

Zeitschrift: Acta Tropica
Herausgeber: Schweizerisches Tropeninstitut (Basel)
Band: 42 (1985)
Heft: 1

Artikel: Correlation of serum zinc levels with resistance of cattle to trypanosomiasis
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DOI: <https://doi.org/10.5169/seals-313451>

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Correlation of serum zinc levels with resistance of cattle to trypanosomiasis

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Summary

Zinc, copper and magnesium levels were determined by atomic absorption spectrophotometry in the serum of 32 cattle (Zebus and Baoulés) which were proven to be sensitive to African trypanosomiasis under field challenge and 45 cattle (Baoulés and Ndamas/Baoulés) which were proven to be resistant. Copper and magnesium levels were similar in all animals but zinc levels were higher in sensitive animals (1.50 ppm) than in resistant ones (1.10 ppm) ($p < 10^{-5}$); the reported normal levels of serum zinc is 1.00 ppm. These differences persisted on repeated measurements and whether individuals were infected with trypanosomes or not. Elevated levels of zinc depressed the stimulation of bovine T cells by trypanosomes in vitro and is reported to inhibit antigen presentation by macrophages. Zinc levels may be an influential factor determining susceptibility or resistance of West African cattle to trypanosomiasis.

Key words: Zinc; African trypanosomes; cattle; trypanotolerance; West African bovine breeds.

Introduction

The role of zinc in the immune response has been reviewed recently (Chandra, 1980; Bach, 1981; Chandra and Tejpar, 1983). Zinc deprivation in rodents impairs antibody responses, especially against T dependent antigens, and the generation of cytotoxic T cells (Fraker et al., 1978; Fernandes et al., 1979; Beach et al., 1980). When serum levels of zinc are depressed by half, mice show an increased incidence of bacterial and parasitic infections (Bach, 1981), also mice

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given a zinc free diet are slightly more sensitive to *Trypanosoma musculi* (Lee et al., 1983). Zinc excess, on the other hand inhibits the motility and phagocytic capacity of neutrophils and macrophages and interferes with antigen presentation (Kiremidjian-Schumacher et al., 1981; Chandra and Tejpar, 1983), moreover it suppresses the stimulation of T cells by ConA and PHA (Zanzonica, 1981).

Some West-African cattle resist infection by African trypanosomes while others are fully sensitive (reviewed in Murray et al., 1982 and in Roelants and Pinder, 1984). We have investigated the levels of zinc, copper and magnesium in the serum of trypanosensitive and trypanoresistant animals.

Materials and Methods

Cattle

We examined 19 Zebus, 48 Baoulés and 10 Ndama/Baoulé crosses. There were 31 females and 46 males all of 3–4 years of age. They were divided in “sensitive” and “resistant” to trypanosomiasis by selection under natural challenge in a heavily *Glossina* infected area as described by Roelants et al., (1983). Briefly, sensitive animals show high parasitaemia, intense anaemia and die within 5 to 12 weeks of exposure unless treated; resistant animals show no or transient parasitaemia, no anaemia and maintain themselves in good condition for at least 31 and as long as 56 weeks, the shortest and the longest periods of time we have followed various animals so far.

In the present study the 19 Zebus and 13 Baoulés were sensitive, 35 Baoulés and the 10 Ndama/Baoulés (which are indigenous to the *Glossina* challenge area) were resistant under the conditions used.

Diet

The animals were maintained at our experimental farm in Banankeledaga (Burkina Faso) where they used the same pastures. In the dry season, food supplement was the same for all animals and consisted of Brachiaria hay, compacted bran, cotton seeds and molasses. Salt licks were provided at all times. All animals had access to the same food but individual diets could not be monitored.

For selection under natural field challenge, the animals were moved to Samandeni (Burkina Faso) near the Black-Volta river, where they had to forage for themselves on very poor land as a single herd.

Mineral analysis

Zinc, copper and magnesium serum levels were determined on a Perkin Elmer model 2380 atomic absorption spectrophotometer at the Projet Minier de Gaoua (Burkina Faso). All determinations were performed in double blind with the same instrument. Individual sera were measured at least twice and often three times and sera from individual animals were measured at least on three occasions and as many as six for eight animals at the experimental farm and during exposure to field challenge. Results remained closely similar for individual sera or animals.

Stimulation of bovine peripheral blood lymphocytes

Stimulation of bovine PBL in vitro by *T. brucei* was performed as described by Fumoux et al. (in press).

Briefly, lymphocytes ($5 \cdot 10^5$ cells in 0.1 ml) were distributed in Linbro tissue culture 96 flat bottom wells plates and to these were added 0.1 ml containing 10^5 trypanosomes. The microplates were incubated at 37°C in 5% CO₂ in air during 5 days. 0.5 µC [5-¹²⁵I] iodo-2-deoxyuridine (Amer-

sham) was added to each well and 16 h later the cells were harvested onto glass fiber filters and counted in a Packard 5360 Autogamma Scintillation Spectrometer. To some cultures various concentrations of zinc in the form of Zn Cl₂ or Zn acetate were added.

Results

The serum zinc, copper and magnesium levels did not vary significantly for individual animals analysed repeatedly at the experimental farm and during field challenge. Only sensitive animals showed intense parasitaemia when exposed to *Glossina* but their mineral levels did not change from those found before they were infected. The overall results are presented in Table 1.

Sensitive animals on the whole (Zebus plus Baoulés) have higher levels of serum zinc (1.50 ppm) than resistant animals (1.10 ppm) and the difference is significant at the 10⁻⁵ level. The normal serum zinc level in European cattle is 1.0 ppm ± 0.3 (Rosenberger, 1977). Differences between breeds could be due to basic variations in mineral serum levels and have nothing to do with resistance, hence, comparison of sensitive (1.52 ppm) and resistant (1.14 ppm) animals *within* the Baoulé breed are the most interesting, the p value is < 10⁻⁴. There is no difference between zinc levels in Zebus and sensitive Baoulé nor between resistant Baoulés and Ndama/Baoulé crosses (p > 0.05). There is no difference between the groups for the two other minerals analysed, copper and magnesium.

It was shown that addition of small amounts of zinc (0.7–3.3 ppm) to culture medium inhibits in vitro generation of direct plaque forming cells against sheep erythrocytes and TNP substituted sheep erythrocytes (Malavé et al., 1983). Zinc also inhibits stimulation of bovine PBL by live trypanosomes in vitro (Fig. 1), a phenomenon that has been shown to be T lymphocyte mediated

Table 1. Levels of zinc, copper and magnesium in the serum of trypanosensitive and trypano-resistant bovids

Animals	Number	Serum levels ppm \bar{x} (SD)		
		zinc	copper	magnesium
<i>Sensitive:</i>				
Zebus	19	1.49 (0.64)	0.79 (0.20)	30.9 (5.9)
Baoulés	13	1.52 (0.41)*	1.03 (0.31)	29.7 (5.1)
Total	32	1.50 (0.40)**	0.90 (0.27)	30.2 (5.5)
<i>Resistant:</i>				
Baoulés	35	1.14 (0.22)*	0.91 (0.29)	26.7 (3.7)
Ndamas/Baoulés	10	0.99 (0.06)	0.69 (0.14)	29.7 (3.3)
Total	45	1.10 (0.20)**	0.86 (0.28)	27.3 (3.8)

* p < 10⁻⁴

** p < 10⁻⁵, Student's t test

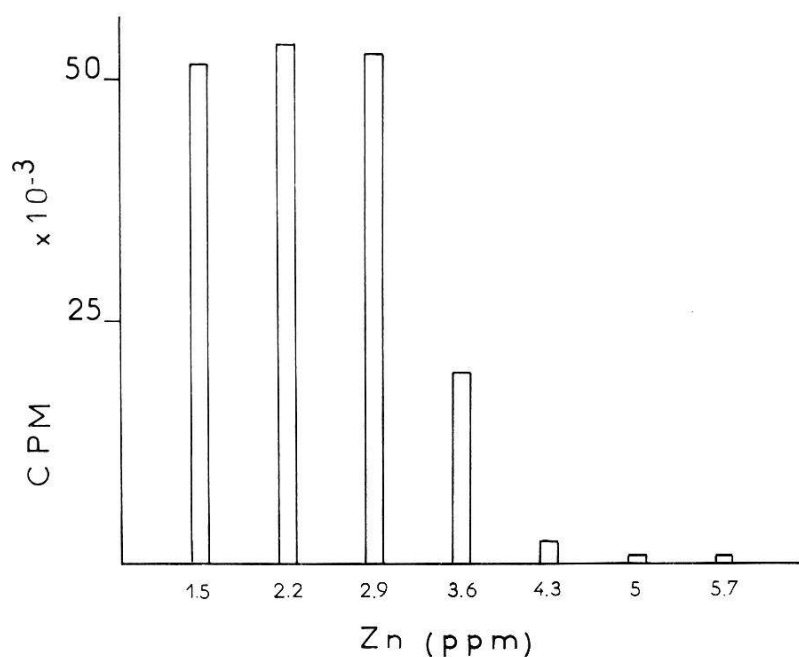


Fig. 1. Influence of zinc on the stimulation of bovine lymphocytes by *T. brucei* in vitro. For technical details see Materials and Methods. The counts are the mean CPM of triplicate cultures. Cultures with zinc concentrations below 2.9 ppm showed stimulations similar to the control without zinc. Cell viability after 5 days in culture was similar at all zinc concentration used (about 60%).

and dependent on monocyte/macrophage type accessory cells (Fumoux et al., in press). The dose effect is narrow since full stimulation or complete inhibition requires only a change of 1.4 ppm in vitro.

Discussion

It appears that elevated levels of serum zinc correlate very significantly with the sensitivity of cattle to trypanosomiasis under natural field challenge.

The reason why zinc levels are different in sensitive and resistant cattle is not known. It does not appear to be obviously a diet difference for all the animals were kept under the same conditions. Furthermore the zinc levels of individual animals did not change significantly whether they were maintained under good nutritional condition in Banankeledaga or very poor ones in Samandeni even during heavy infection with trypanosomes. Zinc levels are the only ones that are different, while copper and magnesium are identical in all groups. This aspect needs further investigation under exactly comparable diet conditions.

There is not a trivial breed difference in the basic level of serum zinc as shown by the analysis of sensitive and resistant Baoulés which are of the same origin and are indistinguishable on a breed basis.

Various facets of the immune response are inhibited by abnormally high zinc levels and this could contribute to the susceptibility of certain West African bovids to trypanosomiasis. The importance in trypanoresistance of antibody

directed against surface antigens of trypanosomes remains equivocal: sensitive and resistant bovids gave the same primary and secondary antibody responses after syringe infection with *T. brucei* (Pinder et al., 1984), but sensitive animals appear to respond less well after fly challenge with *T. congolense* (Pinder M., unpublished) or *T. brucei* (Duvallat G., unpublished). On the other hand, the role of T cells in protection is largely undefined. It is a possibility that zinc acts on the immune response of sensitive animals by interfering with the antigen handling by macrophage-like cells which are known to play an important role in trypanosomiasis (Grosskinsky et al., 1983).

The influence on sensitivity of lowering or enhancing zinc levels in sensitive and resistant animals respectively should allow an evaluation of the importance of zinc in sensitivity to trypanosomiasis.

Acknowledgments

We thank Dr. M. Keuken, Projet Minier de Gaoua, Burkina Faso, for the atomic absorption spectrophotometry readings and Mrs. S. Zerbo for typing the manuscript. – This work was supported by the Deutsche Gesellschaft für Technische Zusammenarbeit, PN 77.2227.5 Federal Republic of Germany and the Institut d'Elevage et de Médecine Vétérinaire des Pays Tropicaux, Maisons-Alfort, France.

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