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Preliminary field survey on *Mycobacterium bovis* infection in cattle herds using caudal fold intradermal tuberculin test in two Northeastern States of Nigeria

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Abstract

Aim: A survey was conducted to determine the prevalence of bovine tuberculosis (bTB) in two states of Northeastern Nigeria, namely Bauchi and Gombe States, between February 2010 and November 2014 using caudal fold tuberculin (CFT) skin test.

Materials and Methods: A total of 5489 cattle were screened using single CFT in Bauchi and Gombe States. Of the 5489 cattle, 2116 cattle were from 189 herds in five testing areas in Bauchi State and 3373 cattle in 217 herds from five testing areas from Gombe State.

Results: On the basis of the CFT, herd prevalence obtained was 56.08% in Bauchi and 55.29% in Gombe State, while the individual animal prevalence was 10.96% in Bauchi and 13.73% obtained in Gombe State. The prevalence based on the testing areas in Bauchi State, Alamari had the highest prevalence with 19.4 % and Disina the lowest with 9.0 %, while in Gombe State, Wakaltu had the highest prevalence with 20.9 % and Poshereng the lowest with 8.0 %. Cows were more likely to have tuberculosis lesions than bulls ($p=0.0035$) in Bauchi State, but there was no significant difference in Gombe State ($p=0.166$). However, a statistically significant association ($p<0.05$) was observed among the cattle age groups with cattle ≥4 years having higher odds for tuberculin reactivity compared to those below the age of 4 years in Bauchi State only.

Conclusion: There is the need to strengthen routine meat inspection and public health awareness programs on the zoonotic nature of bTB among the abattoir workers and the herdsmen.

Keywords: bovine tuberculosis, cattle herds, caudal folds, Northeastern Nigeria, tuberculin test.

Introduction

Bovine tuberculosis (bTB) is a disease caused by a specific type of bacterium called *Mycobacterium bovis*. bTB usually affects animals such as cattle, but it can also affect all mammals causing sickness, coughing, and eventual death. It can be transmitted from animals to humans as well as to other animals [1]. The disease has been reported in 176 countries of the world as one of the important bovine diseases leading to great economic losses [2]. In cattle, exposure to the organism can result in a chronic disease that jeopardizes animal welfare and productivity and in some countries leads to significant economic losses by causing ill health and mortality [3].

Moreover, human TB of animal origin caused by *M. bovis* is becoming increasingly evident in developing countries [4,5]. In Sub-Saharan Africa, humans and animals share the same microenvironment, watering points, feeding facilities, and grazing land. In such countries where bTB is still common, and pasteurization of milk is not practiced, an estimated 10-15% of pulmonary tuberculosis is caused by *M. bovis* [6,7]. Cattle with tuberculosis lose 10-25% of their productive efficiency; direct losses due to the infection become evident by the decrease in 10-18% milk and 15% reduction in meat production [8]. Apart from effects on animal production, it has also significant public health importance [9].

In the developing countries, unfortunately, bTB has received little attention by way of surveillance, control, and/or eradication program. It has been reported by Thoen and Bloom [10] that nearly 85% of cattle and 82% of the human population in the developing countries live in areas where the disease is prevalent and/or only partially controlled. There is a close association between the human and cattle populations. Nevertheless, the available information is limited due to inadequate disease surveillance and lack of better diagnostic facilities [11,12]. Information on genotypic characteristics of *M. bovis*, a strain affecting the cattle population in Nigeria, is limited [13]. It is important to obtain such information to monitor the transmission pattern and spread of the disease among cattle [7]. In developing countries like Nigeria, the socioeconomic

situation and low standard of living areas for both animals and humans are contributing more in TB transmission between human to human and human to cattle or vice versa [14,15].

In Nigeria, the disease had been reported in many parts of the country [16-20]. Despite the lack of effective identification, control, and prevention strategies, bTB is endemic in Nigeria. This study was designed to determine the *M. bovis* infection in cattle herds using caudal fold tuberculin (CFT) test in two Northeastern States of Nigeria.

Materials and Methods

Ethical approval

The purpose of this study was explained to the cattle owners and informed consent was obtained.

Study area

The survey was conducted in Bauchi and Gombe States of Nigeria. Bauchi State lies between latitudes 10° 10́ and 10° 33N and longitudes 9° 40́ and 10° 13́ (Figure-1a). The climate of the states is semi-arid, characterized by a long dry season. The climatic variables vary considerably during the year and are erratic. The temperature regime is warm to hot. The mean annual temperature is about 25°C in the coolest month and 39°C during the hottest month. Evapotranspiration is very high and relative humidity is highest in August (up to 80%) and low in January-March (23-30%) when it is moderated by the harmattan. The year is characterized by marked dry and wet seasons. The wet season is roughly 4 months (June-September), while the dry season is 7-8 months (October-May). The total annual rainfall ranges from "600 mm" in the north to "1000 mm" in the southern part of the state. Most of the state falls within the Sudan Savanna vegetation belt, but traces of Guinea Savanna vegetation are found in the parts of the southern districts (Figure-1b). Bauchi State occupies a total land area of 49,119 Km² representing about 5.3% of Nigeria's total land mass [21]. Gombe State is located between latitude 9° 30′ and 12° 30′N and longitudes 8° 45′ and 11° 45′E of the Greenwich Meridian [22]. The state is in the Northeastern part of Nigeria, with its capital at Gombe town. It is on an altitude of 540 m above the sea level and covers an estimated land mass of 20,265 km² and has a population of 1,820,415 inhabitants [22]. The state has two distinct climates, the dry season (December-March) and the rainy season (April-November) with an average rainfall of "850 mm," with the mean maximum monthly temperature of 37°C and relative humidity of 94% in August and 10% in December.

Study design

This study was a cross-sectional screening test using a caudal fold test in some selected cattle herds in both Bauchi and Gombe States. The field study was composed of primary screening test using SIT in some selected cattle herds in both Bauchi and Gombe States.

Herds selection

A herd in this study is defined as a group of cattle kept together by a pastoralist or on a farm with at least 20 animals, and only herds with a population of at least 20 cattle were selected. All the cattle were tested in each selected herd. Indigenous cattle from the pastoralist herds and a few herds kept by individuals and government-owned institutions were sampled. The multistage sampling procedure as described by Martins *et al*. [23] was used. In the first stage, each of the states, three senatorial districts were used, and

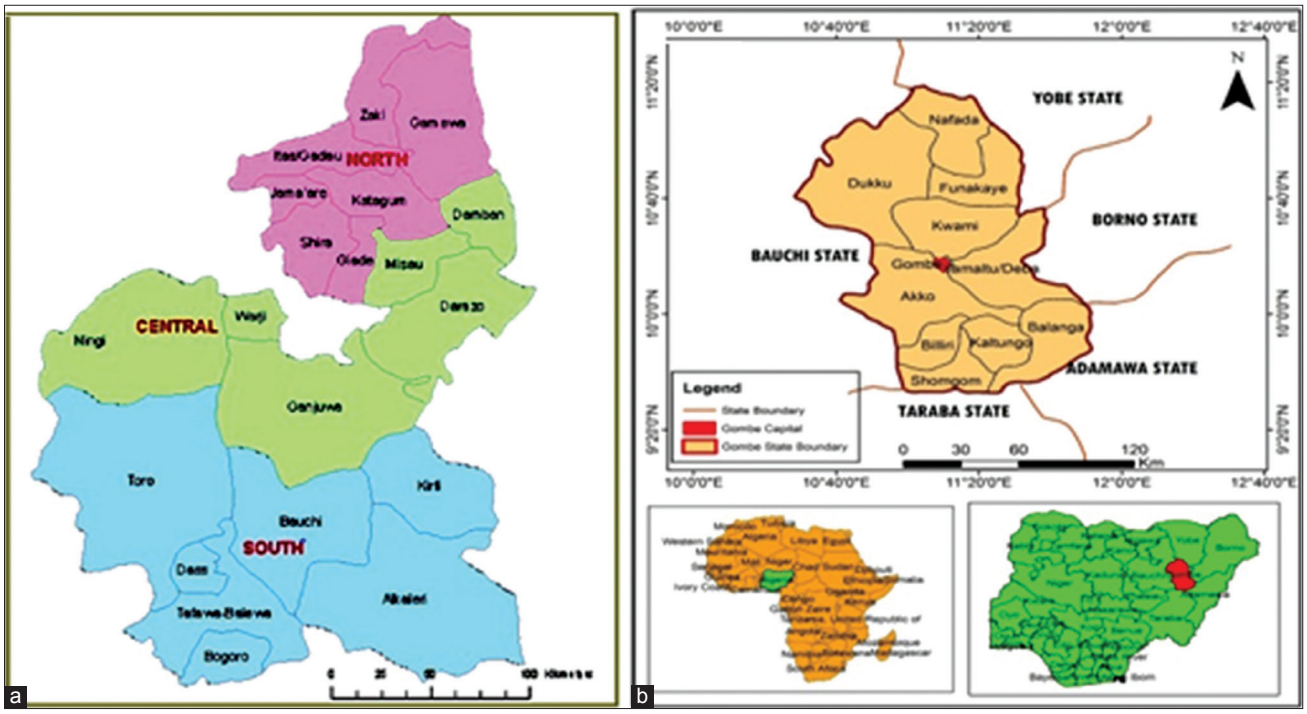

Figure-1: (a) A map showing the study area in Bauchi State, northeastern, Nigeria and (b) a map showing the study area in Gombe State, northeastern, Nigeria.

then three areas with the highest concentration of livestock in each district were selected using simple random sampling as the second stage. In the third stage, 5 herds were selected based on their system of management (Nomadic, Settled, Organized farms and Government farms if any).

Cattle selection

All the 5489 heads of cattle tested were serially numbered using oil-based synthetic enamel paint applied at the back of the animal. The numbering was large and heavy enough so that they were easily read at least 7 days after application. The animals were clinically examined, and information pertaining to the individual animal was collected. These data included an identification number, location (village), and breed, sex, dry, pregnant or lactating (for females), and body condition score (BCS) (poor, fair, and good).

Tuberculin testing of cattle

Each animal was subjected to a single caudal fold test (SCF). The animal was restrained in the field as described by "Alhaji [16]." A site about one-third of the distance from the base of the tail on the right and ventral side of the tail was chosen and cleaned with dry cotton wool. One-tenth 0.1 mL of purified protein derivative of "*M. bovis*" (5000 "i.u/ml") was then carefully deposited intradermally using semi-automatic tuberculin syringe [24]. 72 h post-injection, the site was examined by visualization and palpation to identify reaction based the followings: Any increase in caudal fold thickness, size, or sensitivity either observed or palpated at the injection site was considered response or positive test. The size of responses may vary. Responses may be small, hard, pear-shaped, diffuse, circumscribed, or large responses, and all responses recorded.

Statistical analysis

Data were analyzed using Statistical Package for the Social Sciences (IBM, USA) Version 20.0. Odds ratio (OR) and 95% CI were used to measure the likelihood and strengths of association between variables and bTB (*M. bovis*). Tables were constructed using the Microsoft Excel 2010. Values of $p<0.005$ were considered statistically significant throughout the study.

Results

A total of 5509 cattle were screened using CFT test in Bauchi and Gombe States. Of the 5509 cattle, 2116 cattle were from 189 herds in five testing areas in Bauchi State and 3393 cattle in 217 herds from five testing areas from Gombe State. Regarding the prevalence based on the testing areas in Bauchi State, Alamari had the highest prevalence with 19.4% and Disina was the lowest with 9.0%, while in Gombe State testing areas, Wakaltu had the highest prevalence with 20.9% and Poshereng had the lowest with 8.0% (Table-1). The herd prevalence of bTB in Bauchi State based on this tuberculin test was 56.08% (106/189) while the herd prevalence in Gombe State was 55.29%. With regard to individual animal prevalence, Bauchi State recorded an overall animal prevalence of 10.96% while Gombe State had 13.73% animal prevalence (Table-2). From the study, cows had significantly ($p<0.005$) higher risk of coming down with TB than bulls in Bauchi State while no significant difference was shown in Gombe State (Table-3). Furthermore, it was observed that chances of cattle being infected with bTB increased with age, positivity of cattle 7 years or older have the highest (18.9%) while the least positivity of 10.6% in Bauchi. It was also observed that there was significant ($p<0.005$) relationship among the age groups in Bauchi State and the management systems in Gombe State, respectively. The study has also demonstrated that *White fulani* breeds of cattle had higher chances (22.13%) of coming down with the disease than *Bokoloji* (15.22%) or Crossbreeds (14.67%), but it was non significant ($p>0.005$). With regard to BCS, animals in Bauchi State with poor BCS had the highest lesions (18.0%) followed by those with medium body score (10.4%) and good BCS with 12.2%. In Gombe State, animals with poor BCS had the highest chances of having lesions (16.7%) while medium and good BCS had 11.5% and 10.5%, respectively.

As for herd size, it was observed that, in Bauchi State, herds with more than 11 herds had 60.3% positivity while those with herd size <10 herds had 47.6% positivity. In Gombe State, the trend was observed with herd >11 herds having 57.1% positivity and those with <10 herds having 53.1%. As for management, chances for observing lesions of bTB were highest in

Table-1: Distribution of suspected bovine tuberculosis in five testing areas in Bauchi and Gombe States.

State Bauchi	CFT Resp	Testing areas					Overall
		Tashar leda	Itas	Udugbo	Disina	Alamari	
	Positive	45 (14.1)	60 (11.2)	71 (9.8)	42 (9.0)	14 (19.4)	232 (11.0)
	Negative	275 (85.9)	476 (88.8)	650 (90.2)	425 (91.0)	58 (80.6)	1884 (89.0)
Total		320 (15.1)	536 (25.3)	721 (34.1)	467 (22.1)	72 (3.4)	2116 (100.0)
Gombe		**Poshereng**	**Walama**	**Bodor**	**Wakaltu**	**Hinna**	**Overall**
	Positive	33 (8.0)	107 (19.6)	115 (20.1)	72 (20.9)	136 (9.1)	463 (13.7)
	Negative	379 (92.0)	438 (80.4)	458 (79.9)	273 (79.1)	1362 (90.9)	2910 (86.3)
Total		412 (12.2)	545 (16.2)	573 (17.0)	345 (10.2)	1498 (44.4)	3373 (100.0)

Figures in parenthesis represent percentage response

Table-2: Individual animal and herd level prevalence of bovine tuberculosis in Bauchi and Gombe States.

State	Total screened	Number positive	Prevalence 95% CI	Negative (%)
Bauchi				
Individual animal prevalence	2116	232	10.96	1884 (89.04)
Herd prevalence	189	106	56.08	83 (43.91)
Gombe				
Individual animal prevalence	3373	463	13.73	2910 (86.3)
Herd prevalence	217	120	55.29	97 (44.7)

Table-3: Association between risk factors and tuberculin reactivity of cattle in Bauchi and Gombe States.

Risk factors	Bauchi			Gombe		
	Number tested	Number positive (%)	p value	Number tested	Number positive (%)	p-value
Sex						
Bulls	573	50 (8.7)	0.0035	1136	142 (12.5)	0.166
Cows	1543	182 (11.8)		2237	286 (12.8)	
Age (years)						
<2	350	37 (10.6)	0.035	515	73 (14.2)	0.679
2-4	670	86 (12.8)		945	98 (10.3)	
5-7	520	75 (14.4)		1234	210 (17.1)	
≥7	180	34 (18.9)		679	82 (10.6)	
Breed						
White Fulani	1116	247 (22.13)	0.069	2786	391 (14.0)	0.073
Bokologi	716	109 (15.22)				
Cross	293	43 (14.67)		587	72 (12.3)	
BCS						
Good	253	32 (12.2)	0.063	773	81 (10.5)	0.857
Medium	635	66 (10.4)		986	113 (11.5)	
Poor	1223	220 (18.0)		1614	269 (16.7)	
Herd size						
1-10	63	30 (47.6)	0.067	98	52 (53.1)	0.075
≥11	126	76 (60.3)		119	68 (57.1)	
Management						
Good	201	12 (6.0)	0.083	815	103 (12.6)	0.003
Medium	265	14 (5.28)		920	120 (13.0)	
Poor	1650	206 (12.48)		1638	240 (14.7)	

animals under poor management (14.7%) followed by those with medium and least in animals kept under good management in Gombe States only (Table-3).

Risk factors such as herd size, BCS, and breed were also not significantly ($p>0.05$) associated with tuberculin positivity in cattle.

Discussion

The present study has shown the individual animal prevalence of bTB to be 10.96% and 13.73% in Bauchi and Gombe States, respectively, while the herd prevalences were 56.08% and 55.29%, respectively. The individual animal prevalence reported in this study is in agreement with previous reports from Nigeria by Abubakar [25], Shehu [26], and Jiwa *et al*. [27]. It was, however, observed that the prevalence for the two states was 12.3%, which is from (10.96% [Bauchi State] +13.73% [Gombe State])/2, indicating that there had been steady increase in TB infection rate in Nigeria over the past 40 years as indicated by previous studies, namely Alhaji [16] reported a 4.4% prevalence rate in a study of 529 cattle in four states of the Northern region of Nigeria. Eid [17] reported 1.5% in North Western states, Ayanwale [18] reported 7.8% infection rate in a study in some Southern states of Nigeria, while Shehu [26] reported 11.8% in Kaduna State. Previous studies in both Nigeria and other African countries have shown a similar trend over the years [24, 27, 28]. This may be due to inadequate to none implementation of control policy in the region due to socioeconomic reasons. The herd prevalence obtained in the two states might reflect that bTB infection varies considerably between herds and is widespread in pastoral communities in the study areas. This could be due to the differences in the various communities and other diseases that may be at play with the tubercle bacilli infection.

However, it was observed that fewer animals reacted positively to the tuberculin test among the 2 years old and <2 years of age categories and also in the >4 years age categories. This finding is similar to the findings by other researchers elsewhere in Africa [12,14]. It is possible that the infection may become established in young animals, but as they grow older, there is a delay on set to tuberculin test response. On the other hand, Tizard [29] stated that the other reason for lower response to intradermal tuberculin test in older animals is due to the immune

decline occurring during the old age or poor nutritional level. Some animals could have been missed resulting in an underestimation of the prevalence. There are several other reasons why tubercle bacilli infected animals give false positive or negative results. Reports by Ameni *et al.* [14] indicated that infection of cattle with gastrointestinal parasites such as *Fasciola* and *Strongyle* compromised the immune response of cattle to the tuberculin test. These infections made the immune response to shift from Th1 to Th2, thereby promoting the emergence of more false positive results. In endemic areas, delayed hypersensitivity may develop for 3-6 weeks following infection, and in chronically infected animals with severe pathology, the tuberculin test may be unresponsive [30]. This situation is likely to be found in endemic areas such as those in the study areas and may lead to an increase in false negatives with subsequent underestimation of the prevalence. The higher prevalence seen in animals kept under poor management system compared to medium and good management seen in this study is in agreement with reports of other researchers in Africa [12-14]. Poor management system provides an opportunity for the transmission of infection and development of disease and consequently contributing to the variation in the presence of the disease among animals raised under different husbandry systems [12]. Furthermore, poor husbandry system may lead herdsmen to expose their animals to areas where they can be infected as is the case in communal grazing and during borrowing or lending of bulls for breeding as reported by Kaltungo *et al*. [30].

According to the findings of this study, the infection is more prevalent in cows than in bulls and having poor BCS. Tuberculin reactivity was not significantly affected by body condition of the animal ($p>0.05$). However, the slight differences could be due to the fact that tuberculin reactions rely solely on the immune system, which, in turn, may be linked with the management and less likely body condition of the animals such that those manage under good and better management system are immunocompetent and, therefore, give a better reaction to tuberculin as exogenous. However, animals with poor body condition could be a confounder and hence may not determine tuberculin reactivity [31]. This is in contrary to the work of Lackech *et al.* [32] in which the disease is found to be more prevalent in medium body condition cattle.

From this study, there is a significant association between sex and intradermal skin test positivity ($p<0.05$). The finding concurs with some previous studies in some African countries, which reported similar findings for bTB positivity between bulls and cows [33,34]. Contrary to the findings of this study, other studies have reported no association between sex and intradermal skin positivity [35,36]. For example, Inangolet *et al.* [36] and Cadmus *et al*. [13] reported female cattle being at higher risk of testing positive than males contrary to findings of Kazwala *et al*. [35] and Shehu [26] where male cattle were more affected than female cattle, as male cattle mainly castrated are kept longer and hence more chances of contracting the disease than female cattle.

Our findings showed that there was no significant difference in the prevalence of bTB in the two states between the herds sizes (small and large sizes). This observation was due to the fact that majority of the cattle tested were in small size herds. Therefore, to compare and appreciate the significant difference between small and large herd sizes, a comparable number of animals should be tested. This is in contrast to the findings of Cadmus *et al.* [20] and Cleaveland *et al.* [34] as there was a statistically significant difference between the herd sizes in Nigeria and Tanzania, respectively.

Breed was not statistically significant in this study ($p>0.05$). The tested cattle were mainly of local breeds which were managed under extensive management which might be the reason for the differences in the result, as local breeds are known to be relatively resistant to bTB as compared to Holstein and other western breeds or their crosses that are managed under intensive production system. The result of this study showed that a higher prevalence of bTB was found in Alamari testing area in Bauchi and the lowest was found in Disina. It is assumed that the increased prevalence recorded in Alamari might be due to the presence of Livestock Improvement Breeding Centre which has a great land for pasture and watering points, making pastoralist herds move into the pastures with their cattle in a large number for feeds and water. In Gombe State, the highest prevalence was obtained in Wakaltu, while Poshereng had the lowest. The high prevalence in Wakaltu could be due to the fact that it is an international cattle route as such a lot of herds mixed with each other while passing from one place to another. However, it was recorded that there was no statistically significant difference ($p>0.05$) in prevalence between the testing area, age, breed, and herd sizes.

Conclusion

The prevalence recorded in this survey may serve as a baseline data on the status of bTB in cattle in the two states. There is a need for further studies to determine which strains of *Mycobacteria* species are circulating in those areas between cattle and humans using genetic strain typing methods such as spoligotyping and variable number tandem repeats to characterize them further.

Recommendations

We recommend for a sustainable project focus on the "Animal Disease Monitoring and Surveillance" in the Northeastern part of the country, where livestock population is so dense with a high burden of bTB.

Authors' Contributions

Dr. SI and IAA designed and carried out the research and made the first draft of the article. Drs. BAU, DS, and ASS participated in some field work and contributed in making the final draft, data analyses, and literature searching for the betterment of this article. The overall research was conducted under the supervision of Professor IAA. The author read, finalized, and approved the manuscript.

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Competing Interests

The authors declare that they have no competing interests.

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