Sensitivity of *Trypanosoma vivax* Isolates, Collected From Tsetse Free Areas of Sudan, to Isometamidium chloride (Samorin)

Nadia M. Osman*, Elsagadi, M.E., Ibtisam A. Goreish, and A/Rahman, A.H

Veterinary Research Institute, P. O. Box 8067 Khartoum, Sudan
* Corresponding author
nadia12osman@yahoo.com

ملخص البحث

تمت دراسة حساسية طفيلى المثقبية النشطة (*Trypanosoma vivax*) لعقار الساموريـن. إستخدمت في هذه التجربة أثنان و ثلاثون رأسا من الماعز النوبي حيث تم تقسيم الماعز الى سبعة مجموعات و تمت عدوي الستة مجموعات الأولى بثلاث سلالات مختلفة (سلالة لكل مجموعتين) من طفيلى المثقبية النشطة و هى حلفا , كنانة و سنار. تمت العدوى بالحقن عبر الوريد الوداجي . تم حقن ثلاثة من المجموعات المصابة (كل مجموعة مصابة بسلالة مختلفة) بدواء الساموريـن بجرعة 0.5 ملجم/كلجم من الوزن الحى; بينما ظلت المجموعة السابعة بدون عدوى كمجموعة ضابطة . تم استعمال عدة طرق لتشخيص المرض شملت طريقة تركيز الدم في الأنابيب الشعرية بواسطة الطرد المركزي و طريقة فحص الغشاء الأبيض باستخدام مجهر ذو خلفية معتمة. أثبتت الدراسة أن للماعز النوبي قابلية عالية للإصابة بطفيلى المثقبية النشطة حيث أظهرت الحيوانات المصابة أعراضاً متنوعة و قيم مختلفة في الدم .كذلك أدت الإصابة إلى نفوق بعض حيوانات التجربة . أدت الإصابة إلى إنخفاض معنوي في نسبة حجم الخلايا المتكدسة،العدد الكلي لكريات الدم الحمراء و تركيز الخضاب و العدد الكلي لكريات الدم البيضاء فى الحيوانات المصابة مقارنة بالتى تم علاجها و غير المصابة . فقط ماعز واحد من مجموعة سنار لم يستجيب للعلاج بالساموريـن مما يدل على فعالية الساموريـن لعلاج طفيل المثقبية النشطة.

Summary

This study was carried out to investigate the sensitivity of *Trypanosoma vivax* to isometamidium chloride. In this study, three *T. vivax* stocks were used to infect Nubian goats; these were Halfa, Kenana and Sennar stocks. Thirty-two Nubian goats were used for the experiment. They were divided into seven groups; six were experimentally infected with three stocks of *T. vivax*, two groups with each stock, through the jugular vein. Then three of these infected animal groups, one from each stock-infected groups, were treated with isometamidium chloride at a dose of 0.5 mg/Kg live b.wt, while the seventh group served as uninfected control group. The haematocrit centrifugation technique (HCT) and dark-ground phase-contrast buffy coat technique (BCT) were used for detecting trypanosome infection. The study showed that Nubian goats were highly susceptible to *T. vivax* infection. Infected animals showed variable clinical signs and different values in haematological parameters. Also the infection led to death of five of the infected animals. The infection resulted in a significant increase ($P \leq 0.05$) in body temperature and significant decrease ($P \leq 0.05$) in packed cell volume (PCV), total red blood cells counts (RBCs), haemoglobin concentration (Hb) values and white blood cells counts (WBCs) in all infected animals compared to the treated and uninfected control groups. Relapse was detected after treatment with isometamidium chloride at 0.5 mg/kg bwt in only one animal of treated Sennar isolate-infected group. This suggested that Isometamidium is effective in treatment of *T. vivax*.

Introduction

Trypanocides currently employed for treatment and prophylaxis of animal trypanosomsosis are Homidium salts (Ethidium-Novidium), Quinapyramine, Diminazene aceturate (Berenil), Isometamidium and Cymelarsan. These different drug formulations have different levels of efficacies on trypanosomes; these efficacies have been described in details by Uilenberg (1998). However, trypanocides efficacy has been greatly hampered by developing of drug resistance which is increasingly being recognized as a constraint in livestock production in many parts of Africa (Peregrine, 1994). D' Ieteren *et al* (2000) reported that both *T. vivax* and *T.*

congolense species appeared to express drug resistance to Berenil, Samorin and Ethidium bromide. Isometamidium is expected to provide protection of up to 3 months before another dose is necessary. The period is reduced because the parasite is developing multiple resistance against these drugs (Uilenberg, 1998). Lukins (2000) reported that drug resistance is known to occur in *T. evansi* isolates in several different countries in Africa and Asia.

In Sudan, Mohammed Ahmed *et al* (1992) advised that Ethidium is to be replaced by Berenil in treating infected cattle in areas inside and outside tsetse belt due to resistance. Experimental studies have shown that multiple trypanocidal drug resistance to both Berenil and ethidium bromide exists in *T. vivax* in Sudan (Ragab, 2006).

In the field, Hall *et al* (1983) suggested that resistance to trypanocides might be a principal cause of persistent infections in cattle. A/Rahman *et al* (1997) observed high PCV values and low mortalities in Isometamidium-treated groups of nomadic cattle.

Drug resistance might seriously hamper control of animal trypanosomosis in Sudan. Therefore, distribution and degree of drug resistance have to be carefully monitored. The present study was designed to assess the trypanocidal activity of isometamidium chloride in goats experimentally infected with three *T. vivax* stocks obtained from naturally infected cattle.

Materials and Methods

The parasites were isolated from blood of naturally infected cattle in Sennar town, (Sennar State), Kenana town (White Nile State) and Halfa Eljadieda town (Gedaref State). The infected blood was inoculated intravenously in goats at site of collection before they were transferred to the Veterinary Research Institute at Khartoum. Blood samples were collected from infected goats and preserved in liquid nitrogen till used.

Thirty-two males, 10 to 12- month-old Nubian goats, of nearly similar body weight were purchased from Omdurman livestock market, Khartoum State, transferred to fly-proof premises of the Veterinary Research Institute, Soba and kept for two months as an adaptation period before the start of experiment. The goats were ear-tagged, treated with anthelmintics, antibiotics and anticoccidial drugs and sprayed by an insecticide. Animals were fed dry *Sorghum* hay, wheat bran and groundnut cake in addition to water. They were randomly divided into seven groups. Group one (5 animals) and group two (4 animals) were infected with *T. vivax*, Halfa isolate. Group three (5 animals) and group four (4 animals) were infected with *T. vivax*, Kenana isolate. The goats of group five (5 animals) and group six (4 animals) were infected with *T. vivax*, Sennar isolate while goats of group seven (5 animals) served as uninfected controls. Each goat received 1 ml of infected blood from donor goats. The parasites were inoculated intravenously (I/V) when parasitaemia scores were one (+4). The parasitaemia, using buffy coat smears of the infected donor goat, was estimated according to Murray *et al* (1983).

Experimentally infected animals were observed for changes in body condition and bled daily for detection of the parasite presence and then twice weekly for 16 weeks. Blood was collected from ear veins into heparinised capillary tubes to determine the level of parasitaemia using the haematocrit centrifugation technique (Woo, 1971) and Dark ground phase contrast buffy coat method (Murray, 1977).

Blood for haematology was collected every two weeks from the jugular vein in heparinised vacutainer tubes. Bleeding commenced two weeks pre-infection, at day zero of infection and continued for 16 weeks post-infection. Whole blood and serum were used for haematological investigation and biochemical analysis.

Body weight, was measured using balance for small laboratory animals, one month before treatment, at day of treatment, and then every two weeks after treatment. Body temperature was recorded daily and then twice weekly after treatment.

The infected goats were examined daily for the first eight days and then twice weekly after treatment for a period of 100 days for relapses after treatment. For treatment of the infected goats, Trypamidium–Samorin, Isometamidium chloride hydrochloride powder for injectable solution (Merial, Toulouse - France) was used by deep intramuscular injection at a dose of 0.5 mg/kg bwt. The different blood indices were determined according to Cheesbrough (2000). Statistical analysis was performed using Statistica programme version 5. The adopted level of significance was $P \leq 0.05$.

Results

The infected goats showed weakness, loss of body condition manifested in wastage of the gluteal and crural muscles, pale mucous membranes and rough hair coat, besides mortality of five infected animals.

Trypanosome was eliminated from the circulation 24 hr after treatment. All the infected animals developed parasitaemia 3 to 8 days post-infection (PI). The mean prepatent periods for Halfa, Kenana and Sennar isolates were 3.89 ± 0.11, 4.22 ± 0.15 and 5 ± 0.53 days, respectively. No significant difference was observed among the infected groups ($P \geq 0.05$).

A significant decrease ($P \leq 0.05$) in parasitaemia was observed in Halfa isolate infected group compared with Kenana and Sennar isolates infected groups (Table 1). A relapse was detected in one treated animal from Sennar isolate-infected group, with a ratio of 1/15 (6.67%).

A significant increase ($P \leq 0.05$) in rectal temperatures of infected groups compared to the treated and control ones, was observed (Table 1). No significant change had occurred in the body weight gains of infected groups, compared to the treated and control groups ($P \geq 0.05$).

The haematological changes in blood of all experimental goats are summarized in Table 1. The decrease in these parameters values coincided with the onset of parasitaemia and continued until the fourth week post-infection, and then the values remained in a fluctuating manner until the last week of the experimental period. Compared to the treated and control groups, a significant decrease ($P \leq 0.05$) occurred in PCV % and Hb values of infected groups. Also a significant decrease in PCV and Hb was observed in Halfa and Sennar isolates-infected groups compared to Kenana isolate-infected group. Moreover, a significant decrease ($P \leq 0.05$) in the RBCs and WBCs counts of infected groups, compared to the treated and control groups, was also observed.

Discussion

In this study, three stocks of *T. vivax* obtained from different localities were tested in goats. They proved to be of moderate pathogenicity. A relapse was detected after treatment with isometamidium chloride at 0.5 mg/kg bwt in only one animal from Sennar isolate-infected group. This suggests that Isometamidium is effective in treatment of *T. vivax*. The infection didn't affect the animal body weights during the whole experimental period. This might be due to the good nutritional status of the experimental animals.

Table 1: Mean (±SE) temperature, parasitaemia and haematological values of infected, uninfected and Samorin treated goats experimentally infected with three different *Trypanosoma vivax* isolates isolated from tsetse free areas

Goat group	Mean Rectal Temperature (±SE)	Mean parasitaemia ($x10^4$/ml blood) (±SE)	Mean PCV% (±SE)	Mean Hb (gm/dl) (±SE)	Mean RBCs ($x10^6$)(±SE)	Mean WBCs (±SE)
G1 Treated Halfa	39.02±0.09a	-	26.85±0.99a	8.81±0.41a	11.241±0.93a	10073.15±773.96 a
G2Infected Halfa	39.57±0.08b	3.07±0.792a	18.78±1.49b	6.45±0.54b	7.73±1.24b	8105.83±891.26b
G3Treated Kenana	39.13±0.06a	-	26.06±0.57a	8.66±0.23a	10.58±0.784a	9345.2± 665.07 a
G4Infected Kenana	39.53±.08b	7.302±0.19b	21.48±1.41c	7.39±0.43c	8.039±1.14b	8395± 580.23b
G5Treated Sennar	39.07±0.09a	-	26.33±1.03a	8.69±0.39a	10.66±0.58a	10741.08±761.57a
G6Infected Sennar	39.56±0.095b	7.28±0.152b	18.11±1.70b	6.11±0.58b	7.750±1.31b	8287±634.99b
G7Uninfected control	39.00±0.07a	-	26.98±0.72a	8.85±0.27a	11.03±0.69a	10646.2±612.99a

Values in the same column followed by different letter(s) are significantly different, significant differences were observed at $P \leq 0.05$

The increase in the body temperature (hyperthermia) might have been due to the body defence system response against the multiplying trypanosomes.

All infected animals showed different peaks of parasitaemia; however, a significant decrease in parasitaemia was observed on Halfa isolate-infected group compared to Kenana and Sennar isolates-infected groups ($P \leq 0.05$). This might be due to different parasite kinetics on animals of the same group as one animal in Halfa group showed symptoms of orchitis.

Another prominent sign of the infection was anaemia which was realized here in all infected groups. It is characterized by pale eye mucus membranes and significant decreases ($P \leq 0.05$) in PCV%, Hb concentration and RBCS counts. On the other hand, no signs of anaemia were observed in the treated groups and their PCV had improved. This may be due to elimination of the sensitive population of trypanosomes from the animal body. This finding coincides with that of Holmes and Jennings (1976) who have showed that haematological parameter recovers rapidly after treatment with any trypanocidal drug. No great alterations in these parameters were observed in the uninfected control group. These findings are supported by the findings of Sekoni *et al* (1990) and Silva *et al* (1998) in *T. vivax* and *T. congolense* infections in goats and cattle. The anaemia might be due to the haemolysins induced by the trypanosome itself, different immunological factors, increased erythro-phagocytosis, haemodilution and dyshaemopoiesis as explained in the previous studies (Soulsby, 1982; Murray and Dexter, 1988; Ojok *et al*., 2001). An evidence that the Kenana isolate has a low pathogenicity is produced since its infected animals showed low levels of anaemia.

A significant decrease ($P \leq 0.05$) in WBCs counts was observed in all infected goat groups. This agrees with the findings of Maxie *et al* (1979) and Bengaly *et al* (1993) who encountered leukopenia in experimental trypanosomes infection with both *T. congolense* and *T. vivax* in cattle and small ruminants. The decrease in WBCs may be due to destruction of cells by the haemolysins produced by trypanosomes. It might also be due to the decrease in myeloid: erythroid ratio (M:E), as the bone marrow was tries to compensate the RBCs reduction in the circulation. A similar results were obtained by Biryomumaisho and Katunguka (2007) who have reported

that infections in goats are characterised by reduced peripheral erythrocyte counts and reductions in the M:E ratios compared with the uninfected goats.

Death in goats might also be due to the anaemia or even to heart damage that might lead to congestive heart failure (hydropericardium was observed during postmotem of infected animals); a finding which might be similar to that of Urquhart (1980). Death of infected goats might be also due to the different haematological changes induced by *T. vivax* coupled with the pathological lesions. This is similar to the findings of Anosa (1988) and Brown *et al* (1990).

Finally the results of the present study have clearly indicated that Nubian goats are highly susceptibility to *T. vivax* infection and thus they are suitable as experimental animals for *T. vivax* studies. The relapse observed here in one goat from the treated Sennar isolate-infected group indicates that the relative efficacy of isometamidium chloride at dose of 0.5 mg/kg b.wt, with which it cured infected goats, is obvious. Although there are no indications for drug resistance, it is essential to maintain the efficacy of the currently available drugs as there is a possibility of spread of isometamidium-resistant *T. vivax* strains in tsetse free areas of the Sudan. In the field, a high dose of samorin such as 1mg/kg b.wt will probably be required for treatment and chemoprophylaxis. A/Rahman (2002) recommended that animals inside the tsetse belts should be given Samorin treatments at two monthly intervals throughout their stay in the tsetse area during the dry season. However, for animals spending the rainy season in Radom area, South Darfur State, three treatments of Samorin and anthelmentics are recommended in June, August and late November. Migratory animals should be treated with Samorin at 1 mg/kg b.wt before leaving the endemic areas. Delespaux (2006) developed some new serological and molecular detection tools that enable faster diagnosis of drug resistance than conventional laboratory or field tests. These techniques are required for studying the prevalence of trypanocides resistance and its impact on the production of livestock in the field.

Acknowledgements

The authors are indebted to the Former Director of Veterinary Research Institute (VRI), Sudan, Prof Amal Mustafa for her kind provision of Nubian goats. We would like to express our gratefulness to all staff of Tsetse and Trypanosomosis Control Department, to Mr Mohammed Nagib of the Department of Pathology and to the Head and staff of Department of Laboratory Animals for their great help during the experimental work.

References

A/Rahman, A.H.A. (2002). *Observations on the epidemiology of bovine trypanosomosis in the Sudan*. Ph.D. thesis, University of Khartoum, 144 pp.

A/Rahman, A.H.; Mohammed-Ahmed M.M. and Abdel Karim, E.I. (1997). *Sudan. J. Vet. Sci. Anim. Husb*., **36**: 149-157.

Anosa, V.O. (1988). *Rev. Elev. Med.Vet. Pays. Trop*., **41**: 151-164.

Bengaly, Z.; Clausen, P.H; Poly, H.; Kanwe, A.B. and Duvallet, G. (1993). *Rev. Elev. Med. Vet. Pays. Trop*., **46**: 563-570.

Biryomumaisho, E. and Katunguka, R. (2007). *Vet. Parasitol*. **143**: 354-357.

Brown, C.G.D; Hunter, A.G. and Lukins, A.G. (1990). Diseases caused by Protozoa. Sewell, M.M.H and Brocklesby, D.W. (eds.). **In:** *Hand book on animal diseases in the tropics*. 4th edn. UK. Pp. 205-226.

Cheesbrough, M. (2000). *District Laboratory Practice in Tropical Countries*. Part 2. Cambridge university press, Newyork, USA, 464 pp.

D'Ieteren, G.; Coulibaly, L.; Atse, P.A.; Hecker, P.A.; Krebs, H.A.; Rowlands,

G.J.; Leak, S.G.A. and Nagda, S.M. (2000). ICPTV Newsletter, **2**: 23-24.

Delespaux, V. (2006). *Improved diagnosis of trypanosome infections and drug resistant T. congolense in livestock*. Ph.D thesis. Instituut voor Tropische Geneeskunde Antwerpen–Université Libre de Bruxelles

Hall, M.J.R.; Kheir, S.M.; A/Rahman, A.H. and Noga, S. (1983). *Trop. Anim. Hlth. Prod*., **15**: 191-206.

Holmes, P.H. and Jennings, F.W. (1976). The effect of treatment on the anaemia in African trypanosomiasis. **In:** Soulsby, E.J.L. ed. *Pathophysiology of parasitic Infection*, New York: Academic Press**.** 809 pp.

Lukins, A.G. (2000). ICPTV Newsletter, **2**: 24-26.

Maxie, M.G.; Losos, G.J. and Tabel, H. (1979). *Tropenmed. Parasitol*., **30**: 274-282.

Mohammed Ahmed, A.M.; Abdel Karim, E.I. and A/Rahman, A.H. (1992). *Sudan. J. Vet. Sci. Anim. Husb.,* **36**: 149-157.

Murray, M. (1977). *Trans. R. Soc. Trop. Med. Hyg.,* **71**: 325-326

Murray, M. and Dexter, T.M. (1988). *Acta.Trop.,***45**: 389-432.

Murray, M.; Trail J.C.M.; Turner D.A. and Wissocq, Y. (1983). *Network Training Manual.* ILCA, Addis Ababa, Pp. 45-75.

Ojok, L.; Kaeufer-Weiss, I. and Weiss, E. (2001). *J. Comp. Pathol.,* **124**: 149-158.

Peregrine, A.S. (1994). *Vet. Parasitol*., **54**: 223-248.

Ragab, Salma A. (2006). *The Pathogenicity and drug resistance of T. vivax in calves and goats.* Ph.D, Uinversity of Khartoum, Khartoum, Sudan.

Sekoni, V.O.; Saror, D.I.; Njoku, C.O.; Kumi-Diaka, J. and Opaluwa, G.I. (1990). *Vet. Parasitol.,* **35**: 11-19.

Silva, A.M.S.; Morales, G.; Eulert, E.; Montenegro, A. and Yabnez, R. (1998). *Vet. Parasitol.,* **76**: 153-157.

Soulsby, E.J.L. (1982). *Helminthes, Arthropods and Protozoa of Domesticated Animals*. 7th edn. Bailliere Tindall and Cassell, East Sussex.UK. 809. Pp.

Uilenberg, G. (1998). *A Field Guide for the Diagnosis, Treatment and Prevention of African Animal Trypanosomosis.* FAO, Rome. P. 158.

Urquhart, G.M. (1980). *Trans. R. Soc. Trop. Med. Hyg*., **74**: 726-729.

Woo, P.T.K. (1971). *Acta Trop*., **28**: 298-303.