Full Length Research Article

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

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**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.


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

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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 immunomodulation 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 37°C 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°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).

**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 of 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, 1988a). But Yersinia infections are unusually prevalent in patients receiving desferrioxamine or ingesting large amount of Fe (Robin-Browne and Pripic, 1985, Kpochan, 1973). These reports (Kadurugamuwa et al., 1988, Tai and Holmes 1988a, Robin-Browne and Pripic, 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 \textit{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 \textit{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 \textit{M. bovis} infection will be a reduction in nitric oxide level and an increase in the chances of survival of \textit{M. bovis}.

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


African Journal of Biomedical Research 2007 (Vol. 10) / Cadmus and Arinola


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