Original article

Isolation of *Mycobacterium tuberculosis* from livestock workers and implications for zooanthroponotic transmission in Ibadan, South-western Nigeria

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Keywords

Tuberculosis • Non-tuberculous mycobacteria • Zoonoses • Molecular characterization • Nigeria

Summary

Introduction. *Tuberculosis (TB) remains a public health problem in sub-Saharan Africa coupled with dearth of information about the disease among livestock workers at risk of infection. We determined the prevalence of pulmonary TB infection and factors associated with its occurrence among livestock workers in south-western Nigeria.*
Methods. *A cross-sectional study was conducted using active case-finding among livestock workers with sub-clinical pulmonary TB between August 2014 and March 2015. Sputum samples were cultured and subjected to a two-step multiplex-PCR technique based on genus-typing and genomic regions-of-difference. Interviewer-administered questionnaire was utilized in assessing worker's TB related knowledge and practices. Data were analysed using STATA 12.*
Results. *Overall, 206 livestock workers (traders = 136; butchers = 70) were screened; 5.1% (7/136) of the traders and 7.1% (5/70) of the butchers had positive mycobacterial cultures. Molecular techniques identified one Mycobacterium tuberculosis with six non-tuberculous mycobacteria (NTM) from the traders and four M. tuberculosis with one NTM from the butchers. Participants within the age range of 50 years and above were at higher risk of being infected with TB (OR = 7.7; 95% CI: 1.7-35.6) and majority had poor knowledge and practices regarding TB.*
Conclusions. *We confirmed M. tuberculosis as the cause of pulmonary TB among the livestock workers, with implications for zooanthroponotic transmission of the disease along the human-animal ecosystem interface in Nigeria.*

Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is prevalent worldwide. However, occurrence of the infection is more pronounced in developing countries where surveillance and control measures are inadequate or in some cases nonexistent [1]. Research shows that one-quarter (25%) of the estimated global TB incidence occurs in the African region and majority (70%) of the top thirty countries with the highest burden of the disease are in Africa and Asia [1]. Tuberculosis has great impact on the already exacerbated health problems in sub-Sahara Africa, with estimated TB case fatality rate increasing from 5% to 20% in 2015 [1]. In Africa, the true burden of the disease is unknown largely due to poor and inefficient case reporting and documentation, limited diagnostics and laboratory infrastructure as well as non-commitment of governments [2]. Consequently, the WHO reports are mere underestimation of the true picture of the state of the disease in Africa [1, 3]. Globally, the prevalence of TB among livestock workers is not known, however studies carried out in different parts of the world have reported the isolation of *M. bovis* from livestock workers [2, 4-6]. Considering these pockets of reports of isolation of *M. bovis* from the occupationally exposed, it becomes imperative to investigate the actual prevalence of infection among this group of individuals bearing in mind the key role they play in the disease transmission chain especially in sub-Sahara Africa and other developing countries.

Nigeria, with an estimated population of 196.2 million, ranks 7th among countries with high TB burden, and with South Africa each accounting for 4% of global burden of the disease in 2016 [1]. In Nigeria, like majority of other African countries, despite the exposure of livestock workers (cattle rearers, traders and butchers) to cattle with bovine TB [4, 7], limited studies have investigated possible ongoing zoonotic TB infection among these workers. In addition, due to poor provisions for livestock workers within the healthcare scheme in the country, they are largely neglected and underserved thereby exposing them to a myriad of zoonotic diseases. This constitutes a serious public health concern, since these workers are often in continuous and in some instances very close contact with the general public; thus, serving as a potential source of human to human transmission of zoonotic diseases.

More importantly, these workers are generally uneducated, exhibit poor hygiene practices, indulge in alcoholism, drug addiction and have poor medical care-seeking behavior. A recent study revealed important knowledge gaps with poor practices about zoonotic TB prevention among livestock workers in Nigeria [8]. Coupled with these are the poor state of infrastructures at the cattle markets and abattoirs/slaughter slabs settings where they work [9], which are factors that could facilitate transmission of TB within the human-animal ecosystem interface in Nigeria. We therefore investigated the epidemiology of TB amongst livestock workers operating in a cattle market and an abattoir in southwestern Nigeria. Information obtained will assist in providing concrete control measures against the disease.

Materials and methods

Study location

The study was conducted at the Akinyele Cattle Market (ACM) and Abattoir. Akinyele is located in the northern part of Ibadan metropolis. The market is a major rallying point for cattle brought from various Northern states of Nigeria and neighbouring African countries. Due to poor infrastructure at the cattle market and abattoir, overcrowding and congestion of humans and cattle are common features [4]. Approximately, there are about 800 people within the ACM, comprising mainly of cattle traders, transporters (drivers and those who assist in offloading and loading of cattle) and members of the general public who patronize the traders. Also, present within the ACM vicinity are food vendors, veterinarians and other animal healthcare workers. These veterinarians and animal health workers constituted less than 2% of the population in the market. More importantly, the facilities are managed by largely uneducated workers and are operated under unhygienic conditions [4]. As such; transmission of zoonotic infections between humans and cattle may be easily facilitated.

Study design, sample size and sampling

A cross-sectional study involving active case finding among livestock workers with high risk of sub-clinical pulmonary TB was carried out between August 2014 and March 2015. Using an earlier reported prevalence of 5% among butchers in Ibadan [4], the estimated sample size was 73 participants (at both the market and abattoir). Eligible participants were informed about the aim of the study and verbal/written consent was obtained from willing participants. In all, 136 and 70 participants respectively, were screened at the cattle market (traders) and abattoir (butchers). Sputum samples (one per participant) were simultaneously collected from every study participant using properly labeled sterile plastic sample containers with coded identifiers to maintain confidentiality.

Sample processing

Bacteriology

Sputum samples were processed based on the Becton Dickinson digestion and decontamination procedure [10]. The final concentrate thereby obtained was inoculated onto paired Löwenstein-Jensen slopes with pyruvate and glycerol, respectively and incubated at 37ºC for 12 weeks. Isolates were harvested for genus and deletion typing by scraping the growth from a slope into 200 µl of 7H9 Middlebrook (broth) and heating at 80ºC for 1 h.

Genus typing

The isolates obtained were subjected to genus typing to identify members of the *M. tuberculosis* complex (MTC) as earlier described [11]. Briefly, heat-killed mycobacterial isolates from culture-positive samples were used as a DNA template. DNA amplification was done with the reaction mixture containing 2 µl DNA template, 5 µl of Q-buffer, 10X Buffer, 25 mM MgCl2, 4µl × 10 mM dNTPs, 0.5 µl of each primer (50 pmol/µl; four primer pairs were used) and 0.2 µl GoTaq Flexi DNA polymerase (Promega Madison, USA). The mixture was made up to 25 µl with ultra-pure water and heated in a Thermal Controller (MyGene Series Peltier, Model MG 96+) using the following amplification programme: 95°C for 10 min for enzyme activation, followed by 35 cycles at 95°C for 1 min for denaturation, 61°C for 0.5 min for annealing, and 72°C for 2 min for extension. After the last cycle, the samples were incubated at 72°C for 10 min. Thereafter, PCR amplification products were fractionated by electrophoresis in 3.0% agarose in 1 X TBE pH 8·3 at 6 V/cm for 4 h, and visualized by staining with Gel Red.

Deletion typing

The final products obtained from genus typing were further subjected to deletion typing to identify specific strains of MTC based on the presence or absence of genomic region of difference (RD) as described elsewhere [12]. Each reaction mixture consisted 1µl DNA template, 5 µl Q-buffer, 2.5 µl × 10 buffer, 2 µl 25 mM MgCl2, 4 µl × 10 mM dNTPs, 0.5 µl of each primer (50 pmol/µl), 0.125 µl GoTaq Flexi DNA polymerase (Promega Madison, USA), and was made up to 25 µl with ultra-pure water. The reaction mixture was amplified inside a Thermal Controller (MyGene Series Peltier, Model MG 96+) using; 95°C for 10 min, followed by 45 cycles at 95°C for 1 min, 61°C for 0.5 min and 72°C for 2 min. After the last cycle, the samples were incubated at 72°C for 10 min. The PCR product was finally amplified for visualization as previously described [12].

Questionnaire survey

The knowledge and practices of the respondents about zoonotic TB were investigated using an interviewer-administered structured questionnaire. The questionnaire was pretested and modified to improve clarity. The questionnaire consists of both open and close-ended questions. Information obtained included socio-demographic characteristics of livestock workers as well as factors associated with zoonotic TB transmission. In order to assess the knowledge and practices of respondents, scores between "zero and one" were respectively assigned to each correctly and incorrectly answered question. All questions answered completely and accurately attracted an overall score of 10 and 12 for each of the zoonotic TB prevention knowledge and practices, respectively per respondent. A knowledgeable respondent is regarded as the one who is able to score six or more out of the knowledge questions, and a respondent is said to have exhibited good practices if he/she is able to score eight or more of the practice questions. Each of these scores is equivalent to about 60% or more.

Data analysis

Data were analysed using STATA 12 software (StataCorp, College Station, Tx). Bivariate and multivariate analyses (unconditional logistic regression) were used to test for the association between isolation of mycobacterial species and potential risk factors. Using the isolation of mycobacterial species as the outcome measure, variables with p-value of 0.20 [13] or less in the bivariate analysis and biological plausibility were included in multivariate models. Odds ratios (OR) and 95% confidence intervals (CI) were estimated. All tests were two-tailed and statistical significance was set at $p \leq 0.05$.

Ethical approval

Ethical approval for the study was obtained from the University of Ibadan/University College Hospital Ethics Committee with approval number: NHREC/05/01/2008a. Participants were enrolled after informed consent was obtained from them. Participants who were confirmed culture positive for TB were referred to the nearest Directly Observed Treatment Short Course Centres for treatment according to the Nigerian National Guidelines for management of TB.

Results

Socio-demographic characteristics of study participants

More than half (66.0%) of the livestock workers screened were traders (cattle marketers) and aged between 20 and 49 years (69.9%). Most of the respondents were males (83.5%) while over half (59.7%) had formal education and were more than five years in the business (56.3%) (Tab. I).

Bacteriology and molecular typing

A total of 12 mycobacterial species were obtained using genus typing. Seven (58.3%) were non-tuberculous mycobacteria (NTM) while five were members of the MTC family (Fig. 1). However, deletion typing showed that all the five MTC were *M. tuberculosis* (Tab. II, Fig. 2). In all, one *M. tuberculosis* and six NTM were isolated from the traders, while four *M. tuberculosis* and one NTM were recovered from the butchers (Tab. II).

Knowledge and practice about zoonotic TB

Majority of the livestock workers had poor knowledge (62.6%) and showed poor practices (81.1%) in relation to TB (Tab. I). Only 21% of the respondents knew that man and animals could share common diseases, while 38% knew that TB could be contracted from animals. However, only 22% knew TB is curable (Tab. III). In addition, 58% of the respondents had received BCG vaccination, 18% had been tested for TB while less than half would seek modern medicine in case they have TB (Tab. IV).

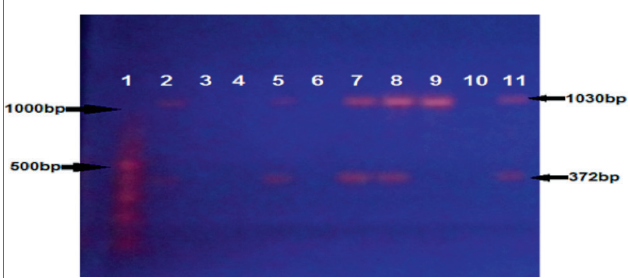
Prevalence and associated factors of TB among study participants

The bivariate analysis revealed association between age and infection with TB. Individuals who were aged 50 years and above were at higher risk of being infected with *M. tuberculosis* (OR = 6.0; 95% CI: 1.6-22.1) (Tab. V). The multivariable logistic regression analysis identified age, educational status and knowledge about TB as positive predictors of mycobacterial species infection among livestock workers (Tab. VI). Our findings revealed that individuals older than 50 years of age were more likely to be infected when compared to those aged between 20-49

Tab. I. Characteristics of livestock workers screened for the study.

Variables	Category	Frequency	Percentage
Population	Traders Butchers	136 70	66.0 34.0
Sex	Male Female	172 34	83.5 16.5
Age	0-19 20-49 ≥ 50	9 144 53	4.4 69.9 25.7
Education status	No formal education Primary Secondary Tertiary	83 50 55 18	40.3 24.3 26.7 8.7
Duration in business	0-5 6-10 ≥ 11	90 66 50	43.7 32.0 24.3
Knowledge	Poor Good	129 77	62.6 37.4
Practices	Poor Good	167 39	81.1 18.9

Fig. 1. Genus typing banding pattern of isolates Lane 1 = DNA ladder; Lanes 2, 5, 7 & 8 = MTC; Lane 9 = NTM; Lane 10 = negative control; Lane 11 = positive control (H37Rv).

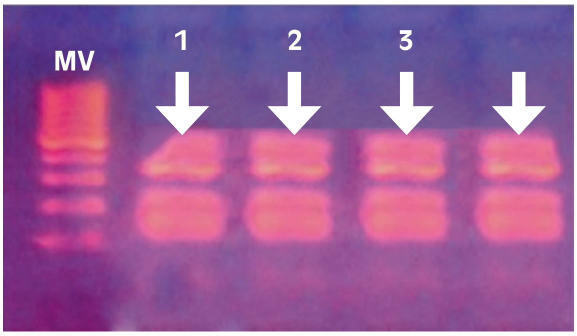


Tab. II. Number of mycobacterial isolates cultured and confirmed from livestock workers.

Diagnostic tools	Traders	Butchers	Total
Culture	7	5	12
Genus typing	1 MTC & 6 NTM	4 MTC & 1 NTM	5 MTC & 7 NTM
Deletion typing	1 Mtb	4 Mtb	5 Mtb

MTC: Mycobacterium tuberculosis complex; NTM: non-tuberculous mycobacteria; Mtb: Mycobacterium tuberculosis.

Fig. 2. Deletion typing banding pattern for MTC isolates. Lane 1, 2, 3 = Mycobacterium tuberculosis; Lanes 4= positive control (H37Rv).



years (OR = 7.7; 95% CI: 1.7-35.6). In addition, those who had secondary school education were less likely to be infected when compared to those without formal education (OR = 0.1; 95% CI: 0.0-0.8). Likewise, individuals with good knowledge of TB were less likely to be infected when compared to those with poor knowledge of the disease (OR = 0.1; 95% CI: 0.0-0.1) (Tab. VI).

Discussion

This study sets out to confirm the possible infection of livestock workers with members of the MTC particularly *M. bovis* given their high risk of exposure to BTB. Coincidentally, instead of *M. bovis*, five cases of *M. tuberculosis* infection were confirmed among livestock traders and butchers with sub-clinical pulmonary TB infections. Since most of these workers work and live under very poor sanitary and environmental health conditions and were unaware of TB, there was increased risk of contracting the disease from infected fellow workers and/or cattle. While we had initially hypothesized *M. bovis* infection among these workers given an earlier report of zoonotic infection in the same setting [4], this was not the case in this study as we only confirmed cases of *M. tuberculosis* infections. This notwithstanding, our findings are of public health importance, since those infected would have remained unidentified and untreated, with the likelihood of spreading the infection to fellow workers and household members. More notably, this becomes important given the poor health care-seeking behaviour in Nigeria and other low-middle income countries [4, 14-16] which may aggravate the burden of TB in these countries.

Poor knowledge of TB has earlier been identified as an important risk factor among hospital patients and the occupationally exposed group [4, 17, 18]. In our study, we observed that majority of the participants had poor knowledge of TB and exhibited practices that could facilitate spread of disease within the abattoir and cattle market settings. Importantly, poor knowledge of TB was significantly associated with *M. tuberculosis* infection among the study participants while individuals who had good knowledge of the disease were less likely to be infected. As observed, a little above half of the respondents had received BCG vaccination despite the fact that this vaccination is recommended for every individual at birth in Nigeria. It is therefore of concern that such a relatively high proportion of these occupationally exposed individuals had not received this vaccination. Worse still, less than half indicated they would seek modern medicine in case they had TB. This suggests that the level of awareness of TB treatment is still low among the occupationally exposed individuals who are at higher risk of the infection.
One major important public health implication of our findings is the possibility of zooanthroponotic infection of cattle and other animals with *M. tuberculosis* in the study setting. Though our current study did not involve simul-

Tab. III. Knowledge of zoonotic TB among livestock workers in Ibadan, Nigeria.

S/N	Questions	Response options	%
B1	Do you know that man and animals can share common diseases?	Yes No	21 89
B2	What type of disease can be contracted?	TB Cancer Ebola AIDS	28 14 36 22
B3	If no, why do you think that man cannot contract disease from animals?	Don't know Not possible	44 56
B4	Do you know that someone can contract TB from animals?	Yes No	38 62
B5	How do you think man can be infected with TB?	Direct contact Consumption of meat and milk products Aerosol	46 37 17
B6	Do you know the clinical signs in processed animals?	Yes No	18 82
B7	What are signs/ symptoms of TB you will observe in a person with TB?	Cough Wasting Sweat in the night Body weakness	36 24 17 23
B8	Is TB curable?	Yes No Don't know	22 48 30
B9	What type of treatment is best for TB?	Modern medicine Traditional medicine Faith healing	46 34 20
B10	Boiling milk before consumption destroy the bacteria causing TB	Yes No Don't Know	24 27 48

Tab. IV. Practices related to zoonotic TB among livestock workers in Ibadan, Nigeria.

S/N	Questions	Response options	%
C1	Have you received BCG vaccination	Yes No	58 42
C2	What do you do to protect yourself from animal infections?	Immunization Use of preventive medicine Good hygiene practices Use of herbs	15 50 25 10
C3	Have you ever been tested for TB	Yes No	18 82
C4	What would you do if you see your slaughtered animal or carcass having TB lesions?	I will sell it I will kill it and bury it I will eat it personally at home	52 26 22
C5	If you have TB where would you go for treatment?	Modern medicine Traditional medicine Faith healing	42 38 20
C6	What type of hygiene practice do you observe to protect yourself from contracting TB from animals?	Putting on protective material while working Limited contact with animals or carcass Washing of hands after touching live or processed animals None of the above	8 12 20 60
C7	Do you boil raw cow milk before drinking?	Yes No	12 88
C8	Do you cohabit with your animals?	Yes No	91 9
C9	Do you pack animal dungs with bare hands	Yes No	89 11
C10	Do you process or handle animal carcasses with unprotected wounds?	Yes No	57 43
C11	What disease condition have you experienced recently?	Cut/sores Cough lasting more than three weeks Wasting None of the above	45 21 14 20
C12	Do you need to go for medical check -up periodically?	Yes No	84 16

taneous screening of cattle for *M. tuberculosis* and other MTC, we cannot preclude the possibility of animal infections with *M. tuberculosis* given the prevalent unhygienic and close human-animal interactions within the setting. More so, earlier reports in Nigeria, Spain, some countries in Africa and Asia [4, 7, 18-20], indicated several cases of animal infections with *M. tuberculosis*. These might not be unconnected with similar deplorable environmental conditions that support zooanthroponotic infections with *M. tuberculosis* given high probability of the risk of pulmonary route of TB transmission/infection among livestock workers and cattle in these settings. More importantly, since the prevalence of TB is high among humans in Nigeria, there is high risk of animal infection and the risk of spill back to humans; thus, posing public health risk via the food chain.

Tab. V. Prevalence of TB among livestock workers screened for the study.

Variables	TB infection		OR	95%CI	P-value
	Positive n (%)	Negative n (%)			
Population					
Traders	7 (58.3)	123 (65.4)	1.0	-	-
Butchers	5 (41.7)	65 (3346)	1.2	0.3-3.8	0.86
Sex					
Male	9 (75.0)	163 (84.0)	1.0	-	-
Female	3 (25.0)	31 (16.0)	1.8	0.4-6.8	0.67
Age					
0-19	0 (0.0)	9 (4.6)	-	-	-
20-49	8 (66.7)	170 (87.6.)	1.0	-	-
≥ 50	4 (33.3)	15 (7.7)	6.0	1.6-22.1	0.01
Education status					
No formal education	8 (66.7)	78 (40.2)	1.0	-	-
Primary	2 (16.7)	48 (24.7)	0.4	0.1-2.0	0.42
Secondary	1 (8.3)	51 (26.3)	0.2	0.0-1.6	0.17
Tertiary	1 (8.3)	17 (8.8)	0.6	0.1-4.9	0.95
Duration in business					
0-5	3 (25.0)	87 (44.9)	1.0	-	-
6-10	4 (33.3)	62 (32.0)	1.9	0.4-8.7	0.67
≥ 11	5 (41.7)	45 (23.2)	3.2	0.7-14.1	0.21
Knowledge					
Poor	10 (83.3)	121 (62.4)	1.0	-	-
Good	2 (16.7)	73 (37.6)	0.3	0.1-1.5	0.24
Practices					
Poor	10 (83.3)	159 (82.0)	1.0	-	-
Good	2 (16.7)	35 (18.0)	1.9	0.5-7.4	0.62

Tab. VI. Multivariable logistic regression analysis of variables significant at 20% level with the main outcome measure (TB infection) in bivariable analysis.

Variables	Category	OR	95%CI	P-value
Age	0-19 20-49 ≥ 50	- 1 7.7	- - 1.7-35.6	- - 0.01
Education	No formal education	1	-	-
Status	Primary Secondary Tertiary	0.3 0.1 1.4	0.1-1.7 0.0-0.8 0.1-15.5	0.17 0.04 0.76
Knowledge	Poor Good	1 0.1	- 0.0 – 0.1	- 0.02

Also, findings from this study revealed that the proportion of mycobacterial species recovered from the abattoir workers was higher than that from the livestock traders. Notably, more *M. tuberculosis* were isolated from the butchers (four) compared to the traders (one). The higher cases among abattoir workers could be attributed to the more congested work place setting obtainable within the enclosed slaughter house facility as compared to the open environment used by the livestock traders, even though overcrowded. Incidentally, this factor has been implicated in the human-to-human transmission of TB in earlier studies [4, 21].

Further, our findings showed that age was a risk factor for mycobacterial infection among study participants with individuals who were 50 years and older having higher likelihood of being infected. Association between age and mycobacterial infection has earlier been reported [22]. Factors associated with old age such as duration of time spent in business and immunocompromised state can predispose this group of individuals to higher risk of infection [4]. In high income countries, it has long been acknowledged that older people are vulnerable to developing TB [23].This fact is seldom recognized in developing countries largely due to factors such as non-specific clinical presentations, knowledge gap about TB, non-consideration of the disease at this extreme age, and late diagnosis [23, 24]. The significance of old age in the epidemiology of TB in humans was reiterated by the 2010 Global Burden of Disease estimates which reported that 57% of all TB deaths worldwide occur among individuals older than 50, with a little more than half of this occurring among 65 and above [23, 25]. In addition, older people (50 years and above) have been identified to account for a large percentage (34%) of Disability-Adjusted Life Years (DALYs) attributable to TB globally [23, 25].

Besides, our results identified educational status as a significant risk factor for TB infection. The individuals with secondary school education were less likely to be infected with TB than those who did not have formal education. Our finding is consistent with previous studies [26, 27] that associated educational status with TB infection. Furthermore, previous reports [28, 29] posited that there exists a direct link between knowledge of TB, which is usually driven by level of education, and risk of being infected. Again, adherence to basic hygiene and protective measures against TB was reported to be common among educationally exposed professionals compared to their non-educated counterparts [17, 27]; thus, making them less exposed to risk of TB infection.

Importantly, we isolated NTM from livestock workers, underscoring the relevance of this group of pathogens in the epidemiology of TB in humans. Several reports have continually linked NTM to pathogenesis of mycobacterial infections in humans causing several clinical manifestations [28-31]. Our finding is of public health concern, especially in Nigeria where laboratory diagnosis of TB is mostly based on smear microscopy which cannot differentiate between MTC and NTM. Consequently, there is a greater tendency of misdiagnosing NTM as MDR-TB since NTM will not ordinarily resolve using the conventional treatment regimen of MTC [29, 32]. Unfortunately, this leads to smear positive patients being placed on MDR-TB second line drugs which are very toxic [33-35].

Despite the findings of this study, there were some limitations. First, we did not carry out molecular typing techniques like spoligotyping and variable number of tandem repeats (VNTR) to further characterize the isolated *M. tuberculosis* and compare them with strains on existing database. Again, we did not carry out drug susceptibility testing on the isolates to confirm their susceptibility patterns. In all, these would have provided some insight into whether they are MDR-TB strains given this emerging public health problem in Nigeria [1]. Also, we did not simultaneously screen cattle from the livestock market and abattoir where the participants were screened. Evidence of direct epi-link on possible ongoing zooanthroponotic transmission of *M. tuberculosis* between the livestock workers and cattle would have been revealed. Though these were beyond the immediate scope of the present study, future epidemiological studies will take care of these gaps.

Conclusions

We report the isolation of *M. tuberculosis* and NTM among livestock workers suspected to have sub-clinical pulmonary TB infections in Ibadan, Nigeria. We also show that *M. tuberculosis* and not *M. bovis* was the incriminating agent responsible for TB among livestock workers in the current study. Furthermore, we identified older age, low educational status and poor knowledge of TB as significant risk factors associated with the infection among livestock workers. Given poor knowledge and practices of these workers towards TB, health threats along the human-animal ecosystem interface may promote zooanthroponotic transmission of *M. tuberculosis* to cattle. On this premise therefore, we advocate a multidisciplinary approach using the One-Health umbrella (i.e. a multidisciplinary platform involving veterinarians and human medics) to provide more insights into zoonotic TB transmission and mitigate its public health threats. There is need for the Ministry of Health in Nigeria to step up awareness campaigns regarding tuberculosis and availability of treatment particularly among the occupationally exposed individuals. Finally, we reiterate the importance of molecular typing methods in the proper identification of MTC and other mycobacterial infections among infected humans and cattle along the human-animal ecosystem interface.

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Conflict of interest statement

None declared.

Authors' contributions

SC conceived the idea of the study and approved the final draft of the manuscript.VA wrote the first draft of the manuscript and carried out the data analysis. AA, NO, MA, JO, HA, EC and VA coordinated screening and sample collection among the livestock workers and writing of the manuscript.

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