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Occurrence of *Mycobacterium bovis* infection in cattle in Kaduna metropolis and its environs

*[1]Tinau, A. T., [1]Alhabib, I. K., [1]Bala, A. G., Hassan, A. H., Bature, M. S. and [2]Usman, B.
[1]*Division of Agricultural Colleges, College of Agriculture and Animal Science, Ahmadu Bello University, Mando, Kaduna State, Nigeria.*
[2]*Veterinary Teaching Hospital, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.*
***Corresponding author:** drtinautijjani@gmail.com; +2348105196175

Abstract

The tedious nature of tuberculin tests in Nigeria, warrants the need for an alternative rapid animal-side means of diagnosis. The aim of this study was to provide information on the epidemiology of Mycobacterium bovis infection in cattle. An experiment was conducted to evaluate the occurrence of M. bovis infection in cattle in Kaduna metropolis and it's environ. A total 239 heads of cattle from 8 herds were randomly selected and tuberculin tested, two herds being from each of the four local government areas involved and pooled milk samples from the eight herds were tested for acid fastness. Also, all heads of cattle totaling 2978 slaughtered at the 2 abattoirs during the month of May, 2012 were examined at postmortem and tissues with lesions compatible with lesions of tuberculosis were collected and tested for acid fastness. The results of the experiment showed that only 23 (9.6%) of the animals tested were classified as reactors and these came from only one of the eight herds studied. A clear swelling was seen in each of the positive cases. Out of the eight herds tested, 6 (75%) were settled and the remaining 2 (25%) were semi- settled. Similarly, 6 (75%) of the herds were for mixed production system and 2(25%) were kept solely as dairy herds. Positive tuberculin test was only observed from among one of the six settled herds 1(17%) while cattle in the remaining 5(83%} settled herds all tested negative. All the two semi-settled herds (100%) tested negative. The tuberculin reaction among different production systems showed that only 1 (50%) of the dairy herds tested positive and all the 6 (100%) mixed herds tested negative. A tuberculin positive reaction of 19 (7.9%) was observed among the females and 4 (1.67%) among the males. The chi-square test of significance between the tuberculin reaction and sex of the animals showed no significant effect (P > 0.05). Hence, it can be concluded that, Mycobacterium bovis was neither cultured nor isolated and therefore, could not be confirmed responsible for the observed reactions. However, its involvement is strongly suggested by both its higher values compared to those for Mycobacterium avium in the single intradermal comparative tuberculin test and the acid-fast bacilli observed in the stained smears of both the milk samples collected and the bovine tissues from the abattoirs.

Keywords: Tuberculin, Lesions, *bovis*, Herds, Tissues

Introduction

The tentative diagnosis of bovine **tuberculosis (**TB) in live animal for more than a century on the tuberculin skin test is based on evoking the cell mediated immune response in infected animals (Radostis *et al*., 2005). The tuberculin was developed over a century ago and has under gone changes to the present day tuberculin-Purified Protein Derivative (PPD), as reported by Monaghan *et al*. (1994). The tedious nature of tuberculin tests, particularly the need for a second handling of the animal, warrants the need for an alternative rapid animal-side means of diagnosis. Many efforts in this direction have led to the development of blood assay tests such as gamma interferon assay

(Quantiferon)-TB Gold) and indirect enzyme-linked immunosorbent assay (ELISA). Recently molecular biology techniques have also been developed (Harris, 2006). Developed nations have been able to drastically reduce cases of bovine TB to very low levels through improved hygiene, surveillance, milk pasteurization and test and slaughter method (Graham, 2003). In Nigeria, reports on *M. bovis* infection rates show the prevalence of bovine TB due to *M. bovis* ranging from 2.5% in 1976 to 14% in 2007 (Alhaji, 1976; Abubakar, 2007). These reports also showed that TB in cattle is on the increase. Raw soured milk, either whole "Kindirmo" or skimmed "Nono", from cattle is used in preparing millet gruel "Fura" which is one of the common staple food of Kaduna metropolis as well as in most communities of northern Nigeria. There have been several reports on the isolation of *M. bovis* from bovine tissues collected from abattoirs and from milk collected from herds and milk markets (Abubakar, 2007). Hence, infected tissues and milk could serve as sources of infection of *M. bovis* to man because it is zoonotic (Charles *et al.,* 2009). The importance of *M. bovis* in Nigeria has generally been underestimated and currently, neither control program nor even public awareness campaigns have been put in place to check its spread (Abubakar, 2007). Findings from this work would contribute to existing knowledge particularly in the areas of public health which will help in the policy formulation by the government in Nigeria. The aim of this study was to provide information on the epidemiology of *M. bovis* infection in cattle in Kaduna metropolis and its environs.

Materials and method

Study area

The work was carried out in Kaduna metropolis and its environs located within $10^{0}20'$-$10^{0}45'$N and $7^{0}20'$-$7^{0}25'$E. It comprises of Kaduna North and Kaduna South local government areas as well as some portions Igabi and Chikun local government areas. The area enjoys a tropical climate, falling within guinea savanna vegetation zone with human population of over 2 million (Bulus *et al*., 2007). Kaduna metropolis has two abattoirs, one at Kawo located at the northern part of the city while the Tudun Wada abattoir is located close to the city centre. In both abattoirs, cattle are the dominant animals slaughtered with a daily average slaughter figure of 150 heads. Other species of livestock such as sheep, goats and occasionally camels are also slaughtered daily in varying numbers. In this study area are found cattle herds of different size and breeds kept under settled and semi-settled management systems for both dairy and dual production systems.

Sample size

A total 239 heads of cattle from 8 herds were randomly selected and tuberculin tested, two herds being from each of the four local government areas involved and pooled milk samples from the eight herds were tested for acid fastness. Also, all heads of cattle totaling 2978 slaughtered at the 2 abattoirs during the month of May, 2012 were examined at postmortem and tissues with lesions compatible with lesions of TB were collected and tested for acid fastness.

Breeds of cattle sampled and their management practices

Cattle breeds examined during the period of the study were dominantly the tropical Zebu and their crosses with *Bos tarus*. The Zebu cattle included both White Fulani (Bunaji) and Sokoto Gudali (Bokolo) which was all humped cattle that are indigenous to northern Nigeria. Some of the crosses included in the study were mainly

of Friesian progeny with Bunaji or Bokolo found in a few farms. The management practices of cattle herds in this study were either sedentary or semi -nomadic. In the former, the animals were provided shelter in the form of half open roofed pens or just shades made of corrugated roofing sheets and were allowed out for free range grazing within short distances. In the dry seasons, when there is usual scarcity of pasture they are given supplementary feeding either before they go out for grazing or when they return. In some cases, the supplementary feeding was limited to cows that calved. In addition, the animals received some veterinary care. In the case of the semi-settled herds which are owned by Fulani Pastoralists, the animals go for free-range grazing over long distances. No supplementary feeding was provided except rarely for the sick ones. In the dry seasons, the entire cattle in the herd were moved southwards to the guinea savanna zone or even to the forest zones in search of pasture except for very young calves, cows with advanced pregnancy and those lactating that were left behind. The production purpose for most of the herds was mixed, for both beef and dairy and only a few herds were kept solely for dairy.

Selection, restrain and identification of animals

In all the herds examined, males and females one year and older were tested. The animals were restrained in crushes where the facility was available or by tying them in pairs by their legs as described by Alhaji (1976), and identified by their names where the herd was small in size or by putting identification numbers using oil paint on their flanks (Shehu, 1988) or by the use of ear tags for those that have been ear tagged.

Test procedures

Bovine Tuberculin- PPD 3000: A purified protein derivative (PPD) was used for the initial TB screening of cattle using the caudal fold test as described by Alhaji (1976) and Monaghan *et al.* (1994). Using the semi-automatic tuberculin syringe, 0.1mL containing 3000 International Units of the PPD was injected intradermally few centimeters from the base of the tail in skin fold of the underside of the tail after cleaning the area to make it free from faeces. Readings of the test was taken 72hrs later. It was considered negative when there was no palpable swelling and positive when there was either diffuse or nodular swelling at the injection site. Suspect reactors were further tested by the single intradermal comparative tuberculin test (SICTT) using avian tuberculin PPD 2500 and the bovine tuberculin. Two locations 12-15cm apart ventrally at the centre of the neck region were shaved and skin thickness of the two sites using a Vanier caliper were taken. After these measurements were taken, 0.1ml of the bovine PPD was injected at the dorsal site while 0.1ml avian PPD was injected at the ventral site. Readings were taken 72hrs later by measuring the skin thickness at the site as done before and the differences in the increase in skin thickness between site of bovine and avian PPDs were recorded in the tuberculin test record Form II. Cases where the differences exceeded 2mm were classified as positive reactors and inconclusive results where the difference was 2mm or less (Monaghan *et al.*, 1994).

Collection and handling of suspected TB abattoir samples

Bovine tissues with lesions compatible with lesions of TB, were collected from Tudun Wada and Kawo public abattoirs and frozen until processed. Data on sex, age, location of lesion on the carcass and origin of animal were recorded for each sample. The milk and tissue samples were processed as described by Alhaji (1976) and Abubakar (2007).

Results

Table 1 shows the results of purified protein derivative tuberculin test carried out in eight herds of cattle. A total of 239 heads of cattle from 8 herds were tested using the caudal fold tuberculin test technique. Only 23 (9.6%) of the animals tested were classified as reactors and these came from only one of the eight herds studied. A clear swelling was seen in each of the positive cases.

Table 1: Purified protein derivative tuberculin test carried out in eight herds of cattle

Farm	Total tested	Purified protein derivative +ve	% +ve*
A	37	0	0.0
B	46	0	0.0
C	16	0	0.0
D	22	0	0.0
E	46	23	9.6
F	30	0	0.0
G	20	0	0.0
H	22	0	0.0
Total	239	23	

Key: * Based on a total population of 239 cattle from 8 herds

Table 2 shows the relationship between prevalence of bovine tuberculosis and type of herd and production system. Out of the eight herds tested 6 (75%) were settled and the remaining 2 (25%) were semi- settled. Similarly, 6 (75%) of the herds were for mixed production system and 2 (25%) were kept solely as dairy herds. Positive tuberculin test was only observed from among one of the six settled herds 1(17%) while cattle in the remaining 5 (83%) settled herds all tested negative. All the two semi-settled herds (100%) tested negative. Table 3 illustrates the tuberculin reaction among different production systems where only one (50%) of the dairy herds tested positive and all the six (100%) mixed herds tested negative.

Table 2: Purified protein derivative tuberculin test reaction according to management system

		Semi-settled		Settled	
Farm	Total tested	+ve	% +ve*	ve+	% +ve*
A	37	0	0	0	0
B	46	0	0	0	0
C	16	0	0	0	0
D	22	0	0	0	0
E	46	0	0	23	9.6
F	30	0	0	0	0
G	20	0	0	0	0
H	22	0	0	0	0
Total	239	0	0	23	9.6

Key: * Based on a total population of 239 cattle from 8 herds

The result of tuberculin test reaction as it relates to the sexes of cattle tested is shown in Table 4. A tuberculin positive reaction of 19 (7.9%) was observed among the females and 4 (1.67%) among the males. The chi-square test of significance between the tuberculin reaction and sex of the animals show no significant effect ($P > 0.05$).

Table 3: Purified protein derivative tuberculin test reaction according to production systems.

		Dairy		Dual	
Farm	Total Tested	+ve	% +ve*	+ve	% +ve*
A	37	0	0	0	0
B	46	0	0	0	0
C	16	0	0	0	0
D	22	0	0	0	0
E	46	23	9.6	0	0
F	30	0	0	0	0
G	20	0	0	0	0
H	22	0	0	0	0
Total	239	23	9.6	0	0

Key: * Based on a total population of 239 cattle from 8 herds

Table 4: Purified protein derivative tuberculin test reaction according to sex.

		Males		Females	
Farm	Total Tested	+ve	% +ve*	+ve	% +ve*
A	37	0	0	0	0
B	46	0	0	0	0
C	16	0	0	0	0
D	22	0	0	0	0
E	46	4	1.7	19	7.9
F	30	0	0	0	0
G	20	0	0	0	0
H	22	0	0	0	0
Total	239	4	1.7	19	7.9

Key: * Based on a total population of 239 cattle from 8 herds

Discussion

This study revealed a prevalence of 9.6% tuberculin positive reaction using the caudal fold tuberculin test. Values obtained earlier in Nigeria include the 2.5% obtained in four northern states by Alhaji (1976), 7.8% in some southern states of Nigeria by Ayanwale (1984), 11.8% in Zaria by Shehu (1988) and 14% in Federal Capital Territory and Kaduna state by Abubakar (2007). This indicates that bTB infection is prevalent in all parts of Nigeria and might suggest the existence of foci of infection in different parts of the country. The spread might be due to the migratory management practiced by the nomads where they seasonally migrate between northern and southern parts of the country in search of pasture. Another contributing factor could be the non-recognition of the importance of the disease which lead to the limited implementation of bovine TB control programme in the country. This therefore shows a dangerous development as the disease will continue to spread among cattle herds and even to humans, *M. bovis* being zoonotic. This is particularly important because of the well-known interaction between TB and HIV infection in humans which has adult prevalence rate of 3.6% in Nigeria (Idoko, 2012).

The only herd where Tuberculin positive animals were observed is both a settled and dairy herd. This agrees with earlier findings that increased animal concentration as promoted by settled husbandry and dairy production both of which bring the animals closer at supplementary feeding points, watering points and the housing (Ayele *et al*., 2004). Findings of this work agree with earlier findings that TB infects a higher proportion of exotic breeds (*Bos tarus*) than

indigenous zebu cattle breeds (*Bos indicus*) as reported by Caffrey (1994). This could be due to prolonged exposure of the indigenous breed to Mycobacterium bovis which is endemic in this area Alhaji (1976) while the disease has long been reduced to very low levels at the western world origin of the exotic breeds (Charles *et al*., 2009). The observance of positive tuberculin reactions in a dairy herd is of great epidemiological and public health importance as milk and its products are known to be a major source of infection to humans (Radostis *et al*., 2005). This is particularly important as large quantities of unpasteurized milk are known to be daily consumed by humans in this area. Higher values of positive tuberculin reaction as observed in the female animals (7.9%) than in their male counterparts (1.67%) is significant and therefore of both epidemiologic and public health importance as the females could spread the disease vertically or/and horizontally to their young ones (Kaneene and Pfeiffer, 2006). The fact that females are more handled (during milking and artificial insemination among others), clean animals could get infected if handled by TB infected attendants thereby spreading the disease within a herd or even to other herds outside at grazing and/or by personnel due to poor hygienic practices. The age range of 3-6 years is the peak of reproductive age of cattle in this part of the world. Therefore, a high prevalence (78%) of tuberculin positive within this age group is also of epidemiological and public health importance because of a wider possibility of spreading the disease as during mating/insemination and milking. Earlier studies have established the transmission of TB through coitus and through contaminated equipment during artificial insemination (Charles *et al*., 2009). In humans, there were reports of people contracting TB from handling or ingesting meat from infected carcasses (Melinda *et al*., 2008).

Conclusion

It can be concluded that, *Mycobacterium bovis* was neither cultured nor isolated and therefore, could not be confirmed responsible for the observed reactions. However, its involvement is strongly suggested by both its higher values compared to those for *Mycobacterium avium* in the single intradermal comparative tuberculin test and the AFB observed in the stained smears of both the milk samples collected and the bovine tissues from the abattoirs.

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