STERILISATION OF BABESIA CANIS INFECTIONS BY IMIDOCARB ALONE OR IN COMBINATION WITH DIMINAZENE

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ABSTRACT

Babesia canis infections were apparently sterilised by a single dose of imidocarb at 7,5 mg kg\(^{-1}\), as well as by a single dose of diminazene at 3,5 mg kg\(^{-1}\), followed by a single dose of imidocarb at 6 mg kg\(^{-1}\) the following day. This was confirmed by subinoculation of blood from these dogs to spleenectomised recipients. Sterilisation of the infection is not recommended in endemic areas; a more rational approach would be to allow a state of premunition to develop in dogs at risk to repeated infections.

Key words: Babesia canis, diminazene, imidocarb, premunition, sterilisation

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INTRODUCTION

Canine babesiosis is an important disease in Southern Africa, involving 10-20% of cases presented for treatment at practices responding to a recent survey\(^1\). Diminazene (Berenil, Hoechst Ag-Vet) is the treatment of choice in the majority of these practices, although imidocarb (Forray-65, Hoechst Ag-Vet) and trypan blue (Trypan Blue SS, Centaur) are also used frequently\(^2\). A large number of respondents reported that relapses are a problem, with diminazene most frequently being incriminated in alleged “drug resistance”. This is apparently due to the misconception that administration of Berenil at the recommended therapeutic dose will sterilise the infection. Perusal of the literature immediately dispels this notion. Doses as low as 0,2-0,5 mg kg\(^{-1}\) body mass administered intramuscularly or subcutaneously lead to clinical recovery but do not eliminate the parasites\(^1\). Even the recommended therapeutic dose of 2,5 - 3,5 mg kg\(^{-1}\) is not claimed to sterilise the infection, although relapses are less frequent\(^2\). At this dose, the majority of dogs remained subclinical carriers of the parasite and therefore entered a state of premunition, which was recommended in endemic areas\(^1\). Sterilisation of the infection occurs in a minority of dogs treated at 4 mg kg\(^{-1}\), and even 5 mg kg\(^{-1}\) resulted in sterilisation of the infection in 40% of the dogs only\(^1\). Sterilisation of the infection in all dogs is achieved at a dose of 12 mg kg\(^{-1}\) administered intramuscularly, which is a toxic level\(^1\).

Some practitioners claim that treating a dog with the recommended therapeutic dose of diminazene, followed by the recommended therapeutic dose of imidocarb diproportionate the next day, prevents relapses. This may imply that the infection has been sterilised.

We could not trace any reference in the literature to chemosterilisation of B. canis by imidocarb. In the registration application for imidocarb (documents on file at the Protozoology Division, Onderstepoort Veterinary Institute) W D Malherbe, previously of the Department of Medicine, Faculty of Veterinary Science, University of Pretoria, was quoted. He had used imidocarb on 200 dogs: at a dose of 5 mg kg\(^{-1}\) relapses occurred, while a dose of 7,5 mg kg\(^{-1}\) prevented relapses from occurring. This could imply sterilisation of the infection, but it was not confirmed by subinoculation of blood from these dogs to spleenectomised recipients. Various French workers reported a curative dose of 3 mg kg\(^{-1}\) imidocarb, while 6 mg kg\(^{-1}\) may result in chemophylaxis for 4-8 weeks\(^4\). The current recommended dose of imidocarb in South Africa is 6 mg kg\(^{-1}\). The registration documents (vide supra) contain a statement of toxicity trials: a dose of 10 mg kg\(^{-1}\) resulted in mild, transient tachycardia; 7,5 mg kg\(^{-1}\), the dose at which Malherbe found no relapses occurring, would therefore appear to be quite safe.

Extreme care should be taken when extrapolating results of investigations done in other countries, as various serologically distinct vector-specific strains which may represent different subspecies or even species have been isolated\(^5\). The South African strain, transmitted by Haemaphysalis leachi, has long been known to be particularly virulent when compared to other strains, particularly the European strain\(^6\). The European strain is transmitted by Dermacentor reticulatus, while the parasite found in North Africa and India is transmitted by Rhipicephalus sanguineus\(^7\). A South African strain was used when the efficacy of trypan blue was determined\(^1\), while both a South African and an East Indian strain were used in the initial experiments.
with diminazene. The European strain is more susceptible to imidocarb than the South African strain.

In view of the empirical and inconclusive data mentioned above, we investigated the possibility of sterilising \textit{B. canis} infections by imidocarb alone (at the dose at which Malherbe reported no relapses) or at the recommended therapeutic dose in combination with diminazene. The experimental dogs were splenectomised to reduce individual variation in immune response to the infection. This also made sterilisation more difficult, as there is a synergy between the immune response and chemotherapy. As a control, an infected dog was treated with trypan blue to confirm that the isolate used was not easily sterilised. The parasites used in this trial were of the so-called Thomas isolate of \textit{B. canis}, which is transmitted by \textit{H. leachi}, but not by \textit{R. sanguineus}.

**MATERIALS AND METHODS**

The parasite material used was from a single batch of blood stabilates, prepared from one reacting dog. Beagles bred under tick-free conditions and therefore not presumed to be naïve for \textit{B. canis} were used for the trial. Before commencement of the trial, serum from each dog was subjected to the Thomas strain \textit{B. canis} indirect fluorescent antibody test.

The dogs were allocated randomly to one of four groups (A, B, Control and Recipient). Dogs in Groups A, B and Control were infected via the vena cephalica with 2 ml blood stabilate thawed at room temperature. Temperature, packed cell volume (PCV) and general habitus were monitored daily from the day following infection and parasitaemia was calculated by preparing thick and thin blood smears of the peripheral circulation. On the day that parasitaemia reached 0.2\% (ca one parasite seen per field in a thin blood smear), the dogs were treated as follows:

- **Group A (2 dogs):** a dose of 7.5 mg kg\(^{-1}\) imidocarb was administered subcutaneously.
- **Group B (4 dogs):** a dose of 3.5 mg kg\(^{-1}\) diminazene was administered subcutaneously.
- **More dogs were included in this group, as therapeutic doses of diminazene may sterilise infections.**

**RESULTS**

None of the infected dogs showed overt clinical signs of babesiosis; temperature and PCV remained within normal limits.

- **Group A (imidocarb only)**
  - Parasites were first seen on thick blood smears on Day 2 and 4, respectively, and the dogs were treated on Days 4 and 6, respectively. On the day following treatment parasites were still present, but were vacuolated. Parasites had disappeared from blood smears 2 or 3 days post-treatment, respectively. Blood smears remained negative until the trial was terminated. The dog receiving pooled blood from Group A dogs showed no clinical signs of babesiosis and remained negative on blood smear even after the second subinoculation.

- **Group B (diminazene plus imidocarb)**
  - Except for one dog whose blood smear became positive on Day 3, blood smears of the other 3 dogs became positive on Day 4. The treatment regimen of 3 dogs was initiated on Day 5, and on Day 6 in one dog. The dog receiving pooled blood from Group B dogs showed no clinical signs of babesiosis and remained negative on blood smear even after the second subinoculation.

**DISCUSSION**

Subinoculation of pooled blood from Groups A and B to splenectomised recipients strongly suggested that both treatment regimens sterilised \textit{B. canis} infection. This method is in general use, but proving that all parasites have been eliminated is virtually impossible. A case has been documented of negative subinoculation from a bovine, which was subsequently found to harbour \textit{Babesia bigemina} in the capillaries of the cerebral cortex. The experimental dogs were splenectomised; sterilisation of infection should be even more likely in spleen-intact dogs.

Treatment was instituted when parasitaemia was low and before the onset of overt clinical symptoms. This was necessitated by the rapid flare-up of symptoms in splenectomised dogs. The longer the duration of clinical signs, the more difficult it becomes to effect a cure.

It has long been known that dogs which recover from babesiosis may remain subclinical carriers of the parasite, on re-infection these dogs generally show no clinical symptoms of babesiosis. Practitioners should therefore consider whether sterilisation of the infection is the desired approach. By promptly and efficiently eliminating the parasite population, the dog is not exposed to antigenic stimulation and therefore does not build up an immunity.

When the dog is returned to its home environment, it runs the risk of re-infection. That this may happen readily enough is confirmed by reports of recurrent bouts of babesiosis in the same dog. In desperation the practitioner often blames the drugs used for no longer being effective. In fact, the exact opposite may be true: the treatment regimen may be so effective that the infection is sterilised, thereby preventing the dog from developing protective immunity.

We would recommend that compounds which do not sterilise the infection are used in endemic areas, but that practitioners should be aware...
that relapses may occur, which would require further treatment. A major drawback is that euflavine (Euflavine, Centaur) and trypan blue, the two compounds currently available in South Africa known not to sterilise infections at therapeutic doses, have to be administered intravenously. Amicarbalide (Diampron, Rhône-Poulenc A.H.), which does not sterilise the infection and is administered intramuscularly, is not currently available in South Africa.

REFERENCES
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