Indian J. Vet. Med. Vol. 40, No. 1, 2020 pp. 11-15

Research Article

Canine trypanosomiasis requires repeated administration of diminazene aceturate

Shanker K. Singh[*]*, Vivek K. Singh, Brajesh K. Yadav and Rakesh K. Singh*

Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, DUVASU, Mathura – 281 001, U.P., India

Abstract

The present study was aimed to investigate the effects of repeated administration of diminazene aceturate against clinical *T. evansi* infection in dogs. Thirteen clinical cases of trypanosomiasis in dogs were randomly divided into two groups. The dogs of Group 2 were treated with diminazene aceturate at a dosage regime of 5.0 mg/kg body weight IM at 24 h of intervals for five times. While Group 1 dogs were treated with the same dose rate but at 48h of intervals for three times. On day 7 post-therapy, remarkable alterations in serum biochemistry was not recorded. The dogs of Group 2 recovered completely and further recurrence was not recorded up to 6 months post-therapy. However, recurrence of clinical disease was recorded in one dog in Group 1 at day 40 post-therapy. Therefore, it can be concluded that diminazene aceturate may be administered repeatedly at 24 h intervals five times for the therapeutic accomplishment over clinical *T. evansi* infection in dogs.

Key words: Diminazene aceturate, Dog, Relapse, *T. evansi*

Trypanosoma evansi (*T. evansi*) is mainly disseminated by *Tabanid* species and can affect both humans and animals (Otto *et al.,* 2010; Desquesnes *et al.,* 2013). Dogs are highly susceptible to *T. evansi*, and they often exhibit severe clinical signs culminating to death especially in untreated stray dogs (Herrera *et al.,* 2004) and sometimes despite treatments (Singh *et al.*, 1993). *T. evansi* must be considered as both a blood and tissue parasite owing to its ability to invade the nervous system, not only in horses and dogs but also in cattle, buffaloes, deer and pigs (Rodrigues *et al.,* 2009). How trypanosomes penetrate the blood-brain barrier (BBB) is unknown, but several mechanisms have been proposed; for instance entrance through sites where the BBB is incomplete, such as sensory ganglia and circumventricular organs; deposition of immune complexes in the choroid plexus, with resultant increase in vascular permeability; and release of toxic substances including cytokines and proteases by trypanosomes that cause opening of intercellular tight junctions in the ependymal lining of the ventricular system (Rodrigues *et al.,* 2009; Singh *et al.,* 2016).

One of the factors that may have contributed to the development of severe encephalitis was the use of subtherapeutic doses of diminazene aceturate and other antitrypanosomal drugs in the affected animals (Rodrigues *et al.,* 2005). Previous studies have well demonstrated that the use of subtherapeutic doses of diminazene aceturate may prolong survival of horses experimentally infected by *T. brucei* spp., but it is associated with subsequent invasion of the central nervous system by trypanosomes and production of necrotizing encephalitis (Rodrigues *et al.,* 2009). Diminazene aceturate clears trypanosomes from tissues except in the central nervous system, because the drug does not cross the BBB. Thus, trypanosomes in the central nervous system survive antitrypanosomal therapy, and with a change in their surface glycoproteins may lead to new parasitemia (Jennings and Gray, 1983). A single dose of diminazene is not effective for horses, mules and dogs as it does not cross BBB. Additionally, there are abundant strains of canine trypanosomes especially *T. evansi* which are refractory to diminazene, thus repeated treatment of infected dogs and constant relapses have been documented (Nwoha, 2013; Nwoha *et al.,* 2013). Therefore, the present study aimed to validate the curative efficacy of multiple dosing therapeutic protocols of diminazene aceturate against canine trypanosomiasis.

Materials and Methods

A total of 13 clinical cases of trypanosomiasis in dogs (Five-Mongrels, Four-German Shepherds, One each of Labrador Retriever, Doberman Pinscher, St. Bernard and Spitz) found positive for trypomastigotes of *T. evansi* on blood smear examination (Fig. 1), were divided randomly into group 1 (n=6) and group 2 (n=7). For the preparation of blood smear, a drop of the blood sample was obtained by aseptic pricking of the ear tips

[1*]Corresponding author: Email: pshankervet@gmail.com

of dogs and spread over a clean slide to prepare the blood smear. All dogs were received and examined in the clinics of College of Veterinary Science and Animal Husbandry, Mathura, India. An ardent clinical examination of all dogs was performed and clinical signs were recorded. With the informed consent of pet owners, three ml blood sample each was collected from all the dogs before the start of any therapy and at day 7 post-therapy and was transferred into a tube containing clot activators to harvest serum. Serum biochemical panels were estimated by using fully automatic serum biochemistry analyzer with kits from Span Diagnostics Ltd. Gujarat, India. Dogs of group 1 were treated with diminazene aceturate (intramuscular injection) using a dose of 5.0 mg /kg at 48h of intervals for three times. While the dogs of group 2 were treated with diminazene aceturate (intramuscular injection) using a dose of 5.0 mg/kg at 24h of intervals for five times. Additionally, supportive medicines were also administered considering the clinical condition of every diseased dog. The data were subjected to paired student t-test to determine differences between pre- and post-treatment values of the same group. The level of statistical significance for all the comparisons made was established at $P \leq 0.05$.

Results and Discussion

Diseased dogs have a history of either close inhabitation with cattle or buffaloes or were kept within a radius of 100 m distance from the cattle or buffaloes dairy farms. The clinical manifestation was patchy and clinical examination revealed major signs of inappetence, apathy, pyrexia, respiratory distress, increased heart rate, emesis, melena, petechial hemorrhages on ear pinna and inguinal region, disinclined to move, posterior weakness, drunken gait, rotation of head, head-butting to wall, aimless wandering, hemiplegia, lacrimation, corneal opacity (Fig. 2), photophobia (hiding in dark place), blindness, pica, pallid mucous membrane and edema of face, scrotum and hind legs.

All the dogs treated with diminazene at 24h of intervals for five times (Group 2) revealed remarkable clinical improvements and were free of *T. evansi* at day 7 post-therapy on blood smear examination. None of the dogs of this group revealed recurrence of the diseases up to six months post-therapy. Moreover, all the dogs treated with diminazene at 48h of intervals for three times (Group 1) also revealed complete clinical recovery at day 7 post-therapy. These dogs were also free of *T. evansi* at day seven post-therapy on blood smear examination. At day seven post-therapy, blood smear examination of one dog of this group revealed the presence of apoptotic or actively phagocytizing leukocytes (Fig. 4). Out of six dogs of this group, recurrence of disease was recorded in one dog at day 40 post-therapy; the dog detected positive for trypomastigotes of *T. evansi* and revealed severe clinical manifestations in comparison to its first episode. At the recurrence of the second episode, the dog revealed a more severe form of the clinical disease including pyrexia, gastritis, unable to stand even after assistance, rotation of the head and pronounced depression; and the dog died within 12 hours despite administration of diminazene and supportive therapies. However, recurrence was not recorded up to 6 months post-therapy in remaining five dogs of group 1. Two dogs showing corneal opacity recovered completely from the opacity within 70 days of therapy (Fig. 3). One dog of group 2 showing the clinical manifestation of hemiplegia on the day of the first presentation started walking without assistance at day 34 post-therapy. While other clinical manifestations of this dog recovered within 14 days of therapy. Another dog of the same group (group 2) showing the clinical manifestation of hemiplegia on the day of the first presentation, started walking without assistance at day 11 post-therapy. Except to ALT activity and total protein level in group 2, remarkable alterations in the biochemical panel on day 7 post-therapy were not recorded in both groups as compared to their day 0 values. No untoward effects were recorded in both groups and biochemical panels of liver and kidney function were within the reference range in all treated dogs at day 7 post-therapy. Two dogs, one from each group revealed immediate evacuation of bowel after each administration of diminazene.

In agreement to our clinical findings, intermittent fever, subcutaneous edema, progressive anemia, blindness, lethargy, cachexia, lymphadenopathy, corneal opacity, blindness, neurologic disturbs, oedema, motor incoordination, paralysis of the pelvic limbs and hemostatic alterations have been reported as the major clinical manifestation of clinical *T. evansi* infection in animals (Da Silva *et al.,* 2012; Desquesnes *et al.,* 2013; Singh *et al.,* 2016). Neurological manifestation of hemiplegia, posterior weakness, drunken gait, apathy and pica in some of the *T. evansi* infected dogs of the current study indicates conceivable dissemination of the parasite into the CNS. Anaemia is a common feature of clinical

Table 1. Biochemical panels of dogs with trypanosomiasis on day 0 and day 7 post-therapy

Biochemical panels	Group 1		Group 2	
	Pre-Therapy	Post-Therapy	Pre-Therapy	Post-Therapy
AST (IU/L)	72.58±20.25	77.51±17.06	65.04±8.98	66.45±7.61
ALT (IU/L)	23.85±5.38	38.96±6.53	30.17±4.46	40.30±4.95*
ALKP (IU/L)	97.00±14.43	113.33±11.67	84.85±6.70	87.70±13.06
Urea (mg/dl)	17.51±3.23	24.86±4.23	28.27±4.59	27.37±4.72
Creatinine (mg/dl)	0.77±0.18	0.79±0.08	0.54±0.12	0.55±0.13
Total Protein (g/dl)	7.71±0.66	7.95±0.63	8.32±0.67	8.64±0.75*
Albumin (g/dl)	2.87±0.23	2.84±0.22	2.56±0.15	2.48±0.14
Cholesterol (mg/dl)	121±16.91	123±15.90	138±17.11	136±17.49
Triglycerides (mg/dl)	70.00±5.53	75.33±4.96	71.71±5.17	77.00±7.46

*Significantly differ as compared to day 0 value of the same group.

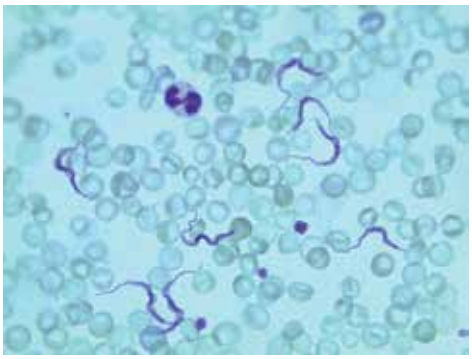

Fig. 1. Trypanomastigotes of *T. evansi* in blood smear examination of diseases dogs.

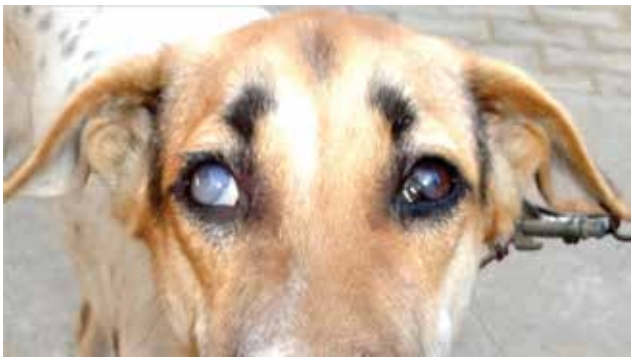

Fig. 2. A diseases mongrel dogs reveling clinical manifestation of corneal opacity

Fig.3. Same Mongrel dog with completely recovered corneal opacity at day 70 post-therapy.

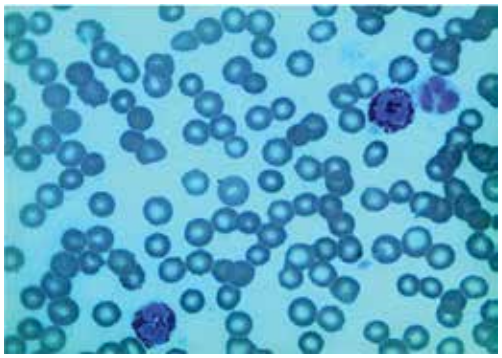

Fig. 4. Blood smears examination revealing presence of apoptotic or actively phagocytizing leukocytes in a dog (Group 1) at day 7 post-therapy.

trypanosomiasis (Habila *et al.,* 2012). Development of anemia in diseased dogs could be the upshot of hemolytic factor such as free fatty acids, immunologic mechanisms, hemodilution, coagulation disorders, and depression of erythrogenesis and release of trypanosomal sialidase (Omer *et al.,* 2007; Adamu *et al.,* 2008; Habila *et al.,* 2012; Singh *et al.,* 2018).

In this study, diminazene aceturate administered at 24h intervals for five times was 100% (7/7) effective, leading to the cure of the nastiest disease. Previously, the same protocol but at lower dose of 3.5 mg/kg body weight was used for dogs with complete recovery without any

relapse and for cats experimentally infected with *T. evansi*, obtaining 85.7% (6/7) of curative efficacy (Da Silva *et al.,* 2009; Howes *et al.,* 2011). On the other hand, in a comparative study of doses of diminazene aceturate in rats infected with *T. evansi*, inefficiency and death of rats treated with a single dose of 3.5 and 7.0 mg/kg were observed. In contrast, the cure in rats occurred when animals received a dose of 3.5 and 7.0 mg kg^{-1} during 5 consecutive days (Da Silva *et al.,* 2008).

However, in the current study, we obtained 83.3% (5/6) curative efficacy of diminazene aceturate administered at 48h interval for three times. The return of the parasitemia after treatment in one dog of this group may be related to the impossibility of the medicament to pass through the blood-brain barrier at this dose regimen or the therapeutic dose is insufficient, which thereby may create a possible refuge for the trypanosomes during the systemic phase of the drug. In agreement with the current study, diminazene refractory *T. evansi* and repeated treatment of infected dogs and constant relapses have been demonstrated in recent past (Nwoha, 2013; Nwoha *et al.,* 2013). We believe that our consecutive five-dose protocol obtained higher efficiency because it might have provided greater passage of drug molecules through the blood-brain barrier, which could eliminate the parasite from the brain.

A relevant aspect to be considered is the absence of toxic effects of the treatment to the dog. The hepatic and renal functions remained normal at day seven post-therapy, similar results were observed in studies with dogs and cats treated with five consecutive doses of diminazene aceturate (Da Silva *et al.,* 2009; Howes *et al.,* 2011). Therefore, nothing prevents the use of this drug in the treatment of dogs infected with *T. evansi* with dose regimen of 5.0 mg/kg at 24h of intervals for five times, though it is advisable to have close monitoring of the animal during the therapy. After treatment, all clinical signs disappeared, physiological parameters returned to normal levels. Thus, it can be concluded that diminazene aceturate dose regimen of 5.0 mg/kg at 24h intervals for five times is effective regimen to cure of trypanosomiasis in dogs. While administrations of the same dose of diminazene aceturate at 48h of intervals for three times is not enough for the complete vanishing of the parasite from diseased dogs. However, it is the discretion of the veterinary clinician to use the therapeutic regimen of the present study, considering the risk versus benefits to the diseased dogs.

References

Adamu, S., Ibrahim, N.D.G., Nok, A.J. and Esievo, K.A.N. 2008. Sialyl transferase activity probably counteracts that of sialidase as one of the possible mechanisms of natural recovery of stabilization of erythrocyte mass in Trypanosome-infected animals-A perspective. *Afr. J. Biotechnol.*, **7:** 4992–5001.

Da Silva, A.S., Tochetto, C., Zanette, R.A., Pierezan, F., Rissi, D.R., Santurio, J.M. and Monteiro, S.G. 2008. Aceturato de diminazeno e dipropionato de imidocarb no controle de infecção por *Trypanosoma evansi* em *Rattus norvegicus* infectados experimentalmente. *Ciência Rural*, **38:** 1357–1362.

Da Silva, A.S., Zanette, R.A., Lopes, S.T.A., Santurio, J.M. and Monteiro, S.G. 2009. Diminazene aceturate in the control of *Trypanosoma evansi* infection in cats. *Vet. Parasitol.,* **165:** 47-50.

Da Silva, A.S., Oliveira, C.B., Bertoncheli, C.M., Santos, R.P., Beckmann, D.V., Wolkmer, P., Gressler, L.T., Tonin, A.A., Graça, D.L., Mazzanti, A., Lopes, S.T. and Monteiro, S.G. 2012. Clinical signs and histopathology of brain, spinal cord and muscle of the pelvic limb of rats experimentally infected with *Trypanosoma evansi*. *Pathol. Res. Pract.,* **208(1):** 39-44.

Desquesnes, M., Holzmuller, P., Lai, D.H., Dargantes, A., Lun, Z.R. and Jittaplapong, S. 2013. *Trypanosoma evansi* and Surra: A Review and Perspectives on Origin, History, Distribution, Taxonomy, Morphology, Hosts, and Pathogenic Effects. *Biomed. Res. Int.* http://dx.doi.org/10.1155/2013/194176.

Habila, N., Inuwa, M.H., Aimola, I.A., Udeh, M.U. and Haruna, E. 2012. Pathogenic mechanisms of *Trypanosoma evansi* infections. *Res. Vet. Sci.,* **93(1):** 13-17.

Herrera, H.M., Davila, A.M.R., Norek, A., Abreu, U.G., Souza, S.S., D'Andrea, P.S. and Jansen, A.M. 2004. Enzootiology of *Trypanosoma evansi* in Pantanal, Brazil. *Vet. Parasitol.,* **125:** 263–275.

Howes, F., Da Silva, A.S., Athayde, C.L., Costa, M.M., Corrêa, M.M.B., Tavares, K.C.S., Miletti2, L.C., Terezinh S., Lopes, A., do Amaral, A.S. and Schmidt, C. 2011. A new therapeutic protocol for dogs infected with *Trypanosoma evansi*. *Acta* Sci. *Vet.Sci.,* **39(3):** 988.

Jennings, F.W. and Gray, G.D. 1983. Relapsed parasitaemia following chemotherapy of chronic *T. brucei* infections in mice and its relation to cerebral trypanosomes. *Contrib. Microbiol. Immun.,* **7:** 147-154.

Nwoha, R.I.O. 2013. A review on trypanosomosis in dogs and cats. *Afr. J. Biotechnol.*, **12(46):** 6432-6442.

Nwoha, R.I.O., Eze, I.O. and Anene, B.M., 2013. Serum biochemical and liver enzymes changes in dogs with single and conjunct experimental infections of *Trypanosoma brucei* and *Ancylostoma caninum*. *Afr. J. Biotechnol.*, **12(6):** 618-624.

Omer, H.O., Mousa, H.M. and Al-Wabel, N. 2007. Study on

the antioxidant status of rats experimentally infected with *Trypanosoma evansi*. *Vet. Parasitol.,* **145:** 142–145.

Otto, M.A., Da Silva, A.S., Gressler, L.T., Farret, M.H., Tavares, K.C.S., Zanette, R.A., Miletti, L.C. and Monteiro, S.G. 2010. Susceptibility of *Trypanosoma evansi* to human blood and plasma in infected mice. *Vet. Parasitol.,* **168:** 1–4.

Rodrigues, A., Fighera, R.A., Souza, T.M., Schild, A.L., Soares, M.P., Milano, J. and Barros, C.S.L. 2005. Surtos de tripanossomı´ase em equ¨ inos no Rio Grande do Sul: aspectos epidemiolo´ gicos, clı´nicos, hematolo´ gicos e patolo´ gicos. *Pesqui. Vet. Bras. ,* **25:** 239–249.

Rodrigues, A., Fighera, R.A., Souza, T.M., Schild, A.L. and Barros, C.S. 2009. Neuropathology of naturally occurring T*rypanosoma evans*i infection of horses. *Vet. Path.* **46(2):** 251-258.

Singh, S.K., Singh, V.K., Yadav, B.K., Nakade, U.P., Kumari, P., Srivastava, M.K., Sharma, A., Choudhary, S., Swain, D. and Garg, S.K. 2016. Potential association of reduced cholinesterase activity with *Trypanosoma evansi pathogenesis in buffaloes. Vet. Parasitol.,* **225:** 29–32.

Singh, S.K., Singh, V.K., Kumari, P., Nakade, U.P. and Garg, S.K. 2018. *Trypanosoma evansi* induces detrimental immuno-catabolic alterations and condition like type-2 diabetes in buffaloes. *Parasitol. Int.*, **67 (2):** 140-143.

Singh, B., Kalra, I.S., Gupta, M.P. and Nauriyal, D.C. 1993. *Trypanosoma evansi* infection in dogs: seasonal prevalence and chemotherapy. *Vet. Parasitol.,* **50(1-2):** 137-141.

Received : 12.12.2019
Accepted : 16.04.2020