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Purification of *Trypanosoma evansi* from mice blood by anion exchange (Diethylaminoethyl cellulose) column chromatography

M Fibi Rani, GS Sreenivasamurthy, M Udaya Kumar and P Kalyani

Abstract
In the current study, a dog strain of *T. evansi* was *in-vivo* passaged in Swiss albino mouse for propagation. 3 DPI, parasitaemia was confirmed by examination of wet blood film of the mouse. Pure and viable *Trypanosoma evansi* were separated from the mouse blood by anion exchange chromatography using the DEAE cellulose minicolumn and were counted using Neubauer's haemocytometer. This is the best method for the purification of *T. evansi* that permits further serological and molecular research investigations.

Keywords: purification, *Trypanosoma evansi*, DEAE cellulose, chromatography, Neubauer's haemocytometer

Introduction
Trypanosoma evansi, an extracellular flagellate is a significant and widely prevalent haemoprotozoa that affects a broad range of domestic and wild animals causing "Surra". It is transmitted mechanically by *Tabanus, Stomoxys, Haematobia* (Radwanska *et al.*, 2018) [1], orally by ingestion of fresh infected meat in carnivores and iatrogenic transmission occurs by using infected syringes. American vampire bat acts as a vector as well as reservoir host (Brun *et al.*, 1998) [2]. Rats and mice are highly susceptible as experimental hosts for detection of subclinical infections (Reid *et al.*, 2001) [3]. Surra is characterized by intermittent fever, loss of appetite and body weight, production losses, nervous symptoms, cachexia and death. Abortions, immunosuppression and increased susceptibility to secondary infections may also be recorded in some cases (Mansfield and Wallace, 1974) [4]. Dogs are highly susceptible to *T. evansi* infection and exhibit signs of intermittent fever, inappetence, reduced body weight, pale mucous membranes, laryngeal edema, corneal opacity, hoarse voice, Hypersalivation, cachexia and hindlimb weakness. Alterations in haemato-biochemical profiles like hypoalbuminemia, hyperglobulinaemia, hyperkalemia and hypoglycemia may be reported (Sarvanan *et al.*, 2005) [5]. Many diagnostic techniques are available for the diagnosis of Surra. Purification of trypanosomes from the blood by minicolumn of DEAE cellulose, followed by centrifugation and microscopic examination is the best concentration and visualizing method that enables laboratory investigations (Lumsden *et al.*, 1979) [6].

Protocol
Preparation of equilibrated slurry and DEAE-cellulose column
Purification of *T. evansi* from host blood components by mini anion exchange column chromatography as suggested by earlier worker [7] with slight modifications. The equilibrated slurry was prepared by suspending 6 g of DEAE cellulose in 25 ml of Phosphate saline glucose buffer (pH 8) and kept undisturbed for 1 hr. 30 mins. Supernatant was decanted and sediment was washed four times with PSG buffer. Later suspension was filtered through No.1 Whatman filter paper and washed. Finally, slurry on the filter paper was collected carefully into a beaker and 25 ml buffer was added, mixed thoroughly and stored at -20 °C.
Glass wool was kept at the bottom of the syringe without a plunger. Equilibrated slurry was mixed thoroughly, loaded uniformly up to 4 cm height into 5 ml syringe and washed twice with elution buffer for equilibration.

Propagation of dog strain of *T. evansi* in mice

Blood was collected aseptically from an ear vein of a mongrel dog which was found positive for *T. evansi* by wet blood film examination and 0.2 ml of positive blood was passaged to the Swiss albino mouse through intraperitoneal inoculation for the propagation of trypanosomes (prior permission was taken from IAEC). The mouse was monitored regularly for the development of parasitaemia by examination of wet blood film. After 72 hrs. of inoculation, peak parasitaemia was noticed, infected mouse was anaesthetized and blood was collected from the heart into a heparinized vacutainer.

Purification and enumeration of *Trypanosoma evansi*

Mouse blood was immediately diluted with chilled PSG buffer in 1:3 ratio in the vacutainer. Diluted blood was charged onto the column and elution buffer was added according to the transit of trypanosomes. Elute was collected into micro centrifuges tubes (fig.1) and examined under 45X for presence of pure trypanosomes (fig.2). Fractions of elute containing *T. evansi* were pelleted at 2,400 g at 4 ℃ and washed twice with PBS. Finally, pellet of pure trypanosomes was resuspended in PBS and stored at -20 ℃ for next laboratory investigation.

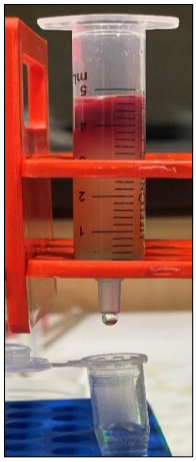

Fig 1: DEAE-cellulose minicolumn for purification of *T. evansi* from mouse blood

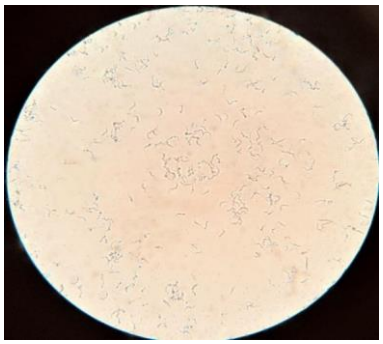

Fig 2: Wet mount examination of elute showing pure, live & motile *T. evansi* (45X)

Pellet of pure trypanosomes was mixed with 1ml of PBS to get a uniform suspension. 10-fold and 100-fold dilutions were made by mixing 10 µl trypanosome suspension with 90 µl trypanosome staining solution (Swathi, 2014) [8]. 10 µl of these dilutions were loaded onto both chambers of Neubauer's haemocytometer and were counted in WBC counting chambers under 45X (fig.3&4). The mean harvest of trypanosomes was 2×10^8 trypanosomes/ ml of elute.

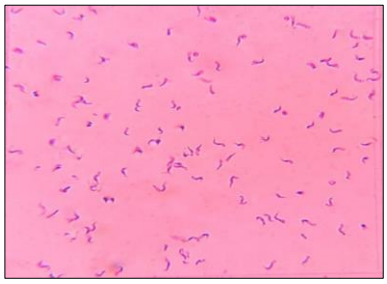

Fig 3: Enumeration of 10-fold dilution of trypanosomes stained by trypanosome staining solution (Neubauer's haemocytometer-45X)

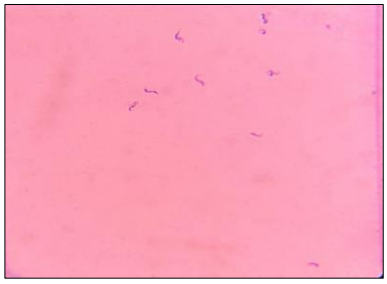

Fig 4: Enumeration of 100-fold dilution of trypanosomes stained by trypanosome staining solution (Neubauer's haemocytometer-45X)

Discussion

Live, motile and pure *T. evansi* were isolated from mouse blood by passing through a DEAE Cellulose, mini anion exchange column. Preparation of DEAE cellulose is a vital step in this process. Washing should be done cautiously to remove fine particles and equilibrate the resin and pH must be adjusted to 8 precisely. All these steps may improve the purification, yield and viability of trypanosomes (Courtois *et al.*, 2019) [9]. Separation of trypanosomes from the blood mainly depends on differences in the surface charges of blood components, trypanosomes and cellulose column. Trypanosome surface charge is less negative than mammalian blood cells. When the infected blood was layered over a mini anion-exchange column and elution buffer (pH 8) was added, more negatively charged blood components were completely adsorbed onto column (positively charged), while the less negatively charged *T. evansi* were eluted out, as previously reported by Lanham and Godfrey, 1970 [7]. Process of separation of pure *Trypanosoma evansi* in our current study was in accordance with the earlier workers [8, 10, 11] who isolated *Trypanosoma evansi* from the mice blood using the DEAE cellulose column chromatography. Around 2×10^8 trypanosomes/ml of elute were counted using Neubauer's haemocytometer, was also observed by Swathi, 2014 [8].

Conclusion

Pure trypanosomes extracted from experimentally infected mice by Diethylaminoethyl Cellulose column chromatography represent a powerful means for further molecular and serological research.

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