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Prevalence of *Besnoitia besnoiti* antibodies in bovine sera and milk in Northern Nigeria

SJ Sambo[1]*, NDG Ibrahim[1], KAN Esievo[1] & HM Kazeem[2]

1. *Department of Veterinary Pathology, Ahmadu Bello University, Zaria-Nigeria*
2. *Department of Veterinary Microbiology, Ahmadu Bello University, Zaria-Nigeria*

*Correspondence: Tel.: 2348080365194, E-mail: sambojames66@gmail.com

Abstract

Besnoitia besnoiti, a re-emergent parasite of cattle in Europe, occurs in many countries of Africa and other parts of the world. Clinical observations and incidental findings of *B. besnoiti* in cattle have been reported in the Southern and Northern regions of Nigeria, but the prevalence of antibodies against this parasite is not yet known. This investigation was designed to determine the seroprevalence of bovine besnoitiosis in Northern Nigeria. A total of 400 cattle were selected at random through cluster sampling of herds from two Local Government Areas (LGA) each, of 5 States in the region (Kano, Kaduna, Katsina, Sokoto and Borno States), between May, 2008 and November, 2009. Sera samples obtained from cows, bulls and calves, and milk from lactating cows with suckling calves were screened with indirect immunofluorescent antibody technique (IFAT) for antibodies to *B. besnoiti*. Out of the 400 samples 321 (80.3%) were positive for antibodies to *B. besnoiti*. Cattle sampled in Borno had the highest (87.5%) prevalence of antibodies to *B. besnoiti*, while those sampled from Katsina State had the least prevalence (62.3%). Wamako LGA of Sokoto State had the highest prevalence of the antibodies (100.0%), while Dan Musa LGA in Katsina had the least prevalence (53.0%) among the ten LGA sampled, however, these differences were not statistically significant ($p > 0.05$). Similarly, the overall prevalence of antibodies to *B. besnoiti* did not vary significantly between bulls (84.0%) and cows (79.0%), or in the dry (83.6%) and wet (77.1%) seasons ($p > 0.05$). The high prevalence of antibodies to *B. besnoiti* in cattle in Northern Nigeria indicates an endemic state of the disease in this region.

Keywords: Antibodies, *Besnoitia*, Cattle, Northern Nigeria, Prevalence

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Introduction

Bovine besnoitiosis is caused by *Besnoitia besnoiti* (Pols, 1960) which has been described as a re-emergent parasite in Europe (Alzieu, 2007; Fernandez *et al.,* 2009). The protozoan parasite is widely distributed in the Middle East (Bargai *et al*., 1980; Goldman & Pipano, 1983; Pipano, 1997; Shkap *et al*., 2002), Asia (Hi-Suk *et al*., 1970; Liu & Wang, 1982; Wang & Liu, 1987) and Europe (Franc *et al*., 1987). Millions of cattle in many African countries including South Africa, Angola, Kenya, Democratic Republic of Congo, Cameroun (Njenga *et al*., 1999), Mozambique (Ferreira *et al*., 1983; Ferreira & Diaz, 1984) and Nigeria (Oduye, 1974; Kumi-Diaka *et al.,* 1981; Sambo *et al*., 2007) had been found infected by the parasite in the past.

The prevalence of besnoitiosis could be determined by gross examination of cattle with the cutaneous or sclero-conjunctival cysts. In a study carried out in Uganda, the typical elephant skin appearance of clinical besnoitiosis was observed among 8.7% of the cattle (Bwangamoi, 1968). Similar clinical signs were seen in 12% of the cattle in South Korea (Hi-Suk *et al*. 1970), but may be in-apparent in some cases. Cysts of *B. besnoiti* were accidentally discovered in skin sections of 4.1% of cattle at Ibadan in South Western Nigeria, although there were no gross lesions or clinical signs of the disease (Oduye, 1974).

Further investigations indicated that 4.6% of cattle in Kaduna State (Sambo *et al.,* 2007) and 8.7% in Borno State (Igbokwe *et al.,* 2009) harbored cysts of the parasite observed in skin sections, with or without gross lesions of the disease. Since the absence of or failure to observe gross lesions and clinical manifestations may allow some cases of the disease to pass undiagnosed, antibody detection is required for screening the population to enhance our understanding of the epidemiology of bovine besnoitiosis.

Serological diagnosis of the disease by Kaggwa *et al*. (1979) established the superiority of immunofluorescent antibody technique (IFAT) to enzyme linked immunosorbent assay (ELISA). It has been shown that 68.7% of cattle found with sclero-conjunctival cysts of *B. besnoiti* clinically were positive for the antibodies to the parasite when analyzed with ELISA, but 81.7% were positive to IFAT (Janitschke *et al*., 1984). In the same study, 45.7% of sera samples from asymptomatic cattle were positive with ELISA while 49.4% of the same samples were positive with IFAT. This clearly shows that IFAT is highly sensitive and could be very reliable in the determination of seroprevalence of bovine besnoitiosis. A survey in Israel had shown that 50% of beef cattle were IFAT positive for *B. besnoiti,* while 36% of young bulls which were initially negative at the time of importation into the country developed recognizable titers after grazing for less than a year with indigenous herds (Goldman & Pipano, 1983).

The prevalence of antibodies to *B. besnoiti* is unknown in the Northern region of Nigeria and the epidemiology of the disease is poorly understood in the country. Since clinical observations (Kumi-Diaka *et al*., 1981; Sekoni *et al*., 1992), diagnostic technique (Sannusi, 1991) and histopathology of bovine skin infected with the parasite (Sambo *et al.,* 2007; Igbokwe *et al*., 2009) have been reported in the region, a serological survey is a relevant step in understanding besnoitiosis in the population. In this study, sera and milk samples were screened to establish the prevalence of antibodies to *B. besnoiti* among cattle in the Northern region of Nigeria which is endowed with the highest concentration of livestock in the country.

Materials and methods

The study area and period

The Northern region of Nigeria is located in the guinea savannah zone. Five States from this region, namely: Borno, Kaduna, Kano, Katsina and Sokoto (Lat. 9^0 03" - 13^0 58" N; Long. 4^0 08" - 15^0 00" E) were chosen at random for the study. Two Local Government Areas (LGAs) were chosen from each of the 5 States based on availability of resident herds. The herds sampled were chosen at the convenience of owners' willingness to allow sampling of the cattle during the study, which covered a 19-month period between May, 2008 and November, 2009 including both the dry and wet seasons. Samples were not collected during the wet season in Borno State and during dry season in Katsina State.

Sample collection

Samples of blood, 7ml each without anticoagulant were obtained through the jugular veins of 336 cattle, using sterile hypodermic 19 gauge needles and allowed to coagulate in centrifuge tubes to yield serum. The cattle here include 64 suckling calves (3 day-old to 7 months) and 22 weaner calves (8 months to 1½ years old). A total of 64 milk samples (10 ml) were obtained, each in a centrifuge tube, from lactating cows with suckling calves and transported on ice packs to Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria-Nigeria. These milk samples include 4 colostra. Blood samples for sera were not obtained from these lactating cows. The milk samples were centrifuged as described by Tellez *et al*. (2003). Aliquots of the milk and sera were stored at -20^0C until use.

Determination of cut-off titer for the IFAT

Positive and negative control sera prepared at four-fold dilutions of 1:1, 1:64, 1:256 and 1:1024 were used for staining *B. besnoiti* antigen on one slide to determine the cut-off titer. During this preliminary staining it was observed that 1:256 dilution produced the brightest fluorescence and was, therefore, chosen for assaying all the test samples.

Screening of sera and milk samples

The sera and aliquots of milk were screened with the same immunofluorescent antibody technique (IFAT) for antibodies to *B. besnoiti* using the method of Goldman and Pipano (1983). A total of 45 slides, each with 16 wells (on 2 rows) of the *B. besnoiti* antigen were used for the IFAT screening throughout the study. The positive and negative control sera (20µl each) were applied onto the first wells on each row and test samples (20µl each) onto the other wells. The wet chamber was improvised with a sterile Petri-dish lined with cotton wool soaked in phosphate buffered saline (PBS) and overlaid with filter paper. A 20µl of 1:600 dilution of the conjugate

(IgG-Fluorescein isothiocyanate from Sigma®) was used throughout the analysis. The IFAT reactions were examined with Leilt® Dialux 22EB model of fluorescence microscope, in the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria.

Statistical analyses

The data generated from this study were presented as proportions and summarized into tables. Prevalence of antibodies to *B. besnoiti* was calculated according to category and sources of samples, sexes and seasons. The differences in prevalence were tested for level of association using Chi-square test on Graphpad Prism version 4.0 for windows and values of $p < 0.05$ were considered significant.

Results

Sample prevalence of B. besnoiti antibodies

Out of a total of 400 cattle sampled 321 (80.3%) were positive for antibodies to *B. besnoiti* (Table 1). There were 62 (96.8%) milk samples found positive for the antibodies, which was not significantly higher than 144 (75.8%) positive sera from cows ($p > 0.05$). A total of 49 (76.6%) sera from suckling calves, 17 (77.3%) weaner calves and 49 (81.7%) bulls were positive for the antibodies. The differences in prevalence of the antibodies among samples from these categories were not statistically significant ($p > 0.5$).

Source prevalence of antibodies to B. besnoiti

It was observed that all the samples (100%) from cattle sampled in Wamako LGA of Sokoto State were positive for the antibodies (Table 2). The lowest (53.0%) prevalence of the antibodies was recorded among cattle from Dan Musa area of Katsina State, and consequently, the least (62.4%) prevalence was observed among samples obtained from Katsina State. Samples from Borno State had the highest (87.5%) seroprevalence of *B. besnoiti* during the study. These differences were, however, not statistically significant ($p > 0.5$).

Seasonal prevalence of antibodies to B. besnoiti

The overall prevalence of *B. besnoiti* antibodies was not significantly higher in the dry (89.7%) than in the wet season (70.4%). The seroprevalence of *B. besnoiti* appeared to be higher in the dry than wet season in 3 States (Table 3). The prevalence was highest (81.8%) during the wet season in Kano State and lowest (62.3%) in Katsina State. On the other hand, the highest (98.0%) prevalence during the dry season was recorded in Sokoto State and the least (83.7%) was recorded in cattle from Kaduna State. These differences in the seasonal prevalence of the antibodies within the States were not statistically significant ($p > 0.05$).

Sex specific prevalence of antibodies to B. besnoiti

The overall prevalence of the antibodies among females (79.0%) was lower than in males (84.0%), but the difference was not statistically significant ($p > 0.05$). The prevalence of antibodies to *B. besnoiti* was higher among bulls (62.5% to 92.3%) than among cows (60.0% to 85.5%) in 4 out of 5 States. In Kaduna State, however, the reverse was the case where 61 (79.2%) cows and 11 (73.3%) bulls were seropositive for antibodies against *B. besnoiti* (Table 4).

Table 1: Sample prevalence of *Besnoitia besnoiti antibodies* in Northern Nigeria

Samples	Category of cattle	Number Sampled	Number Positive (%)
Sera	Cows	190	144 (75.8)
	Bulls	60	49 (81.7)
	Suckling calves	64	49 (76.6)
	Weaner calves	22	17 (77.3)
Milk	Cows	64	62 (96.8)
Total		400	321 (80.3)
$\chi^2 = 0.7$, $P > 0.05$			

Table 2: Source prevalence of antibodies to *B. besnoiti* among cattle in five Northern States of Nigeria

LGA	State	No. Sampled	No. Positive (%)
Sabon Gari	Kaduna	43	31(72.1)
Lere	Kaduna	49	41(83.7)
Kaita	Katsina	35	25(71.4)
Danmusa	Katsina	34	18(53.0)
Jere	Borno	62	54(87.1)
Maiduguri	Borno	18	16(88.9)
Kano	Kano	55	45(81.8)
Bunkure	Kano	23	21(91.3)
Sokoto	Sokoto	61	50(82.0)
Wamako	Sokoto	20	20(100.00)
Over all		400	321(80.3)

Table 3: Seasonal prevalence of *B. besnoiti* antibodies among cattle in five northern States of Nigeria

State	Number Sampled		IFAT Positive (%)	
	Dry	Wet	Dry	Wet
Borno	80	0	70 (87.5)	NS
Kaduna	49	43	41(83.7)	31 (72.1)
Kano	23	55	21 (91.3)	45 (81.8)
Katsina	0	69	NS	43 (62.3)
Sokoto	52	29	51 (98.0)	19 (65.5)
Total	204	196	183 (89.7)	138 (70.4)

Note: NS = Not sampled $\chi^2 = 0.5$, $P > 0.05$

Table 4: Sex specific prevalence of *B. besnoiti* antibodies among cattle in five northern States of Nigeria

State	Number Sampled		Number Positive (%)	
	Males	Females	Males	Females
Borno	25	55	23 (92.0)	47(85.5)
Kaduna	15	77	11(73.3)	61(79.2)
Kano	26	51	24(92.3)	42(76.4)
Katsina	16	53	10(62.5)	33(60.0)
Sokoto	24	58	21(87.5)	49(84.5)
Over all	106	294	89 (84.0)	232 (79.0)

$\chi^2 = 0.9$, $P > 0.05$

Discussion

This study has shown a high prevalence of antibodies to *B. besnoiti* and may indicate that the parasite is endemic in all the five States. It is known that in an endemic situation the disease agent, the vector and host are all present and interact in such a way that immunity is acquired sufficiently early in the life of majority of host animals (Cantu-Martinez *et al*., 2008) and clinical disease may rarely occur. The high prevalence of the antibodies observed here, suggests that the cattle population have been previously exposed to the parasite and those at the risk of having clinical besnoitiosis in the Northern region of Nigeria might be few, in view of the fact that a solid immunity against bovine besnoitiosis might occur (Pols, 1960) following an active primary or anamnestic response.

The observed prevalence of antibodies to *B. besnoiti* among cattle in the study area was higher than the

values reported in Israel three decades ago (Goldman & Pipano, 1983). This could probably be reduction of exposure to biting insects appeared to reduce the rate of transmission of *B. tarandi* among reindeers. Therefore, if the husbandry practices did not include adequate control of insect bites, it is possible that this might have allowed the transmission of *B. besnoiti* and hence the high prevalence in the study area, where most of the cattle population are essentially nomadic. It has also been stated that the emergence of clinical signs of bovine besnoitiosis coincided with summer when the blood-sucking arthropods became active and new cases of the disease occurred during the warmer-moist months (Glover *et al*., 1990; Alzieu, 2007; Fernandez *et al*., 2009), but this study was not designed to investigate vector activity.

The present study seemed to have a higher prevalence of antibodies to *B. besnoiti* in cattle during the dry season, although the difference was not statistically significant. The possibility of a significant seasonal variation might have existed in the surveyed States especially during the wet season, which Glover *et al*. (1990) believed could favour insect transmission of the parasite. However, it was not possible to differentiate the old from new cases of besnoitiosis in this study and hence the insignificant seasonal variation of the antibodies among the screened cattle.

The study also observed that prevalence of the antibodies did not vary significantly between the bulls and cows. This differed from the observation of Kumi-Diaka *et al*. (1981) that more bulls than cows were involved in the outbreak of besnoitiosis in Kano State. According to Igbokwe *et al*. (2009), the difference between prevalence of cutaneous cysts of *B. besnoiti* was significantly higher in bulls than in the cows. The sex specific prevalence of the antibodies appears to be inconsistent with these previous studies which emanated from the same region, possibly because the methods of diagnosis differed. The present study employed IFAT which is highly sensitive and could recognize asymptomatic cases of besnoitiosis (Goldman & Pipano, 1983; Shkap *et al*., 1995), which gross examination or histopathology in previous investigations (Kumi-Diaka *et al*., 1981; Sambo *et al*., 2007; Igbokwe *et al*., 2009) could not identify.

The antibodies to *B. besnoiti* were demonstrated both in milk and sera samples from cows and in the sera from their new born calves. The antibodies might have been passed to these calves through due to more aggressive vector activity in Northern Nigeria. Glover *et al*. (1990) had observed that suckling of colostrum. It has been established that biologically stable IgA and IgM could be transferred to infants through natural milk for protection against several disease agents including protozoa (Lawrence & Pane, 2007) and could confer solid immunity to new born humans (Tellez *et al*., 2003) and animals (O'Handley *et al*., 2003).

The presence of antibodies to *B. besnoiti* in sera from calves at the dilution of 1:256, observed here, is in tandem with an established record (Shkap *et al*., 1994) in which the new born calves in Israel had antibody titers ranging from 1:64 to 1:1024. The Israeli calves were born seronegative but became positive the next day when they had suckled colostra that were positive for antibodies to *B. besnoiti*. In the present study the time of appearance of the antibodies in sera was not investigated among day-old calves, but the four seropositive cases of 3 – 5 days old is suggestive of colostral transfer of the antibodies and gives credence to the previous reports. Most of the seropositive calves examined in this study were 2 to 7 months old and it's not clear whether the antibodies were maternal or due to field exposure. On the other hand, the weaner calves of over 7 months old might have been exposed to the parasite in the field, and might have developed the anti-*Besnoitia* antibodies, since the maternal immunity should have waned at that age to very low levels. It's a known fact that neonatal B-cells could produce IgM and limited amounts of IgG subclasses, the levels of which could rise slowly to reach adult levels for IgG_1 and IgG_3 at the age of 12 months in humans (Tellez *et al*., 2003).

In conclusion, there was a high prevalence of antibodies to *B. besnoiti* in sera and milk which suggests that the parasite is endemic among cattle in the surveyed Northern States of Nigeria. Investigations on vectors and transmission of the parasite in the country may be very useful for further understanding of the epidemiology of bovine besnoitiosis in the country and are hereby recommended.

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