# Evaluation of the Combined Formulation of Parvaquone and Frusemide (Fruvexon) in the Treatment of Experimental Tropical Theileriosis

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## ABSTRACT

A combined formulation of parvaquone and frusemide (Fruvexon, Bimeda, Ireland) was evaluated for treatment experimental tropical theileriosis infection of calves. A total of Friesian dairy calves were infected with *T. annulata* sporozoite stabilate. Following infection with an equivalent of five infected *Hyalomma excavatum* ticks per calf all animals, developed severe clinical theileriosis. Fruvexon failed to cure or reduce severity of the infection. In contrast, buparvaquone (Butalex, Schering-Plough Animal Health, UK) was highly effective when administered at late stages of infection, and appears to remain the drug of choice against tropical theileriosis.

## INTRODUCTION

Tropical theileriosis caused by hemoprotozoan parasite *Theileria annulata* is a tick-transmitted cattle disease mortality ranging from 30 to 90% in exotic cattle breeds in areas from the Mediterranean basin to China.[1-4] There are three developmental stages infective for cattle:

• Sporozoites, which develop in ticks and transmit the disease in nature

• Schizonts that develop in macrophages (monocytes)

• Erythrocytic merozoites

*T. annulata* are transmitted by ticks of the genus *Hyalomma*. Upon blood meal, sporozoites from infected ticks penetrate mononuclear cells and develop into schizonts, which are detectable in biopsy smears from superficial lymph nodes and liver from day 6 to 28 post infection. Schizont-infected cells spread to other internal organs, including kidneys, lungs, abomasum, adrenal glands, myocardium, and later, to the brain. Within 2-3 days after the first appearance of schizonts, merozoites are detected in the peripheral blood smears.5 Schizonts found in biopsy material of lymph nodes or liver is a characteristic for acute tropical theileriosis, caused by T. annulata, and for East Coast Fever, caused by T. parva.

There are important differences between the two diseases. The severity of tropical

***Table 1**:Response of calves experimentally infected with T. annulata sporozoites to treatment with combination of parvaquone and frusemide (Fruvexon, Bimeda, Ireland), and buparvaquone (Butalex, Schering-Plough Animal Health, UK)*

| No of Calf | Clinical response | | | | Treatment on DPI with: | | Outcome |
|---|---|---|---|---|---|---|---|
| | Fever ° C/ from DPI[1] | Schizonts detected on DPI | Max. % schizonts in tissues[2] | Max.PPE[3]/ on DPI | Fruvexon | Butalex | |
| 491 | 40.7 / 8 | 9-19 | 10.0 | 5.0 / 14 | 8-10 | 14 | Survived |
| 510 | 40.8 / 8 | 9-14 | 9.0 | 11.0 / 14 | 10-12 | 14 | Survived |
| 511 | 41.0 / 8 | 9- 19 | 3.0 | 5.0 / 14 | 10-12 | 14 | Survived |
| 512 | 41.0 / 7 | 10-21 | 11.0 | 12.0 / 16 | 10-12 | 15 | Died on DPI 24 |
| 530 | 40.0 / 7 | 7-20 | 25.0 | 28.0 / 20 | 6-11 | | Died on DPI 21 |
| 539 | 39.5 / 6 | 7-14 | 15.0 | 15.0 / 19 | 6-11 | | Died on DPI 20 |
| 540 | 40.0 / 6 | 7-15 | 25.0 | 10.0 / 19 | 6-11 | | Died on DPI 23 |
| 406 | 41.1 / 9 | 9-16 | 10.5 | 29.0 /16 | | 16 | Survived |
| 430 | 40.4 / 7 | 9- 15 | 12.5 | 60.0 / 14 | | 14-15 | Survived |
| 439 | 40.6 / 8 | 8 -12 | 4.0 | 20.0 / 13 | | 13-14 | Survived |
| 442 | 41.0 / 9 | 9-15 | 4.5 | 15.0 / 18 | | 15-16 | Survived |
| 436 | 41.8 / 8 | 8-15 | 20.0 | 46.0 / 15 | | 12-13 | Survived |

*[1] Days post-infection*
*[2] Number of schizonts counted per 200 lymph node or liver biopsy cells*
*[3] Percent infected erythrocytes, at least 500 erythrocytes counted in Giemsa stained blood smears*

theileriosis is associated with high piroplams level resulting in anemia, while in ECF anemia is low, but the pulmonary edema is profound.[6] In *T. annulata* infection, the anemia has been described as a result of removal of erythrocytes by phagocytosis, although as suggested by Uilenberg,[1] autoimmune responses may contribute. The pathogenesis of lesions observed in internal organs is poorly understood. It has been suggested that pulmonary congestion and edema are associated with lymphocytosis of infected cells in lungs.[7] Tropical theileriosis can be controlled by drug therapy with long-acting oxytetracycline,[8-10] halofuginone lactate,[11] parvaquone and buparvaquone.[12-16] Fruvexon, the most recently reported combination of parvaquone and the diuretic frusemid (Bimeda, Dublin, Ireland), has been shown to be an effective treatment of East Coast Fever (ECF).[17,18] In the present study efficacy of Fruvexon was evaluated in calves experimentally infected with *T. annulata* sporozoites.

## MATERIALS AND METHODS

All the experiments with cattle were performed in compliance with the requirements of the Animal Welfare Committee of Kimron Veterinary Institute. A total of 12 susceptible Friesian dairy calves aged 3-6 months and serologically negative by indirect antibodies test (IFA) for *T. annulata* antibodies19 were housed in tick-proof units. The calves were infected with *T. annulata* sporozoites by subcutaneous injection (SC) of frozen *Hyalomma excavatum* stabilate. Each calf received an equivalent of five infected *Hyalomma excavatum* whole male tick suspension, shown to produce clinical theileriosis.[20] *T. annulata* sporozoite-infected calves were treated with Fruvexon (Bimeda, Dublin, Ireland) and Butalex (Schering-Plough Animal Health, Middlesex, UK) at doses recommended by the manufacturers (Table 1).

Fruvexon was injected at the rate of 1.0 ml per 30 kg body weight (5.0 mg parvaquone, 1.8 mg of frusemide per kg); Butalex was administered at the rate of 1.0 ml per 20 kg (2.5 mg per kg body weight).

Following infection with sporozoites, all calves were monitored daily for rectal temperature and peripheral blood parasitemia. Lymph node and liver biopsies for the detection of schizonts were performed according to Sergent et a.[1,2] Blood films and biopsy smears were stained with Giemsa and examined for presence of parasites. Post-mortem examinations of dead calves were performed at the Division of Pathology of the Kimron Veterinary Institute.

Three calves, # 530, 539 and 540 were given Fruvexon for 6 consecutive days, starting from day 6, when fever of 39.5º C and higher were first recorded. In a group of four calves (# 491, 510, 511 and 512), treatment with Fruvexon began from day 8 to day 10, or from day 10 to 12 post infection (DPI), in order to assess whether the drug would be effective when administered 2-3 days after calves showed fever >40.0ºC, and countable schizonts found in lymph node smear preparations. All four calves in this group treated for 3 successive days, showed no sign of cure, and clinical reaction severity increased. Therefore, to prevent death these calves received a single dose of Butalex on days 14 and 15 post infection.

Five additional calves, # 406, 430, 439, 442, 436 were treated with Butalex only. Calf # 406 received a single dose of Butalex on day 16, and the other four were treated for two consecutive days (Table 1) - days 12 and 13 (calf # 436), 13 and 14 (calf # 439), 14 and 15 (calf # 430), 15 and 16 (calf # 442) - which is about 1 week after the recorded onset of high fever, and consistent detection of schizonts in tissues for 4-7 days.

## RESULTS

All 12 sporozoite-infected calves developed characteristic symptoms and pathology. Initially, 4 calves, # 510, 491, 511, and 512 were treated with Fruvexon for 3 days. In these calves treatment was started on various days: on the first appearance of elevated fever (calf # 491); 2-3 later, or on the day following detection of schizonts in the biopsy smears from enlarged lymph nodes (calves # 510, 511 and 512) (Table 1). No cure was observed after any of these three treatments, and in all four calves severe clinical theileriosis progressed. A fever over 40ºC was maintained, schizonts from 3 to 11%, were detected up to days 14-21 post infection, and there was continuous increase in parasitemia level. Therefore, to prevent death, three calves received a single injection of Butalex on day 14 post infection; the only calf treated with Butalex on day 15 –i.e., # 512 - succumbed to the infection 24 DPI.

All three calves in a group that received Fruvexon for six successive days - # 530, 539 and calf # 540 – died 20 to 23 DPI. The five calves - # 406, 430, 439, 442, 436 - given two injections of Butalex at the late stages of the infection survived (Table 1). Despite the severe anemia the calves # 430 and

# 436 developed, negative prognosis associated with large numbers of schizonts detected (12.5 and 20%, respectively), high parasitemia (60 and 46%, respectively), these animals recovered.

At post-mortem examination, gross-pathology of dead calves showed typical lesions of lymphoid tissues, numerous petechiae in subcutaneous tissues, enlarged spleens, swollen and friable livers, and slightly edematous lungs. There were excessive amounts of serous fluids in body cavities, and numerous red patches were observed on the mucous membranes of the abomasums, small and large intestines.

## DISCUSSION

It is well documented that in ECF lymphocytosis of infected cells within the lungs results in the development of a prominent pulmonary edema, with abundant froth in bronchi, and tracheal and nasal passages. Death in ECF is associated with pulmonary edema that is not resolved within the early stages the infection.[7,22] Tropical theilerio-

sis, in contrast to ECF, is characterized by marked and profound anemia and jaundice. Pulmonary edema does develop, but appears to be less prominent, and death is attributed to severe anemia.[1,6, ,23,24]

Development and the extent of pulmonary edema in tropical theileriosis are vaguely described. A number of drugs have been employed to control tropical theileriosis, some, eg, halofuginone, are of limited practical use because of the narrow margins between therapeutic and toxic effect,[25] and others, such as parvaquone need to be applied in the early stages of the disease, or might require repeated administration. Application of the highly efficacious buparvaquone, considered as the drug of choice, and shown to be effective against both schizont and merozoite stages,[26] has a major disadvantage of being highly costly, not affordable, or not available for small-scale farmers.

Application of Fruvexon, a combination of parvaquone, effective against the pathogenic schizont stage and the diuretic frusemide, was reported to provide 89% cure rate and 77% survival rate in treatment of ECF.[18] However, the present study, designed to examine the efficacy of Fruvexon against experimentally induced *T. annulata* infection, showed that the drug failed either to reduce severity of the infection, or cure tropical theileriosis. According to the manufacturer, in the case of ECF, two injections of Fruvexon were sufficient to resolve clinical symptoms, although parasitic cure required at least four daily injections. As shown here, in *T. annulata* infection, neither three nor six treatments with Fruvexon resolved the disease. All calves that were treated with Fruvexon for 6 successive days starting immediately on the onset of the prepatent period died of tropical theileriosis. Four calves treated with Fruvexon during the early stages of the infection, ie, on the day following appearance of high fever, or on the day of detection of schizonts, did not improve. According to Sing et al,[25] parvaquone alone given at 20 mg per kg had a marked suppressive effect on *T. annulata* infection, but buparvaquone was highly effective. Treatment of eight infected calves (two groups of four) with 10 mg per kg parvaquone at days 10 to 12 resulted in clinical cure of three animals. One died of theileriosis in each group. Total parasitological cure was not achieved in any of the infected calves.[12] Unsuren et al[27] reported that 10 mg/kg parvaquone given twice at 48 hours interval resolved infection in 11 out of 13 naturally infected cattle. It appears that even six treatments with Fruvexon recommended injecting at the rate of 5 mg parvaquone per kg body weight were not sufficient to provide recovery from the *T. annulata* acute infection. On the other hand, a single or double injection of buparvaquone was highly effective even administered during the late stages of the disease. These results are in accordance with those reported by Singh et al.[25] In ECF comparative evaluation of chemotherapeutic activities of parvaquone (Parvexon) and the parvaquone-plus-frusemide combination (Fruvexon) showed 93.3 and 80.8% cure, respectively,[17] and as shown in our present experiments, buparvaquone appears to remains the drug of choice against tropical theileriosis.

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