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ZOONOTIC RISKS AND TRANSMISSION OF *MYCOBACTERIA* SPECIES FROM COWS' MILK AND SLAUGHTERED CATTLE TO MAN IN IBADAN: ROLE OF BUTCHERS

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SUMMARY

To ascertain the zoonotic risks associated with the handling, processing and consumption of milk and meat products in respect to bovine tuberculosis in Ibadan. This study was conducted by simultaneous screening of 105 unpasteurised cows' milk samples and 587 slaughtered cattle some of which showed gross lesions suggestive of tuberculosis. Samples from the milk and suspected tuberculous lesions were cultured on Lowenstein-Jensen media while nitrate and niacin tests were carried out to classify the isolated *Mycobacteria* species. Prevalence rates of 5.7% and 4.3% were confirmed from the milk and cattle samples screened respectively. Based on the biochemical tests, three isolates of *Mycobacterium tuberculosis*, one of *M. bovis* and one of *M. africanum* were identified from the milk samples; while six *M. tuberculosis*, fourteen *M. bovis*, two *M. africanum* and three unclassified *Mycobacteria* species were obtained from the tuberculous cattle. The unhygienic handling and processing of these animal products by butchers may lead to the zoonotic transmission of *M. tuberculosis* complex to the public and a source of occupational exposures to the butchers.

KEYWORDS: Zoonoses, Food-products, *Mycobacterium tuberculosis*, Butchers, Nigeria

INTRODUCTION

Tuberculosis is an endemic problem in the human and cattle populations in Nigeria (Cadmus *et al.*, 2004; 2006; WHO, 2004). Evidence of zoonotic transmission of *Mycobacteria* species between humans and cattle have been reported in Nigeria (Idrisu and Shnurrenberger, 1977; Idigbe *et al.*, 1986; Cadmus *et al.*, 2006). The pulmonary form of zoonotic tuberculosis (TB) caused by *Mycobacterium bovis* is indistinguishable from that caused by *M. tuberculosis* (Dankner *et al.*, 1993; Cosivi *et al.*, 1998), however, both species belong to the *M. tuberculosis* complex group, which also includes *M. africanum*, *M. microti*, *M. caprae* and *M. canettii* (Brosch *et al.*, 2003; Smith *et al.*, 2006). Cattle derived tuberculosis in man is attributed to *M. bovis* and occasionally *M. tuberculosis* and *M. africanum* (Kazwala, 1998; Cadmus *et al.*, 2006).

Mycobacterium bovis has the widest host range including animals and humans (Acha and

Syrups, 1987; Sreevatsan *et al.*, 1997). *M. bovis* has been a historical source of TB in humans infected through drinking of contaminated unpasteurised milk or inhaling aerosols produced by diseased farm animals (Kleeberg, 1984; Cosivi *et al.*, 1998), and to some extent through consumption of improperly cooked infected meat and meat products.

In countries with a relatively high prevalence of bovine TB in cattle, abattoir and farm workers are the professional groups mostly exposed to infection (Ayele *et al.*, 2004). In Nigeria, the degree of zoonotic transmission of tuberculosis from animals to humans is not fully known, however, cultural practices exist that could facilitate transmission between cattle and humans. For example, prior to sale, cattle are raised and fattened in close proximity to farmers' home. After being sold at markets, cattle are often slaughtered in nearby abattoirs, where the butchers wear minimal protective clothing and process offal from diseased carcasses with bare hands. The close association between farmers and cattle is exemplified by the Fulani herdsmen, who live their entire lives with their cattle, offering ample opportunity for zoonotic transmission of infection (Cadmus *et al.*, 2006).

Cows meant for sale and slaughter at the cattle markets and abattoirs are sometimes milked; the milk samples are consumed by some of the Fulanis'/Hausas' believing that these serve as nutritional or medicinal supplements. However, recent study from a local setting in Ibadan through molecular characterization of *Mycobacteria* species from human isolates revealed that approximately 13% of the disease in humans was caused by strains of *M. africanum* and *M. bovis* rather than *M. tuberculosis* (Cadmus *et al.*, 2006).

The purpose of this study was therefore to establish whether *Mycobacteria* species were being secreted in milk of cows' slaughtered at the abattoir and if the cattle slaughtered were also infected with these organisms. This is with a view to ascertain the zoonotic risks involved in handling and consuming these food products.

MATERIALS AND METHODS

Study site

The field work was carried out at the Bodija Municipal Abattoir, Ibadan, Oyo State in South-Western Nigeria. It is the largest abattoir in the state and most of the cattle slaughtered here came from the northern parts of the country as well as the neighboring African countries of Benin Republic, Burkina Faso, Cameroon, Chad and Niger. In addition, a few of the cattle bred within the premises of the abattoir were also slaughtered in the abattoir. Like many other abattoirs in the country, there was minimal facility and personnel for proper ante-mortem and post-mortem inspection. This was compounded by a deplorable water supply system, lack of a functional effluent disposal system and heap of wastes from human and animals left over the years.

Duration of study

The entire study spanned a period of two months from June 1st to July 31st 2006. During this period, collections of samples were made on week days (Monday-Friday) between 8.00 a.m. and 12.00 noon (i.e. the peak period of slaughtering).

Milk sample collection

Milk samples were collected from 105 different breeds of cows awaiting slaughter in Bodija abattoir. The choice of cows from which milk was collected was based on the cooperation of animal owners. Milk samples were collected aseptically from udders into 50 ml sterile universal containers and then placed in clean cooler packs. Samples were later refrigerated at 4°C in the laboratory prior to culturing.

Collection and storage of suspected tuberculous lesions

The animals inspected were identified based on sex, age and breed (Tables I, II and III). Post-

mortem examination was carried out on 587 breeds of slaughtered cattle with different tissue samples, organs and lymph nodes inspected for suspected lesions of TB. From these, only 25 animals had suspected lesions of TB. About 50g to 150g of the infected tissues were collected aseptically and kept individually in well labeled and sealed sampling bags in cooler packs in the abattoir before being transported to a -4C freezer in the laboratory prior to processing. The samples collected included lungs (n=17), mediastinal lymph node (LN) (n=2), mesenteric LN (n=2), other LN (n=3), parenchymatous organs (n=2), spleen (n=2), aorta (n=1) heart (n=1), muscles (n=3) (Table I).

Laboratory work

All the laboratory work was done in the Tuberculosis Laboratory of the Department of Veterinary Public Health & Preventive Medicine, University of Ibadan, Ibadan, Nigeria.

i) Processing of samples for detection of *Mycobacteria:* The processing of milk samples and lesions was based on the Becton Dickinson digestion and decontamination procedure (Anonymous, 1999). The same procedure was carried out for processing both the milk and suspected lesions (for the lesions, grinding with pestle and mortar was first done with the addition of sterile distilled water before the procedure). The digestion and decontamination procedure entailed using a sterile 15 ml centrifuge tube; equal amounts of specimen and activated NALC (N-acetyl-L-cysteine)-NaOH of about 5 ml each were added. The centrifuge tube was capped and mixed on a vortex-type mixer until the specimen was liquefied. The mixture was allowed to stand at room temperature for 15 min with occasional gentle shaking. Prepared phosphate buffer was added to the 15 ml mark on the centrifuge tube and mixed, followed by centrifugation for 15 to 20 min at 3,000 x g. The supernatant was decanted, and 2 ml of phosphate buffer of pH 6.8 was added to re-suspend the pellet.

ii) Microscopic examination: In all, sediments from 33 suspected tuberculous lesions (i.e. tissues, organs and lymph nodes) from 25 animals and 105 milk samples (Table I and II) were examined microscopically using the Ziehl Neelsen staining technique for the detection of acid fast bacilli.

iii) Cultural examination: The Lowenstein-Jensen (L-J) medium was used to culture sediments from 33 suspected tuberculous lesions from 25 animals and 105 milk samples after the decontamination and digestion method according to the Becton Dickinson procedure. Samples were cultured at 37°C for 8 to 12 weeks on paired L-J media enriched with pyruvate (L-J-P medium) or with glycerol (L-J-G medium) as earlier described by Cadmus *et al.* (2004).

iv) Biochemical tests

Niacin test: An extract from 3-4 weeks old *Mycobacterium* culture was prepared by adding 1.5 ml of sterile distilled water to the culture. The surface growth was gently scraped off using a pipette and a stab was made through the growth into the medium to permit extraction of the niacin employing the use of a 1ml sterile pipette. The tube was slanted such that the medium was covered with the liquid and was left in this position for about 25 minutes. Then, approximately 0.6ml of the extract was removed with a sterile capillary pipette fitted with a bulb and transferred into a test tube and was covered with a stopper. A negative control was prepared by adding 0.6ml of distilled water in place of the extract. Using a pair of flamed forceps, a BBL Taxo TB Niacin Test Strip (Becton, Dickinson and Company, Sparks, Maryland 21152 USA) with arrow downward was dropped into each tube and covered with a stopper immediately. The tubes were shaken gently to mix the fluid with the reagent on the bottom of the strip. This was repeated after 5-10 minutes. The colour of the extracts was then compared after 12 15 minutes. Yellow colour in the extract was considered positive for *Mycobacterium tuberculosis*; suggestive of *M. africanum,*

while no colour change is suggestive of *M. bovis*.

Nitrate test: 0.5ml of distilled water was added to a clean screw-cap tube into which two clumps of growth was added using a 1 ml pipette. The growth was dispersed. Using a pair of flamed forceps, a BBL Taxo TB Nitrate Test Strip (Becton, Dickinson and Company, Sparks, Maryland 21152 USA) with arrow downward was dropped into each tube and the tubes held vertically. The tubes were then capped and incubated at 37°C for 2 hours. The tubes were gently shaken at the end of the first and second hours of incubation. After the 2 hours of incubation, the tubes were carefully tilted back and forth six times to wet the entire strip and were then slanted at room temperature to cover the strip with the liquid and were left to remain in this position for 10 minutes. A change of colour at the top portion of the strip to light or dark blue indicated nitrate reduction; thus implying *Mycobacterium tuberculosis* while no colour change indicated a negative reaction suggestive of *M. africanum* or *M. Bovis*.

RESULTS

Cultural examination

From the milk samples, isolations were made from two (4.35%) White Fulani, one (7.14%) Red Bororo and three (7.9%) Sokoto Gudali breeds of cattle giving a prevalence rate of 5.7% (Table II). However, all the 33 suspected tuberculous lesions from the 25 cattle were culture positive, giving an overall prevalence rate of 4.3%. The highest prevalence rate of 8.4% was recorded among the White Fulani breed followed by 3.0% in the Red Bororo breed and 1.5% among the Sokoto Gudali breed (Table II). The adult cattle were the most affected (5.1%) (Table III).

Microscopic examination

All the isolates had typical microscopic appearances of *Mycobacteria* upon acid-fast staining.

Biochemical tests

Results from the biochemical tests revealed three isolates of *Mycobacterium tuberculosis* (presence of nitrate reduction and positive niacin production), one of *M. bovis* (absence of both nitrate reduction and niacin production) and one of *M. africanum* (absence of nitrate reduction and slightly positive niacin production) from the milk samples based on the related interpretations used by Kallenius *et al.* (1999) and Niobe-Eyangoh *et al.* (2003); while six *Mycobacterium tuberculosis*, fourteen *M. bovis*, two *M. africanum* and three unclassified *Mycobacteria* species were identified from the isolates of the slaughtered cattle (Table IV).

TABLE I: Predilection sites of suspected tuberculous lesions collected

Animal no	Breed	Sex	Age	Lesions collected									Total lesions collected per animal
				Lungs	Mediastinal ln	Mesenteric ln	Hepatic ln	Liver	Spleen	Aorta	Heart	Muscles	
I	WF	F	A	*		*							2
II	WF	F	A	*									1
III	WF	F	Y.A	*					*				2
IV	WF	F	A	*			*					*	3
V	WF	F	A									*	1
VI	WF	M	A	*									1
VII	WF	F	A				*						1
VIII	WF	F	A	*					*				2
IX	WF	F	Y.A								*		1
X	WF	F	A	*									1
XI	WF	F	Y.A	*						*			2
XII	WF	M	A	*									1
XIII	WF	F	A		*								1
XIV	WF	F	A					*					1
XV	RB	M	A	*									1
XVI	WF	F	A				*						1
XVII	WF	F	A	*									1
XVIII	WF	F	A	*								*	2
XIX	WF	M	A	*									1
XX	WF	F	A	*	*								2
XXI	WF	F	A			*							1
XXII	RB	F	A	*									1
XXIII	SG	M	A					*					1
XXIV	RB	F	A	*									1
XXV	WF	F	Y.A	*									1

KEYS:
WF: White Fulani; RB: Red Bororo; SG: Sokoto Gudali
M: Male; F: Female
A: Adult; YA: Young Adult
***: Predilection site from which lesion was collected**

TABLE II: Breed distribution of culture positive milk samples and slaughtered cattle lesions

	MILKING COWS			SALUGHTERED CATTLE		
BREED	**TOTAL EXAMINED**	**CULTURE POSITIVE**	**% POSITIVE**	**TOTAL EXAMINED**	**CULTURE POSITIVE**	**% POSITIVE**
WHITE FULANI	46	2	4.35	251	21	8.40
RED BORORO	14	1	7.14	101	3	3.00
SOKOTO GUDALI	38	3	7.90	66	1	1.50
KURI	-	-	-	38	-	-
MIXED	7	0	0.00	64	-	-
BOKOLO	-	-	-	67	-	-
TOTAL	105	6	5.7	587	25	4.3

TABLE III: Sex and age distribution of culture positive slaughtered cattle

		TOTAL EXAMINED	**CULTURE POSITIVE**	**% POSITIVE**
SEX	MALE	138	5	3.6
	FEMALE	449	20	4.5
AGE	<1YR	4	–	–
	1-3YRS	174	4	2.3
	>3YRS	409	21	5.1
TOTAL		587	25	4.3

TABLE IV: Results of the biochemical tests

ISOLATES FROM MILK SAMPLES				**ISOLATES FROM CATTLE LESIONS**			
No of Samples	**Nitrate Test**	**Niacin Test**	**Interpretation**	**No of Samples**	**Nitrate Test**	**Niacin Test**	**Interpretation**
3	Dark blue	Yellow	*M. tuberculosis*	6	Dark blue	Yellow	*M. tuberculosis*
1	NVR	NVR	*M. bovis*	14	NVR	NVR	*M. bovis*
2	NVR	Yellow	*M. africanum*	2	NVR	Yellow	*M. africanum*
0	Dark blue	NVR	Unclassified	3	Dark blue	NVR	Unclassified

NVR: No Visible Reaction

DISCUSSION

The prevalence rate of 5.7% obtained from the milk samples is lower than the previous work by Cadmus and Adesokan (2007) in which 11.3% was obtained from 53 unpasteurised cows' milk samples in the same abattoir. Another major difference between the two studies is that unlike in the previous work where *M. bovis* was the only species isolated; *M. tuberculosis* and *M. africanum* were also identified in this study based on the available biochemical tests. Since there was no molecular typing of these isolates when compared to the studies by Kallenius *et al.* (1999) and Niobe-Eyangoh *et al.* (2003) particularly regarding the identification of *M. africanum*, conclusions made were based on extrapolations of biochemical analyses carried out in these two previous studies.

Compared to the work done by Alhaji (1976), our result was lower than the 54.5% he obtained from 11 pooled milk samples from markets in the northern states of Nigeria. However, he also isolated *M. bovis*, *M. tuberculosis* and other unclassified *Mycobacteria* species. The reason that could be given for the lower prevalence rate in our present work when compared to other works cited may not be unconnected with the larger sample size in our present study.

As regards the results from the slaughtered cattle, the prevalence rate obtained was lower than the 8.8% by Cadmus *et al.* (unpublished data) in 2004 in the same abattoir. In the same vein our result is also lower than the 8.2% and 10.5% prevalence obtained in a private beef cattle herd in Ibadan by Cadmus *et al.* (2004) and by Wekhe and Berepubo (1989) in the eastern abattoir of the country respectively. However, one important finding from this work is the isolation of different *Mycobacteria* species; results which are similar to published work by Cadmus *et al.* (2006).

The results from both the milk and cattle screened can be summarized as follows: **i.** Different *Mycobacteria* species were incriminated in the occurrence of bovine tuberculosis in this abattoir **ii.** The disease was found mostly in the White Fulani breed of cattle which were also the majority of cattle slaughtered. **iii.** The most affected age group was the over three year olds (adult). **iv.** All the suspected animals with tuberculous lesions were confirmed positive by culture.

Judging from the summary above, there are some zoonotic risks the cattle marketers, meat inspectors, butchers and other people directly involved in the cattle industry in this abattoir are exposed to. This submission is drawn from the following realities:
i. About 50% -70% of animals examined at this abattoir are emaciated, weak and unthrifty. **ii.** There are no proper infrastructural facilities to separate diseased and healthy animals. **iii.** The setting in this abattoir does not allow for the opportunity to carry out detailed meat inspection. **iv.** Cows brought for slaughter are sometimes milked for human consumption. **v.** There are no protective wares used while carrying out postmortem examination despite the cases of bovine tuberculosis recorded in the abattoir. **vi.** Most butchers use their bare hands to process carcasses, even those showing TB suspect lesions. **vii.** Butchers who have cuts on their hands still go ahead to process infected carcasses. **viii.** Improperly washed hands are also used by butchers to eat while slaughtering is going on. **ix.** Children and food sellers are always present in the slaughter slabs while slaughtering and meat processing is going on. **x.** The slaughter slabs are always over congested with humans and cattle. **xi.** Gutters meant for easy passage of effluents within the slabs are sometimes used for urinating. **xii.** Drainages inside the slabs are often blocked through stuffing of hidden infected tuberculous tissues and other diseased organs. **xiii.** Water supply is grossly inadequate and often non-potable water is used for meat processing. **xiv.** The slaughter slabs are always in an un-hygienic state and slaughtering is done on bare floors. **xv.** The same knives and other processing materials are used to handle healthy and

infected/contaminated carcasses. xvi. Condemned meat and offal due to TB are also regularly smuggled out of the slabs by butchers and other meat processors to be sold to unsuspecting buyers.

The consequences of the above findings and practices in the abattoir therefore support the transmission of bovine tuberculosis to most workers involved in meat processing in this abattoir, together with the general consuming public. Therefore, our observations and findings support the assertion made by Ayele *et al.* (2004) that in the countries with a relatively high prevalence of bovine TB in cattle, abattoir and farm workers are the most exposed to infection.

As earlier confirmed, in countries where animal TB is uncontrolled, most human cases occur in young persons and results from the drinking or handling of contaminated milk or milk products (Acha and Syzfres, 1987; Cosivi *et al.*, 1998) and close association with infected livestock (Cadmus et al., 2005). The increasing trend of pulmonary and extra-pulmonary TB in children in Ibadan has been confirmed by Akang *et al.* (1993) and Osinusi (1998) and this may not be unconnected with the endemicity of bovine TB in both farm and slaughtered cattle in Ibadan (Cadmus *et al.*, 2004, 2006).

The isolation of *M. tuberculosis* from the milk samples and the lesions of the slaughtered cattle further confirm the zoonotic nature of tuberculosis. In an earlier work by Cadmus *et al.* (2006), *M. tuberculosis* was also isolated from cattle in this abattoir. This confirms that due to the close co-habitation of humans and cattle and the endemicity of tuberculosis due to *M. tuberculosis* in the human population, man has also been found to infect cattle and other animals.

From the above scenarios, more cases of *M. tuberculosis*, *M. bovis* and *M. africanum* infection may spread to the larger society through the food chain and close contact with abattoir workers since they are highly exposed to the *M. tuberculosis* complex. The human to human transmission of *M. bovis* is therefore likely to be facilitated through the deplorable lifestyles and living conditions of most butchers in Ibadan. Majority of the butchers are known to have multiple sexual partners, hence vulnerability to human immunodeficiency virus (HIV). Some of them are also involved in drug abuse coupled with the habit of heavy drinking of alcohol while working within the abattoir premises. Due to these risk factors, the butchers are more at risk of being infected with these *Mycobacteria* species either through the pulmonary or extrapulmonary route. Hence, once infected through the pulmonary route, they are therefore more likely to infect others through contacts made over long or short periods.

Based on all the above, vis-à-vis the unwholesome activities in meat processing and risk factors the butchers are exposed to, coupled with their lifestyles, it therefore becomes evident that they are a special group that is highly at risk with the *Mycobacterium tuberculosis* complex infection. Hence, the butchers are a group that needs to be studied in the epidemiology of bovine tuberculosis in Nigeria.

CONCLUSION

Bovine tuberculosis remains a major zoonotic problem in animals slaughtered in Nigerian abattoirs. Evidences have shown that *M. bovis*, *M. tuberculosis* and *M. africanum* are found in milk and organs of slaughtered cattle; hence humans are exposed to these pathogens through the food chain. However, there is a need to complement biochemical tests with molecular typing to conclusively characterize species and strains of *Mycobacteria* from infected cattle in order to better understand the epidemiology of the disease in the country. The butchers and all those involved in the trade cattle industry are at high risk of exposure to tubercle bacilli and could therefore serve as sources of spread to other humans. In conclusion, government should step

up bovine TB control programmes in the country as well as incorporate stakeholders in the livestock industry in the national control of tuberculosis in Nigeria.

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REFERENCES

ACHA, P.N. and SZYFRES, B. (1987): Zoonotic tuberculosis. Zoonoses and Communicable Diseases Common to Man and Animals. 2nd ed. Washington: Pan American Health Organization/World Health Organization; Scientific Publication No. 503.

ALHAJI, I. (1976): Bovine tuberculosis in four northern states of Nigeria. PhD Thesis, Ahmadu Bello University, Zaria, Nigeria; 236.

AKANG, E.E.U., EKWEOZOR, C., PINDIGA, H.U. and ONYEMENEM, T.N. (1993): Childhood infections in Nigeria: an autopsy study. *J. Trop. Med. and Hyg.*, **96**: 231-236.

ANONYMOUS (1999): Becton Dickinson. BBL™ MycoPrep™ Specimen digestion/decontamination kit manual for processing of Mycobacterial specimens; 2.

AYELE, W.Y., NEILL, S.D., ZINSSTAG, J., WEISS, M.G. and PAVLIK, I. (2004): Bovine tuberculosis: an old disease but a new threat to Africa. *Intern. J. Tuberc. Lung Dis.*, **8**: 924-937.

BROSCH, R., GORDON, S.T., MARMIESSE, M., BRODIN, P., BUCHRIESER, C., EIGLMEIER, K., GARNIER, T., GUTIERREZ, C., HEWINSON, G., KREMER, K., PARSONS, L.M., PYM, A.S., SAMPER, S., VAN, SOOLINGEN, D. and COLE, S. (2002): A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proc. Natl. Acad. Sci, USA*, **99**: 3684-3689.

CADMUS, S. I. B. and ADESOKAN, H. K. (2007): Phenotypic characterization and spoligotype profiles of *Mycobacterium bovis* isolated from unpasteurized cow milk in Ibadan, *Nig. Trop. Vet.* **25**(2): 65-72.

CADMUS, S.I.B., PALMER, S., OKKER, M., DALE, J., GOVER, K., SMITH, N., JAHANS, K., HEWINSON, R.G. and GORDON, S.V. (2006): Molecular analysis of human and bovine tubercle bacilli from a local setting in Nigeria. *J. Clin. Microbiol.*, **44**.1: 29-34.

CADMUS, S.I.B., OLUWASOLA, O.A. and GORDON, S.V. (2005): Molecular methods for the accurate assessment of *Mycobacterium bovis* infection. *Intern. J. Tuberc. Lung Dis.*, **9**: 705.

CADMUS, S. I. B., ATSANDA, N.N., ONI, S.O. and AKANG, E.E.U. (2004): Bovine tuberculosis in one cattle herd in Ibadan in Nigeria. *Vet. Med. Czech.*, **49**: 406-412.

COSIVI, O., GRANGE, J.M., DABORN, C.J., RAVIGLIONE, M.C., FUJIKURA,T., COUSINS, D., ROBINSON, R.A., HUCHZERMEYER, H.F.A.K., DE KANTOR, I. and MESLIN, F.X. (1998): Zoonotic Tuberculosis due to

Mycobacterium bovis in Developing Countries. *Emerg. Infect. Dis.*, **41**: 59-70.

DE KANTOR, I., PEREIRA, J., MIQUET, J. and ROVERE, R. (1979): Pouuvoir pathogene experimental de *Mycobacterium africanum* pour les bovins. *Bull. de L'Académie Vétérinaire de France*, **52**: 499-503.

IDIGBE, E.O., ANYIWO, C.E. and ONWUJEKWE, D.I. (1986): Human pulmonary infections with bovine and atypical mycobacteria in Lagos, Nigeria. *J. Trop. Med. Hyg.*, **89**:143-8.

IDRISU A. and SCHNURRENBERGER P. (1977): Public health significance of bovine tuberculosis in four northern states of Nigeria: a mycobacteriologic study. *Nig. Med J.*, **7**: 384-7.

KALLENIUS, G., KOIVULA, T., GHEBREMICHAEL, S., HOFFNER, S.E., NORBERG, R., SVENSSON, E., DIAS, F., MARKLUND, B.I. and SVENSON, S.B. (1999): Evolution and clonal traits of *Mycobacterium tuberculosis* complex in Guinea-Bissau. *J. Clin. Microbiol.*, **37**:3872-3878.

KAZWALA, R.R., DABORN, C.J., KUSILUKA, L.J., JIWA, S.F., SHARP, J.M. and KAMBARAGE, D.M. (1998): Isolation of *Mycobacterium* species from raw milk of pastoral cattle of Southern Highlands of Tanzania. *Trop. Anim. Hlth. Prod.*, **30**: 233-239.

KLEEBERG, H.H. (1984): Human tuberculosis of bovine origin in relation to public health. *Revue Scientifique et Technique, O. I. E.* **3**: 11-76.

NIOBE-EYANGOH, S.N., KUABAN, C., SORLIN, P., CUNIN, P., THONNON, J., SOLA, C., RASTOGI, N., VINCENT, V. and GUTIERREZ, M.C. (2003): Genetic biodiversity of *Mycobacterium tuberculosis* complex strains from patients with pulmonary tuberculosis in Cameroon. *J. Clin. Microbiol.*, **41**(6): 2547-2553.

OSINUSI, K. (1998): Clinical and epidemiological features of childhood tuberculosis in Ibadan. *Nig. J. Paediatr.*, **25**:15-9.

SREEVATSAN, S., PAN, X., STOCKBAUER, K.E., CONNEL, N.D., KRIESWIRTH ,B.N., WHITTAM, T.S. and MUSSER, J.M. (1997): Restricted structural gene polymorphism in the *Myconbacterium tuberculosis* complex indicates evolutionary recent global dissemination. *Proc. Natl. Acad. Sci. USA*, **94**: 9869-9874.

WEBER, A., REISCHL, U. and NAUMANN, L. (1998): Demonstration of Mycobacterium africanum in a bull from North Bavaria. *Berl. Munch. Tierarztl. Wochenschr.*, **111**: 6-8.

WEKHE, S.N. and BEREPUBO, N.A. (1989): Prevalence of bovine tuberculosis among trade cattle in southern Nigeria. *Trop. Anim. Hlth. Prod.*, **21**:151-152.

WORLD HEALTH ORGANIZATION. (2004): posting date. Global TB control Report: Nigeria annex [Online] World Health Organization. Geneva, Switzerland http://www.who.int/tb/publications/global _report/2004/en/Nigeria.pdf.