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TREATMENT AND THROMBOCYTE LEVELS IN EXPERIMENTALLY INDUCED CANINE EHRLICHIOSIS AND CANINE BABESIOSIS

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ABSTRACT

VAN HEERDEN, J., REYERS, F. & STEWART, C. G., 1983. Treatment and thrombocyte levels in experimentally induced canine ehrlichiosis and canine babesiosis. *Onderstepoort Journal of Veterinary Research*, 50, 267–270 (1983).

Three dogs which were carriers of *Babesia canis* were infected with *Ehrlichia canis*. These animals developed clinical signs and haematological evidence of ehrlichiosis and made an uneventful recovery, despite the fact that they were subsequently treated with doxycycline only.

Three control dogs which were also carriers of *B. canis* were clinically normal, despite the fact that they showed a distinct drop in the thrombocyte levels associated with increases in the numbers of parasitized red cells.

INTRODUCTION

The clinical importance of mixed infections of *Babesia canis* and *Ehrilichia canis*, as well as the diagnostic difficulties encountered with such mixed infections in the dog, have been reported by several investigators (Malherbe, 1947; Ewing & Buckner, 1965; Ewing, 1969; Van Heerden & Immelman, 1979; Van Heerden, 1982a).

Dogs with mixed infections are often diagnosed as having babesiosis only and are therefore treated with babesiacides. Subsequently they are presented as either "chronic biliary fever" or so-called "biliary-relapse" cases. If a mixed *B. canis*/*E. canis* infection is diagnosed, the patient is then usually treated with both a babesiacide and an agent effective against *E. canis* (Van Heerden & Immelman, 1979; Van Heerden, 1982b).

Casual unpublished observations, however, indicated that such further treatment with a babesiacide may not be necessary (Van Heerden, unpublished data, 1981).

This investigation was undertaken in an attempt to give some guidelines to the treatment of mixed infections in the dog, especially in the case of so-called "biliary-relapse" cases.

MATERIALS AND METHODS

Blood smears were made from the first drop of blood obtained from pricking the anterior border of the ear of the experimental dogs. The smears were stained with Diff-Quik (Harleco) and 100 oil immersion fields (10 × 100 magnification) were examined along the borders and in the feather ends of the smears. The number of parasitized cells (red blood cells in the case of *B. canis* and monocytes or lymphocytes in the case of *E. canis*) per 100 fields was recorded.

Cell counts and indices were obtained with an electronic cell counter (Coulter Model FN*) and a haemoglobin meter, utilizing the cyanmethaemaglobin technique. Thrombocyte counts were obtained with a Sysmox (Model PL 110**) electrode platelet counter.

The thrombocyte counts for each individual dog within the control group (i.e. the *Babesia*-infected group) were classified into 2 groups: counts obtained with low levels of parasitaemia, i.e. 0–1 parasitized cell per 100 fields, and counts obtained with higher levels of parasitaemia, i.e. more than 1 parasitized red cell per 100 fields.

Donor: The isolate of *E. canis* was obtained from a 1-year-old male Dobermann Pinscher which had acquired a natural infection. The animal was presented in a mildly depressed febrile state, with mild enlargement of peripheral lymph nodes, slight petechiation of the gums and a history of partial anorexia and mild mass loss. Examination of a blood smear demonstrated the presence of morulae of *E. canis* in the cytoplasm of monocytes. No trophozoïtes of *B. canis* were seen. The dog was treated intramuscularly once only with diminazene†, at a dosage rate of 3,5 mg/kg body mass. Four mℓ of venous blood was collected in heparin 24 hours after treatment with diminazene and injected intravenously into experimental Beagle X.

The donor dog was subsequently treated orally with doxycycline†† for 10 days at a daily dosage rate of 10 mg/kg body mass.

Beagle X: This dog was a physically healthy, 6-month-old, female Beagle, kept on concrete and treated weekly with an acaricide. Haematological investigations were undertaken serveral times before inoculation with infected blood. Immediately after the dog was given infected blood, it was treated intramuscularly once only with diminazene at a dosage rate of 3,5 mg/kg body mass. Physical examination of the dog as well as examination of blood smears was performed daily after the intravenous inoculation of infected blood.

On the 17th day after infection 4 mℓ aliquots of venous heparinized blood collected from this dog was injected intravenously into Beagles 5, 7 and 15.

Beagle X was subsequently treated orally with doxycycline for 10 days at a daily dosage rate of 10 mg/kg body mass.

Babesia *carriers:* These dogs (No. 4, 5, 7, 10, 15 and 17) were all infected with *B. canis* prior to this experiment as previously described (Stewart, 1983). Briefly, all dogs were inoculated with a virulent strain of *B. canis* 107 days before the commencement of this experiment. Treatment with various drugs was carried out to control severe infection and all dogs were challenged with homologous parasites 62 days later. Parasitaemia with *B. canis* was patent in all the dogs before inoculation with *E. canis*.

These animals were housed on concrete floors and kept tick-free by weekly treatment with an acaricide.

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Haematological investigations were undertaken before and after the initiation of this experiment. Blood smears were examined daily (Fig. 1 & 2).

Dogs 5, 7 and 15 were injected intravenously with 4 mℓ aliquots of *E. canis* infected blood (*Ehrlichia*- and *Babesia*-infected group).

Dogs 4, 10 and 17 served as a control group (*Babesia*-infected group).

Dogs 5 and 7 were treated orally 32 days after infection and Dog 15, 57 days after infection with doxycycline for 10 days at a daily dosage rate of 10 mg/kg body mass.

No treatment was given to Dogs 4, 10 and 17.

RESULTS

Donor of E. canis: The dog responded favourably to treatment with doxycycline and made an uneventful recovery. Eight months later it was still in good physical condition.

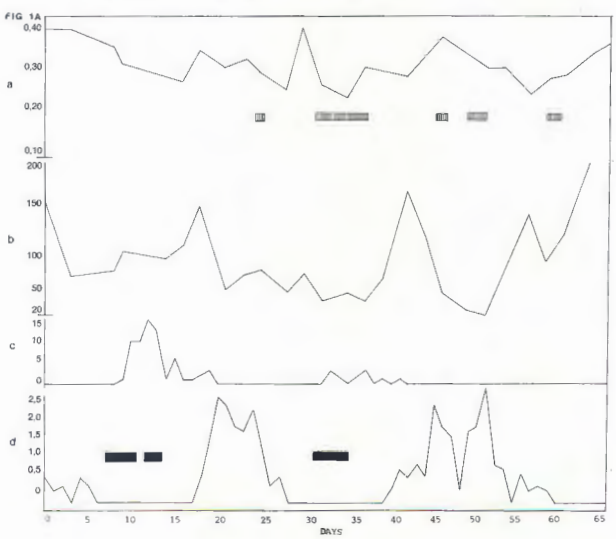
Beagle X: Physical and haematological investigations prior to artificial infection failed to demonstrate any signs of disease. No parasites were seen on examination of stained peripheral blood smears.

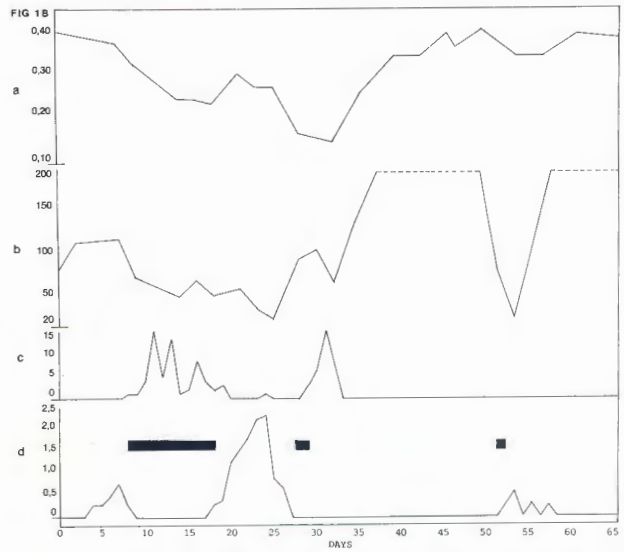
On the 12th day post-infection the dog showed a fever of 40 °C. Examination of blood smears revealed the presence of morulae of *E. canis*, and it remained positive for 5 days.

The dog responded promptly to treatment with doxycycline and made an uneventful recovery.

Babesia *carriers:* Physical and haematological investigations performed showed these animals were physically healthy. Trophozoites of *B. canis* were seen in blood smears of Dogs 4, 5, 10 and 17 on Day 0 of the experiment. Dogs 7 and 15 showed trophozoites of *B. canis*, respectively, 27 and 23 days before commencement of the experiment.

All the Beagles in the *Ehrlichia*- and *Babesia*-infected group (Dogs 5, 7 and 15) showed a fever reaction and the presence of morulae of *E. canis* in blood smears within 8 days of infection. All the dogs also showed a drop in haematocrit and/or a drop in the thrombocyte counts associated with patent parasitaemia with *E. canis*. This was demonstrable for a short period only, but it was followed in all cases by severe parasitaemia with *B. canis*. This latter infection was associated with a marked thrombocytopaenia (Fig. 1A, B & C).





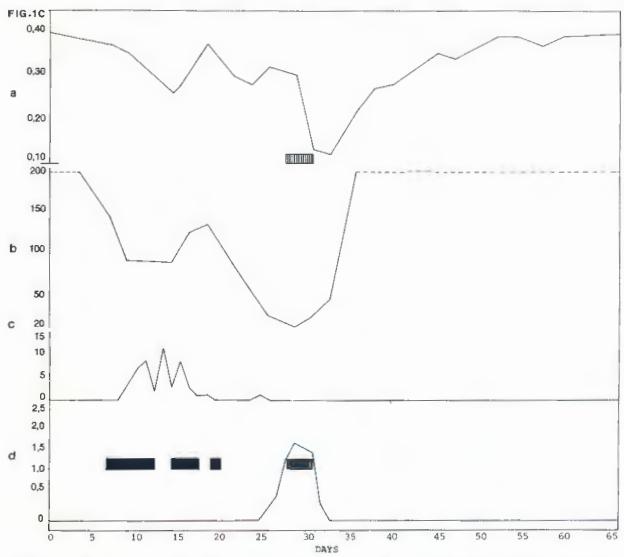


FIG. 1 Haematocrit, thrombocyte count, leucopaenia, fever-reaction, number of parasitized white and red blood cells of experimental dogs infected with *E. canis* (*B. canis* + *E. canis*-infected group)

a = Haematocrit

b = Thrombocyte count × $10^9/\ell$

c = Number of morulae per 100 fields

d = Log [(number of parasitized red blood cell per 100 fields) + 1]

▥ = Leucopaenia

■ = Fever-reaction

FIG. 1A : Dog No. 5

FIG. 1B : Dog No. 15

FIG. 1C : Dog No. 7

The alternating parasitaemia with *B. canis* and *E. canis* in these animals is clearly demonstrated in Fig. 1A, B & C.

By post-infective Day 32, severe depression, very pale mucous membranes and anorexia were evident in Dogs 5 and 7. They were subsequently treated with doxycycline only at a daily oral dosage rate of 10 mg/kg body mass for 10 days. A babesiacide was not given in spite of the presence of *B. canis* in the blood smears. Dog 15 developed milder clinical signs of disease, but was nevertheless treated on post-infective Day 57 in the same way as Dogs 7 and 15.

All 3 dogs responded favourably to treatment and made an uneventful recovery.

The control group (i.e. *Babesia*-infected group), Beagles 4, 10 and 17, showed no fever reaction, nor any other clinical sign of disease, nor was there a drop in haematocrit. Peaks of parasitaemia with *B. canis* were often characterized by drops in the thrombocyte count (Fig. 2A, B & C). The only evidence of the laboratory confirmed state of a thrombocytopaenia was the excessive bleeding that occurred after the dogs' ears had been pinpricked for the preparation of blood smears.

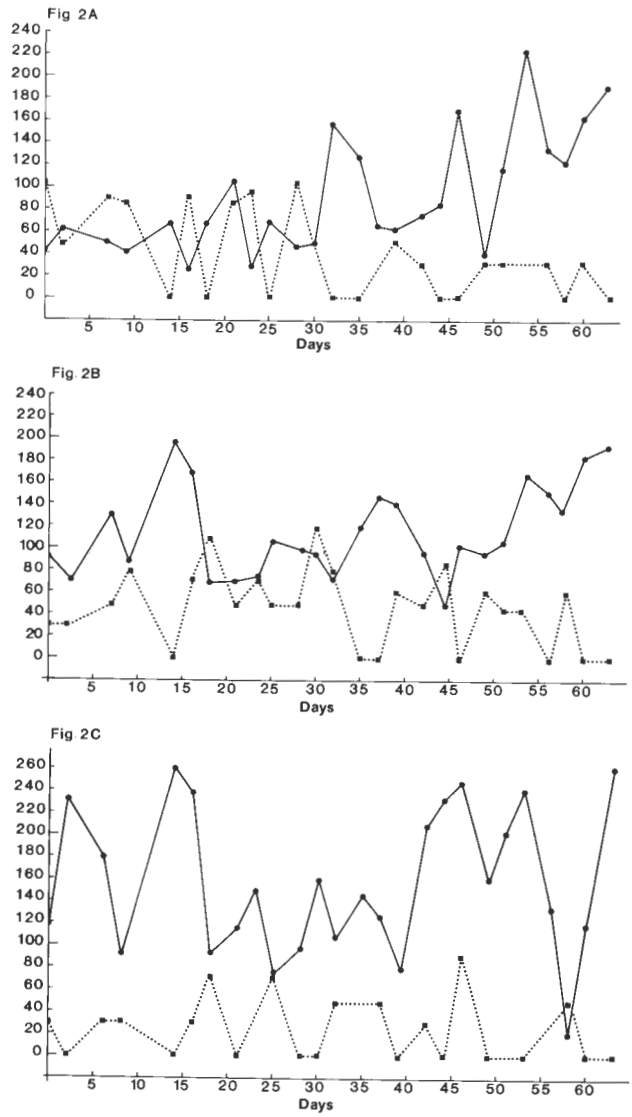


FIG. 2 Thrombocyte count and number of parasitized red cells of control dogs (*B. canis*-infected group)

——— = Thrombocyte count × $10^9/\ell$

---------- = Log [(number of parasitized red blood cells per 100 fields) + 1] × 100

FIG. 2A : Dog No. 4

FIG. 2B : Dog No. 10

FIG. 2C : Dog No. 17

DISCUSSION

All 3 experimental Beagles that were carriers of *B. canis* and that were subsequently infected with *E. canis*, developed clinical signs and haematological evidence of disease. Subsequently, all 3 animals were successfully treated with doxycycline only.

Mixed *B. canis* and *E. canis* infections in dogs, when diagnosed for the first time, are usually (and apparently justifiably) treated with both a babesiacidal drug and a tetracycline. According to the findings in this investigation in cases where *E. canis* infections were overlooked initially and where they were then subsequently presented as "chronic biliary fever" or "biliary-relapse" cases, treatment could consist of a tetracycline only. In a practical situation this would depend, however, on the physical state of the animal and would require careful monitoring of the patient to ensure that acute babesiosis did not develop. Avoiding another administration of a babesiacidal drug to a dog could be advantageous in that a) possible immunity against *B. canis* is not interfered with and b) the repeated use of potentially harmful drugs such as phenamidine isethionate is avoided (Naudé, Basson & Pienaar, 1970).

The diagnosis of canine ehrlichiosis, when based on the demonstration of morulae of *E. canis* in monocytes in peripheral blood smears, is a time-consuming and frustrating undertaking. This investigation confirms the fact that the chances of demonstrating morulae in peripheral smears are extremely good only during the initial reaction when a dog is running a fever (Fig. 1A, B & C).

The possibility of overlooking the *E. canis* component in mixed *E. canis*/*B. canis* infections in dogs is a real problem for 2 reasons: a) trophozoites of *B. canis* are usually relatively easily seen on peripheral smears, and as a result the examination of the smear is often terminated, and b) the cyclic alternating appearance of *B. canis* trophozoites and morulae of *E. canis*, as observed in the current mixed experimental infection.

The diagnosis of canine ehrlichiosis, in the absence of serological methods, should thus be based on: a) anamnesis [recent prior treatment(s) against canine babesiosis should arous suspicion], b) clinical signs, c) haematological investigation (red cell count, white cell count, thrombocyte count), d) serum protein electrophoresis (hypergammaglobulinaemia) (Van Heerden, 1982) and e) the results of an examination of a peripheral blood smear.

In the present investigation thrombocytopaenia was present in both groups of experimental dogs, that is, in dogs with mixed *B. canis* and *E. canis* infection as well as in dogs that were carriers of *B. canis*.

Thrombocytopaenia in canine babesiosis has been ascribed to disseminated intravascular coagulapathy (Moore, 1978). Thrombocytopaenia has also been described in other protozoal diseases (Allen, Frerichs & Holbrook, 1975; Robins-Browne, Schneider & Mets, 1975; Skudowitz, Katz, Lurie, Levin & Metz, 1973; Wright & Goodger, 1977). In human trypanosomiasis, the thrombocytopaenia has been ascribed to possibly one or more of the following mechanisms: hyperplasia of reticuloendothelial elements in the spleen, with increased phagocytosis of normal and immunologically-altered platelets from the blood by the reticuloendothelial system; consumption of platelets as part of disseminated intravascular coagulation (Robins-Browne *et al.*, 1975). The mechanisms involved in thrombocytopaenia in bovine and equine babesiosis have not been described.

There is probably little doubt that disseminated intravascular coagulopathy does occur in some dogs with babesiosis. This, however, is usually associated with clinical signs of disease in dogs with advanced babesiosis. The findings in this study of a thrombocytopaenia in dogs with no indication of clinical signs of desease, indicate that disseminated intravascular coagulopathy is probably not the only mechanism which may produce a thrombocytopaenia in canine babesiosis.

This preliminary experimental investigation into thrombocyte numbers in dogs that are carriers of *B. canis* and into treatment in dogs with mixed *E. canis*/*B. canis* infections, had the following shortcomings: a) a small number (6) of experimental animals was investigated; b) thrombocyte counts were not done daily; c) an *E. canis*/*B. canis* negative control group was not investigated; d) parameters for activated intravascular coagulopathy were not investigated, and e) the effect of tetracyclines on *B. canis* was not evaluated.

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