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Clinical management of babesiosis in dogs with homeopathic *Crotalus horridus* 200C

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Homeopathic *Crotalus horridus* 200C was evaluated in 13 clinical cases of babesiosis in dogs, compared with another 20 clinical cases treated with diminazine. Babesiosis is an important tropical tick-borne haemoprotozoan disease in dogs clinically manifested by anorexia, dehydration, temperature, dullness/depression, diarrhoea/constipation, pale mucosa, hepatomegaly, vomiting/nausea, splenomegaly, distended abdomen/ascites, yellow coloured urine, emaciation/weight loss, and occular discharge. The diagnosis of babesiosis was based on cytological evidence of *Babesia gibsoni* in freshly prepared blood smears. The dogs were treated with oral *C. horridus* 200C, 4 pills four times daily for 14 days ($n = 13$) or diminazine aceturate 5 mg/kg single intramuscularly dose ($n = 20$). All the dogs were administered 5% Dextrose normal saline at 60 ml/kg intravenously for 4 days.

Initial clinical scores were similar in both groups and showed similar progressive improvement with the two treatments over 14 days. Parasitaemia also improved in both groups, but haematological values showed no change.

No untoward reactions were observed. It appears that *C. horridus* is as effective in causing clinical recovery in moderate cases of canine babesiosis caused by *Babesia gibsoni as* the standard drug diminazine. Large scale randomized trials are indicated for more conclusive results. *Homeopathy* (2007) **96,** 90–94.

Keywords: Babesiosis; *B. gibsoni*; *Crotalus horridus*; dog

Introduction

Canine babesiosis, a tick borne haemoprotozoan disease of domestic dogs in tropical and subtropical regions of the world, is caused by the species of *Babesia* (*B. canis*, *B. gibsoni* and *B. vogeli*). In India both *B. canis*[1,2] and *B. gibsoni*[3–6] are prevalent. The prevalence of canine babesiosis varies and has been reported as 0.66%,[5] to 8.9% in referral canines[7] in Uttar Pradesh, India, to 21.7% in Assam, India. The pathogenesis of canine babesiosis varies in different regions,[8] possibly owing to strain or species variations of *Babesia* and various ecological conditions.[9]

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The drug management of canine babesiosis is complicated by the wide variety of host factors and *Babesia* spp. with differing degrees of drug tolerance and susceptibility.[10] Small *Babesia* species e.g. *B. gibsoni*, are more refractory to treatment than larger *Babesia* e.g. *B. canis*.[11] Babesiosis is effectively treated by a number of babesiocidal compounds, diminazine aceturate and imidocarb dipropionate are most often used. Diminazine aceturate given once intramuscularly or subcutaneously at 3.5–5.0 mg/kg has been reported effective against *B. canis*.[12] But for treatment of *B. gibsoni* infection higher dose rates such as 3.5 mg/kg on two consecutive days or 7.5 mg/kg as a single dose have been recommended. But because of its toxicity to kidney, brain and liver, the drug is not approved by FDA (Food and Drug Administration) in the USA.[13] Diminazine aceturate reduces parasitaemia, but does not cure *B. gibsoni* completely.[14] Dogs remain at risk of recurrence and may become a reservoir for infection.[15] Imidocarb at 5 mg/kg

intramuscularly or subcutaneously once or twice at 24 h interval is effective in dogs infected with *B. canis*,[16] but its high cost and systemic side effects even at therapeutic dose, limits its use in practice. These drugs are toxic and their use is not recommended in extremely debilitated patients. We have observed deaths owing to acute hepatic and renal failures in dogs that received diminazine aceturate at the dose rate of 5 mg/kg single intramuscularly dose.

Thus, a need was felt to find an effective and less toxic therapeutic agent for the treatment of canine babesiosis particularly caused by *B. gibsoni*. In homeopathy, *Crotalus horridus*, *Phosphorus*, *Ficus religiosa*, *China officinalis* and *Millefolium* have been recommended for the treatment of babesiosis in cattle.[17] But none of these has been evaluated in dogs. *C. horridus* is indicated for fluid haemorrhages, yellow skin and black vomit. It affects blood, heart, and liver and producing profound nervous shock with trembling and prostration. There is a similarity between the symptoms of *C. horridus* and babesiosis caused by *B. gibsoni* in dogs.

The present investigation was carried out to evaluate the therapeutic efficacy of *C. horridus* in dogs with clinical babesiosis, caused by natural infection of *B. gibsoni*.

Materials and methods

Animals

Thirty three dogs of different breeds and of varying age (4–53 months) referred to the Referral Veterinary Polyclinic of the Indian Veterinary Research Institute formed the patient group for this clinical trial. All the dogs included in this study were cytologically positive for *Babesia gibsoni* in their freshly prepared peripheral blood smears (see Figure 1). The clinical signs included anorexia/poor appetite, dehydration, temperature, dullness, diarrhea or constipation, pale mucosa, hepatomegaly, vomiting, splenomegaly, distended abdomen/ascites, emaciation/weight loss and occular discharge. The presence of at least three of these clinical signs with cytological detection of *B. gibsoni* in the freshly prepared blood smears were the criteria for inclusion of the dogs in this study. Dogs having concurrent infection of with *Ehrlichia canis*, *Ehrlichia platys*, *Trypanosoma evansi*, or *Dirofileria immitis* or in critical condition having blood haemoglobin less than 5 g/dl were not included in the study.

Drugs

Group A was treated with *C. horridus* 200C, 4 pills four times a day orally for 14 days and Group B with diminazine aceturate at 5 mg/kg intramuscularly single dose. All the dogs were administered 5% Dextrose normal saline at 60 ml/kg intravenously for 4 days.

Clinical trial design

The number of dogs was not fixed initially. A prospective study was performed on 33 dogs diagnosed with babesiosis, caused by *B. gibsoni* during 2005–2006. All the dogs were client-owned. The decision to assign the dogs to treatment with *C. horridus* or diminazine aceturate was made following the choice of the owners after explanation of the pros and cons of both the treatments. The owner's agreement was

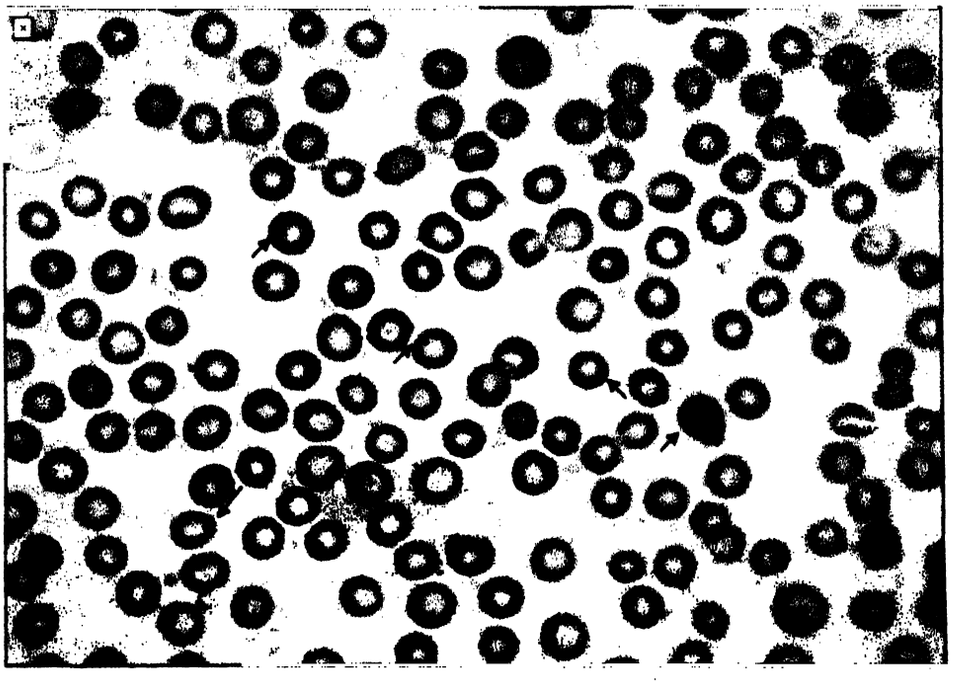

Figure 1 Blood smear of a dog showing *Babesia gibsoni* in the erythrocytes (arrows). Giemsa stained × 1000.

obtained to treat the (13 dogs) with homeopathy. The study was approved by Joint Director Academic, Indian Veterinary Research Institute, Izatnagar. The duration of treatment was fixed as 14 days. Except fluid therapy no other drug was given during the treatment period.

Evaluation criteria

The dogs were thoroughly examined clinically and subjected to haematological examinations. The diagnosis of canine babesiosis was based on cytological detection of *B. gibsoni* in the fresh peripheral blood smears (see Figure 1) prepared from ear tips. The therapeutic efficacy was evaluated using clinical score, peripheral blood smear examination and haematological indices (Hb, PCV and TEC) on day 0, 3, 7 and 14.

Clinical scoring

Each dog was clinically examined in detail. Clinical scoring was based on presence of anorexia/partial appetite, dehydration, temperature, dullness/depression, diarrhoea/constipation, pale mucosa, ticks on body, hepatomegaly, vomiting/nausea, splenomegaly, rapid thready pulse, nasal discharge, ataxia/CNS signs, distended abdomen/ascites, dysponea, dark yellow coloured urine, emaciation/weight loss, epistaxis, occular discharge and edema. Each of the features was assigned 01 mark each. A clinical score for each dog was computed based on the presence/absence of these clinical signs.

Cytological examination

Thin blood smears prepared from the ear tips were fixed immediately with methanol, and stained with Giemsa (1:10) for 45 min and examined under oil immersion for the presence of *Babesia gibsoni*. At least 1000 infected red blood cells in each smear were screened to evaluate the state of parasitaemia on day 0, 3, 7 and 14 post therapy.

Collection of blood

Approximately 2 ml blood from each dog was collected in the vial containing EDTA (1 mg/ml blood), for haematological study on day 0, 3, 7 and 14 post therapy.

Haematological examinations

The following haematological parameters were studied by standard techniques.[18] Haemoglobin was estimated by acid haematin method using Sahli's haemoglobinometer. Packed cell volume was measured by Wintrobe haematocrit method. Total erythrocyte count was monitored by using Neubauer Haemocytometer and Hayem's solution (RBC diluting solution) by the standard technique.[18]

Statistical analysis

The data were analysed statistically using paired '*t*' test between days post therapy.

Results

History revealed that anorexia, vomiting, weakness, recurrent pyrexia and tick infestation as a common complaint in these dogs. At the time of referral, the dogs showed anorexia/partial appetite, dehydration, temperature, dullness/depression, diarrhoea/constipation, pale mucosa, ticks on body, hepatomegaly, vomiting/nausea, splenomegaly, distended abdomen/ascites, yellow coloured urine, emaciation/weight loss, and occular discharge. Anorexia was characterized by complete loss of appetite and dogs declined the food when offered. Blood smears were positive for *B. gibsoni* in all cases. Initial blood haematocrit values indicated anaemia and parasitaemia (1.3–8.38%) (see Table 1).

On day 0, the mean clinical score (on 20 point scale) showed a progressive significant decline in both groups on days 3, 7, 14. Mean clinical score revealed that numbers of clinical signs reduced significantly ($P<0.05$) on day 14 post therapy with both *C. horridus* and diminazine aceturate.

The number of parasitized erythrocytes also reduced significantly ($P<0.05$) after treated with *C. horridus* on day 14 ($1.94\pm0.35\%$) from $3.34\pm0.53\%$ on day 0 and with diminazine aceturate on day 3 ($3.39\pm0.41\%$), 7 ($2.80\pm0.35\%$) and 14 ($1.83\pm0.33\%$) from $4.38\pm0.37\%$ on day 0.

Haemoglobin, packed cell values and erythrocyte count values in dogs did not improve significantly on days 3, 7, and 14 from initial values.

Discussion

Clinical manifestations in these cases were in agreement with those described for canine babesiosis caused by *B. gibsoni*.[13,19] *B. gibsoni* is difficult to treat in comparison to *B. canis* infection.[20] Diminazine aceturate is the most frequently used drug. Itoh *et al* observed that diminazine aceturate was not effective against *B. gibsoni* infection in splenectomized dogs.[21] Because of the toxicity of diminizine aceturate it has not been approved by FDA, USA for clinical use.[13] In homeopathy, *C. horridus* has been recommended in the treatment of babesiosis in cattle.[17] It is a drug of choice for haemorrhagic diathesis.[22] These properties of *C. horridus* matched well with the clinical manifestations of babesiosis in the present study.

Therefore, the present therapeutic trial was conducted to evaluate the efficacy of *C. horridus* v diminazine in the clinical management of canine babesiosis, caused by *B. gibsoni*, on the basis of clinical score, parasitaemia, and haemogram. On the clinical score, *C. horridus* and diminazine aceturate showed

Table 1 Therapeutic efficacy of *Crotalus horridus vs* diminazine aceturate in dogs with babesiosis (Mean ± SE)

Parameters	*Group*	*Days post treatment*			
		0	3	7	14
Clinical score	A	6.49 ± 0.63^{a} (3–10)	4.23 ± 0.43^{b} (2–7)	2.40 ± 0.43^{c} (1–5)	1.36 ± 0.34^{d} (0–4)
	B	6.40 ± 0.52^{a} (2–11)	4.93 ± 0.61^{b} (1–8)	2.00 ± 0.43^{c} (0–5)	$1.33 \pm 0.36^{c\ d}$ (0–4)
Parasitaemia (PE) (%)	A	3.34 ± 0.53^{a} (1.3–8.38)	3.23 ± 0.51^{a} (1.04–8.14)	$2.74 \pm 0.52^{a\ b}$ (0.9–6.3)	1.94 ± 0.35^{b} (0.64–4.07)
	B	4.38 ± 0.37^{a} (1.85–8.43)	3.39 ± 0.41^{b} (1.16–5.39)	$2.80 \pm 0.35^{b\ c}$ (1.04–4.52)	1.83 ± 0.33^{d} (0.77–4.25)
Haemoglobin (Hb) (mg/dl)	A	10.88 ± 0.6 (7–14)	10.77 ± 0.53 (7–13)	11.00 ± 0.60 (8–13)	11.59 ± 0.35 (10–13)
	B	10.48 ± 0.72 (5–14)	10.81 ± 0.98 (5–14)	10.64 ± 0.88 (4–14)	11.21 ± 0.61 (6–14)
Packed cell volume (PCV) (%)	A	32.46 ± 1.74 (21–42)	32.31 ± 1.59 (21–39)	33.30 ± 1.71 (26–39)	35.18 ± 1.11 (30–40)
	B	33.95 ± 2.17 (15–43)	32.38 ± 2.93 (15–42)	31.91 ± 2.62 (12–42)	33.29 ± 1.88 (18–42)
Total erythrocyte count (TEC) (million/mm^3)	A	5.17 ± 0.28 (2.83–6.88)	5.13 ± 0.30 (2.71–7.12)	5.25 ± 0.40 (2.99–7.02)	5.58 ± 0.34 (3.13–7.28)
	B	4.86 ± 0.35 (1.82–6.73)	4.67 ± 0.40 (1.99–6.19)	4.59 ± 0.37 (2.18–6.11)	4.72 ± 0.33 (2.61–6.29)

Figures in parentheses indicate range.
a–d mean with dissimilar superscript in a row differ significant ($P < 0.05$).
Group A—animals treated with *Crotalus horidus*.
Group B—animals treated with diminazine aceturate.
PE—parasitized erythrocytes.
Group A: Mean age—24.77 months (median 24months); mean weight—11.81 kg (median 10 kg).
Group B: Mean age—40.68 months (median 37months); mean weight—15.78 kg (median 15 kg).

progressive and significant regression in clinical signs on day 3, 7 and 14 post therapy (see Table 1), there was no significant difference between the groups. Similarly, there was progressive decline in the percentage of parasitized erythrocytes in both the groups. However, significant reduction in parasitized erythrocytes in case of *C. horridus* was evident only on day 14, but parasitized erythrocytes reduced significantly from day 3rd onwards in case of diminazine aceturate. Complete cytological clearance was not attained in either group as reported earlier by other workers using diminazine aceturate or clindamycin.[14,23] Though clinical and parasitological recovery was evident in dogs given *C. horridus* or diminazine; improvement in haemogram was not statistically significant. The effectiveness of *C. horridus* has been described in cattle babesiosis but it has never been evaluated in canine babesiosis.[17] The results showed an encouraging response of *C. horridus* in the management of canine babesiosis, caused by *B. gibsoni*. The clinical efficacy of the homeopathic drug was comparable with modern allopathic drug diminazine aceturate. The use of homeopathic drugs in the management of babesiosis in dogs has not previously been reported. Our findings regarding clinical recovery of the dogs with babesiosis with *C. horridus* are encouraging. The results are particularly interesting in less severe cases of canine babesiosis where there was no impending danger of collapse.

From the present study it seems that *C. horridus* can be an effective alternative in uncomplicated case of babesiosis.

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