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PRESENCE OF *TRYPANOSOMA LEWISI* IN *RATTUS NORVEGICUS* IN SRI LANKA

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ABSTRACT

Natural infection of the haemoflagellate, *Trypanosoma lewisi,* in a laboratory colony of mixed bred rats, *Rattus norvegicus*, is reported for the first time in Sri Lanka. Some aspects of parasitology and immunology on this host-parasite association were studied. The length measurements of the parasite, the cyclic development of *T. lewisi* in *R. norvegicus* and in the rat flea, *Xenopsylla cheopis,* confirmed the identification of this haemoflagellate. Fluctuations of parasitaemia of *T. lewisi* in these infections ranged between 0.065% - 12.4%. Nine and eight parasite proteins were detected by SDS-PAGE analysis of high and low parasitaemic infections, respectively. Six of these proteins (60, 63, 72, 75, 78 and 79 kD) were shared while three (42, 46 and 106 kD) and two (56 and 112 kD) proteins were specific to high and low parasitaemic infections, respectively. Of these specific proteins, the 42 and the 56 kD antigens were detected on immunoblots indicating the recognition of these proteins by specific host antibodies. This may indicate that antibodies against the 42 kD protein may be responsible for the decline of the high parasitaemic status of *T. lewisi* infection in rats while anti- 56 kD antibodies may lead to the complete elimination of *T. lewisi* infection in rats. A significant increase in neutrophils and monocytes in high parasitaemic rats may indicate a possible role of cellular response.

Key words: *T. lewisi, R. norvegicus,* Haemoflagellate, Stercorarian trypanosome, Sri Lanka

INTRODUCTION

Trypanosoma lewisi is a stercorarian trypanosome that naturally infects rats. Its presence in large numbers in the blood does not affect the health of the host, as it is a non-pathogenic parasite (Smyth, 1962). It has a cyclic development which is completed in the alimentary tract of the vector, the Indian rat flea, *Xenopsylla cheopis* (Smyth, 1962).

Much work has been published in the West on *T. lewisi* with respect to its biology, parasitology and host immune responses (Cheng, 1964; Lumsden, 1965; Smyth, 1994). However, studies on *T. lewisi,* in Sri Lanka are scanty and limited to two preliminary reports. The presence of *T. lewisi* was first reported in house-frequenting rats, *Mus decumanus*, in Colombo, by Castellani and Willey in 1904. Since then, this observation has neither been reconfirmed by other workers nor its presence reported in laboratory rats. Subsequently, Dissanaike (1963) reported the presence of *T.lewisi* in bandicoots, *Bandicota malabarica*, examined from Colombo and the suburbs.

In the course of another experiment, we detected natural infections of *T. lewisi* in a laboratory-bred colony of a mixed strain of rats (*Rattus norvegicus),* maintained in the

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animal house of the Department of Zoology, University of Colombo. To our knowledge, this finding has not been previously reported in Sri Lanka. As a result of this finding, investigations on some aspects of parasitology and immunology on natural infections of *T. lewisi* in *R. norvegicus* were undertaken. Thus, the life cycle stages of *T. lewis* were studied in the rat (*R. norvegicus)* and the rat flea *(X. cheopis).* Fluctuations of parasitaemia of *T. lewisi* were investigated in relation to the presence of specific parasite antigens. Immune responses of the host were also studied.

MATERIALS AND METHODS

A total of 245 rats (laboratory bred mixed strain of *R. norvegicus)* from the animal house of the Department of Zoology, University of Colombo, were screened for *T. lewisi* by microscopically examining both fresh wet mounts of blood prepared from tail pricks of rats and thin blood films stained with Giemsa's stain. The rats were housed in plastic cages under controlled animal house conditions of light (approximately 12 hrs light and 12 hrs dark), humidity (50-55%) and temperature (28-30^{0}C) with free access to pelted food (Master Feed, Colombo, Sri Lanka) and tap water.

The following measurements were made from dried thin blood smears obtained from 20 rats, measuring 100 parasites (on average 5 parasites from each rat) from drawings made with a camera lucida (Olympus, Tokyo, Japan), in order to confirm their identification: total length (including and excluding free flagellum), length from the posterior end to the nucleus, width at the position of the nucleus.

In order to establish the life cycle of this parasite, both wet and dry blood smears as well as tissue smears prepared from the heart, kidneys, liver and the spleen of *R. norvegicus* were microscopically examined at the ascending peak of parasitaemia at an average value of 4%. Further, the Indian rat flea, *X. cheopis,* ectoparasitic on *T. lewisi*-infected rats were examined for life cycle stages of *T. lewisi*. Rat fleas (n=26) were collected, placed in phosphate buffered saline (PBS), *p*H 7.4, on a glass slide and dissected under a dissecting microscope (Nikon, Tokyo, Japan) at a magnification of 10 X 3.2. A cover slip was lowered on to the gut of the dissected flea, which was first examined intact and then ruptured by applying gentle pressure to the coverslip for further observation. For detailed studies the alimentary tract was first cut into midgut and hind gut (rectal) regions and were then separately teased apart, air dried, fixed and stained with 10% (v/v) Giemsa's stain and examined for intracellular and extracellular parasite stages under oil immersion.

The level of parasitaemia of rats positive for *T. lewisi* infections (n=24) was assessed by examination of thin blood smears, daily, over a period of 14 days. Out of these 24 rats the course of parasitaemia of 10 rats representing 5 high parasitaemic and 5 low parasitaemic infections were selected for this study. Percent parasitaemia was calculated by counting *T. lewisi* (both slender and stumpy forms) in 10 microscopic fields for a given thin smear and was expressed as a % value of the average number of red blood cells present in a given microscopic field.

White blood corpuscle (WBC) differential counts of infected rats (n=16) and of controls (n=18) were made using thin blood smears stained with Giemsa's stain. Trypanosomes were purified from infected rat blood using the Lymphoprep preparation according to the manufacturer's instructions (Nycomed Pharma As, Norway). Briefly, 500 µl of infected rat blood was collected into anti-coagulant (heparin 50 units/ml) and was diluted in an equal volume of PBS. This was carefully layered over 1 ml of Lymphoprep in a 15 ml centrifuge tube and was centrifuged at 1214 x g for 20 minutes at room temperature (30 - 32^{0}C). The distinct layer of trypanosomes was removed from the interface and the harvested fraction was diluted up to 10 ml with PBS and was centrifuged for 15 minutes at 1214 g for clarification. The supernatant was discarded and the pellet of trypanosomes was collected.

Thereafter, the parasites were immobilized and counted using an improved Neubauer haemocytometer (WHO, 1991).

Purified *T. lewisi* parasites from both high and low parasitaemic infections were subjected to SDS-PAGE analysis (Laemmli, 1970) using normal rat blood cells as the control. The gels were stained with Coomassie Brilliant Blue R-250 (Bio-Rad, USA) to reveal protein profiles of parasites. In the case of immunoblots (Towbin *et at.*, 1979), immediately after separation by SDS-PAGE, proteins in the gel were electrophoretically transferred on to nitro-cellulose paper (NCP). Thereafter, NCP strips were incubated with 1:1000 dilution of noninfected and infected rat sera (collected from high and low parasitaemic infections, on average at 10% and 5% parasitaemia, respectively) in PBS. Five sera samples were pooled from each category of non-infected and infected rats. The NCP strips were then incubated with 1:1000 dilution of horse radish peroxidase conjugated anti-rat immunoglobulins (BioRad, USA). The colour reaction was visualised by adding the substrate 4-Chloro-1-Naphthol (Bio-Rad, USA) onto blot strips.

The results were expressed as means $\pm$ SD. Statistical evaluations were made using the Mann-Whitney U-test. Significance level was set at $p<0.05$.

RESULTS

Out of a total of 245 rats investigated, 54 rats (26 adults and 28 sub-adults; sex ratio 2[male]: 3 [female]) were blood positive for *T. lewisi* as detected by screening both thin blood films and wet mounts. In wet mounts the presence of *T. lewisi* parasites were detected by their wriggling movements among the blood cells (Manter, 1950).

The mean total length of the trypomastigotes (n=100) inclusive of the free flagellum was 28.75 $\pm$ 1.77 µm and was 22.1 $\pm$ 0.493 µm excluding the free flagellum. The mean length from the posterior end to the nucleus measured 13.88 $\pm$ 0.357 µm while the cell width at the position of the nucleus was 2.67 $\pm$ 0.108 µm. The accentric nucleus is situated anterior to the centre of the body. The rod-shaped kinetoplast is placed at some distance from the sharp posterior end. The identification of the parasite was confirmed by Professor A. S. Dissanaike, Emeritus Professor, Department of Parasitology, Faculty of Medicine, University of Colombo.

Examination of the stained thin blood films showed pleomorphic trypomastigote forms; 92% of the stages present in the blood of all rats were of the long narrow form, while thick stumpy forms comprised 8%. Flagellates were present in the hind gut and the rectal region (the rectum, intestinal lumen, intestinal wall and rectal lumen) of infected rat fleas. However, their haemolymph was parasite free. It was also evident that the parasites within the vector flea (*X. cheopis)* were present both as extracellular and intracellular forms.

Of the ten rats subjected to daily blood screening, the infection in 5 rats showed peak parasitaemias fluctuating from 5.27%-12.4% (6.44% $\pm$ 3.26) (Figure 1). In the other 5 rats, parasitaemia fluctuated below 5, ranging from 0.065% - 3.65% (1.71% $\pm$ 1.27). The mean parasitaemias of these two groups were found to be significantly ($p<0.001$) different. Rats with parasitaemias below 5% were considered as having low parasitaemic infections while those with parasitaemias above 5% were considered as having high parasitaemic infections.

T. lewisi-infected rats with high parasitaemic levels demonstrated significantly ($p<0.001$) higher neutrophil counts compared to both low parasitaemic and uninfected rats. However, the low parasitaemic rats had a significantly ($p=0.009$) low neutrophil count than the controls. A significant ($p<0.001$) decrease of lymphocyte counts in rats with high parasitaemic status was also detected (Figure 2).

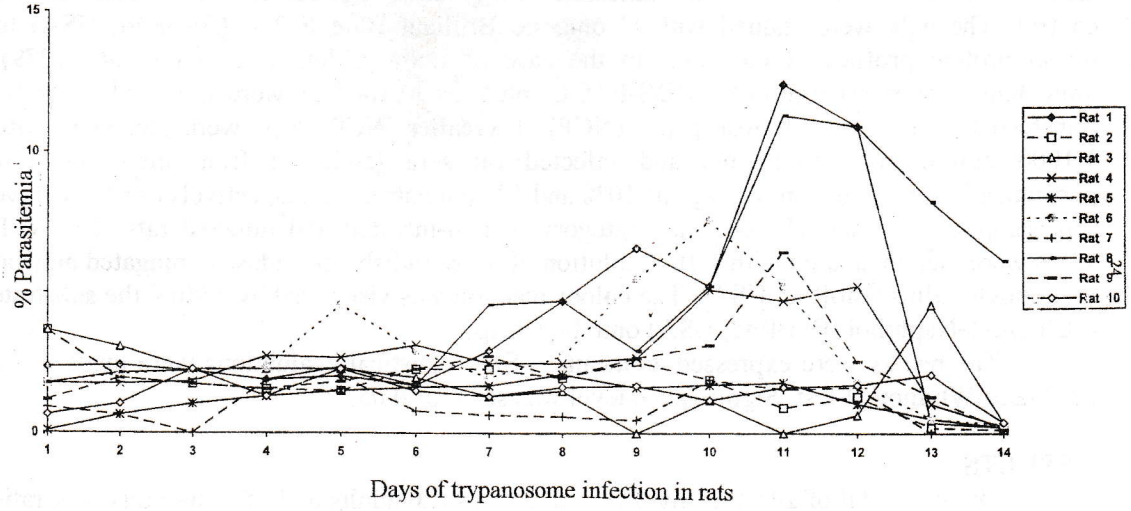

Figure 1. Fluctuations of parasitaemia of *T. lewisi* infections in naturally infected rats, *R. norvegicus* (n=10) in relation to time

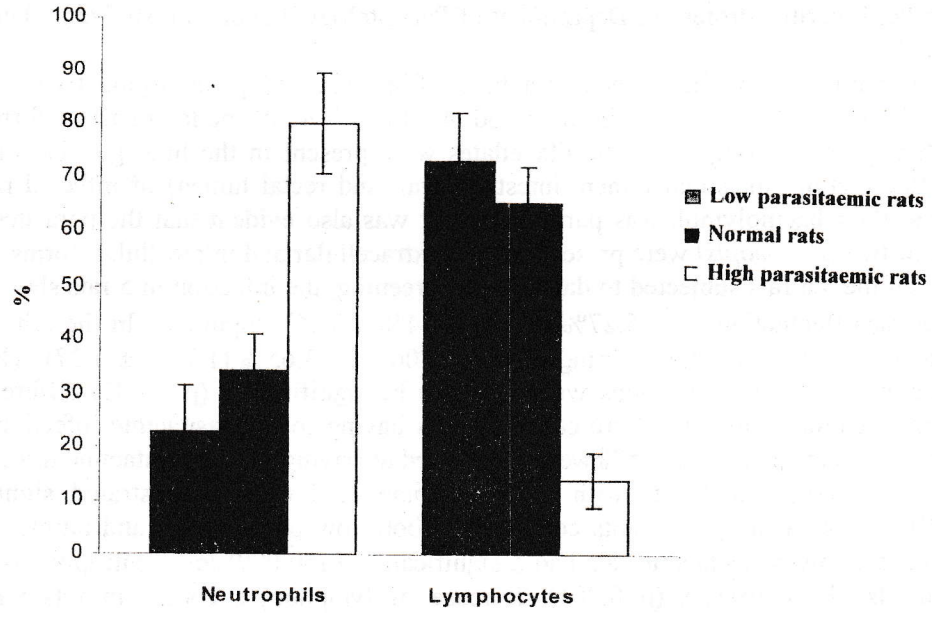

Figure 2. Differential white blood cell counts (neutrophils and lymphocytes) of *T.lewisi* infections of high and low parasitaemic rats compared with normal rats

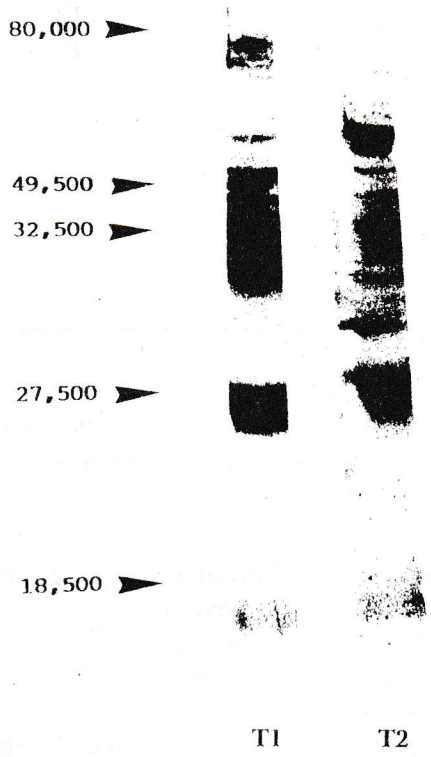

Figure 3. An immunoblot of purified *T. lewisi* extracts separated on a 7% SDS-PAGE gel probed with pooled sera, from low parasitaemic rats (lane T1) and from high parasitaemic rats (lane T2). Molecular weight markers (in daltons) are indicated on the left margin

The *T. lewisi* protein profiles analysed by SDS-PAGE, of high and low parasitaemic infections revealed the presence of 9 and 8 protein bands, respectively (Figure 3 and Table 1). Comparison of these profiles revealed that 6 proteins were shared, while 3 proteins of 42, 46 and 106 kD and two of 56 and 112 kD were specific to parasites from high and low parasitaemic infections, respectively. Normal rat erythrocytes were used as controls in these experiments.

Of the three specific proteins (42,46 and 106 kD) from high parasitaemic infections, the 46 and 106 kD proteins escaped detection by rat antibodies. Only the 42 kD protein was detected on immunoblots. Of the two parasite antigens (56 and 112 kD) specific to low parasitaemic status, only the 56 kD protein was detected by immunoblotting (Table 1 & Figure 3).

TABLE I

Analysis of *Trypanosoma lewisi* proteins from parasites isolated from high and low parasitaemic infections by SDS-PAGE analysis.

Proteins from high parasitaemic status	Proteins from low parasitaemic status
* 106	Δ 112
80	79
78	78
76	75
74	72
63	63
60	60
* 46	Δ ⊗ 56
* □ 42	

*Proteins specific to high parasitaemic status.

ΔProteins specific to low parasitaemic status.

□ Proteins reacted with immune serum from high parasitaemic sera on immunoblots.

⊗ Proteins reacted with immune serum from low parasitaemic sera on immunoblots.

DISCUSSION

This is the first report of *T. lewisi* occurring in rats (*R. norvegicus)* documented in Sri Lanka, where 54 rats of 245 animals screened were positive for *T. lewisi.* The presence of *T. lewisi* was first reported in Colombo, by Castellani and Willey in 1904 in house-frequenting rats which were documented as *Mus decumanus* by these authors. However, house frequenting rats are likely to be of the genus *Rattus*.

As reported by Baker (1969), *T.lewisi* has a length of 21-36 μm, average breadth of 2 μm, a large, rod-like kinetoplast that is not terminal, pointed long posterior end, long free flagellum and slightly anterior nucleus. The morphological characteristics and the measurements of this parasite confirmed its identification as *T.lewisi*.

Pleomorphic trypomastigotes of *T. lewisi* were observed in the rat. The cyclic development of this parasite was completed in the rat flea (*X. cheopis*) as was evident by the presence of epimastigotes in the 'gut' epithelium and infective 'metacyclic' trypomastigotes in the rectum of the rat flea.

In naturally infected rats subjected to daily blood screening, it was evident that there were minor fluctuations in parasitaemia prior to attaining peak parasitaemias (Figure 2). Cheng (1964), observed similar patterns of fluctuations in parasitaemias in naturally infected rats. The plausible explanation for this may be that, though stercorarian typanosomes lack antigenic variation, *T. lewisi* may exhibit antigenic variation of a very minor degree (Vickerman & Luckins, 1969). In experimental *T. lewisi* infections, the duration of the infection was shown to be 36 days (Taliaferro, 1932). However, the current study shows that duration of natural infections is on average 14 days (Figure 1). This difference may be due to the ability of this out bred rat population to mount effective immunity to natural *T. lewisi* infections.

Some aspects of the host immune responses to *T. lewisi* were studied using immunoblots and differential counts of rat white blood cells. A significant increase in neutrophils and monocytes in high parasitaemic rats may indicate a possible role of cellular

response while a marked decrease in lymphocytes in these rats may imply immunosuppression. However, in the low parasitaemic rats, the lymphocyte population seemed to be in par with the normal rats. But the neutrophil count was significantly lower than in that of the normal controls.

According to Taliaferro and Stabler (1969) and D' Alesandro (1970), three different antibodies may be involved in trypanosome infection that would lead to the elimination of the infection. It has now been shown by Giannini and D' Alesandro (1984) that only two antibodies are involved; a trypanostatic IgG that interferes with the active transport of nutrients and the crisis due to the trypanocidal antibody of IgM isotype.

Of the three proteins (42, 46 and 106 kD) specific to high parasitaemic status of *T. lewisi* in *R. norvegicus,* only the 42 kD protein could be detected on immunoblots. This may indicate the recognition of this protein by specific host antibodies that may be responsible for the decline of high parasitaemic status of *T. lewisi* infection in rats. *T. lewisi* parasites from low parasitaemic status also presented a protein (56 kD), against which host antibody response is mounted that may possibly lead to complete elimination of *T. lewisi* infection in *R. norvegicus.* Detection of these two proteins warrant further studies as these may probably be associated with the trypanostatic and trypanocidal antibodies described by Giannini and D'Alesandro (1984).

These results may indicate complex and balanced interactions between antigenic expression of *T. lewisi,* and immune mechanisms of rats that would lead to the non-pathogenic nature of these infections in *R. norvegicus.*

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