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EFFICACY OF BUPARVAQUONE IN PCR CONFIRMED CONCURRENT INFECTION OF *Trypansoma evansi* AND *Theileria annulata* IN BUFFALOES

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ABSTRACT

Five buffaloes, 5 to 7 years old in the month of July 2015 with history of high rise of temperature, nasal discharge, exophthalmia, excitement, lacrimation, salivation, dyspnea, generalised lymphadenopathy, especially in the prescapular lymph nodes were attended at a farm in Mahakaushal region of Madhypradesh. By blood smear and polymerase chain reaction analysis, it was determined that all the buffaloes had mixed infections of *T. evansi* and *T. annulata*. The treatment of all five buffaloes was initiated with single deep intramuscular injection of diminazine acceturate 3.5 mg/kg body weight along with three dosages of long acting oxytetracycline 20 mg/kg b.wt. on alternate day. A single injection of buparvaquone on the fourth day (in 3 cases) and two doses of buparvaquone (2.5 mg kg^{-1}) at 48 h intervals (in 2 cases) were administered. Only 3 buffaloes showed uneventful recovery after a single injection with buparvaquone. When the blood samples of all the five buffaloes were again analyzed by PCR and found all the samples were negative for *T. evansi* and *T. annulata* except two buffaloes (Two cases hading two doses of buparvaquone) which were positive for *T. annulata* in spite of giving the second dose of buparvaquone. The study indicated that there might be reduced efficacy or resistance of *T. annulata* against buparvaquone.

Keywords: *Bubalus bubalis*, buffaloes, *Trypansoma evansi*, *Theileria annulata*, buparvaquone, PCR

INTRODUCTION

Trypansoma evansi called "surra" (a rotten-sounding Hindi word; salivarian trypanosome) and *Theileria annulata* are the most economically important haemoprotozoan diseases of bovine which are transmitted mechanically by tabanid flies

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and *Hyalomma anatolicum*, respectively (Soulsby, 1982). The most common method for diagnosis of these blood protozoans is the examination of stained blood smear but inherited with limitation of sensitivity and specificity. Further in both cases clinical symptoms are similar type. Therefore, confirming the parasites via a molecular method is imperative. The overall molecular prevalence of surra in buffalo varies from 28% to 51% in India (Aregawi *et al*., 2019). The reported prevalence of *T. evansi*, *T. annulata* and *B. bigemina* is 13.40%, 14.43% and 1.72%, respectively in bovine in Mahakaushal region of Madhya Pradesh (Agrawal, 2016). Peculiar clinical symptoms of surra is fluctuating parasitaemia, sever anaemia, edematous swelloing on legs, intermittent nature of fever, abortion and uncoordinated movement (Gill, 1991) despite the fact that theileriosis is marked by recognisable clinical symptoms such as halted rumination, tearing, corneal opaqueness, diarrhea, end stage dyspnea marked with a foamy discharge from the nasal cavity, high temperature, mucous membrane petechial haemorrhages, and anaemia, these symptoms may not always occur in the same way. (Fukasawa, 2003). Both of the infection in acute phase are highly fatal in nature if not treated timely. Due to the sever clinical illness in infected animals, both of these diseases result in substantial monetary losses. At a dosing rate of 2.5 mg kg^{-1} for theileria, parvaqunone compounds, particularly buparvaquone, are regarded as the preferred medication. In most cases, a one dose of this drug is sufficient to treat theileriosis, but a second dose may also be required (Wilkie *et al*., 1998). Due to lack of literature on mixed clinical infection of these two causative agents has prompted authors to undertake this investigation along with failure of treatment with buparvaquone eventually resulting in the death of buffaloes.

MATERIALS AND METHODS

In the month of July 2015, five buffaloes, 5 to 7 years old from a farm in the Mahakaushal region of Madhypradesh with a history of high temperature, nasal discharge, exophthalmia, excitement, tearing, salivation, dyspnea, generalized lymphadenopathy, especially in the prescapular lymph nodes were examined.

Blood collection

A 3 ml blood sample was taken aseptically from the ear vein of a suffering buffalo in a blood collection vial containing anticoagulant EDTA. At the time of collection, a peripheral thin blood smear was prepared from the animal and immediately fixed with methanol. Blood samples were brought to the Department of Veterinary Parasitology, College of Veterinary Science and Animal Husbandry, Jabalpur, and blood smears were immersed in diluted Giemsa stain for 30 to 45 minutes before being washed with distilled water to remove excess stain. The slides were air dried before being examined for the presence of any haemoprotozoan parasites using an oil immersion lens (Coles, 1986).

Molecular assay

Blood samples were used to extract genomic DNA according to the instructions on the DNA isolation kit (DNeasy blood and tissue kit, Qiagen). The optical density ratio at 260:280 nm was used to determine the purity of DNA. PCR was performed on samples with admissible purity (ratio 1.8 to 2.0). *T. evansi* and *T. annulata* were diagnosed using buffalo blood samples. Amplification was carried out using a set of primers specific to the *T. evansi* repetitive DNA sequence and *T. annulata* as mentioned by Wuyts *et al*. (1994); d'Oliveira *et al*.

(1995), respectively.

RESULT AND DISCUSSION

Clinical symptoms and gross pathology

Clinical symptoms in animals included rapid increase in temperature, nasal discharge, exophthalmia, excitement, tearing, salivation, dyspnea, and widespread lymphadenopathy, namely in the prescapular lymph nodes. Clinical signs were suggestive of haemoprotozoan infection. The presence of intraerythrocytic *T. annulata* piroplasm and exoerythrocytic *T. evansi* confirmed the diagnosis by Giemsa-stained blood smears (Figure 1).

Other most commonly haemoprotista i.e., *Babesia* or *Anaplasma* was not detected. Further it was also confirmed by PCR (Figure 2 and 3).

Faecal examination was done for presence of any endoparasitic infection, results of which were positive for mild infection (+) of strongyle infection although presence of mild infection of strongyle has no significant effect on observed clinical symptoms. Treatment of mixed infection of *T. evansi* and *T. annulata* is a very challenging task. In present case, treatment was initiated with single injection of diminazine acceturate 3.5 mg/kg body weight deep intramuscular along with three dosages of long acting oxytetracycline 20 mg/kg b.wt., on alternate day. A single injection of buparvaquone on the fourth day (in 3 cases) and two doses of buparvaquone (2.5 mg kg^{-1}) at 48 h intervals (in 2 cases) were administered. Additionally, supportive therapy with iron preparations, multivitamin, folic acid, and cyanocobalamin was also used as it can fasten the recovery rate in theileriosis and trypanosomiosis infected animals (Ramyadevi and Ponnuswamy, 2020). Only 3 buffaloes showed uneventful recovery after a single injection with buparvaquone. On day 7th after the start of treatment, again PCR was conducted. The report revealed that all the samples were found negative for *T. evansi* and *T. annulata* except two buffaloes (found positive for *T. annulata*) in which second injection of buparvaquone were used (Figure 4 and 5).

Postmortem examination revealed the punched-out ulcer in abomasuma secondarged and edematous lymph node and there was presence of white foci of various sizes on kidney (Figure 6).

The current study aimed to uncover the treatment for *T. evansi* and *T. annulata* mixed infections. Due to limitation of blood smear examination, it is imperative to diagnose the blood sample by molecular method also. Although the gold standard technique for diagnosis of blood protozoa is blood smear examination but having inherent limitation. There is every possibility that due to immunosuppression by *T. evansi* by which the infection of theileriosis flares up. Buparvaquone is 92% effective in a single injection at a dose rate of 2.5 mg kg^{-1}. (McHardy, 1991). Buparvaquone is responsible for destroying the schizont and piroplasm stages of *T. annulate* (Mhadhbi *et al*., 2010) but in our study, blood smears from buffaloes that had been given two doses of buparvaquone showed that the ring-shaped piroplasm in their blood was normal and had not changed. The thickness of the bands on the gel was about the same, which means that the amount of *T. annulata* DNA did not change before or after the treatment. Resistance could happen for a number of reasons, including underdosing and incomplete treatment of active infections. The misuse of prophylactic medications, as well as the rapid rate of parasite proliferation brought on by the parasites' ability to adapt at the genetic and metabolic levels, are

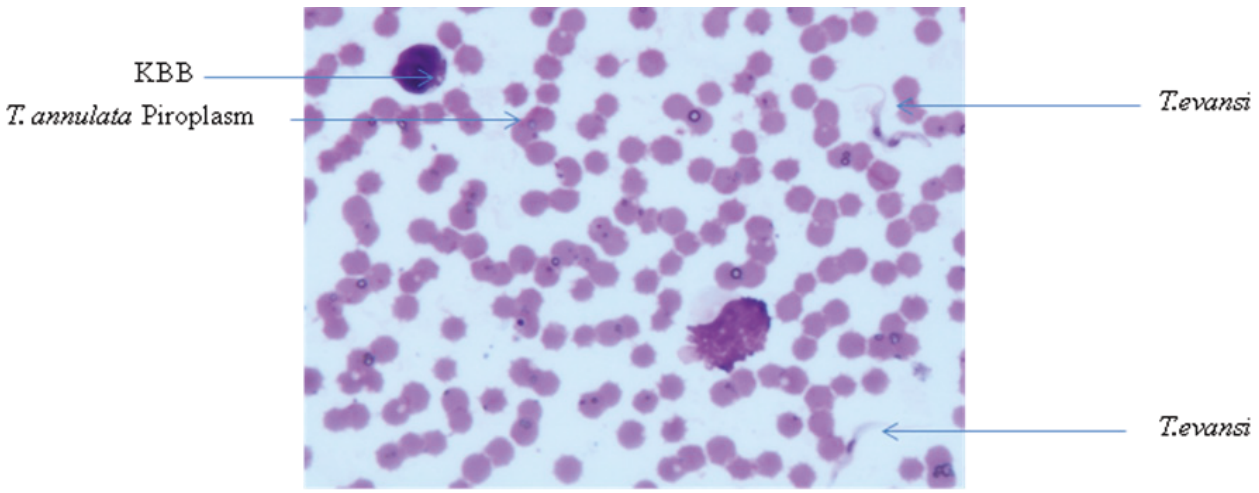

Figure 1. Photomicrograph of blood smear showing, *T. evansi*, Koch's blue body in lymphocyte, Piroplasm of *T. annulata* within RBC (1000x).

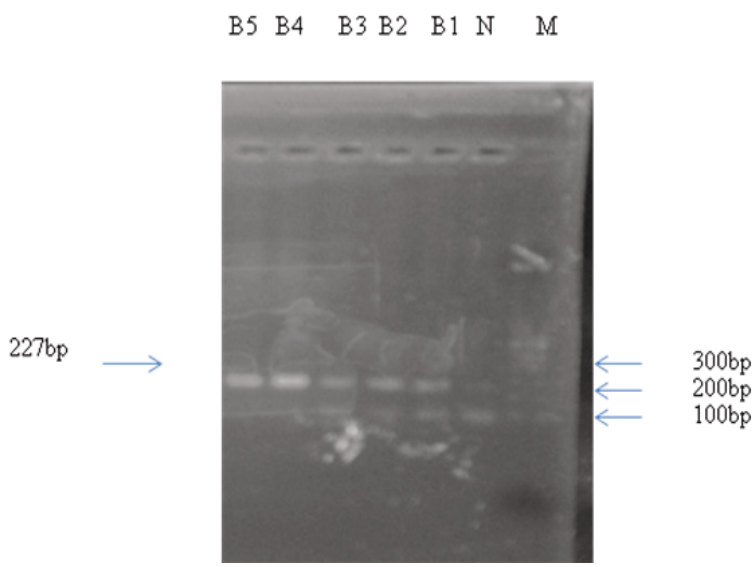

Figure 2. Agarose gel electrophoresis (1.5%) showing the intact band of 227 bp fragment from genomic DNA of *Trypanosoma evansi* before injection of diminazine acceturate.
Lane M: 100 bp DNA ladder;
Lane N: Negative control (No template);
Lane B1 to B5: Positive processed filed samples.

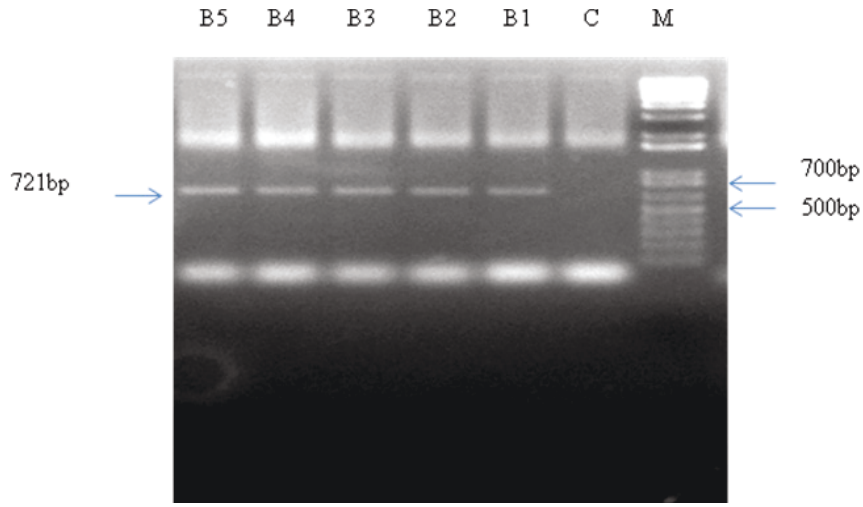

Figure 3. Agarose gel electrophoresis (1.5%) showing the band of 721 bp fragment from genomic DNA of *Theileria annulata* before injection of bupavaquone.
Lane M: 100bp DNA ladder;
Lane C: Negative control (No template);
Lane B1 to B5: Positive processed filed samples.

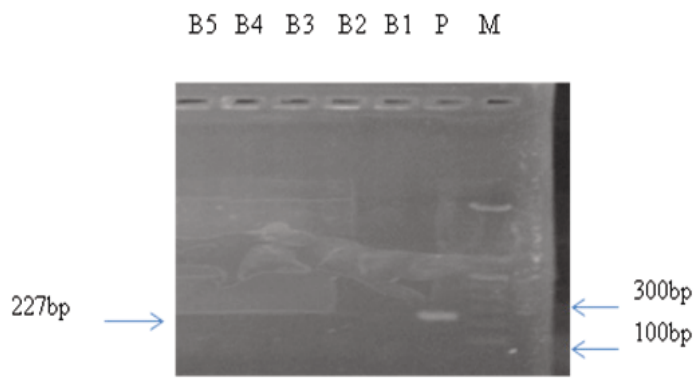

Figure 4. Agarose gel electrophoresis (1.5%) showing the intact band of 227 bp fragment from genomic DNA of *Trypanosoma evansi* after injection of diminazine acceturate.
Lane M: 100 bp DNA ladder;
Lane P: Positive control;
Lane B1 to B5: Negative processed filed samples.

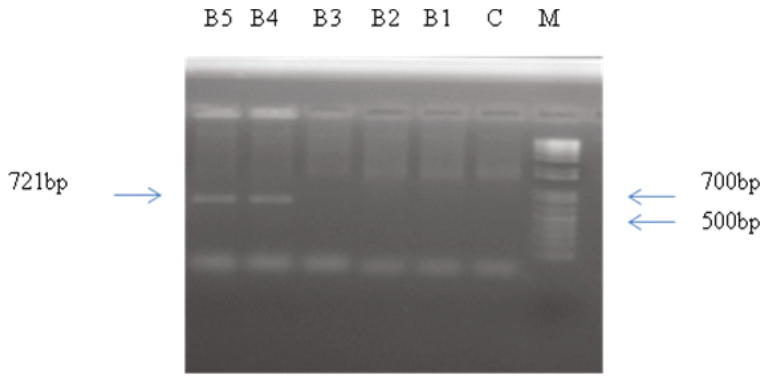

Figure 5. Agarose gel electrophoresis (1.5%) showing the band of 721 bp fragment from genomic DNA of *Theileria annulata* after injection of bupavaquone.
Lane M: 100 bp DNA ladder
Lane C: Negative control (No template)
Lane B4 and B5: Positive processed filed samples
Lane B1 to B3: Negative processed filed samples

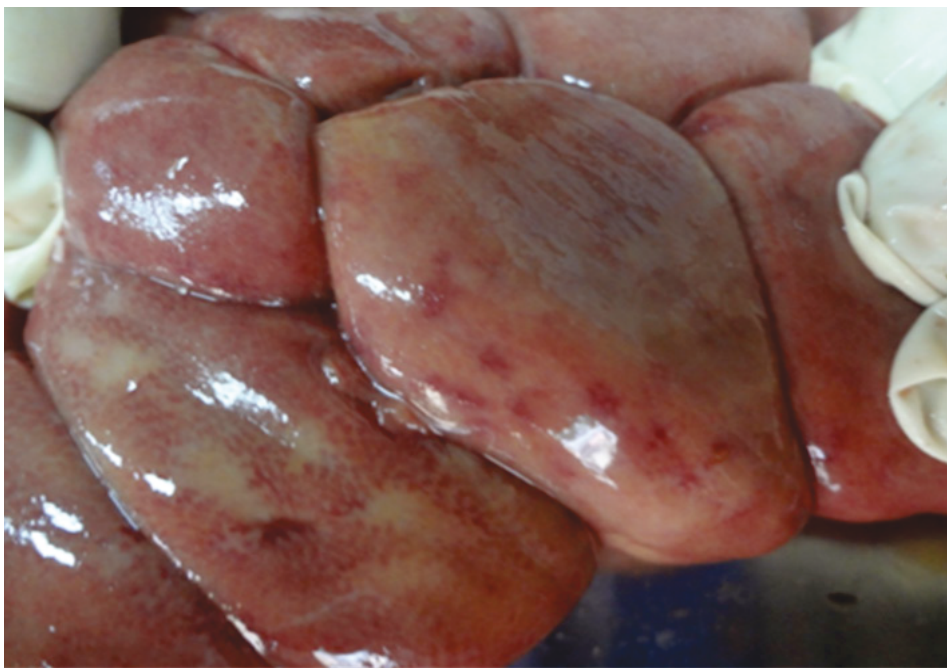

Figure 6. White foci of various sizes on kidney of dead buffalo.

further causes of apicomplexan parasite resistance (Hyde, 2007). The susceptibility of native cattle and buffaloes varies, and cattle infected at a subclinical stage may be a major factor in the transfer of buparvaquone-resistance to buffaloes. This might be the first report where two animals were not responded to while three were responded by buparvaquone in a single herd. So, tracking and assessing how the vector changes with the seasons, using vaccination prophylactic regimens for exogenous animals, and using buparvaquone and tetracycline chemicals simultaneously during treatment may all help to slow down or stop the development of resistance.

CONCLUSION

Failure of treatment by using the buparvaquone against theileriosis is a major concern for field veterinarian. To fully comprehend the significance of co-infection, the existence of any interactions between the two infections, and the effectiveness of buparvaquone against *T. annulata*, more research is required. Since the clinical symptoms of both infections overlap, veterinarians should take the likelihood of co-infection into account.

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