

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

Varda Shkap, PhD,

Benjamin Leibovich, DVM

Yuri Krigel, DVM

Lea Fish, MSc

U. Orgad, DVM

*Kimron Veterinary Institute,  
P. O. Box 12, Bet Dagon 50250, Israel*

**KEY WORDS:** *Theileria annulata*, cattle, treatment, frusemide, buparvaquone

## 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.<sup>1-4</sup> 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.<sup>5</sup> 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 <sup>1</sup>	Schizonts detected on DPI	Max. % schizonts in tissues <sup>2</sup>	Max.PPE <sup>3</sup> / 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

<sup>1</sup> Days post-infection

<sup>2</sup> Number of schizonts counted per 200 lymph node or liver biopsy cells

<sup>3</sup> Percent infected erythrocytes, at least 500 erythrocytes counted in Giemsa stained blood smears

theileriosis is associated with high piroplasm level resulting in anemia, while in ECF anemia is low, but the pulmonary edema is profound.<sup>6</sup> In *T. annulata* infection, the anemia has been described as a result of removal of erythrocytes by phagocytosis, although as suggested by Uilenberg,<sup>1</sup> 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.<sup>7</sup> Tropical theileriosis can be controlled by drug therapy with long-acting oxytetracycline,<sup>8-10</sup> halofuginone lactate,<sup>11</sup> parvaquone and buparvaquone.<sup>12-16</sup> 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).<sup>17,18</sup> 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* antibodies<sup>19</sup> 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.<sup>20</sup> *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 al.<sup>1,2</sup> 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 39.5°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 >40.0°C, 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 40°C 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.<sup>7,22</sup> 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.<sup>1,6, 23,24</sup>

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,<sup>25</sup> 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,<sup>26</sup> 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.<sup>18</sup> 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,<sup>25</sup> parvaquone alone given at 20 mg per kg had a marked sup-

pressive 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.<sup>12</sup> Unsuren et al<sup>27</sup> 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.<sup>25</sup> 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,<sup>17</sup> and as shown in our present experiments, buparvaquone appears to remain the drug of choice against tropical theileriosis.

## REFERENCES

1. Uilenberg G. Theilerial species of domestic livestock. In: Irvin A, Cunningham M, Young A, ed. *Advances in the Control of Theileriosis*. The Hague, Martinus Nijhoff Publishers, 1981:4-37.
2. Flach E, Ouhelli H. The epidemiology of tropical theileriosis (*Theileria annulata* infection in cattle in an endemic area of Morocco. *Vet Parasitol* 1992; 44:51-65.
3. Luo J, Lu W. Cattle theileriosis in China. *Trop. Anim Hlth Prod* 1997;29: 4S-7S.
4. Shkap V, DeVos A, Zwegarth, E, et al: Attenuated vaccines for tropical theileriosis, babesiosis and heatwater: the continuing necessity. *Trends arasitol* 2007; 23: 420-426.
5. Pipano E, Shkap V. *Theileria annulata* theileriosis. In: Goetzer J, Thompson G, Tustin R, eds. *Infectious Diseases of Livestock with Special Reference to Southern Africa*. Vol 4. Oxford University Press; 2002:486-497.
6. Bolter N, Hall R. Immunity and vaccine development in the bovine theileriosis. *Adv Parasitol* 2000; 44: 41-97.
7. Irvin A, Morrison W. Immunopathology, immunology and immunoprophylaxis of Theileria infection. In: Soubry E. ed. *Immune Response in Parasite Infections: Immunology, Immunopathol-*

- ogy and Immunoprophylaxis*. Protozoa. CRC Press;1987:223-274.
8. Gill B, Bhattacharyulu Y, Kaur D, Singh A. Chemoprophylaxis with tetracycline drugs in the immunization of cattle against *Theileria annulata* infection. *Int J Parasitol* 1978;8:467-469.
  9. Dhar, S., Mnalhotra, D., Bhushan, C., Gautam, O. Chemoprophylaxis against bovine tropical theileriosis in young calves: a comparison between buparvaquone and long-acting oxytetracycline. 1990. *Res Vet Sci* 49;110-112.
  10. Singh J, Gill J, Kwatra M, et al: Treatment of theileriosis in crossbred cattle in the Punjab. *Trop Anim Hlth Prod* 1993;25:75-78.
  11. Mbwapo H, Mkonyi, P, Sondi J, et al: Chemotherapy of field cases of East Coast Fever using halofuginone lactate. *Acta Tropica* 1986; 43: 401-406.
  12. McHardy N, Morgan D, Treatment of *Theileria annulata* infection in calves with parvaquone. *Res Vet Sci* 1985; 39:1-4.
  13. Bansal G, Sharma N. Prophylactic efficacy of buparvaquone in experimentally induced *Theileria annulata* infection in calves. *Vet Parasitol* 1989;3: 219- 224.
  14. Hashemi-Fesharki R. Chemotherapeutic value of parvaquone and buparvaquone against *Theileria annulata* infection in cattle. *Res Vet Sci* 1991; 50:204-207.
  15. Osman S, Al-Gaabary A. Clinical, hematological and therapeutic studies on tropical theileriosis in water buffaloes (*Bubalus bubalis*) in Egypt. *Vet Parasitol* 2007;146: 337-340.
  16. Wilkie G, Brown C.G.D, Kirvar E, Thomas M, et al: Chemoprophylaxis of *Theileria annulata* and *Theileria parva* infections of calves with buparvaquone. *Vet Parasitol* 1998; 78:1-12.
  17. Mbwapo H, Sudi F, Mkonui P, Mfinanga J, et al: Comparative studies of the efficacy of parvaquone and parvaquone-plus-frusemide in the treatment of *Theileria parva* infection (East Coast Fever) in cattle. *Vet Parasitol* 2002;108:195-205.
  18. Musoke R, Tweyongyere R, Bizimenyera E, Waiswa C, et al: Treatment of East Coast Fever of cattle with a combination of parvaquone and frusemide. *Trop Anim Hlth Prod* 2004; 36: 233-245.
  19. Shkap V, Pipano E, Rasulov I, Azimov D, et al: Proteolytic enzyme activity and attenuation of virulence in *Theileria annulata* schizont-infected cells. *Vet Parasitol* 2003;115:247-255.
  20. Samish M, Ziv M, Pipano E. Preparation of suspensions of excavatum ticks infected with *Theileria annulata*. *Vet Parasitol* 1983 ;13 :267-272.
  21. Sergent E, Donatien A, Parrot L, Lestoquard F. Etudes sur les Piroplasmoses Bovines. *Inst Pasteur Alge*, 1945 ;96 :723.
  22. Lawrence J, Perry B, Williamson S. East Coast fever. In: Coetzer J, Tustun R, eds. *Infectious Diseases of Livestock*. Oxford University Press; 2004:448-467.
  23. Hooshman-Rad P. The pathogenesis of anemia in *Theileria annulata* infection. *Res Vet Sci* 1976; 20:324-329.
  24. Gill B, Bhattacharyulu Y, Kaur D. Symptoms and pathology of experimental ovine tropical theileriosis (*Theileria annulata* infection). *Ann Parasitol Hum Comp* 1977; 52:597-608.
  25. Singh D, Thakur M, Varshney B. Chemotherapeutic trials with four drugs in crossbred calves experimentally infected with *Theileria annulata*. *Res Vet Sci* 1993; 54(1):68-71.
  26. McHardy, N: Butalex (buparvaquone): a new therapeutic for theileriosis. In: Dolan T, ed. Recent Developments in The Research and Control of *Theileria annulata*. Proc 1992 Workshop ILRAD Nairobi Kenya 1990.
  27. Unsuren H, Kurtdede A, Goksu K. Effectiveness of parvaquone in cattle infected with *Theileria annulata*. *Trop Anim Hlth Prod* 1988; 20 (4): 256-258.