Prevalence of *Mycobacterium bovis* in some cattle breeds in the aids era: An abattoir survey in Ogun State, Nigeria

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Abstract

Eight breeds of cattle totalling 15520 heads and comprising of 3784 bulls and 11736 cows were examined during slaughter in the two largest abattoirs in Ogun State, Nigeria between January and December, 2007. Samples of lung, liver, spleen, kidney, udder, skin and pleural lymph nodes with gross lesions suspected to be those of tuberculosis were obtained from the slaughtered animals. Each of the samples was processed according to the standard method and thereafter stained by Ziehl-Neelsen's technique and was microscopically examined for acid fast organisms. Samples containing acid fast bacilli were cultured on Lowenstein-Jensen (LJ) medium with pyruvate or glycerol. *M. bovis* was identified by growth rate, pigment production, colony and cell morphology and biochemical characteristics. A total of 41 heads of cattle comprising of 9 bulls and 32 cows from 7 breeds were positive for *M. bovis*. No isolate of *M. bovis* was obtained from Keteku breed and no seasonal distribution of the organism was observed. Among the organs with lesions, the lung had the highest number (18) of *M. bovis*. The number of *White Fulani* cattle with *M. bovis* was highest (13) among the breeds examined.

Keywords: Prevalence, *Mycobacterium bovis*, Cattle, Abattoir, Ogun State

Introduction

Some members of the Genus *Mycobacterium* cause tuberculosis in human and animals. Although there are over 80 named species of mycobacteria only members of the *Mycobacterium tuberculosis* complex cause tuberculosis in man and animals (McFadden, 1992). This complex includes *Mycobacterium tuberculosis* (*M. tuberculosis*), *Mycobacterium bovis* (*M. bovis*), *Mycobacterium africanum* (*M. africanum*) and others (Grage, 2002).

According to Grage (2002) members of the complex are closely related species which on generic analysis are variant of single species. Record of tuberculosis as contained in the Assyrian King Assanbanipal's library (668-626BC) had it

that *M. bovis* had been causing tuberculosis in animals before invading human (Esse, 2001). According to history, it was after domestication of cattle that there was evidence of tuberculosis in human through milk ingestion (Esse, 2001).

Bovine tuberculosis caused by *M. bovis* occurs mainly via the respiratory route leading to a primary disease of the lungs but can be disseminated to cause lesions in other organs. Bovine tuberculosis can be transmitted to human through respiratory route, consumption of unpasteurized infected milk, meat and meat products (Radostits *et al.*, 2007). Punctured wounds and cuts of butchers and other related workers can also be contaminated by bovine tuberculosis organs.

By 2003, not less than 17 million people are

reported to be infected with tuberculosis (WHO, 2003) and these may represent either reactivation or new infections and the former is thought to be more common. It is estimated that almost one third of the world's population have latent tuberculosis infection (Sepkowitz *et al.*, 1994). It has also been estimated that progression from latent tuberculosis infection to clinical disease occurs in 5% of non-HIV infected individuals (Allen *et al.*, 1992) but this increases to 30% in HIV infected persons (McFadden, 1992). Persons co-infected with HIV and tuberculosis have an estimated 10% annual risk of developing fatal tuberculosis (Allen *et al.*, 1992). Whereas human tuberculosis caused by *M. bovis* have been eradicated in most of the developed world (McFadden, 1992), *M. bovis* accounted for 20% of cases in Nigeria (Garba *et al.*, 2004). Since *M. bovis* infection in human is likely to be acquired through animal sources, control of the disease in cattle should form a major part of the tuberculosis eradication programme. A pre-requisite to the control/eradication of tuberculosis is its surveillance among domestic and wild animals. In this study, we reported the prevalence of *M. bovis* among some cattle breeds slaughtered for human consumption in Ogun State, Nigeria.

Materials and Methods

Collection of Samples

Samples suspected to be tuberculosis lesions comprising of lung, liver, kidney, spleen, skin, peritoneal lymph node and udder were collected from a total of fifteen thousand, five hundred and twenty (15520) heads of cattle from eight breeds of cattle (Table 1) during slaughter in the two largest abattoirs in Ogun State, Nigeria. These abattoirs are those located in Lanfewa, Abeokuta and Ijebu-Igbo. The samples which were collected between January and

December 2007 were transported in ice pack to the laboratory immediately after collection and stored under refrigeration at 4°C until processed.

Laboratory examination of the samples

Processing of the samples: Each sample was ground in sterile mortar and pestle with sterile sand. The homogenized sample was then treated with 10ml of 4% sodium hydroxide containing phenol red (15mg/litre) with constant shaking for 20 minutes at room temperature. This was centrifuged at 300 rpm for 15 minutes. The supernatant was poured off into dettol® in water (disinfectant) and the sediment restored to neural pH by adding 25% hydrochloric acid (HCl) drop by drop.

Direct examination of the sample: Smears of sediments from each of the samples were made on microscopic glass slides and stained by Ziehl-Neelsen's technique (Barrow and Felthan, 1993) for presence of acid fast organisms.

Isolation and cultural procedures: Sediment from the sample was mixed well and 0.1ml from it was inoculated onto triplicate slants of LJ medium with pyruvate or with glycerol. These slants were incubated overnight at room temperature in a horizontal position, then at 37°C in upright position and examined weekly for 8 to 12 weeks. When there was growth, the colony was dropped on a microscope glass slide by means of a coarse inoculating needle with a flat end. A smear was made and stained by Ziehl Neelsen's technique. The stained smear was then examined under an oil immersion objective (x100) of a light microscope (Olympus, Germany).

Test to detect pigment production by the isolates: This was carried out by sub-culturing the isolate into two tubes of LJ medium. One tube was incubated in light

and the second wrapped in aluminium foil and incubated in darkness all at 37°C.

Biochemical tests: Production of niacin, nitrate reductase and catalase production by the isolates were determined according to the methods of Barrow and Feltham (1993).

Results

All the smears prepared directly from the suspected samples and stained by Ziehl-Neelsen's stain technique were positive for acid fast bacilli (AFB). The colonies which are dry, sparse appeared within 15-19 days post incubation. The cells are small, slender and acid fast. No pigment was produced in light and darkness. The isolates did not produce niacin, catalase and nitrate reductase and were therefore identified as *M. bovis*. The *M. bovis* was not isolated from Keteku of all the 8 breeds of cattle that were investigated. All the other 7 breeds were positive for *M. bovis* with the prevalence (0.1%) lowest in Red Bororo and highest (0.5%) in Adamawa breed (Table 1).

The total number of Nigerian cattle with *M.*

bovis was 41 (0.26%) comprising of 32 (0.21%) cows and 9 (0.06%) bulls (Table 2). The number of cows with *M. bovis* was significantly higher ($P<0.05$) than the bulls (Table 2).

In those breeds of cattle that carried *M. bovis*, the organism was disseminated in not less than two organs (Table 3) with the lung (18 cases) being the most affected organs followed by the pleura (7 cases). More cow than the bull lungs (14 vs 4) were affected. The udder accounted for 4 cases. Only the cow pleural lymph nodes carried *M. bovis*.

Except for the month of March, when no cases were encountered, the monthly occurrence of the organism in cattle did not show any consistent pattern in relation to the season of the year. The usual climate pattern in Nigeria is that the dry season occurs in November through to March while the rainy season is between April and October every year. However, the prevalence of *M. bovis* in cattle was highest in January which is within the dry season period (Table 4). The mean monthly occurrence of *M. bovis* is significantly

Table 1: Status of *M. bovis* in Nigerian breeds of cattle

Breed	No examined	Bull	Cow	No (%) with <i>M. bovis</i>	Mean ($\pm$ SE*)
Ndama	997	251	746	03 (0.30)	0.25 ^{bc} (0.13)
Muturu	1321	285	1036	04 (0.30)	0.33 ^{bc} (0.14)
Kuri	1230	385	845	05 (0.41)	0.50 ^b (0.19)
White Fulani	3626	859	2767	13 (0.36)	1.08 ^a (0.23)
Red Bororo	4011	881	3130	04 (0.10)	0.33 ^{bc} (0.14)
Sokoto Gudali	2186	640	1546	07 (0.32)	0.58 ^b (0.15)
Adamawa	980	211	769	05 (0.51)	0.42 ^{bc} (0.15)
Keteku	1169	272	897	0 (0.00)	0.00 ^c (0.00)
Total	15520	3784	11736	41 (0.26)	

*mean of occurrence of *M. bovis* in breeds of cattle

^{a,b,c} means along the same column having different superscript are different ($P<0.05$)

Table 2: Sex distribution of *M. bovis* in Nigerian breeds of cattle

Sex	Number of Animals with <i>M. bovis</i>									n (±SEM*)	Mean (±SEM*)
	Nd	Mt	Kr	WF	RB	SG	Ad	Kt	Total		
Male	1	1	2	2	1	2	0	0	9	8 (0.06%)(0.30) ^b	1.13
Female	2	3	3	11	3	5	5	0	32	8 (0.21%)(1.15) ^a	4.00
Total	3	4	5	13	4	7	5	0	41		

*mean of sex occurrence of *M. bovis* in breeds of cattle

^{a,b} means along the same column having different superscript are different (P<0.05)

Nd= Ndama

Mt= Muturu

Kr= Kuri

WF=White Fulani

RB= Red Bororo

SG= Sokoto Gudali

Ad= Adamawa

Kt= Keteku

N=No of breeds

higher (P<0.05) in White Fulani than in the other breeds. The mean monthly occurrence is significantly lower (P<0.05) in Ndama, Muturu, Red Bororo and Adamawa than in the other breeds that were examined (Table 4).

Discussion

The detection of AFB by acid fast staining directly from the samples suggests that these samples carry approximately not less than 10⁴ bacilli per gramme of the sample (Gording and Slutkin, 1990). The identification of the isolates as *M. bovis* was based on the cultural characteristics, cell

Table 3: Dissemination of *M. bovis* in organs of Nigerian breeds of cattle

Breed	N	n	Organs from which <i>M. bovis</i> was isolated						
			Lung	Liver	Spleen	Kidney	PLN	Udder	Skin
Nd	997	3	1M 1F	0	0	0	1F	0	0
Mt	1321	4	2F	0	1M	0	1F	0	0
Kr	1230	5	1F	0	1M	1M	1F	0	1F
WF	3626	13	2M4F	2F	0	2F	2F	1F	0
RB	4011	4	1F	0	0	1M	1F	0	1F
SG	2186	7	1M2F	0	1M	0	0	3F	0
Ad	980	5	3F	1F	0	0	1F	0	0
Kt	1169	0	0	0	0	0	0	0	0
Total	15520	41	4M14F	3F	3M	2M2F	7F	4F	2F

N=No of Animals examined

n=No with tuberculosis

PLN= Pleural Lymph Node

M=Male

F=Female

Nd= Ndama

Mt= Muturu

Kr= Kuri

WF=White Fulani

RB= Red Bororo

SG= Sokoto Gudali

Ad= Adamawa

Kt= Keteku

Table 4: Prevalence of *M. bovis* in slaughtered cattle in Abeokuta and Ijebu Igbo abattoirs

Month	N	n	Monthly occurrence of <i>M. bovis</i>							
			Nd	Mt	Kr	WF	RB	SG	Ad	Kt
January 1508	5	0/94	1/127	0/51	1/361	1/446	1/211	1/87	0/131	
February	1337	2	0/84	0/113	0/45	1/320	0/397	0/186	1/78	0/114
March	1423	0	0/89	0/120	0/48	0/342	0/421	0/200	0/81	0/122
April	722	2	0/33	0/118	1/107	1/116	0/71	0/145	0/89	0/43
May	1665	6	1/101	1/108	1/206	1/355	1/420	1/216	0/119	0/140
June	1974	10	1/151	1/186	2/243	3/450	1/350	1/227	1/169	0/196
July	1284	5	0/77	0/83	1/130	2/360	1/320	1/140	0/72	0/102
August	1205	4	1/84	0/80	0/246	1/257	0/250	1/258	1/24	0/06
September	188	1	0/14	0/20	0/07	0/51	0/61	0/04	1/13	0/18
October 1265	2	0/73	1/98	0/39	1/280	0/447	0/161	0/67	0/100	
November	1496	2	0/94	0/127	0/51	1/358	0/442	1/209	0/85	0/130
December	1453	2	0/103	0/141	0/57	1/376	0/386	1/229	0/96	0/67
Total	15520	41	3/997	4/1321	5/1230	13/3626	4/4011	7/2186	5/980	0/1169
Mean ($\pm$ SE*)			0.25 ^{bc} (0.13)	0.33 ^{bc} (0.14)	0.50 ^b (0.19)	1.08 ^a (0.23)	0.33 ^{bc} (0.14)	0.58 ^b (0.15)	0.42 ^{bc} (0.15)	2.00 ^c (0.00)

*mean of monthly occurrence of *M. bovis* in breeds of cattle

a,b,c means along the same column having different $\pm$ superscripts are different ($P < 0.05$)

N=No of Animals examined

Nd= Ndama

Mt= Muturu

n=No with *M. bovis*

Kr= Kuri

WF=White Fulani

RB= Red Bororo

SG= Sokoto Gudali

Ad= Adamawa

Kt= Keteku

morphology and staining reaction and the results of the biochemical tests that were performed.

In the absence of more rapid methods (Shawar *et al.*, 1993) culture, staining and biochemical tests are approximately equal in sensitivity in the identification of Mycobacteria (Kolo, 1991).

Although the percentage of carriers of *M. bovis* among the breeds of cattle examined in this study was low (0.26%) the spread of the organism among them was wide as seven out of 8 breeds carried *M. bovis* (Table 1). The absence of *M. bovis* in the Keteku can be assumed to be due to the natural resistance of the breed to many infections but this cannot be substantiated in the present study. The distribution of *M. bovis* in many body organs of the affected

animals lends credence to the usual characteristics of *M. bovis* infection. This facilitates the transmission of the infection through ingestion of the affected organs and products (milk) or by inhalation through respiratory discharge of *M. bovis* from infected animals (Radostits *et al.*, 2007). Involvement of *M. bovis* in human infection has been reported in many countries of the world (Griffith, 1937; Christensen *et al.*, 1981; Silber *et al.*, 1987) including Nigeria (Idigbe *et al.*, 1986; Kolo, 1991). Apart from the practice of close association between man and its livestock in some parts of Nigeria, ingestion of infected or contaminated meat product (suya), raw milk and some local milk products (fura) can facilitate the transmission of *M. bovis* to humans. Most of the slaughtered cattle in

Nigeria are derived from those reared by the herdsmen.

These tuberculosis reservoir cattle are extensively reared by the herdsmen who move from one state to the other in Nigeria. Furthermore, these herdsmen are known to be closely associated with their animals (Garba, 2002). All these practice enhance spread of the organism in the environment and its transmission to the herdsmen.

In the developing countries like Nigeria, tuberculosis is spreading rapidly because of many reasons such as neglect by government in finding of cases and treatment, increasing urbanization with people living in overcrowded houses in densely populated communities. Other reasons include increasing poverty with malnutrition and increasing air pollution from infected animals and humans. It is speculated that about 75% of the world tuberculosis cases occur in developing countries (WHO, 2003). For effective control of bovine tuberculosis in Nigeria, education of those at risk of the disease is important. The key facts to be understood include how

- i. bovine tuberculosis is caused and spread between animals and humans
- ii. poor ventilation and over overcrowding hasten the spread of the disease.
- iii. poor hygiene and malnutrition enhance the spread of the disease.
- iv. effective cooking of food from animal sources will reduce or eliminate bovine tuberculosis.

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