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Polypeptide Profiles of Diminazene Aceturate Resistant *Trypanosoma evansi* Organisms Isolated from a Buffalo

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Abstract

Trypanosomosis was recorded in a buffalo during the month of September 2014 in Proddatur of Andhra Pradesh, India. Disease was confirmed by the presence of *Trypanosoma evansi* organisms in peripheral blood smears. Buffalo was treated with intra muscular administration of diminazene aceturate at the rate of 3.5 mg per kg body weight along with supportive therapy. After two days of therapy, re-examination of blood smear revealed the presence of live *Trypanosoma evansi* organisms. To determine the profiles of diminazene resistant *Trypanosoma evansi* organism's whole blood was collected and processed. Whole cell lysate antigen was prepared from the host cell free *Trypanosoma evansi* parasites. A total of 8 polypeptide bands with relative molecular weight of 34, 48, 53, 55, 64, 74, 80 and 98 kDa were identified. Buffalo was successfully treated with sub cutaneous administration of antrycide prosalt, at the dose rate of 7.5 mg per kg body weight along with supportive therapy.

Introduction

Trypanosomosis is an important haemoprotozoan disease of domesticated animals. Among the several species of trypanosomes, *Trypanosoma evansi* is the most common species in India. It causes anaemia, lowering milk yield which leads to economic losses to the small farmers [1]. Cattle and buffaloes are considered as reservoir of parasite for other domestic as well as pet animals. Due to stress reservoir hosts may also suffer with clinical trypanosomosis [2]. In India, diminazene aceturate, isometamidium chloride and quinapyramine sulphate and chloride are currently available drugs for the treatment of trypanosomosis [3]. Diminazene aceturate is one of the most common drugs used in the treatment of trypanosomosis and babesiosis in animals. Previous studies shown that, diminazene and isometamidium are therapeutically effective against clinical *T. evansi* infection [4]. Consistent use of low doses of diminazene aceturate leads to the development of resistant strains of trypanosome species and relapses of infection was observed few days of post treatment [5]. Hence present study was conducted to record the protein profiles of diminazene aceturate resistant *Trypanosoma evansi* organisms.

Materials and Methods

A 9 nine years old milch buffalo with history of anorexia, chronic emaciation, reduction in milk yield and occasional excitement (Figure 1) was referred to the Teaching Veterinary Clinical Complex, College of Veterinary Science, Proddatur during the month of September, 2014. Up on clinical examination, buffalo showed pale mucus membranes, corneal opacity (Figure 2), pyrexia (103.2 °F), heart rate of 82 bpm, respiratory rate of 26/min, enlarged sub mandible lymph nodes and oedema of the fore limbs. Electrocardiography and examination with metal detector was carried out to detect other abnormalities [6-8]. Whole blood, peripheral blood smears, serum, urine and dung samples were collected for laboratory analysis according to the previous studies [9]. Peripheral blood smear revealed the presence of live *Trypanosoma* spp. organisms. Giemsa stained blood smears confirmed the morphology of *Trypanosoma evansi* organisms (Figure 3). Initially buffalo was treated with intra muscular administration of diminazene aceturate at the dose rate of 3.5 mg/kg body weight along with fluid and supportive therapy [10,11]. Diagnosis of *Trypanosoma evansi* organisms was done by the wet blood film examination and it was confirmed by the examination of stained blood smear [12].

After two days of therapy partial improvement in the condition was noticed. Again blood samples were collected for laboratory analysis and which was positive for live *Trypanosoma evansi* organisms. Whole blood with addition of EDTA was collected for further studies. Multiplication of *Trypanosoma evansi* was done in a rat and host cell free *Trypanosoma evansi* parasites was separated by anion-exchange chromatography using a Diethylaminoethyl cellulose column with phosphate buffer saline glucose solution. The whole

Figure **1:** Buffalo suffering with clinical Trypanosomosis.

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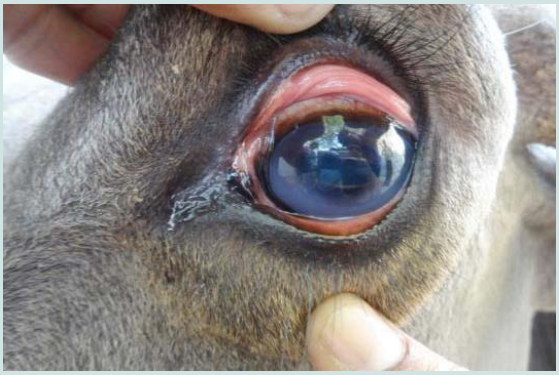

Figure **2:** Presence of corneal opacity.

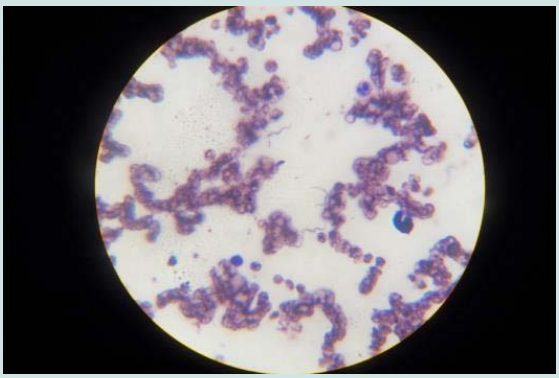

Figure **3:** Presence of Trypanosoma evansi organism in stained blood smears.

cell lysate antigen was prepared from the parasites and its protein concentration was adjusted to 1.0 mg/mL in PBS. Polypeptide profile of WCL of *T. evansi* was determined by SDS-PAGE according to the previous studies [13,14]. Buffalo was again treated with sub cutaneous administration of antrycide prosalt at the dose rate of 7.5 mg/kg body weight along with supportive therapy [15].

Results and Discussions

After two days of therapy with antrycide prosalt buffalo was free from live *Trypanosoma evansi* organisms and improvement in the condition was noticed which indicates the presence of diminazene aceturate resistant *Trypanosoma evansi* organisms in the present case.

Polypeptide profiles of whole cell lysate antigens from the diminazene aceturate resistant *Trypanosoma evansi* was shown in Figure 4. In the present study a total of 8 polypeptides were observed when resolved in 10% SDS-PAGE and stained with Commassie brilliant blue. The approximate molecular weights of these polypeptides were ranged from 34 to 98 kDa with relative molecular weight of 34, 48, 53, 55, 64, 74, 80 and 98 kDa. Whole cell lysate antigen of *T. evansi* isolate was prepared according to the previous studies [2]. Recorded polypeptide profiles of diminazene aceturate resistant isolates of *T. evansi* revealed the presence of different bands from the previous studies which indicates the presence of variations in the isolates. Previously 8 polypeptide bands size of 25, 34, 37, 42, 53, 60, 74 and 85 kDa recorded from isolate of cattle. Similarities between the cattle isolate and present isolate was noticed at the band size of 34, 53 and 64 kDa molecular sizes [2].

Previously Laha et al. recorded the 11 dominant polypeptide bands with relative molecular weight ranging from 95 to 13 kDa. Among the 11, seven were major polypeptides with relative molecular weight ranges between 86-87, 74-75, 61-62, 51-53, 39, 34-35, 13 kDa and four minor polypeptides ranges 93-95, 46-47, 28-29, 25-26 kDa appeared as common to all *T. evansi* of different hosts origin [16].

Loss of sensitivity of a trypanosome to a drug which it had previously been susceptible is called as trypanocidal drug resistance. There is increase in the resistance of trypanosomes to the regularly used trypanocidal drugs in animals. Lack of appropriate usage and under dosing is the main predisposing factors for the development of drug resistance [17]. Diminazene aceturate was used as both trypanocidal and babesiacidal drug for domestic livestock. Because of rapidly excreted activity of Diminazene aceturate it is recommended only for therapeutic use. Diminazene binds to trypanosomal kinetoplast DNA and inhibits the synthesis of RNA primers, resulting in accumulation of replicating intermediates, there by inhibiting kDNA replication [18]. According to the previous studies, reduction in the number of trypanosomes immediately after treatment and complete clearance of trypanosomes can be noticed within eight hours of treatment with diminazene aceturate [19].

According to the previous studies, trypanosomes have developed significant resistance to the regularly used diminazene aceturate and isometamidium chloride [3]. In the present study, buffalo had the history of multiple injection of diminazene aceturate whenever suffering with non specific fever. Therapeutic dose rate of diminazene aceturate was given at the dose rate of 3.5 to 7 mg/kg by intramuscular route. But, often use dose of 3.5 mg/kg to get rid of the clinical signs, but is most likely unable to cure the infection [11]. In Japan, acquired resistance to diminazene aceturate in a cloned isolate of *Trypanosoma evansi* [15].

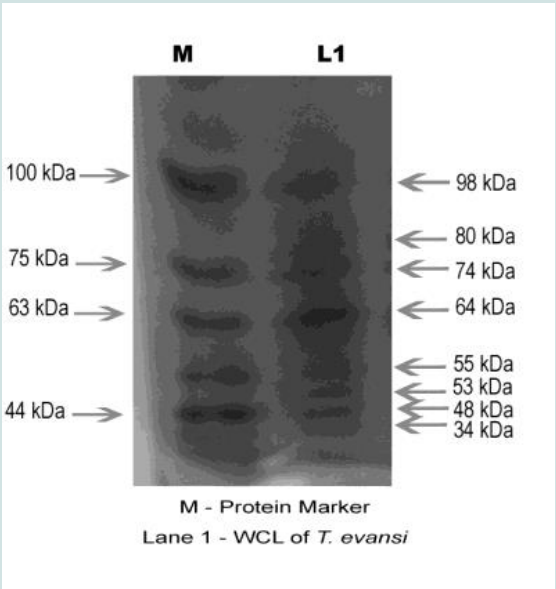

Figure **4:** Polypeptide profiles of diminazene resistant Trypanosoma evansi by SDS-PAGE.

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Contrast to the present study, in Nigeria self limiting phenomena was recorded in West African dwarf goats infected with Sokoto (Northern Nigeria) isolate of *Trypanosoma evansi* [20]. But in African countries, animal trypanocidal drug resistance has been reported and they were developed some new molecular detection tools enabling faster diagnosis of drug resistance [21]. Extensive use of the same trypanocidal drug results in appearance of trypanosome strains resistant to the drugs. Previously, in the Mindanao area of Philippines resistance to isometamidium was noticed and its therapeutic dose was increased to 20 mg/kg from its normal curative dosage (10 mg/kg). Dose rate of diminazene was also increased in the present country to 10 mg/kg [22].

In the present study, recorded diminazene aceturate resistant *T. evansi* might be due to chronic misuse of diminazene aceturate. Diminazene aceturate resistant *T. evansi* organisms were collected to record the antigenic variation by the parasites from other *T. evansi* collected in this geographical region. Significant difference was noticed at the 98 kDa molecular weight size. Present study was useful for the characterization of antigenic profile of diminazene aceturate resistant *T. evansi* and to record the antigenic variations in India. In India recently reported the diminazene aceturate resistant *T. evansi* in a farm. But, no other study was conducted on the diminazene aceturate resistant *T. evansi* organisms [23].

Previously some of the authors isolated the *Trypanosoma evansi* organisms from cattle, camel and identified the polypeptide profiles of isolates with molecular weight ranging from 195 to 26 kDa from cattle isolates and molecular weight ranging from 180 to 24 kDa from camel isolates [24]. By conducting the Western blotting, different polypeptides of *T. evansi* with molecular weight ranging from 74 to 38 kDa was identified in experimental animals include bovines, donkeys, dogs and coatis. Among the all 48-46 and 38 kDa bands were mainly recognized in chronic phase of infection. The antigen with apparent molecular weight of 66 kDa, was revealed by antibodies from all experimental animals. According to their study, 48-46 kDa polypeptide was identified by antibodies from all naturally infected animals [25]. In the present study, identified the 48 kDa molecular weight polypeptide indicative of chronic type of infection and molecular weight 64 kDa was noticed which was common in all the *T. evansi* infected animals.

Diamidines molecules bind to the minor groove of DNA at AT-rich sites. They exert their biological activity by primarily binding to DNA and then inhibiting one or more of the DNA dependent enzymes or by directly impeding the transcription process. The selectivity of diamidines is primarily due to the selective accumulation by the pathogen rather than by host cells. Diminazene aceturate do not cross the blood-brain barrier [26]. Diamidines are actively taken up by transporters; alterations of the transporters can cause development of drug resistance. There are numerous reports of resistance to diminazene aceturate in different countries and in several *Trypanosoma* species. In any case, resistance seems to be limited to highly endemic areas where the use of this drug is very common. Cross resistance to diminazene aceturate with isometamidium and pentamidine have been reported [27]. Drug resistance to diminazene aceturate is less widespread than isometamidium, but increasingly there are reports of multiple drug resistance. Importance of the P2-type purine transporter in the uptake of arsenical diamidines by *T. evansi* and the consequences of inhibition was described and a novel gene, *Te*DR40, might be a factor contributing to high diminazene aceturate-resistance in *T. evansi*.

Conclusion

In conclusion, the *Trypanosoma evansi* isolate was resistant to diminazene aceturate and showed the different protein profile when compare with the previous studies.

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