*Chemotherapeutic Value of Parvaquone and Buparvaquone Against Thileria annulata Infection of Cattle**

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Abstract: Parvaquone (BW993C), 2-cyclohexyl-3-hydroxy-1,4-naphthoquinone, and buparvaquone (BW 720C) 2-(trans-4-t-butylcyclohexyl-methyl)-3-hydroxy-1,4-naphthoquinone, were evaluated to determine their therapeutic efficacy in the treatment of theileriosis caused by Theileria annulata infection in cattle in Iran. One hundred and fifty-nine pure and crossbred Bos taurus cattle, experimentally or naturally infected with T annulata were treated. Parvaquone was injected into 86 animals with up to three doses of 20 mg kg^{-1} or 10 mg kg^{-1} at intervals of 48 hours between doses. Buparvaquone was injected into 73 animals. Up to three doses of 2.5 mg kg^{-1} were injected with an interval of 48 hours between doses. The recovery rate of animals treated with parvaquone was 60.7 per cent and with buparvaquone it was 88.7 per cent. No significant side effects or relapse of disease were observed following the use of either compound. It is concluded that buparvaquone at a dose of 2.5 mg kg^{-1} has a satisfactory therapeutic index and is a more effective treatment of T annulata infection than parvaquone. The prophylactic use of schizont tissue culture vaccine and chemotherapy with buparvaquone could be the most promising means of controlling theileriosis in Iran.

Keywords: *Bovine theileriosis / Theileria annulata / Parvaquone / Buparvaquane*

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THEILERIOSIS

Caused by *Theileria annulata* infection represents a major threat to crossbred and purebred cattle in Iran. during the last three decades scientists in the Razi and other Institutes throughout the world have worked to find a potent compound to cure theileriosis. A wide range of antibacterial and antiprotozoal drugs has been tested. Brown et al (1977), Neitz (1953) and Wilde (1967) all reported that the efficacy of tetracyclines in the treatment of *T parva* infection is limited. Hashemi-Fesharki (1974) stated that oxytetracycline hydrochloride alone has no therapeutic value in the treatment of *T annulata* infection. The discovery of the antitheilerial activity of the hydroxynaphthoquinones (McHardy et al 1976)indicated that effective treatment for theileria infection is possible, and a series of hydroxynaphthoquinones has been tested since then for antibacterial activity against *T parva, T annulata* (McHardy et al 1983, 1985, Dolan et al 1984, 1988, Dhar et al 1988) at *T sergenti* (Minami et al 1985). Among this series, parvaquone and buparvaquone were the most effective (McHardy et al 1985).

The objective of this study was to compare the therapeutic effect of parvaquone and buparvaquone in the treatment of bovine theileriosis due to *T annulata* infection in Iran.

Materials and Methods

Experimentally infected calves

Thirty-seven Holstein-friesian calves, three to four months old, were used. The animals were obtained from a farm on which strict acaricidal treatment was practised and where no recent history of theileriosis had been recorded. They were maintained in a tickfree stable. Blood smears were checked microscopically for one month before the study to ensure freedom from pathogenic haemoprotozoa.

Twenty-four of the calves were infected by subcutaneous inoculation of 40 to 45 ml of blood from a donor calf infected with a virulent Iranian strain of *T annulata.* The strain was isolated from a locally infected calf at the Razi Institute and has been maintained by successive blood passage in calves and storage at -70°C. it provides a mortality rate of more than 80 per cent in Holstein-Friesian cattle. The strain has lost the ability to produce intraerythrocytic piroplasms in

recipient calves (Hashemi-Fesharki 1988).

The remaining 13 calves were not infected but were used for drug toxicity tests, as described below.

Naturally infected cattle

One hundred and forty purebred and crossbred Holstein-Friesian cattle, clinically affected by theileriosis, were brought to the Institute for treatment. They were naturally infected with wild and uncharacterised strains of *T annulata.* The severity of their infections ranged from mild, with few piroplasms evident, to advanced, with piroplasm parasitaemia up to 80 per cent. They were treated as described below and then returned to their owners. Their subsequent response to treatment was monitored on their owners' farms for one month, both clinically and by the examination of lymph and blood smears.

Test drugs

The compounds under test were parvaquone, 2-cyclohexyl-3-hydroxy-1,4-naphthoquinone, formulated as a solution for injection (Clexon; Pitman-Moore) containing 150 mg parvaquone ml^{-1}, and buparvaquone 2-(trans-4-t-butylcyclohexylmethyl)-3-hydroxy-1,4-naphthoquinone, as a solution for infection (Butalex; Pitman-Moore) containing 50 mg buparvaquone ml^{-1}. Both drugs were administered by deep intramuscular injection in the neck muscles.

Observations

Experimentally infected calves. Rectal temperatures were taken daily. Jugular blood and prescapular lymph node biopsy smears were taken starting on the day that the animal's temperature rose to 39.5°C or higher, and daily until rectal temperature, heematocrit and total white blood cell count had returned to within normal ranges, and lymph node and blood smears, respectively, were free of schizonts and piroplasms. They were then taken twice weekly, until the end of the observation period. The smears were stained with Giemsa and checked microscopically for the presence of *Theileria* species schizonts. The parasitic reaction was expressed as a schizont score 0 to 4, in 50 microscopic fields of lymph node biopsy smears. The scoring system was: 1 to 5 schizonts in 50 fields – 1+; 5 to 10 schizonts in 50 fields – 2+; 10 to 15 schizonts in 50 fields – 3+;more than 15 schizonts in 50 fields –4+. Blood smears were also examined for the presence of schizonts. Schizonts were usually not seen in blood smears until there were more than 10 in each microscopic field of lymph node biopsy

smears. In these cases, disease was acute, and usually fatal. The general condition of calves was monitored daily and a post mortem examination was conducted on all calves which died.

Naturally infected cattle: For naturally infected animals, similar observations were made and the piroplasm parasitaemia in blood smears was also determined. It was expressed as a percentage of red blood cells parasitised, on examination of 1000 erythrocytes.

Table1: *Uninfected calves used for toxicity tests*

Number of calves	*Compound*	*Dose, mg kg^{-1} bodyweight, injected intramuscularly*			
		O h	*48 h*	*96 h*	*25 days*
Tolerance tests					
3	*Parvaquone*	*20*	*20*	*10*	–
4	*Buparvaquone*	*2.5*	*2.5*	–	–
2	*Untreated*	–	–	–	–
anaphylaxis tests					
2	*Parvaquone*	*20*	*10*	–	*10*
2	*Buparvaquone*	*2.5*	*2.5*	–	*2.5*

Calves for toxicity tests. The 13 uninfected calves used for toxicity tests were treated as shown in Table1.

Jugular blood samples were taken from the calves immediately before and at weekly intervals for six weeks after the first drug injection to estimate white blood cell (WBC) and red blood cell (RBC) count, haemoglobin (Hb)concentration and packed cell volume (PCV) using Schalm's (1975) methods. The day of first treatment was 'day 0'.

Results

Therapeutic effect of parvaquone and buparvaquone in experimentally infected calves

Of the 24 calves infected experimentally with *T annulata,* 12 were treated when they became clinically sick with theileriosis, with two injections of parvaquone each of 20 mg kg^{-1}, followed by a third injection of 10 mg kg^{-1}, with an interval of 48 hours between doses. Six animals recovered and six died of acute theileriosis.

Seven calves were treated with two injections of buparvaquone, each of 2.5 mg kg^{-1}, with an interval of 48 hours; six recovered and one died.

Five experimentally infected calves served as untreated controls; all five died of theileriosis.

Naturally infected cattle

Seventy-four animals naturally infected with *T annulata* were treated with parvaquone. With the exception of one animal, which received a single dose of 20 mg kg^{-1}, all received two or three injections of the drug at the recommended dose of 10 mg kg^{-1} over a period of five to six days (Table 2). Twenty four cattle had a piroplasm parasitaemia of more than 10 per cent at the time of treatment and 13 of them recovered. The remaining 11 died due to acute theileriosis. Among the 50 whose parasitaemia was less than 10 per cent, and which all received two injections of 10 mg kg^{-1} parvaquone, 37 recovered and 13 died of the disease. Fifteen per cent of animals treated with parvaquone showed painless swelling at the site of injection which disappeared within two to four days.

Sixty-six naturally infected cattle were treated with buparvaquone. These animals received one, two or three injections of 2.5 mg buparvaquone kg^{-1} with an interval of 48 hours between doses (Table 2). Among 28 animals with piroplasm parasitaemia above 10 per cent, 24 recovered and four died of the disease. Of 38 animals with less than 10 per cent parasitaemia, all of which received only a single injection of buparvaquone, 36 recovered and two died of theileriosis.

Table 2: Treatment of field cases of T annulata infection with parvaquone or buparvaquone

Treatment	Number of cattle	Piroplasm parasitaemia	Number died	Number recovered	Percentage recovery
Parvaquone					
10 mg kg^{-1}x2	50	<10%	13	37	74.0
10 mg kg^{-1}x2	15	>10% (mean 22.0%)	5	10	66.7
10 mg kg^{-1}x3	8	>10% (mean 37.0%)	5	3	37.5
20 mg kg^{-1}x1	1	>10% (50%)	1	0	–
Total	74		24	50	67.6
Buparvaquone					
2.5 mg kg^{-1}x1	38	<10%	2	36	94.7
2.5 mg kg^{-1}x1	16	>10% (mean 20.2%)	0	16	100
2.5 mg kg^{-1}x2	10	>10% (mean 18.9%)	2	8	80.0
2.5 mg kg^{-1}x3	2	>10% (mean 40.0%)	2	0	–
Total	66		6	60	90.9

* Majority had no detecable piroplasm parasitaemia and were mot anaemic

Piroplasm elimination and relapse of the disease

The level of parasitaemia in the naturally infected animals which recovered began to fall 24 hours after treatment with both drugs. Degenerate piroplasms of anaplasmoid form were observed. These disappeared within two to three days after administration of drugs. A low parasitaemia of piroplasms of normal appearance remained in a few animals treated with parvaquone and persisted for 30 days after treatment, but following buparvaquone treatment, blood smears became negative for piroplasms. The temperature of treated cattle also fell to normal within one or two days of treatment. No relapse of disease was observed in animals treated with either drug.

Short-term tolerance and toxicity tests

All the treated calves tolerated the drugs well during the 28-day period of observation, and no signs of anaphylaxis were observed. No clinical signs of toxicity were observed, and no significant haematological changes in RBC, WBC, Hb or PCV were seen.

Discussion

The results of both laboratory and field studies show that parvaquone and buparvaquone administered intramuscularly are effective against theileriosis caused by *T annulata* infection under Iranian conditions. Buparvaquone was very efficacious. Parvaquone caused some transient swelling at the site of injection but the administration of buparvaquone revealed no local or generalised abnormal effects.

Chema et al (1988) and Mbwambo et al (1987) observed that the treatment of field infections of *T parva* with parvaquone at the beginning of the febrile and parasitic reaction was very effective. The present study also showed that in the early stages of disease caused by *T annulata* infection, parvaquone was effective, and recovery rates were fairly high. In animals with more advanced disease, in which piroplasm parasitaemia was more than 10 per cent, recovery rates were reduced.

Both drugs were active against both lymphocytic and erythrocytic forms of *T annulata,* although piroplasms persisted in blood smears of some cattle treated with parvaquone. Although persistent piroplasm parasitaemia was not seen in

this study following buparvaquone treatment, N. McHardy (unpublished observation) has noted that buparvaquone does not usually eliminate the infection totally. While a persistent low level piroplasm parasitaemia is likely to produce premunity against homologous strains, it may also be a source of transmission of the disease through the tick vector. This risk, following treatment with buparvaquone may, however, be less than with parvaquone.

The study revealed that no relapse of disease occurred in the animals treated with either drug. While Kilani and Bouattour (1984) showed that parvaquone could cure 80 per cent of field cases of *T annulata* infection in Tunisia, this study in Iran showed that the recovery rate was 67.6 per cent with parvaquone and 90.9 per cent with buparvaquone.

At the moment the use of schizont tissue culture vaccine is the best way to minimise the incidence of *T annulata* infection in Iran (Hashemi-Fesharki 1988); nevertheless, chemotherapy, especially with buparvaquone, could become important. It is suggested that a programme of vaccination of exotic cattle and treatment of sick animals with buparvaquone may offer an effective procedure to prevent the spread of theileriosis in Iran. However, since the possibility of drug resistance developing in the future must be taken into consideration, research should be continued to find further compounds for the treatment and control of theileriosis.

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