VETERINARSKI ARHIV 76 (1), 11-18, 2006

Clinical studies on experimental gambian trypanosomosis in vervet monkeys

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ABENGA, J. N., V. O. ANOSA: Clinical studies on experimental gambian trypanosomosis in vervet monkeys. Vet. arhiv 76, 11-18, 2006.

ABSTRACT

Vervet monkeys (*Cercopithecus aethiops*) were inoculated with two different strains of *T. brucei gambiense* in order to determine their clinical effects. Both strains of parasite behaved alike in the monkeys, producing a virulent disease course resulting in their death 10 to 15 weeks post-infection (PI). Clinical features exhibited in the monkeys included pyrexia, progressive mass loss, mild anaemia, oedema of hind quarters and central nervous system (CNS), disturbances which did not occur until week 8 PI, corresponding to onset of late-stage sleeping sickness in man. Clinical changes in the late stage of the infection in monkeys included somnolence, ataxia and uncontrolled shaking of the head. A significant drop in the packed cell volume (PCV) and body mass occurred in the late stage of the disease. It was concluded that a relationship exists between CNS pathology, PCV and mass loss in Gambian trypanosomosis. The course of infection observed in the monkeys also suggests that many strains of *T. b. gambiense* may be more virulent in both man and animals than has hitherto been known.

Key words: gambian trypanosomosis, monkeys

Introduction

Human African trypanosomosis (HAT, sleeping sickness) is a complex and debilitating disease in man. This disease still ravages several parts of sub-Saharan Africa despite decades of efforts aimed at its control (ANONYM., 1998; JANNIN, 2000; KABAYO, 2002). Human trypanosomosis now presents an emerging public health crisis in several countries, including Nigeria (AIRAUHI et al., 2001; WAISWA et al., 2003). The disease has been identified as a major health risk to tourists travelling to tropical Africa (SABBAH et al., 1997; CONWAY-KLAASSEN et al., 2002; JELINEK et al., 2002).

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ISSN 0372-5480
Printed in Croatia

In West and Central Africa, HAT is caused by *Trypanosoma brucei gambiense*, transmitted principally by tsetse flies, *Glossina palpalis* and *G. tachinoides* resulting in a devastating, chronic form of the disease described as Gambian trypanosomosis (SCOTT, 1970). Two stages of the disease are recognized: the early or haematolymphatic stage, and the late or meningoencephalic stage which is characterised by CNS disturbances (POLTERA, 1985; ANONYM., 1998). The trypanosomes of man and animals are generally characterized by haematological and biochemical aberrations, the severity of which are often determined by the level of parasitaemia, species of infecting trypanosome and host species (POLTERA, 1985; ANOSA, 1988).

Conflicting reports of observations in animal modes have posed an obstacle to the proper understanding of the factors associated with the pathogenesis of the Gambian form of HAT and chemotherapy and clinical management of the disease in man. Pigs (NKININ et al., 2002; WAISWA et al., 2003) are known animal reservoir hosts, while other animals such as dogs (GIBSON et al., 1978), sheep (MOLYNEUX, 1973), cattle (MEHLITZ et al., 1982), and monkeys and baboons (KAGERUKA et al., 1991) are also readily infected by *T. brucei gambiense*. Model studies involving *T. brucei* have been attempted in sheep (BOUTEILLE et al., 1988a, BOUTEILLE et al., 1988b) and mice (KEITA et al., 1997).

In this study we describe clinical changes and disease course precipitated by *T. b. gambiense* in vervet monkeys (*Cercopithecus aethiops*).

Materials and methods

A total of four vervet monkeys with an average body mass of 2.8 ± 0.8 kg were used in the study. The monkeys were acclimatized for a period of six months before use and were fed cooked beans, bananas, pawpaw leaves and pawpaw fruits, while water was provided ad libitum. All animals gained mass during the acclimatization period. Two monkeys were randomly selected and inoculated with NITR/Abraka strain of *Trypanosoma brucei gambiense*, while the remaining animals were inoculated with IL3250 strain. Both strains of parasites were obtained from patients suffering from sleeping sickness. A total of 2×10^6 parasites were used to infect each of the monkeys intraperitoneally, while pre-infection data collection for four weeks served as control for each animal.

The daily body temperature of the monkeys was taken using a clinical thermometer. Temperature was taken orally for monkey numbers 01, 02 and 03, while for monkey N^o 04 this could only be achieved via the rectum. The body mass of the monkeys was measured weekly using a weighing balance (Henson®, Gallenkamp, England). To determine parasitaemia after infection, wet blood film made daily from the ear vein was examined by light microscopy. Blood for determination of PCV and reticulocyte count was obtained through venipuncture of the femoral vein. The site for venipuncture was aseptically prepared by shaving and cleaning with 70% ethanol. In order to obtain blood for haematology or

mass determination, the monkeys were put under general anaesthesia by injecting ketamine hydrochloride (Parke-Davies, England) at a dose of 10.0mg/kg intramuscularly. PCV and reticulocyte counts were determined as described by DACIE and LEWIS (1984). During the course of infection a daily physical examination of the monkeys was undertaken. Analysis of variance (ANOVA) was used for statistical interpretation to determine level of significance.

Results

After infection, both strains of *T. b. gambiense* behaved similarly in the monkeys and they were therefore treated as one group.

Table 1. Changes in mean body mass, packed cell volume and reticulocyte counts of *T. b. gambiense*-infected monkeys

	Pre-infection	Weeks after infection						
		2	4	6	8	10	12	14
Body mass (kg)	3.7 ± 1.4	3.5 ± 1.1	3.2 ± 1.1	3.0 ± 0.7	2.5 ± 0.5	2.2 ± 1.6	1.2 ± 1.4	2.0 ± 0.0
PCV(%)	41.0 ± 0.6	35.02 ± 6	32.7 ± 7.2	36.5 ± 2.6	32.2 ± 2.6	29.0 ± 5.3	22.0 ± 4.7	27.5 ± 0.7
Reticulocytes (%)	0.3 ± 0.0	1.9 ± 0.8	2.7 ± 0.6	3.7 ± 1.2	5.7 ± 0.9	5.7 ± 0.5	4.2 ± 3.1	4.8 ± 0.2

Monkeys became parasitaemic 3 to 4 days post-infection (PI). Parasitaemia was first recorded on day three in monkeys numbers 01, 02 and 04, while the remaining monkeys became positive by day four. Following parasitaemia, the animals exhibited fluctuating pyrexia, anorexia, intermittent diarrhoea, progressive emaciation and pale mucous membranes of the eyes. One monkey showed oedema of the hind legs and scrotum. Later, from week 8 PI, the primates became more emaciated, extremely dull and sleepy, while monkeys numbers 01 and 02 developed CNS disturbances characterized by unsteady gait and uncontrolled shaking of the head, corresponding to late-stage symptoms in man. By weeks 10 and 12 PI, monkeys numbers 02 and 01 died, respectively. At weeks 11 and 13 the animals became too weak to be put under general anaesthesia for bleeding. The remaining monkeys developed CNS signs at week 12 PI and were euthanized at sternal recumbency by week 15 PI.

Changes in mean body mass, PCV and reticulocyte counts of the monkeys are summarized in Table 1. Average body mass of monkeys before infection was 3.73 ± 1.4

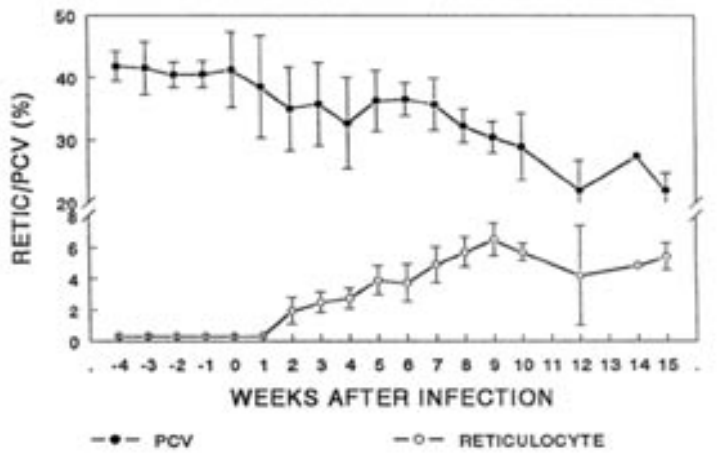

Fig. 1. Changes in mean PCV and reticulocyte values of monkeys infected with *T. brucei gambiens*e

kg. After infection, the animals showed no significant change in body mass until week 12 PI, when mass fell to 1.2 ± 1.4 kg, and then to 2.00 ± 0.0 by week 14 PI. The pre-infection PCV of the monkeys was 41.06 ± 0.6%. After infection, the PCV showed fluctuating decrease in the first seven weeks of infection, with the highest decrease to 32.7 7.7% by week 4 PI. From the eighth week the drop in PCV became progressively marked, with values ranging from 32.2 ± 2.6% from week 8 PI to 22.0 ± 2.8% by week 15 PI (Fig. 1. $P \leq 0.05$). The reticulocyte counts of the monkeys was 0.3 ± 0.0 pre-infection but increased progressively after infection, attaining maximum increase of 6.3 ± 1.0% (Fig. 1. $P \leq 0.05$) by the fifteenth week.

Discussion

Trypanosoma brucei gambiense showed marked pathogenicity in vervet monkeys resulting in death 10 to 15 weeks PI. This does not agree with the typically chronic nature of the disease in man (SCOTT, 1970; ANONYM, 1998) and previous observations on the course of *T. b. gambiense* experimental infection in monkeys and baboons (GODFREY and KILLICK-KENDRICK, 1967; YESUFU, 1971). YESUFU (1971) observed that vervet monkeys infected with *T. b. gambiense* looked apparently healthy and added mass one year after infection, although parasites could still be detected in the blood. Similarly, chimpanzees infected with *T. b. gambiense* achieved self-cure (YESUFU, 1971). However, the findings in this study are closer to the observations of MOLYNEUX (1973) in which *T. b. gambiense* resulted in an acute disease leading to death of monkeys 45 days post-infection. Although

T. b. gambiense typically causes chronic disease, while *T. b. rhodesiense* causes acute disease in man, both acute forms of Gambian sleeping sickness and chronic forms of Rhodesian sleeping sickness do occur (APTED, 1970; SCOTT, 1970). The virulence of *T. b. gambiense* in vervet monkeys observed here probably arose from the high antigenic nature of the parasite strains. A virulent course of sleeping sickness following an outbreak of the disease in Abraka endemic focus, Delta State of Nigeria, has been described (EDEGHERE et al., 1989; ENWEZOR and UKAH, 2000; AIRAUHI et al., 2001).

The sequence of clinical disease observed in the monkeys was, however, similar to those observed in man (WELLDE et al., 1989a; ANONYM., 1998). The CNS signs characterized by uncontrolled shaking of head and somnolence, which did not occur until weeks 8 to 12 PI, correspond to the late stage of the disease in man (POLTERA, 1985; ANONYM., 1998) following CNS invasion by trypanosomes. KEITA et al. (1997) demonstrated late stage disease in *T. brucei*-infected mice. Similar CNS signs were also described in *T. brucei*-infected goats (WELLDE et al., 1989c) and *T. b. rhodesiense*-infected cattle (WELLDE et al., 1989b) which included uncontrolled movement of the head, ataxia, circling, aimless wandering and convulsions. Both the early and late stages of clinical sleeping sickness were therefore observable in the vervet monkeys.

POLTERA (1985) described CNS histopathological changes associated with late HAT which included meningoencephalitis, destruction of neurons, microglial reaction, diffuse scattering of inflammatory cells in the neuropil, and demyelination.

Mild anaemia, characterized by a slight drop in PCV and accompanying reticulocytosis in the early stage of the disease in monkeys, was in consonance with the observation of EMERIBE and ANOSA (1991) in *T. b. gambiense*-infected rabbits and natural sleeping sickness in man (POLTERA, 1985).

However, in the late stage, the anaemia characterized by significant drop in PCV, and body mass of the monkeys, was more marked than that observed by EMERIBE and ANOSA (1991). WELLDE et al. (1989c) observed similar marked anaemia and mass loss in *T. b. rhodesiense* infected cattle with CNS disturbances, suggesting that there is a relationship between CNS pathology, PCV and mass loss in late-stage sleeping sickness.

Although anaemia is a consistent pathological feature of African trypanosomosis, it is relatively less prominent in man (POLTERA, 1985). Also, *T. b. rhodesiense* causes more severe anaemia in man than *T. b. gambiense* (POLTERA, 1985; ANOSA, 1988) due to acute fulminating parasitaemia in the Rhodesian disease. Similarly, anaemia in chimpanzees infected with *T. b. rhodesiense* was found to be more severe than those infected with *T. b. gambiense* (GODFREY and KILLICK-KENDRICK, 1967). To judge from the outcome of *T. b. gambiense* infection in vervet monkeys, the observations obtained here appeared to compare well with those arising from *T. b. rhodesiense*. Also, with the exception of somnolence,

clinical observations in *T. gambiense*-infected monkeys did not differ from those described in *T. brucei*-infected animals (LOSOS and IKEDE, 1972; POLTERA, 1985).

It is concluded that even though *T. b. gambiense* precipitated a virulent course resulting in the death of the monkeys 10 to 15 weeks post-infection, clinical signs mimicked those of natural Gambian trypanosomosis in man. This fact makes vervet monkeys suitable animal models for clinical and chemotherapeutic investigation into human African trypanosomosis. The outcome of infection in the primates suggests that many strains of *T. b. gambiense* may be more virulent in man than has hitherto been known.

Acknowledgements

We express our gratitude to Prof. S.O. Akpavie, Drs. O.O. Ogunsanmi and V.O. Taiwo and the entire staff of the National EC Trypanosomosis Project Laboratory, University of Ibadan, Nigeria, for their numerous supporting activities, and to the Director General, Nigerian Institute of Trypanosomosis Research, Kaduna, for funding the research.

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Received: 19 August 2004
Accepted: 10 January 2006

ABENGA, J. N., V. O. ANOSA: Kliničko istraživanje pokusne gambijske tripanosomoze u zelenih zamoraca. Vet. arhiv 76, 11-18, 2006.

SAŽETAK

Zeleni zamorci (*Cercopithecus aethiops*) bili su invadirani s dva različita soja *T. brucei gambiense* u svrhu utvrđivanja kliničkih znakova bolesti. Oba soja parazita slično su se ponašala izazivajući teški oblik bolesti što je dovelo do smrti 10 do 15 tjedana poslije invazije (PI). Klinički znakovi očitovali su se povišenom temperaturom, progresivnim gubitkom težine, blagom anemijom, edemom stražnjih ekstremiteta i poremećajima središnjeg živčanog sustava (CNS), koji su se pojavili 8. tjedna poslije invazije, što nalikuje kasnom stadiju bolesti spavanja u ljudi. Kliničke promjene u kasnom stadiju invazije u majmuna očitovale su se pospanošću, ataksijom, nekontroliranim pokretima glave, a došlo je i do značajnog pada vrijednosti staničnog volumena (PCV) i gubitka težine. Može se zaključiti da postoji veza između promjena u središnjem živčanom sustavu, padu vrijednosti PCV i gubitku težine u gambijskoj tripanosomozi. Tijek bolesti u majmuna također pokazuje da mnogi sojevi *T. brucei gambiense* mogu biti virulentniji u čovjeka i životinja nego što se dosada mislilo.

Ključne riječi: gambijska tripanosomoza, majmuni