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ARTICLE

Mycobacterial Species Identification and Public Health Implications of Tuberculosis Among Nomadic Pastoralists in Three Local Governments of Plateau State, North Central Nigeria.

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SUMMARY

Bovine and human tuberculosis is endemic in Nigeria, and apart from meat inspection at the abattoir, which is not very effective, no control measures are currently practiced against the disease in Nigerian livestock. A study was conducted to determine the level of awareness and knowledge of the public health implications of tuberculosis among pastoralists in some selected Local Government Areas of Plateau State. Majority of the respondents in the study area were aware of tuberculosis and they consumed both raw and boiled milk. However, despite their knowledge of tuberculosis, very few of them vaccinate their children against the disease. Five persons admitted being infected with tuberculosis and 3 (three) of these five were receiving treatment as at the time of questionnaire administration. Smear microscopy and deletion analyses were deployed to detect *Mycobacterium species*. All specimens were however negative by both techniques.

KEY WORDS: Tuberculosis, Nomadic, Cattle, Pastoralists, Awareness

INTRODUCTION

The genus *Mycobacterium* comprises of bacteria which are aerobic, non-motile, and rod-shaped with two distinguishing characteristics; acid-fastness and slow growth (Grange, 1998). Only few species are pathogenic and these include *Mycobacterium tuberculosis* (*M. tuberculosis*) which causes tuberculosis in humans, *Mycobacterium bovis* (*M. bovis*) which causes the disease in cattle, humans and other animals, and *Mycobacterium avium* (*M. avium*)which is pathogenic to birds and some animals such as pigs (Duguid *et al.*, 1980). *Mycobacterium tuberculosis* and *Mycobacterium bovis* are very closely related and are referred to as members of the *Mycobacterium tuberculosis* complex (MTC) of organisms which also includes Bacillus Calmette –Guerin (BCG), *Mycobacterium africanum*, *Mycobacterium microti*, *Mycobacterium canetii* and some nontuberculous *Mycobacteria* (Fifis *et al.*, 1991; Perez *et al.*, 2008). Tuberculosis remains a major health problem affecting millions of people all over the world (Sugawara *et al.*, 2007). It is the world's second commonest cause of death from infectious disease, after HIV/AIDS and most cases are in people aged 15-49 years (Frieden *et al.*, 2003). *Mycobacterium tuberculosis* is the most frequent cause of human tuberculosis but some cases are caused by *Mycobacterium bovis* (WHO, 1994). The prevalence of human tuberculosis due to *Mycobacterium bovis* has been estimated at 3.1% of all human tuberculosis cases worldwide, accounting for 2.1% and 9.4% of pulmonary and extra-

pulmonary tuberculosis cases respectively (Ayele *et al*., 2004). Bovine tuberculosis, caused by *Mycobacterium bovis*, is a highly significant zoonotic disease that can be transmitted through aerosols and by ingestion of unpasteurized milk (OIE, 2005). Infections of humans from cattle are caused by close contact with infected animals or by the consumption of unpasteurized milk or cheese made with raw milk (Martinez *et al*., 2007). In rural areas, livestock farmers live in close contact with cattle which if infected with the agent are sources of preventable infection (Cook *et al*., 1996). Farmers, abattoir workers, butchers and veterinarians are especially at risk of acquiring the disease from animals (Milian-Suazo *et al*., 2002). The high prevalence of human tuberculosis may be due to the high prevalence of tuberculosis in slaughtered cattle and their products consumed by humans (Asiak *et al*., 2007). Frequent cattle movements across borders favor the dissemination of the aetiologic agent (Njanpop-Lafourcade *et al*., 2001). Rapid and reliable diagnostic techniques for the identification of *Mycobacterium bovis* and for its differentiation from other members of the *MTC a*re desirable for accurate diagnosis (Cobos-Marin *et al*., 2003).Because of lack of laboratory facilities in developing countries to effectively isolate and diagnose *Mycobacterium bovis*, its role in the human tuberculosis epidemic fostered by HIV/AIDS is not clearly known (Hilty *et al*., 2005). The incidence of the disease in slaughter house workers and rural people suggests that contact with infected cattle may be responsible (Cobos-Marin *et al*., 2003). In many countries, the epidemiological situation of bovine tuberculosis is unknown, with no surveillance or control implemented; hence there is the need to undertake vigorous control and eradication programmes using the appropriate and recommended methods (Benkirane, 2004). Although bovine tuberculosis is known to be common in Africa, control policies have not been enforced in many countries due to cost implications, lack of capacity, and infrastructure limitations (Muller *et al*., 2009). Nigeria is reported to have the fourth highest burden of human tuberculosis in the world, with an incidence of 304 cases per 100,000 and a mortality rate of 89 per 100,000 in 2002 (Cadmus *et al*., 2006). A prevalence rate of 1.52% was reported from cattle slaughtered at Maiduguri abattoir and older animals were the most affected especially those above 5 years (Aliyu *et al*., 1992). A seroprevalence of 45.7% in slaughtered cattle and 13% from sedentary cattle was also reported in south western Nigeria (Asiak *et al*., 2007).
This study was conducted to determine the knowledge of tuberculosis and its zoonotic implications among nomadic pastoralists in three local government areas of Plateau State, to suggest measures of controlling the disease in cattle and humans in the study areaand to genetically characterize the mycobacterial species circulating in the area.

MATERIALS AND METHODS

Study area and Study group

Plateau State is located in the north central zone of Nigeria within latitude 80° 22" North and 100° 24" North and longitude 80° 32" East and 100° 24" East. The three local governments (LGs) selected for this study are located in the upper Plateau where the weather is coolest. Thirty one households that kept cattle from three LGs (Jos North, Jos South, Barkin Ladi) in Plateau State were selected for the study. Ten households each were selected in Jos North and Barkin Ladi LGs while in Jos South LG 11 households were selected. Closed-ended questionnaires were designed and administered to herd owners.

Chairmen of Miyetti Allah Cattle Breeders Association and community leaders were used as entry points in the communities.

Interviews

Oral interviews were also conducted with individual herd owners, butchers and cattle traders. Simple questions regarding livestock and family health as well as the knowledge of tuberculosis were asked. Prior to the administration of the questionnaires and conduction of interviews, detailed explanations were given to the respondents and interviewees regarding the purpose of the study.

Sample Collection (Milk)

Milk samples were collected randomly from ninety nine (99) lactating cows in the three local government areas that made up the study area. Three (3) samples were randomly collected from each of the thirty one (31) selected herds. The milk samples (10ml each) were collected directly from properly cleaned and disinfected teats of the selected cows into sterile top screw cap plastic specimen containers. The samples were labelled properly and placed on ice packs before transportation to the laboratory for acid fast test.

Sample Collection (Sputum)

One hundred (100) sputum samples were collected in the study area from abattoir workers, butchers and herd owners from the thirty one (31) selected herds into sterile plastic containers with top screw caps and labeled for proper identification. The samples were transported on ice to the laboratory for acid fast test. Approximately 1ml of sputum and a drop of milk was taken from each of the samples (milk and sputum) and used for acid- fast test (Ziehl-Neelsen Test) while the remaining portions were stored at -20°C for PCR.

Ziehl-Neelsen Test

Acid Fast Test (Ziehl-Neelsen Test) was carried out according to the method described by Baker and Silverton (1985). Smears were prepared from a small portion of each of the samples using clean frosted microscope slides and these were fixed by heating with a gas flame. Following fixation, slides were flooded with carbol fuchsin this was followed by heating until the stain began to steam but was not allowed to boil. Slides were washed with water and decolorized with 3% acid alcohol before methylene blue was applied. This was followed by washing again for about 2 minutes; slides were air-dried and observed using a light microscope with ×100 objective using oil immersion for the presence of acid-fast bacilli and the results recorded accordingly.

DNA Extraction from Milk and Sputum

DNA was extracted using kit extraction (Zymo Research, U.S.A, distributed by Inqaba Biotechnical Industries, South Africa) following manufacturer`s instructions. Samples stored at -20°C were allowed to thaw at room temperature and 100µl of each transferred into 1.5ml microcentrifuge tube. About 95µl of 2X digestion buffer and 5µl of proteinase K (20mg/ml) were added and the tubes incubated at 55°C for 20 minutes. 700µl of genomic lysis buffer was added and mixed by vortexing. The mixture was transferred to a spin column in a collection tube and centrifuged at 10,000 rpm for one minute. Then 200µl DNA- pre wash buffer was added to the spin column in a new collection tube and centrifuged at 10, 000 rpm for one minute. 400µl of g- DNA buffer was then added to the spin column and centrifuged at 10,000 rpm for one minute. The spin column was transferred to a new, clean, well labeled 1.5ml microcentrifuge tube and 70µl of DNA elution buffer added.

The tubes were incubated for 5 minutes at room temperature before centrifugation at 14,000 rpm for 30 seconds. The eluted DNA (70µl) was stored at -20^{oC} until needed for molecular analysis.

Deletion Analysis

Deletion analysis was carried out as described by Warren *et al.* (2006), for the purpose of speciation using four primers namely RD1, RD4, RD9 and RD12.

Table I: Primer sequences used for deletion analysis

Primer sequence	RD	M. tb	M. bovis	M. africanum
AAGCGGTTGCCGCCGACCGACC	1	present	present	present
CTGGCTATATTCCTGGGCCCGG	1	(146bp)	(146bp)	(146bp)
GAGGCGATCTGGCGGTTTGGGG	1			
ATGTGCGAGCTGAGCGATG	4	present	absent	present
TGTACTATGCTGACCATGCG	4	(172bp)	(268bp)	(172bp)
AAAGGAGCACCATCGTCCAC	4			
CAAGTTGCCGTTTCGAGCC	9	present	absent	absent
CAATGTTTGTTGCGCTGC	9	(235bp)	(108bp)	(108bp)
GCTACCCTCGACCAAGTGTT	9			
GGGAGCCCAGCATTTACCTC	12	present	absent	present
GTGTTGCGGGAATTACTCGG	12	(369bp)	(306bp)	(369bp)
AGCAGGAGCGGTTGGATATTC	12			

The PCR reaction contained 2.5µl green Gotag® flexir buffer (Promega, U.S.A), 2.5µl 25mM $MgCl_2$ 0.5µl 10mM dNTPs, 0.5µl each of primers (10pmol/ml) forward, reverse, and internal), 14.25µl of Nuclease- free water, and 1µl of template DNA, making a final volume of 25µl. Amplification was initiated by initial denaturation at 96°C for 15minutes and this was followed by 40 cycles of 96°c for 1minute, 62°c for 1minute, and 72°c for 1minute. This was followed by incubation for a final extension at 72°c for 10 minutes. The PCR was carried out in a Thermal cycler (Applied Biosystems- Gene Amp PCR system 2700). PCR products were fractionated electrophoretically in 2% agarose gel in 1x TBE buffer, pH 8.3 for 1hour 30 minutes, and visualized under UV light after staining with ethidium bromide. The staining was done by immersing the gel in a solution of ethidium bromide in a shaker for 15 minutes. The size of the amplicons was determined by comparison with a 100bp DNA ladder.

RESULTS

Interviews and Questionnaire Administration

Table II: Level of awareness of tuberculosis among herdsmen in 31 households interviewed

Parameter	Yes	(%)	No	(%)	Sometimes
Drinking of raw milk	16	(51.6)	5	(16.1)	10 (32.3)
Vaccination against TB	6	(19.4)	25	(80.6)	Nil
Awareness of TB	28	(92.3)	3	(9.7)	Nil
Recorded cases of TB	5	(16.1)	26	(83.8)	Nil
Currently on treatment	3	(9.7)	28	(92.3)	Nil

Key: TB =Tuberculosis

Table III: Distribution of respondents from households in the study area

	Local government areas								
	Jos north			Jos south			Barkin ladi		
Parameter	**Yes (%)**	**No (%)**	**S/T (%)**	**Yes (%)**	**No (%)**	**S/T (%)**	**Yes (%)**	**No (%)**	**S/T (%)**
Drinking of raw milk	6 (60)	1 (10)	3 (30)	6 (54)	2 (18.2)	3 (27.3)	4 (40)	2 (20)	4 (40)
Vaccination against TB	1 (10)	9 (90)	2 (18.2)	9 (81.8)	3 (30)	7 (70)			
Awareness of TB	9 (90)	1 (10)		10 (90.9)	1 (9.1)		9 (90)	1 (10)	
Recorded cases of TB	-	10 (100)		3 (27.3)	8 (72.7)		2 (20)	8 (80)	
Cases on treatment	-	10 (100)		1 (9.1)	10 (90.9)		2 (20)	8 (80)	

Key: TB = Tuberculosis, S/T =

Sometimes. Acid Fast Test and Deletion Analysis

All Samples (milk and sputum) were negative by all laboratory tests, both Microscopy and PCR- based molecular technique (Deletion Analysis) used in this study.

DISCUSSION

Nigeria has a cattle population of over 14 million (Biu and Wakawa, 2004) majority of which are concentrated in the Northern part of the country. Nomadic pastoralists own the greater part of these livestock and raise them exclusively on extensive management system. All the 31 households selected for this study practiced purely extensive management system and herds from different communities usually meet at grazing and watering points as well as through cattle routes. This facilitates contacts between herds and favours disease transmission. Cattle owners in the study area live in close association with their animals and men interact more with the animals than women because they are responsible for milking, de-ticking as well as taking the animals out for grazing. Meanwhile, milk (mostly raw) serves as a staple food for the cattle owners especially their children and this may be a potential source of mycobacterial infection to them and their families. Nomads usually keep their animals close to their homes and mostly use cow dung for cooking and as manure for farming, all of these serving as potential sources of zoonotic tuberculosis. Nomadic pastoralists in Africa generally have an obsessive attachment to their animals and their diet which is mostly raw milk, occasionally meat, and in some parts of east Africa, fresh blood, are regarded as sources of infection (Oloya *et al.*, 2007). The milk is usually processed for either family or commercial uses and at this point, the milk is boiled and subsequently fermented in most cases before being taken to the market for sale to the public. Unpasteurized milk and milk products are regarded as the main sources of bovine tuberculosis in humans especially in countries where bovine tuberculosis is prevalent and control measures are patchy or non- existent (Recardo de la Rau-Domenech, 2006). Families, who do not have enough milk from their herds for commercial purposes, usually buy from other households to supplement their own. In this case, milk from different sources are pooled together and this is an avenue of contamination if one of the sources is contaminated. Similarly, during occasions such as wedding and naming ceremonies, families, friends, and neighbours also contribute milk among other household items which is usually served to guests and if such milk come from an infected herd it will serve as a source of infection to all

exposed individuals. Hassanain *et al.* (2009) reported that most human tuberculosis cases caused by *Mycobacterium bovis* occur in young individuals and these result from drinking or handling of contaminated milk. Animals slaughtered during ceremonies can also be a source of tuberculosis to consumers if such animals are infected. Unpasteurized milk, poorly heat- treated meat and close contact with infected animals are regarded as the main sources of infection (Hilty, 2006). Despite the fact that tuberculosis is a well known condition among the high risk group especially the nomadic pastoralists, very few vaccinate their children against the diseases and majority of those affected do not ordinarily seek medical attention. Unfortunately however, most of the members of the households that participated in the study admitted to the consumption of raw milk. The level of awareness of tuberculosis and non-vaccination of children against the disease by pastoralists in the three local governments that made up the study area were similar. More than 50% of the pastoralists in the study area drink raw milk and 80% do not vaccinate their children against tuberculosis despite the fact that over 90% of them are aware of the disease (Table: II). The percentage of those that drink raw milk in Jos north, Jos south, and Barkin Ladi local governments was 60%, 54%, and 40% respectively (Table: III). More people (30%) vaccinate their children against tuberculosis in Barkin Ladi local government compared to only 10% and 18.2% in Jos north and Jos south local governments respectively. The drinking of raw milk and with majority of pastoralists not vaccinating their children against tuberculosis as well as few of them seeking medical attention when affected is an indication that most of the pastoralists are not aware of the zoonotic implications of the disease. Meanwhile, none of the 5 reported cases tested positive for tuberculosis with any of the laboratory diagnostic techniques applied during the period of this study. This could be as a result of treatment with anti-tuberculosis drugs as at the time of sampling even if at the end of the day the required period of treatment of between 6 to 9 months was not complied with. The practice of buying of unscreened replacement stocks by farmers and the returning of unsold animals to the herds are favourable factors for the disease transmission across the country. Uncontrolled movement of cattle across the country either for the purposes of grazing, commercial activities, or as a result of ethnic conflicts is an important factor that affect the epidemiology of tuberculosis in the country.

In Nigeria, the use of tuberculin test and slaughter in the control of bovine tuberculosis is not practiced and this may be due to economic reasons and perhaps lack of availability of purified protein derivative (PPD) for bovine tuberculosis. Test and slaughter control programmes that have successfully reduced the prevalence of bovine tuberculosis in developed countries have been increasingly questioned by farmers due to economic reasons (Mitchel *et al.*, 2010). The burden of zoonotic tuberculosis in Africa is largely unknown or under diagnosed due to lack of adequate laboratory equipments (Hilty, 2006). Efforts to establish tuberculosis cases caused by *M. bovis* and other mycobacterial infections derived from cattle in Plateau State and Nigeria at large should be intensified. Few studies in some parts of the country especially in south western Nigeria (Cadmus *et al.*, 2006) have indicated that *M. bovis* is responsible for quite a number of tuberculosis cases in humans. In developed countries, zoonotic tuberculosis has been successfully controlled through the pasteurization of

milk ((Recardo de la Rau- Domenech, 2006) but the situation is completely different in developing countries especially in sub- Saharan Africa. In Nigeria in particular, consumption of raw milk and other dairy products is a common practice among nomadic pastoralists. This can expose them and their families not only to tuberculosis but other zoonoses and the chain of transmission may spread to the rest of the public. Even though clinical cases of human tuberculosis were encountered in some of the households that participated in this study, none of the samples collected was positive for tuberculosis including those collected from persons diagnosed of tuberculosis. Apart from effects of anti-tuberculosis drugs that were already in use prior to sampling, effects of temperature on the DNA during transportation to South Africa for molecular analysis could be responsible for the negative results obtained. From this study it is evident that tuberculosis is a major health risk and of public health importance especially in the communities under study and the trend may increase with the increased urbanization of infected young herdsmen who migrate to urban cities.

CONCLUSION

Tuberculosis is a well known disease among the nomadic pastoralists in the study area even though most of them are not aware of its zoonotic implications and very few vaccinate their children against the disease. This constitute a great health risk to the nomads considering their close association with their animals and for the fact that milk (usually raw) and other animal products form a major part of their diet. Creating awareness among these people on the zoonotic significance of tuberculosis and the control measures usually applied against the infection will go a long way in reducing the risks tuberculosis poses to the nomadic communities in the study area and Nigeria generally.

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