Sahel J. Vet. Sci. Vol. 9, No. 1, pp. 41 - 46 (2010)

1605-8954/10/$25.00 + 00

Sahel Journal of Veterinary Science

Prevalence and Public Health Potentials of *Mycobacterium bovis* in Excretions of Slaughter Cattle in Makurdi, Nigeria

R. A. Ofukwu[*1], C. A. Akwuobu[2], and S. I. Oboegbulem[3]

[1]Department of Veterinary Public Health and Preventive Medicine, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria; [2]Department of Veterinary Pathology and Microbiology, College of Veterinary Medicine, University of Agriculture, Makurdi, Nigeria and [3]Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Nigeria, Nsukks, Nigeria

ABSTRACT

The prevalence of *Mycobacterium bovis* in excretions of 255 randomly selected trade cattle in Makurdi, Nigeria, was determined from October 2003 to September 2004. The standard methods of acid-fast microscopy, culture, and biochemical tests were used. *Mycobacterium bovis* was present in 13 (5.1%) nasal secretions, 5 (2.0%) faeces and 2 (0.8%) urine samples. Generally, higher number of cattle were *M. bovis* positive during the dry season than in rainy season. Sex specific prevalence was significantly ($p<0.05$) higher in females than in males, even though 61.2% of the examined cattle were male. Age specific prevalence increases with increasing age, with more than 60.0% of the positive cattle aged above 6 years. Breed specific prevalence also varied significantly ($p<0.05$) amongst the breeds of cattle screened. Mycobacteria laden excretions present serious public health problems as it contaminates the environment.

Key words: Epidemiology, *Mycobacterium bovis*, slaughter cattle, excretions, Makurdi, Nigeria

INTRODUCTION

Tuberculosis is a fatal zoonotic bacterial disease characterized by persistent hacking dry cough, myalgia, weakness and inappetence, has become a resurgent public health problem (Idigbe *et al*., 1986; Parry and Davis, 1996).

Humans contact this disease principally by aerosol droplet inhalation, consumption of unpasteurised milk and food contaminated by *Mycobacterium tuberculosis* or *Mycobacterium bovis* which unfortunately present indistinguishable clinical signs (Collins and Grange, 1987; Cook *et al*., 1996; Cosivi *et al.*, 1998; Idigbe *et al*., 1986; Ravenel, 1992; Grange and Yates, 1994).

Nigeria has the forth largest burden of human tuberculosis in the world, with a prevalence rate of over 2.1% and 368,000 cases in 2002 alone. Globally, 3.1% of all human tuberculosis is due to *M. bovis*; 7.1% pulmonary and 9.4% extra pulmonary (Cosivi *et al*., 1995). A rising prevalence of *M. bovis* infection of man has been reported in several parts of Nigeria (Idrisu and Schurrenberger, 1977; Idigbe *et al*., 1986; Ayanwale *et al*., 1991; Cadmus *et al*., 1999; Garba, 2001; Cadmus *et al*., 2003).

The increasing prevalence of human tuberculosis is attributed to several factors amongst which are poor sanitation, overcrowding and sharing of contaminated drinking water sources with cattle and other animals in periods of scarcity. This scenario resulted in a situation where humans in close contact with cattle, drink from water sources which may be contaminated by excreted urine, faeces and nasal secretions laden with *Mycobacterium* (Francis, 1972).

Mycobacterium bovis, has been isolated from some of these excretions and secretions of cattle in other parts of Nigeria (Ojo and Unigbo, 1987; Cosivi *et al*., 1998; Kolo, 1991; Cadmus, 2003). The isolation of this pathogen in excretions of cattle has, however, never been done in Benue State.

Benue State with a 75% agrarian population living under poor housing conditions, with HIV/AIDS prevalence of about 13%, in an environment housing about 70% of nomadic cattle herds in dry seasons (Uza, 1998), presents

*Author for correspondence; *E-mail:* ofu_kwu@yahoo.com

a serious potential public health problem. The objective of this study is to determine the prevalence and epidemiology of *M. bovis* in excretions of slaughter cattle in Makurdi, Nigeria. This was a view to highlighting its public health importance as sources of human tuberculosis infection.

MATERIALS AND METHODS

Study area

Makurdi, capital city of Benue State, Nigeria, with a population of 500, 000 (NPC, 1991), lies on the luxuriant flood plane of Benue river in the Southern Guinea savannah belt. It is located on latitude 7°44' North and longitude 8°54' East. It has a relative humidity of 60 - 80%, with an annual rainfall of 1000 - 1,500 mm and temperature of 24°C.

Makurdi has two major seasons, rainy season (April to October) and dry season (November to March). The dry season normally witnesses periods of heat, drying up of natural sources of water and influx of about 800, 000 migratory cattle herds, from the drier northern parts of the country (Uza, 1998). These herds of cattle usually graze at the peri-urban areas of the town living closely with the natives at the dry season periods.

Sample collection and analysis

Two hundred and fifty-five (255) each of nasal secretions, faeces and urine samples were collected from 255 randomly selected slaughter cattle at Makurdi abattoir from October 2003 to September 2004. For each visit one in every six slaughter were selected and examined for presence of *Mycobacterium bovis*. For every visit, 4 - 7 samples each were collected. The breed, sex and age of each animal were recorded as described by Alhaji (1989) before sample collection. The samples (nasal secretion, faecal and urine) were analysed using the methods of Kent and Kubica (1985) as modified by Kolo (1991) as described briefly.

Nasal secretion

A sterile cotton swab (EVEPON®) was used to swab the secretion in the nasal orifice of each selected cattle and transported in an ice box to the laboratory. In the laboratory each sample was washed into 10 ml of 15% phenol red in a 50 ml screw capped tubes. Drops of 2 N NaOH was added slowly until pale pinkish colour emerged. This was centrifuged at 3 000 RCF for 15 minutes and the supernatant discarded into 10% formaldehyde. The sediment was then used for acid-fast microscopy. Acid-fast negative samples were discarded. Acid-fast positive samples were cultured on slants of Lowenstein-Jensen media with glycerol and incubated at 37°C for 4 - 8 weeks. Slants that showed small, moist, smooth, flat growth (positive samples) were subjected to biochemical tests (nitrate reduction and niacin production) to identify *M. bovis*.

Faecal sample

Approximately 20- 30 g of fresh faeces from each cattle were collected before slaughter, using gloved hands into sterile beaker. The samples were similarly to the laboratory. In the laboratory, 5 g of faeces from each sample was placed in sterile McCartney bottles and mixed with 5 ml of sterile water and again thoroughly mixed in 50 ml of sterilized physiological saline by stirring. The mixture was stored in the refrigerator at 4°C for 18 hours. Ten ml of the fluid at the liquid-solid interphase was pipetted out and decontaminated in a screw capped tube with 10 ml of 10% oxalic acid. This was vibrated using Votex mixer and centrifuged at 3 000 RCF for 20 minutes. The supernatant was discarded into 10% formalin. The sediment was used for acid-fast staining. Acid-fast positive samples were subjected to culturing and biochemical tests as earlier described for nasal sample. The acid-fast negative samples were discarded into 10% formalin.

Urine sample

Ten ml of urine were collected from each identified cattle before slaughter or shortly after slaughters using sterile needle and syringe into sterile screw capped tubes. The samples were transported in ice-pack to the laboratory and stored at 4°C until processed according to Kent and Kubica (1985). Ten ml of each sample were diluted to 40 mls using buffered saline and centrifuged in a 50 ml screw capped tube at 3 000 RCF for 20 minutes. The supernatant was discarded into 10% formalin. The sediment was then re-suspended in 15 ml of 4% NaOH and again centrifuged at 3 000 RCF for 20 minutes. The resultant sediment was analyzed as already described for nasal and faecal samples.

RESULTS

Mycobacterium bovis was isolated from the three excretory samples (nasal secretions, faeces and urine) processed in this study. The prevalence rates of *M. bovis* were 5.1%, 2.0% and 0.5% for nasal, faecal and urine samples, respectively (Table 1). The prevalence rates for nasal and faecal samples were significantly higher ($p<0.05$) than that of urine.

Table 1. Seasonal and monthly distribution of positive *Mycobacterium bovis* in faecal, urine and nasal excretions of slaughter cattle in Makurdi, Nigeria (October 2003 - September 2004).

Period	No. of cattle examined	No. cattle with positive samples			
		Faeces (%)	Urine (%)	Nasal secretion (%)	Total (%)
Dry season					
October 2003	17	0 (0.0)	0 (0.0)	1 (5.9)	1 (5.9)
November	28	1 (3.6)	0 (0.0)	1 (3.6)	2 (7.1)
December	16	0 (0.0)	0 (0.0)	1 (6.3)	1 (6.3)
January 2004	20	1 (5.0)	0 (0.0)	2 (10.0)	3 (15.0)
February	18	1 (5.6)	0 (0.0)	1 (5.6)	2 (11.1)
March	24	1 (4.2)	1 (4.2)	2 (8.3)	4 (16.7)
Subtotal	123	4 (3.3)	1 (0.8)	8 (6.5)	13 (10.6)
Rainy season					
April 2004	24	1 (4.2)	0 (0.0)	1 (4.2)	2 (4.3)
May	20	0 (0.0)	1 (5.0)	1 (5.0)	2 (10.0)
June	16	0 (0.0)	0 (0.0)	1 (6.3)	1 (6.3)
July	28	0 (0.0)	0 (0.0)	1 (3.6)	1 (3.6)
August	18	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
September	26	0 (0.0)	0 (0.0)	1 (3.8)	1 (3.8)
Subtotal	132	1 (0.8)	1 (0.8)	5 (3.8)	7 (5.3)
Total	255	5 (2.0)	2 (0.8)	13 (5.1)	20 (7.8)

Note: $\chi^2 = 2.4$; $p>0.1$

Seasonal and monthly occurrence of *M. bovis* in excretions of slaughter cattle are shown in Table 1 and Fig. I. Even though dry season recorded higher isolation rate (10.6%) compared to rainy season (5.3%), there was no association between the disease and the seasons ($\chi^2 = 2.4$; $P>0.1$). There were variations in the seasonal isolation of *M. bovis* from nasal secretions and faecal samples but there was no change in the seasonal isolation of *M. bovis* from urine samples (Table 1). Monthly isolation rates of the bacterium ranged from 0 to 6.3% in the rainy season and 3.6% to 10.0% in the dry season. The month of August, within the study period, recorded no isolation (Table 1 and Fig. I).

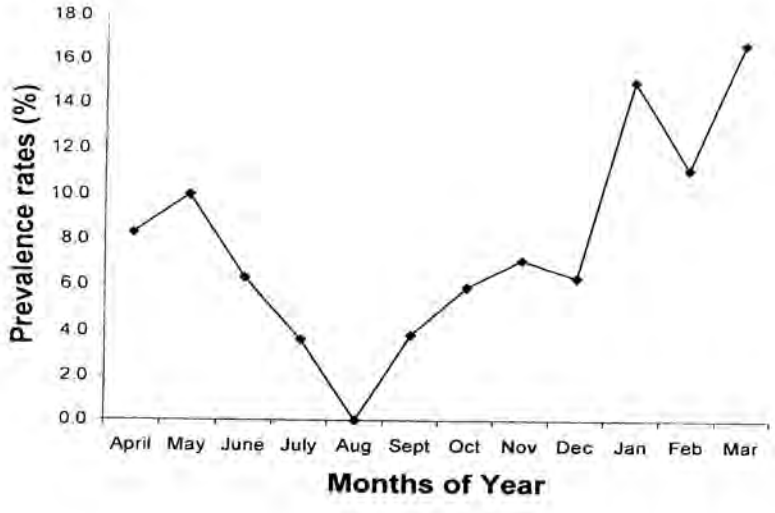

Fig. I. Monthly prevalence rates of *M. bovis* in excretions of slaughter cattle in Makurdi, Nigeria (October 2003 - September 2004).

Sex-specific prevalence was significantly ($p<0.05$) higher in females (12.1%) than in males (5.1%) even though 61.2% of the sampled cattle were male (Table 2). Table 2 also revealed that *M. bovis* was isolated from the 3 samples in both sexes.

Age-specific prevalence increases generally with increase in age, with more than 60% of the positive cattle aged above 6 years (Table 3).

Table 2. Sex-specific prevalence of *Mycobacterium bovis* in excretions of slaughter cattle in Makurdi, Nigeria (October 2003- September 2004).

Sex	No. of cattle examined	Number of cattle with positive			
		Faeces (%)	Urine (%)	Nasal secretions (%)	Total (%)
Male	156	2 (1.3)	1 (0.6)	5 (3.2)	8 (5.1)[a]
Female	99	3 (3.0)	1 (1.0)	8 (8.1)	12 (12.1)[b]
Total	255	5 (2.0)	2 (0.8)	13 (5.1)	20 (7.8)

Note: The figures in the same column with different superscripts are significantly different ($\chi^2 = 4.1$; $p<0.05$)

Table 3. Age-specific prevalence of *Mycobacterium bovis* in excretions of slaughter cattle in Makurdi, Nigeria (October 2003 - September 2004).

Age (years)	No. of cattle examined	Number of cattle with positive			
		Faeces (%)	Urine (%)	Nasal secretions (%)	Total (%)
0 – 2	97	0 (0.0)	0 (0.0)	1 (1.0)	1 (1.0)
3 – 4	29	1 (3.4)	1 (3.4)	1 (3.4)	3 (10.3)
5 – 6	28	1 (3.6)	0 (0.0)	2 (7.1)	3 (10.7)
> 6	101	3 (3.0)	1 (1.0)	9 (8.9)	13 (12.9)
Total	255	5 (2.0)	2 (0.8)	13 (5.1)	20 (7.8)

Note: $\chi^2 = 10.6$; $p<0.05$

Significant breed-specific prevalence rates of *M. bovis* were recorded in 4 of the 6 different breeds screened in this study (Table 4). Ndama and Muturu breeds recorded no isolation of *M. bovis*. Crosses recorded the highest rate (19.2%) followed by Sokoto Gudale (8.6%). The prevalence rate for White Fulani was 6.4% even though 67.1% of the number examined was White Fulani.

Table 4. Breed-specific prevalence of *Mycobacterium bovis* in excretions of slaughter cattle in Makurdi, Nigeria (October 2003 - September 2004).

Breeds	No. of slaughter cattle examined	Number of cattle with *M. bovis* positive			
		Faeces (%)	Urine (%)	Nasal secretions (%)	Total (%)
White Fulani	171	3 (1.8)	1 (0.6)	7 (4.1)	11 (6.4)
Sokoto Gudale	35	1 (2.9)	0 (0.0)	2 (5.7)	3 (8.6)
Adamawa Gudale	18	0 (0.0)	0 (0.0)	1 (5.6)	1 (5.6)
Ndama	4	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Muturu	1	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Crosses	26	1 (3.8)	1 (3.8)	3 (11.5)	5 (19.2)
Total	255	5 (2.0)	2 (0.8)	13 (5.1)	20 (7.8)

DISCUSSION AND CONCLUSION

The different prevalence rates obtained for nasal secretion, faeces and urine samples corroborated the work of Ojo and Unigbo (1987), Kolo (1991), and Smith (1998) who reported various prevalence rates in respect of different samples of nasal secretion, faeces and urine samples. The highest rate of 5.1% obtained from nasal secretions may be due to the fact that at least 75% of tuberculosis in cattle are pulmonary in nature and hence the agents are more likely to be shed through the nose or cough.

The seasonal variation (more in dry season) may be due to high congregation of cattle in search of pasture and water (Idrisu, 1976; Cook *et al*., 1996). This high congregation and the need to sell off unmanageable or sick ones may inadvertently lead to high prevalence within the herd. However further study on the association between the disease and the seasons in Benue State needs to be done.

The females were more affected than the males even though the sex-specific prevalence in both cases were lower than that reported by Aliyu *et al*. (1993). The higher prevalence observed in females may be due to the fact that the females, which are breeders, stay longer in the herd. This allows for insidious development of the chronic disease.

The trend in age-specific prevalence increasing with age agrees with reports of other workers (Alhaji, 1976; Ayanwale *et al*., 1991; Garba, 2001). This again may be due to the long presence of the animal in the herd allowing for the gradual development of the infection (Fadiran *et al*., 1998) and long incubation period in hosts.

Breed-specific prevalence rate was significantly ($P<0.05$) higher in the crosses than in other breeds. This may suggests that crosses are more susceptible than other breeds of cattle to *M. bovis* infection. This however needs further investigation as the number sampled was not large. The absence of *M. bovis* infection in Ndama and Muturu breeds may not indicate resistance to the infection as this might also be due to few numbers of the breeds screened. No significant ($P>0.05$) differences in the prevalence rates existed between White Fulani, Sokoto Gudale and Adamawa Gudale. This finding does not agree with other studies (Ayanwale *et al*., 1991; Idigbe *et al*., 1986) as they reported that Adamawa Gudale has higher breed-specific prevalence than Sokoto Gudale. The result in this study might be due to the fact that very few (18) of Adamawa Gudale were examined compared to 35 of Sokoto Gudale that were also older in age. However, more work on this needs to be done. The *Mycobacterium* laden excretions present serious public health problems (Collins and Grange, 1987; Cosivi *et al*., 1998; Grange and Yates, 1994)

It is concluded that tuberculous cattle in Makurdi could shed the infectious agent in its excretions. This agent when inhaled or consumed presents a serious public health problem as it could result in tuberculosis in humans. This becomes more worrisome considering the high prevalence rate of HIV/AIDS in Makurdi which increases the chance of infection by *Mycobacterium* species. There is need therefore to intensify public enlightenment on personal hygiene and sanitation for humans who have contact with cattle. Cattle should be prevented from sharing water holes or sources of drinking water with humans to avoid infections.

ACKNOWLEDGEMENTS

The assistance of the 2003/2004 National Diploma students of Akperaoshi College of Agriculture, Yandev, Nigeria who were on attachment at the abattoir for transporting most of the samples to Mkar. Also acknowledged are the technologists at the NKST Tuberculosis and Leprosy Hospital at Mkar for their assistance in analysis of the samples.

REFERENCES

Alhaji, A. I. (1989). Infectious diseases of livestock in Nigeria. *Bull. Anim. Hlth. Prod*. 24 (1): 17-28.

Alhaji, I. (1976). Bovine tuberculosis, a general review with special reference to Nigeria. *Vet. Bull*. 46 (2): 829-841.

Aliyu, M. M. and Kalra, D. S. (1993). Prevalence of tuberculosis in cattle slaughtered in Maiduguri abattoir. *Ann. Borno* 10: 182-187.

Ayanwale, F. O., Ogundipe, G. A. T. and Adeyemi, I. G. (1991). Characterization of *Mycobacterium* species isolated from slaughtered food animals in southwest Nigeria (1988 - 1991). *Trop. Vet*. 9: 181-185.

Cadmus, S. I. B. (2003). Tuberculosis in food animals. *Nig. Vet. J.* 24 (3): 72-75.

Cadmus, S. I. B., Olugasa, B. O. and Ogundipe, G. A. T. (1999). The prevalence and zoonotic importance of tuberculosis in Ibadan. Proceedings of the 36th Annual Conference of the Nigerian Veterinary Medical Association, 1999 October 25th – 31st; Kaduna, Nigeria. 199: pp. 8-10.

Cadmus, S. I. B., Thomas, J. O. and Oluwasola, O. A. (2003). Isolation of *Mycobacterium bovis* in human tuberculosis patient suffering from cervical adenitis in Ibadan, Nigeria. *Proceedings of the International Conference on Emerging Zoonoses* at Ames, Iowa, USA. September 18th – 21st, 2003.

Collins, C. H. and Grange, J. M. (1987). Zoonotic implications of *Mycobacterium bovis* infection. *Iran. Vet. J*. 41, 363-366.

Cook, A. J. C., Tuchili, L. M., Bure, A., Foster, S. D., Godfrey-Faussett, P., Pandey, G. S. and McAdam, K. P. W. J. (1996). Human bovine tuberculosis in the Manze district of Zambia (Cross-sectional study). *Br. Vet. J*. 152: 37-46.

Cosivi, O., Grange, J. M., Daborn, C. J., Raviglione, M. R., Fiyikura, J., Cousins, D., Robinson, R. A., Huchzermeyir, H. F. A. K., Denkantor, J. and Melin, F. X. (1998). Zoonotic tuberculosis due to *Mycobacterium bovis* in developing countries. *Emerg. Infect. Dis*. 4, 59-70

Cosivi, O., Meslin, E. X., Dabora, C. J. and Grange, J. M. (1995). The epidemiology of *Mycobacterium bovis* infection in animals and humans with particular reference to Africa. *OIE Sci. Tech. Rev*. 14: 733-746.

Fadiran, O. A., Kintan, B. and Oluwole, S. F. (1998). Tuberculosis orchitis co-existing with tuberculosis of the sternum – case report. *Cent. Afr. J. Med*. 45 (2), 45 – 47.

Francis, T. J. (1972). Abdominal tuberculosis in Nigeria; Clinicopathology. *Cent. Afr. J. Med*. 24: 232-239.

Garba, H. S. (2001). Tuberculosis in humans and livestock in Sokoto State Nigeria. Ph.D. Thesis Usman Danfodio University, Sokoto, Nigeria.

Grange, J. M. and Yates, M. D. (1994). Zoonotic *Mycobacterium bovis* infection. *Vet. Microbiol*. 40 (1/2): 137-151.

Idigbe, E. O., Anyam, C. E. and Onwujekwe, D. I. (1986). Human pulmonary infection with bovine and atypical mycobacteria in Lagos, Nigeria. *J. Trop. Med. Hyg*. 89: 143-145.

Idrisu, A. A. (1976). Tuberculin test survey of cattle in four northern states of Nigeria. *Bull. Anim Hlth Prod*. 24 (1): 6-9.

Idrisu, A. and, Schurrenberger, P. (1977). Public health significance in four northern states of Nigeria. A mycobacteriologic study. *Nig. J. Vet. Med*. 7: 384-387.

Kent, T. P. and Kubica, G. P. (1985). Public health mycobacteriology. A guide for level III laboratory. United States. Human Health Services. Centre for Disease Control, Atlanta, Georgia 30: 333.

Kolo, I. (1991). Bovine and human tuberculosis in Zaria, Nigeria. A mycobacteriological study. Ph.D Thesis, Department of Microbiology, A.B.U. Zaria. Pp. 58-59.

National Population Commission (1991). Nigeria and its people. Workshop series, 11:5.

Ojo, O. A. and Unigbo, J. A. (1987). Genital tuberculosis in the University of Benin Teaching Hospital. A nine year review. *Cent. Afr. J. Med*. 33 (5): 129-132.

Parry, C., Davies, P.D.O. (1996). The resurgence of tuberculosis. *J. Appl. Bacteriol*. 81: 23-26.

Ravenel, M. P. (1992). Intercommunicability of human and bovine tuberculosis. *J. Comp. Path. Therapeut*. 15: 112-143.

Smith, T. (1998). A comparative study of bovine tubercle bacilli and human bacilli from sputum. *J. Exp. Med*. 3: 451-511.

Uza, D. V (1998). Volume of livestock trade in Benue State and implication for disease control. Published research report. pp. 4- 6.