Journal of Animal and Veterinary Advances 6 (7): 883-886, 2007
ISSN: 1680-5593

The Use of Elisa in the Detection of Bovine Tuberculosis in Slaughtered Trade Cattle and Sedentary Herds in South West Nigeria

[1]I.E. Asiak, [1]O.G. Ohore, [1]B.O. Emikpe, [1]O.O. Abatan and [2]M.A. Ockiya
[1]Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria
[2]Department of Livestock Production Technology, Faculty of Agricultural Technology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

Abstract: The control of bovine tuberculosis is aimed at detection and removal of infected animal to prevent the spread of infection both within and between farms, the diagnosis of TB is often made at necropsy, using Tuberculin skin test and by Mycobacterium isolation. These methods are not suited for mass screening hence the need for sensitive and reliable diagnosis method especially in a developing economy. In this study, sera were collected from 247 cattle from three major cosmopolitan abattoirs in three southwestern states of Nigeria; Lagos, Oyo, Osun states while 100 cattle were also sampled form sedentary herds with history of endemic tuberculosis confirmed by intradermal tuberculosis test and cultural technique. The sera were screened using the ELISA Technique. A total seroprevalence of 36.3% was observed with seroprevalence of 45.7% in slaughtered cattle and 13% of sedentary herd in Southwestern Nigeria. This shows a higher prevalence when compared with previous studies in the same environment. Comparing the clinical signs of TB and some gross lesions with the sero-positivity indicates the high sensitivity of the antibody ELISA. An observation of 4.8% prevalence among cattle with apparently good body condition indicates that some cattle incubate the disease without exhibiting clinical signs. The comparative study of sex relationship in bovine TB, showed no sex preference as both sexes are at equal risk of infection. There is the need for combination of test methods including ELISA for mass screening of animals' population for bovine tuberculosis especially in developing nations with less sophisticated diagnostic tools used in detection and control of the zoonosis.

Key words: Bovine, tuberculosis, ELISA, south, west, Nigeria

INTRODUCTION

Bovine Tuberculosis (TB) is an infections disease of cattle mainly caused by *Mycobacterium bovis*. It is characterized by progressive development of tubercles in any tissue or organ of the body (Clarke, 1998). The control of the disease is aimed at detection and removal of infected animal to prevent the spread of infection both within and between farms (Morrison *et al*., 2000). The incidence of bovine TB has been observed to be rising in many parts of the world where the standard of living is poor especially in Asia and Africa (Kochi, 1991; Collin, 1993; Cadmus *et al*., 1999; Ameni *et al*., 2003). This may be attributed to lack of organized and practicable test methods suitable for mass screening.

In many animal species, the diagnosis of TB is often made at necropsy. Tuberculin skin test and Mycobacterium isolation designed for use in animals (Ameni *et al*., 2003) are not suited for mass screening as the techniques are laborious, time consuming and highly expensive. Although TB involves cell-mediated immune response, serological reactions in animals have also been reported to be of diagnostic importance (Wood *et al*., 1992) as high positive titers reflect active stage of the disease.

Previous surveys of Bovine TB in Africa had been based on necropsy finding at meat inspection with fewer reports based on single intradermal tuberculin test and isolation of Mycobacterium sp (Ameni *et al*., 2001, 2003). The prevalence in East Africa ranged between 7.9-14.2% (Ameni and Roger, 1997; Asseged *et al*., 2000; Ameni *et al*., 2001, 2003) while in Nigeria, it varies between 0.49-2.8% (Drisai and Abdullahi, 1994; Cadmus *et al*., 1999; Igbokwe *et al*., 2001). Although there has been use of some serological tests such as complement fixation, haemagglutination test, indirect immuno fluorescent antibody test and IFN-Y assay (Wood *et al*., 1991; 1992), the potential value for mass screening is rather little in a developing economy, hence the need to employ the use of reliable, sensitive technique which is effective for mass screening in the detection diagnosis of Bovine TB.

Corresponding Author: B.O. Emikpe, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria

Enzyme Linked Immunosorbent Assay (ELISA) had been observed to be sensitive and reliable in the diagnosis of disease of animals especially bovine TB (Ayanwale, 1998) but there is paucity of information on the use of ELISA in Nigeria. This study was carried out to assess the use of ELISA in the detection of bovine TB in slaughter cattle and known TB herds in southwestern part of Nigeria.

MATERIALS AND METHODS

Animals/sera: Sera were collected from 247 cattle from three major cosmopolitan abattoirs in three southwestern states of Nigeria; Lagos, Oyo, Osun states (Fig. 1) while 100 cattle were sampled form a sedentary herd with a history of endemic tuberculosis confirmed by intradermal tuberculosis test and culture technique.

ELISA Technique was as described by Robbe-Austerman *et al*. (2006). Following the chequer board titration, the optimal dilution: Antigen 1:1000, sera 1:100 and conjugate 1:5000 were obtained. The commercial bovine tuberculosis PPD (Vet. Lab. Agency surrey KT15 3NB, UK) was used as ELISA coating antigen. The negative control was a calf serum confirmed by a random chequer board titration of some supposedly negative samples.

The positive control used was a confirmed positive serum for a known TB infected sedentary herd.

Test validation: The test was considered valid only when the different between the positive and negative control mean was greater than 0.30,in addition to the mean optical density value of the negative control being less than or equal to 0.35.

Data presentation and analysis: Mean optical densities and samples to positive ratios.

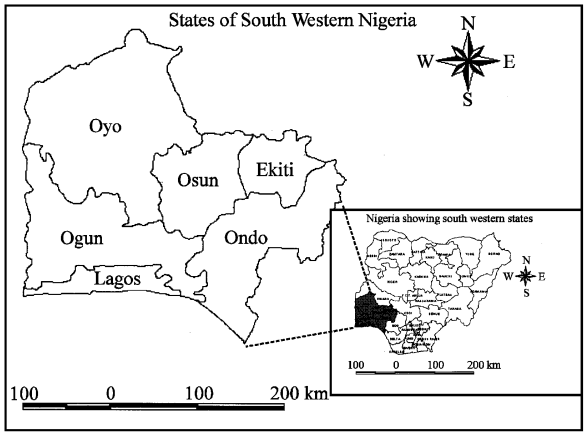

Fig. 1: Showing the states where samples were taken

The Samples to Positive Ratio (SPR) were determined from the mean optical densities of the test serum samples based on the formula:

$$\text{Samples to positive ratio} = \frac{\text{S-NCX}}{\text{PCX-NCX}}$$

Where,

S : Mean optical density of test sample
NCX : Mean optical density of the Negative control
PCX : Mean optical density of the positive control

Prevalence: To obtain the prevalence, the cut-off value for the samples to positive ratio was determined by the formula:

$$\text{Cut off} = \frac{2\text{NCX}}{\text{PCX -NCX}}$$

Where,

Ncx : Mean optical density of negative control
Pcx : Mean optical density of positive control

Samples with values of SPR grater than the cut-off were designated as positive. Otherwise, it was designated as negative.

The prevalence per group of bird was calculated thus:

$$\text{Prevalence (\%)} = \frac{\text{No. of positive sample in group} \times 100}{\text{Total number of samples in-group}}$$

Statistics: Data was analyzed using the SAS GLM mode statistical package (SAS, 1992) Chi-square analysis was used to determine significant difference between the seroconversion rates. Significance is reported at the $p<0.0.5$ level.

RESULTS

The relative prevalence of antibodies to *M. bovis* is as shown in Table 1. There was significant difference in the prevalence observed for the slaughter cattle and sedentary herds, especially those from Lagos and Osun states.

The relative prevalence of antibodies to *M. bovis* in slaughter cattle with various ante mortem and post mortem lesions are showed in Table 2. A significant prevalence of antibodies was observed in animals with cases as lymphadenitis, granulomatous pneumonia and marked emaciation.

Table 1: The relative prevalence of antibodies to *M. bovis* in slaughtered cattle and sedentary herds

Location	No sampled	No positive	ELISA units Mean+SD
Lagos	99	84 (84.9%)	30.4±13.7
Oyo	100	10 (10.0%)	50.8±25.8
Osun	48	19 (39.6%)	30.6±19.8
Total	247	113 (45.7%)	
Sedentary herds	100	13 (13.0%)	56.1±27.5
Total	347	126 (36.3%)	

Table 2: Relative prevalence of antibodies to *M. bovis* in slaughtered cattle with various gross lesions

Gross lesion	No sampled	No positive	(%)
Severe emaciation	27	4	14.8
Slight emaciation	38	5	13.2
Caseous lymph nodes	2	2	100
Apparently normal body condition	21	1	4.8
Skin lesion	3	0	0
Granulomatous pneumonia	2	2	100
Total	93	14	

Table 3: Prevalence of antibodies to *M. bovis* in male and female slaughtered cattle in Oyo state

Sex	No sampled	No positive	(%)
Female	60	6	10
Male	40	4	10
Total	100	10	10

The prevalence of antibodies to *M. bovis* in both male and female animal sampled at the abattoir in Oyo state is as shown in Table 3. There was no significant difference in prevalence of antibodies in both sexes.

DISCUSSION

In this study, a total seroprevalence of 36.3% was observed with 45.7% in slaughtered cattle and 13% of sedentary herd in Southwestern Nigeria. This shows a higher prevalence when compared with previous studies in the same environment, which showed a prevalence of 1.22 and 1.44%, respectively (Cadmus *et al*., 1999). This is also higher than previous reports in intensive diary farm in Ethiopia (Kiros, 1998), Eritrea (Omer *et al*., 2001) and smallholder farm in Ethiopia (Asseged *et al*., 2000; Ameni *et al*., 2001, 2003). This remarkably difference may be due to the method of diagnosis as previous studies mostly used gross pathological changes, which may not be able to detect sub clinical infection. However, the use of ELISA, which is a highly specific and sensitive method, produces a more realistic result. The prevalence shown by the slaughter cattle indicated exposure to the infections agent in the various herds from which they were obtained. This is an indication of high prevalence of tuberculosis in cattle in Nigeria and this may be connected to the high prevalence seen in humans in Nigeria (Cadmus *et al*., 1999).

Comparing the clinical signs of TB and some gross lesions with the sero-positivity indicates the high sensitivity of the antibody ELISA. This results support the inference that clinical signs of progressive emaciation, weakness and lymphodenopathy are some of the general signs associated with bovine TB (Clarke, 1998). There is significance correlation of the prevalence with occurrence of caseous lymphadenitis (Morrison *et al*., 2000) and frequency of lung lesion (McClroy *et al*., 1986). In this study however, an observation of 4.8% prevalence among cattle with apparently normal body condition indicates that some cattle incubate the disease without exhibiting clinical signs especially in early infections (Cousins, 2001). It is also worthy to note that the disease is chronic and the onset of clinical signs or gross pathology may take some time before being observed (Radiostits *et al*., 2000). The early detection by ELISA technique can aid in proper screening and elimination.

The comparative study of sex relationship in bovine TB, showed no sex preference as both sexes are at equal risk of infection. This is in agreement with other workers (Ameni *et al*., 2003).

The prevalence of TB in this study had been observed to be higher than previously reported indicating possible less sensitive diagnostic methods used in previous studies. There is need for combination of test methods including ELISA for mass screening of animals' population. The relative high prevalence of human TB as observed by previous workers in the same environment (Cadmus *et al*., 1999) may be related to the high prevalence of TB among slaughter cattle consumed by human population despite the present use of post mortem examination as the diagnostic tool of detecting Bovine TB.

There is need to control Bovine TB and accurate diagnosis is essential as Mycobacteria are among the known opportunistic infections associated with AIDS pandemic and the combination of the two infections have been referred to as a " cursed duet" as 5.6 million or 40% of the 14 million people with AIDS were infected with TB (Chrétien, 1990). Recent reports also showed that human TB were from animals especially where people are exposed to diseased cattle or their products (Ameni *et al*., 2003) hence the use of ELISA along with other diagnostic methods can be recommended to aid the detection of TB in slaughter cattle especially in developing nations so as to control the zoonosis.

CONCLUSION

This study clearly showed that with ELISA technique the true prevalence of bovine TB can be obtained as subclincal infection can be detected early. This technique is also cheap and affordable in a developing economy.

REFERENCES

Ameni, G. and F. Roger, 1997. Epidemiology of bovine tuberculosis in East Shewa (Debre Zeit and Zeway). In: Proc. 12th Ann. Conf. Ethiopia Vet. Assoc. Addis Ababa, Ethiopia, pp: 13-19.

Ameni, G., K. Miorner, F. Roger and M. Tibbo, 2000. Comparison between comparative tuberculin and gamma interferon tests for the diagnosis of bovine tuberculosis in Ethiopia. Trop. Anim. Health Prod., 32: 267-276.

Ameni, G., A. Ragassa, T. Kassa and G. Medhin, 2001. Survey on bovine tuberculosis and its public health implications to cattle raising families in Wolaita Soddo, Southern Ethiopia. Ethiop J. Anim. Prod., 1: 55-62.

Ameni, G., P. Bonnet and M.A. Tibbo, 2003. Cross-sectional study of bovine tuberculosis in selected dairy farms in Ethiopia. International Journal of Applied Research Veterinary Medicine Vol. 1 (www.jarvm.com/articles/Vol1Iss1/AMENIJVM.htm)

Asseged, B., A. Lubke-Becker, E. Lemma, K. Taddele and S. Britton, 2000. Bovine tuberculosis: A cross-sectional and epidemiological study in and around Addis Ababa. Bull. Anim. Health Prod. Afr., 48: 71-80.

Ayanwale Olalekan, F., 1998. Isolation of mycobacteria from nasal secretion of cattle. Zariya Veterinarian, 4: 77-79.

Clarke, C.F., 1998. Tuberculosis. In: The Merck Veterinary Manual, (8th Edn.), Philadelphia: Merck, pp: 489-493.

Collin, F.M., 1993. Tubercullosis: The return of an old enemy. Microbiology, 19: 1-6.

Cadmus, S.I.B., B.O. Olugasa and G.A.T. Ogundipe, 1999. The prevalence and zoonotic importance of tuberculosis in Ibadan. Proceedings of Nigerian Veterinary Medical Association Conference, Kaduna.

Chrétien, J., 1990. Tuberculosis and HIV. The cursed duet. Bull. Union Int. Tuberculosis, 65: 25-28.

Cousins, D.V, 2001 *Mycobacterium bovis infection* and control in domestic Livestock. Rev. Sci. Tech., 20: 71-85.

Drisai D.H.M. and D.A. Abdullahi, 1994. Current status of bovine tuberculosis at Sokoto abattoir. Trop. Vet., 12: 134-137.

Igbokwe, I.O., I.Y. Madaki, S. Darburan, J.A. Ameh, M.M. Aliyu and C.O. Nwosu, 2001. Prevalence of pulmonary tuberculous lesion in cattle slaughtered in Northeastern Nigeria Revue d elevage et de medicine veterinaire des pays tropicaux 54: 191-195.

Kiros, T., 1998. Epidemiology and zoonotic importance of bovine tuberculosis in selected sites of Eastern Shoa, Ethiopia. MSc Thesis, Faculty of Veterinary Medicine, Addis Ababa University and Free University of Berlin.

Kochi, I., 1991. The global tuberculosis situation and a new control strategy of the World Health Organization, Tubercle, 72: 1-6.

Radostits, D.M., D.C. Blood and C.C. Gay, 2000. Veterinary Medicine, A Textbook of Diseases of Cattle, Sheep, Pigs, Goats and Horses, (8th Edn.), London: Bailliere Tindal., pp: 748-785.

McClroy, S.G., S.D. Neill and R.M. McCrackeen, 1986. Pulmonary lesions and *M.bovis* excretion from the respiratory tract of tuberculin reacting cattles. Vet. Rec., 118: 718-721.

Morrison, W.T., F.J. Bourne, D.R. Cox, C.A. Donnally, G. Gettinby, J.P. McInerny and R. Woodroffe, 2000. Pathogenesis and diagnosis of infections with *M.bovis* in cattle. Vet. Rec., pp: 236-242.

Omer, M.K., E. Skjerve, Z. Woldehiwet and G. Holstad, 2001. A cross-sectional study of bovine tuberculosis in dairy farms in Asmara, Eritrea. Trop. Anim. Health Prod., 33: 295-303.

Robbe-Austerman, S., I.A. Gardner, B.V. Thomsen, D.G. Morrical, B.M. Martin, M.V. Palmer, C.O. Thoen and C. Ewing, 2006. Sensitivity and specificity of the agar-gel-immunodiffusion test, ELISA and the skin test for detection of paratuberculosis in United States Midwest sheep populations. Vet. Res. 37: 553-64.

Wood, P.R., L.A. Corner, J.S. Rothel, C. Baldock and D.B. Cousins, 1991. Field comparison of the gamma IFN assay and intradermal tuberculin test for the diagnosis of bovine Tuberculosis. Aus. Vet. J., 68: 286-290.

Wood, P.R., L.A. Corner, J.S. Rothel, J.L. Ripper, T. Fifis, B.S. McCormick, B. Francis, L. Melville, K. Small, K. Dewilte, K. Tolson, T.J. Ryan, G.W. Delisle, J.C. Cox and S.I. Jones, 1992. A field evaluation of serological and cellular diagnostic tests for bovine tuberculosis. Vet. Microbiol., 31: 71-79.