2.0 REVIEW OF LITERATURE

The literature on the epidemiological and immunological aspects of *Trypanosoma evansi* infection in bovines in different parts of the world in general and India in particular has been reviewed.

2.1 Epidemiological aspects of *T. evansi* infection in bovines

2.2 Conventional methods of diagnosis of *T.evansi*

Comparative studies of diagnostic tests in the diagnosis of Surra was reported by Goel and Singh (1971) and it was found that the thick smear method of examination for the detection of *T. evansi* was three times more effective than the thin smear method. The biological test was more effective than the fresh smear and serological examinations in suspected cases of surra in bovines. The formol-gel test gave 100 per cent positive results in experimentally infected donkeys, buffalo calves and dogs, whereas in suspected cases it was 75 per cent positive in camels and 20 per cent each in donkeys and buffaloes.

Abortion due to *T. evansi* in she-buffalo was reported by Paikne and Dhake (1972) from Nagpur and the blood films from the aborted foetus revealed the presence of *T. evansi* with 14 to 16 organisms in each microscopic field. The wet blood films of foetal blood showed actively moving trypanosomes but no *T. evansi* was detected in the blood smear of the dam four hours after abortion.

Mandal *et al.* (1977) reported an outbreak of Surra in bovines from Krishna district, Andhra Pradesh wherein the disease occured in 183 bovines viz., cattle 12 (29.2%) and buffaloes 171 (36.9%). Among these 41 cattle and 463 buffaloes were diagnosed by blood smear examination after Giemsa staining.

In an experimental *T. evansi* infection in 10 buffaloes and 5 cow calves, Verma and Gautam (1977) found that the passive haemagglutination test was reliable, and sensitive and was positive at an early stage (fourth day after infection) whereas indirect fluoroscent antibody test was positive in one buffalo calf on the eighth day and in the other 14 animals between the 12th and 16th day.
Chhabra et al. (1978) reported an outbreak of bovine trypanosomosis in flood affected district of Haryana. The wet film examination and Giemsa staining method revealed 28.5 and 20.2 per cent positivity in buffaloes and cattle respectively.

Saarma (1979) reported high incidence of trypanosomosis by blood smear examination in local cows as well as buffaloes and horses in the mountainous areas of Jammu and Kashmir.

Verma and Gautam (1979) observed pathological changes in experimental Surra in bovines and recorded emaciation, cachexia, atrophy of gluteal muscles, enlargement of prescapular and other superficial lymph nodes with petechial haemorrhages, dark tarry coloured partially coagulated blood, excess of fluid in the peritoneal cavity, congestion of the liver and spleen and occasionally of lungs, petechial haemorrhages at the base of the heart, gelatinisation of the pelvis of kidneys and base of the heart and extensive emphysema of lungs.

Balani and Chandiramani (1980) examined 2094 animals comprising of buffaloes, bullocks, cows, calves, horses, mules, donkeys and camels from Rohtak and Panipat districts of Haryana state and 339 of the animals were found positive by stained blood smear examination and mouse inoculation methods.

Blood smears from 985 clinical cases of pyrexia of unknown origin in bovines from Kurukshetra and Ambala districts of Haryana were examined by Galhotra and Chandiramani (1980) who found that 119 cases were positive for trypanosomosis.

Patel (1980) screened 528 blood smears of cattle and 115 blood smears of equines and found 21 and 29 positive for *T. evansi* in cattle and equines, respectively in Kheda and Panchamahal districts of Gujarat state.

Shanta (1980) reported the occurrence of haemoproteozan infections in West Malaysia in purebred and crossbred animals and recorded *T. evansi* along with other haemoproteozans viz., *Babesia bigemina, Babesia argentina, Anaplasma marginale, Theileria mutans*. 
In an epizootiological survey, Shaw and Mir (1980) reported the incidence of trypanosomosis sporadically in some localities in Jammu province.

Mallick and Dwivedi (1981) described the clinical symptoms in *T. evansi* infected bovines ranging from weakness, staggering gait, signs of nervous involvement and exhaustion. The body temperature varied between 99.0 and 104.4°F. The peripheral blood smears revealed the presence of *T. evansi* and the intensity of parasitaemia ranging from ++ to ++++ and blood glucose estimation revealed marked hypoglycemia in nine animals, out of 12 acute cases which had temperature within normal range whereas the remaining animals had high temperature with no reduction in blood glucose level.

In the swamp buffaloes of Thai, Patchimasiri *et al.* (1983) conducted a survey of *T. evansi* infection by screening 200 adult swamp buffaloes by mouse inoculation test and recorded 50 per cent prevalence (3 out of 6) in Roi Et province and 66 per cent (7 out of 11) in Chachoengsao province.

Raisinghani *et al.* (1983) reported a case of Surra in a pure Jersey bull from Udaipur district of Rajasthan and found excitement, somnolence, charging against walls, kicking around, lacrimation, nasal discharge, drooping salivation, laboured respiration with frequent lying down and getting up movements, exhaustion, sweating and listlessness with temperature 104.5°F and the blood film examination revealed *T. evansi* infection which was treated successfully with Antrycide prosalt.

Singh and Gaur (1983) recorded clinical and blood cellular changes associated with *T. evansi* infection in experimentally infected buffalo calves. It was found that the calves developed anaemia that coincided with the onset of parasitaemia and there was no alteration in TLC, but eosinophilia was noticed with no changes in monocyte count.

In the Mandya district of Karnataka state, Muraleedharan and Srinivas (1985) attributed abortions in a buffalo to *T. evansi* infection based on blood examination. Similarly, in a crossbred cow also the infection was confirmed by examining the aborted foetal liver and heart impression smears.
Muraleedharan *et al.* (1985) reported *Trypanosoma theileri* infection in a six-year-old Hllikar bullock in Mandya district of Karnataka. The serosanguineous fluid stained with Giemsa method and identified the parasite on the basis of its morphological characters.

Lohr *et al.* (1985) reported 20 per cent *T. evansi* infection in buffaloes in Thailand based on the blood smear examination and the infection rate was found to be high during the rainy season.

Bansal *et al.* (1986) reported a clinical case of Surra in a buffalo at Bareilly, Uttar Pradesh based on clinical symptoms, wet film and Giemsa stained blood smears and it was treated successfully with Tribexin at the rate of three grams dissolved in 10 ml distilled water by s/c route.

In North-East Thailand, Lohr *et al.* (1986) conducted blood smear examination to diagnose the *T. evansi* infection in buffaloes and found its prevalence in 22 to 60 per cent of the infected animals.

Renuka Prasad and Gokak (1988) reported the occurrence of *Trypanosoma theileri* in buffaloe and bullock from Uttara Kannada district of Karnataka by Giemsa staining method and identified the parasites based on morphological findings.

Wellde *et al.* (1989) surveyed Zebu cattle in the Lambwe valley and reported 70 per cent infection rate of *T. evansi* followed by *T. congolense*. The organisms were detected in cerebrospinal fluid and central nervous system disorders were observed in the infected cattle.

Bidyanta *et al.* (1990) reported a sudden outbreak of trypanosomosis in cattle at Fulia in West Bengal by examining the stained blood smear. The animals were treated successfully with Berenil and prophylactic treatment with Tribexin also proved effective.

Veerasingh and Raisinghani (1990) reported clinical observations in experimental Surra in buffalo calves which include intermediate pyrexia, dullness, depression, slight
enlargement of prescapular lymph nodes, anaemic intermandibular edema, conjunctivitis with white mucoid discharge from the eyes.

Bhoop Singh and Joshi (1991) screened a total of 265 adult female buffaloes at Parbhani for the prevalence of *T. evansi* infection by Giemsa’s method of staining and 53 (20%) were found positive. The prevalence of surra was highest from August to November (84.9%) in recently calved buffaloes.

Muraleedharan *et al.* (1991) reported the prevalence of *T. evansi* infection in buffaloes from Mandya and Mysore districts of Karnataka and observed that the incidence of *T. evansi* infection was high in the months of August and September. South-west monsoon period showed a higher incidence of 2.98 per cent than north-east monsoon (1.31%) and it was further noted that the buffaloes of four to eight years age group were commonly affected.

Batra *et al.* (1994) reported a total of eight outbreaks of trypanosomosis (3 in buffaloes, 2 in cattle and 3 in both cattle and buffaloes) of Surra in bovines from Haryana state. The diagnosis was made by wet film and Giemsa stained blood smear examination and the mortality ranged from 21.66 to 100.0 per cent among the affected animals.

Kalra *et al.* (1994) reported trypanosomosis in a 23 day old cow calf by Giemsa stained blood smear examination and it was treated successfully with Triquin @ 0.025 ml/kg body weight subcutaneously.

Kulkarni *et al.*, (1994) screened she-buffaloes at Akola with a complaint of complete or partial anorexia and sudden stoppage or drop in milk yield by wet and stained blood smears and found them to be positive for *T. evansi*.

Kulkarni *et al.* (1996) recorded surra in she-buffaloes at Akola, Maharastra with symptoms like partial or complete anorexia and sudden drop in milk yield which was treated successfully with berenil and glucose for three days.

Darunee *et al.* (1997) reported cerebral trypanosomosis in naive cattle with nervous symptoms including circling, excitation, jumping aggressive behaviour, lateral
recumbency, convulsion and finally death. Out of 39 blood samples from cattle examined for the presence of *T. evansi* by mouse inoculation test and indirect fluroscent antibody test, 16 were found positive. *T. evansi* was detected on impression smears of organs from three cattle which died with nervous symptoms and also in smears made from their cerebrospinal fluid. In addition, trypanosomes were isolated from the cerebrum, cerebellum, pons and spinal cord by mouse inoculation.

Prasad *et al.* (1997) undertook a survey in and around Gudivada of Krishna delta in Andhra Pradesh for the detection of Surra in buffaloes. In all, 299 (4.05%) animals were found positive by wet blood film examination out of 7377 screened. The seasonal prevalence of infection recorded was 5.08, 2.78 and 2.20 % in rainy, summer and winter seasons respectively.

Das *et al.* (1998) reported a prevalence of Surra in bovines in Guntur district of Andhra Pradesh. The examination of blood smears and lymph fluid after Leishman or Giemsa staining revealed that 1.42 % cattle and 2.71 % buffaloes were positive. The seasonal prevalence recorded was 34.13, 32.21, 24.51 and 9.13 % in monsoon, post-monsoon, winter and summer seasons respectively.

An investigation into the epidemiology of *T. evansi* infection in crossbred cattle was conducted by Cheah *et al.* (1999) in Malaysia. It was reported that the prevalence of parasitaemia was highest in lactating animals (13.4%) followed by those in dry herd (8.8%), late pregnant animals (8.1%), early pregnant animals 4.7%), calves (0.3%) and heifers (0.2%).

Sero-epidemiological studies were carried out by Dhami *et al.* (1999) in Punjab to detect Surra in bovines by using card agglutination test. The incidence of surra was found to be 40.62, 40.00, 63.33 and 25.00 percent in spring, summer, rainy and autumn seasons respectively. The rainy and post rainy seasons showed high incidence due to high prevalence of the vectors.
Nalinikumari et al. (2000) reported a mixed infection of theileriosis and trypanosomosis in a crossbred cow by examination of stained blood smear. It was treated successfully with chloroquin phosphate and Berenil based on improvement in alertness, appetite, milk yield and haematological values in the treated animal.

Rajguru et al. (2000) reported the transplacental transmission of *T. evansi* in neonatal cow calves at Parbhani by wet and Giemsa stained blood films.

Panduranga Rao et al. (2001) reported transplacental transmission of trypanosomes in a female buffalo calf which was of 20 days old and in a comatose condition. The blood smears stained with Giemsa and wet blood smears revealed trypanosomes in newborn calf and dam.

The prevalence of trypanosomosis in domestic animals based on data obtained from 23 districts of Karnataka was reported by Krishnappa et al. (2002). The information was based on the symptoms exhibited, response to therapy and laboratory examination and reported that cattle was the commonly affected species followed by buffaloes and goats. The age group of 9-10 years affected (38.46 %) followed by 1-2 years (37.14 %) and the lowest in the age group of 10 years and above. The season wise prevalence of trypanosomosis was found to be highest in rainy season followed by summer and winter seasons.

Serodiagnosis by the passive haemagglutination test was conducted by Krishnappa et al. (2002) which indicated a seroprevalence of *Trypanosoma evansi* in bovines from different parts of Karnataka. Out of the 608 sera samples 170 (27.98 %) were found to be positive.

Shinde et al. (2003) reported a prevalence of 3.06 and 4.36% of *T. evansi* infection in cattle and buffaloes respectively among 736 bovines screened for the blood protista. Mixed infection of *Trypanosoma* and *Ehrlichia* was found to be 0.87 % in the screened population was observed.

Sunilkumar Modi (2003) reported a case of concurrent clinical trypanosomosis and subclinical mastitis in a seven year old murrah she-buffalo at Jabalpur which was
treated with Intamox-2 intramammary infusion @ 250 mg/quarter and Nilbery @ 1.0 ml/15 kg body weight.

The prevalence of haemoproteozoan infections in domestic animals at Nagpur was reported by Awandkar et al. (2004). The prevalence of 1.73 % of Trypanosoma spp. was recorded out of 580 blood smears of cattle, buffaloes, goats and dogs screened. The incidence of infection was highest in monsoon (22.07%) followed by post-monsoon (12.93%), winter (0.17%) and summer (0.05%) seasons.

Bhaskar Rao and Varaprasad (2004) reported mixed infection of trypanosomosis and microfilariosis in a she-buffalo of third lactation at Kakinada in Andhra Pradesh.

Bhaskar Rao and Varaprasad (2005) reported the prevalence of trypanosomosis in buffaloes as 7.28 % in East Godavari district of Andhra Pradesh based on blood smear examination after Leishman’s or Giemsa’s staining methods.

Narladkar et al. (2004) reported the clinical cases of trypanosomosis in bovines by wet smear and Giemsa stained blood smear examination. The symptoms such as severe convulsions, tremors, continuous twitching of eyelids, circling movements and attacks followed by recumbency and the haematological examination revealed leucocytosis, lymphopenia, neutrophilia, eosinophilia and moderate monocytosis.

Rajanish Kumar et al. (2004) screened the blood samples of 101 cattle at Pantnagar and recorded the prevalence of T. evansi as 11.11 %.

Roy et al. (2004) reported the prevalence of trypanosomosis in Chattisgarh state, based on Giemsa stained blood smear examination. The season wise prevalence recorded was 13.47, 31.68, 20.13 and 12.70 % in summer, monsoon, post-monsoon and winter seasons respectively. The highest prevalence of trypanosomosis was recorded in the age group of 1-3 years (33.54 %) followed by 3-6 years (23.95 %) and was lowest in the age group of 6 years (9.61 %)

Jindal et al. (2005) reported the prevalence of Surra in bovines in Haryana state and the disease was reported to occur throughout the year. However, the number of
outbreaks were more in the winter season (246 out of 480) followed by rainy (121) and summer seasons (113).

Muraleedharan et al. (2005) reported the incidence of *T. evansi* of 0.40 per cent by blood smears examined after Giemsa staining among the cattle of the project area of Mysore Cooperative Milk Producers Union of Karnataka state. Further, they found that *T. evansi* infection rate was high in south west monsoon followed by north east monsoon and the lowest incidence was recorded in 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 years of age of cattle and was least in six months to one year old animals and nil in calves between zero to six months of age.

Bidhya Sankar Sinha et al. (2006) reported the incidence of bovine trypanosomosis in Bihar state and observed that the incidence was found higher in cattle (58.86%) compared to buffaloes. The incidence was higher in females than males in both cattle and buffaloes and the incidence recorded was 52.69 per cent in cattle and 52.31 per cent in buffaloes during monsoon followed by winter (cattle 29.03% and buffaloes 33.85%) and least during summer season (cattle 18.28% and buffaloes 13.84%).

The incidence of haemoproteozoan diseases was reported by Harish et al. (2006) from different regional animal disease diagnostic laboratories located at Bangalore, Belgaum, Bellary, Gulbarga, Davanagere, Mangalore and Mysore of Karnataka based on blood smear examination. The incidence of trypanosomosis reported was 89.35% in cattle and 4.18% in buffaloes and 3.42% in horses.

### 2.2.1 Immunological diagnosis of *T.evansi*

Zwart et al. (1973) conducted a seroprevalence survey using the indirect fluorescent antibody test in cattle, sheep and goats at Kiboko area of Kenya and reported that 80 per cent of cattle, 39 per cent of sheep and 44 per cent of goats had antibodies against *T. congolense*, *T. brucei* and *T. vivax*. The routine trypanosome detection methods like wet, thick and thin blood films combined with inoculation of blood in to the mice, 15 per cent of the cattle were found positive. In sheep and goats lymph node smears were
made in addition to the standard trypanosome methods and 10 per cent of the sheep and 6 per cent of the goats were found to be infected.

Three different serodiagnostic tests were evaluated by Verma and Gautam (1977) in experimental bovine Surra viz., passive haemagglutination, gel diffusion and indirect fluorescent antibody tests in 10 buffaloes and 5 cow calves. The PHA test was reliable and sensitive and could detect infection by the fourth day post-infection. In the gel diffusion test, the precipitating antibodies were detected on eighth day post-infection in two infected buffalo calves and in the remaining 13 animals between 12\textsuperscript{th} and 20\textsuperscript{th} day whereas indirect fluorescent antibody test was found positive in one buffalo calf on eighth day post-infection and in the remaining 14 animals between 12\textsuperscript{th} and 16\textsuperscript{th} day.

Luckins \textit{et al.} (1979) compared five diagnostic tests such as mercuric chloride test, formol-gel test, quantitation of IgM levels, IFAT and ELISA for infection with \textit{T. evansi} in camels in the Sudan. The correlation of positive results obtained by assays of IgM levels, the mercuric chloride and the formol-gel test during active infection was unsatisfactory, but there was a good correlation between results obtained using IFAT and ELISA.

Comparative studies on the serodiagnosis of experimental \textit{T. evansi} infection in rabbits was conducted by Sabah and Abbassy (1980). It was found that the complement fixation test affords a relatively high level of specificity and sensitivity than the indirect haemagglutination test.


Hu \textit{et al.} (1985) adopted Indirect Hemagglutination Test to diagnose \textit{T. evansi} infection in three experimentally infected buffaloes. It was found that one of them was positive on 7\textsuperscript{th} day, whereas two were found positive on 21\textsuperscript{st} day of infection.
In a comparative study for the diagnosis the *T. evansi* infection in buffaloes at Shanghai, China by Shen *et al.* (1985) it was found that ELISA, IHA and CFT could detect 96.2%, 78.0% and 82.3% percent infections respectively.

Caille (1987) conducted a serological survey on the prevalence and seasonal incidence of different haemoprotozoan diseases in livestock in Somalia using ELISA and found 57.6 per cent of camels, 65.2 per cent cattle, 53.8 per cent of sheep and 45.7% goats positive.

Paikne and Dhake (1972) reported *T. evansi* infection in a she-buffalo which had aborted and the foetal blood revealed the presence of *T. evansi*.

Singh and Misra (1988) compared the agar gel diffusion test and passive haemagglutination test for the diagnosis of *T. evansi* infection in experimentally infected calves and found that the efficacy of agar gel diffusion test ranged from 20.00 to 86.66%, whereas passive haemagglutination test ranged from 6.66 to 100.00%.

In Indonesia, Payne *et al.* (1991) screened cattle, buffaloes and horses for the diagnosis of *T. evansi* by Micro-Haematocrit Centrifugation Technique and an enzyme linked immuno-sorbent assay for the detection of antibodies to *T. evansi* and reported that the prevalence rates in buffaloes were higher than the cattle in the same area while in horses the infection rates were much lower than cattle and buffaloes and found that age dependent prevalence rate was seen in buffaloes and cattle with the highest rates noticed in animals older than two years.

A parasitological and serological survey for *T. evansi* infection was conducted by Ray *et al.* (1992) in cattle and buffaloes in an organised dairy farm and in a few villages around Calcutta. Two hundred randomly selected animals were tested with Giemsa’s stain, but did not reveal any trypanosomes. One mouse was inoculated with 0.8 ml of heparinised blood and mouse inoculation test detected 5.7%, 4.3 and 8.0% infection in crossbred cattle, zebu cattle and buffaloes respectively. The Indirect Fluorescent Antibody Test was found positive in 45.7% crossbred cattle, 22.6 per cent zebu cattle and 24.0% buffaloes.
Swarnkar et al. (1993) screened 110 cattle and 50 buffaloes and found only one (0.91%) case of cattle and two (4.00%) buffaloes to be positive for *T. evansi* by wet blood film examination. The double immuno diffusion and counter immuno electrophoresis test failed to detect trypanosomal antigen, whereas sandwich ELISA detected *T. evansi* antigen in 16 (14.54%) cattle and 4 (8.00%) buffaloes.

Nessiem (1994) evaluated the silicone centrifugation technique (SCT) in the detection of *T. evansi* infection in camels and experimental animals and opined that the SCT is as sensitive as the other concentration methods for detection of low parasitaemia.

Baghel et al. (1995) reported the indirect ELISA to determine the antibody levels in the sera for *T. evansi* and the Ab-ELISA values of sera of control calves showed no significant variation and ranged between 0.152±0.013 to 0.282±0.050, whereas the mean Ab-ELISA values of sera of infected buffalo calves increased from 0.257±0.019 (pre-inoculation level) to 1.032±0.134 on 63 day post-infection and found that a two to five fold increase in the antibody levels of sera of infected calves in comparison to sera of control calves was observed throughout the period of study.

Saseendranath et al. (1995) evaluated passive haemagglutination test for the diagnosis of *T. evansi* infection in sheep. This test detected specific antibodies at 21 days post-infection and it was observed that the antibody titre tended to increase from 3rd week of infection in subacute and chronic cases and declined by 13th week post-infection.

Veer Singh et al. (1995a) employed ELISA for the detection of *T. evansi* in serum samples of field cases of buffaloes and horses in northern India. In 323 naturally infected/suspected buffaloes, circulating antigen was detected in 180 (55.72%), whereas parasitemia by wet blood smear examination was found in 62 (19.19%). The antigen-ELISA was positive in 47 of the 62 parasitologically proven cases and in 86 of the 116 cases with anti trypanosome antibodies detected by ELISA. Of the 80 horses examined antigen-ELISA was positive in 45 (56.75%) sera. The antigen assay was positive in 14 of the 19 parasitemic cases, whereas the antibody assay was positive in 18 of the 30 parasitemic cases.
Comparative evaluation of various parasitological and immuno-diagnostic techniques in buffalo calves experimentally infected with *T. evansi* was carried out by Baghel *et al.* (1996). It was observed that among the immunological techniques, the trypanosome antigen was first detected by sandwish-ELISA followed by CIEP and DID. Indirect ELISA detected antibody in 76.53 % among 298 serum samples but DID and CIEP could not detect the antibodies. By parasitological techniques 13.26% of 490 blood samples examined were found positive for *T. evansi*, whereas the immunodiagnostic techniques detected trypanosomal antigen in 73.81 % and antibodies in 25.51 % of the 294 serum samples examined.

Boid *et al.* (1996) opined that although direct demonstration of trypanosomes in the infected animal gives conclusive proof of infection, the limitations of parasitological diagnosis has been the driving force for research into alternative techniques like immuno-diagnostic techniques which provide indirect evidence of infection. it was emphasized that tests such as the IFAT and ELISA directly measure the interaction between antigen and antibody rather than relying on a secondary reaction consequent upon the initial binding.

Boid *et al* (1996) evaluated three methods for detecting trypanosomes in the experimentally infected sheep and goats. The wet film examination detected 5 out of 80 (6 %) whereas thick wet film examination could detect 25 out of 80 (31 %)and the the microhaemotocrit centrifugation could detect 67 out of 80(84 %).

Greiner *et al.* (1997) screened a total of 457 serum samples from cattle kept under moderate tsetse challenge in Uganda and 86 sera from cattle in Germany were tested for Trypanosoma antibodies using Enzyme Linked ImmunoSorbent Assay with antigen obtained from blood stream form (BSF) and *in vitro* cultivated procyclic (PRO) trypanosomes.It was observed that the diagnostic agreement between both assays was significant and they recommended the PRO-ELISA to be used for sero-epidemiological surveys since *in vitro* cultivation of procyclic trypanosomes allows a continuous and standardised preparation of test antigen.
Jithendran and Rao (1997) diagnosed the subclinical *T. evansi* infection in experimentally infected calves by antibody detecting ELISA and concluded that ELISA may be considered a potential screening test for the detection of latent trypanosomosis in bovines.

Elamin *et al.* (1998) conducted a survey for the prevalence and infection pattern of *T. evansi* in camels in mid-eastern Sudan by Ag-ELISA in conjunction with parasitological examination of blood and reported 5.4 % based on parasitological examination and 31.3 % based on Ag-ELISA and also found that the infection rate was higher during the dry period (November to May) than the wet season (June to October).

Ghorui and Srivatsava (2000) found that the weekly collected sera from experimentally *T. evansi* infected rabbits were assayed for detection of anti-trypanosomal antibody by IFAT and antibody capture assay (dot-ELISA) and detected the anti-trypanosomal antibody seven days after infection and concluded that these two antibody assay systems were found to be rapid, sensitive and specific.

In Malawi, Van den Bossche *et al.* (2000) surveyed the distribution and epidemiology of bovine trypanosomosis by parasitological and serological methods and found 1.96 % of the 186 cattle examined to be positive.

In Vietnam, Verloo *et al.* (2000) evaluated different diagnostic antibody detection methods to detect infections of *T. evansi* in water buffaloes and reported the diagnostic sensitivity and specificity as 98, 82 and 95 % for direct card agglutination test, indirect card agglutination test and antibody detection ELISA, respectively.

Reid and Copeman (2002) found that an antibody ELISA using five different crude antigens of *T. evansi* and an IgM-ELISA, were highly specific but had a low to moderate sensitivity when evaluated with serum from Indonesian cattle naturally infected with *T. evansi* and in uninfected Australian cattle.

Enzyme linked immuno-sorbent assay was standardized by Jeyabal *et al.* (2003) to detect circulating immune complexes (CIC) of *T. evansi* in cattle and buffaloes. It was observed that 39.64 and 36.1 % buffaloes out of 111 cattle and 225 buffaloes screened
were positive. CIC-ELISA was compared with conventional methods and the positivity of cattle and buffaloes for *T. evansi* with wet blood film was 3.6 and 2.35 % in comparison to 4.50 and 2.35 with microhaematocrit centrifugation technique respectively.

Dwivedi (2004) detailed the recent advances in diagnosis and therapy of trypanosomosis observed in humans and animals in tropical and sub-tropical regions of the world. The diagnostic methods for *T. evansi* infection particularly in subclinical or carrier state of infection included dark-ground phase contrast buffy coat examination, detection of trypanosomes in haemolysed blood, anion exchange technique for detection of trypanosomes and animal inoculation test.

Narender Singh *et al.* (2004) screened a total of 1161 camels for *T. evansi* infection by parasitological (wet blood film, stained thin blood smears), immuno-diagnostic (double antibody sandwich ELISA) and DNA amplification by polymerase chain reaction (PCR) and noted a prevalence of *T. evansi* in 17.05, 9.67, 4.60 and 4.14 % by PCR, Ag-ELISA, TBS and WBF with a sensitivity of 100.0, 56.75, 27.02 and 24.32 per cent, respectively.

Reeta Rani Jeena *et al.* (2004) recorded the incidence of trypanosomosis in cattle and buffaloes in Rajasthan by screening a total of 200 serum samples comprising 100 from buffaloes by indirect fluroscent antibody test and found a total of 51 per cent cattle in the test were sero reactive to *T. evansi*, whereas an overall incidence in buffaloes sero-reactive to *T. evansi* was 48 per cent.

### 2.2.2 Separation and purification of Trypanosomes

Salivary trypanosomes were separated by Lanham and Godfrey (1970) from infected blood by adsorbing the particulate blood components onto DEAE-cellulose columns and eluting the trypanosomes and concluded that the separation depends fundamentally on differences in surface charge since the DEAE-cellulose adsorbs the
more negatively charged blood components while the less negatively charged flagellates are eluted.

Isolation of the trypomastigote form of *T. evansi* from a mixture of the trypomastigote and epimastigote forms of the parasite was carried out by Al-abbassy *et al.* (1972) by the use of a DEAE-cellulose column. The percentage of the trypomastigotes in the original mixture was approximately 19 per cent and the percentage of this form after the first passage was 76 per cent and the percentage after the second passage was 98 per cent.

Jatkar and Purohit (1977) separated *T. evansi* organisms from the blood of infected rats and mice for preparing the antigen. Antigen free from red blood cells and serum was obtained by mixing infected blood with phosphate buffer containing EDTA and normal dog serum. During subsequent refrigeration, the red blood cells settled to the bottom and centrifugation of supernatant fluid at various speeds and repeated washing of the sediment with phosphate buffer produced clear trypanosome preparations.

Ahuja *et al.* (1985) adopted phytohaemagglutination for purification of *T. evansi* from the blood of experimentally infected rats. Exposure of *T. evansi* to prolonged and/or high concentrations (4-14 mg/ml blood) of phyto-haemagglutinin killed and degenerated the trypanosomes. The quick removal of the trypanosomes from phytohaemagglutinin medium and suspension in phosphate saline glucose (pH 8.0) medium prevented killing and degeneration of trypanosomes.

The trypanosomes separated through DEAE cellulose columns were dried in air, fixed in methanol and stained with Giemsa stain by Srivatsava *et al.* (1987). The parasites were found to be intact and the internal structures were clearly visible after staining.

Srivatsava *et al.* (1988) employed DEAE-cellulose columns of 5 and 20 cm height containing cellulose slurry treated with phosphate saline glucose (6:4) for separation of *T. evansi* from blood of rats and observed that when the height of column was more, the parasites took more time to come in the elute and recovery rate was up to 40 to 50 % and
when the height of the column was reduced to 5 cm, 80 to 90 % trypanosomes were collected in elutes in less time.

Ghorui and Srivastava (1999) studied the immunochemical studies of variant surface glycoproteins of *T. evansi* and the trypanosomal soluble proteins were resolved in low ionic strength phosphate buffer through anion exchanger diethyl amino ethyl cellulose columns and found that the unbound protein revealed a polypeptide mass of 66.67 kDa on sodium dodecyl sulphate polyacrylamide gel electrophoresis and this antigen showed a strong precipitin in immuno-electrophoresis with homologous hyperimmune serum.

### 2.3 SDS-PAGE and Enzyme Immuno Transfer Blot (EITB) studies of *T. evansi*

Gibson *et al.* (1978) studied the comparative analysis of iso enzyme soluble proteins, poly peptides and free amino acids of ten isolates of *T. evansi* by SDS-PAGE and showed inter species differences due to polypeptides in the molecular weight range of 40-70 kDa.

Pathak *et al.* (1993) analysed the crude somatic antigen of *T. evansi* of camel origin and identified the presence of protein bands ranging from 14 to 65 kDa.

Comparative data on the poly peptide profiles of seven stocks whole cell lysate antigen of *Trypanosoma evansi* were studied by Veer Singh *et al.* (1994). It was indicated that the maximum number of bands (21-36) were found in the range of 81 to 166 kDa, followed by 33 to 81 kDa (15-19 bands) and the minimum number of bands (9-15) were found in the in the range of 14 to 33 kDa.

Veer Singh *et al.* (1995b) studied the Western blot analysis of cell membrane preparations of *Trypanosoma evansi* by employing the hyper immune serum against whole cell lysate antigen which revealed 11 major bands where as flagellar preparations detected 3-14 bands in the range of 17.2 to 98.2 kDa.

Polypeptides and antigens of whole cell lysate, cell membrane and flagellar preparations of seven different stocks of *T. evansi* were studied by Veer Singh *et al.* (1995b) by SDS-PAGE. The major poly peptide bands were found in the range of 33 to
81 kDa in whole cell lysate antigen and 17.6 to 80.2 kDa in flagellar antigen and the cell membrane preparations had 2 to 5 polypeptides in the molecular weight range of 48.4 to 80.2 kDa.

Veer Singh et al. (1997) by Western blotting of whole cell lysate preparations of *Trypanosoma evansi* isolated from buffaloes, equids and camels from different locations of northern India, detected major antigens ranged between 17 and 30 and most of which were in the range of 14.6 to 99.5 kDa when tested with hyper immune serum.

Xie-chao et.al. (1997) used the western blot technique to analyse the anti sera of excreted/secreted antigen of *T.evansi* which manifested 5 bands with molecular masses ranged from 105 to 52.8 kDa whereas anti sera of soluble antigen revealed molecular masses ranged from 71.6 to 52.8 kDa.

Jithendran and Rao (1999) by SDS-PAGE observed the common and major *T. evansi* surface component associated proteins in the molecular weight range of 95 to 97, 83 to 89, 72 to 75, 63 to 67, 42 to 48, 32 to 38 and 19 to 23 kDa.

Polypeptide profile of *T. evansi* isolate of camel and cattle origin were studied by Pareek et al. (1999) of which the polypeptides of camel isolate revealed a complex protein band pattern ranging from 180 to 24 kDa, while the molecular weight of cattle isolate varied from 195 to 26 kDa.

Uzcanga et al. (2002) with western blot revealed a high immunological cross reaction between *T.evansi* and *T.vivax* and observed that an antigen with 64 kDa was responsible for the cross reactivity.

Rocio et al. (2004) purified two additional cross-reacting antigens with molecular masses of approximately 51 and 68 kDa from the cytosolic fraction of the *T. evansi* isolate by sequential chromatography on DEAE-sepharose and sephacryl S-300 and reported that sera obtained from animals infected with *T. evansi* or *T. vivax* recognised with purified proteins, suggesting their potential use as diagnostic reagents.
Western blot analysis conducted by Camargo et al. (2004) using sera from bovines infected with *T. evansi* recognized the poly peptide pattern of molecular weight ranging from 14-109 kDa.