African Journal of Biomedical Research, Vol. 10 (2007); 77 - 82
ISSN 1119 – 5096 © Ibadan Biomedical Communications Group

Full-text available at
http://www.ajbrui.com
http://www.bioline.br/md

Full Length Research Article

Reduced Plasma Levels of Essential Trace Elements in *Mycobacterium bovis* Infected Cattle In Nigeria.

Cadmus S.I.B.[1], Arinola O.G.[2]

[1]*Department of Veterinary Public Health and Preventive Medicine, University Of Ibadan, Ibadan, Nigeria.*
[2] *Department Of Chemical Pathology and Immunology, University Of Ibadan, Ibadan, Nigeria.*

Received:
July, 2006

Accepted (Revised):
December, 2006

Published
January, 2007

ABSTRACT

Ninety-four (94- 39 infected, 55 un-infected with M. bovis) cattle were screened at an abattoir and a farm in Ibadan, Southwestern, Nigeria over a period of eight weeks for bovine tuberculosis. The levels of essential trace elements (Zn, Fe, Se, Cu and Mn) in the plasma collected from 39 *Mycobacterium bovis* infected cattle and 55 *M. bovis* free cattle were measured using atomic absorption spectrophotometry. The result shows significant reduction between $p<0.01$ and $p<0.001$ in the levels of all the nutritionally essential trace elements except manganese in the M. bovis infected cattle compared with the un-iinfected ones. Mineral supplementation is therefore advocated in cattle herds in areas where bovine tuberculosis is endemic.
(Afr. J. Biomed. Res. 10: 67 - 72, January 2007)

Keywords: Bovine tuberculosis, trace-elements, reactive oxygen species, immune system, Africa.

**Address for Correspondence :(e-mail): sibcadmus@yahoo.com*
Tel : 234 8023751093; fax : 234 02 8103043

Abstracted by:
African Index Medicus (WHO), CAB Abstracts, Global Health Abstracts, Asian Science Index, Index Veterinarius,

INTRODUCTION

Bovine tuberculosis (TB) caused by *Mycobacterium bovis* and *M. caprae* (Pavlik et al., 2002a,b, Erler et al., 2004) is a chronic bacterial disease mainly of cattle and other animals including man (Pavlik et al., 2003). The severity of TB was linked with nutritional status. The finding that mineral deficiencies may increase the risk of TB infection in cattle was documented in UK (Udris, 1983). In related direction, a report from Republic of Ireland documented an increase in certain diseases of cattle, which were attributed to mineral deficiencies and poor animal husbandry (Griffins et al., 1993).

Apart from the above findings, there is a strong epidemiological association between malnutrition and infection with *Mycobacterium* (HMSO, 1997). Among the nutritional deficiencies that have been implicated in the impaired response to tuberculosis are micronutrients (Stead and Dutt, 1988). Deficiencies of trace elements especially Zn, Fe, Se, and Cu have been demonstrated to impair cell-mediated immunity. Cell-mediated immunity is known to be important in the successful response to the control of infection with tubercle bacilli (Kaufmann, 2003). Despite the implicated importance of trace elements in immuno-modulation of *Mycobacterium* infection, the levels of trace elements and other micronutrients had not been determined in bovine tuberculosis in Nigeria. Previous studies in bovine tuberculosis in Nigeria were centered on epidemiology, pathology, and control (Ayanwale, 1984, Cadmus *et al*., 2004). However, little is known about the level of trace elements in tuberculous cattle as compared to non-infected ones in Nigeria. The aim of this study is to establish the levels of certain essential trace elements (Zn, Fe, Se, Cu, and Mn) in culture positive cattle for *M. bovis* in Nigeria.

MATERIALS AND METHODS

Study site: A municipal government abattoir in Bodija and the University of Ibadan, Teaching and Research farm, both in Ibadan, South western, Nigeria were visited for this study over a period of eight weeks. 10ml of blood each was collected from a total of 94 cattle.

Single Intradermal Comparative Tuberculin Test (SICCTT): Fifty-five cattle were screened using the single intradermal comparative cervical tuberculin test (SICCTT) as described by Rothel et al. (1993) with bovine purified protein derivative (B-PPD) and avian purified protein derivative (A-PPD) obtained from the Veterinary Laboratories Agency Weybridge, (U.K). Measurements of skin thickness were taken before and after the inoculation following the OIE (2000) procedure.

Laboratory diagnosis of *Mycobacterium bovis*

Gross pathology*:* This was carried out 39 in animals showing gross lesions indicative of tuberculosis from the abattoir using different tissue samples and lymph nodes. This was done by visual examination and palpation for the presence or absence of tubercles.

Histopathology*:* The gross pathology findings were confirmed by the histological investigations using Haematoxylin and Eosin (H-E) and Ziehl-Neelsen (ZN) staining techniques.

Detection of mycobacteria*:* From each tuberculous animal, randomly selected tuberculous lesions were examined microscopically by ZN staining technique for the detection of acid fast bacilli (AFB) and cultured using the Lowenstein –Jensen (L-J) medium after the decontamination/digestion procedure was carried out using the Becton Dickinson **(USA)** method. The tissue suspension was inoculated onto Lowenstein-Jensen slopes with pyruvate and /or glycerol and incubated at 37ºC for between 8 and 12 weeks.

Blood collection: 10ml of venous blood was withdrawn from each subject into bottles containing an anticoagulant (lithium heparin). The plasma was separated soon after collection

and preserved at –20^{0}C until required for analysis.

Determination of trace elements: Trace elements were determined from the preserved plasma with atomic absorption spectrophotometer (AAS). Zinc was analysed based on the method carried out by Smith et al, (1979); the method of Zettner et al., (1966) was used for Fe; selenium was analysed using the method of Pleban et al (1982); copper through the method of Osheim (1983), while manganese was analysed through the method of Versieck and Cornelis (1980). Meticulous attention and strict adherence to standard procedures of trace elements analysis were duly followed.

Statistical analysis: The results were expressed as mean + 1 S. D. Comparisons were made using Student t – test. P value less than 0.05 was considered significant.

RESULTS

Thirty-nine animals screened from the abattoir were confirmed to have TB based on positive cultural analysis coupled with gross pathological lesions and histological findings. The remaining 55 cattle were certified free from TB based on their being negative on the single intradermal comparative cervical tuberculin test (SICCTT) carried out before the blood collection (Table 1). The values of most of the trace elements were significantly reduced in the TB infected animals compared to those un-infected. Copper was significantly reduced ($p<0.001$) in infected compared to the un-infected; Fe was also highly significantly reduced in infected cattle compared to un-infected ($p<0.01$). Manganese was, however, slightly lower in infected cattle compared to un-infected and did not reach statistical significance ($p>0.05$). Finally, selenium and zinc were also significantly lower in infected cattle compared to the un-infected ($p<0.01$) (Table 2).

Table 1:
Methods of confirming TB status of cattle sampled

	TB-infected abattoir cattle	Un-infected farm cattle
No. sampled	39	55
Methods of confirming TB status	Gross pathology, histology and culture	Tuberculin test

Table 2:
Levels of Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu) and Selenium (Se) in *M. bovis* infected and uninfected cattle

	Infected cattle (n=39)	Un-infected cattle (n=55)	t-value	P-value
Cu(ug/dl)	53.56 ± 10.67	65.33 ± 8.46	5.72	P < 0.001
Fe(ug/dl)	50.62 ± 20.34	63.53 ± 10.86	3.62	P < 0.01
Mn(ug/dl)	44.95 ± 27.68	50.22 ± 9.25	1.14	P > 0.05
Se(ug/dl)	47.72 ± 20.01	60.98 ±11.37	3.20	P < 0.01
Zn(ug/dl)	16.08 ± 5.71	19.43 ± 2.95	3.36	P < 0.01

Values are mean ± SD.

DISCUSSION

Anecdotal evidence that trace elements deficiencies in soils are related to susceptibility of cattle to tuberculosis has been presented on more than one occasion (DEFRA, 2004). It is postulated that deficiencies in trace elements such as selenium, copper and iodine result in a compromised immune response. In turn this leads to increased disease susceptibility (DEFRA, 2004). Many micronutrients play important roles

in key cellular and metabolic processes. It is not surprising that micronutrient deficiencies would alter immune response that would in turn determine resistance to disease or disease progression.

Zn deficiency causes abnormal functions of lymphocytes atrophy and reduced thymulin activity (Chandra, 1980). Zn deficiency also results in decreased ingestion and phagocytosis by macrophages. Macrophages and T-lymphocytes are important effector cells in the control of *Mycobacterium* spp, thus low level of Zn in bovines with *M. bovis* may explain the observed pathology. Zn is also known to react with oxygen to generate a product highly toxic to ingested pathogens (Spencer et al., 1990), thus low level of Zn in bovines may be responsible for the failure to mount effective antituberculosis drug response by infected cattle.

The humoral and cellular arms or the immune system are suppressed by Cu deficiency (Lukasewycz and Prohaska, 1990), since it is known that decreased cupper level and neutropenia will impair phagocytosis. Previous studies have revealed that dietary Cu deficiency decreased the relative percentage of splenic T-lymphocytes and T-helper (CD4+) cells in mice (Lukasewycz et al., 1985). Several laboratories have also reported decreased mitogenic responsiveness of splenocytes from Cu-deficient nutrients (Lukasewycz and Prohaska, 1983, Vyas and Chandra). Together, these data suggest that the development and function of T lymphocytes may be particularly sensitive to low Cu. Evidence exists to indicate depletion of CD4+ and CD8+ T-cells exacerbates experimental tuberculosis (Prohaska and Lukasewycz, 1981). It may be reasoned that low plasma Cu in bovine with tuberculosis increases their proneness to *M. bovis* and other intracellular parasites.

It was shown that certain bacteria grow *in vivo* under Fe- limiting condition (Kadurugamuwa et al., 1988), even toxin production is maximal *in vitro* under condition of Fe-deprivation (Tai and Holmes, I988a). But *Yersinia* infections are unusually prevalent in patients receiving desferrioxamine or ingesting large amount of Fe (Robin-Browne and Prpic, 1985, Kpochan, 1973). These reports (Kadurugamuwa et al., 1988, Tai and Holmes I988a, Robin- Browne and Prpic, 1985, Kpochan, 1973) showed the double edge effects of Fe in regulating infections. In the present study, significantly low level of Fe was found in bovines with tuberculosis. This may be due to ingestion of Fe containing materials by mononuclear phagocytes. Uptake of Fe-containing substance or the phenomenon of "reticuloendothelial cell blockade" reported in *Histoplasma capsulatum* infection (Bagg and Neilands, 1987). *Mycobacterium* spp is also a macrophage invading pathogen like *Histoplasma capsulatum*.

Cu, Zn, Mn, Fe and Se are nutritionally essential minerals, which are components of enzymes including antioxidant enzymes (superoxide dismutase, catalase and glutathione peroxidase) required for the removal of reactive oxygen intermediates in metabolically active cells like phagocytes (Stead and Dutt. 1988). These essential elements (Zn, Cu, Mn, Fe, and Se) are low in cattle with tuberculosis, suggesting that there may be an increase in the level of reactive oxygen species especially in *M. bovis* infected macrophages. Thus, this implies a state of oxidative stress that may enhance the activities of the pathogens and probably make the cattle more vulnerable to other diseases. Reactive oxygen species have been associated with damage to cells and tissues of the immune system through formation of free radicals (Machlin, 1987). Free radical is a highly reactive, transient chemical species characterized by the presence of unpaid electrons. This transient nature and reactivity of free radicals confer on them the ability to exert positive or negative effects on biological system at a high rate. In biologic systems, free radicals may be centred on carbon, oxygen, or sulphur atoms. However, most researches have focused on oxygen centered radicals because of the ability of molecular oxygen in aerobic organisms and its ability to readily accept electrons (Machlin and Bendich, 1987). There are numerous intracellular reactions that generate free radicals and are important components of the oxidative burst

reaction of macrophages and neutrophils. The bactericidal, virucidal, and tumouricidal capacity of reactive oxygen species is essential in combating pathogenic infections and killing tumour cells (Machlin, 1987). However, the non-specific and excessive production of these reactive molecules may be responsible for the structural and functional damage to macrophages invaded by *M. bovis*. This is in support of a previous finding that free radical releases arachidonic acid from cell membranes, resulting in its damage (Machlin, 1987).

Killing of intracellular *Mycobacterium* spp by mononuclear phagocytes is primarily accomplished by reactive nitrogen intermediates (Stead and Dutt, 1988). Fe deficiency has been found to impair the production of interferon (IFN) and IL-1 (Tai and Holmes, 1988b, Bendich, 1990). IFN is involved in macrophage activation for the production of nitric oxide (a reactive nitrogen intermediate) from L-arginine (Griffin et al., 1993). IL-1 plays an important role in T-cell dependent processes. Bacterial killing by neutrophils is under partial control of IL-1 (Muller et al., 1987). The implication of low concentration of Fe in cattle with *M. bovis* infection will be a reduction in nitric oxide level and an increase in the chances of survival of *M. bovis*.

Conclusion: The conclusion from this study is that nutritionally essential micronutrients (Zn, Fe, Se and Cu) are low in cattle with *M. bovis*, thus dietary supplementation of these trace elements in *Mycobacterium* infections is advocated.

Acknowledgments: *We sincerely appreciate the immense help of Mr. Ojo Williams of the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria for assisting with the statistical analysis. Our profound gratitude also goes to Dr. Ivo Pavlik of Veterinary Research Institute, Brno Czech Republic for reading through the manuscript and making useful corrections.*

REFERENCES

Ayanwale, F.O. (1984). Studies on the epidemiology of bovine tuberculosis in some States of Southern Nigeria. [PhD Thesis.] University of Ibadan, Ibadan, Nigeria. 184 pp.

Bagg, A. & Neilands J.B. (1987). Molecular mehanism of regulation of siderophore mediated iron assimilation. Microbiology Review. 51, 509-518.

Bendich, A. (1990). Antioxidant micronutrients and immune responses. Annals of New York Academy of Science. 587, 169-180.

Cadmus, S.I.B., Atsanda, N.N., Oni S.O. & Akang, E.E.U. (2004). Bovine tuberculosis in one cattle herd in Ibadan in Nigeria. Veterinarni Medicina. 49, 406-417.

Chandra, R.K. (1980). Acrodermatitis enteropathica; Zinc levels and cell-mediated immunity. Paediatrics, 66, 789-791.

DEFRA. (2004). Bovine TB: Government Reply to the Committee's Report. Seventeenth Special Report.

Deni, M. (1991). Involvement of cytokines in determining resistance and acquired immunity in murine tuberculosis. Journal of Leukemia Biology. 50, 495-501.

Erler, W., Martin, G., Sachse, K., Naumann, L., Kahlau, D., Beer, J., Bartos, M., Nagy, G., Cvetnic, Z., Zolnir-Dovc, M. & Pavlik I. (2004). Molecular fingerprinting of Mycobacterium bovis subsp. caprae isolates from Central Europe. Journal of Clinical Microbiology. 42, 2234-2238.

Griffins, J.M., Hahesy, T., Lynch, K., Salman, M.D. & Hurley T. (1993). The association of cattle husbandry practices, environmental factors and farmer characteristics, with the occurrence of chronic bovine tuberculosis in dairy herds in the Republic of Ireland. Preventive Veterinary Medicine. 17, 145-160.

HMSO. (1997). The Report of the Chief Veterinary Officer: Animal Health (1997): London: The Stationery Office.

Kadurugamuwa, J.L., Anwar, H., Brown, W., Hengstler, B., Kunz, S. & Zak O. (1988). Influence of cephalosporin and iron on surface protein antigens of Klebsiella pneumoniae in vivo. Infection and Immunity. 32, 364-368.

Kaufmann, S.H.E. (2003). Immunity to intracellular bacteria. In: William E.P. (eds). Fundamental Immunology. 5th ed. Lippincott Williams, Wilkins, Philadelphia. 1229-1261.

Kpochan, I. (1973). The role of iron in bacterial infections with special consideration of host -tubercle bacillus interaction. Current Topics in Microbiology andImmunology. 60, 1-30.

Lukasewycz, O.A., Prohaska, R.J., Meyer, S.G., Schmidtke, J.R., Hatfield, S.M. & Marder P. (1985). Alterations in lymphocyte subpopulations in copper-deficient mice. Infection and Immunity. 48,

644-647.

Lukasewycz, O.A., Prohaska, R.J. (1983). Lymphocytes from copper-defiecient mice exhibit decreased mitogen reactivity. Nutrition Research. 3, 335-341.

Lukasewycz, O.A. & Prohaska, R.J. (1990). The immune response in copper deficiency. Annals of New York Academy of Science. 587, 147-159.

Machlin, L.J. (1987). Free radical tissue damage: Protective role of antioxidant nutrients. Journal of the Federation of American Society for Experimental Biology. 1, 441-445.

Muller, I., Cobbold, Sp., Waldmann, H. & Kaufmann, S.H. (1987). Impaired resistance against Mycobacterium tuberculosis infection after selective in vivo depletion of L3T4+ and Lyt2+ T cells. Infection and Immunity. 55, 2037-2041.

O.I.E. (2000). Bovine tuberculosis. Manual of standards for diagnostic tests and vaccines. 4th ed. Paris, France. 359-370.

Osheim, D.C. (1983). Atomic absorption determination of copper: a collaboratory study. J. Assoc Off Anal Chem. 66, 1140-1142.

Ottaway, J.M. & Halls, D.J. (1986). Determination of manganese in biological materials. Pure & Appl. Chem. 58, 1307-1316.

Pavlik, I., Ayele, W. Y., Havelkova, M., Svejnochova, M., Katalinic-Jankovic, V. & Zolnir Dovc, M. (2003). Mycobacterium bovis in human population in four Central European countries during 1990-1999. Veterinarni Medicina. 48, 90-98.

Pavlik, I., Bures, F., Janovsky, P., Pecinka, P., Bartos, M., Dvorska, L., Matlova, L., Kremer, K. & Van Soolingen, D. (2002a). The last outbreak of bovine tuberculosis in cattle in the Czech Republic in 1995 was caused by Mycobacterium bovis subspecies caprae. Veterinarni Medicina. 47, 251-263.

Pavlik, I., Dvorska, L., Bartos, M., Parmova, I., Melicharek, I., Jesenska, A., Havelkova, M., Slosarek, M., Putova, I., Martin, G., Erler, W., Kremer, K. &Van Soolingen, D. (2002b). Molecular epidemiology of bovine tuberculosis in the Czech Republic and Slovakia in the period 1965-2001 studied by spoligotyping. Veterinarni Medicina. 47, 181-194.

Pleban, P.A., Munyani, A., Beacham, J. (1982). Determination of selenium concentration and glutathione peroxidase and erythrocytes. Clin. Chem. 28, 311-316.

Prohaska, R.J. & Lukasewycz, O.A. (1981). Copper deficiency suppresses the immune response of mice. Science. 213, 559-561.

Robin-Browne, R.M. & Prpic, J.K. (1985). Effects of iron and desferrioxamine on infections with Yersinia enterocolitica. Infection and Immunity. 47, 774-779.

Rothel, J.S., Corner, L.A. & Wood, P.R. 1993. Bovine tuberculosis: immunodiagnosis. Australian Standard Diagnostic Techniques for Animal Diseases. CSIRO for the Standard Committee on Agriculture and Resource Management. L.A. Corner. and T.J. Bagust. Eds: East Melbourne, Australia. 1-18.

Smith, J.C.Jr., Butrimovitz, G.P. & Purdy, W.C. (1979). Direct measurement of zinc in plasma by atomic absorption spectroscopy. J. Clin Chem. 25, 1487.

Spencer, H., O'sullivian, V. & Sontag, S. J. (1990). Zinc Balances and White Blood Cell and Lymphocytes Counts in Different States of Zinc Metabolism in Man. Annals of New York Academy of Science. 587, 316 – 318.

Stead, W.W. & Dutt, A.K. (1988). In Mycobacterium tuberculosis Interactions with the Immune System. M. Bend 'nelli., H. Friedman. (eds). Plenum Publishing Corp. New York, New York. 371-377.

Tai, S.S. & Holmes, R.K. (1988a). Iron regulation of the cloned diptheria toxin promoter in Escherichia coli. Infection and Immunity. 56, 2430-2436.

Tai, S.S. & Holmes R.K. (1988b). Iron regulation of the cloned diptheria toxin promoter in Escherichia coli. Infection and Immunity. 56, 2430-2436.

Udris, G.A. (1983). Trace elements in the preventive treatment of bovine tuberculosis. Veterinary Moscow. 2, 17-20.

Versieck, J. & Cornelis, R. (1980). Normal levels of trace elements in human blood plasma. Anal. Chim **Acta**. 116, 217.

Vyas, E. & Chandra, K. (1983). Thymic factor activity, lymphocyte stimulation response and antibody-producing cells in copper deficiency. Nutrition Research. 3, 343-349.

Zettner, A., Sylvia, L.C. & Capacho- Delpado, L. (1966). The determination of serum iron and iron-binding capacity by atomic absorption spectroscopy. Amer. J. Clin. Nutr. 45, 533.