Polypeptide profiles of South Indian isolate of *Trypanosoma evansi*

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Abstract The field isolates of *Trypanosoma evansi* was collected from the infected cattle and it was propagated in rats. *Trypanosoma evansi* parasites were separated from the blood of infected rats by using diethylaminoethyl cellulose column chromatography. Whole cell lysate antigen (WCL) was prepared from purified trypanosomes by ultrasonication and centrifugation. The prepared WCL antigen was further purified by 50 % ammonium sulphate precipitation. Protein concentration of WCL antigen of *T. evansi* was 60 mg/ml. Protein concentration was adjusted to 1.0 mg/ml in PBS, pH 8.0 and stored at -20°C . Polypeptide profiles of WCL antigen of *T. evansi* was determined by sodium dodecyl sulphate polyacrylamide gel electrophoresis. A total of eight polypeptide bands of the size ranging from 25 to 85 kDa in WCL antigen of *T. evansi* were obtained. Five prominent bands with molecular weight of 74, 60, 53, 42 and 37 kDa and three light bands with molecular weight of 85, 34 and 25 kDa were observed.

Keywords Cattle · Polypeptide profiles · SDS-PAGE · *Trypanosoma evansi* · Whole cell lysate antigen

Introduction

Trypanosomosis or Surra caused by *Trypanosoma evansi* which is one of the important vector borne diseases occurring in tropical and subtropical countries including India. Trypanosomosis ranks high in importance amongst animals due to its devastating effects on the livestock health leading to severe economic losses to the dairy industry (Laha et al. 2008). *Trypanosoma evansi* causes anaemia, leucopaenia, abnormal changes in serum biochemical parameters and histopathological changes in experimental and natural infections (Sivajothi et al. 2013a, 2014a, 2014b). Diagnosis of trypanosomes by direct parasitological techniques is not reliable, especially for naturally occurring field cases, where parasitaemia is often low and sporadic. Serological tests for circulating antibody, ELISA and IFAT, have been intensively employed, showing good sensitivity (OIE. 2008; Sivajothi et al. 2012; Sivajothi et al. 2013b). But antibody detection assays are often hampered by the lack of specificity, the result of strong cross-reactions with other pathogenic trypanosomes (Uzcanga et al. 2002). Antigen detection ELISA have been used in various animal species (Kashiwazaki et al. 1998). However, proper evaluation of the diagnostic parameters in these assays showed poor results (Davison et al. 1999). Surra has a wide host spectrum, the main host species varies with the geographical region. The disease is most severe in horse, donkey, mules, camels, dogs and cats. Within the hosts, the chemical composition of outer layer the variable surface glycoprotein (VSG) of *T. evansi* changes throughout the course of infection. The parasite has

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the ability to undergo such antigenic changes frequently and is known as antigenic variation. As intra species differences in terms of antigenicity of *T. evansi* of different stocks have been established (Zheng et al. 1990). Isolates from different geographical origins might undergo some variations during the stages of evolution, therefore Jithendran and Rao (2001) advocated for a need to analyze various *T. evansi* stocks from various geographical regions. So the present investigation was planned to study the polypeptide profiles of *T. evansi* isolated from cattle of southern region of India by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE).

Materials and methods

Preparation of WCL Ag of *T. evansi*

A cattle isolate of *T. evansi* was maintained in the Wistar rats for bulk harvest of parasites for various applications. At the height of parasitaemia, the rats were bled by heart puncture and the blood sample was immediately diluted with chilled phosphate buffered saline glucose (PBS-G), pH 8.0. The separation and purification of *T. evansi* from infected blood was accomplished by using diethyl aminoethyl cellulose (DEAE) anion exchange column chromatography. Purified Trypanosomes were used for preparation of Whole cell lysate antigen (Fig. 1). The whole cell lysate antigen (WCL Ag) was partially purified after precipitating with 50 percent saturated ammonium sulphate followed by extensive dialysis against PBS, pH 7.4. Protein concentration of the WCL Ag of *T. evansi* prepared in the present study was estimated (60 mg/ml). Protein concentration was adjusted to 1.0 mg/ml in PBS, pH 8.0 and stored at -20°C in 1.0 ml aliquots (Sivajothi et al. 2014c). Polypeptide profile of WCL of *T. evansi* was determined by SDS-PAGE as described by Laemmli (1970).

Gel preparation procedure

The vertical slab gel unit was assembled in the casting mode with 1 mm spacers. The resolving gel solution was prepared (Table 1) and mixed well and poured into the sandwich to a level of 4 cm from the top. Then 3 ml of distilled water was gently added along the wall of the sandwich to form uniform gel surface after polymerization. A very sharp water gel interface will be visible when the gel is polymerized. The water layer was poured off and wiped out using filter paper. Stacking gel was prepared (Table 1) and overlaid on the resolving gel. The comb was inserted into the sandwich of stacking gel and was allowed to polymerize for 15 min.

Fig. 1 *Trypanosoma evansi* parasites purified by DEAE cellulose column chromatography and observed after Leishman's staining (1,000X)

Sample preparation

50 μl of sample (WCL of *T. evansi*) was mixed with equal volume of Laemmli's sample buffer and boiled for 3 min in a water bath and cooled rapidly.

Loading and running the gel

The lower and upper chambers of the tank were filled with electrode buffer. The comb was slowly removed from the gel after polymerization without any disturbance to the well dividers. The wells were loaded with the samples and marker protein by using a Hamilton syringe. The electrophoresis unit was connected to the power pack and the voltage was initially adjusted from 60 to 100 V. The power supply was disconnected when the tracking dye reached the other end of running gel.

Staining and destaining

The gel mould was carefully disassembled after completion of electrophoresis and the gel was immersed into the staining trough containing Coomassie brilliant blue R-250 staining solution (CBBR) for 2 h. Later the gel was destained in destaining solution until the background became clear. After destaining the gel was transferred to storage solution.

Table 1 Composition of the resolving and stacking gel solutions for SDS-PAGE

Stock solutions	12 % resolving gel (10 ml)	4 % stacking gel (5 ml)
30 % Acrylamide	4 ml	0.65 ml
1.5 M Tris-HCl, pH 8.0	2.5 ml	–
1 M Tris-HCl, pH 6.8	–	1.25 ml
Distilled water	3.33 ml	3.02 ml
10 % SDS	100 μ l	50 μ l
1 % APS	50 μ l	25 μ l
TEMED	20 μ l	10 μ l

Molecular weight determination

The markers used for electrophoresis were standard molecular weight markers (MWM) (Genei, Bangalore) with molecular weights ranging between 98 and 16 kDa. The distance migration from the origin to the middle of each protein band of samples and marker proteins were measured. A curve was drawn by tacking distance migration on X-axis and molecular weights on Y-axis for marker proteins on semi log graph paper. A straight line was drawn passing through maximum points. The molecular weight of the proteins was calculated by this standard curve.

Results and discussion

Polypeptide profiles of WCL antigen of *T. evansi* determined by SDS-PAGE are shown in Fig. 2. Approximate molecular weights (kDa) of polypeptides of WCL of *T. evansi* are presented in Table 2. A total of eight polypeptide bands of the size ranging from 25 to 85 kDa in WCL of *T. evansi* were obtained. Five prominent bands with molecular weight of 74, 60, 53, 42 and 37 kDa and three light bands with molecular weight of 85, 34 and 25 kDa were observed.

Trypanosoma evansi causes severe economic losses in India. Different diagnostic methods were developed to diagnose *T. evansi* in the animals at the field level also (Sivajothi et al. 2012; 2013b; 2014c). The variation of the antigen can cause development of variable severity of the infection and changes in the development of the symptoms. Differences in antigen expression among parasite isolates or immunogenetic variations among hosts could determine the ability for antigen recognition by the various animal species. The identification and characterization of pool of antigens could serve not only for diagnosis, but also for prophylaxis and chemotherapy against animal Trypanosomosis. The present study identified the

Fig. 2 Polypeptide profile of *Trypanosoma evansi* by SDS-PAGE. Lane 1 and 2: WCL of *Trypanosoma evansi*. Lane M: Protein marker

Polypeptide profiles of WCL antigen of *T. evansi* which was isolated from the cattle suffering with clinical *Trypanosomosis*. Polypeptide profile of WCL was resolved in the SDS-PAGE. A total of 8 polypeptides in WCL could be detected in Coomassie's brilliant blue R-250 stained gel. The approximate molecular weights of these polypeptides (8) were ranged from 25 to 85 kDa. Laha et al. (2008) observed eleven polypeptides with molecular weights from 13 to 95 kDa from WCL of *T. evansi* of cattle origin. Rayulu (2008) found polypeptides (16) ranged from 11.93 to 91.31 kDa in WCL of *T. evansi* field isolate of cattle. Singh et al. (1994) found 13–14 major bands in the range of 14.6–102 kDa in seven different stock of *T. evansi* collected from three buffaloes, two horses, one mule and one camel. Eight identical polypeptide bands of camel and cattle ranging from 26 to 110 kDa were reported by Pareek et al. (1999). Size range between 14 and 65 kDa of WCL of camel isolate of *T. evansi* was reported by Pathak et al. (1993). Giardina et al. (2003) observed 25 polypeptides of molecular weight between 97 and 14.8 kDa. The present findings reported in the study are more or less in agreement with the findings of these authors. The parasites were maintained separately in rats by serial passage, hence relapse of parasitaemic waves were expected to be different for three different isolates. But in the present study similarity in polypeptide profiles were observed which might be due to nonsignificant antigenic diversity between stocks of *T. evansi*.

Table 2 Approximate molecular mass (kDa) of polypeptides in WCL antigen of *Trypanosoma evansi*

Polypeptide band No	Polypeptide size (kDa) in <i>T. evansi</i> whole cell lysate
1	25
2	34
3	37
4	42
5	53
6	60
7	74
8	85
Total no. of bands	8

However the variations in the profiles of the polypeptides of *T. evansi* preparations might be due to not only the strain variation but also antigenic variation of different isolates in different species. Further this may also due to variations in the technique followed and possibly due to some degradation of the isolates studied. The differences might be due to parasite factors, which include the amino acid sequence on the surface of the parasite, which is reported to be influenced the exposure as well as physical differences in between different parasite environment. This might affect the release of the soluble form of VSG.

Conclusion

It can be concluded from the results of the present study that antigenic variation due to change in VSG of *T. evansi* is not a constant phenomenon. The consistent antigenic characterization of the protein would indicate it as a good candidate to be assessed for diagnosis purposes. However, further studies must evaluate additional parameters related to the proposed antigen before it may be considered a reliable tool in serodiagnosis of *T. evansi* infection in animals.

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