular eco-destinations in West Africa and attracts tourists from different parts of the world (Yankari National Park, 2000). However, there is paucity of information about the actual prevalence of bTB in wildlife population at YGR and these poses a risk to other livestock living around the Game Reserve, tourism economy, and wildlife conservation (Michel *et al*., 2010). Hence, this study was carried out to determine the seroprevalence of *M. bovis* in wildlife in YGR and cattle living in settlements around the game reserve.

Materials and Methods

Study area

The Yankari Game Reserve (YGR) is one of the largest wildlife parks in Nigeria, situated in the heartland of the West African savannah with characteristic savannah vegetation that includes swamps with river floodplains, grasslands and thick forest (Odunlami, 2000). It is located in the south-central part of Bauchi State in the North-East zone of Nigeria. It lies between latitude 9.750000 North and longitude 10.500005 West, and covers an area of about 2,244 km². The Game reserve is home to several natural springs, as well as to a wide variety of flora and fauna.

Sampling procedure

Convenience and purposive sampling techniques were used to select cattle and wildlife species respectively. Herds of cattle living around the YGR were identified and herd with 10 cattle and above were identified, and blood samples were collected from cattle above 6 months of age. The ages, sexes and breeds of cattle sampled in each herd were recorded. Age estimation was done by identification of the permanent incisors teeth as described by Pace and Wakeman (2003), while the identification of different breeds of cattle was done based on the body characteristics of the cattle as described by Mason (1996), Tawah and Rege (1996) and Rege and Tawah (1999) for Red Bororo, Sokoto Gudali and White Fulani breeds of cattle respectively. For the wildlife species, identified animals were darted in order to collect blood samples. Sick wildlife under the care of the resident veterinarian and those captured by the wildlife staff and hunters were also sampled. Based on these, samples from 750 cattle and 250 different wildlife species were collected.

Sample collection from cattle

Each of the sampled animals was physically restrained and 5mL of blood was collected from the jugular vein using a sterile disposable 10mL syringe with 18 gauge needle attached. The blood sample was emptied into a sterile plain sample bottle that was appropriately labelled with an acronym number, place and date of collection. The blood sample was kept in a slanting position and allowed to coagulate to produce sera according to the methods described by Okeudo *et al*. (2003). The serum was separated from the blood and kept in a separate appropriately labelled vial and stored at -20^0 C until further analysis.

Sample collection from wildlife species

The wild animals in the YGR irrespective of age and sex were chemically restrained using etorphine via dart gun administered by wildlife rangers. Blood samples were then taken from the wild animals either through the jugular or recurrent tarsal vein using a 23 gauge needle mounted on a 5 mL while the animal is still sedated. The blood sample was emptied into a sterile plain sample bottle that was appropriately labelled with an acronym number, place and date of collection. The blood sample was kept in a slanting position and allowed to coagulate

to produce sera according to the methods described by Okeudo *et al*. (2003). The serum was separated from the blood and kept in a separate appropriately labelled vial and stored at -20^0 C until further analysis.

Test kits

The Immunochromatography test kits. Rapid bTB Antibodies (RbTBAb) test kit - Bionote Incorporated (Seogu-dong, Hwaseong-si,Gyeonggi-do, South Korea) was used for the detection of *M. bovis* antibodies in the serum samples of both the cattle and wildlife species. The RbTBAb test kit is based on a chromatographic immunoassay for the quantitative detection of IgG and IgM antibodies against *M. bovis* in serum, plasma, or whole blood. The MPB70 is a specie-specific protein produced by *M. bovis* and is a major antigen from culture filtrate protein of *M. bovis.* It has a sensitivity of 90% and a specificity of 98% (Wiker, 2009).

Laboratory analysis

Serum analysis for *M. bovis* antibodies:

The test kit has a sample well and a developing buffer well. The serological test was carried out according to the manufacturer's instructions as follows;

(a) Stored sera were removed from the freezer and allowed to thaw to room temperature

(b) One drop of the test serum was added to the sample well (s) using a capillary tube and after 1 minute, 3 drops of the developing buffer was added into the developing buffer hole

(c) The result was interpreted within 20 minutes, and beyond 20 minutes the result was considered invalid

Interpretation of the test results

Positive results:

The presence of two red colour bands ('T' band and 'C' band) within the result window no matter which band appeared first indicated a positive result. Even if the intensity of the red band colour was faint, it was interpreted as positive result.

Negative results:

The presence of only 1 red colour band within the ''C'' result window indicated a negative result (Plate II).

Data analysis

Data obtained were expressed as percentages in tables and graphs where necessary. Chi square test was used to test for association between presence of antibodies and the age, sex, breed of cattle and wildlife type. GraphPad Prism Version 4.0 for Windows (SanDiego, California, USA) was used for the data analysis. A confidence interval of 95% and 5% significant level (P<0.05) were considered.

Results

Out of the 1000 sera samples collected from the 750 cattle living around YGR and the 250 wildlife in the YGR for the purpose of screening for *M. bovis* antibodies, an overall sero-prevalence of 11.73% (88/750) and 12.0% (30/250) was seen in cattle and the wildlife respectively. There was no significant difference in sero-prevalence of *M. bovis* between cattle living around YGR and the wildlife (Table 1) (Odd Ratio = 0.9748; *p* = 0.9101 and CI= 0.6-1.5).

Both male and female cattle leaving around YGR were sero-positive for *M. bovis* infection. A sero-prevalence of 11.79% was found in both male (19/165) and female (66/585) cattle (Table 2) and there was no significant difference in the prevalence of *M. bovis* among the cattle of different sex living around YGR (OR =0.9232; *p*=1.00; 95% CI = 0.5670-1.670). Based on age category, the prevalence of *M. bovis* in cattle between 6 months to 2 years, 2 to 5 years and above 5 years were 11.76% (14/119), 12.53% (45/359) and 10.66% (29/272) respectively. There was no significant difference in the sero-prevalence of *M. bovis* among the different age groups of the cattle living around YGR (Table 3) ($\varkappa^2$ = 0.6103; df = 2; p = 0.7370). The sero-prevalence of *M. bovis* in different breeds of cattle living around YGR showed that 11.36% (80/704), 18.51% (5/27), and 15.79% (3/19) sero-positive for *M. bovis* were observed among White Fulani, Sokoto Gudali and Red Bororo breeds of

Table 1: Prevalence of *M. bovis* in cattle and wildlife at the surrounding and in YGR

Animal type	Total number sampled	No positive (%)
Cattle	750	88 (11.73)
Wild life	250	30 (12.0)
Total	1000	118 (11.8)

OR; 0.9748, CI; 0.6268-1.516, P = 0.9101

Table 2: Prevalence of *M. bovis* by sex in cattle living at the surrounding of YGR

Sex	Total number sampled	No positive (%)
Male	165	19 (11.5)
Female	585	69 (11.8)
Total	750	88 (11.73)

OR; 0.9732, CI; 0.5670-1.670, P = 1.00

cattle respectively. (Table 4). There was still no significant difference in the sero-prevalence of *M. bovis* among the breeds of cattle living around YGR ($\varkappa^2$=1.595, df=2 and p=0.4505). In the different wildlife species sampled for this study, 6 (19.38%) Zebras, 2 (10.0%) elands, 3 (7.6%) antelopes, 4 (10.0%), baboons, 5 (15.0%) African giant rats, 3 (12.0%) hares and 6 (30.0%) Grass cutters were sero-positive for *M. bovis* (Table 5). However, there was no significant difference in the sero-prevalence of *M. bovis* among the different wildlife species sampled in YGR ($\varkappa^2$=13.75, df=9, p=0.1315).

Discussion

The presence of lush pastures, streams and water springs in YGR are the major reasons behind cattle encroachment into the Game Reserve, especially during the dry season (Yankari National Park, 2000). Contact between cattle and wildlife at the grazing and watering sites, or indirect contact with feaces, urine and wound discharges contaminated with *M. bovis* may serve as source of transmission of *M. bovis* between cattle and wildlife.

The prevalence of *M. bovis* in both cattle and wildlife found in this study could be due to their interaction at the grazing land or water points. This usually happens mostly during the dry season when the grazing land around the communities surrounding the YGR becomes scarce or dry. More so, wildlife has been observed moving beyond the borders of the Game Reserve into communities surrounding the Game Reserve. Furthermore, the cross infection observed in this study could be as a result of the scavenging habit of wildlife on dead infected carcasses of cattle as reported by Norton *et al.* (2005). Cattle may also be exposed to *M. bovis* through, sniffing or licking of discharges from dead infected or dying wildlife as was reported by Zuckerman (1980) and Griffin *et al.* (1996). Phillips *et al.* (2003), De Lisle *et al.* (2002) and Delahay *et al.* (2007) whom in their separate studies, reported *M. bovis* infection both cattle and wildlife.

The seropositivity of *M. bovis* in cattle of all ages and sexes is in agreement with the reports of Bonsu *et al.* (2000). The fact that more cows were sampled in the

Table 3: Prevalence of *M. bovis* according to age group of cattle living at the surrounding of YGR

Age	Total number sampled	No positive (%)
6 months to 2 yrs	119	14 (11.76)
2 -5 years	359	45 (12.53)
> 5 yrs	272	29 (10.66)
Total	750	88 (11.73)

$\mathbf{x}^2$; 0.6103, df; 2, P= 0.7370

Table 4: Prevalence of *M. bovis* According to Breeds of Cattle living at the surrounding of YGR

Breed	Total number sampled	No. Positive (%)
Red Bororo	19	3 (15.79)
Sokoto Gudali	27	5 (18.52)
White Fulani	704	80 (11.36)
Total	750	88 (11.73)

$\mathbf{x}^2$; 1.595, df; 2, P = 0.4505

Table 5: Prevalence of *M. bovis* in different species of wildlife in YGR, Bauchi State

Wildlife Species	Total No. sampled	Number positive (%)	Total positive (%)
Zebra	31	6(19.35)	2.4
Western Heart beast	10	0(0.0)	0.0
Water bucks	15	0(0.0)	0.0
Elands	20	2(10.0)	0.8
Antelopes	39	3(7.6)	1.2
Baboons	40	4(10.0)	1.6
African Giant Rat	40	5(15.0)	2.0
Hares	25	3(12.0)	1.2
Grass cutters	20	6(30.0)	2.4
Total	250	30(12.0)	12.0

$\mathbf{x}^2$; 13.75; df; 9, P = 0.1315

herds studied, could be due to their greater number in the herds since bulls are normally sold, and the cows maintained for the purpose of breeding and milk production (Zanini *et al., 1*998). Infection in calves could have resulted as a result of pseudo vertical transmission, consumption of contaminated milk or close contact with infected dams. These potential routes of transmission could be supported by the reports of Phillips *et al.* (2003) and Ozyigit *et al.* (2007) who explained how calves could be infected from their dams through grooming and also congenitally. The duration of exposure of susceptible animals to infected ones will certainly trigger the infection as shown by Munroe *et al.* (1999) in a study in Ethiopia.

The detection of *M. bovis* in some wildlife in YGR is of significant zoonotic importance. For example, the antibody to *M. bovis* detected in the baboons pose a zoonotic risk to tourists coming to YGR from all over the world. The Baboons in YGR might have been infected either by feeding on infected carcasses or coming into contact with contaminated feeds and water since all wildlife use the same watering points in the reserve for their source of water. Similar findings were also made by Sapolsky & Else (1987) in Masai Mara Game Reserve in Kenya, and Thoen *et al.* (1977) in the Biological Research Laboratory Primate Facility in the University of Illinois USA in Baboons.

Zebras in this study were also found to be seropositive for *M. bovis* infection. They could have been infected through feeding on contaminated pastures or indirectly through close contact at the watering points and grazing land. Infected Zebras could serve as source of *M. bovis* infection to their predators such as lion, cheaters, leopards etc

The seropositivity of hares and grass cutters to *M. bovis* revealed in this study is of great public health concern as this wildlife is usually hunted for and their meat consumed as delicacies. The Hares and Grass cutters might have been infected through consumption of contaminated grasses (Hares are gregarious in their feeding habits) at the surrounding of the YGR. This is because dead infected wildlife can still release the organism in nature (De Kantor & Ritacco*,* 1994). Hares were previously reported to be infected with *M. bovis* in Argentina and New Zealand (De Kantor & Ritacco, 1994). *Mycobacterium bovis* detected in the serum of Elands and Antelopes are of great concern especially to individuals that keep such wildlife in their homes as pets (Daborn *et al.,* 1997).

In conclusion, the study revealed the presence of *M. bovis* in cattle and wildlife in YGR. The finding is of public health significance especially to people living in close proximity to the Game Reserve, hunters and people consuming wildlife/bushmeat due to possibility of transmission of this microbe to humans. Cattle reared in settlements close to the Game Reserve need to be screened for bTB and positive animals should be isolated from the herds. Further study is required for isolation and molecular characterization of *M. bovis* in cattle and wildlife in YGR.

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