Bovine tuberculosis in one cattle herd in Ibadan in Nigeria

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ABSTRACT: Bovine tuberculosis was investigated in one private herd with 171 cattle after five cases were suspected to be tuberculous following *post mortem* examination. Using the intradermal comparative cervical tuberculin test 18 (10.5%) animals (ages from 2 to 12 years) were positive: 11 animals of N'dama breed and seven animals of White Fulani (i.e. Bunaji) breed; 17 female and one male animal. In all 11 randomly selected positive reactors, a spectrum of tuberculous lesions affecting the lungs, spleen, heart, liver, and the lymph nodes were observed. All the smear samples obtained were positive for acid-fast bacilli; cultural isolation confirmed the growth of mycobacteria on pyruvate-enriched Loewenstein-Jensen medium, which were identified by molecular typing to be *Mycobacterium bovis*. This study demonstrates widespread infection in this cattle herd and potential risk of infection for the human population with *M. bovis*.

Keywords: Africa; zoonosis; tuberculous lesions distribution

Bovine tuberculosis is a chronic bacterial disease of animals and humans caused by *Mycobacterium bovis*, slow growing and non-photochromogenic an acid-fast bacillus (AFB). The disease has a worldwide distribution including Africa, and in many countries, bovine tuberculosis remains a major chronically infectious disease among cattle, other domesticated animals, and certain populations of wildlife (Ayele et al., 2004). Bovine tuberculosis is a zoonosis and the transmission to humans constitutes an important public health problem (Thoen and Steele, 1995; O.I.E., 2000).

Infected cattle with *M. bovis* are the main source of infection for other cattle. *M. bovis* is excreted through aerosol, in sputum, faeces (from both intestinal lesions and swallowed sputum from pulmonary lesions), milk, urine, vaginal and uterine discharges, and discharges from open peripheral lymph nodes (Radostits et al., 1994). In the early stages of the disease, before any lesions are visible, cattle may also excrete viable mycobacteria in nasal and tracheal excretions (McIlroy et al., 1986).

Inhalation is the almost invariable portal of entry in housed cattle, and even in those at pasture, it is considered the principal mode of transmission. Infection by ingestion is obviously more likely at pasture, when faeces contaminate the feed and communal drinking water and feed troughs; while under natural conditions, stagnant drinking water may cause infection up to 18 days after its last use by a tuberculous animal (Radostits et al., 1994; Thoen and Steele, 1995; Ayele et al., 2004).

After infection, non-vascular nodular granulomas known as tubercles may develop. Characteristic tuberculous lesions occur most frequently in the lungs, the retropharyngeal, bronchial and mediastinal lymph nodes. Lesions are also commonly found in the mesenteric lymph nodes, liver, and

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spleen, on serous membranes and in other organs (Thoen and Steele, 1995). According to Radostits et al. (1994), housing predisposes to the disease, as does zero grazing, so that the disease is more common and serious where these forms of husbandry are practised. The closer the animals are packed together, the greater is the chance that the disease will be transmitted (Thoen and Steele, 1995; Ayele et al., 2004).

The first report of the existence of bovine tuberculosis in Nigeria based on the tuberculin test, followed by *post mortem* and laboratory examination was made by Manley in 1929 (Alhaji, 1976). This was reported from the former British Cameroon among pure-bred and half-bred stock kept indoors. It was concluded that the infection probably originated from a herd imported from Germany. Cases of bovine tuberculosis in both private resident and Fulani herds have been reported previously (Alhaji, 1976; Ayanwale, 1984). Recent and old reports from Nigerian abattoirs have confirmed the presence of bovine tuberculosis in all parts of Nigeria (Hall, 1932; Alhaji, 1976; Ayanwale, 1984; Dusai and Abdullahi, 1994; Cadmus et al., 1999). Alhaji (1976), however, reported that most of the tuberculous cattle slaughtered in southern Nigeria came from the Northern provinces and from the neighbouring francophone countries of Cameroon, Niger, and Chad.

Nigeria according to relevant data gathered over the years had roughly about 13.9 million heads of cattle in 1990; 11.5 million heads of cattle were kept with the pastoralists (commonly referred to as the Fulanis) and 2.4 million heads of cattle were kept in villages with vast majority of these resident in the northern part of the country. Over the years there have been a lot of crossbreeds produced; however, the most common breeds are: (1) the zebus (i.e. Bunaji or White-Fulani, Sokoto Gudali and Rahaji), which make up about 90% of the entire cattle population and (2) the taurines (i.e. the Keteku, Kuri, Muturu, and N'dama) making up about 115 000 heads of cattle. Bunaji's breed makes up about 7.7 million heads of the entire cattle population (Anonymous, 2003).

In Nigeria majority of these cattle are of dual-purpose productivity; however, because of the inadequate disease monitoring and surveillance system regarding zoonotic diseases (especially tuberculosis), the people are at risk of exposure to contacting bovine tuberculosis through the food chain and direct co-habitation with these animals (in the case of the Fulani's).

The aim of this study was to confirm the presence of bovine tuberculosis in the herd investigated employing both clinical signs, and different diagnostic tools including tuberculin test, morphological studies and molecular technique. Attempts were also made to show whether breed, sex, and age played any role in the incidence of the disease in this herd.

MATERIAL AND METHOD

History of the studied herd

This work was conducted in a resident cattle herd with 171 animals in South-Western Nigeria, which was suspected to have been exposed to bovine tuberculosis when it was initially kept in a suspected infected Fulani cattle settlement with *M. bovis*. In five animals tuberculous lesions were found in different organs. Animals in the studied herd were of two major breeds: 77 animals were of N'dama and 30 animals were of White Fulani breeds; the remaining were crossbreeds between Brown-Swiss and N'dama; Brown-Swiss and White Fulani; N'dama and White Fulani. From the gender point of view, in the herd 55 male and 116 female animals were reared. Consecutive commissioned investigation was therefore to ascertain the authenticity and incidence of bovine tuberculosis in the herd as a follow-up to the earlier single intradermal cervical tuberculin test done nine months before the commencement of this study.

Skin testing

All the cattle were examined using the skin test with of bovine purified protein derivative (B-PPD; 2 500 IU/ml; 0.1 ml *per dose*) and with 0.1 ml of avian purified protein derivative (A-PPD; 2 500 IU per ml; 0.1 ml *per dose*), which were obtained from the Central Veterinary Laboratory (Weybridge, UK). Tuberculin was applied intradermally into the skin fold of the neck and the injection site measured with calliper. The results were determined from the combination of clinical symptoms and measurements of skin thickness taken at the time before and after the skin testing. The results were evaluated according to Rothel et al. (1993) and O.I.E. (2000). For experimental purposes the reactions were read after the 48, 72, and 96 hours.

Laboratory diagnostics of mycobacteria

Gross pathology. Gross pathology was carried out in 11 (Nos. I to XI) randomly selected cows in different tissue samples and lymph nodes.

Detection of mycobacteria. From each animal, randomly selected tuberculous lesions were examined microscopically by Ziehl-Nielsen (Z-N) staining technique for the detection of AFB and cultured using the Lőwenstein-Jensen (L-J) medium after the decontamination/digestion procedure was carried out using the Becton Dickinson method (Anonymous, 1999). A total of 20 randomly selected tissue samples from 11 animals were cultured at 37°C for 8 weeks on paired L-J media enriched with pyruvate (L-J-P medium) and enriched with glycerol (L-J-G medium).

Molecular typing of isolates. Molecular confirmation of four isolates was done by spoligotyping at the Veterinary Laboratory Agency (UK) according to previously described technique (Njanpop-Lafourcade et al., 2001).

RESULTS

Eighteen (10.5%) animals were considered positive to bovine tuberculin between 48 to 96 hours (Table 1): 10, 12 and 14 animals were positive by 48, 72, and 96 hours, respectively. Of the 18 positive cattle to bovine tuberculin 17 were female and one male animal; seven (9.1% of all animals of this breed in herd) animals were of N'dama and 11 (36.7% of all animals of this breed in herd) animals were of

Table 1. Reaction in 18 animals after the 48, 72, and 96 hours after the simultaneous skin test

Animal			48 hours		72 hours		96 hours		No. Animal (slaughtered)[3]
No.	Breed	Age	B[1]	A[2]	B[1]	A[2]	B[1]	A[2]	
1	N	11	20.0	10.5	21.0	9.5	21.0	11.0	746 (I)
2	N	12	18.5	10.0	21.0	12.0	20.0	11.0	1 301 (II)
3	N	12	12.5	8.0	16.0	8.0	14.0	8.0	1 313 (III)
4	N	7	10.5	9.0	15.5	8.5	14.0	9.0	678 (IV)
5	B	5	15.5	10.0	17.0	11.0	15.0	11.5	1 306 (V)
6	B	5	14.0	8.5	17.5	9.0	16.0	9.5	598 (VI)
7	N	2	8.0	6.0	12.0	5.0	9.5	6.0	1 315 (VII)
8	B	7	16.5	11.0	15.0	8.5	16.0	10.0	668 (VIII)
9	B	11	12.0	8.0	14.0	8.0	14.5	8.0	706 (IX)
10	N	7	12.0	7.5	15.5	8.0	15.0	7.5	1 303 (X)
11	B	7	13.5	8.0	14.0	8.5	14.0	8.0	1 307 (XI)
12	N	11	14.0	12.0	16.0	15.0	16.5	15.0	1 512 (XII)
13	B	5	12.0	8.0	14.0	9.5	15.0	8.5	1 348
14	B	N/A	12.0	8.0	10.0	9.0	11.5	8.0	1 342
15	B	5	12.0	7.0	11.0	6.0	11.0	6.0	1 344
16	B	3	11.0	7.0	11.5	7.0	12.0	7.0	901
17	B	N/A	8.5	8.0	9.5	9.5	11.0	8.0	682
18	B	8	7.0	8.0	10.0	7.0	12.0	7.0	704

Explanations:
N/A = not available; N = N'dama; B = Bunaji (i.e. White Fulani)
[1]reaction number in mm to bovine tuberculin; [2]reaction number in mm to avian tuberculin; [3]distribution of tuberculous lesions in each cow see in Table 2

Table 2. Distribution of the tuberculous lesions (TB) in 11 skin test positive cows

Animal No.	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	Total No. of animals with TB
Breed	N	N	N	N	B	B	N	B	B	N	B	
Tissue/Age	11	12	12	7	5	5	2	7	11	7	7	
Lung	–	+	–	+	+	+	+	+	+	+	+	9
Liver	–	–	–	–	–	–	+	–	+	+	–	3
Heart	–	–	–	–	–	–	–	–	+	–	–	1
Spleen	–	–	–	–	–	–	+	–	+	–	–	2
Kidney	–	–	–	–	–	–	–	–	–	–	–	0
Head ln1	+	+	+	+	+	+	+	+	+	+	+	11
Thoracic ln2	–	+	+	+	+	+	+	+	–	+	+	9
Abdominal ln3	–	–	–	–	–	–	+	–	+	+	–	3
Other ln4	+	+	+	+	+	+	+	+	–	–	–	8
Total tissues affected/cow	2	4	3	4	4	4	7	4	6	5	3	

Explanations:
ln = lymph nodes; + = tuberculous lesions detected; – = tuberculous lesions not detected
[1]parotid, mandibular, lateral, and medial retropharyngeal ln; [2]apical, bronchial, and mediastinal ln; [3]hepatic, renal, splenic, and mammary ln; [4]prefemoral and prescapular ln

the White Fulani breeds. Their age ranged between 2 to 12 years (average age was 7.8 years) and they represent 1.8 % of male and 14.7 % of female animals (Table 2).

Eleven randomly selected positive cows to bovine tuberculin were culled for *post mortem* examination. Tuberculous lesions were observed in pulmonary tissue in all 11 culled cows while extra-pulmonary infection was found in only three cows. It is noteworthy that in all these examined 11 cows lesions were found in most of the lymph nodes of the head, apical, bronchial, and mediastinal lymph nodes (Table 2).

The majority of these lesions, which were found in all animals, consisted of firm, enlarged lymph nodes, which on incision contained one or more areas of yellow-grey caseous necrosis, well encapsulated by fibrous tissue (Figure 1). In some cases these foci contained calcified matter detectable by palpation and grittiness. In the most severely af-

Figure 1. Tuberculous lesion in firm, enlarged mediastinal lymph node; on incision it showed yellow-grey caseous necrosis, well encapsulated by fibrous tissue

Figure 2. Spoligotype of four-*M. bovis* isolates obtained from the herd. N-1: Nigerian Type-1 *M. bovis* spoligotype was found in four of the isolates typed

Nigerian *M. bovis* Spoligotype

Spoligotype	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
H37Rv	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■			■
N-1	■	■		■	■	■	■	■		■	■	■	■	■	■		■	■	■	■	■	■
Spoligotype	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	
H37Rv	■	■	■	■	■	■	■	■	■	■					■	■	■	■	■	■	■	
N-1	■	■	■	■	■	■	■		■	■	■	■	■	■	■	■						

fected cases, multiple, well-encapsulated nodules from 3 to 20 mm in diameter were found throughout the lungs. They were distributed mainly in the dorsal portions of the caudal lobes. Their contents were soft and caseous with numerous gritty foci. In addition, many of the regional lymph nodes in these cases were totally replaced by caseous material enclosed in thick fibrous capsules.

Histologically the lesions consisted of characteristic epithelioid cell granulomas with central caseation and focal variable calcification, enclosed in a fibrous capsule infiltrated by lymphocytes and plasma cells.

All the samples were positive for AFB. Only one of the 20 pairs of L-J media had growth on both L-J-P and L-JG. The other 19 pairs of LJ had growth only on the LJ with pyruvate without glycerol. The presence of these positive growths was confirmed by demonstrating AFB on each isolate. However, the conclusive confirmation of *M. bovis* infection in the herd was done by spoligotyping; which showed the same spoligotype of *M. bovis* in the herd (Figure 2).

DISCUSSION

The distribution of tuberculous lesions (Table 2) strongly suggested that these animals were mainly infected through inhalation, though infection through ingestion could have occurred in three cases. Another interesting result of the post-mortem findings was that the youngest animal culled (i.e. a two year old female N'dama) had the most generalized spread of the lesions. Therefore, it may be postulated that: (i) this animal had an early exposure to the disease, (ii) was infected by more doses of the pathogen or (iii) that this animal had lower immunity when compared to other animals in combating the disease.

The gross lesions were generally encapsulated foci in isolated lymph nodes to extensive, soft, caseous nodules in the dorsal areas of the lung, spleen, liver, heart, and some regional lymph nodes. All these findings are consistent with bovine tuberculosis in cattle (Jubb et al., 1985; Radostits et al., 1994; Thoen and Steele, 1995). The histological findings presented an array of very simple to extremely complex picture indicating a combination of the early commencement of the disease in some of the animals affected; and the animals' immunologically readiness to respond strongly in warding off the infection (Jubb et al., 1985; Thoen and Steele, 1995).

The percentage of affected animals that were females was 94.4%. The majority of the cows affected were between 7 to 12 years with an average age of 7.8 years (Table 2). It could be suggested that since most of them had calved for more than three times, they may not be immunologically as active as the males because of the stress of pregnancy (Pavlik et al., 2002b).

Eleven (61.1%) of the N'dama and seven (38.9%) of the White Fulani were affected. This could principally have been due to the ratio of N'dama to White Fulani (roughly 2.5 : 1) in the herd. Therefore, breed may not play much role in the occurrence of the disease in these animals. As also shown in Table 2, the spread of the lesions was not restricted to any particular breed. The spread and distribution of *M. bovis* infection in this herd becomes worrisome when one considers the fact that the animals in this herd and other similar herds are sold to the public for consumption since there is no organised formal governmental monitoring and surveillance mechanism or screening to detect animals with bovine tuberculosis.

CONCLUSIONS

The incidence of bovine tuberculosis in cattle herds in Nigeria will continue to increase unless there is a concrete effort by the Nigerian government to formulate a comprehensive policy on the control of bovine tuberculosis as is done in the developed countries, for example in Central Europe (Pavlik et al., 1998, 2002a,c,d, 2003; Erler et al., 2004). If this is not done, the zoonotic spread to humans as documented by Cadmus *et al*. (1999) will increase and this may have a very devastating effect on the people when one considers the epidemic of *HIV/AIDS* in the country.

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