INTRODUCTION

1.1. Introduction

*Trypanosoma*, a flagellated parasitic protozoon with corkscrew-like motion was derived from the Greek *trypano* (borer) and *soma* (body). *Trypanosoma evansi* is known for causing disease termed as ‘trypanosomosis’ and is mostly transmitted through a vector such as, *Tabanus*, *Stomoxys*, and *Lyperosia*. Trypanosomes are mainly distributed in South America, Asia and inter Tropical Africa and affect wide variety of hosts such as, humans and animals. A broader range of trypanosome species including *Trypanosoma brucei*, *Trypanosoma evansi*, and *Trypanosoma equiperdum* causes trypanosomosis in humans and animals. The fatal Human African Trypanosomosis (HAT) or sleeping sickness is caused by two distinct sub species of *Trypanosoma brucei* named *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* and is transmitted by haematophagus biting flies through blood meal. This is an endemic disease in South America and Africa. Further, Chagas disease or American trypanosomosis is caused by *Trypanosoma cruzi* and is transmitted by the infected feces of reduviid bugs.

The transmission of trypanosomes are either cyclical or mechanical (non-cyclical) and are transmitted mainly by blood sucking insects from one vertebrate host to another. Cyclical transmission within the insects may culminate in two sites, the foregut also known as ‘anterior station development’ and hindgut also known as ‘posterior station development’. Members of the subgenus ‘Trypanozoon’ have considerable potential for antigenic variation as the surface antigens change in every few days resulting specific antibodies produced by the host to be ineffective. These molecules are named as variant surface glycoprotein (VSG) and cover the entire surface of trypanosomes. Trypanosomes cause severe and prolonged infection due to their unique ability to evade the immune response of the vertebrate host by altering antigenic composition of their surface glycoprotein coat.

*Trypanosoma evansi*

*Trypanosoma evansi* was identified by Griffith Evans (1880) and was the first trypanosome to be pathogenic for mammals. The disease caused by *Trypanosoma evansi* is called ‘Surra’ or trypanosomosis. ‘Surra’ has been derived from the Hindi word ‘rotten’ and is used for infection in all kinds of hosts. The infection is called ‘Tibersa’ in camels in India, ‘Delebab’ in Algeria, ‘Gufar’ in Chand and ‘Mbori’ in Sudan. Infection in
horses is called ‘Murrina’ in Panama and ‘Derrengad’ era in Venezuela. *Trypanosoma evansi* has wide geographical distribution in India, Malaysia, Pakistan, South America, Indonesia, Central America, southern parts of China, northern Africa, Russia, Egypt, Asia Minor, Israel, Somalia, Syria, Iraq, Mauritius, Turkey, Iran and Bulgaria.

*Trypanosoma evansi* affects wide range of domestic and wild hosts including camel, donkey, horse, cattle, buffaloes, pig, goat, elephant, mule, sheep, dog, deer, tiger, lion, fox etc. Cattle and water buffaloes are considered to be the main reservoirs of the infection for equines. Debilitated and young animals suffer severely. Moreover, buffaloes have higher incidence of infection than cattle and the infection is also severe in horses and dogs. The affected animals in acute disease appear dull and sleepy with hard and sleepy breathing, eyes staring and wide open, nervous excitement, apparent blindness, bellowing, groaning, and shivering of the body followed by coma, circulating movements collapse and finally death in 6-12 hours. In chronic and sub acute cases, the animals are sleepy, dull and have bilateral lacrimation. There are intermittent attacks of fever, progressive emaciation, oedema of legs, rapid pulse, diarrhea and death from exhaustion. The parasitaemia is usually low during the course of the disease. Abortions have also been reported in buffaloes. Moreover, now *T. evansi* is not only restricted in animal hosts but also reported its adaptations in human host (Joshi *et al.*, 2005), leading to its importance of zoonotic potentiality.

*Trypanosoma evansi* is mechanically transmitted by the biting flies e.g. species of *Tabanus, Stomoxys, Lyperosia, Haematopota, Chrysops* and *Hippobosca*. The animals acting as a carrier or suffering from the disease are the usual source of infection. In India, *T. evansi* infections were found to be common in the environmental areas where the breeding of insect vectors like tabanid flies are most prevalent. The occurrence of the infection also coincides with rain, floods and inundations. Incidence of the infection has been reported from West Bengal, Maharashtra, Assam, and Madhya Pradesh along with worst affected areas such as Punjab, Uttar Pradesh, Gujarat and Rajasthan. The incidence of the infection is considerably low in other states. The disease is more prevalent from the ends of August and remains till mid-winters. India possesses a significant number of domestic animal population – e.g. buffalo population (108.70 million), sheep population (65.07 million) and goat population (135.17 million). While the cattle population (190.90 million) is the second largest in the world. Camel, equine (horses and ponies) and pig population of the country is respectively, 0.40, 0.62 and 10.29 million (DADF 2014).
Besides, India also houses a large number of wild & domestic carnivores and herbivores and the exact economic loss due to ‘surra’ has not been estimated so far. In earlier study it has been shown that if executed properly the benefit for a typical village in a moderate/high risk area can be equivalent to ₹ 96,00,000/ year/ village as the impact of the disease on host fertility and mortality are key factors in determining the economic losses and the net-profit incurred from the control regimes (Dobson et al., 2009). In terms of the value added to the domestic livestock this was estimated to be ₹ 5280, ₹ 5040, ₹ 9060, ₹ 420, ₹ 6840 per animal/year for buffaloes, cattle, horses, goats/sheep and pigs, respectively (Dobson et al., 2009).

The animal serves as a carrier of the disease for years by exhibiting low levels of fluctuating parasites even after recovery. Many diagnostic tools have been developed in the past for the detection of carrier status of infection for trypanosomosis such as, several serological test like indirect fluorescent antibody test (IFAT) or enzyme linked immunosorbant assay (ELISA) (Sengupta et al., 2014; Ligi et al., 2015; Ligi et al., 2016a), nucleic acid detection by PCR (Sengupta et al., 2010; Rudramurthy et al., 2013), card agglutination test (CATT/T.evansi) and parasitological diagnostic tests like thick or wet blood smear examination tests. The improved serological tests using monoclonal antibodies against trypanosome antigens for the detection of antigen/antibody have been used (Ligi et al., 2016b). Further, the diagnostic techniques have their own limitations when applied to field. (Hide et al., 1991).

Diagnosis and treatment of trypanosomosis for the carrier and infected animals play a very important role in the control regime programme. Trypanosomosis can be controlled effectively by treating the infected animals with the trypanocidal drugs such as suramin, Iso-metamidium chloride, diminazene aceturate (Berenil®, Surraplex® and Trypan®), melarsomine (cymelrsan®) and Quinapyramine salts etc. which are considered to be safe in all animal species. Further, the control of fly by spraying insecticidal spray in the vicinity of the animal shelter and sheds also helps in the control regime programme of the disease.
1.2. Present Study

1.2.1. Rationale

Several flagellar pocket-associated proteins have been identified and found to contribute to trafficking and virulence (Field and Carrington, 2009) and are involved in the binding of macromolecules and interactions with the host, it is clear that flagellar pocket region has to be considered as a privileged site for possible immunological intervention (Radwanska et al., 2000).

Though, according to literature survey, there is no much information available on the use of flagellar antigen in serodiagnosis of surra, however, the flagellar antigen has been reported in having tremendous antigenic potentiality which can be used for the development of many diagnostic tests, viz. Antibody detecting competitive inhibition ELISA and double antibody sandwich ELISA.
1.2.2. Objectives

i) To isolate and purify the flagellar protein of *T. evansi*.

ii) To develop monoclonal antibody against purified flagellar protein.

iii) Characterization of the purified protein and developed monoclonal antibody.

iv) To develop a suitable diagnostic test.

v) To develop antibody detecting competitive inhibition ELISA using monoclonal antibody.

vi) To develop antigen detecting ELISA using monoclonal antibody.

vii) Validation of the test and field screening for population survey.