The effect of experimental *Trypanosoma vivax* infection on the thyroid gland in Zebu bulls

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ABSTRACT

Thyroid gland dysfunction was studied in Zebu cattle experimentally infected with *Trypanosoma vivax,* based on the levels of serum thyroxine (T_4) and occurrence of pathological lesions at 13 weeks post infection (p.i.). There were statistically significant declines in the T_4 levels in the infected group at 3 weeks p.i. ($P<0.05$), 6 weeks p.i. ($P<0.01$) and from 9 to 11 weeks p.i. ($P<0.05$); when compared to the normal T_4 levels that were observed in the uninfected, control bulls throughout the period of study. The thyroid glands of both infected and control bulls showed no gross lesions and the mean weights were not statistically significant ($P>0.05$) between the groups. Histopathological lesions observed in the thyroid glands of the infected bulls were focal in occurrence and include; squamous metaplasia of follicular epithelium with fibroplasia and widened interfollicular connective tissue. The control bulls showed no histopathological lesions in the thyroid glands. It was concluded that thyroid gland dysfunction occurs in trypanosomosis due to *T. vivax* in cattle and it is suggested that this could contribute to the weakness associated with trypanosomosis.

Key words: thyroid gland, dysfunction, Zebu cattle, *Trypanosoma vivax*

Introduction

Trypanosomoses are serious, often fatal parasitic diseases of animals and humans which occur throughout the tropical regions of Africa, the Middle East, Asia and Latin America (MUTAYOBA et al., 1994). The disease is an important constraint to livestock and mixed crop-livestock farming in tropical Africa, with the greatest socio-economic impacts of this disease occurring in sub-Saharan Africa where a suitable environment exists for the survival of tsetse fly, which is the vector responsible for cyclical transmission of both human and animal trypanosomes (KRISTJANSON et al., 1999).

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The major trypanosomes of ruminants are *Trypanosoma vivax*, *T. congolense* and *T. brucei* which have been classified into haematic and tissue groups. *Trypanosoma vivax* and *T. congolense* constitute the haematic group while *T. brucei* falls into the tissue group (LOSOS and IKEDE, 1972). In West Africa, *T. vivax* predominates and *T. congolense* in East Africa (ITARD, 1989) whereas *T. brucei* is generally distributed in tropical Africa (SOULSBY, 1982). The presence of *T. vivax* in both Africa and South America, easy mechanical transmission and virulence for economically important animals make it the most important animal trypanosome (DE GEE, 1980).

Endocrine dysfunctions have been recorded in both human and animal trypanosomoses. *Trypanosoma brucei* infection has resulted in adrenal insufficiency, hypopituitarism, hypogonadism and hypothyroidism in humans and polyglandular endocrine failure in animals due to local inflammation of the pituitary, thyroid, adrenal and gonadal glands (REINCKE et al., 1998). Similarly, some endocrine dysfunctions were reported in *T. congolense* infected-goats (MUTAYOBA et al., 1988), -cattle (OGWU et al., 1992), and rams (MUTAYOBA et al., 1994). There is dearth of such reports on *T. vivax* infection.

The present study was conducted to investigate the pathological changes in the thyroid gland, based on circulating thyroxine profiles and thyroid glandular lesions in cattle experimentally infected with *T. vivax*.

Materials and methods

Experimental animals. Four White Fulani Zebu bulls of between 3 to 4 years of age, with mass ranging from 180 to 200 kg were purchased from Anchau, in the Kubau Local Government Area of Kaduna State, located in a tsetse fly-free zone of northern Nigeria. The animals had no record of exposure to trypanosomal infection. On arrival at Zaria, the animals were acclimatized for at least 3 months during which they were dewormed with albendazole (Pantex Holland B.V.), sprayed with chlorphenvinphos (Pfizona, Pfizer). The animals were kept in cowsheds, grazed daily and given groundnut hay supplement. Water was provided *ad libitum*. Blood samples were collected and screened for haemoparasites.

Parasite. The primary isolate of *Trypanosoma vivax* was obtained from a naturally infected cow in Kudaru, the Lere Local Government Area of Kaduna State. The parasite was identified as pure *T. vivax* isolate at the Department of Veterinary Parasitology and Entomology, Ahmadu Bello University, Zaria. A donor bull was inoculated with 5 mL of the infected cow blood intravenously (i.v.). The parasitaemia was monitored regularly up to the peak level.

Animal treatment. The experimental animals were placed into two groups of two animals each. The groups were housed separately and blood samples were collected

from each bull prior to infection, to determine the thyroxine (T_4) levels. The groups were treated as follows:

Group A. This was the infected group where each bull was infected with 3×10^6 *T. vivax* primary isolate, using 6 mL of the donor bull's blood. Two milliliters of blood was collected daily into McCartney bottles containing ethylene diaminetetra acetic acid (EDTA) and examined, until parasitaemia was established. Determination of their PCVs was done using the haematocrit method (COLES, 1986) and parasitaemia was monitored and estimated by the dark ground/phase contrast buffy coat technique-DG (MURRAY et al., 1983). Subsequently, 3 mL of weekly blood samples were collected from each animal out of which serum samples were harvested (BUSH, 1975) and stored at -20 °C until required for T_4 assay.

Group B. These were the uninfected, healthy control bulls from which blood samples were collected at the same time and treated as in Group A, until the experiment was terminated at the thirteenth week p.i.

Thyroxine assay and data analysis. Thyroxine was assayed by the competitive protein binding (CPB) technique, using a solid-phase enzyme immunoassay method (Microwell T_4 EIA kit, Syntron Bioresearch, U.S.A.). The technique is a quantative method for T_4 determination. The intra-assay coefficient of variation (CV) was 8.6% (n = 15) at 8.92 µg dL^{-1}, 7.2% (n = 15) at 7.65 µg dL^{-1} and 8.5% (n = 15) at 16.9 µg dL^{-1}. The inter-assay CV for 15 different assays were 11.9% at 1.94 µg dL^{-1}, 9.0% at 8.2 µg dL^{-1} and 6.1% at 17.5 µg dL^{-1}. Sensitivity by this assay is 0.5 µg dL^{-1} and specificity is ≥100% at 8µg dL^{-1} for both L-thyroxine and D-thyroxine.

The weekly means (±SEM) were calculated for both groups. The Student's *t*-test (PHILLIPS, 1978) was used to compare the two means for any significant difference.

Necropsy. Postmortem examination was conducted promptly on the carcasses of the sacrificed bulls on termination of the experiment at the thirteenth week p.i. The thyroid glands were excised, trimmed and weighed. Representative sections were obtained and fixed in 10% neutral buffered formalin for at least 48 h (BUSH, 1975). The thyroid tissues were dehydrated in ethanol and embedded in paraffin. Sections were made at 5 μm and stained with haematoxylin and eosin (H&E) stains.

Results

Packed cell volume and parasitaemia. The preinfection mean PCV in the infected group was 29.5 ± 0.7% and 28 ± 1.0% in the uninfected group. At termination of the experiment the mean PCVs were 18.5 ± 1.5% and 27.0 ± 1.5% in the infected and uninfected groups respectively, which were significantly different (P<0.05). Parasites were detected on day 3 p.i. and the first parasitaemic peak occurred on day 6 p.i. in the infected group.
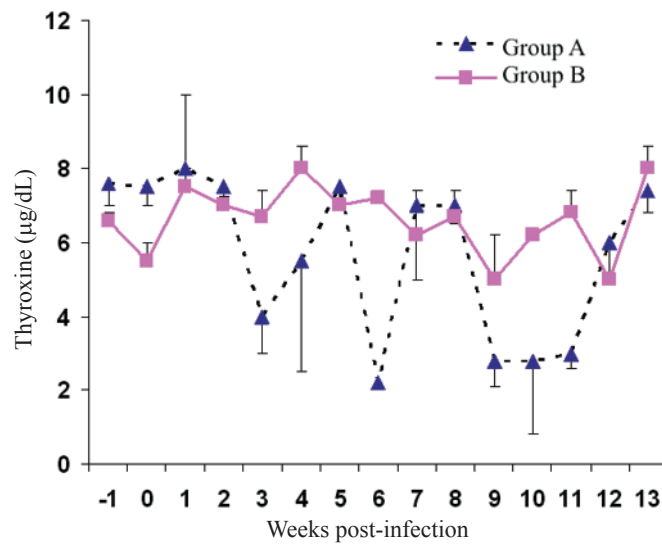
Fig. 1. Mean (± SEM) serum thyroxine in *Trypanosoma vivax* infected (Group A) and uninfected (Group B) Zebu bulls
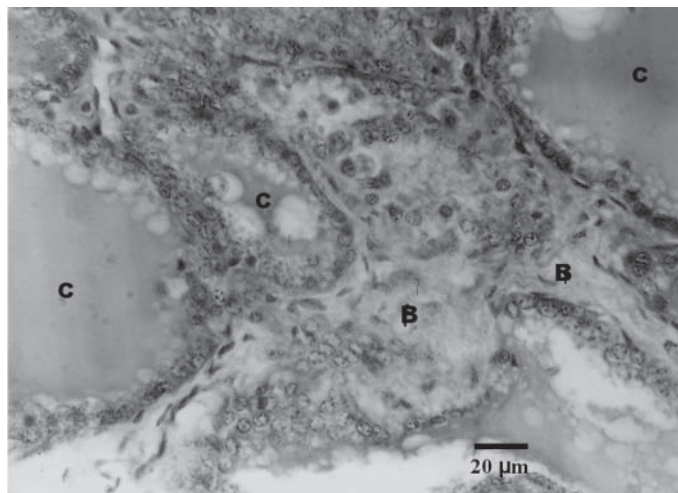

Fig. 2. Photomicrograph of the thyroid gland of a bull infected with *Trypanosoma vivax*. Note peripheral vacuolations of the colloid (C) and fibrosis in the stroma (B). H&E.
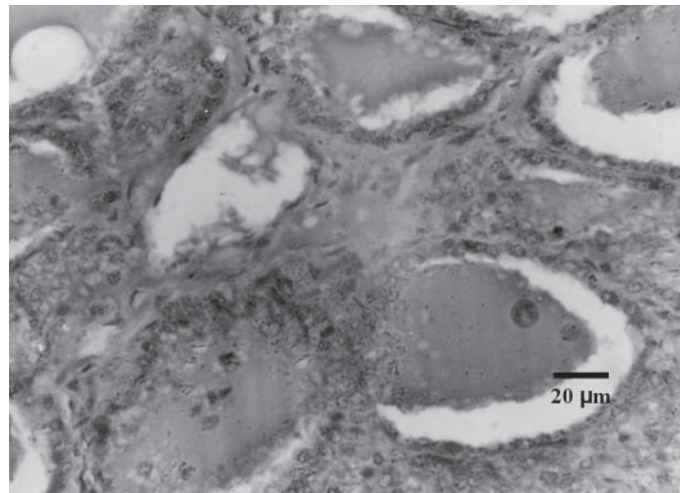
Fig. 3. Photomicrograph of the thyroid gland from a *Trypanosoma vivax*-infected bull. Widened interfollicular tissue with fibroplasia. H&E.
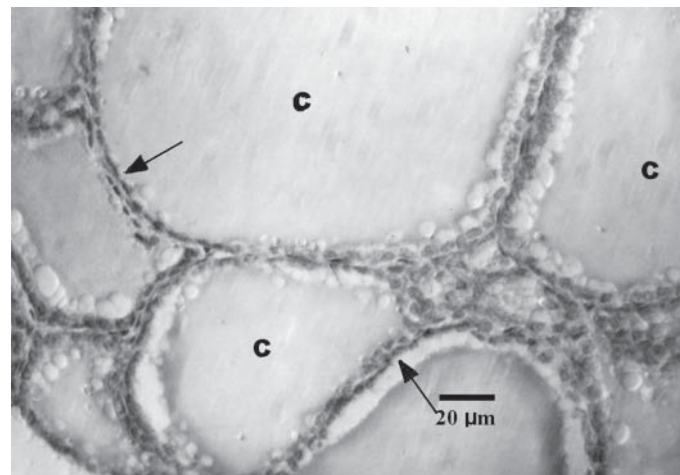

Fig. 4. Photomicrograph of the thyroid gland of a bull infected with *Trypanosoma vivax*. Note peripheral vacuolations of the colloid (C) and squamous metaplasia of epithelial cells (arrows). H&E.

Serum thyroxine concentrations. The mean weekly serum thyroxine (T_4) levels in the *T. vivax*-infected and uninfected bulls are presented in Fig. 1. The mean T_4 levels ranged from 2.0 to 8.0 μg dL^{-1} and 5.0 to 8.0 μg dL-^{1} in the *T. vivax*-infected and uninfected bulls respectively. There was a significant fall ($P<0.05$) in the mean T_4 level of the infected bulls, 3 weeks p.i. The lowest levels of T_4 occurred in the infected bulls 6 weeks p.i which was significantly ($P<0.01$) below the T_4 levels in the uninfected bulls. Subsequent declines in serum T_4 occurred from the ninth to the eleventh weeks p.i. which were significantly different ($P<0.05$) from the T_4 levels in control bulls. The T_4 levels in the uninfected group remained within the normal range throughout the duration of the experiment.

Gross observations. The thyroid glands of both the infected and uninfected animals showed no gross lesions. The mean mass of the thyroid glands of the infected bulls was 10.15 ± 0.1 g compared to 9.74 ± 0.6 g in the control bulls. The mean weights were not statistically significant ($P>0.05$).

Histopathological observations. In the *T. vivax*-infected bulls, the thyroid glands showed focal occurrence of lesions which include: distended follicles, containing pale-staining colloid, with numerous peripheral vacuolations of the colloid. There was squamous metaplasia of the follicular epithelium and fibroplasia in the widened stroma (Figs. 2 to 4). The uninfected (control) bulls showed no histopathological lesions in the thyroid glands.

Discussion

The significant declines, below the normal level recorded in the serum thyroxine (T_4) on the third, sixth and nineth to eleventh weeks p.i. in the *T. vivax*-infected bulls, unlike in the control bulls which remained normal throughout the period of study, could be attributed to effects of the infection. This fall in the levels of T_4 could partly explain the weakness (ITARD, 1989) exhibited in bovine trypanosomosis. The aetiology and classification of hypothyroidism have been given (COLES, 1986). The decrease in T_4 levels coupled with the pathological lesions obtained in the infected bulls were features of primary hypothyroidism as supported by COLES (1986). A 50% prevalence of hypothyroidism has been reported in natural *T. brucei* infection in humans (REINCKE et al., 1998).

The numerous and large peripheral vacuolations observed in *T. vivax*-infected bulls in this study were indicative of active resorption of colloid from the follicles (CURRAN and CROCKER, 2000). This is possibly a response to subnormal declines in T_4 levels as revealed by the serum concentrations, and could explain the compensatory higher T_4 level on the fifth, seventh and eighth weeks in the infected, compared to the uninfected groups. However, in *T. congolense*-infected heifers, OGWU et al. (1992) observed progressive decreases in serum T_4 levels from the third to the eleventh weeks when the fall became significant. This difference could be attributed to a faster response to the falls in T_4 in the

infected bulls in this study since the follicular vacuolations and widened interfollicular tissue observed in the thyroid glands were not reported in the previous study. The squamous metaplasia of the follicular epithelium observed in the *T. vivax*-infected bulls, although not seen in the uninfected (normal) bulls in this study, may also be indicative of a resting gland (DELLMANN, 1998).

Fibrosis in the stroma of thyroid glands of *T. vivax*-infected bulls as similarly encountered in heifers infected with *T. congolense* (OGWU et al., 1992), could occur due to reparative or reactive processes and consequently affect the secretive functions of the gland.

The trypanosomosis-induced decreases in the levels of circulating thyroxine reported here and previously due to other species of trypanosomes (MUTAYOBA et al., 1988; OGWU et al., 1992; VAN DAM et al., 1996; REINCKE et al., 1998) could be multifactorial in causation. Primarily, the pathological lesions produced in the thyroid gland directly affect the ability of the follicular epithelial cells to produce thyroglobulin (colloid). Indirectly, the thyroid plasma membrane for thyrotropin contains gangliosides and glycoproteins which contain sialic acids (JEANLOZ and CODINGTON, 1976; SCHACTER and ROSEMAN, 1980). Perhaps the sialic acids are also cleaved by trypanosomal sialidases (ESIEVO, 1979) as earlier established on erythrocytes (DUROCHER et al., 1975; SEAMAN et al., 1977; ESIEVO et al., 1982) which renders the thyroid membranes insensitive or less sensitive to thyrotropin stimulation from the anterior pituitary gland, and results in diminished production of thyroid hormones. Studies in human African trypanosomiasis (REINCKE et al., 1998) demonstrated central (hypothalamic/pituitary) and peripheral (thyroid and adrenal glands, gonads) defects in hormone secretion, that were reversed partly by specific therapy, which restored adrenal and thyroid functions. The authors inferred that the correlation of hypopituitarism with high cytokine concentration (TNF, IL-6) together with direct parasitic infiltration of endocrine glands, could be factors involved in pathogenesis of sleeping sickness-associated endocrine dysfunction.

The present study which has demonstrated dysfunction and pathology in the thyroid gland of bulls infected with *T. vivax*, complements available reports on *T. congolense* infection in goats (MUTAYOBA et al., 1988) and cattle (OGWU et al., 1992). It further shows that the haematic group (LOSOS and IKEDE, 1972) of trypanosomes (*T. congolense* and *T. vivax*) could produce significant tissue damage.

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SAŽETAK

Istraživana je disfunkcija štitnjače u zebu goveda pokusno invadiranih protozoonom *Trypanosoma vivax* na osnovi određivanja razina serumskoga tiroksina (T4) i pojave patoloških promjena 13 tjedana nakon invazije. Razine T4 bile su statistički značajno manje u invadiranoj skupini životinja tri tjedna nakon invazije (P<0,05), 6 tjedana nakon invazije (P<0,01) i od 9. do 11. tjedna nakon invazije (P<0,05) u usporedbi s fiziološkim razinama T4 ustanovljenima u neinvadiranih kontrolnih bikova tijekom pokusa. Patoanatomske promjene štitnjača nisu bile ustanovljene ni u pokusnih ni u kontrolnih životinja, a nije ustanovljena ni statistički značajna razlika tjelesnih masa (P>0,05) među skupinama. U štitnjačama pokusno invadiranih bikova ustanovljene su žarišne patohistološke promjene uključujući skvamoznu metaplaziju folikularnoga epitela s fibroplazijom i proširenim interfolikularnim vezivnim tkivom. Kontrolni bikovi nisu pokazivali patohistološke promjene u štitnjačama. Zaključeno je da se poremećaj štitaste žlijezde javlja kod tripanosomoze uzrokovane nametnikom *T. vivax* u goveda što doprinosi općoj slabosti invadiranih životinja.

Ključne riječi: štitnjača, disfunkcija, zebu govedo, *Trypanosoma vivax*