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EPIDEMIOLOGICAL AND CLINICO - THERAPEUTIC STUDIES ON BOVINE TRYPANOSOMOSIS

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Thesis submitted in partial fulfilment of the requirement for the degree of

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2005

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DECLARATION

I hereby declare that the thesis, entitled **"EPIDEMIOLOGICAL AND CLINICO-THERAPEUTIC STUDIES ON BOVINE TRYPANOSOMOSIS"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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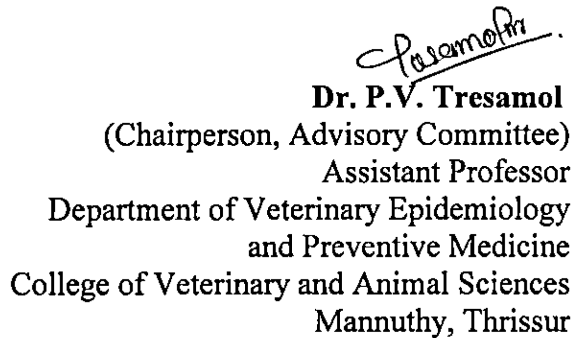
CERTIFICATE

Certified that the thesis, entitled **"EPIDEMIOLOGICAL AND CLINICO - THERAPEUTIC STUDIES ON BOVINE TRYPANOSOMOSIS"** is a record of research work done independently by **Dr. Smitha. P.S.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.



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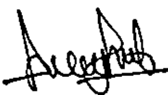

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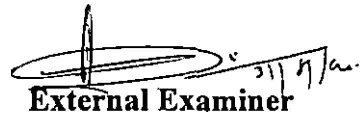

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Introduction

1. INTRODUCTION

Trypanosomosis is a major constraint to livestock production in tropical and subtropical countries. The causative agent, *Trypanosoma evansi* (*T.evansi*) is a cosmopolitan parasite that affects a wide range of hosts in Asia, Middle and East Africa, and Central and South America. *Trypanosoma evansi* is transmitted mechanically by haematophagous biting flies of the family Tabanidae, of which the most important is Tabanus. Inspite of being considered a haemoflagellate, *T.evansi* is a protozoan parasite of both intra and extra vascular fluids (Sukanto *et al.*, 1990).

There are considerable differences in the severity of symptoms caused by *T.evansi* infections in different geographical areas of its occurrence depending on virulence of strain and susceptibility of the host (Herrera *et al.*, 2004). Cattle and buffalo act as reservoirs of *T.evansi*. They run a subclinical or latent course of infection and clinical signs are precipitated on exposure to adverse climatic conditions, stress, vaccination or concurrent diseases. These carrier animals may remain as a source of infection to other animals including dogs and cats (Rode, 2002).

Clinical signs when exhibited include dullness, depression, anaemia, respiratory distress, staggering gait, circling movements and drop in milk production. The ability of trypanosomes to periodically switch their major surface glycoprotein coat produces relapses of parasitaemia resulting in intermittent fever in the affected animals.

The diagnosis of *T.evansi* infections by clinical signs is difficult because of the varied and non-specific clinical signs and the mild chronic form of infection in bovines. The routine diagnosis is mainly based on the detection of flagellates in wet films or microhaematocrit test. These methods though specific are less sensitive in

low levels of parasitaemia. Serological tests based on antigen and antibody detection can effectively complement the parasitological techniques and are used for mass screening of susceptible animals. Molecular methods based on DNA amplification are currently gaining popularity as highly sensitive and specific diagnostic tools.

Therapy and prophylaxis of surra still remain a problem because of the limited number of compounds available in the market, emergence of drug resistance and toxicity and relapses after chemotherapy. Vector control is a difficult task in India. With the range of trypanosomosis expanding because of the constant movement of livestock and ubiquitous biting flies, early detection and treatment of animals is a formidable task in the hands of veterinarians.

The present study was conducted with the following objectives:

1. To assess the prevalence of trypanosomosis among cattle and buffalo.
2. To study the epidemiological factors associated with trypanosomosis.
3. To compare the efficacy of selected parasitological tests for the diagnosis of trypanosomosis, and
4. To assess the efficacy of antitrypanosomal drugs in trypanosomosis.

Review of Literature

2. REVIEW OF LITERATURE

2.1. INTRODUCTION

Gill (1965) stated that surra was an important disease of domestic animals in most countries of Asia.

Trypanosomes were ranked high in importance amongst parasites of man and animals by Gill (1977).

According to Soulsby (1982), *T.evansi* was transmitted by biting flies (Tabanidae) and affected a wide range of domestic species.

Trypanosomosis or surra in farm animals was caused by *T.evansi* and was transmitted by tabanid flies (Kaur and Ahuja, 2002).

Rode (2002) described trypanosomosis as an important haemoprotozoan infection occurring throughout the tropical and subtropical regions of the world.

Gupta *et al.* (2003) reported that trypanosomosis or surra was a widely prevalent disease among different domestic and wild animals in India.

2.2. ETIOLOGY

In majority of infections, *T. evansi* was monomorphic in character, but polymorphism occurred sporadically. The typical form was 15-34 µm in length (mean 24 µm), the kinetoplast was sub-terminal, the undulating membrane was well developed and there was a substantial free flagellum (Hoare, 1972).

The earliest workers to detect trypanosomes were Laveran and Mesnil in the year 1807 in the blood of a trout, *Salmo farcio*. Gruby in 1843 gave the name

trypanosome to the organism described in a frog. *Trypanosoma evansi*, the first trypanosome to be demonstrated as pathogenic to domestic livestock was recognized by Evans in 1880 from the blood of horses and camels at Derra Ismail Khan, which is now in Pakistan (Gill, 1977).

Rottcher *et al.* (1987) described *T.evansi* to be a cosmopolitan parasite that affected a wide range of hosts in which it caused illness.

Biometrical observations on different strains of *T. evansi* by John *et al.* (1992) revealed that buffalo, bovine and canine trypanosomes varied in length from posterior end to kinetoplast, kinetoplast to mid-nuclear part, mid-nuclear part to anterior end, and the free part of the flagellum and these differences were highly significant ($P < 0.01$). The buffalo strain in general had a greater length of free flagellum, total length and width ($P < 0.01$) compared to the canine strain. However, the buffalo and bovine strains did not differ significantly ($P > 0.01$).

2.3. EPIDEMIOLOGY

2.3.1. Incidence

2.3.1.1. *Global*

Payne *et al.* (1991b) reported natural cases of surra among bovines in Indonesia based on an epidemiological study using parasitological and serological methods.

Tuntasuvan *et al.* (1997) described 42 cases of nervous form of trypanosomosis among cattle of 13 farms in Bangkok. The symptoms included circling, excitation, jumping, aggressive behaviour, lateral recumbency, convulsions and finally death.

The prevalence of trypanosomosis due to *T.evansi* in the Brazilian Pantanal was described by Seidla *et al.* (1998).

The prevalence of *T.evansi* infection among camels and horses in Jordan was studied by Abo-Shehada *et al.* (1999) using thick blood smears and inoculation techniques with mice and rats. The clinically affected camels were positive by thick blood smear and mouse and rat inoculation; while in horses, mouse and rat inoculation revealed greater number of positive cases than thick blood smears.

Cheah *et al.* (1999) investigated the epidemiology of *T. evansi* infection in crossbred dairy cattle for a period of 12 months on a dairy cattle farm in Penninsular Malaysia. It was concluded that the prevalence of parasitaemia was highest in lactating animals (13.4 per cent), followed by those in the dry herd (8.8 per cent), late pregnant animals (8.1 per cent), early pregnant animals (4.7 per cent), calves (0.3 per cent) and heifers (0.2 per cent).

Pholpark *et al.* (1999) studied the effect of sub-clinical *T.evansi* infections on the milk yield of newly introduced Holstein-Friesian cattle in Loei province of north-east Thailand. Antigen-Enzyme linked immuno-sorbent assay (Ag-ELISA) detected trypanosome antigen in 40 per cent of the screened samples and the study suggested that sub-clinical trypanosomosis caused decreased milk production in dairy cattle.

The prevalence of *T.evansi* infection among camels in Morocco, Nigeria was reported by Atarhouch *et al.* (2003). A total of 1460 serum samples were collected and tested by a card agglutination test for trypanosomosis (CATT) and antibody (Ab) -ELISA. The overall sero-prevalence was 14.1 per cent by CATT and 18.2 per cent by Ab- ELISA.

2.3.1.2. *India*

In India, the first authentic record of a natural outbreak of surra among cattle was made at Karnal by Lingard (Gill, 1977).

Das *et al.* (1998) studied the prevalence of bovine surra during the period from 1992 to 1996 in Guntur district of Andhra Pradesh. Among 492 cattle and 7411 buffalo examined, seven cattle (1.42 per cent) and 201 buffalo (2.71 per cent) showed parasitaemia in their blood while the overall prevalence of the parasite in bovines was 2.63 per cent.

Jayathangaraj *et al.* (1999) reported a case of trypanosomosis in a captive wild dog kept at Vandalur zoo in Tamilnadu. The dog was suffering from anorexia, dullness, fever and did not respond to therapeutic management with antibiotics.

The occurrence of surra in buffalo and ponies in R.S.Pura and Samba Tehsil areas of Jammu Division was described by Raina *et al.* (2000) and it was concluded that buffalo mainly suffer from the sub-acute form of trypanosomosis and act as carriers.

Shukla *et al.* (2000) described a case of trypanosomosis due to *T. evansi* in a mare aged three and a half years in Dhar district. The mare was presented with a history of inappetance, nasal and ocular purulent discharge, corneal opacity, swaying of hindquarters and responded to treatment with antrycide prosalt and intra-venous administration of dextrose.

An outbreak of trypanosomosis in bovines in Punjab due to *T.evansi* after dexamethasone administration characterized by symptoms of high fever (105-106°F), muscle twitching, anorexia, increased salivation and acute abdominal pain manifested by kicking at the belly was described by Gupta *et al.* (2003).

Rajkhowa *et al.* (2003) recorded the occurrence of trypanosomosis in mithuns in Nagaland.

Trypanosomosis due to *T.evansi* among 96 equines in Jammu region screened for haemo-protozoan infections was reported by Soodan *et al.* (2003) based on wet blood film, thin blood smear and mouse inoculation tests.

Singh *et al.* (2004) used parasitological, serological and DNA based amplification methods for diagnosis of natural *T.evansi* infection in camels. The prevalence was detected as 17.05, 9.67, 4.6 and 4.14 per cent by polymerase chain reaction (PCR), Ag-ELISA, blood smear and wet film examinations respectively.

2.3.1.3. *Kerala*

Balakrishnan *et al.* (1994) reported three clinical cases of trypanosomosis due to *T.evansi* in dogs brought to the University Veterinary Hospital, Kokkalai, Thrissur during the period from 1988 to 1993.

Canine trypanosomosis with jaundice in a one-year-old dachshund dog brought to the University Veterinary Hospital, Mannuthy, Thrissur was described by Baby *et al.* (2000).

Tresamol *et al.* (2003) reported a case of trypanosomosis in crossbred cattle of a private dairy farm in Thrissur district.

Ajitkumar *et al.* (2004) reported a case of trypanosomosis in a five-year-old Murrah cross-bred she-buffalo with profuse nasal bleeding for four days.

2.3.2. Hosts

Trypanosoma evansi affected camels, buffalo, cattle, pigs and horses in tropical and subtropical areas of the world (Songa and Hames, 1987).

In a study of surra among bovines of Indonesia, Payne *et al.* (1991a) found a higher infection rate among buffalo. It was postulated that this could be associated with the environmental factors like their use for land preparation in areas of irrigated rice fields, which favour high tabanid population. The infection with *T.evansi* was seen in each of the seven breeds of cattle encountered in the study.

Bovines acted as reservoirs of infection and so the latent form of infection in cattle and buffalo posed serious problem in the spread of infection to more susceptible species (Kaur and Ahuja, 2002).

In an epidemiological study of trypanosomosis in Durg district of Chhattisgarh state, Agrawal *et al.* (2003) reported higher prevalence of trypanosomosis in buffalo (9.33 per cent) as compared to cattle (6.57 per cent).

According to Kaur and Juyal (2003), cattle and buffalo acted as reservoir hosts for *T.evansi* due to sub-clinical nature of the disease.

2.3.3. Vectors

According to Oldryoyd (1954), favourable sites for transmission of surra were those that facilitated high concentrations of both animals and vectors.

A high positive association among *T.evansi* infection rates, tabanids and rainfall had been described for Sudan and India (Mahmoud and Gray, 1980).

Transmission of surra was by biting flies such as Tabanus, Stomoxys and Lyperosia. No cyclical development occurred in these. An essential factor in mechanical transmission was interrupted feeding on the part of flies, which went from one host to the other to become replete. Trypanosomes did not survive for more than ten to fifteen minutes in the proboscis of a fly (Soulsby, 1982).

The most frequent mechanical vectors of trypanosomes were hematophagous insects like Tabanidae and Stomoxyinae and sometimes Hippoboscidae and in these vectors, trypanosomes could survive only for a very short time of a few seconds or minutes. Female Tabanidae had mouthparts that were adapted for biting and feeding on blood and usually attacked large domestic or wild mammals whereas males were harmless (Shah-Fischer and Ralph Say, 1989).

According to Seidla *et al.* (1998), the incidence of *T.evansi* infections in the Brazilian Pantanal correlated positively with the population of Tabanidae.

2.3.4. Season

Trypanosomosis existed most in areas with considerable rains and good grass cover, which serve as suitable breeding environment for tabanids (Gill, 1977).

Silva *et al.* (1995) opined that the increased incidence of trypanosomosis caused by *T. evansi* among livestock in the Brazilian Pantanal during rain and flood season coincided with the high Tabanidae population during the season.

Surra was encountered after the onset of monsoon due to the presence of large biting flies from July to November with the peak from August to October (Prasad *et al.*, 1997).

An epidemiological study of bovine surra in Guntur district of Andhra Pradesh by Das *et al.* (1998) revealed that the maximum infection of the disease in bovines was found during monsoon season (34.13 per cent), followed by post-monsoon (32.21 per cent).

Trypanosoma evansi infections in ruminants in Vietnam occurred especially during the spring-winter season when conditions were harsh due to shortage of fodder and low atmospheric temperatures (Holland *et al.*, 2001a).

Agrawal *et al.* (2003) reported that the increased prevalence of trypanosomosis in rainy season (10.4 per cent) was accounted for by the decreased host resistance due to climatic stress in rainy season, which precipitated subclinical cases into apparent infections.

In a study on recrudescence of trypanosomosis after dexamethasone administration in a dairy farm in Ludhiana district, Gupta *et al.* (2003) reported that hot and humid season in September after rains was favourable for spread of the disease since it was the breeding season of tabanid fly, the vector of surra.

Trypanosomosis due to *T.evansi* was mainly recorded during rainy season because of the availability of vectors (Soodan *et al.*, 2003).

Increased risk of surra among camels of Chad was seen during dry season. More acute forms of the disease seen during dry season appeared to relate to malnutrition, with abundant pasture and water available only in the rainy season (Delafosse and Doutoum, 2004).

2.3.5. Age

In a study based on the sero-diagnosis of surra among 2145 bovines of Indonesia, Payne *et al.* (1991b) found that an ascending age related prevalence was seen in both cattle and buffalo with the highest rates seen in animals older than two years of age and the lowest in animals in their first year of life.

According to Das *et al.* (1998), based on an epidemiological study of bovine surra in Guntur district of Andhra Pradesh, adult buffalo had higher average prevalence of the disease (3.11 per cent) than young ones (1.16 per cent).

From an epidemiological study of surra among camels of Chad, Delafosse and Doutoum (2004) found out that the risk of infection increased with age, with the acute disease being more common in young animals.

2.3.6. Management

Stress, like hard work and immunization precipitated relapses of dormant infections of trypanosomosis (Gill, 1977).

Transmission of trypanosomosis occurred easily when the animals were closely confined and put together in animal fairs (Dwivedi, 2000).

Large-scale movement of animals increased the risk of infection due to an increase in the probability of contact between camels and parasites traversing certain environments (Delafosse and Doutoum, 2004).

2.4. PATHOGENESIS

2.4.1. Anaemia

Naylor (1971) observed that anaemia in trypanosomosis caused degenerative changes in skeletal and cardiac muscles.

Coating of erythrocytes, leucocytes, platelets and other cells with trypanosome antigen, antibody, immune complexes and autoantibodies damaged them and predisposed them to phagocytosis (Mackenzie *et al.*, 1978).

Based on experimental infection of sheep with *T.evansi*, Bouteille *et al.* (1988) concluded that a level of haemoglobin (Hb) below eight gram per cent was first attained about sixth week after inoculation and it tended to worsen and reached six gram per cent during the final stages. The anaemia was found to be *normochromic.*

The causes of anaemia and immunosuppression in trypanosomosis was worked on by Esievo and Saror (1991). According to them, the mechanisms of anaemia in trypanosomosis were debatable but the contributory factors included haemolysis, haemodilution, dyshaemopoeisis, non-specific immunological mechanisms, complement factors and reduction in the life span of erythrocytes due to removal of surface sialic acid by sialidase produced by the trypanosomes.

Igbokwe (1994) observed that living and dead trypanosomes produced a number of biologically active substances like proteases, neuraminidases and phospholipases that damaged the vascular endothelium and blood cellular components. The undulating fever associated with trypanosomosis contributed towards erythrocyte destruction by causing increased osmotic fragility, membrane permeability and decreased plasticity of the erythrocyte membrane.

Walia *et al.* (1996) stated that *T.evansi* infection decreased the sialic acid content of erythrocyte membranes and the desialylation of host cells during infection indicated the initiation of pathogenic changes in blood cells and nervous tissues.

Haematological studies on *T.evansi* infection by Das *et al.* (1998) revealed that the Hb content and the erythrocyte numbers were reduced to 25 per cent in the infected animals.

Audu *et al.* (1999) hypothesized that anaemia in *T.evansi* infection might be due to involvement of specific immunoglobulins against *T.evansi*, which form

complexes with the antigen and complement on the surface of red blood cells leading to their sequestration and destruction in the reticulo-endothelial system.

Erythrophagocytosis was an important mechanism leading to anaemia in the pathology of *T.congolense* infection in Zambian goats (Witola and Lovelace, 2001).

Rajkhowa *et al.* (2003) opined that anaemia in trypanosomosis affected mithun might result from inhibition of erythrocyte formation in bone marrow or their lysis by endotoxins liberated by trypanosomes and observed decreased Hb (8.2 ± 0.2 g per cent), packed cell volume (PCV) (38.5 ± 0.5 per cent) and total erythrocyte count ($5.65 \pm 0.15 \times 10^{6}$ /µl) in the affected animals, suggestive of anaemia.

2.4.2. Immunosuppression

Thymus dependent suppressor cells were involved in non-specific suppression of immunity in mice experimentally infected with *T. brucei* (Jayawardena and Waksman, 1977).

Jayawardena *et al.* (1978) using S42 strain of *T.brucei* in mice found that the ability of spleen cells from infected mice to mount primary antibody response to sheep red blood cells was drastically reduced and also spleen cells from infected mice when co-cultured with normal spleen cells suppressed the ability of the normal cells to respond to antigens.

Sollod and Frank (1979) stated that immunodepression in pathogenesis of natural field infection with trypanosomes was undefined, but the implications were clearly understood: the immunodepressed state prolonged the primary infection, allowed the development of secondary infections and rendered vaccination procedures for non-related diseases ineffective.

Investigations on the effect of animal trypanosomosis on Rinderpest vaccination under field conditions by Twinamasiko and Kakaire (1994) revealed that the pathogenic trypanosomes caused a delay in the antibody response to Rinderpest vaccination.

Ouma *et al.* (1997) studied experimental *T.evansi* infection in five dromedary camels and demonstrated that complement concentration increased in the infection initially, followed by a decrease as the infection progressed to chronicity, leading to immunosuppression.

In *T. evansi* infected sheep, a decline in number of T cells was observed (Onah *et al.*, 1998).

According to a study by Holland *et al.* (2001b) based on the immune responses against heterologous antigens in *T.evansi* infected water buffalo, it was concluded that humoral and cell mediated immune responses against heterologous antigens were suppressed in *T.evansi* infected animals.

2.4.3. Haemato-biochemical changes

In rats experimentally infected with *T.brucei* and *T.equiperdum,* an increase of globulin, a decrease of albumin and altered albumin-globulin (A-G) ratio was observed. Kidney damage was suggested as the probable cause for the altered ratio (Okechukwu, 1946).

In pigs experimentally infected with *T.evansi*, Srivastava and Ahluwalia (1973) noted a fall in serum albumin with simultaneous rise of globulins until the normal ratio was reversed. Hypoglycemia and hypochromic anaemia were features of the disease.

Trypanosomes secreted or excreted hydrolytic enzymes, mainly proteases into the extra-cellular space, which reduced the total plasma proteins. High glucose consumption by trypanosomes was responsible for the fatty degeneration of liver in the infected animals (Raisinghani *et al.*, 1980).

Mallick and Dwivedi (1981) observed marked hypoglycemia in nine animals out of twelve acute cases of surra in cattle and buffalo.

Fall in blood glucose level was noted even in aparasitaemic days during *T. evansi* infection by Kathiria and Avsatthi (1985). The biochemical changes noticed in experimentally infected buffalo calves included gradual fall in blood glucose level in the acute phase of the disease and an irregular or fluctuating fall in the chronic phase. No significant deviation in total serum protein was observed, but an increase in serum gamma–globulin was noticed.

According to Otesile *et al.* (1991), *T.brucei* infections in boars led to a significant decrease in both serum total proteins and albumin levels.

No significant difference in basal plasma glucose level was observed by Abebe (1992) in *T. congolense* infected cattle.

Haematological studies by Sangwan *et al.* (1993) on natural *T.evansi* infection in buffalo in a surra endemic area of Eastern Haryana revealed low levels of PCV, Hb concentration, folic acid and vitamin B_{12} in the positive animals.

Circulating trypanosomes and their by-products caused haemolysis and consequent fall in PCV in sheep experimentally infected with *T.evansi.* Also, the decrease in total plasma protein concentration was as a result of increased protein break down or urea loss, haemodilution and serum extravasation, which caused oedema in infected sheep (Audu *et al.*, 1999).

Onah *et al.* (1999) concluded that infection with *T.evansi* resulted in significant alterations in the number of T and B lymphocytes in infected sheep and *T.evansi* specific IgM and IgG were detected in all the positive cases.

Trypanosoma evansi secreted proteases, which degrade plasma proteins and this accounted for the hypoproteinaemia in trypanosomosis (Kaur and Ahuja, 2002).

Kaur and Juyal (2003) noted significant decrease in Hb, PCV and total erythrocyte count in cow calves infected experimentally with *T. evansi*. He also noted significant increase in total plasma protein on days 28 and 35 post infection.

Haemato-biochemical changes seen in trypanosomosis in mithuns due to *T.evansi* included decreased Hb content, PCV, total erythrocyte count and blood glucose. Leucocytosis associated with lymphopaenia, neutrophilia and eosinophilia were also observed (Rajkhowa *et al.*, 2003).

2.5. CLINICAL SIGNS

According to Gill (1965), commonest manifestation of trypanosomosis due to *T.evansi* in cattle and buffalo was the chronic form of the disease, which was accompanied by very low parasitaemia.

In a study involving acute cases of surra among 12 cattle and buffalo, Mallick and Dwivedi (1981) observed that the clinical symptoms involved weakness, staggering gait, signs of nervous involvement and exhaustion.

The major clinical effects of surra in camels included mortality, loss of body condition, abortion and agalactia (Rottcher *et al.*, 1987).

The clinical signs noticed by Singh and Misra (1988) in experimentally infected cow-calves included a mild rise of temperature on the fourth day post infection followed by an afebrile period, enlargement of prescapular lymph nodes, progressive reduction in body weight, conjunctivitis, bilateral mucopurulent discharge from eyes coupled with sunken eyes and testicular swellings in two of the five infected calves.

Based on experimental *T. evansi* infection in rams, Ngeranwa *et al.* (1991) concluded that infection caused severe pathology in the testicles and subsequent deterioration in the semen quality.

According to an epidemiological study on surra by Payne *et al.* (1991a) among bovines in Indonesia based on parasitological and serological tests, no clinical disease owing to *T.evansi* infection was seen in any of the sampled areas, despite the high infection rates encountered in many instances.

The pathogenic effects of trypanosomosis were varied including anaemia, immunosuppression, retarded growth, weight loss and poor reproductive performance, resulting in a delay in oestrus, abortion and still birth (Luckins, 1992).

Saseendranath (1993) observed facial oedema, swollen eye lids, conjunctivitis, purulent lachrymal discharge, anaemia, progressive loss of weight, swollen lymphnodes, orchitis and abortion to be the prominent symptoms in experimental *T.evansi* infection in sheep.

Kandavel and Nedunchelliyan (1994) inoculated *T.evansi* into nine male buffalo calves subcutaneously and noted temperature rise from the fourth day post-infection. A peak rise was noticed between sixth and tenth day post-inoculation followed by return to normal temperature within three to four days.

Fall in body weight in sheep experimentally infected with *T.evansi* was an indication of the wasting nature of trypanosomosis (Sachey, 1998).

Audu *et al.* (1999) opined that metabolic disorders due to the presence of circulating trypanosomes and their by-products played a significant role in pyrexia.

Trypanosoma evansi caused fatal surra in equines and decreased productivity in bovines (Kaur and Ahuja, 2002).

According to Rajkhowa *et al.* (2003), the clinical signs in trypanosomosis-infected mithuns included intermittent fever, dullness, emaciation, anaemia and mucopurulent discharge from the eyes.

Ajitkumar *et al.* (2004) reported profuse bilateral nasal bleeding for four days and fever in a she-buffalo suffering from trypanosomosis.

2.6. DIAGNOSIS

Animal trypanosomosis presented special problems with regard to diagnosis. The clinical signs were not pathognomonic and the standard techniques for the detection of trypanosomes were not sufficiently sensitive. A high proportion of infections remained undetected, as the chronic, more common form of the disease was often aparasitaemic (Nantulya, 1990).

2.6.1. Examination of blood

Killick-Kendrick and Godfrey (1963) regarded thick blood film to be more efficient than wet film examination for the detection of *T.evansi* infections.

According to Luckins *et al.* (1978), parasitological diagnosis of trypanosomosis was easy in animals with acute or sub-acute infection when trypanosomes were present in very large numbers in the peripheral circulation, but was more difficult in latent or chronic infections in which parasitaemia was intermittent or low.

Paris *et al.* (1982) stated that the sensitivity of parasite detection techniques in trypanosomosis was limited because of low parasitaemia in cattle.

According to Boid *et al.* (1985), the diagnosis of *T.evansi* infection by microscopical examination of Giemsa stained blood smears was insensitive and led to underestimation of the prevalence of infection.

Comparison of six parasitological methods in the diagnosis of *T.evansi* infection by Monzon *et al.* (1990) revealed the sensitivity of wet blood film examination and Giemsa stained blood smears to be 53.8 per cent and 45.6 per cent respectively.

In two replications of experimental *T.evansi* infection in sheep by Saseendranath (1993), it was found that wet blood film detected parasitaemia in 51.21 ± 6.5 and 36.24 ± 8.67 per cent samples when the animals were having a febrile reaction and 28.69 ± 6.5 and 21.69 ± 3.45 per cent samples when animals were having normal body temperature. The blood smear examination detected trypanosomes in 61.18 ± 13.6 and 58.54 ± 7.66 per cent of the examinations.

In low parasitaemia, a drop of blood concentrated and spread into a smear of 1.0 to 1.25 cm area, stained with Giemsa for 25 minutes using PBS, pH 7.2 as diluent helped to locate the organism quickly (Dwivedi, 2000).

Wet blood film examination detected trypanosomes in peripheral blood of buffalo calves from day six to fourteenth day post-challenge in experimental *T.evansi* infection (Singh *et al.*, 2002).

Definitive diagnosis of a current infection with *T.evansi* relied on the demonstration of the parasite in the blood or tissue fluids of infected animals (Singh *et al.*, 2004).

2.6.2. Concentration techniques

Trypanosomes were readily separated from blood cells and platelets by passing blood from infected mice and rats through a column of the anion exchanger, Diethyl Amino Ethyl (DEAE) cellulose. The separation depended fundamentally on differences in surface charge; the DEAE cellulose adsorbed the more negatively charged blood components while the less negatively charged flagellates were eluted (Lanham, 1968).

Lanham and Godfrey (1970) used DEAE cellulose to isolate salivarian trypanosomes from blood of man and other animals. They opined that the separation of trypanosomes using DEAE cellulose was very simple and useful quantities of trypanosomes could be collected from the hosts that exhibited only low parasitaemia.

Srivastava *et al.* (1988) found that when *T.evansi* infected rat blood was passed through DEAE cellulose column of five-centimeter height and two-centimeter diameter, it resulted in 80-90 per cent recovery of trypanosomes.

Micro-haematocrit centrifugation technique provided information on the number of animals with patent parasitaemia at the time of sampling. Such information would help in assessing the risk of infection, as one of the principal

requirements for successful mechanical transmission was the presence of animals with relatively high number of trypanosomes in the blood (Payne *et al.*, 1991b).

Dwivedi (2000) opined that micro-haematocrit centrifugation of blood and examination of buffy coat were good methods to detect sub-clinical or carrier state of trypanosomosis.

Diagnosis of experimental *T.evansi* infection in horses by Wernery *et al.* (2001) revealed that micro-haematocrit centrifugation technique was the most sensitive and detected parasites between one and three days post-infection.

Based on an epidemiological study of surra among camels in Chad, Delafosse and Doutoum (2004) concluded that the specificity of buffy coat technique was unequivocal but sensitivity was low because the method could not detect parasitaemia below thousand trypanosomes per millilitre.

2.6.3. Serological diagnosis

The most useful tests for detection of anti-trypanosome antibodies were the indirect fluorescent antibody test (IFAT), enzyme immunoassay (ELISA) and the card agglutination test for trypanosomosis (CATT). However, the shortfalls of these serological tests included the use of ill-defined antigens, inapplicability under field conditions and inability to indicate an existing infection (Nantulya, 1990).

According to Masake and Nantulya (1991), antibody detection could provide only a presumptive diagnosis of trypanosomosis as it could not differentiate between current and past infections; they were more useful as epidemiological tools rather than as diagnostic procedures.

Payne *et al.* (1991b) confirmed the suitability of ELISA as an indicator of infection with *T.evansi* in that ELISA classed 95 per cent of the trypanosome positive animals as antibody positive.

Saseendranath *et al.* (1994a) observed that Ab-ELISA could detect *T.evansi* antibodies from fourteenth day of infection onwards in 83.6 per cent of the samples from experimentally infected sheep.

An ELISA was standardized by Reyne-Bello *et al.* (1998) for the detection of anti-*T.evansi* antibodies in naturally and experimentally infected horses. A relative sensitivity of 98.39 per cent and specificity of 95.12 per cent were determined.

Molina *et al.* (2000) used an indirect immunoassay for the detection of specific anti-*T.evansi* IgG to assess the sero-prevalence of *T.evansi* infections in Canary Islands. He concluded that ELISA was one of the best serological methods to detect *T.evansi* infected camels because of the ability to measure specific antibodies and antigens in serum samples.

According to Lejon *et al.* (2003), ELISA was a useful method to complement the parasitological diagnosis of trypanosomosis in goats.

An Ab-ELISA for *T.evansi* infection in camels standardized by Shahardar *et al.* (2004) using crude solubilised antigen and detergent solubilised antigen had sensitivity of 62.22 per cent and 68.88 per cent respectively.

2.6.4. Dot – ELISA

The dot-ELISA was an easily performable and interpretable test, which was reagent conservative, cost effective and field portable for the diagnosis of various human and animal protozoan parasites (Pappas, 1988).

Based on experimental *T.evansi* infection in sheep, Saseendranath *et al.* (1994b) concluded that dot-ELISA detected trypanosomal antibodies in 83.6 per cent of samples from infected animals. He opined that dot-ELISA could be one of the best qualitative and more specific tests than Agar Gel Immuno diffusion (AGID) and Counter Immuno Electrophoresis (CIEP) and can be performed under field conditions.

Singla *et al.* (1996) described dot-ELISA for detection of antibodies to *T.evansi* infection in rabbits.

A dot-ELISA was optimized for detection of antibodies against *T.evansi* in dromedary camels by Shahardar *et al.* (2002). Assay of 90 field collected camel sera revealed 46.66 per cent sero-reactivity for *T.evansi.*

2.6.5. Comparison of diagnostic tests

In a comparative study of six parasitological methods for the diagnosis of *T.evansi* infection in 165 horses, Monzon *et al.* (1990) found that mouse inoculation test gave a sensitivity of 88.2 per cent, haematocrit centrifugation technique, 71.1 per cent; buffy coat method, 63.4 per cent; wet blood films, 53.8 per cent and Giemsa stained smears 45.6 per cent. They opined that the haematocrit centrifugation technique, mouse inoculation of blood and Giemsa stained smears were the most effective diagnostic combinations for *T. evansi.*

According to Payne *et al.* (1991b) the lower infection rates obtained with parasitological-based methods like micro-haematocrit centrifugation technique was due to continuous fluctuations in trypanosome numbers. The early appearance and persistence of trypanosome antibodies in the infected animal increased the probability of its detection by serological methods like ELISA.

Based on a study on *T. evansi* among cattle and buffalo in Calcutta, West Bengal; Ray *et al.* (1992) found out that Giemsa stained blood smears failed to reveal the infection. Mouse inoculation of the suspected blood samples detected parasitaemia in 5.7 per cent, 4.3 per cent and 8.0 per cent of crossbred cattle, zebu cattle and buffalo respectively. Indirect fluorescent antibody test (IFAT) showed positivity in 45.4 per cent of the crossbred cattle, 22.6 per cent of the zebu and 24 per cent of the buffalo.

Olaho-Mukani *et al.* (1993) compared antigen and antibody immunoassays for the diagnosis of *T.evansi* infection in camels and concluded that use of Ag-ELISA alone or in combination with buffy coat examination could be a more preferred approach in assessing patent infections than the use of Ab-ELISA.

Comparative evaluation of various parasitological and immunodiagnostic techniques in buffalo calves experimentally infected with *T.evansi* by Baghel *et al.* (1996) revealed that mini-anion exchange chromatography (mAEC) and micro-haematocrit centrifugation techniques (MHCT) detected trypanosomes in blood samples earlier (6.6 ± 0.4 days post-infection) than wet film, thin and thick blood smears (6.8 ± 0.37 days post-infection). The rate of detection of trypanosomes in 98 blood samples was 17.35 per cent for mAEC, 15.31 per cent for MHCT and 11.22 per cent, 12.24 per cent and 10.20 per cent for wet film, thick and thin blood smears respectively.

According to Omer *et al.* (1998), serological techniques were more sensitive for the detection of trypanosome-infected animals than parasitological methods.

Gutierrez *et al.* (2000) examined 745 dromedary camels using the card agglutination test (CATT/ *T.evansi*) and parasitological methods. Trypanosomes were detected in seven animals, while 36 animals yielded CATT positive results. All parasitologically positive animals were found to be CATT positive.

According to Molina *et al.* (2000), the prevalence of *T.evansi* in dromedary camels from Canary Islands using Ab-ELISA was higher than that using a parasitological method.

Comparison of five parasitological methods and a polymerase chain reaction (PCR) for diagnostic sensitivity of *T. evansi* in water buffaloes by Holland *et al.* (2001a) showed that the combined sensitivity of PCR was the highest (78.2 per cent), closely followed by mouse inoculation (74 per cent), micro-hematocrit centrifugation technique (69.6 per cent) and mini-anion exchange centrifugation technique (62.4 per cent). The sensitivity of the buffy coat technique was much lower.

Various parasitological tests were used for the evaluation of *T. evansi* infections by Reid *et al.* (2001). He recorded that mouse inoculation (MI) with buffy coat was the most sensitive test followed by mini-anion exchange centrifugation technique (MAECT) using buffy coat, MI with whole blood, MAECT with whole blood and haematocrit centrifugation technique (HCT).

Singh *et al.* (2004) detected presence of *T. evansi* infection in camels in 17.05, 9.67, 4.60 and 4.14 per cent cases by PCR, Ag-ELISA, blood smear examination and wet blood film with a sensitivity of 100, 56.75, 27.02 and 24.32 per cent respectively.

2.7. TREATMENT

Ray in 1957 observed a mucopolysaccharide like substance in the cytoplasm of trypanosomes when treated with quinapyramine (Gill, 1977).

Meshnick (1978) opined that quinapyramine salts act by inhibiting the nucleic acid biosynthesis in the trypanosomes.

Uche and Jones (1992) observed that Berenil at the rate of seven milligram per kilogram body weight was effective in the diminution of trypanosome nuclear material from spleen, liver and vulval tissues in rabbits experimentally infected with *T.evansi.*

Diminazene aceturate was the most commonly used compound for the treatment of trypanosome infections at the rate of seven milligram per kilogram body weight with 100 per cent efficacy (Silva *et al.*, 1995).

According to Seidla *et al.* (1998) diminazene aceturate has not been recommended as a prophylactic agent for trypanosomosis as it was rapidly excreted and retained effectiveness only over a short period of time.

From experimental studies on the trypanocidal activity of diminazene aceturate in sheep by Bengaly *et al.* (2000), it was concluded that one or two days post-treatment, no trypanosome DNA could be detected in the sheep and a clinical improvement as measured by PCV was observed.

Dwivedi (2000) opined that quinapyramine prosalt was an established chemo-prophylactic agent against surra and was effective in the treatment of clinical cases at the dose of 7.4 mg/kg body weight.

On the basis of estimation of haemato-biochemical parameters like Hb, PCV, blood glucose, total protein, albumin, globulin, A-G ratio, serum creatinine and serum urea nitrogen, Rajguru *et al.* (2000) concluded that a combination therapy consisting of a single dose of quinapyramine sulphate at the dose of 5.64 mg/ kg body weight subcutaneously along with suramin sodium ten per cent at the dose of 1.88 mg/kg as a single intravenous injection was more effective in the treatment of *T. evansi* infection in goats.

In experimental studies on *T.evansi* in water buffalo by Holland *et al.* (2001a), the PCR signals disappeared within 24 hours after treatment with diminazene aceturate.

Clinical trials on *T.evansi* infections in buffalo using quinapyramine, diminazene and samorin by Joshi and Bhoopsingh (2001) revealed a marked increase in Hb, PCV, total erythrocyte count, blood glucose, total plasma proteins and cholesterol levels.

Quinapyramine prosalt conferred protection for a period of 70 days against *T.evansi* infection in buffalo calves (Singh *et al.*, 2002).

Based on a study on the effect of quinapyramine treatment on ELISA antibody levels, Monzon *et al.* (2003) concluded that successful trypanocidal therapy showed a progressive decrease of antibody levels at 2.3, 4.1, 10.5, 12.8 and 22.6 months post treatment; but when the cure was only temporary, an increase in the antibody titres was obtained. Relapses occurred in horses after treatment with trypanocidal drugs due to drug resistant trypanosomes, the occult location of trypanosomes in spinal fluid or due to challenge with heterologous parasite population.

Triquin at 4.17 mg/kg subcutaneously and Berenil at 3.5 mg/kg as intramuscular injection were found to be effective in the treatment of horses infected with *T.evansi* along with parenteral administration of glucose (Soodan *et al.*, 2003).

Diminazine aceturate at 3.5 mg/kg body weight as deep intramuscular injection was found to be effective in the treatment of a she-buffalo suffering from trypanosomosis (Ajitkumar *et al.*, 2004).

Materials and methods

3. MATERIALS AND METHODS

The study was carried out at the department of Veterinary Epidemiology and Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala during the period from January 2004 to June 2005.

3.1. SCREENING OF ANIMALS

A total of 530 samples from animals brought to the University Veterinary Hospital, Mannuthy; the animals of the University Livestock Farm, Mannuthy; Livestock Research Station, Thiruvizhamkunnu; Cattle Breeding Farm, Thumburmuzhy; private farms in and around Thrissur and suspected random cases from different districts of Kerala formed the samples for the present study. The animals were screened for trypanosomosis using parasitological methods like wet film examination, micro-haematocrit centrifugation technique and Giemsa stained whole blood smears. To detect the presence of trypanosome antibody, dot-ELISA was carried out with the serum samples. In positive cases, biochemical parameters like the blood glucose level, the total protein content and albumin-globulin ratio were assessed. Also, PCV, Hb content, total erythrocyte counts and differential leucocyte counts were determined to assess the degree of anaemia. The infected animals were treated with diminazine aceturate or quinapyramine prosalt alternatively as the case was encountered. Blood samples were collected from ten apparently healthy control animals and were subjected to the estimation of haematological and biochemical parameters.

3.2. SCREENING BASED ON PARASITOLOGICAL TECHNIQUES

3.2.1. Wet blood film examination

Materials

1. Glass slides
2. Coverslips
3. Microscope

Method

Screening for parasitaemia was done by wet film examination as per the method of Cabrera and Lui (1956). The ear tip was swabbed with alcohol and peripheral blood was obtained from each animal by puncturing a small vein. A small drop of blood was placed on to a clean glass slide and covered with coverslip to spread the blood as a thin layer of cells. This was examined under the low power and high power objective of the microscope for the presence of motile trypanosomes.

3.2.2. Blood smear examination

Materials

1. Glass slides
2. Geimsa stain

Giemsa stain (stock solution)	: 1ml
Distilled water	: 9 ml

Method

The blood smears made from the peripheral blood were stained by Giemsa staining technique (Benjamine, 2001) and examined under oil immersion objective of the microscope for detecting the presence of trypanosomes.

3.2.3. Micro-haematocrit centrifugation technique (MHCT)

Materials

1. Heparinised capillary tubes
2. Micro-haematocrit centrifuge
3. Glass slides
4. Coverslips
5. Wax
6. Microscope

Method

Micro-haematocrit centrifugation technique was done following the method of Woo (1969).

Blood collected in citrated vials from the jugular vein was drawn into heparinised capillary tubes (75mm x 1.5mm), sealed at one end with wax and centrifuged at 4500 rpm for three minutes with the sealed ends facing outwards. The tubes were broken at the buffy coat area where the trypanosomes concentrate and expressed on to a glass slide, covered with coverslip and examined for motile trypanosomes. Smears were also prepared from the buffy coat layer, stained with Giemsa stain and observed under oil immersion objective of the microscope for the presence of trypanosomes.

3.3. SEROLOGICAL SCREENING

3.3.1. *Trypanosoma evansi* isolate

Trypanosoma evansi strain (Fig.1) was isolated from a natural case of tyrpanosomosis in a dog brought to the University Veterinary Hospital, Mannuthy

during January 2004 and the species was identified at the Department of Veterinary Parasitology, College of Veterinary and Animal Sciences, Mannuthy. The primary isolate of *T.evansi* was maintained in albino mouse, white rats, rabbits and guinea pigs by syringe passage.

3.3.2. *Trypanosoma evansi* antigen preparation

Materials

1. Sterile syringes (10ml)
2. Beakers (50 ml)
3. Sterile serum vials
4. Glass wool
5. Heparin
6. DEAE Cellulose slurry

Diethyl amino ethyl cellulose	: 10 g
Distilled water	: 100ml
pH adjusted to 8.0 with phosphoric acid	

7. Phosphate buffered saline (PBS)

i. PBS stock (10X)

Sodium chloride	: 80g
Potassium chloride	: 2.0 g
Disodium hydrogen phosphate	: 11.33g
Potassium dihydrogen phosphate	: 2 g
Distilled water to make	: 1000 ml
pH was adjusted to 7.2	

ii. PBS (pH 7.2)

PBS (10X)	: 100 ml
Distilled water to make	: 1000 ml

iii. Phosphate buffered saline glucose (PBSG, pH 8.0)

Disodium hydrogen phosphate	: 13.48 g
Sodium dihydrogen ortho phosphate	: 0.78 g
Sodium chloride	: 4.25 g
Distilled water to make	: 1000ml

Four parts of the stock solution of PBSG was diluted with six parts of distilled water and the final glucose concentration was adjusted to one per cent.

Method

Trypanosoma evansi was separated from heavily parasitaemic rat and mouse blood using the mini-anion exchange method described by Lanham and Godfrey (1970) (Fig.2).

3.3.2.1. Mini-anion exchange method

The mini-anion exchange technique was carried out as per the method of Lanham and Godfrey (1970) with modifications. A five-milliliter sterile plastic syringe without plunger was placed on a test-tube rack with the nozzle facing downwards. A thin layer of glass wool was placed covering the inside of the syringe at the region of the nozzle. Five milliliters of the DEAE cellulose slurry was poured into the syringe and allowed to pack. The slurry was washed thrice with PBSG. The blood collected was mixed with thrice the amount of PBSG and added at the rate of ten drops at a time followed by washing with equal amount of PBSG. First few drops of the filtrate were discarded. The elute was centrifuged at 1000 rpm for 15 minutes, washed thrice with PBS, and stored at –20°C for antigen preparation.

3.3.2.2. Trypanosoma evansi antigen

Trypanosoma evansi was separated from heavily parasitaemic rat and mouse blood using the mini-anion exchange method described by Lanham and

Godfrey (1970). The organisms were washed thrice in PBS (pH 7.2) and repeatedly frozen and thawed for disrupting the cell membrane and release of the soluble antigens of trypanosomes. The parasitic debri were removed by high-speed centrifugation (10000 rpm for one hour at 4° C). The antigen solution was aliquoted into 0.5 ml amounts and stored.

3.3.2.3. Sera collection

Materials

1. Sterile glass test tubes
2. Sterile serum vials

Method

Blood was collected from the jugular vein of the animals into sterile test tubes. The blood was allowed to clot at room temperature and later transferred into refrigerator and kept overnight at 4°C. The serum was separated, inactivated at 56°C for 30 minutes and stored at –20°C for carrying out dot-ELISA and estimation of biochemical parameters.

3.3.3. Dot- Enzyme linked immunosorbent assay (Dot-ELISA)

Materials

1. Nitrocellulose combs (DRDE, Gwalior)
2. Micropipettes
3. PBS – Tween 20

Tween 20	: 0.5 ml
PBS (10X)	: 100 ml
Distilled water to make	: 1000 ml

4. Milk powder solution (5 per cent) (AMUL, INDIA)

Milk powder	: 5g
PBST to make	: 100ml

5. Peroxidase – conjugated Rabbit Anti Bovine Immunoglobin (1: 500) solution (GENEI, Bangalore)

Peroxidase – conjugated rabbit anti bovine immunoglobulin	–	1 μ l
PBST	_	0.49ml

6. DAB Substrate (GENEI, Bangalore)

3'3 – Diamino benzidine tetrahydrochloride	: 6 mg
Phosphate buffered saline	: 10 ml
30 percent Hydrogen peroxide	: 10μl

The solution was freshly prepared just before use.

Method

The dot- ELISA was carried out as per the method of Hawkes (1986) with minor modifications.

3.3.3.1. Standardisation of dot- ELISA

Nitro-cellulose (NC) membrane bound to plastic strips in the form of combs (Fig.3) were used in the present study. *Trypanosoma evansi* antigen was prepared. A serial two fold dilution of the antigen was made in PBS (pH 7.2) to provide 1:10 to 1:80 dilution and 1:10 dilution was found to give the optimum results.

3.3.3.1.1. Peroxidase conjugated rabbit anti-bovine immunoglobulin

Different dilutions of peroxidase conjugated rabbit anti-bovine immunoglobulin were prepared in PBS (pH 7.2) viz., 1: 250, 1:500, 1:1000 and 1: 500 dilution was found to give the optimum results.

3.3.3.1.2. Sera samples

Sera samples were diluted in PBS (pH 7.2) with ranges from 1:10, 1:50, 1:100 and dot-ELISA was performed with these serum samples. The serum dilutions of 1:10 was found to give the optimum results.

3.3.3.2. Test procedure

One µl of optimum dilution of antigen (1:10) of *T.evansi* antigen in PBS (pH 7.2) was dotted on to the NC membrane using a micropipette. The NC membrane was allowed to dry at room temperature and incubated at 37°C for 30 minutes. Unsaturated sites of the NC membrane were blocked by a five per cent solution of milk powder in PBST solution (pH 7.4) for one hour at 37°C. The membrane was rinsed in PBST solution and incubated at 37°C for 45 minutes in the test serum (dilution 1:10). The unbound proteins were removed by rinsing the membrane thrice for ten minutes each time in PBST solution. The membrane was then dipped in peroxidase conjugate (1:500) for 30 minutes at 37°C and subsequently washed thrice for ten minutes each time in PBST solution. The membrane was then dipped in freshly prepared Diamino benzidine tetrahydrochloride (DAB) substrate solution for three minutes. The NC combs were washed under tap water, air-dried and stored.

3.3.3.3. Interpretation of the results

Development of a brownish dot at the site of antigen coating on the NC membrane was taken as a positive reaction.

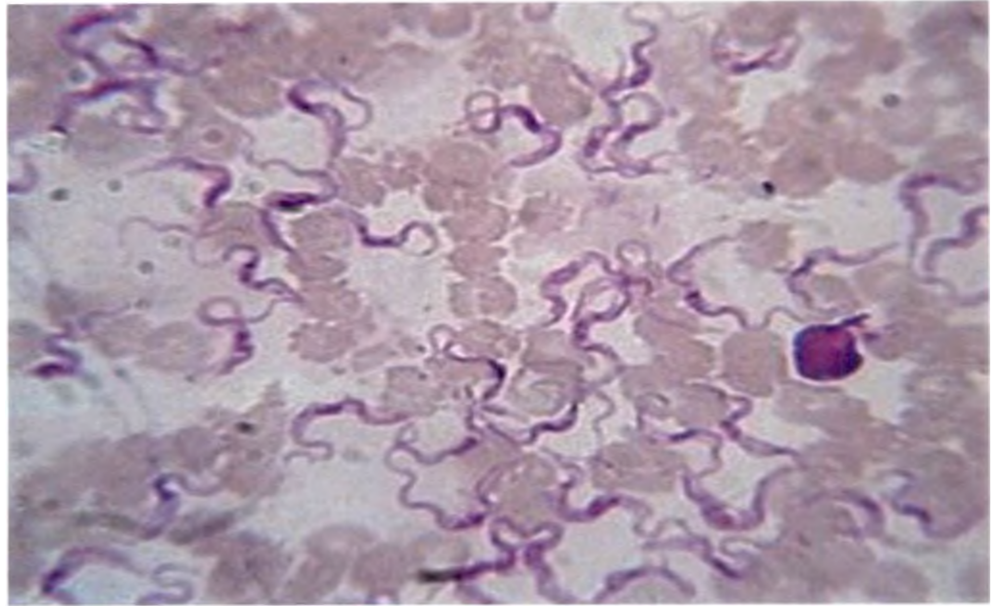


Fig.1. ***Trypanosoma evansi*** **in rat blood smear (100X)**

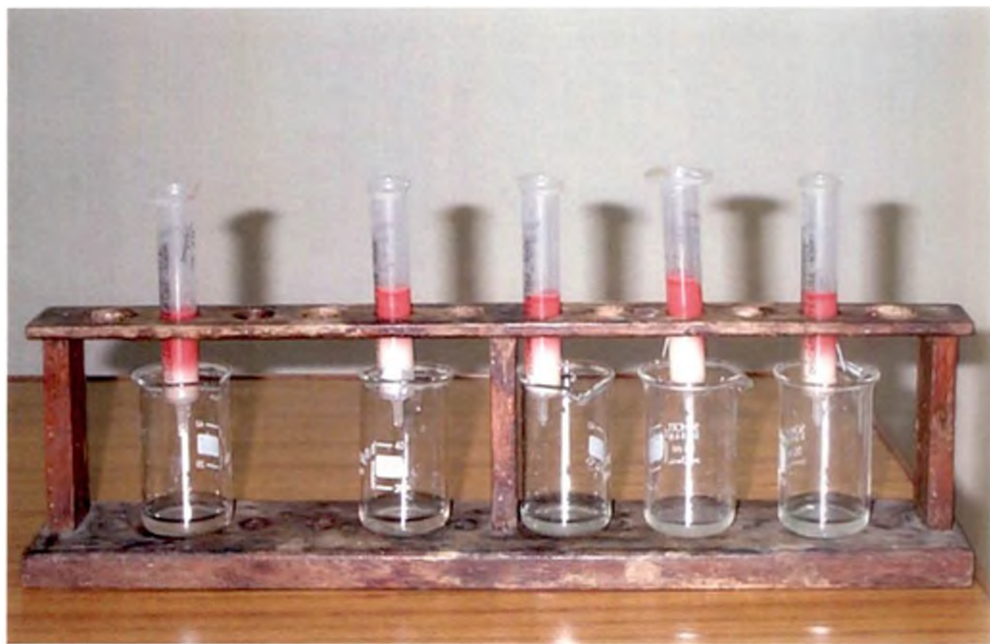


Fig.2. Mini-anion exchange method for separation of *Trypanosoma evansi*

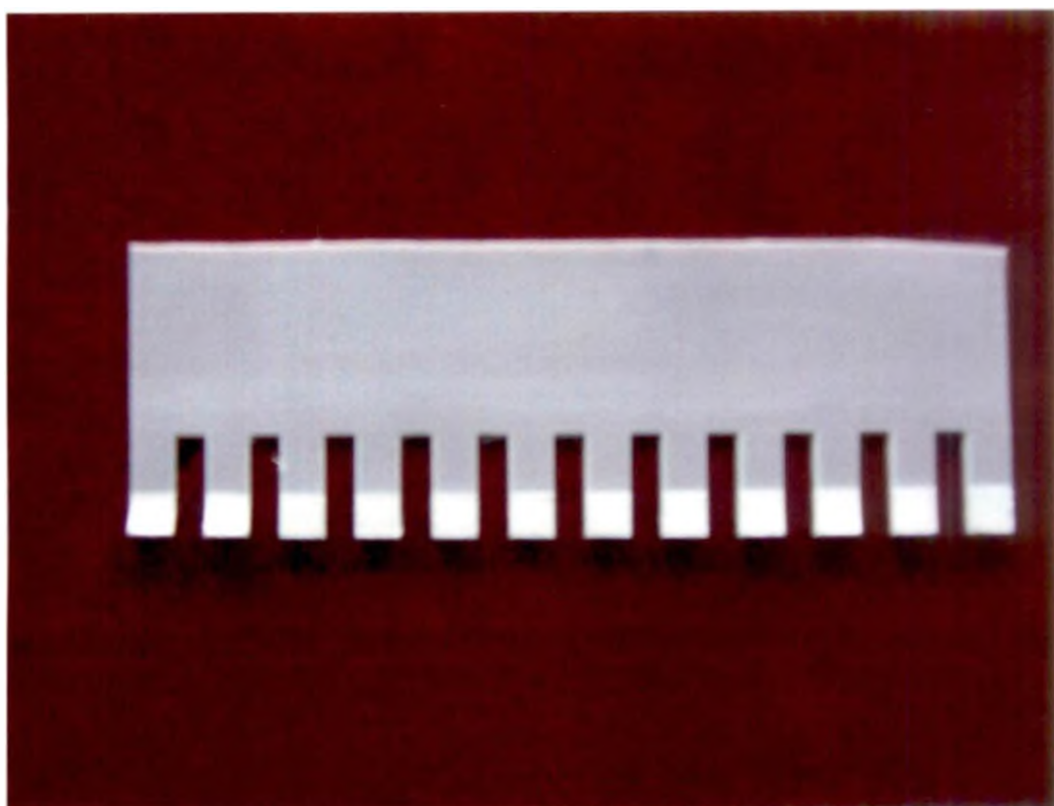


Fig.3. Twelve tip Nitrocellulose (NC) comb for dot-ELISA

3.4. IN POSITIVE CASES

3.4.1. Haematological studies

Materials

1. Glass slides
2. Coverslips
3. Citrated vials
4. Microscope
5. Haemocytometer
6. Haemoglobinometer
7. Micro-haematocrit centrifuge

Method

The blood from positive cases and control animals was subjected to haematological studies. The PCV, total erythrocyte count, Hb content and differential leucocyte counts were estimated using standard procedures (Benjamine, 2001).

3.4.2. Biochemical analysis

Materials

Glucose estimation kit (Merckotest, M/s MERCK)
Protein estimation kit (Agappe's diagnostics, Maharashtra)

Method

The blood glucose, total protein content and albumin-globulin ratio of the samples were estimated by Spectrophotometry in Merck 200 Spectrophotometer.

3.4.2.1. *Blood glucose*

Blood glucose was estimated by glucose oxidase method as described by Sacks (2001).

3.4.2.2. *Total protein*

The total protein concentration of the sera was determined by direct Biuret method (Gormall *et al.*, 1949).

3.4.2.3. Albumin

Albumin concentration of the sera was determined by Bromocresol green method (Doumas *et al.*, 1971).

3.4.2.4. Globulin

Globulin concentration was derived from the total protein and albumin values (Benjamine, 2001).

3.4.2.5. Albumin-globulin ratio

The albumin-globulin ratio was calculated by dividing the albumin concentration with the globulin concentration (Benjamine, 2001).

3.4.3. Treatment

3.4.3.1. Quinapyramine prosalt

TEVANSI® (RANBAXY)

Each vial contains:

Quinapyramine sulphate 1.5g

Quinapyramine chloride 1g

15 ml sterile water for injection

3.4.3.2. Diminazene aceturate

BERENIL® VET 7 % RTU (INTERVET)

Each ml contains:

Diminazene aceturate	70 mg
Phenazone BP	375 mg
Water for injection	q.s.

The positive case was treated with quinapyramine prosalt at the dose rate of 7.5 mg/kg body weight subcutaneously. The treatment response was monitored on the third day by examination of wet film, blood smear and micro-haematocrit centrifugation technique. Blood collected after one week was subjected to examination of haematological and biochemical parameters.

3.5. STATISTICAL ANALYSIS

The data were subjected to student's t-test as per the procedures of Snedecor and Cochran (1994).

Results

4. RESULTS

4.1. SCREENING OF ANIMALS

In the present study 510 cattle and 20 buffalo were screened for trypanosomosis using parasitological and serological methods. The parasitological methods used for the detection of trypanosomes included wet film examination of blood, Giemsa stained blood smears and micro-haematocrit centrifugation technique. Serum samples were subjected to dot-ELISA for detection of *T.evansi* antibodies.

4.1.1. Screening based on parasitological techniques

4.1.1.1. Wet blood film examination

Among the 510 cattle screened for trypanosomes, only one animal was found positive for trypanosomosis using wet film examination of blood. All the twenty buffalo screened, gave a negative result for the presence of trypanosomes using wet film examination.

4.1.1.2. Blood smear examination

Giemsa stained whole blood smears revealed parasitaemia in one out of the 510 cattle screened for trypanosomosis. No trypanosomes could be detected by blood smear examination in any of the twenty buffalo screened.

4.1.1.3. Micro-haematocrit centrifugation technique (MHCT)

The technique yielded positive result in one out of the 510 cattle and none among the 20 buffalo screened for trypanosomosis.

4.1.2. Serological screening

4.1.2.1. Isolation and maintanence of Trypanosoma evansi

The *T. evansi* strain, isolated from a natural case of trypanosomosis in a dog brought to the University Veterinary Hospital, Mannuthy was maintained in albino mouse, white rats, rabbits and guinea pigs by syringe passage.

4.1.2.2. Dot-ELISA

Serum samples from 510 cattle and 20 buffalo were subjected to dot-ELISA (Fig. 4) for detecting anti-*T. evansi* antibodies. Among the 510 serum samples from cattle, 12 samples were found positive for trypanosome antibodies using dot-ELISA. Six out of the 20 samples from buffalo gave positive results for dot-ELISA.

4.2. EPIDEMIOLOGY

4.2.1. Species

Trypanosomes were identified in one cow out of the 510 cattle and none of the buffalo screened. Sero-prevalence was detected in 12 cattle and 6 buffalo. Buffalo showed higher prevalence of infection due to *T. evansi* (30 per cent) compared to cattle (2.35 per cent) (Table 1, Fig.5).

Table 1. Sero-prevalence of *T. evansi* infection among cattle and buffalo using dot- ELISA

Species	No. of animals screened	Positive for *T. evansi* infection using dot-ELISA	
		Number	%
Cattle	510	12	2.35
Buffalo	20	6	30.00
Total	530	18	3.40

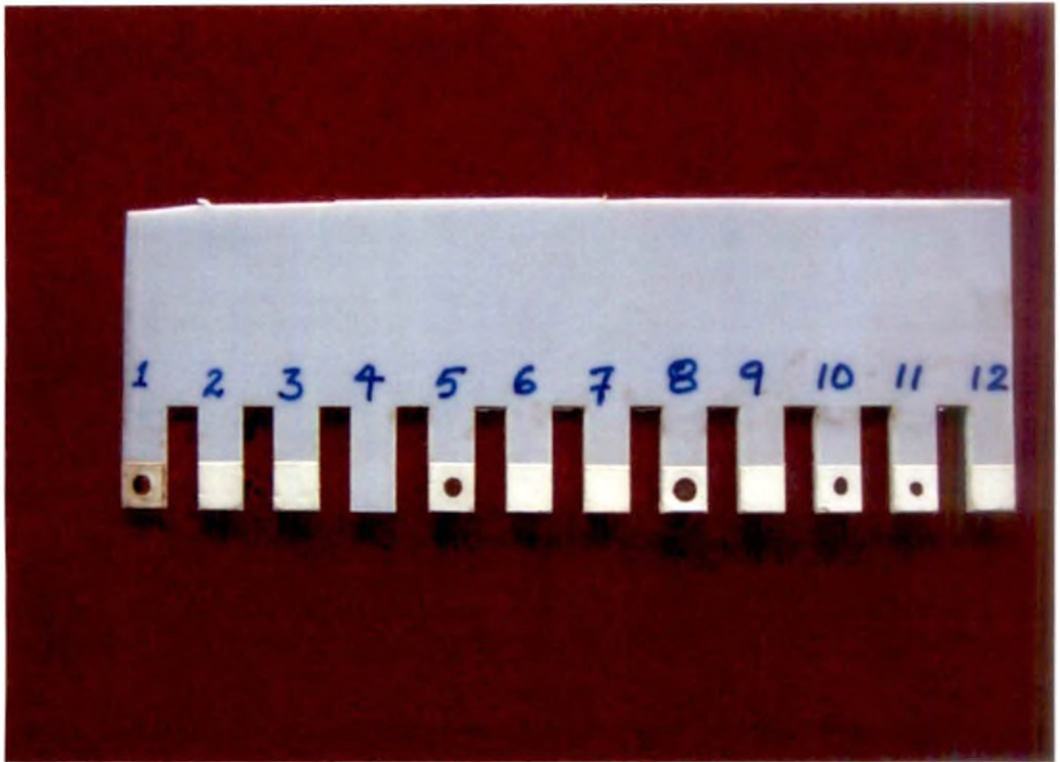


Fig.4. Dot-ELISA for detection of anti-*Trypanosoma evansi* antibodies

1 - Positive control
2 - Negative control
5,8,10,11 - Positive test sera
3,4,6,7,9,12 - Negative test sera

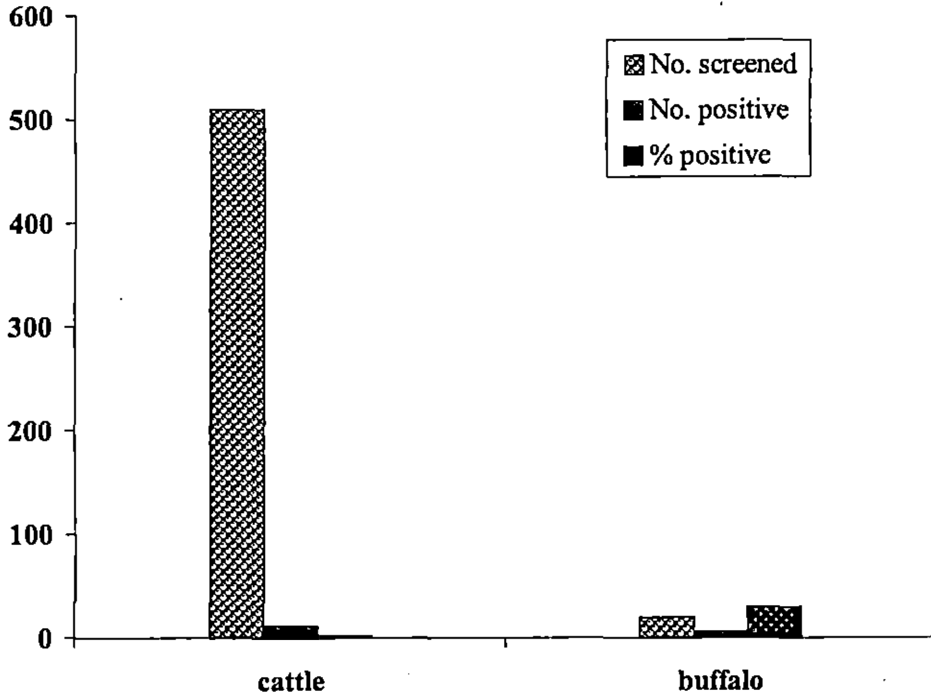


Fig. 5. Sero-prevalence of *Trypanosoma evansi* infection among cattle and buffalo screened using dot-ELISA

4.2.2. Breed

Trypanosomosis was observed in different breeds of cattle, viz., crossbred Jersey (CBJ), crossbred Holstein Friesian (CBHF), crossbred Brown- Swiss (CBBS) and non-descript desi cattle (ND). No purebred exotic cattle were screened during the present study. All the buffalo screened for the present study belonged to the Murrah breed.

The rate of infection in CBHF was 1.96 per cent, in CBJ 2.86 per cent, CBBS 2.05 per cent and 1.92 per cent in ND cattle (Table 2 and Fig. 6).

Among the total positive cases, CBJ contributed the highest (50 per cent) followed by CBBS (25 per cent), CBHF (16.67 per cent) and ND cattle (8.33 per cent) (Table 3 and Fig. 7).

Table 2. Breed-wise sero-prevalence of *T. evansi* infection in cattle using dot-ELISA

Breed	No. of animals screened	Positive for *T. evansi* infection using dot-ELISA	
		Number	%
CBHF	102	2	1.96
CBJ	210	6	2.86
CBBS	146	3	2.05
ND	52	1	1.92
Total	510	12	2.35

Table 3. Breed-wise sero--prevalence of *T. evansi* infection among total positive cases in cattle using dot-ELISA

Sl. No.	Breed	No. of positive cattle	%
1	CBJ	6	50
2	CBHF	2	16.67
3	CBBS	3	25
4	ND	1	8.33
	Total	12	100

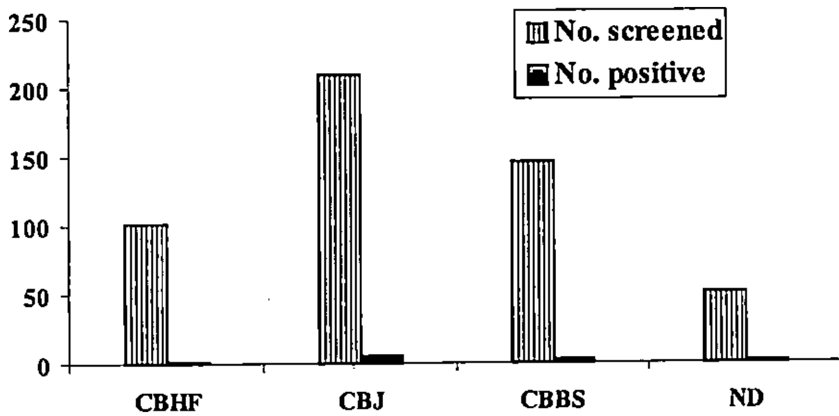


Fig.6. Breed-wise sero-prevalence of *Trypanosoma evansi* infection in cattle using dot-ELISA

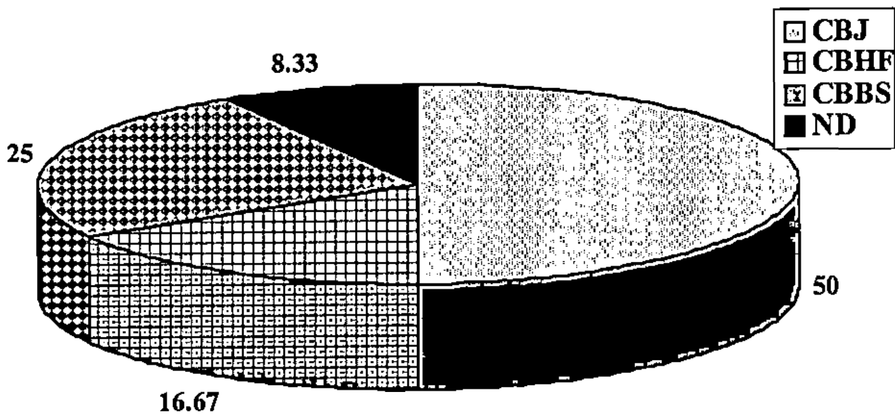


Fig.7. Breed-wise sero-prevalence of *Trypanosoma evansi* infection among total positive cases in cattle using dot-ELISA

4.2.3. Age

The cattle and buffalo screened for the present study included animals of all age groups ranging from six months to twelve years.

The highest rate of infection was noticed in animals of 1 to 3 years of age (7.5 per cent) followed by 3 to 5 year age group (3.5 per cent) and above 5 years (1.5 per cent). No animal below 1 year of age was found positive for trypanosomosis (Table 4 and Fig. 8). Clinical trypanosomosis was encountered in a four-year-old cow.

Out of the positive cases, cattle of 1 to 3 years of age contributed 50 per cent followed by 3 to 5 years (38.89 per cent) and above five years (11.11 per cent) (Table 5 and Fig. 9).

Table 4. Age wise sero-prevalence of *T. evansi* infection in bovine using dot-ELISA

Age	No. of animals screened	Positive for trypanosomosis using dot-ELISA	
		Number	%
Below 1 year	80	0	0
1 to 3 years	120	9	7.5
3 to 5 years	200	7	3.5
Above 5 years	130	2	1.5
Total	530	18	3.40

Table 5. Age wise sero-prevalence of *T. evansi* infection among total positive cases in bovine

Age	No. of animals positive by dot-ELISA	%
Below 1 year	0	0
1 to 3 years	9	50
3 to 5 years	7	38.89
Above 5 years	2	11.11
Total	18	100

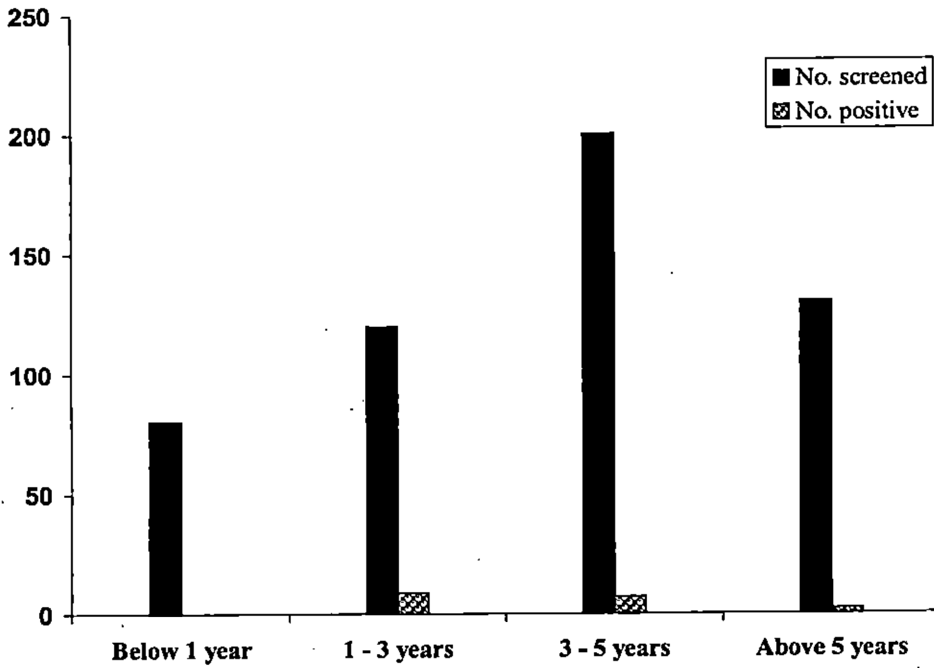


Fig. 8. Age wise sero-prevalence of *Trypanosoma evansi* infection in bovine using dot-ELISA

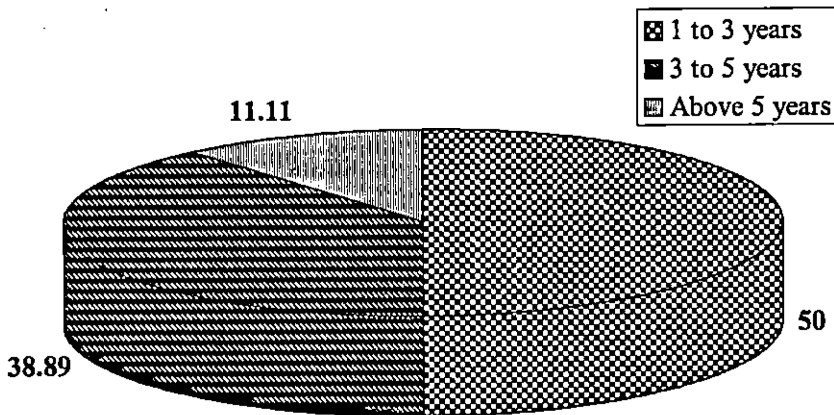


Fig. 9. Age wise sero-prevalence of *Trypanosoma evansi* infection among total positive cases in bovine using dot-ELISA

4.3. CLINICAL SIGNS

Among the 510 cattle and 20 buffalo screened for trypanosomosis, the clinical signs and parasitaemia due to *T. evansi* infection was detected only in one case presented at the University Veterinary Hospital, Mannuthy. The cow aged four years had a history of snoring, inappetance, decreased milk yield and wasting for a considerable period of time before being presented at the clinics. Clinical examination revealed pyrexia (temperature 104° F) and pale mucous membranes. The wet film and blood smear examination revealed trypanosomes.

4.4. HAEMATOLOGICAL STUDIES

The haematological parameters of the sero-positive and control group of animals are presented in table 6.

4.4.1. Packed cell volume

The mean PCV of the positive animals (17.667 ± 5.156 per cent) was significantly ($P < 0.05$) lower than that of the control group (32.600 ± 4.719 per cent).

4.4.2. Haemoglobin concentration

The positive animals had a lower mean value of Hb (6.600 ± 1.129 g/dl) than the control animals (10.860 ± 2.133 g/dl). Statistical analysis revealed significant difference ($P< 0.05$) in the Hb concentration of the infected and control animals.

4.4.3. Total erythrocyte count

The mean total erythrocyte counts of the positive animals (3.460 ± 1.234 x $10^{6}/mm^{3}$) showed a significant decrease ($P< 0.05$) than that of the control animals (6.230 ± 1.771 x $10^{6}/mm^{3}$).

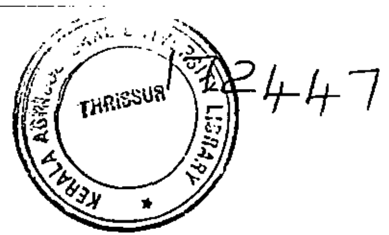
4.4.4. Differential leucocyte count

Significant variations were noticed in the lymphocyte and neutrophil counts of the positive and control group. The mean lymphocyte count of the positive animals (51.944 ± 7.083 per cent) was significantly (P< 0.05) lower than the control group (63.500 ± 7.933 per cent), whereas, the mean neutrophil count of the positive animals (45.444 ± 6.002 per cent) was significantly (P< 0.05) higher than the control group (34.000 ± 5.598 per cent). No significant variations were observed in the mean monocyte and eosinophil counts. The mean monocyte count of the infected group was 3.112 ± 2.342 per cent, whereas, that of the control group was 3.500 ± 3.434 per cent. The infected group showed mean eosinophil count of 1.332 ± 3.440 per cent. The corresponding value for the control group was 1.213 ± 4.567 per cent.

Table 6. Haematological parameters of *T. evansi* infected and control animals

Haematological parameters	Mean values ± SD		t values
	Infected group n = 18	Control Group n = 10	
Packed cell volume (per cent)	17.667 ± 5.156	32.600 ± 4.719	7.559**
Haemoglobin (g/dl)	6.600 ± 1.129	10.860 ± 2.133	5.873**
Total Erythrocyte counts ($10^6/mm^3$)	3.460± 1.234	6.230 ± 1.771	4.855**
Neutrophils (per cent)	45.444 ± 6.002	34.000 ± 5.598	4.947**
Lymphocyte (per cent)	51.944 ± 7.083	63.500 ± 7.933	3.965**
Monocyte (per cent)	3.112 ± 2.342	3.500 ± 3.434	0.146^{NS}
Eosinophil (per cent)	1.332 ± 3.440	1.213 ± 4.567	0.475^{NS}

**- Significant at P < 0.05
^{NS} - Non- significant



4.5. BIOCHEMICAL STUDIES

The biochemical parameters of the positive and control group of animals are presented in table 7.

4.5.1. Blood glucose

The mean blood glucose showed a significant ($P<0.05$) decrease in the positive animals (18.056 ± 3.780 mg/dl) compared to the control group (53.200 ± 5.653 mg/dl).

4.5.2. Total protein

The animals positive for trypanosomosis showed significantly ($P<0.05$) higher total protein concentration in their serum (9.600 ± 1.858 g/dl) compared to the animals in the control group (6.040 ± 1.219 g/dl).

4.5.3. Albumin

The mean albumin concentration of the positive animals (2.411 ± 0.386 g/dl) was significantly ($P<0.05$) lower than that of the control group (2.940 ± 0.450 g/dl).

4.5.4. Globulin

The infected animals showed a significantly ($P<0.05$) higher globulin concentration in their serum (7.206 ± 1.902 g/dl) compared to the control group (3.040 ± 1.184 g/dl).

4.5.5. Albumin-globulin ratio

Significantly ($P<0.05$) lower values were observed for the Albumin–globulin (A-G) ratio in the infected animals (0.381± 0.127) compared to the control group (1.187± 0.653).

Table 7. Biochemical parameters of *T. evansi* infected animals and the control group

Biochemical parameters	Mean values ± SD		t values
	Infected group n = 18	Control group n = 10	
Blood glucose (mg/dl)	18.056 ± 3.780	53.200 ± 5.653	19.726**
Total protein (g/dl)	9.600 ± 1.858	6.040 ± 1.219	5.420**
Albumin (g/dl)	2.411 ± 0.386	2.940 ± 0.450	3.252**
Globulin (g/dl)	7.206 ± 1.902	3.040 ± 1.184	6.253**
Albumin-globulin ratio	0.381 ± 0.127	1.187 ± 0.653	3.859**

** Significant at P < 0.05

Table 8. Haemato-biochemical parameters of the clinically positive animal before and after treatment

Haemato-biochemical parameters	Before treatment	After treatment
Packed cell volume (%)	16.0	22.0
Haemoglobin (g/dl)	4.6	5.2
Total erythrocyte count ($10^6/mm^3$)	3.3	6.0
Blood glucose (mg/dl)	26.0	53.0
Total protein (g/dl)	8.5	7.9
Albumin (g/dl)	2.3	2.5
Globulin (g/ dl)	6.9	4.4
Albumin-globulin ratio	0.33	0.56

4.6. TREATMENT

The clinically positive case was treated with quinapyramine prosalt (TEVANSI®, RANBAXY) at the dose rate of 7.4 mg/kg body weight sub-cutaneously. Wet blood film, Giemsa stained blood smears and micro-haematocrit centrifugation technique revealed parasitaemia on the next day post-treatment. No trypanosomes could be detected from the peripheral blood from the second day post treatment onwards. PCV, Hb content and total erythrocyte counts were noted a week after the therapy. There was marked improvement in PCV, Hb and total erythrocyte counts (Table 8).

Discussion

5. DISCUSSION

5.1. SCREENING OF ANIMALS

Parasitological and serological methods were used to screen 510 cattle and 20 buffalo from various farms, veterinary hospitals and farm households for trypanosomosis. The parasitological methods used for diagnosis of trypanosomosis included wet blood film examination, Giemsa stained whole blood smears and micro-haematocrit centrifugation technique. These methods have been reviewed by Dwivedi (2000) and Varadharajan (2000). Dot-ELISA was used to screen for the presence of antibodies against *Trypanosoma evansi.*

5.1.1. Screening based on parasitological techniques

The wet blood film examination, Giemsa stained whole blood smears and micro-haematocrit centrifugation detected parasitaemia only in one and the same animal, which was clinically positive out of the 530 samples tested . The low infection rates obtained with parasitological methods is due to continuous fluctuations in trypanosome numbers. Each fall in parasite numbers is brought about by the action of host antibodies, the subsequent rise being due to proliferation of parasites that differ antigenically from their predecessors (Payne *et al.*,1991a).

As the general clinical signs of various forms of *T. evansi* infections are not pathognomonic, diagnosis of *T.evansi* infections is mainly based on the microscopical examination of blood and sero-biochemical tests. The isolation of *T. evansi* from the blood of the infected hosts is the only true gold-standard diagnostic test available. However, these techniques are insensitive mainly because of the periodically cryptic nature of the parasitaemia that results in long periods during which the host has no detectable parasites in its blood (Reid *et al.*, 2001).

5.1.1.1. Wet blood film examination

In the present study, the wet blood film examination detected parasitaemia only in one out of the 530 samples tested. The low diagnostic sensitivity of wet film examination in trypanosomosis is well documented (Killick-Kendrick and Godfrey, 1963). Pathak and Khanna (1995) had opined that a single examination of wet blood film from affected animals may result in missing 40 per cent of the infections. The diagnosis by wet film examination is satisfactory in animals with acute infection or high intensity of parasitaemia, but is inconsistent in chronic or latent disease when parasitaemia may be very low and detects only 50 per cent of the infected animals (Saseendranath and Ramakrishna, 1995). *Trypanosoma evansi* inhabits the deep blood vessels in case of low parasitaemia. So, it is recommended that blood for diagnosis be obtained from both the peripheral and deep blood vessels (Dwivedi, 2000).

5.1.1.2. Blood smear examination

In the present study, Giemsa stained smears identified trypanosomes in one out of 530 animals screened. Giemsa stain is best for diagnosing *T.evansi* (Batra *et al.*, 1994., Saseendranath and Ramakrishna, 1995). A long flagellum and centrally placed nucleus is characteristic of *T. evansi* (Kuppuswamy, 1941). Giemsa stained blood smears, although of less sensitivity, maintained their usefulness for the identification of species or in those cases when blood samples reached the diagnostic laboratory in a time limit exceeding the viability period of the parasites (Monzon *et al.*, 1990).

5.1.1.3. Micro-haematocrit centrifugation technique

The micro-haematocrit centrifugation technique detected trypanosomes in a single case in the present study. Kelley and Schillinger (1983) described micro-haematocrit method to be extremely useful in the diagnosis of *T.evansi* infection. Monzon *et al.* (1990) opined that micro-haematocrit centrifugation technique was the most effective technique after mouse inoculation, but not significantly better than buffy coat and wet film methods.

5.1.2. Screening based on serological methods

5.1.2.1. Dot-Enzyme Linked Immunosorbent Assay (Dot-ELISA)

Anti-*T.evansi* antibodies were detected in 2.35 per cent of the cattle samples and 30 per cent of the serum samples from buffalo by dot- ELISA. Based on experimental *T.evansi* infection in sheep, Saseendranath *et al.* (1994b) had concluded that dot-ELISA was one of the best qualitative field tests for the diagnosis of trypanosomosis. Luckins *et al.* (1979) had confirmed the suitability of ELISA as an indicator of infection with *T. evansi* in that 95 per cent of the trypanosome-positive animals in his study were classified as antibody positive by ELISA. Dot-ELISA had also been employed by Pappas (1988), Singla *et al.* (1996) and Shahardar *et al.* (2002) for detection of anti-trypanosomal antibodies in various species of animals. The early appearance and persistence of trypanosomal antibodies in the infected animals increased their probability of detection by diagnostic methods. An inherent problem with serological tests is the inability to distinguish past and present infections (Luckins *et al.*, 1979, Olaho-Mukani *et al.*, 1993). Antibodies to trypanosomes persist for a period of three months even after successful chemotherapy (Waitumbi and Nantulya, 1993).

5.2. EPIDEMIOLOGY

5.2.1. Species

The present study revealed higher prevalence of infection in buffalo (30 per cent) compared to cattle (2.35 per cent). The higher prevalence in buffalo encountered in the present study could be due to the small sample size. Payne *et al.* (1991a) and Agrawal *et al.* (2003) had reported higher incidence of trypanosomosis in buffalo compared to cattle based on epidemiological studies. The higher infection rates in buffalo could be associated with environmental factors rather than host factors and related to the differences in feeding preference of tabanids (Payne *et al.*, 1991b). Cattle and buffalo act as reservoir hosts for *T.evansi* due to sub-clinical nature of the disease. The latent form of infection in cattle and buffalo posed serious problems in the spread of infection to more susceptible species (Kaur and Ahuja, 2002).

5.2.2. Breed

No breed predisposition was observed in the present study. The positive cases were encountered in crossbred cattle viz., CBHF (1.96 per cent), CBBS (2.05 per cent), CBJ (2.86 per cent) and ND cattle (1.92 per cent) and Murrah breed of buffalo. Payne *et al.* (1991b) had observed trypanosomosis in each of the seven breeds of cattle he used for epidemiological studies. He also observed that *T.evansi* infection in cattle had no breed preference. Griffin (1978) had observed that trypanosome infection produced a marked erythropoiesis in indigenous animals, but only a slight increase in exotic indigenous cross-bred cattle. He opined that erythropoietic ability of exotic breeds of cattle may be lower than that of indigenous breeds, thereby contributing to their greater susceptibility to the pathogenic effects of trypanosomes.

5.2.3. Age

In the present study, the highest rate of infection was noticed in animals of one to three years of age followed by three to five year age group. No animal below one year of age was found positive for trypanosomes. Payne *et al.* (1991a) had observed an ascending age related prevalence of trypanosomosis due to *T.evansi* in both cattle and buffalo. The highest serological infection rates were usually seen in animals older than two years of age and the lowest in animals in their first year of life. Stephen (1986) had recorded that calves are more resistant to trypanosome infection than adult animals. Increased prevalence of infection in older animals was also found in studies by Das *et al.* (1998), Delafosse and Doutoum (2004) and Gutierrez *et al.* (2000). Singh *et al.* (2004) had described the lack of maternal immunity in older animals to be a cause for the increased susceptibility. According to Delafosse and Doutoum (2004), greater sensitivity of calves to trypanosomosis and poor capacity to control parasitaemia led to their rapid mortality.

5.3. CLINICAL SIGNS

Trypanosomosis is generally latent or sub-clinical in bovines and epizootics occur due to stress from adverse climatic conditions, work or presence of intercurrent diseases. In the present study, wherein 510 cattle and 20 buffalo were screened, only one animal exhibited clinical symptoms of trypanosomosis. The apparent lack of any obvious disease due to *T. evansi* infection in the sampled animals suggested that a form of stability existed in most endemic areas which served to ameliorate the effect of *T. evansi* infection. The clinically positive animal exhibited pyrexia, paleness of the mucous membranes, inappetance, dullness, emaciation and decreased milk production. Pyrexia may be due to the presence of circulating trypanosomes and their by-products. Also, the fluctuations in temperature coinciding with parasitaemic rises are due to the phenomenon of antigenic variation in trypanosomosis (Audu *et al.*, 1999). These findings agreed

with those reported by Singh and Misra (1988), Das *et al.* (1998), Gupta *et al.* (2003), Rajkhowa *et al.* (2003) and Tresamol *et al.* (2003). Bouteille *et al.* (1988) had observed marked weight loss in sheep experimentally inoculated with *T. evansi* from the first week onwards and observed a continuous diminution with the animals weighing 20 per cent less than their initial weight at the end of the observation period (till the time of death).

5.4. HAEMATOLOGICAL STUDIES

Haematological and biochemical tests are not specific for *T.evansi* infection, but they reveal the pathological consequences of infection. In areas where the disease is endemic, such tests help to monitor the results of chemotherapy.

5.4.1. Packed cell volume

The mean PCV of the positive animals (17.667 ± 5.156 per cent) was significantly lower than that of the control group (32.600 ± 4.719). In *T. evansi* infection, decreased PCV suggestive of anaemia had been described by Raisinghani *et al.* (1980), Manohar *et al.* (1984), Ngeranwa *et al.* (1991), Audu *et al.* (1999), Joshi and Bhoopsingh (2001) and Witola and Lovelace (2001). Anaemia is one of the most significant symptoms of trypanosomosis. Most reports describe a normochromic, normocytic anaemia (Losos and Ikede, 1972, Clarkson, 1968). Jennings (1976) had opined that attachment of trypanosome antigen to the surface of red cells, their subsequent combination with anti-trypanosome antibody in the plasma and eventual sequestration by reticulo-endothelial cells led to anaemia.

5.4.2. Total erythrocyte count

The mean total erythrocyte counts of the positive animals (3.460 ± 1.234x10 ^{6}/mm^{3}) showed a significant decrease (P< 0.01) than that of the control

animals (6.230 ± 1.771 x 10^6 /mm$^{3)}$. Das *et al.* (1998), Joshi and Bhoopsingh (2001), Soodan *et al.* (2003) and Rajkhowa *et al.* (2003) had described decreased erythrocyte counts in *T.evansi* infected animals. According to Esievo and Saror (1991), sialidase produced by trypanosomes cleave erythrocyte surface sialic acid during bovine trypanosomosis exposing new antigenic sites. Antibodies produced against these antigenic sites then render them suitable for erythrophagocytosis. Hemolytic factors play a role in the pathogenesis of anaemia in trypanosomosis. The hemolytic factors include proteases, neuraminidases, phospholipases and free fatty acids, which in circulation damage vascular endothelial cells and blood cellular components (Igbokwe, 1994).

5.4.3. Haemoglobin concentration

In the present study, the positive animals had a lower mean value of Hb (6.600 ± 1.129 g/dl) than the control animals (10.860 ± 2.133 g/dl). Statistical analysis revealed significant difference (P< 0 .05) in the Hb concentration of the infected and control animals. Similar findings were reported by several workers in animals infected with different trypanosome species (Ikede *et al.*, 1977., Raisinghani *et al.*, 1980 and Ngeranwa *et al.*, 1991). Anosa and Isoun (1980) had reported that the level of anaemia was closely related to parasitaemia. A small rise in temperature can increase and accelerate haemolysis through decreased plasticity of erythrocytes and hence, the undulating fever associated with trypanosomosis play a significant role in anaemia. Also, trypanosomes cause direct mechanical injury to the erythrocytes and other cells by the lashing action of their flagella and microtubule reinforced bodies (Igbokwe, 1994).

5.4.4. Differential leucocyte count

Significant variations were noticed in the lymphocyte and neutrophil counts of the positive and control groups. The mean lymphocyte count of the positive animals (51. 944 ± 7.083 per cent) was significantly lower than the

control group (63.500 ± 7.933 per cent), whereas, the mean neutrophil count of the positive animals (45.444 ± 6.002 per cent) was significantly higher than the control group (34.000 ± 5.597 per cent). No significant variations were observed in the mean monocyte and eosinophil counts of the infected and control group of animals. These observations are in accordance with the findings of Joshi and Bhoopsingh (2001) and Ajitkumar *et al.* (2004). The lymphopaenia is presumably due to the depopulation of the lymphoid nodules and the transformation of the lymphocytes to morula cells. Lymphopaenia may contribute to immunosuppression which co-exists with trypanosomosis (Anosa and Isoun, 1980).

5.5. BIOCHEMICAL STUDIES

5.5.1. Blood glucose

In the present study, hypoglycemia was observed in the eighteen positive cases. The mean blood glucose showed a significant decrease in the positive animals (18.056 ± 3.780 mg/dl) compared to the control group (53.200 ± 5.653 mg/dl).

According to Richardson (1948), hypoglycemia in trypanosomosis is associated with exhaustion of the glycogen reserve of the body which may be due to failure of the liver to lay down the glycogen reserve than to an abnormal consumption of sugar by trypanosomes. Hypoglycemia during trypanosomosis has also been reported by Kathiria and Avsatthi (1985).

5.5.2. Plasma proteins

The animals positive for trypanosomosis showed significantly higher total protein concentration in their serum (9.600 ± 1.858 g/dl) compared to the animals in the control group (6.040 ± 1.219 g/dl). Significantly lower values were

observed for the A-G ratio in the infected animals (0.381 ± 0.127) compared to the control group (1.187 ± 0.653).

The change in serum proteins is due to the degeneration of the kidney observed in pathogenic trypanosome infections. The damage done to the kidney injure the selective excretory mechanism by which the kidney aids in maintaining a constant blood plasma protein level. With the loss of proteins, chiefly albumin, the serum A-G ratio falls. In the plasma, the concentration of globulin fraction may exceed that of the albumin fraction, the normal ratio is inverted (Ikejiani, 1946). Increased globulin may also be due to persistent antigenic stimulation of the B-cells in trypanosome infected animals leading to the development of abnormal plasma cells with increased globulin production (Igbokwe, 1994).

5.6. TREATMENT

In the present study, quinapyramine prosalt (TEVANSI®, RANBAXY) was used in the clinical case of trypanosomosis encountered. Quinapyramine prosalt was administered at the dose of 7.4 mg/kg body weight sub-cutaneously. It cleared trypanosomes from the peripheral blood from the second day post-treatment. No trypanosomes could be detected by the wet film, Giemsa stained blood smears and micro-haematocrit centrifugation technique from the second day of therapy.

Quinapyramine prosalt (mixture of soluble dimethyl sulphate and the less soluble chloride salt) is known as a valuable chemoprophylactic agent against almost all pathogenic trypanosomes (Singh *et al.*, 2002). Meshnik *et al.* (1978) found that quinapyramine salts inhibited the nucleic acid synthesis of trypanosomes. When treated with quinapyramine, a mucopolysaccharide like substance was found in the cytoplasm of trypanosomes (Gill, 1977).

A week after the therapy, marked improvement in PCV, Hb, and total erythrocyte count was noted. These findings are in agreement with those of Dwivedi (2000), Singh *et al.* (2002), Soodan *et al.* (2003) and Singh *et al.* (2004) who had reported the therapeutic efficacy of quinapyramine prosalt in tyrpanosomosis. Monzon *et al.* (2003) had revealed progressive decline in antibody levels at 2.3, 4.1, 10.5, 12.8 and 22.6 months post-treatment. Also, it has good prophylactic activity against subsequent infection. Chaudhri *et al.* (1996) had reported that cross-bred calves could resist repeated challenge inoculation of trypanosomes till thirty five days post chemoprophylaxis. Singh *et al.* (2002) had reported the period of protection to be seventy days after quinapyramine administration in buffalo calves.

Summary

6. SUMMARY

In the present study, 510 cattle and 20 buffalo were screened for *Trypanosoma evansi* infection using parasitological and serological techniques. The samples were collected from animals brought to the University Veterinary Hospital, Mannuthy; the animals of the University Livestock Farm, Mannuthy; Livestock Research Station, Thiruvizhamkunnu; Cattle Breeding Farm, Thumburmuzhy; private farms in and around Thrissur and suspected random cases from different districts of Kerala.

Trypanosoma evansi was isolated from a dog brought to the University Veterinary Hospital, Mannuthy during January 2004 and maintained in albino mouse, white rats, rabbits and guinea pigs through syringe passage. Trypanosomes were separated from highly parasitaemic rat blood using mini-anion exchange centrifugation method. The antigenic material was centrifuged, washed, repeatedly frozen and thawed, aliquoted and stored at -20°C. Using the antigen prepared, dot-ELISA was performed on the serum samples collected to detect anti-*Trypanosoma evansi* antibodies.

The parasitological techniques employed in the present study included wet film examination of blood, Giemsa stained blood smears and micro-haematocrit centrifugation technique. Among the 510 cattle and 20 buffalo screened, only one animal was found positive for *Trypanosoma evansi* infection using these techniques.

Sero-prevalence was detected in twelve cattle and six buffalo using dot-ELISA. Buffalo showed a higher prevalence (30 per cent) of infection than cattle (2.35 per cent). No breed predisposition was observed for trypanosomosis among cattle. There was no significant ($P<0.05$) variation in the infection rates of different breeds viz., CBHF (1.96 per cent), CBJ (2.86 per cent), CBBS (2.05 per

cent) and ND cattle (1.92 per cent). Observations on the agewise prevalence of *Trypanosoma evansi* infection in bovines revealed the highest rate in cattle of one to three years of age (7.5 per cent), followed by three to five years of age (3.5 per cent) and above five years (1.5 per cent). No animal below one year of age was found positive for *Trypanosoma evansi* infection.

The clinical signs of trypanosomosis were encountered in a four year old cow brought to the University Veterinary Hospital, Mannuthy with a history of snoring, inappetance, decreased milk yield and wasting for a considerable period of time. Clinical examination revealed pyrexia (temperature 104°F) and pale mucous membranes. Trypanosomes were detected in the infected animal by wet film examination of blood, Giemsa stained blood smears and micro-haematocrit centrifugation technique. The PCV, Hb concentration and total erythrocyte counts were below the normal values.

The haemato-biochemical values were noted in the positive cases. The mean packed cell volume of the sero-positive animals was significantly lower than that of the control group. The mean Hb concentration and the total erythrocyte counts also showed significant decrease compared to the control group. There was significant lymphopaenia and neutrophilia in the sero-positive animals. The sero-positive animals showed marked reduction in mean blood glucose concentration. The total protein concentration in the serum of the infected animals was significantly lower than that of the control group. The mean albumin concentration and the mean albumin-globulin ratio of the sero-positive animals showed a significant decrease when compared to the control.

The clinically positive case was treated with quinapyramine prosalt (TEVANSI, RANBAXY) at the dose rate of 7.4 mg/kg body weight sub-cutaneously. Parasitaemia was absent in the infected animal from the second day post-treatment. Marked clinical improvement and increase in PCV, Hb and total erythrocyte counts were noted a week after therapy.

In the present study dot-ELISA was found to be a useful test for sero-diagnosis of *T.evansi* infection in bovines. It is cost-effective, easy to perform and can be adopted as a diagnostic tool in the early detection of trypanosomosis. Quinapyramine prosalt was found to be an effective drug in the treatment of bovine trypanosomosis. It has curative and prophylactic properties making it an ideal drug for *Trypanosoma evansi* infection in bovines.

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EPIDEMIOLOGICAL AND CLINICO - THERAPEUTIC STUDIES ON BOVINE TRYPANOSOMOSIS

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ABSTRACT

The present study was undertaken to assess the epidemiological and clinico-therapeutic aspects of *Trypanosoma evansi* infection in bovines. A total of 530 animals (510 cattle and 20 buffalo) from various University farms, University Veterinary hospitals and farm households were screened for trypanosomosis using parasitological and serological techniques. Wet film examination of blood, Giemsa stained blood smears and micro-haematocrit centrifugation technique could detect parasitaemia only in one animal (0.19 per cent). Dot-ELISA detected anti-*Trypanosoma evansi* antibodies in twelve cattle and six buffalo screened for trypanosomosis. Buffalo showed a higher rate of infection (6 per cent) than cattle (2.35 per cent). No breed predisposition was observed among cattle for trypanosomosis. A higher prevalence of infection was observed among animals of one to three years of age. The mean packed cell volume, haemoglobin and total erythrocyte counts of the sero-positive animals (17.667 ± 5.156 per cent, 6.600 ± 1.124 g/dl and 3.467 ± 1.234 $x10^6/mm^3$ respectively) were significantly lower than that of the control group (32.600 ± 4.719 per cent, 10.860 ± 2.133 g/dl and 6.230 ± 1.772 $x10^6$ $/mm^3$ respectively). Significant lymphopaenia and neutrophilia were observed in the infected animals. The clinically positive animal exhibited snoring, inappetance, reduced milk yield, emaciation and anaemia. Quinapyramine prosalt at the dose rate of 7.4 mg/kg body weight sub-cutaneously provided effective clinical cure and marked improvement in haemato-biochemical parameters a week after therapy. The study revealed dot-ELISA to be a highly sensitive, cost-effective and easy to perform test that can be adopted as a diagnostic tool in trypanosomosis.

Appendix

PROFORMA FOR COLLECTION OF DATA ON EPIDEMIOLOGY OF BOVINE TRYPANOSOMOSIS

Case No / Sl. No. **Date:**

1. Name and address of the owner

2. DETAILS OF THE ANIMAL

Species :
Breed :
Age :
Sex :
Colour :
Parity :
Stage of the animal : Heifer / Lactating / Dry / Pregnant
Milk Yield (liters) :
If vaccinated : Yes / No
If yes : FMD, HS, BQ

3. CLINICAL HISTORY

Date	Diseases encountered in the past	Treatment adopted

4. CLINICAL OBSERVATION

1. Respiration (rate/minute) :
2. Pulse (rate/minute) :
3. Temperature (Celsius) :
4. Mucous membrane :

5. Rumen motility :
6. Lymph nodes :
   a. Prescapular
   b. Prefemoral

5. CLINICAL SIGNS : Present/Absent

1. Fever : Intermittent/ Persistent
2. Malaise :
3. Loss of condition :
4. Oedema : Present/Absent
   If yes, part of the body involved :
5. Urticarial plaques :
6. Mucosal hemorrhages :
7. Hair coat : Normal/ Dry

6. FEEDING HABITS

1. Feed intake :
2. Frequency of feeding : Increased/Decreased
3. Preferential feeding if any : Yes/No
   If yes, specify

7. PRODUCTION PERFORMANCE

1. Milk yield :
   Current
   Previous
2. Characteristics of milk :
   Colour
   Consistency
3. Weight gain :
4. If pregnant
   1. Any abnormal vaginal discharge :
   2. Abortion :
   3. Still birth :

8. HEMATOLOGICAL PARAMETERS

1. PCV (%) :
2. Hb (g%) :
3. Blood glucose (mg/dl) :
4. RBC Count (10^6/ml) :
5. Differential count (10^3/ml) :
6. Total protein (g/dl) :
7. Albumin-Globulin Ratio :

9. Wet Film Examination : Positive/Negative

10. Blood Smear Examination : Positive/ Negative

11. Microhaematocrit centrifugation : Positive/ Negative

12. Result of Dot-ELISA : Positive/ Negative

13. If treated for current illness : Yes/No
If yes, specify

14. <u>IN POSITIVE CASES OF TRYPANOSOMOSIS</u>

1. Treatment given

Drug	Dose	Quantity administered	Route	Frequency

2. Response to treatment

1. <u>Clinical Observations</u>

1. Respiration
2. Pulse
3. Temperature
4. Mucous membrane
5. Rumen motility

2. <u>Clinical Signs</u>

1. Fever :
2. Malaise :
3. Oedema :
4. Urticarial plaques :

3. **<u>Feeding Habits</u>**

Feed Intake :

4. **<u>Production Performance</u>**

Milk Yield :

5. **Wet film examination** : Positive/Negative

6. **Blood Smear Examination** : Positive/Negative

7. **Microhematocrit Centrifugation** : Positive/Negative

8. **Serum Biochemistry and Haematology**

1. PCV (%) :
2. Hb (g %) :
3. Blood Glucose (mg/dl) :
4. RBC Count (10^6/ml) :
5. Differential Leukocyte Count :
6. Total Protein (g/dl)
7. Albumin Globulin Ratio :