5.0 DISCUSSION

Trypanosomosis or Surra caused by *Trypanosoma evansi* is one of the important vector borne diseases occurring in tropical and subtropical countries including India. Introduction of purebred exotic animal germplasm for crossbreeding with indigenous animals was done to improve the livestock productivity. Although, this could be achieved it lead to the increased incidence of haemoprotezoan infections.

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 (Kulkarni *et al.*, 1996). The pyrexial stage of trypanosomosis in bovines in characterised by dullness and depression along with irregular appetite. This is followed by apyrexia during which time the temperature returns to normal and the animal starts feeding normally. The animals continue to develop weakness and manifests loss of condition. Progressive anaemia whether as severe anaemia due to destruction of red blood cells or inhibition of their production is not definitely known but it is presumably due to an inhibitory effect of the toxins liberated by the trypanosomes and during the nervous stage the animals show weakness, incoordination of muscular movements, staggering gait, paralysis of the hind quarters, prostration, inability to feed and drink and may lead to death (Pathak and Narendra Singh, 2005).
The economic losses due to trypanosomosis are attributed to loss of production and decreased efficiency in draught animals to be more specific and precise, the impact of trypanosomosis is exhibited in the form of infertility, abortion, reduced milk yield, loss of weight and poor work output leading to adverse effect on agricultural produce in livestock managed agricultural sector.

Diagnosis of trypanosomosis is based on clinical signs and demonstration of the parasites in the blood supplemented by haematological, biochemical and serological tests.

The clinical manifestations of Surra although indicative, are not pathognomonic enough to confirm the disease without the laboratory methods. When there is high parasitaemia, the examination of wet blood films, stained blood smears and lymph node materials should reveal the trypanosomes but in chronic cases such as the carrier status, examination of thick blood smears as well as methods of parasite concentration and the inoculation of laboratory animals are recommended (OIE, 2000)

Despite the fact that trypanosomosis is one of the most economically important haemoprototzoan disease of bovines in India, a comprehensive work on the epidemiological status and diagnostic methods is lacking particularly in southern parts of India in general and Karnataka and Andhra Pradesh in particular and most of the
reports are based on the parasitological diagnostic methods like wet blood film and blood smear staining methods and response to chemotherapy.

There has been no systematic work on the prevalence and diagnostic methods of *T. evansi* infection in bovines in Karnataka and Andhra Pradesh. Considering the above aspects, the present study was undertaken to observe the prevalence of *T. evansi* infection in bovines based on wet blood film examination, blood smear staining methods, buffy coat technique and serodiagnosis with Indirect ELISA and Enzyme Immune Transfer Blot. Protein profile of whole cell lysate antigen of *T. evansi* was studied by SDS-PAGE along with the comparison of other laboratory diagnostic methods for the diagnosis of *T. evansi* infection in bovines.

Prevalence of trypanosomosis in bovines has been reported from various countries throughout the globe with emphasis on epidemiological observations and economic losses to the livestock industry with different percentages based on parasitological methods including the serological techniques. Shen *et al.* (1985) reported that the prevalence of trypanosomosis was 96.2 % in buffaloes based on ELISA.

Lohr *et al.* (1985) reported 20.0 % *T. evansi* infection in buffaloes in Thailand based on the blood smear staining examination and observed the high rate of infection during the rainy season. Using the buffy coat
technique, Maitioli et al. (2001) reported a prevalence of 3.0 % in N’Dama cattle of 19 to 28 months in the Gambia. Mahama et al. (2004) reported 8.0 per cent prevalence of bovine trypanosomosis using the buffy coat technique in the Savelugu and West Mampursi districts of northern Ghana. Hilali et al. (2004) reported 24.0 % prevalence of trypanosomosis with card agglutination test in water buffaloes in Egypt.

In India, *T. evansi* infection was reported from many parts in bovines. Chhabra et al. (1978) reported on outbreak of trypanosomosis in Haryana state and found 28.5 and 20.2 % prevalence in cattle and buffaloes, respectively by wet blood film examination and Giemsa staining methods.

In Kheda and Panchamahal districts of Gujarat state Patel (1980) reported a prevalence of 3.9 % trypanosomosis in cattle by blood smear staining method. Bhoop Singh and Joshi (1991) found trypanosomosis is buffaloes at Parbhani with a prevalence rate of 20.0 % and the disease was most common from August to November months.

Muraleedharan et al. (1991) reported a prevalence of trypanosomosis in buffaloes from Mandya and Mysore districts of Karnataka and recorded the higher incidence (2.98%) in south-west monsoon followed by north-east monsoon (1.31%) and observed that the buffaloes of four to eight years age group were commonly affected. Krishnappa et al. (2002) reported a prevalence of trypanosomosis in
domestic animals in Karnataka and analysed that cattle was the commonly affected species followed by buffaloes and goat and further reported that 38.46 per cent occurrence of trypanosomosis was recorded in the age group of 9 to 10 years followed by 37.14 % in one to two years and the lowest in the age group of 10 years and above. The incidence of Surra in bovines recorded by Dhami et al. (1999) was 40.62, 40.00, 63.33 and 25.00 % in spring, summer, rainy and autumn seasons and opined that rainy and post rainy seasons showed high incidence due to high prevalence of the vectors. Krishnappa et al. (2002) indicated 27.98 per cent prevalence of trypanosomosis in bovines by passive haemagglutination test. Awandkar et al. (2004) reported a prevalence of 1.73 % of Trypanosoma spp. in cattle and buffaloes and found it to be highest in monsoon (22.07%) followed by post-monsoon (12.93%), winter (0.17%) and summer (0.05%) seasons. The seasonal prevalence of trypanosomosis in bovines was reported by Roy et al. (2004) in Chattisgarh state based on staining of blood smear by Giemsa method and revealed 13.47, 31.68, 20.13 and 12.70 per cent in summer, monsoon, post-monsoon and winter seasons respectively. The highest prevalence of trypanosomosis was recorded in the age group of one to three years (33.54%) followed by 23.95 per cent in three to six years and lowest (9.61%) in the age group of six years. Muraleedharan et al. (2005) reported 0.04 % of trypanosomosis in bovines by staining of blood smear with Giemsa’s method in Karnataka and further recorded that
trypanosomosis was high in south-west monsoon followed by north-east monsoon and the lowest during the hot weather followed by cold weather. The age-wise incidence was found to be high between four to eight years of age and above eight years of age followed by one to four year old cattle and no prevalence was recorded in calves between zero to six moths of age. The incidence of trypanosomosis in cattle was found to be 89.35 and 4.18 % in buffaloes in Karnataka as per Harish et al. (2006).

Harish et al. (2006) reported 89.35 and 4.18 % prevalence of *T. evansi* infection in cattle and buffaloes, respectively by different regional animal disease diagnostic laboratories located at Bangalore, Belgaum, Bellary, Gulbarga, Davanagere, Mangalore and Mysore of Karnataka.

In three districts of Karnataka, in the present investigation, EITB detected highest per cent prevalence 22.22 and 8.75 of *T. evansi* infection in buffaloes and cattle, respectively, wherein ELISA detected 8.00 and 4.76 per cent in buffaloes and cattle, respectively. Buffy coat technique revealed 3.63 and 4.00 per cent in buffaloes and cattle, respectively and Giemsa’s blood smear staining revealed 1.22 and 1.12 per cent, respectively in buffaloes and cattle. This findings indicated that the serodiagnosis by EITB is a highly specific and sensitive method when compared with the other parasitological and concentration methods and also other serodiagnostic tests like ELISA, EITB can be effectively used as
a diagnostic test for the reliable diagnosis of *T. evansi* infection in bovines.

Chhabra et al. (1978) reported an outbreak of *T. evansi* infection in Haryana state and found 28.5 and 28.2 per cent prevalence in cattle and buffaloes, respectively. Patel (1980) reported 3.90 % of *T. evansi* infection in cattle in Gujarat state. Krishnappa et al. (2002) indicated sero prevalence of *T. evansi* infection of 27.98 % in bovines in Shimoga district of Karnataka state and reported 47.05 % infection in bovines followed by 9.72 % prevalence in Chitradurga district in bovines and based on the analysis of data, Krishnappa (1999) indicated 50 per cent occurrence of *T. evansi* infection in bovines from Davanagere district. The present findings are similar to the findings of Patel (1980) who reported a prevalence of 3.90 % in cattle in West Bengal. Muraleedharan et al. (1991) reported 0.04 % prevalence of *T. evansi* infection in bovines in Karnataka state. The present findings are in agreement with that of Krishnappa et al. (2002) with little variation which could be due to the stage of the infection while collecting the blood because parasites could be observed in peripheral circulation only in acute infection (Gill, 1991) and the district-wise variation could be due to variation in the exposure to the vector during grazing and geographical locations like forest area and rainfall and nutritional status and susceptible host population. On the other hand, the present study revealed much lower percentage of infection both in cattle and buffaloes which may due to random sampling
and larger sampling size and perhaps due to constant vigilance and proper preventive measures, if not due to dormant state of *T. evansi* infection especially in symptoms less carriers. The geoclimatic factors in the south transzone and central dry zone of the state could also be one of the important factors.

Bhoop Singh and Joshi (1991) observed 84.90 per cent prevalence of Surra during August to November in buffaloes. Muraleedharan *et al.* (1991) recorded the highest prevalence of *T. evansi* infection in the months of August and September and during the south-west monsoon 2.98 % prevalence was recorded whereas 1.31 % was reported in north-east monsoon in Mandya and Mysore districts of Karnataka. Sero-epidemiological studies by Dhami *et al.* (1999) revealed 40.62, 40.00, 63.33 and 25.00 % of *T. evansi* infection in bovines in spring, summer, rainy and autumn seasons, respectively and opined that rainy and post-rainy seasons the high incidence could be due to the more prevalence or abundance of the vector population. Krishnappa *et al.* (2002) observed by analysing the data that prevalence of *T. evansi* infection in bovines was found to be highest in rainy season followed by summer and winter seasons in Karnataka. Awandkar *et al.* (2004) recorded 22.07, 12.93, 0.17 and 0.05 % of *T. evansi* infection in monsoon, post-monsoon, winter and summer seasons, respectively in bovines. The season-wise prevalence of *T. evansi* infection in bovines recorded by Roy *et al.* (2004)
included 13.47, 31.68, 20.13 and 12.70 %, respectively in summer, monsoon, post-monsoon and winter seasons in Chattisgarh.

Muraleedharan et al. (2005) observed the highest prevalence of *T. evansi* infection in bovines during south-west monsoon followed by north-east monsoon and the lowest in hot weather followed by cold weather in the Mysore Cooperative Milk Producers Union of Karnataka state.

The ELISA in the present study indicated that in cold weather the per cent prevalence of *T. evansi* infection was 5.00 and 6.25 in buffaloes and cattle, respectively whereas in hot weather 7.14 and 7.40 % prevalence was observed in buffaloes and cattle, respectively. During the south-west monsoon 8.77 and 4.08 % prevalence of *T. evansi* was observed in buffaloes and cattle, respectively and during the north-east monsoon 8.88 and 3.77 per cent prevalence was found in buffaloes and cattle, respectively. The present findings in relation to the seasonal prevalence of *T. evansi* infection in bovines is in agreement with Muraleedharan et al. (1991) and Krishnappa et al. (2002). In the present study, it was concluded that the prevalence of *T. evansi* infections in the Indian subcontinent in different seasons and increase in its prevalence in the monsoon season and reaching its peak in October and November could be due to the Tabanid fly breeding at the highest level and the incidence was lowest in April and May when water resources are dried up.
and fly breeding reduced to large extent. However, *T. evansi* infection was recorded throughout the year (Jindal *et al.*, 2005). The variations in the prevalence pattern of *T. evansi* infections in bovines could be due to the vector population increase in considerable number during rainy and post-rainy seasons than the winter and summer seasons in a year. Inclement weather such as hot and humid climate in the months of monsoon and thereafter has also been incriminated to depress the body defense mechanism thereby resulting in the exacerbation of *T. evansi* infection in bovines.

In relation to gender-wise prevalence of *T. evansi* in bovines, the present study recorded 4.34 and 1.35 per cent prevalence in male buffaloes and cattle, respectively whereas 9.61 and 6.61 % prevalence was observed in female buffaloes and cattle, respectively.

Bidhya Shankar Sinha *et al.* (2006) reported that the incidence of *T. evansi* infection is higher in female than male cattle and buffaloes. Verma and Gautam (1978) observed heavy mortality (80.00%) in cow calves in experimentally induced surra. Das *et al.* (1998) reported 0.36 and 3.25 % prevalence of *T. evansi* infection in male and female buffaloes, respectively. Elamin *et al.* (1998) observed that there was no significant difference in the *T. evansi* infection rates of male and female calves in mid-eastern Sudan. Mallick and Dwivedi (1981) found that the
intensity of parasitaemia with *T. evansi* is more in she buffalo than the bullock.

Age-wise prevalence of *T. evansi* infection in bovines recorded was highest of 2.27 % in buffaloes followed by 1.61 per cent in cattle in the age group of six to eight years. In the age group of three to six, 1.19 and 1.28 % prevalence was found in buffaloes and cattle, respectively. In the age group between one to three years, 0.80 and 1.47 % prevalence of *T. evansi* was recorded in buffaloes and cattle, respectively. In the age group of above eight years, 1.92 and 1.33 per cent prevalence was recorded in buffaloes and cattle, respectively whereas no *T. evansi* organisms were found in the age group below one year of buffaloes and cattle. Krishnappa *et al.* (2002) reported the highest prevalence 38.46 % prevalence of *T. evansi* infection in bovines in age group of 9 to 10 years and a lower prevalence of 20.00 % in the age group of above 10 years.

Roy *et al.* (2004) recorded the highest prevalence of 33.54 % of *T. evansi* infection in bovines in the age group of one to three years followed by 23.95 % in the three to six year age group and a lower 9.61 % in the age group above six years. The age-wise prevalence of *T. evansi* infections in cattle was reported by Muraleedharan *et al.* (2005) who found high prevalence between four to eight years of age and above eight years of age followed by one to four years of age and least in six months to one year old animals, which was nil in calves below six months of age.
Muraleedharan et al. (1991) noted that the buffaloes of four to eight years age group were commonly affected with *T. evansi* infection in Mandya and Mysore districts of Karnataka state. The present findings of prevalence of *T. evansi* infection in bovines in relation to sex of the animal species is in agreement with the findings of Mallick and Dwivedi (1981) where the females were found to be infected more than the male species of animals and the reasons could be due to the stress factors like lactation and pregnancy which will made the animals predisposed for the infection under peculiar conditions apart from exposure to the biting flies which are primary source for the transmission of the infection among the livestock. The age-wise prevalence of *T. evansi* infection in bovines is in agreement with the observations of Krishnappa et al. (2002), Roy et al. (2004) and Muraleedharan et al. (2005). The animals in the age group between six to eight years of age seem to be best suited for infection due to demands of production and reproduction including Immunological and nutritional status of the animals which play a vital role and could be attributed to the old age susceptibility because of the hypofunction of the immune system.

In three districts of Andhra Pradesh, in the present study, EITB detected 47.77 and 31.66 % infection of *T. evansi* in buffaloes and cattle, respectively wherein the ELISA could detect a much lower prevalence of 13.95 and 14.28 % in buffaloes and cattle, respectively. Buffy coat technique indicated positivity in 8.69 and 7.14 % of buffaloes and cattle,
respectively. Giemsa blood smear staining revealed 8.72 and 5.58 % in buffaloes and cattle respectively whereas wet blood film examination could detect only 1.09 and 0.46 per cent prevalence of *T.evansi* organism in buffaloes and cattle respectively. A seasonal prevalence of *T. evansi* infection in bovines reported by Prasad *et al.* (1997) in Gudivada of Krishna district of Andhra Pradesh was found to be 5.08, 2.78 and 2.20%, respectively in rainy, summer and winter seasons. Awandkar *et al.* (2004) reported the highest prevalence of *T. evansi* infection in bovines during monsoon (22.07%) followed by post-monsoon (12.93%), winter (0.17%) and summer (0.05%) in Maharashtra.

Roy *et al.* (2004) observed the seasonal prevalence of *T. evansi* infection in bovines in Chattisgarh which showed the highest infection during monsoon followed by post-monsoon and was lowest during winter and summer seasons. Bidhya Sankar Sinha *et al.* (2006) reported 52.31% prevalence of *T. evansi* infection in buffaloes and 56.29 % in cattle during monsoon followed by 29.03 and 33.85 % in cattle and buffaloes during winter and was least during summer season in 18.28 and 13.84 %, respectively in cattle and buffaloes.

During the winter season, ELISA revealed 11.76 and 9.61 % prevalence of trypanosomosis in buffaloes and cattle, respectively whereas at summer it was 11.29 and 15.68 % was found in buffaloes and cattle, respectively. In the monsoon season 17.64 and 16.66 %
prevalence of *T. evansi* was detected in buffaloes and cattle, respectively. The present findings are in agreement with the findings of Prasad *et al.* (1997) and Das *et al.* (1988) and similar to that of Awandkar *et al.* (2004).

Krishnappa *et al.* (2002) reported the high prevalence of *T. evansi* infection in bovines during rainy and post-rainy seasons, which could be due to high abundance and breeding of the vectors responsible for transmission of *T. evansi*. Muraleedharan *et al.* (1991) observed that the prevalence of *T. evansi* infection was found to be high in the months of August and September, whereas during south-west monsoon it was 2.98 and 1.31 % during north-east monsoon. The present findings regarding the seasonal prevalence of *T. evansi* infection in bovines is in agreement with the findings of Das *et al.* (1998); Prasad *et al.* (1997) and also is in agreement with the observations of Muraleedharan *et al.* (1991) and Krishnappa *et al.* (2002). The higher prevalence of *T. evansi* infection in bovines during the south-west and north-east monsoon could be due to the availability of the vector population. The breeding and wide spread distribution of the vector population in the rainy season and decreased host resistance due to climatic stress results in the precipitation of the infection.

The lowest prevalence during the winter and summer seasons could be due to the unfavourable conditions like drying of the water channels, less rainfall received and nutritional status of the susceptible
host population thereby reducing the breeding and abundance of vector population in the Krishna Godavari zone of Andhra Pradesh.

The highest sex-wise prevalence of *T.evansi* infection recorded was 20.20 % in female buffaloes followed by 14.81 % in male cattle. In the female cattle, 13.82 % prevalence was recorded followed by 8.62 % in male buffaloes.

The present findings regarding the prevalence of *T.evansi* infection between sexes is in agreement with the findings of Bidhya Shankar Singh *et al.* (2006) and Das *et al.* (1998).

The age group of four to eight year old buffaloes were commonly affected with *T.evansi* infection as reported by Muraleedharan *et al.* (1991) is almost similar to observations in the present study in relation to age of the animal. However, Krishnappa *et al.* (2002) reported that the age group of 9 to 10 year old bovines had 38.46 % of *T.evansi* infection. Payne *et al.* (1991) reported that an age-dependent prevalence rate of *T.evansi* infection was seen in bovines with highest rates in animals older than two years.

In relation to age-wise prevalence of *T.evansi*, 28.12 per cent was recorded in the age group of above eight years of cattle followed by 17.5 per cent in the cattle of six to eight years. A per cent prevalence of 17.18 and 3.38 was recorded in buffaloes and cattle of 3-6 years, respectively.
In the age group 1-3 years, cattle and buffaloes had 13.63 and 8.77 per cent infection respectively. In the age group of cattle and buffaloes, less than one year of age, 2.50 and 1.96 per cent prevalence of *T.evansi* was found. Differences were observed between areas not only in the proportions of animals infected in each age group, but also in the relationships between the different age groups. The variations in the prevalence of *T.evansi* infection in bovines in different age groups could be due to relation lack of stress probably accounted for the absence of parasitaemia in calves and heifers and more stress factors like lactation, pregnancy, impaired immunological status and nutritional status and frequent exposure during grazing and exposure to the potential vector population contributed to the prevalence of *T.evansi* infection in adults.

In *Trypanosoma evansi* infection in bovines, the clinical signs are not pathognomonic and laboratory diagnosis is required when there is high parasitaemia. The examination of wet blood films, stained blood smears reveal the trypanosomes. In chronic cases such as the carrier state, the examination of thick blood smears as well as methods of parasite concentration and the inoculation of laboratory animals are recommended. Alternate methods of diagnosis of *T.evansi* infections therefore have been explored and several serological tests have been described for the detection of specific antibodies against *T.evansi* in experimental and naturally infected animals.
Dhami et al. (1999) employed the Giemsa’s stained method and wet blood film examination for the diagnosis of *T.evansi* infection in bovines which revealed out 75 animals 1.34 % to be positive, whereas wet blood film could not detect parasitaemia.

Das et al. (1998) investigated into prevalence of bovine Surra in Guntur district of Andhra Pradesh by wet blood film examination and Giemsa or Leishman’s staining method, which manifested 2.63 % prevalence of *T.evansi* infection. Rajesh Agarwal et al. (2003) reported 7.49 % prevalence of *T.evansi*, infection in bovines in Chattisgarh state by Giemsa blood smear staining method.

Batra et al. (1994) carried out wet blood film examination and Giemsa blood smear staining method for the diagnosis of *T.evansi* in bovines in Haryana and reported 76.92 % and trypanosomes could not be observed in wet blood film examination but after inoculating two to five ml of blood into mice and found numerous parasites in a few days in the blood of mice and thereby confirmed the disease.

Muraleedharan et al. (2005) reported 0.4 % prevalence of *T.evansi* infection in bovines by blood smears staining method in Karnataka. Harish et al. (2006) based on blood smear staining method detected 89.35 and 4.18 % prevalence of *T.evansi* infection in bovines in Karnataka.
In the present study, wet blood film examination revealed 0.77 % *T.evansi* infection followed by 1.75 % with Giemsa blood smear staining method in bovines. The present findings are in agreement with that of Muraleedharan *et al.* (2005) and slightly lower than the findings of Harish *et al.* (2006). The variation in the detection of *T.evansi* infection could be due to the random sampling, sample size and as per the observations of Killick Kendrick (1968), who stated that the conventional parasitological diagnostic techniques for the detection of parasites in the blood are not always effective since trypanosomes are frequently absent from peripheral blood.

The application of parasite concentration methods like buffy coat techniques are recommended (OIE, 2000) to diagnose the *T.evansi* infection as an alternative method including the serological techniques. Dwivedi (2004) stressed the importance of buffy coat technique for the identification of subclinical or carrier state of *T.evansi* infection in bovines.

Cheah (1999) adapted the buffy coat technique for the detection of *T.evansi* organisms in Malaysia in crossbred dairy cattle. Elamin *et al.* (1998) detected 5.6 % prevalence of *T.evansi* infection in camels using the buffy coat technique in mid-eastern Sudan. Levine *et al.* (1989) evaluated *Trypanosoma brucei rhodesiense* infection using quantitative buffy coat technique in experimentally infected rats and indicated that
this technique was sensitive for the diagnosis of haemoparasitic diseases including *Trypanosoma, Babesia* and *Theileria* infection. In the present study, buffy coat technique detected 2.45% prevalence of *T.evansi* infection in bovines. The buffy coat technique detected more number of cases of *T.evansi* infection compared with the wet blood film and Giemsa stained blood smears examination. It could attributed to the reason that in most of the hosts, *T.evansi* can induce mild clinical or subclinical carrier infections with low parasitaemia and in such conditions concentrations methods like buffy coat technique become necessary. In the present study quantitative buffy coat method revealed *T.evansi* organisms in 25 blood samples out of 25 examined of experimentally infected mice.

The diagnosis of *T.evansi* infection in bovines relies on the detection of trypanosomes either by parasitological or concentration methods but serodiagnostic methods such as ELISA and EITB which aid in the detection of specific antibodies to *T.evansi* are necessary in low grade infections, are more sensitive and are of immense value to study the epidemiology of diseases. Although, direct demonstration of trypanosomes in the infected animal gives conclusive proof of infection, the limitations of parasitological diagnosis has lead to consider alternative methods such as serodiagnostic techniques which provide indirect evidence of infection.
In the present study, the antigen developed for sero-diagnosis of *T.evansi* infection in bovines included the whole cell lysate antigen prepared from *T.evansi* organisms. The utility of whole cell lysate antigen has been used by many workers (Veer Singh *et al.*, 1997; Jithendran and Rao, 1999; Reid, 2002). Payne *et al.* (1991) reported 42.7 % prevalence of antibodies against *T.evansi* out of 1522 cattle screened and 48.0 % out of 276 screened by ELISA in Indonesia. Shahardar *et al.* (2002) reported 46.66 per cent sero-positivity for *T.evansi* antibodies using DOT-ELISA.

Jithendran *et al.* (1997) reported that an Indirect ELISA for the detection of antibodies against *T.evansi* may be considered as a potential screening test for the detection of latent infections in bovines. Raina *et al.* (1986) observed that counter immunoelectrophoresis and agar gel precipitation test gave promising results to detect antibodies against *T.evansi* infection in experimental buffalo calves. Baghel *et al.* (1996) reported that indirect ELISA detected antibodies against *T.evansi* in 76.53 % among 298 serum samples screened. Krishnappa *et al.* (2002) indicated 27.98 % prevalence of *T.evansi* infection in bovines in Karnataka by passive haemagglutination test.

In the present study, by indirect ELISA *T.evansi* infection of 4.55 % in bovines was observed. It was concluded that serological tests will be of immense value in the sensitive detection of *T.evansi* infections in bovines than the parasitological methods and prevalence of antibodies against
*T. evansi* indicated that animals are being constantly exposed to infection throughout the year or are in the latent phase of Trypanosomosis in bovines in endemic regions of some districts in both the states included in the present study.

By western blot analysis Camargo *et al.* (2004) recognised that the polypeptide pattern of molecular weight ranged from 14 to 198 kDa in the serum of bovines infected with *T. evansi*. Uzcanga *et al.* (2002) by Western blot revealed a high immunological cross reaction between *T. evansi* and *T. vivax* and observed that an antigen with 64 kDa is responsible for the same between these two parasites.

Xie-Chao *et al.* (1997) used western blot technique to *T. evansi* the antiserum of excreted / secreted antigen of *T. evansi* which showed five bands with molecular masses of 105.0, 100.2, 95.4, 62.6 and 52.8 kDa and the antiserum of soluble antigen revealed two bands with molecular weights of 71.6 and 52.8 kDa.

In the present study, the Enzyme Immuno Transfer Blot revealed, polypeptides which recognised five bands when reacted with hyperimmune serum with molecular weights of 50.12 to 15.85 kDa, whereas with the known positive serum three bands were recognised, in the range of 50.12 and 19.95 kDa. The overall prevalence of *T. evansi* infection in bovines by Western blotting was 5.39 % and this method was highly sensitive compared to ELISA in the present investigation which
clearly indicates that the Western blot technique can be employed for the specific diagnosis of *T.evansi* infection in bovines. Veer Singh *et al.* (1995) by Western blot analysis of *T.evansi* whole cell lysate antigen found 11 bands in the range of 17.2 to 98.2 kDa with hyperimmune serum. The results indicated that Western blot technique can be effectively used for the diagnosis of *T.evansi* infection in bovines in field serum samples and in turn could curtail pathogenic effects on livestock thereby enhancing the health status and productive and reproductive efficiency of livestock particularly in bovines.

The protein profile of whole cell lysate antigen of *T.evansi* by SDS-PAGE indicated by Pareek *et al.* (1999) ranged from 195 to 26 kDa. Jithendran and Rao (1999) observed the 16 polypeptide bands in the range of 16.98 to 97.72 kDa with the soluble antigen of *T.evansi* by SDS-PAGE. Veer Singh *et al.* (1995) by SDS-PAGE studied, the polypeptide profile of whole cell lysate antigen of *T.evansi* which indicated the molecular weights ranging from 33 to 81 kDa. Pathak *et al.* (1993) analysed the crude somatic antigen of *T.evansi* and identified the prevalence of protein bands ranging from 14 to 65 kDa with SDS-PAGE analysis. Xie-Chao *et al.* (1997) revealed the protein profile of soluble antigen of *T.evansi* which consisted of 13 bands ranged from 71.6 to 22.1 kDa by SDS-PAGE.
In the present study, the polypeptide profile of whole cell lysate antigen of *T.evansi* revealed 10 bands and the molecular weight ranged from 69.18 to 15.85 kDa. The role of preparation of antigen purification and the percentage of resolving gel may slightly influence the separation of polypeptides based on the molecular size of polypeptides.