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Comparative polypeptide profiles of whole cell lysate antigens of *Trypanosoma evansi* isolated from three different hosts of eastern India

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ABSTRACT

Trypanosoma evansi from buffalo, cattle and horse isolates were purified by Diethylaminoethyl cellulose column chromatography. The purified trypanosomes from each sources were used separately for preparation of whole cell lysate antigens. A comparative study on polypeptide pattern of whole cell lysate antigens of these three isolates of *T. evansi* was studied. It showed a total of 11 dominant polypeptide bands with relative molecular weight ranging from 95 to 13 kDa, when resolved in 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis and stained with Coomassie brilliant blue. Except some minor differences between the relative molecular weight of isolates in a particular region, the polypeptide profiles of these three isolates did not resolve any significant differences between them. Seven major polypeptides of 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 and these were shared by all the isolates. A doublet band of relative molecular weight ranging between 86-87 kDa and 74-75 kDa appeared in the area between 66 and 97 kDa and a doublet band of relative molecular weight 39 kDa and ranges 34-35 kDa also appeared in the area between 43 and 29 kDa in these three isolates. It is opined from the result that antigenic variation due to change in VSG of *T. evansi* is not a constant phenomenon. It is relative, depending on some intrinsic factors related to the physiological environment of host and parasite.

Key words: buffalo; cattle; horse; polypeptide profiles; *Trypanosoma evansi*

INTRODUCTION

The haemoprotozoan parasite, *Trypanosoma evansi,* is the causative agent of one endemic disease in domestic animals popularly known as ‘surra’ in India. The disease is widely prevalent throughout India and causes huge economic loss to the livestock owners (Seidl *et al.,* 1998). 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 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) and isolates from different geographical origins might undergo some variations during the stages of evolution, therefore Jithendran and Rao (2001) advocated for a need to

analyse various *T. evansi* stocks from various geographical regions. In the present plan of work, the polypeptide profiles of three isolates of *T. evansi* isolated from buffalo, cattle and horse of eastern region of India, have been planned to be studied by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) to evaluate the antigenic diversity of the whole cell lysate (WCL) antigens, if any. This type of study simultaneously involving three different isolates from three different hosts buffalo, cattle and horse, have not yet been done before.

MATERIALS AND METHODS

Isolation of *Trypanosoma evansi* from different hosts

Isolates of *T. evansi* were obtained from naturally infected cattle, buffaloes and horses of different geographical regions of West Bengal, India. Then the parasites were expanded by inoculating 0.5 ml of such infected blood intraperitoneally into six adult Swiss albino mice (two mice for each host origin). Further expansions of the parasites were carried out by inoculating 0.25 ml of infected mice blood containing about 10^8 numbers of parasites into other rats. The same method was followed for expansion of trypanosomes of three different hosts like cattle, buffalo and horses, separately. When the number of parasites reached about 10^6 - 10^8 trypanosomes per ml, the blood was extracted from the rats by cardiac puncture using Ethylene diaminetetra acetic acid (EDTA) as anticoagulant. The concentration of parasites was determined by counting in the Neubauer haemocytometer.

Trypanosomes were separated from the blood by anion-exchange chromatography using a Diethylaminoethyl (DEAE) cellulose column (Lanham and Godfrey, 1970), with phosphate-saline-glucose (PSG) solution, pH 8.0, as eluting buffer. Parasites eluted from the column were collected by centrifugation @10,000 rpm. for 15 minutes, at 4^0C and were washed 3 times with PBS (pH 7.4). The final cell pellet was suspended in PBS (pH 7.4) and was kept frozen at -70^0C until further use.

Preparation of whole cell lysate antigens

The WCL antigens (Ags) of *T. evansi* separated from different hosts were prepared following the standard method of Giardina *et al.* (2003). In brief, samples containing *T. evansi* of different hosts origin were suspended in electrophoresis sample buffer (ESB) containing 50 mmol/L Tris HCL, pH 7.5, 2.0% SDS, 2.0% B-mercaptoethanol, 15% glycerol, 0.01% bromophenol blue) separately, boiled for 5 min and cooled on ice. The supernatant was used as WCL antigen of *T. evansi.*

Polypeptide profiles of WCL preparations of trypanosomes of buffalo, cattle and horse origin were analysed by SDS-PAGE containing 10% gel, with a 5% polyacrylamide stacking gel (Laemmli, 1970) under protein denaturing condition. All gels were run at constant current (20 mA/gel), using Tris-glycine (pH 8.3) as running buffer. A protein mixture containing proteins of 205.0, 97.0, 66.0, 43.0, 29.0, 20.0 and 14.0-kDa molecular weight was used as molecular weight marker protein standards (PMWB1, Bangalore Genei) and were run simultaneously.

The gel apparatus was connected to the power pack and carried out at constant current until the tracking dye reached the positive pole i.e. when the tracking dye front reached approximately 5 mm above the bottom edge of the gel. After completion of run, power supply was switched off; plate was removed and separated gently with the help of a scalpel. The stacking gel was then removed and the gel was stained with

Coomassie brilliant blue. The polypeptides appeared in respective lanes of cattle, buffalo and horse isolates were compared with standard molecular weight protein marker.

The molecular weights for the trypanosomal proteins that were resolved by SDS-PAGE were estimated by a Soft ware programme named KODAK 1D Image Analysis Software.

Table 1. Polypeptide profiles of crude whole cell lysate antigens of *T. evansi* isolated from buffalo, horse and cattle origin resolved in 10% SDS-PAGE and stained with Coomassie brilliant blue

Standard Molecular Wt. Marker (kDa)	Buffalo	Horse	Cattle	Range
205	95	93	95	93-95
97	**87**	**87**	**86**	**86-87**
66	**75**	**75**	**74**	**74-75**
43	**61**	**62**	**61**	**61-62**
29	**51**	**53**	**51**	**51-53**
20	46	47	46	46-47
14	**39**	**39**	**39**	**39**
	34	**35**	**35**	**34-35**
	28	29	28	28-29
	26	26	25	25-26
	13	**13**	**13**	**13**
Total	11	11	11	

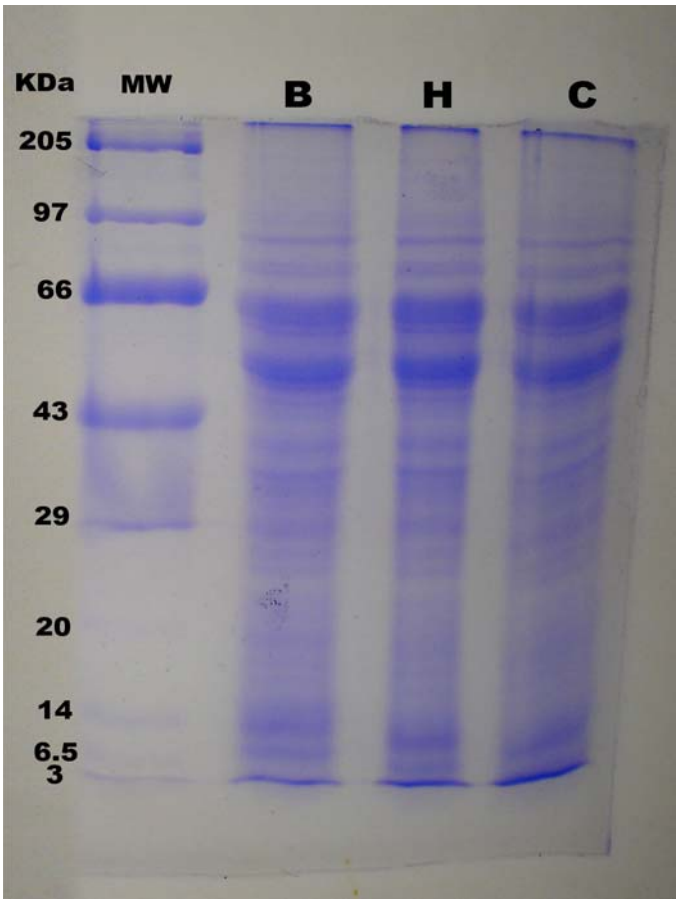

Fig. 1. Polypeptide profiles of crude whole cell lysate antigens of *T. evansi* isolated from buffalo (B), horse (H) and cattle (C) origin resolved in 10% SDS-PAGE and stained with Coomassie brilliant blue

RESULTS AND DISCUSSION

The comparative polypeptide profiles of WCL Ags prepared from the *T. evansi* of buffalo, horse and cattle origins have been shown in Table 1 and Fig. 1. Trypanosomes of buffalo, cattle and horse origin showed total 11 polypeptides when resolved in 10% SDS-PAGE and stained with Commassie brilliant blue, with relative molecular weight *(M_r)* ranging from 95 to 13 kDa. M_r of 11 polypeptides resolved from *T. evansi* of buffalo origins were 95, 87, 75, 61, 51, 46, 39, 34, 28, 26, and 13 kDa. Out of which seven polypeptides i.e. 87, 75, 61, 51, 39, 34, and 13 kDa were resolved as major polypeptides while rest 4 were considered as minor polypeptides. M_r of 11 polypeptides resolved from *T. evansi* of horse origins were 93, 87, 75, 62, 53, 47, 39, 35, 29, 26 and 13 kDa. Out of which seven polypeptides of M_r 87, 75, 62, 53, 39, 35, and 13 kDa were resolved as major polypeptides and rest 4 were minor polypeptides. Eleven polypeptides of M_r 95, 86, 74, 61, 51, 46, 39, 35, 28, 25 and 13 kDa were resolved from *T. evansi* of cattle origins. Among them seven polypeptides of M_r 86, 74, 61, 51, 39, 35, and 13 kDa were resolved as major polypeptides and rest 4 were considered as minor polypeptides.

Hence, from the present findings it is observed that there is a similarity in respect of number of polypeptide bands resolved in 10% SDS-PAGE of WCL Ags of *T. evansi* isolated from buffalo, horse and cattle origins, although there were minor differences between the M_r of individual polypeptides resolved in a

particular region. But, instead of analyzing specific molecular mass of polypeptide profiles, if we consider the distribution of polypeptide bands in certain ranges, then comparative polypeptide profiles of WCL preparations of *T. evansi* Ags, isolated from the parasites of buffalo, horse and cattle origin revealed the distribution of seven major polypeptides in the M_r ranges of 86-87, 74-75, 61-62, 51-53, 39, 34-35 and 13 kDa. And the distribution of four minor polypeptides in the M_r ranges of 93-95, 46-47, 28-29 and 25-26 kDa. A doublet band of M_r range 86-87 kDa and 74-75 kDa appeared in the area between 66 and 97 kDa for all these three isolates. In the area between 43 and 29 kDa, a doublet band of M_r ranged 39 kDa and 34-35 kDa were also observed in these three isolates.

Analysis of polypeptides in 10% SDS-PAGE of WCL Ags prepared from buffalo, cattle and horse isolates revealed a complex protein band pattern with the appearance of almost similar molecular weight ranged polypeptides as common to all *T. evansi* of different hosts origin. Hence, from the present findings it could be concluded that the comparative polypeptide profiles of the cattle, buffalo and horse isolates of *T. evansi* did not show any significant differences between them when WCL Ags were resolved in 10% SDS-PAGE separately. Most of the polypeptides were equally shared by all three isolates.

The parasites were maintained separately in mice 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*. Thus it is different from tsetse transmitted trypanosomes as supported by Jithendran and Rao (2001). In cloned *T. evansi* populations of buffalo origin they observed some degree of serological cross reactivity within a high degree of variable antigenic composition. They suggested that it might represent a state of limited diversity and there is the need to analyse large number of *T. evansi* stocks from different geographical regions in the Indian subcontinent. Follow's their recommendations in the present study non-significant diversity in polypeptide profiles of *T. evansi* isolated from three different Indian hosts have been observed. Using crude sonicated Ags of *T. evansi* of cattle and camel isolates of northern India, Pareek *et al.* (1999) observed eight polypeptide bands common in both isolates in 12% SDS-PAGE. This is in conformity with the present findings of polypeptide profiles of *T. evansi,* isolates from three different hosts.

Irrespective of different hosts, trypanosomes isolated from the same geographical zone, they showed similarity in polypeptide profiles when resolved by 10% SDS PAGE. Although a few very minor differences in respect of kDa were observed between the isolates of trypanosome (Table 1). This 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 variable surface glycoprotein (VSG) as also expressed by various workers (Uche, 1992). Using the same method of preparation of Ags, Giardina *et al.* (2003) observed 25 polypeptides of MW between 97 and 14.8 kDa, but they did not used *T. evansi* from different hosts. Although they did not mention the *Mr* of all 25 polypeptides, but it seems that 97, 72, 62-64, 57, 53, 48, 42, 40, 37, 31, 30, 27, 22 and 14.8 kDa polypeptides were resolved by them and most of them are in close conformity with the polypeptides appeared in the present study. The difference in geographical distribution and thereby strain variation might be responsible for this difference. A doublet appeared in between 66 to 97 kDa and 29 to 43 kDa as observed in the present study are in agreement with them. 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. It is relative, depending on some intrinsic factors related to the physiological environment of host and parasite.

REFERENCES

Giardina, S., Paganico, G., Urbani, G. and Rossi, M. 2003. A Biochemical and Immunological Comparative Study on *Trypanosoma equiperdum* and *Trypanosoma evansi*. Vet. Res. Commun. 27: 289-300.

Jithendran, K.P. and Rao, J.R. 2001. Antigenic characterization of blood stream variant of an Indian isolates of *Trypanosoma evansi* from buffaloes. J. Vet. Parasitol. 15: 17-23.

Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227: 680-685.

Lanham, S.M. and Godfrey, A.G. 1970. Isolation of salivarian trypanosomes from man and other mammals using DEAE-cellulose. Exp. Parasitol. 28: 521-534.

Pareek, R.K., Pathak, K.M.L., Kumar, S., Garg, R. and Kapoor, M. 1999. Polypeptide profile of *Trypanosoma evansi* isolates of camel and cattle origin. J. Vet. Parasitol. 13: 53-54.

Seidl, A., Moraes, A.S., Aguilar, R. and Silva, M.S. 1998. A financial analysis of treatment strategies for *Trypanosoma evansi* in Brazilian Pantanal. Prev. Vet. Med. 33: 219-234.

Uche, E.1992. Identification of the surface components of *Trypanosoma evansi*. Res. Vet. Sci. 53: 252-253.

Zheng, R.J., Sheng, J., Qui, Q.P. and Wang, H.A. 1990. Comparison of protein components and antigenicities of various strains of *Trypanosoma evansi* from Zhejiasg, Yunan and Anhui provinces. Chinese J. Vet. Sci. Technol. 2: 7-9.