Chemotherapy of *T b brucei* infection: Use of DFMO, diminazene aceturate, alone and in combination

P. A. Onyeyili and S. M. Anika

Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria, Nsukka

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ABSTRACT

The therapeutic activity of diminazene aceturate, difluoromethylornithine (DFMO) and a combination of the two agents was investigated in experimental *Trypanosoma brucei brucei* infections in mongrel dogs. The criteria used in the assessment of the trypanocidal effect of these compounds included the examination of the blood for the parasite, as well as clinical and haematological changes at intervals following treatment. Diminazene aceturate (7 mg/kg intramuscularly), DFMO (300 mg/kg/day orally in three divided doses for six days) and the combination of diminazene aceturate (7 mg/kg intramuscularly) and DFMO (300 mg/kg/day orally for six days) produced an intermittent aparasitemia in the dogs. Relapse infection occurred in all the three groups, but the period of aparasitemia produced by the combination of the agents was longest. The packed cell volume, haemoglobin concentration and red cell count values decreased after the dogs were inoculated with the parasite. The values improved slightly following the treatments with the agents or their combination. The total white blood cell counts in the infected dogs indicated leucocytosis, but this improved with drug treatment.

INTRODUCTION

Trypanosomiasis is recognised as one of the major diseases of economic importance to man and domestic animals in Africa. For two-and-a-half decades there has been very little progress made in the drug control of animal trypanosomiasis, although a lot of work has been done on the efficacy of trypanocides in various animals (Wilson 1958, Fairclough 1963, Hoeve and Cunningham 1964, Verma and others 1973, Wilson and others 1975). Due to the reported cases of relapse which occur with the routinely used trypanocides (Chukwu and others 1989) it has become necessary to assess new compounds for both prophylactic and therapeutic activity against species and strains of trypanosomes at all stages of infection, and in addition to periodically reassess the therapeutic potentials of the routinely used trypanocides. In Nsukka and environs, trypanosomiasis is most often encountered in dogs (Omamegbe and others 1984).

Diminazene aceturate is one of the conventional trypanocides available for the treatment of trypanosomiasis in Nigeria. Difluoromethylornithine (DFMO) is an irreversible inhibitor of ornithine decarboxylase; an enzyme that catalyses the synthesis of polyamines from ornithine (Metcalf and others 1978). DFMO has been successfully used in the treatment of both animal and human trypanosomiasis (Bacchi and others 1980, Nathan and others 1981, Bacchi 1981, Schillinger and Gorton 1984; Van Nieuwenhove and others 1985). Furthermore, DFMO was found to be highly synergistic with several standard trypanocides when examined in the acute *T b brucei* model. The agents include suramin (used in humans for early-stage trypanosomiasis); pentamidine (early stage human disease); diminazene aceturate (a veterinary trypanocide) (Bacchi and McCann 1987). In addition, Clarkson and others (1984) have shown that a combination of suramin and DFMO is curative in the treatment of late-stage African trypanosomiasis. This study reports the therapeutic activity of diminazene aceturate, DFMO and a combination of both agents in mongrel dogs experimentally infected with *T b brucei*. The trypanosome isolate used in this study has been shown to cause relapses in dogs (Chukwu and others 1989).

MATERIALS AND METHODS

Experimental animals

Eighteen mongrel dogs of both sexes, weighing

between 3·4 and 6 kg and eight to 10 months of age were used for the study. The dogs were randomly selected and certified clinically healthy at the onset of the experiment. The animals were purchased from Orie-Orba, a local market located close to Nsukka. The dogs on arrival were dewormed with fenbendazole (Panacur; Hoechst) at an oral dosage of 100 mg/kg bodyweight and disophenol (DNP: American Cyanamid Company) subcutaneously at the rate of 0·2 ml/kg bodyweight. They were kept in fly-proof house in separate cages and fed twice daily with food procured from the students' cafeteria. Water was provided ad libitum. Before the commencement of the experiments, each dog was physically examined and screened for the presence of trypanosomes and other blood parasites using the wet mount, Giemsa stained blood films and haematocrit buffy-coat technique (Murray and others 1977).

Trypanosome stock

T b brucei strain 8/18 was used for the study. The trypanosome was obtained from the Nigeria Institute For Trypanosomiasis Research, Vom. The dogs were infected by intravenous injection of diluted rat blood containing 5×10^5 *T b brucei* organisms. Trypanosomes were enumerated in haemocytometer using the method of Brown and Losos (1977).

Trypanocidal drugs

The agents used for the treatment were diminazene aceturate (Berenil; Hoechst) and DFMO (Merrel Dow Research Centre, USA). Diminazene aceturate was prepared according to the specification of the manufacturer. Four per cent DFMO solution was utilised for the study.

Experimental groups

Fifteen dogs were infected with *T b brucei* and when parasitaemia was established they were divided into four treatment groups.

Group 1: DFMO, 300 mg/kg/day orally in three divided doses for six days, (four dogs).

Group 2: Diminazene aceturate, 7 mg/kg instramuscularly, (four dogs).

Group 3: Diminazene aceturate (7 mg/kg intramuscularly) plus DFMO (300 mg/kg/day orally in three divided doses for six days), (four dogs). Diminazene aceturate was administered three hours after the initial DFMO treatment.

Group 4: Infected untreated controls, (three dogs).

The remaining three dogs were neither infected nor treated and were used to monitor the occurrence of other diseases. Treatment was initiated 15 days after infection when parasitaemia was well established on assessment with the haematocrit buffy-coat technique (Murray and others 1977) and examination of blood films.

Parameters for assessing therapeutic activity

The dogs were examined daily. Active infections with the trypanosomes were detected by examination of smears every five days made from the femoral venous blood using EDTA as anticoagulant. Wet blood films, Giemsa-stained thin blood smears and the haematocrit buffy-coat technique were used for the detection of the trypanosomes. Packed cell volume, haemoglobin concentration, red blood cell counts, white blood cell counts were measured every five days. Packed cell volume was determined by a micromethod, using a Hawksley microhaematocrit centrifuge. Haemoglobin concentration was determined by the cyanomethaemoglobin method. The red blood cell and total white blood cell counts were done using the improved Neubauer haemocytometer.

RESULTS

Clinical findings

All mongrel dogs infected with *T b brucei* exhibited the following clinical signs: fever which was intermittent, oedema of the face and jaw; purulent ocular discharges, emaciation, weakness, anaemia and enlargement of the peripheral lymph nodes. These signs were reversed with subsequent treatments, although there was reoccurrence following the relapse of parasitaemia in the dogs. Some of the dogs treated with DFMO and its combination with diminazene aceturate developed, during the course of therapy, diarrhoea and vomition which disappeared when treatment was stopped.

Parasitological findings

The non-infected control dogs showed no signs of trypanosomiasis or other infections throughout the period of experimentation. The comparative trypanocidal efficacy of DFMO, diminazene aceturate and a combination of the two drugs in *T b brucei* infected mongrel dogs are shown in Table 1. The infected animals developed teeming parasitaemia within five days after inoculation with the parasite. There was no detectable level of *T b brucei* in the blood of dogs 24 hours after treatment with diminazene aceturate and the combination of diminazene aceturate and DFMO (not indicated in Table 1). The same was observed in DFMO treated dogs six days following administration. Relapse parasitaemia however occurred in

groups 2 and 3 at 30th and 35th day, respectively, after treatment; and in group 1, at the 10th day after treatment. Animals in group 4 exhibited progressive parasitaemia that resulted in early deaths.

Haematological findings

Fig 1 shows the mean packed cell volume of dogs infected with *T b brucei* and subsequently treated with DFMO, diminazene aceturate and a combination of both. Before treatment, the infected dogs demonstrated a significantly reduced packed cell volume, but following treatment, there was an improvement in the packed cell volume of the dogs as compared to the non-infected controls, though it further decreased with the onset of relapse. The mean haemoglobin concentration (Fig 2) of infected dogs seem to follow the same trend as the packed cell volume before and after the drug treatments. There was some improvement in the red blood cells of diminazene aceturate treated dogs following treatment. Treatment with DFMO alone resulted in a further decrease in the red blood cells of the infected animals, and only improved with the termination of the treatment. All the infected dogs showed leucocytosis which improved with treatment.

DISCUSSION

The present study shows that *T b brucei* strain 8/18 can produce within three days, parasitaemia associated with gradual loss of condition, lymph node enlargement, oedema of the face and jaw and weakness in mongrel dogs. The clinical signs observed in this study are found to be similar to those reported in acute cases of *T brucei* infection of dogs (Losos and Ikede 1972), and in mice (Anika and others 1987). The subcutaneous oedema may have occurred as a result of the release of vasoactive substances as reported by van den Ingh and others (1977).

The parasitological findings show that when the condition is treated with diminazene aceturate, DFMO, or a combination of the two agents, at the dosage levels employed, there is an obvious aparasitaemia. The combination of DFMO and diminazene aceturate seems to have a slight advantage over diminazene aceturate alone, as no parasite could be detected in the blood of the dogs at 24 hours after treatment and relapse parasitaemia occurred in the group at the 35th day after treatment (as against day 30 when diminazene aceturate was used alone). One dog in group 2 died on the 17th day after treatment of unknown cause.

The relapse infection occurring in diminazene aceturate treated dogs in the present study was in agreement with the finding of Jennings and others (1977), who observed that treatment of infected mice 14 days after inoculation resulted in relapse. Similarly the relapse in DFMO treated dogs in this study was in agreement with the observations of other investigators (McCann and others 1981, Clarkson and others 1983). In the infected un-

Table 1. Comparative trypanocidal efficacy of DFMO, diminazene aceturate and a combination of DFMO and diminazene aceturate in *T b brucei* infected dogs

	Parasitaemia* ‡Days after infection												
Treatment	1	5	10	15†	20	25	30	35	40	45	50	55	60
DFMO**	0	4	4	4	4	0	0	4	4	4	4	3	2
	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	4	4	4	4	4	4	4	4	4	4	3	2
Diminazene††	0	4	4	4	0	0	0	0	0	1	2	2	1
aceturate	–	–	–	–	–	–	–	–	–	–	–	–	–
	4	4	4	4	4	4	4	3	3	3	3	3	2
DFMO plus	0	4	4	4	0	0	0	0	0	0	1	3	3
Diminazene	–	–	–	–	–	–	–	–	–	–	–	–	–
aceturate	4	4	4	4	4	4	4	4	4	4	4	4	4
Infected**	0	3	3	3	3	3	3	3	0	0	0	0	0
untreated	–	–	–	–	–	–	–	–	–	–	–	–	–
control	3	3	3	3	3	3	3	3					
Uninfected control	0	0	0	0	0	0	0	0	0	0	0	0	0

* Number of animals positive/number infected
† Immediately before drug treatment (0 day after treatment)
‡ Day 1 = day after inoculation, Day 60 = 60 days post-inoculation
** Two animals in the DFMO group and all the animals in the infected untreated control group died due to trypanosomiasis
†† One animal died 32 days after inoculation from an unknown cause while another died 56 days after inoculation due to trypanosomiasis

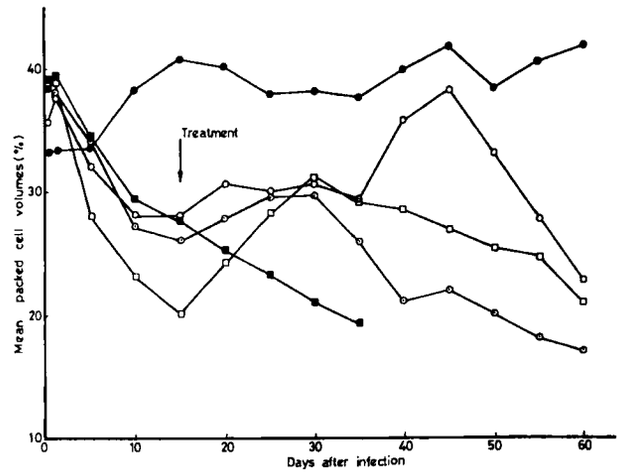

FIG 1. Mean pack cell volume of dogs infected with *T b brucei* and subsequently treated with DFMO, diminazene aceturate alone and in combination. Dogs were infected on day zero and treatment started 15 days after inoculation. Key: ⊙ DFMO monotherapy, ○ diminazene aceturate, □ combination, ■ infected untreated, ● uninfected controls

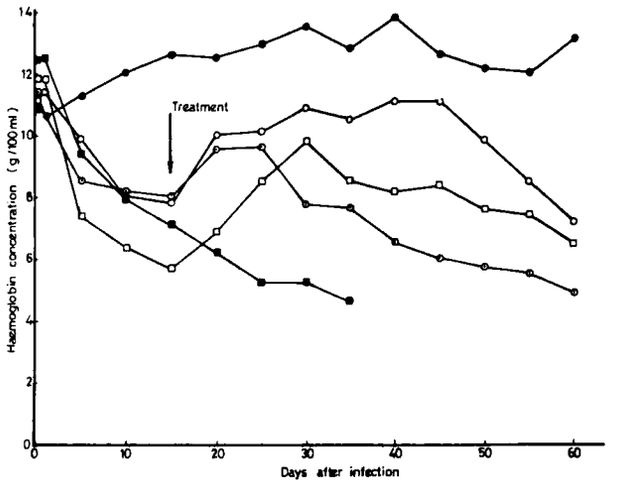

FIG 2. Mean haemoglobin concentrations of dogs infected with *T b brucei* and treated with DFMO, diminazene aceturate alone and in combination. Dogs were infected on day zero and treatment was instituted on day 15 postinoculation. Key: ⊙ DFMO monotherapy, ○ diminazene aceturate, □ combination, ■ infected untreated, ● uninfected controls

treated controls, there was progressive parasitaemia (Table 1) which resulted in early death.

The relapse in the treated animals is perhaps an indication of the central nervous system involvement, as *T brucei* are known to infect this organ. Trypanosomes in these areas may have evaded the action of the trypanocidal agents, and thus produced the relapse infection.

The haematological findings (Figs 1, 2 and 3) showed that the packed cell volume, haemoglobin and red blood cell levels fell after infection with *T b brucei*. Some earlier investigators (Jennings and others 1977, Mackenzie and others 1978, Anika and others 1987, Anene 1987), have observed a progressive fall in packed cell volume, red cell and haemoglobin values appear to improve in diminazene aceturate treated group on day 5 after treatment. In the groups treated with DFMO and the combination of DFMO with diminazene aceturate, there was further decrease in red cell until the treatment was stopped. The further decrease in red cell may be due to the inhibitory effect of DFMO on the red cell production. Abeloff and others (1984), observed a similar decrease in erythrocyte counts of about 30 per cent to a maximum of 53 per cent in some of the patients treated with DFMO.

The infected dogs showed leucocytosis, which decreased to normal range with the disappearance of the parasites following chemotherapy. The leucocytosis seen in the present study appear to be in agreement with the findings of Anene (1987) and Kagwa and others (1984) in infected dogs, though the findings are in contrast with those of Anika and others (1987), who observed leucopenia in mice infected with *T brucei*.

Diarrhoea and vomition was noticed in the groups treated with DFMO and the combination of DFMO with diminazene aceturate. The diarrhoea and vomition noticed in this study is in agreement with the observations of Abeloff and others (1984) and Schechter and Sjoerdsma (1986) with oral DFMO therapy in human patients. These gastrointestinal disturbances may have occurred due to irritation within the gastrointestinal tract and in order to by-pass the problems associated with oral DFMO therapy Schechter and Sjoerdsma (1986) have recommended the use of the intravenous route of administration. The vomition and diarrhoea, may have limited the effective amount of the drug absorbed for therapy, hence the short aparasitaemic period observed in DFMO therapy alone.

The failure of the DFMO/berenil combination to achieve a more effective and prolonged therapy might be due to the way DFMO was given. Further

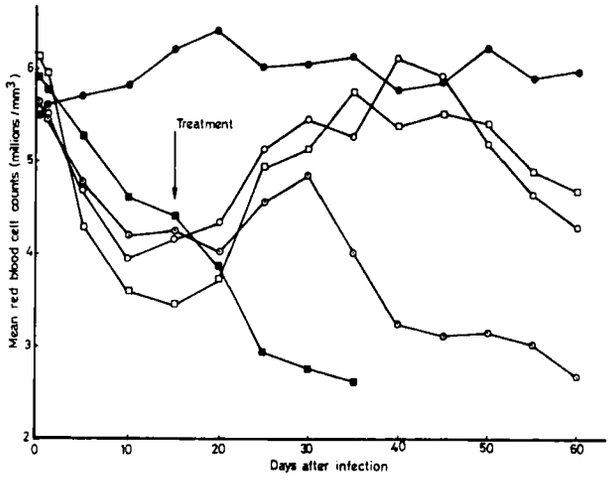

FIG 3. Mean red blood cell counts of dogs infected with *T b brucei* and treated with DFMO, diminazene aceturate alone and in combination. Infection of dogs occurred on day zero and treatment was started on day 15 after inoculation. Key: ⊙ DFMO monotherapy, ○ diminazene aceturate, □ combination, ■ infected untreated, ● uninfected controls

studies will elucidate the efficacy of the combination when DFMO is administered throughout a 24 hour period. In addition, the timing of the berenil administration in relation to DFMO may also be critical as most successful experiments have given the berenil at least three days after the start of the DFMO regimen. This will also be taken into consideration in further work.

In conclusion DFMO appears to potentiate the chemotherapeutic activity of diminazene aceturate in dogs infected with relapsing strain of *T b brucei*. There appeared to have been some gastrointestinal disorders following oral administration of DFMO and this may have partially inhibited its effectiveness. It is probable that a six day treatment period for *T b brucei* infection using DFMO orally was insufficient for the effective control of the infection. Further study will evaluate the therapeutic effect of DFMO following intravenous administration.

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REFERENCES

ABELOFF, M. D., SLAVIK, M., LUK, G. D., GRIFFIN, G.A., HERMANN, J., BLANC, O., SJOERDSMA, A. & BAYLIN, S. B. (1984) Phase I trial and pharmacokinetic studies of α-difluoromethylornithine – an inhibitor of polyamine biosynthesis. *Journal of Clinical Oncology* **2,** 124-130

ANENE, B. M. (1987) Immunosupression in canine trypanosomiasis. Master's Science Thesis. University of Nigeria, Nsukka

ANIKA, S. M., SHETTY, S. N., ASUZU, I. U. & CHIME, A. B. (1987) Effects of some trypanocides and anti-inflammatory agents in experimental *Trypanosoma brucei* infection in mice. *Zariya Veterinarian* **2,** 9-15

BACCHI, C. J. (1981) Contents, synthesis, and function of polyamines in trypanosomatids: relationship to chemotherapy. *Journal of Protozoology* **28,** 20-27

BACCHI, C. J. & MCCANN, P. P. (1987) Parasitic Protozoa and Polyamines. In: Inhibition of polyamine biosynthesis. Eds P. P. McCann, A. E. Pegg and A. Sjoerdosma. Academic Press, Orlando Florida. p322

BACCHI, C. J., NATHAN, H. C., HUTNER, S. H., MCCANN, P. P. & SJOERDSMA, A. (1980) Polyamine metabolism: A potential therapeutic target in trypanosomes. *Science* **210,** 332-334

BROWN, L. A. & LOSOS, G. J. (1977) A comparative study of the responses of the thymus, spleen, lymphnodes and bone marrow of the albino rat to infection with *Trypanosoma congolense* and *Trypanosoma brucei*. *Research in Veterinary Science* **23,** 196-203

CHUKWU, C. C., ANENE, B. M., ONUEKWUSI, K. O. & ANIKA, S. M. (1989) Relapse infection after chemotherapy in dogs experimentally infected with *Trypanosoma brucei brucei*. *Journal of Small Animal Practice*

CLARKSON, A. B., BACCHI, C. J., MELLOW, G. H., NATHAN, H. C., MCCANN, P. P. & SJOERDSMA, A. (1983) Efficacy of combinations of difluoromethylornithine and bleomycin a mouse model of central nervous system African trypanosomiasis. *Proceedings of the National Academy of Science USA* **80,** 5729-5733

CLARKSON, A. B., BIENEN, E. J., BACCHI, C. J., MCCANN, P. P., NATHAN, H. C., HUTNER, S. H. & SJOERDSMA, A. (1984) New drug combination for experimental late-stage African trypanosomiasis: DL-α-difluoromethylornithine (DFMO) with suramin. *American Journal of Tropical Medicine and Hygiene* **33,** 1073-1077

FAIRCLOUGH, R. (1963) A comparison of metamidium, samorin, berenil, and ethidium bromide under field conditions in Kenya. *Veterinary Record* **75,** 855-858

VAN HOEVE, K. & CUNNINGHAM, M. (1964) Prophylactic activity of berenil against trypanosomes in treated cattle. *Veterinary Record* **76,** 260

INGH VAN DEN, T. S. G. A. M., SCHOTMAN, A. J. H., VAN DUIN, C. TH. M., BUSSER, F. J. M., TEN HOEDT, E. & DE NEYS, M. H. H. (1977) Clinicopathological changes during *Trypanosoma brucei* infection in the rabbit. *Zentralblatt fur Veterinia-medizin* **B24,** 787-797

JENNINGS, F. W., MURRAY, P. K., MURAY, M. & URQUHART, G. M. (1974) Anaemia in trypanosomiasis: Studies in rats and mice infected with *Trypanosoma brucei*. *Research in Veterinary Science 16,* 70-76

JENNINGS, F. W., WHITELAW, D. D. & URQUHART, G. M. (1977) The relationship between duration of infection with *Trypanosoma brucei* in mice and the efficacy of chemotherapy. *Parasitology* **75,** 143-153

KAGWA, E., MUNYUA, W. K. & MUGERA, G. M. (1984) Pathogenecity of *Trypanosoma brucei brucei* in the dog. *Bulletin of Animal Health and Production in Africa* **32,** 360-368

LOSOS, G. J. & IKEDE, B. O. (1972) Review of pathology of diseases in domestic and laboratory animals caused by *Trypanosoma congolense, T vivax, T brucei, T rhodesience* and *T gambiense*. *Veterinary Pathology* **9** (supplement), 1-71

MACKENZIE, P. K. I., BOYT, W. P., NESHAM, V. W. & PIRIE, E. (1978) The aetiology and significance of the phagocytosis of erythrocytes and leucocytes in sheep infected with *Trypanosoma congolense* (Broden 1904). *Research in Veterinary Science* **24,** 4-7

MCCANN, P. P., BACCHI, C. J., CLARKSON, (JR) A. B., SEED, J. R., NATHAN, H. C., AMOLE, B. O., HUTNER, S. H. & SJOERDSMA, A. (1981) Further studies on difluoromethylornithine in African trypanosomes. *Medical Biology* **59,** 434-440

METCALF, B. W., BEY, P., DANZIN, C., JUNG, M. J., CASARA, P. & VEVERT, J. P. (1978) Catalytic irreversible inhibition of mammalian ornithine decarboxylase (EC4.1.1.17) by substrate and product analogues. *Journal of American Chemical Society* **100,** 2551-1553

MURRAY, M., MURRAY, P. K. & MCINTYRE, W. I. M. (1977) An improved parasitological technique for the diagnosis of African trypanosomiasis. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **71,** 325-326.

NATHAN, H. C., BACCHI, C. J., HUTNER, S. H., RESCIGNO, D., MCCANN, P. P. & SJOERDSMA, A. (1981) Antagonism by polyamines of the curative effect of α-difluoromethylornithine in *Trypanosoma brucei brucei* infections. *Biochemical Pharmacology* **30,** 3010-3013

NIEUWENHOVE VAN, S., SCHECHTER, P. A., DECLERCQ, J., BONE, G., BURKE, J. & SJOERDSMA, A. (1985) Treatment of gambiense sleeping sickness in the Sudan with oral DFMO (DL-α-difluoromethylornithine), an inhibitor of ornithine decarboxylase; first field trial. *Transactions of the Royal Society of Tropical Medicine and Hygiene* **79,** 692-698

OMAMEGBE, J. O., ORAJAKA, L. J. E. & EMEHELU, C. O. (1984) The incidence and clinical forms of naturally occurring canine trypanosomiasis in two veterinary clinics in Anambra State of Nigeria. *Bulletin of Animal Health and Production in Africa* **32,** 23-29

SCHECHTER, P. J. & SJOERDSMA, A. (1986) Difluoromethlornithine in the treatment of African trypanosomiasis. *Parasitology Today* **2**, 223-224

SCHILLNGER, D. & GORTON, E. (1984) Efficacy of difluoromethylornithine upon a drug-resistant *Trypanosoma congolense* strain in mice. *Drugs and Experimental Clinical Research* **10**, 677-679

VAN DEN INGH, T. S. G. A. M., SCHOTMAN, A. J. H., VAN DUIN, C. TH. M., BUSSER, F. J. M., TEN HOEDT, E. & DE NEYS, M. H. H. (1977) Clinicopathological changes during *Trypanosoma brucei* infection in the rabbit. *Zentralblatt fur Veterinia-medizin* **B24**, 787-797

VERMA, B. B., GAUTAM, O. P. & MALIK, P. D. (1973) Diminazene acetuarate in the treatment of experimental *Trypanosoma evansi* infection in buffalo calves. *Veterinary Record* **93**, 465-467

WILSON, S. G. (1958) Animal trypanosomiasis in Northern Nigeria. In: Symposium on animal trypanosomiasis, Luanda, CCTA Publication 45, 37-52

WILSON, A. J., LE ROUX, J. G., PARIS, J., DAVIDSON, C. R. & GRAY, A. R. (1975) Observations on a herd of beef cattle maintained in a tsetse area. I. Assessment of chemotherapy as a method for the control of trypanosomiasis. *Tropical Animal Health and Production* **1**, 187-199

ABSTRACTS

Medical management of canine viral enteritis

CORONAVIRUS, parvovirus, distemper virus and rotavirus can cause acute enteritis in dogs, with acute vomiting and diarrhoea. The treatment is supportive only and aims to stop the clinical signs, replace fluid and electrolyte losses, maintain hydration until oral fluid and food can be tolerated and control secondary bacterial infections. Dehydration should be corrected by intravenous lactated Ringer's solution administered over four to six hours; subsequently the composition of maintenance fluids should be adjusted in accordance with daily determinations of blood electrolytes. Injectable antibiotics are preferable and should be effective against both aerobic and anaerobic bacteria. Food should be withheld for at least 48 hours and anti-emetics such as chlopromazine or metoclopramide may be necessary. The initial diet should be easily digestible, low-residue and low in fat; rice and low-fat cottage cheese is ideal. The efficacy of intestinal protectants is doubtful but, empirically, bismuth subsalicylate seems more effective than kaolin and pectin.

NELSON, R. W. (1988) Proceedings of the 13th World Congress of the World Small Animals Veterinary Association, Barcelona. p 10

Canine sinonasal neoplasm: A clinicopathological study

SIXTY per cent were of epithelial origin with 31·5 per cent being adenocarcinomas. Non-keratinising squamous cell carcinomas were more common than keratinising squamous and carcinoma. The commonest non-epithelial neoplasm was chondrosarcoma. Carcinoids and adenocarcinoids were also found. Chondrosarcomas were seen in dogs between one and eight years old. Most dogs with the other tumour types were over nine years old. Males predominated in all groups. Clinical signs and breed varied according to histological type. In 50 per cent of the dogs the neoplasm involved nasal or frontal sinuses bilaterally and more than one anatomical site was involved in 50·1 per cent. The overall rate of metastases varied according to histological type of neoplasm; the non-epithelial tumours having the lowest rate.

PATNAIK, A. K. (1989) *Journal of American Animal Hospital Association* **25**, 103-113

Lymphosarcoma with osseous involvement in a dog

FIVE-year-old male Airedale terrier was lame in the left stifle. He also had an enlargement of the right tonsil. Radiography revealed extensive lysis of the distal part of the left patella, with a pathological fracture. Biopsies of the tonsil, patella and popliteal lymph node demonstrated lymphosarcoma. Subsequently multiple osseous lesions developed. Initially, chemotherapy was unsuccessful but after a change in regime remission occurred. The dog died four years later from unrelated causes.

TURNWALD, G. H., PECHMEN, R. D., SHIRES, P. K., SNIDER, T. G. & BURICE, J. H. (1988) *Journal of the American Animal Hospital Association* **24**, 350-354

Portosystemic vascular shunts in American cocker spaniels

FOUR American cocker spaniel bitches between 10 and 19 months had multiple acquired extrahepatic shunts, associated with portal hypertension, demonstrated by radiographic studies. Presenting signs in three cases were diarrhoea and weight loss, plus ascites in one. The youngest bitch had no clinical signs other than being smaller than normal. Two of the animals were little sisters and the other two were closely related to the sisters.

RAND, J. S., BEST, S. J. & MATHEWS, K. A. (1988) *Journal of the American Animal Hospital Association* **24**, 265-272