CAN CHANGES IN THYROID HORMONES BE USED AS AN INDICATOR OF TRYPANOTOLERANCE IN GOATS?[1]

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SUMMARY

No definitive and simple markers have been found for characterization of trypanotolerant and trypanosusceptible animals, though trypanotolerance trait is currently being looked upon as one of the potential means of increasing livestock productivity in tsetse-endemic areas. Since thyroid hormones are thought to play a key role in mammalian adaptive processes to various stressful environments it was the objective of this study to find out whether their variations in trypanosome-infected animals could be used as markers for trypanotolerance. Plasma thyroxine (T4) levels were measured in experimentally *Trypanosoma congolense* infected small East African female goats obtained from three tsetse-endemic areas i.e Lambwe Valley in Kenya, Arusha and Morogoro in Tanzania and one tsetse-free area i.e Imbo in Kenya. T4 levels declined rapidly and progressively after one week of parasitaemia in all infected goats and remained low during the six months infection period. A positive correlation was obtained between decline in T4 levels and the state of health of the goats as measured by loss of body weight ($r = 0.62$; $P<0.001$) and PCV ($r = 0.75$; $P<0.001$); that is, change in T4 levels being less severe in trypanotolerant goats and vice versa. Similarly, trypanotolerant goats with reduced T4 depression had intermittent and low parasitaemia and reduced reproductive impairment. It is suggested that T4 levels should be considered an additional index which can be used for assessing trypanotolerance in goats and possibly in other livestock breeds residing in vast tsetse-endemic areas of the tropics.

INTRODUCTION

Total thyroxine (T4) levels in blood have long been recognised as important indicators of thyroid status; abnormally high or low levels being indicative of thyroid malfunction (Evered 1974). Thyroid damage is a frequent outcome of chronic trypanosomiasis in both humans (Hawking and Greenfield 1941) and animals (Fiennes 1970; Stephen 1970; Ikede and Losos 1975; Mutayoba *et al.* 1987; 1988a), and is usually accompanied by an early decline in plasma T4 levels (Mutayoba *et al.* 1988b).

Since thyroid hormones are thought to play a key role in mammalian adaptive processes to various stressful environments (Riis 1987), their variations which occur in trypanosomiasis may directly or indirectly be related with the change in life performances of infected animals. Trypanosomiasis is known to cause marked stress in infected animals (Stibbs *et al.* 1984a; 1984b). Marked differences in susceptibility of the small East African breed of goats from different localities of East Africa to an

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experimental *T. congolense* infection has recently been reported (Mutayoba *et al.* 1989). Goats from a tsetse-endemic region of Morogoro in Tanzania were found to be more tolerant than goats obtained from Arusha in Tanzania and Lambwe Valley in Kenya (also tsetse-endemic areas). However, goats from Imbo, a tsetse-free enclave near Lake Victoria in Central Nyanza, Kenya were most susceptible.

Plasma T4 levels were measured in the same groups of infected goats in this study to find out whether trypanotolerance might be associated with minimal changes in thyroid secretory function; which indirectly might account for better performance following infection. An attempt has been made to correlate the measured T4 levels with changes in individual and group goat health performances as assessed by parasitaemia indices, changes in body weight, haemogram (PCV) and reproductive function. The use of thyroid hormones as possible markers of trypanotorelance is discussed.

MATERIALS AND METHODS

Animals

A total of 52 normally cycling Small East African female goats aged 2-3 years and weighing between 16-24 kg were used in this study. Goats were divided into experimental and control groups according to their origin. Experimentals comprised of four groups obtained from three tsetse-endemic areas namely Morogoro in Tanzania (n=10), Lambwe Valley in Kenya (n=10), Arusha in Tanzania (n=9) and one tsetse-free area Imbo in Kenya (n=10). Thirteen goats from all the four areas above served as a control group. The study was conducted at Chiromo Campus, University of Nairobi, Nairobi, Kenya. Prior to experimental infection, goats were acclimatized in their new environment for 1½ months, dewormed and screened for haemoprotozoa. All were found to be free from blood parasites. The detailed managemental regimen for these goats throughout the experimental period have been described elsewhere (Mutayoba *et al.* 1989).

Infection with *T. congolense*

One month after the acclimatization period, all experimental goats were infected through the jugular vein with approximately 20,000 *T. congolense* (strain EATRO 1753) obtained from Kenya Trypanosomiasis Research Institute (KETRI). The strain previously stored in capillary tubes was passaged twice in outbred BALB/c mice before being transferred to goats. The infection period lasted 24 weeks.

Bleeding regimen

Bleeding (5 ml of blood/goat) commenced at the end of the acclimatization period once every other day for a period of one month to provide pre-infection values of progesterone (P4) and oestradiol-17ß (E2). The PCV% and T4 levels were measured from weekly blood samples. The same bleeding regimen continued throughout the infection period and the plasma obtained was stored at -20° C for later hormonal analyses.

Determination of T4 and other parameters

Total plasma T4 levels were determined by I^{125}-Radioimmunoassay (RIA) kits marketed as Amerlex MT4 RIA (Amersham, England). Since the kit was designed to measure total T4 levels in human serum, its validity when applied to goat plasma was first carried out (Mutayoba 1988b). The intraassay and interassay coefficient of variations were 4.5% (n=20) and 13.1% (n=23), respectively. The sensitivity of the assay at 95% confidence interval was 2.2 nmol/l.

Other parameters determined included changes in weekly body weight (kg), Packed Cell Volume (PCV%) and the onset, level and duration of parasitaemia. Teaser bucks were used to monitor overt and silent oestrus. Plasma P4 and E2 levels were measured by respective specific ^{3}H-RIA (WHO, 1985).

Statistical analysis

The PCV, body weight and T4 levels obtained during the infection period were analysed on the bases of changes from individual goat baseline pre-infection levels. Group data was compared by one way analysis of variance, linear and multiple regressions. Comparisons between means were done by Kramer's test (Kramer, 1956).

RESULTS

Clinical observations

The infected goats in all groups became parasitaemic within 5-8 days. The first peak of parasitaemia occurred within a week following patency and was significantly higher (P<0.01) in the Imbo and Lambwe goats (5 x 10^6 trypanosomes/ml) than in the Arusha and Morogoro goats (5 x 10^3 trypanosomes/-ml). The first peak was followed by intermittent and scant parasitaemia throughout the course of infection in the Morogoro goats and to lesser extent in other goat groups. Higher body temperatures (P<0.01), fluctuating between 38.5-41.5° C, were recorded in the infected goats vs the control values of 37.2-38.5° C. The Imbo group suffered heavy mortalities with the loss of 70% of the infected goats by Week 22 post-infection. The Morogoro group only lost 30% and the rest infected goats maintained fairer body conditions than goats from other localities. Mortality rates in the Lambwe and Arusha goats were 40% and 44%, respectively.

PCV changes

Changes in the PCV levels are shown in Figure 1a. The values declined significantly (P<0.01) within one week of parasitaemia in all infected goat groups and remained low, thereafter, up to Week 20 when slight recovery was observed. The decline in PCV values was, however, most marked in the Imbo (P<0.01) goats. The Arusha and Lambwe group values were intermediate, whilst the Morogoro variety was least affected.

Body weight changes

Figure 1b summarises the body weight changes in the infected and control groups. Though all infected goats started losing weight progressively from the second week of the infection, this was more marked (P<0.05) in the Lambwe and Imbo groups than the Morogoro group. Changes in the Arusha group were only significantly higher (P<0.05) than the Morogoro group from Week 2 to 18. The changes in Arusha group values were consistently lower but not significantly different from the Imbo and Lambwe groups.

Whereas, the changes in body weights in the Imbo, Arusha and Lambwe groups became significantly lower than the control group soon after infection (P<0.01), the Morogoro group values became significantly lower (P<0.01) only from Week 10 onwards. A closely similar pattern in the change in PCV and body weight between the infected and control groups was observed (see Figure 1a and 1b). Weekly regression of body weight and PCV changes in all groups gave a highly significant correlation (r=0.68; P<0.001) indicating a strong dependency of body weight changes on PCV levels or vice versa.

Changes in T4 levels

The normal pre-infection levels and weekly episodic variations in T4 were not

significantly different between goats from all localities and ranged from 68.5–124.4 nmol/l. Changes in T4 levels during the infection period in the infected and control groups are shown in Figure 1c. Rapid drop in T4 levels ($P<0.05$) was observed in the Lambwe, Arusha and Imbo groups within one week of infection, during which time the Morogoro and control group values were still normal. But the decline became highly significant

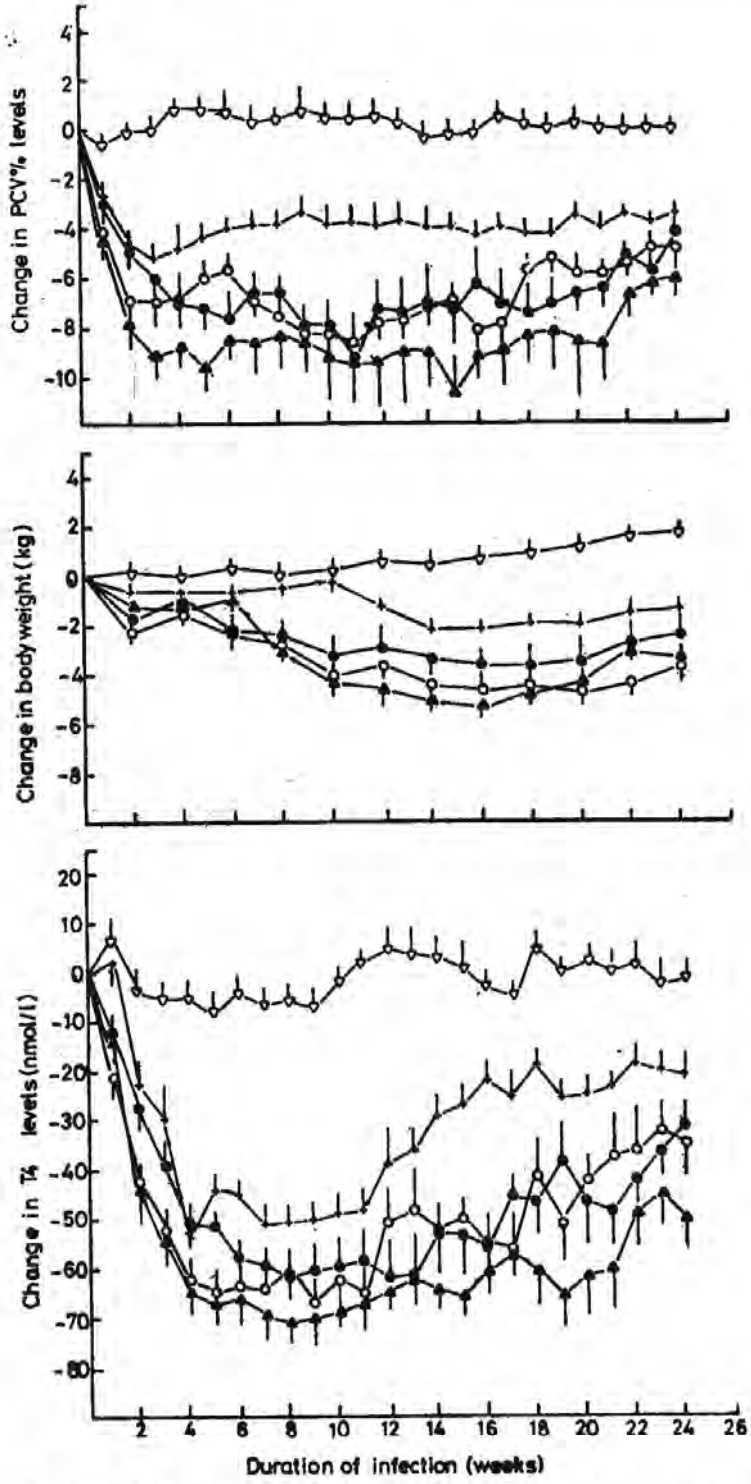

Figure 1. Mean changes in (a) PCV% (b) body weight and (c) plasma T4 levels in the experimental (o) Lambwe, (o) Arusha, (+) Morogoro, ( ) Imbo and ( ) control goats.

(P<0.01) in all infected groups from Week 2 onwards. Slight recovery in T4 levels were observed in all infected groups from week 16 post-infection. Weekly changes in T4 levels strongly correlated with the changes in PCV (r=0.75; P<0.001) and body weights (r=0.62; P<0.001)

Changes in reproductive performance

Trypanosome infection was accompanied by irregular and shorter oestrus cycles which ceased within 2 cycles post-infection in the more susceptible Imbo goats and 4th cycle in the more resistant Morogoro goats. Decline in ovarian hormones occurred concurrently with degeneration of the ovaries and impairment of the pituitary gonadotrophs. Resumption of the cycles were noted in resistant goats towards the end of a 6-month infection period.

DISCUSSION

T. congolense infection resulted in marked decline in plasma T4 levels in all infected goats within one week of parasitaemia and in all subsequent weeks of the infection. Decline in T4 levels in the infected goats in this study positively correlated with clinical parameters of health as measured by changes in PCV (r = 0.75; P<0.001), body weight (r = 0.62; P<0.001) and reproductive function. Changes in T4 levels were less drastic in the Morogoro goats which were found to be more tolerant to an experimental *T. congolense* infection than the Arusha, Lambwe Valley and Imbo goats; in that order.

Although the cause of rapid thyroid hypofunction was not investigated in this study, we are postulating that this might be due to stress associated rapid rise in brain monoamines which suppresses Thyroid Releasing Hormone and possibly other hypothalamic hormones. This may lead to rapid hypo- or hyperfunction of respective trophic glands. Trypanosomiasis imparts considerable stress in infected animals resulting in marked neurosecretory aberrations (Stibbs 1984a,b) and may interfere with the secretory and release pattern of several pituitary hormones including Luteinizing Hormone, Follicle Stimulating Hormone and possibly Thyroid stimulating Hormone (O'hara *et al.* 1985; Mutayoba *et al.* 1988a). Whereas, the infection stress may be responsible for early thyroid hypofunction, further impairment is probably brought about by slow degeneration of the thyroid gland which seems to be a common sequelae of chronic trypanosomiasis (Hawking and Greenfield 1941; Stephen 1970; Ikede and Losos 1975; Mutayoba *et al.* 1988b).

Whichever mechanisms are involved in the pathogenesis of trypanosome-induced thyroid hypofunction, trypanotolerance seem to moderate or limit the onset, level and duration of these changes. Reduced susceptibility to trypanosomiasis seem to be an adaptive process inherited or acquired by individuals to prevent or suppress the detrimental effects of this disease. Minimum decline in plasma T4, PCV, body weight and other clinical parameters were observed in trypanotolerant Morogoro group than in other groups.

Trypanotolerance is believed to be a genetically inherited trait (Murray *et al.* 1982). Most of research efforts are now directed towards the identification of genetic and non-genetic marker systems for characterization of trypanotolerant and trypanosusceptible animals that are easy and cheap to monitor and that could in addition be used as selection criteria for trypanotolerance (Hoste 1987). Some compromising results seem to have been obtained in comparing haemotypes i.e polymorphism of serum protein (albumin and transferrin); erythrocytes (blood types, haemoglobin, purine nucleoside phosphorylase, carbonic anhydrase, malate dehydrogenase) and leucocytes (major histocompatibility complex, CRTA 1986). The predictive possibilities of

using PCV changes as selective criteria for trypanotorelance has also been investigated and seem to have the sufficing repeatability of around 0.32 (ILCA, 1986). It is suggested from this study that measurements of plasma thyroid hormones during the course of infection could also serve as an additionalgoodindexintrypanotolerance research programmes.

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