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Seroprevalence of Besnoitiosis and Associated Risk factors in Apparently Healthy Cattle Presented for Slaughter at the Maiduguri Central Abattoir

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Abstract

Bovine besnoitiosis is viewed as a growing chronic and fatal infection, with isolated reports of subclinical and clinical cases in Europe. *Besnoitia besnoiti* has been found in cattle in Nigeria's southern and northern regions, according to reports. Apparently healthy cattle that are slaughtered at Maiduguri Central Abattoir in Borno State, Nigeria, were the subject of this study, which looked into the seroprevalence of besnoitiosis in that region. Besnoitia indirect 2.0 ELISA technique with positive and negative controls utilizing a *Besnoitia besnoiti* purified antigen extract was employed in the current study. Out of 176 tested samples a seroprevalence of 25% (44/176) was reported positive for *Besnoitia besnoiti*. Out of which, 26.04% (25/96) of cows were positive and 23.75% (19/80) of bulls were positive, with no significance ($p > 0.05$) difference between cows and bulls. Similarly, out of the 176 samples tested, 151 were adults while 25 were calves. 25.83% (39/151) of the adults were positive while 20.0% (5/25) of the young cattle were positive, indicating no significant ($P > 0.05$) difference between *Besnoitia besnoiti* infestation and age. Out of the sample tested, Abore breed had a seroprevalence of 26.97% (24/89) while the Kuri breed recorded a seroprevalence of 27.27% (3/11). However, Bokoloji appears to be a resistant breed with zero seroprevalence, indicating no significant association between *Besnoitia besnoiti* infestation and breeds of cattle.

Keywords: Besnoitiosis, ELISA, Seroprevalence, Cattle, Abattoir.

Introduction

A parasite called *Besnoitia besnoiti*, which belongs to the family Sarcocystidae, subfamily Toxoplasmatidae, and phylum Apicomplexa, causes the illness known as bovine besnoitiosis, also known as bovine elephantiasis or bovine anasarque, in cattle [1]. Widespread in various African nations, *Besnoitia besnoiti* is an obligate intracellular protozoan parasite of cattle. Asia, Germany, Italy, Switzerland, Hungary, Portugal, within Europe and in the south western regions of Europe [2-14]. The chronic form of the condition, known as elephant skin disease, manifests both topically and internally [14,15]. By the parasite's sporozoites, which are present on the mouth portions of the insects, the illness is mechanically propagated by biting flies, furthermore, iatrogenic

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transmission is possible via hypodermic needles and is most likely to occur during close animal contact [16,17]. It is believed that mechanical transmission between cattle or transmission by hematophagous insects serve as the major routes of transmission [18]. All breeds, ages, and sexes of cattle are affected by the parasite, however clinical signs in calves less than 6 months of age are rare [19]. In Africa and the Mediterranean areas, the high morbidity rate of besnoitiosis results in significant losses for the cattle industry. During the chronic phase, a lot of cysts develop, mostly in the subcutaneous tissues [20]. The most frequent clinical symptom is pyrexia, which can last for a week or more. Additional clinical symptoms include persistent inappetence, significant weight loss, total anorexia, and serous nasal and ocular discharge. Photophobia, tachycardia, tachypnea, reluctance to move, potential recumbency, subcutaneous edema, and hyperemia of the skin, especially on the muzzle, periorbital skin, and orchitis (European Food Safety Authority, EFSA, 2010) [21]. Animals who have contracted the chronic sickness are always carriers. Individuals could recover from acute symptoms and exhibit no or few clinical symptoms, but they could still spread illness to other people. There will be some animals with serious chronic illness. Bovids are the intermediate host in the life cycle of *Besnoitia besnoiti*, and carnivores are the ultimate hosts, as is the case with other cyst-forming coccidia [22]. Economically speaking, this disease might result in lower hide prices, worse-quality carcasses, and either temporary or permanent sterility in breeding bulls who have survived the acute and chronic stages of the illness [23].

Materials and Methods

Study area

The investigation was carried out in Maiduguri, the capital of Borno State, in Nigeria's northeast, specifically in the Central Abattoir. The city of Maiduguri is located 300 meters above sea level and is located between latitudes 11.46°N and 11.54°N and longitudes 13.04°E and 13.14°E. With an estimated 1,112,449 residents and a total area of 72,609 square kilometers, it is the largest city in the state. Northeastern, northern, and eastern borders of the state are shared with the Republics of Chad, Niger, and Cameroon. Along with Adamawa State to the south, Yobe State to the west, and Gombe State to the southwest, it also borders these states.

The primary livestock market, in Gamboru Ward, next to the abattoir, acts as a center for producing, dealing, and transiting livestock, particularly cattle. The majority of breeds sold at the market are kuris, which are native to Chad and Cameroon, as well as the breeds Rahaji and Bunaji from Niger, Mbala, and Abore are from Borno State. Cattle are large domesticated cloven-hooved herbivore farm animals that are raised for their meat, milk, hides, or draft purposes.

According to Babayemi et al., the most prevalent breeds of cattle in Nigeria are the White Fulani, Red Bororo, Sokoto Gudali, Adamawa Gudali, Wadara, Azawak, Muturu, Keteku, N'dama, and Kuri [24]. The N'Dama, West African short-horned cow, and Kuri are the three most prevalent foreign cattle breeds seen in Maiduguri's cattle market. The majority of the livestock are owned by the nomadic Fulani, Shuwa Arab, and certain Kanuri speaking inhabitants of the state. The majority of the year in Maiduguri is hot, dry, windy, and dusty, and the people's main jobs include farming, raising cattle, and fishing (NIMET, 2018) [25]. The primary objective of the abattoir is to provide a location for the slaughter of cattle so that inhabitants of Maiduguri and the surrounding area can obtain meat.

Sample size determination

The number of samples was calculated by using Thrusfield (2005) formula:

$$n=\frac{1.962\times Pexp\ (1-Pexp)}{0.052}$$

Description

n = required sample size

d^2= allowable error = 5% $(0.05)^2$

P_{exp} = prevalence of 6.7% [16]

The sample size=96.0

To increase the precision, the number of samples was increased to 176.

Sample collection and transportation

Following appropriate restraint, ten milliliters of blood were aseptically drawn from each animal's jugular vein using a plain vacutainer tube and needle. The sex, age, and breed of each sample were listed together with a special identification number on the label. The ice-packed cooler was used to deliver the samples to the lab. The clot-filled blood samples were centrifuged for 5 minutes at 3000 rpm to separate the sera, which were then extracted and stored at -20°C until needed.

Serological analysis

Besnoitia indirect 2.0, a commercially available bovine test kit made by Innovative Diagnostics in Grabels, France, was utilized. It uses an indirect ELISA approach and *Besnoitia besnoiti* purified antigen extract as the positive and negative controls. Results are considered negative if the S/P% value is less than or equal to 25, positive if the S/P% value is more than or equal to 30, and uncertain if the S/P% value is between 25 and 30.

Data analysis

Seroprevalence of *Besnoitia besnoiti* was calculated using the following formula:

$$\frac{\text{Total number of positive animals}}{\text{Total number of animals Examined}}\times 100$$

Statistical Package for Social Sciences (SPSS) version 23.0 was used to examine the data that were produced. To find the association, descriptive statistics, odds ratios, 95% confidence intervals, and chi-square tests were performed. Throughout the investigation, significance was set at P< 0.05. According to sex, age, and breed, the results were grouped and summarized in tables. The Chi-Square test on SPSS 23.0 for Windows was used to determine the degree of

association between the variations in prevalence, and values of P< 0.05 were regarded as significant.

Result

Seroprevalence of *Besnoitia besnoiti* in cattle

Out of 176 samples that were tested for *Besnoitia besnoiti* in cattle, the results showed that 44 (or 25%) of them were positive. A total of 176 samples were collected from cattle, of which 96 samples came from cows and 80 samples came from bulls. Bulls only had a 23.75% overall seroprevalence compared to cows' 26.04% (25/96). Bulls were 1.1 times more likely to have the infestation (OR=1.1, 95% CI: 0.56-2.135) compared to cows (Table 1). There was no significant (p = 0.866) association of *Besnoitia besnoiti* infestation between bulls and cows.

Seroprevalence of *Besnoitia besnoiti* based on age in cattle

Of the overall sample evaluated, 151 were adults and 25 were members of the younger demographic. The seroprevalence of *Besnoitia besnoiti* was 39 (25.83%) in adults while the seroprevalence in the young population was 5 (20. 0%). The young cattle were 1.3 times more likely to have the infestation (OR=1.3, 95% CI: 0.46-3.6) compared to adult cattle. There was no significant (p = 0.620) association of *Besnoitia besnoiti* infestation between adult and young cattle (Table 2).

Seroprevalence of *Besnoitia besnoiti* in different breed of cattle

A total of 89 Abore, 13 Bokoloji, 11 Kuri, and 63 Wadara were found in the sample analyzed. Breeds of Abore and Wadara both have a seroprevalence of 26.98%. Kuri was 27.27% seroprevalent. Bokoloji, however, seems to be a resistant breed with zero seroprevalence. Wadara breed was 1.01 times more likely to have the infestation (OR=1.01, 95% CI: 0.25-4.54) compared to Kuri and Abore. There was no significant (p = 0.307) association between Besnoitia besnoiti infestation and breeds of cattle (Table 3).

Discussion

The 25.0% seroprevalence reported in this study is relatively higher than the 6.7% and 2.0% reported in cattle by Igbokwe et al., and Zango et al., respectively [16,26]. The observed disparity may be explained by the differences in the technique used and the sample used in the study.

While earlier studies used gross examination or histopathology in their investigations, which could not detect asymptomatic cases of besnoitiosis, the current study used the highly sensitive Besnoitia indirect 2.0, ELISA method [16,27-29].

However, there was no significant association between the infestation and sex. This agrees with the study conducted by Sambo et al. but contrary to that of Igbokwe et al. who reported a significant association between infestation and sex [16]. This might also be a result of the relatively small sample size in this study which might be unable to determine the relationship. The study also revealed that sex, age, and breeds were not significant risk factors for Besnoitiosis. This study showed that cows had a slightly higher (26.04%) seroprevalence compared to bulls (23.75%). This may be attributed to the fact that cows stayed longer in the herd exposing them to various parasitic challenges. This is contrary to the findings of Igbokwe et al., who recorded 13.6% and 2.7% seroprevalence in bulls and cows respectively [16]. This disparity may be a due sampling of symptomatic bovine with *B. besnoiti* cutaneous cysts, unlike this study which used antibodies against *Besnoitia besnoiti*.

Sex	Sample Tested	Sample positive	Seroprevalence (%)	OR	95% CI	χ^2	P-value
Cow	96	25	26.04	Ref		0.074	0.866
Bull	80	19	23.75	1.0965	0.56-2.135		
Total	**176**	**44**	**25.00**				

Table 1: Seroprevalence of *Besnoitia besnoiti* in bulls and cows.

Age	Sample Tested	Sample positive	Seroprevalence (%)	OR	95% CI	χ^2	P-value
Adult	151	39	25.83	Ref		0.241	0.620
Young	25	5	20.00	1.3	0.46-3.6		
Total	**176**	**44**	**25.00**				

Table 2: Seroprevalence of *Besnoitia besnoiti* in adult and young Cattle.

Breed	Sample Tested	Sample positive	Seroprevalence (%)	OR	95% CI	χ^2	P-value
Kuri	11	3	27.27	Ref		3.454	0.307
Wadara	63	17	26.98	1.01	0.25-4.03		
Abore	89	24	26.97	1.0114	0.2612-3.92		1
Bokoloji	13	0	0.00				
Total	**176**	**44**	**25**				

Table 3: Seroprevalence of *Besnoitia besnoiti* in Different Breeds of Cattle.

Conclusion

This study revealed an overall seroprevalence of 25.0% (44/176) of *Besnoitia besnoiti* in slaughtered cattle in Maiduguri central abattoir. This study showed that cows and adults had a higher seroprevalence of 26.04% and 25.83% compared to Bulls and Young with 23.75% and 20.0% respectively. This study also showed that Bokoloji recorded zero seroprevalence of *Besnoitia besnoiti* among slaughtered cattle in Maiduguri central abattoir. This study revealed no significant association between age, sex, breeds, and *Besnoitia besnoiti* infestation in slaughtered cattle in Maiduguri central abattoir. In comparative terms, Besnoitia indirect 2.0, ELISA used in this present study is more sensitive in detecting asymptomatic infestation than using histopathological cutaneous lesions of besnoitiosis in previous studies.

Recommendations

1. Adoption of indirect ELISA for the active surveillance of antibodies against *Besnoitia besnoiti* because of its fastness and sensitivity in the detection of antibodies.

2. Pastoralists should be enlightened about the significance of vector control and biosecurity measures toward the control and prevention of besnoitiosis in the study area.

3. Molecular studies should be conducted to explore the reason for the inability of a single Bokoloji breed against *Besnoitia besnoiti* to develop antibodies against *Besnoitia besnoiti* in the study area

Authorship

Falmata Kyari, Ali Mohammed, Chahari Alfred Midala, and Salamatu Mohammed Tukur conceptualized and planned the idea; Otsahyel Stephen collected the data. The study was written by Falmata Kyari, and Lawan Adamu examined it, statistically analyzing and interpreting the data.

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Ethical statement

The University of Maiduguri, Faculty of Veterinary Medicine, gave its approval for the collection of blood samples and the handling of animals.

Declaration of Competing Interest

No conflicting interest to declare.

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