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# *Mycobacterium bovis* infection in livestock workers in Ibadan, Nigeria: evidence of occupational exposure

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## SUMMARY

**SETTING:** Bovine tuberculosis (TB) is endemic in the cattle population in Nigeria. Livestock workers are at risk of *Mycobacterium bovis* infection and unaware of their health status.

**OBJECTIVE:** To determine the occurrence of pulmonary *M. bovis* infection among livestock workers.

**DESIGN:** A cross-sectional study of livestock traders was conducted for TB through screening of sputum samples using a simple random sampling method coupled with oral interview on the assumption of sub-clinical pulmonary TB infection. Specimens were cultured, and the isolates analysed using molecular typing techniques.

**RESULTS:** Overall, 10% (7/70) of the livestock traders had a positive culture indicative of *M. tuberculosis* complex, which were differentiated into *M. bovis* ($n = 2$) and *M. tuberculosis* ($n = 5$) using deletion typing. Further spoligotyping analyses of the *M. bovis* and two available *M. tuberculosis* isolates classified the strains as SB1432 and SB09444 and LAM_10 CAM and T1 using respectively www.mbovis.org and spotclust databases. Prolonged cough and >3 years in the livestock trade were risk factors for infection.

**CONCLUSION:** We confirm that there is undetected pulmonary *M. bovis* infection among livestock traders in Nigeria. Further studies on the role of occupationally exposed workers in the transmission of *M. bovis* infection to the larger community are required.

**KEY WORDS:** transmission; tuberculosis; zoonoses; Nigeria



TUBERCULOSIS (TB) remains a major cause of death worldwide and a serious public health threat. More than 95% of TB deaths occur in the developing world.[1] *Mycobacterium tuberculosis* is known to account for most cases of human TB; however, the proportion due to *M. bovis* is unknown.[2] TB caused by *M. bovis* has been reported to cause disease in humans in widespread regions of the world.[3] Although most reports indicated ≤1% cases of zoonotic TB in humans,[4–6] these studies were not conducted in areas with a high burden of bovine tuberculosis (BTB), nor were factors facilitating transmission and breakdown to disease considerable. Earlier reports have, however, disclosed that factors such as close proximity to cattle, consumption of unpasteurised milk, overcrowded settings, high incidence of human immunodeficiency virus (HIV), malnutrition and poverty,[7] all of which are common in most developing nations such as Nigeria, enhance the prevalence of BTB. Cleaveland et al., in a study carried out in Tanzania, reported a 10.8% prevalence of *M. bovis* infection in human TB, indicating that it is a high BTB risk country.[8]

With a population of over 150 million, Nigeria is rated thirteenth globally among countries with the highest TB burden.[9] This is further aggravated by the prevalence of BTB, which is estimated between 8.8%[10] and 10.5%[11] among trade and farm cattle, respectively. Furthermore, cultural practices that can facilitate the transmission of *M. bovis* infection between cattle and humans also exist. Some of these include close proximity of cattle to the homes of farmers, processing of offal from diseased carcasses with bare hands, close association between farmers and animals, as exemplified by the Fulani herdsmen who live their entire lives with their animals, and the consumption of unpasteurised milk by herders.[10] Coupled with these problems are other health and social problems such as HIV/AIDS (acquired immune-deficiency syndrome), smoking, drug addiction and alcoholism, which are prevalent among livestock workers, many of whom are unaware of their health status, leading

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to considerable delays in seeking health care. These problems are further complicated by the lack of BTB control measures in Nigeria, facilitating transmission of zoonotic infection.

To explore the vulnerability of occupationally exposed groups to pulmonary *M. bovis* infection, livestock traders in Ibadan, south-western Nigeria, were screened for TB using mycobacterial culture and molecular identification techniques. Our aim was to provide important insights into the incidence of zoonotic TB among livestock workers and identify possible control measures.

## STUDY POPULATION AND METHODS

### *Location of the study*

The present study was carried out at Akinyele Cattle Market in Ibadan, south-western Nigeria. The Akinyele Cattle Market is the main trading point for cattle brought from different parts of northern Nigeria and neighbouring African countries to Ibadan metropolis. The study site is characterised by overcrowding of cattle and humans, which can facilitate transmission of BTB from cattle to humans through aerosol spread (Figure 1).

### *Sample size, sample collection and data analyses*

Based on an earlier report of 5% prevalence of *M. bovis* infection among humans in Nigeria,[12] our estimated sample size was 73 participants. Participants were informed of the study aims, and due consent was obtained from those willing to participate. Sputum samples were randomly collected from one of every four potential participants using sterile, well-labelled plastic sample containers until the desired sample size was reached, over a period of 6 months. One on-the-spot sputum sample was collected from each participant and transported in a cool transport flask to the laboratory, where it was refrigerated prior to processing. Oral interviews were also conducted among the participants to obtain socio-demographic data, including age, occupation and duration of involvement in livestock trade and probable symptoms of TB.

**Figure 1** Overcrowded setting with cattle and livestock traders in close proximity at the Akinyele Cattle Market.

Ethical clearance for the study was obtained from the Oyo State Ministry of Health Ethical Review Board.

The $\chi^2$ test was used to test for associations between infection and duration of involvement in livestock trade, and TB symptoms. Correlation analysis was used to analyse the correlation between infection rates and the age groups of the participants.

### *Processing of samples*

Sputum samples were processed using the Becton Dickinson digestion and decontamination procedure (BD, Sparks, MD, USA).[13] The concentrate thus obtained was inoculated onto Löwenstein-Jensen slopes with pyruvate and/or glycerol and incubated at 37°C for 12 weeks. Isolates were harvested for deletion typing and spoligotyping by scraping the growth from a slope into 200 µl of 7H9 Middlebrook (broth) and heating at 80°C for 1 h.

### *Deletion analysis*

The isolates obtained were subjected to deletion typing to identify *M. tuberculosis* complex (MTC) isolates by the polymerase chain reaction (PCR) amplification of genomic regions of difference (RD), as described elsewhere.[14] Briefly, two primer sets were used; each set consisted of two flanking primers and an internal primer. The first set of primers included the RD4 and RD9 primers and the second set the RD $1^{mic}$ and RD $2^{seal}$ primers. RD9 deletion was used to discriminate *M. tuberculosis* from other MTC. Those with a deletion at this region were further investigated with primers targeted at RD4. This reaction discriminates between *M. bovis, M. caprae* and other MTC. The PCR mix contained 12.5 µl of Hotstart Taq multiplex master mix (Qiagen, Hilden, Germany), 5 µl of Q-solution (Qiagen), 3.5 µl of RNAse free water and 0.5 µl of each 50 pM primer. The reaction was run at a denaturation temperature of 95°C for 15 min, 40 cycles at 94°C for 1 min, at 62°C for 1 min and at 72°C for 1 min, with a final elongation step at 72°C for 10 min and a holding step at 4°C until use. PCR products were then separated by electrophoresis using 3% agarose gel at 10 V/cm for 2 h.

### *Spoligotyping*

Four of the seven isolates earlier identified by deletion analysis as belonging to MTC were further analysed by spoligotyping,[14] following a standardised international method described elsewhere[15] using a commercially available kit (Isogen Biosciences BV, Maarsen, The Netherlands). *M. tuberculosis* H37Rv, *M. bovis* bacille Calmette-Guérin and sterile distilled water were used as controls.

**Table** Results of deletion typing with respect to occupation and age range, length of involvement in livestock trading and presence of prolonged cough

| | Deletion typing *M. bovis* *n* | *M. tuberculosis* *n* | Total *n* (%) | |
|---|---|---|---|---|
| Occupation, age group, years | | | | |
| Livestock trader | | | | |
| 0–19 | 0 | 0 | 0 | $r = 0.999$ |
| 20–39 | 1 | 1 | 2 (28.6) | $P < 0.01$ |
| 40–59 | 0 | 3 | 3 (42.9) | (2-tailed) |
| ⩾60 | 0 | 1 | 1 (14.3) | |
| Other | | | | |
| 0–19 | 0 | 0 | 0 | |
| 20–39 | 0 | 0 | 0 | |
| 40–59 | 1 | 0 | 1 (14.3) | |
| ⩾60 | 0 | 0 | 0 | |
| Length of involvement in livestock trading, years | | | | |
| Livestock trader | | | | |
| <1 | 0 | 0 | 0 | $P = 0.000$ |
| 1–3 | 0 | 1 | 1 (14.3) | |
| >3 | 1 | 4 | 5 (71.4) | |
| Others | | | | |
| <1 | 0 | 0 | 0 | |
| 1–3 | 0 | 0 | 0 | |
| >3 | 1 | 0 | 1 (14.3) | |
| Prolonged cough | | | | |
| Livestock trader | | | | |
| Cough | 1 | 3 | 4 (57.1) | $P = 0.006$ |
| No cough | 0 | 2 | 2 (28.6) | |
| Others | | | | |
| Cough | 1 | 0 | 1 (14.3) | |
| No cough | 0 | 0 | 0 | |
| Total | 2 | 5 | 7 | |

## RESULTS

Among the 73 livestock traders screened, samples from only 70 participants were viable for mycobacterial investigation and processed in this study. In all, 10% (7/70) of those screened were positive for acid-fast bacilli by culture. Molecular analysis using deletion typing identified five *M. tuberculosis* and two *M. bovis* isolates (Table); further characterisation by spoligotyping identified two *M. bovis* and two *M. tuberculosis* isolates from the four isolates available for this analysis out of the initial seven (Figure 2).

The 70 participants screened were all males and most (98.57%) were adults; 90% (63/70) of the participants were involved in the livestock trade, while the others 10%, although living and working in the cattle market, had different but associated occupations. Furthermore, 91% of the participants had been engaged in livestock trade for more than 3 years; the age groups most affected were 20–39 years and 40–59 years, with a positive correlation between infection and age ($r = 0.999$, $P < 0.01$; Table). In addition, 85.71% (6/7) of those infected had been involved in the livestock trade for >3 years, a factor that was statistically significant ($P = 0.000$; Table, Figure 3).

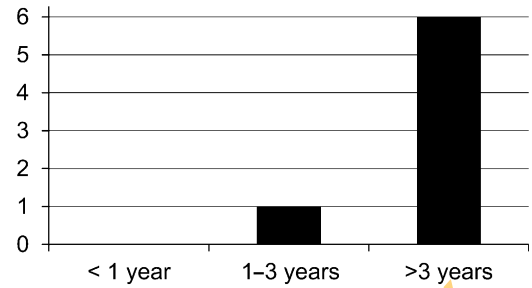


**Figure 3** Number of individuals infected with tuberculosis based on the length of involvement in livestock trading.

A significantly higher proportion (71%) of those who tested positive (57% of livestock traders and 14% of non-livestock traders) had a history of cough of ⩾3 weeks ($P = 0.006$; Table).

## DISCUSSION

The results of this study reiterate the zoonotic nature of BTB and further highlight the pulmonary route as the main route of *M. bovis* transmission in occupationally exposed individuals. Our findings also re-emphasise the risks posed to occupationally exposed persons, particularly livestock traders, butchers, meat processors and animal health workers. This is in agreement with previous studies where *M. bovis* was isolated from occupationally exposed individuals.[16–20]

In our study, the two *M. bovis* strains isolated showed distinct spoligopatterns (Figure 2), suggesting that they originated from different sources. The spoligotype of strain LVT1 is identical to SB1432, per the www.mbovis.org database, while LVT2 had spoligotype SB0944, previously reported in cattle in Nigeria,[10] Chad, Cameroon,[21] Mali,[22] France[23] and the United States.[24] These spoligotypes belong to the

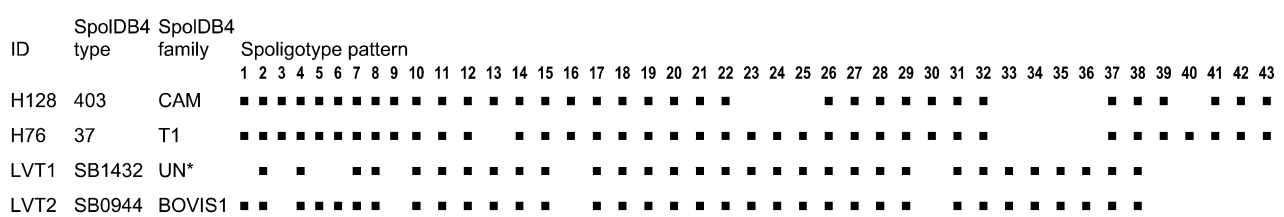


**Figure 2** Spoligotype patterns of *M. tuberculosis* and *M. bovis* isolates from livestock workers in Ibadan. *Spoligopatterns not reported on the SpolDB4 database.

African 1 (Af1) clonal complex, which is characterised by the absence of spacer 30,[25] with the SB0944 spoligotype reported to be prevalent and ubiquitous in West Africa.[25] The isolation of these *M. bovis* strains from affected individuals unequivocally confirms the zoonotic transmission of BTB among those working and living in livestock markets in Nigeria. Furthermore, the SB0944 spoligotype had earlier been isolated from infected sedentary and trade cattle in Ibadan.[10,11] It should be noted, however, that there are divergent spoligotypes of *M. bovis* in the local cattle population in Nigeria.[11,26]

The two *M. tuberculosis* strains confirmed by spoligotyping were identified as belonging to the LAM_10 CAM and T1 families using spotclust database. Earlier reports showed that LAM_10 CAM strains have been isolated from humans in Nigeria[10] and other countries of the world, including Burkina Faso,[27] Sierra Leone,[28] Niger and Ivory Coast as well as many parts of Europe,[23] with the T1 family earlier reported in Nigeria.[29,30]

The 10% prevalence of TB among those screened was relatively high, and likely attributable to the congested communal lifestyle among these workers, a factor that may fuel human-to-human transmission of infection. Most livestock traders live within the same micro-environment characterised by overcrowding, which may further facilitate spread of infection from diseased to healthy individuals. As the majority (86%) of those infected had been involved in the livestock trade for more than 3 years ($P = 0.000$; Table, Figure 3), the long duration of interactions with infected animals also seems to increase the risk of zoonotic transmission of infection. In the light of the earlier report by Cadmus et al.[10] of *M. tuberculosis* isolation from cattle, coupled with human and animal co-habitation in the same study area, this increases the risk of infection through the pulmonary route, rather than by ingestion. A cursory look at those infected reveals that the majority (86%) were in the age groups 20–39 years and 40–59 years ($r = 0.999$, $P < 0.01$; Table). These age groups are also most vulnerable to HIV and social vices such as smoking and drug addiction, which potentiate TB infection. However, as information about these risk factors was not captured in this present study, little can be said about their role in this setting; other reports have implicated their role in exacerbating the progression of *M. bovis* infection to full-blown disease.[16,31]

Our results also show that cough of >3 weeks was highly significant for TB infection ($P = 0.006$; Table). This finding is in agreement with current World Health Organization guidelines which stipulate that cough of >2 weeks is symptomatic of TB.[32] However, as there was very little public health awareness about TB symptoms and signs among this group of individuals, study subjects failed to visit nearby health care facilities despite the availability of free anti-tuberculosis treatment in Nigeria. Based on this finding, it would therefore be expedient to initiate active TB case finding among livestock workers and improve knowledge about infectious diseases in the population.

An interesting finding of this study is the isolation of *M. bovis* from one of the participants who was not directly involved in livestock trade, but who had been in close proximity with those engaged in the business. This finding suggests possible human-to-human transmission of the disease. However, as the strain isolated from the other participant is different from this patient, it cannot be proved that both individuals were infected by the same animal. Our results show that, due to the presence of different strains of *M. bovis* among infected cattle in Nigeria, individuals can be directly infected either by sick animals or by the humans who are exposed to these infected animals. Further investigation of human-to-human transmission of *M. bovis* in this setting is needed.

Some of the limitations of this study include the fact that clinical parameters that could indicate suspicion of ongoing extra-pulmonary TB, such as chronic lymphadenopathy (especially cervical), ascites with lymphocyte predominance or joint inflammation (monoarticular) with negative bacterial cultures, were not obtained in this study. The two cases of *M. bovis* infection reported were due to pulmonary TB. Hence infection was through inhalation, rather than the generally assumed extra-pulmonary route associated with the consumption of unpasteurised milk and other infected meat or dairy products. This therefore suggests possible direct animal-to-human or human-to-human transmission of *M. bovis* infection. Our findings are in line with earlier reports from a study in the United Kingdom on person-to-person transmission among six clusters of cases.[33] As approximately 90% of TB infections in cattle affects the respiratory route,[34] TB spreads primarily by the aerogenic pathway among cattle, and those directly in contact with them are more likely to develop pulmonary disease than an alimentary form.[35]

## CONCLUSIONS

The occurrence of pulmonary *M. bovis* infection among livestock traders is a matter of concern as it may lead to further disease transmission to the community at large, in addition to extra-pulmonary infection due to consumption of contaminated food. This may have serious public health consequences given the soaring HIV/AIDS prevalence,[7] poor living conditions and inadequate health care services likely to compromise patient care in Nigeria. We therefore urgently recommend intensive public health awareness about BTB and its zoonotic implications in endemic settings so as to provide optimal diagnosis and treatment of those infected with *M. bovis*.

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## RÉSUMÉ

**CONTEXTE :** La tuberculose (TB) bovine est endémique dans le bétail au Nigéria. Les éleveurs encourent un risque d'infection par *Mycobacterium bovis* et ne sont pas conscients de leur statut de santé.

**OBJECTIF :** Déterminer la présence d'une infection par *M. bovis* chez les éleveurs.

**SCHÉMA :** On a mené chez les marchands de bétail une étude transversale concernant la TB par le dépistage d'échantillons de crachats en utilisant une méthode d'échantillonnage simple au hasard couplée avec une interview dans la supposition d'une infection tuberculeuse pulmonaire subclinique. Les échantillons ont été mis en culture et les isolats analysés par des techniques de typage moléculaire.

**RÉSULTATS :** Au total, une culture positive indicatrice d'un complexe *M. tuberculosis* a été découverte chez 10% (7/70) des marchands de bétail. Ces souches ont été différenciées par typage des délétions en *M. bovis* ($n$ = 2) et *M. tuberculosis* ($n$ = 5). Des analyses complémentaires par spoligotypage de *M. bovis* et de deux isolats disponibles de *M. tuberculosis* ont classifié les souches respectivement comme SB1432 et SB09444 et LAM_10 CAM et T1 au moyen des bases de donnee www.mbovis.org et de spotclust. Les facteurs de risque d'infection ont été une toux prolongée et un travail de plus de 3 ans dans le commerce de bétail.

**CONCLUSION :** Nous confirmons une infection pulmonaire par *M. bovis* non notée jusqu'ici chez les marchands de bétail du Nigéria. Des études complémentaires sont nécessaires au sujet du rôle des travailleurs exposés professionnellement dans la transmission de l'infection par *M. bovis* vers une collectivité plus large.

## RESUMEN

**MARCO DE REFERENCIA:** La tuberculosis (TB) bovina es endémica en el ganado vacuno de Nigeria. Las personas que trabajan con este ganado están en riesgo de contraer la infección por *Mycobacterium bovis* y desconocen su estado de salud.

**OBJETIVO:** Determinar la presencia de infección pulmonar por *M. bovis* en las personas que trabajan con el ganado vacuno.

**MÉTODOS:** Se llevó a cabo un estudio transversal de los comerciantes de ganado, en el cual se practicó una investigación exhaustiva de la TB en las muestras de esputo mediante un método de muestreo aleatorio, además de entrevistas presenciales, con base en la hipótesis de una infección pulmonar tuberculosa asintomática. Se cultivaron las muestras y los aislados se tipificaron mediante técnicas moleculares.

**RESULTADOS:** El 10% de los comerciantes de ganado (7/70) presentó cultivos positivos para el complejo *M. tuberculosis*; el genotipado por deleción detectó cepas de *M. bovis* ($n$ = 2) y *M. tuberculosis* ($n$ = 5). Los análisis por espoligotipado de los aislados de *M. bovis* y de los dos aislados disponibles de *M. tuberculosis* permitieron clasificar las cepas como SB1432 y SB09444 y LAM_10 CAM y T1 a partir de las bases de datos de www.mbovis.org y spotclust, respectivamente. Los factores de riesgo de contraer la infección fueron la tos prolongada y más de 3 años de trabajo en el comercio del ganado.

**CONCLUSIÓN:** Se confirmó la presencia desapercibida de infección pulmonar por *M. bovis* en los comerciantes de ganado en Nigeria. Se precisan nuevos estudios que examinen la contribución de estos trabajadores con exposición ocupacional a *M. bovis* a la trasmisión de la infección en la comunidad general.