THEILERIA INFECTIONS AMONG LIVESTOCK - AN OVERVIEW

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Abstract

Theileria annulata and T. orientalis are the two recognized species of Theileria of bovines of Karnataka state. Rod-shaped erythrocytes forms found in higher numbers in carriers and clinically recovered cattle from theileriosis had been confirmed as only transitional stages of T. annulata, but not of T. mutans as considered earlier. A downward trend on the incidence of T. annulata observed at present is a result of prompt remedial measures. The variations in the haematological norms of infected animals were correlated with appropriate parameters. The sudden spurt of anaemia with reduction in infected erythrocytes due to erythrophagocytosis was noticed in clinical cases. The biochemical changes in the blood in T. orientalis were recorded. Development of disease in experimentally infected calves was pursued. The efficacy of various serological tests was assessed and molecular level studies were undertaken. Transmission of theilerial infections through vector ticks was attempted. Among sheep, T. hirci and T. ovis infections were noticed. A transformation in chemotherapy is evident in clinical front.

Keywords: *Theileria, prevalence, diagnostic techniques, molecular studies, Karnataka*

Theileriosis is a tick-borne haemo-parasitic diseases causing great economic loss to the livestock farmers of Karnataka state. Four species *of Theileria, viz*, *T. annulata* and *T. orientalis* in cattle and buffaloes and *T. hirci* and *T. ovis* in sheep have been recognized. Though great success has been achieved in augmenting the milk production by the genetic improvements of indigenous zebu cattle (*Bos indicus*) by continuous and intense cross-breeding with high yielding exotic cattle (*Bos taurus*), their susceptibility to theileriosis has been enhanced. Introduction of improved technology cleared the existed confusion on the speciation of Theileria in bovines of Karnataka. The epidemiology, haematology, serology and molecular aspects of the disease caused by them were studied. In short, this review is an assemblage of old and new concepts of various aspects of theilerial infections in bovines as well as ovines of Karnataka state.

Theileria species of large ruminants

The intra-erythrocytic stages of the Theileria showed great pleomorphism and appeared in annular, oval, rod, comma, dot and comet-shaped forms in stained blood smears and earlier identification of species was purely based on the proportion of different morphological forms. Three species were identified based on this concept were *T. annulata* which predominated the presence of annular and oval shaped forms (80%) *T. mutans* comprising of almost equal number of ring and rod forms and *T. parva*, the one consisting majority of rod forms, amidst other forms mentioned above (Setty, 1969). The existence of *T. parva* in cattle of Bengaluru was

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also reported by Rahiman (1972). But detailed and repeated studies based on by using wet smear technique, Setty *et al*. (1985) had drawn the conclusion of the existence of only two species of Theileria in bovines, *viz*., *T. annulata* and *T. orientalis* in Karnataka.

Identification of species by wet smear technique

Setty (1975) and Setty (1983a) earlier demonstrated the live-morphology of Theileria in hypotonized erythrocytes by wet mount technique (WMT). The organisms appeared as bright spherical bodies varying size from 0.5µ to 1.5µ in diameter showing motility. Larger forms showed brighter cresentic margin and smaller forms represented the comma and rod shaped forms seen in stained smears. The addition of vital stain, 0.5% new methylene blue helped for the better understanding of the live morphology. The motility and the presence of cresentic mass (nucleus) of the spherical bodies were clearly observed (Setty, 1983b). Setty *et al*. (1985) ultimately recognized them as they belonged to two types, Type I and Type II. The type I showed no additional structures attached with the parasite as observed in *T. annulata* while the Type II had bar-like structure, slender and rectangular bar-like attached to the parasite, later confirmed as *T. orientalis*. The presence of *T. annulata* could be readily detected in Giemsa stained blood smear, but not so in the case of *T. orientalis*, as the stained haemoglobin (Hb) masked the intracellular inclusions associated with parasites. These observations were confirmed by Ramananda (1996) Ramananda and Setty (1996) and Setty (2002).

Conventional methods used for diagnosis

Among the conventional methods, Giemsa-stained blood smear examination (GBSE), wet mount technique (WMT), buffy coat analysis (BSA) and lymph-node aspiration smears examination are employed for the detection of theilerial species. GBSE is the most commonly used for routine examinations of haemoparasites. With respect of *T. orientalis* infection, Gowda (1993) and Gowda and Setty (1994) reported that WMT gave highest positive results of 34.76% and GBSE gave 21.95% while Ramesh (1995) and Ramesh *et al.* (2003) demonstrated 48.0% and 15.73% infection respectively indicating the superiority of WMT.

Theileria annulata

Morphology

Wide variations in the morphological forms of *T. annulata* infection in carrier cattle was observed by Renukaprasad (1978) and Renukaprasad and Hiregoudar (1987) and these forms were classified into 4 groups such as Group I comprising ring and oval forms; Group II consisting rod, comet and pinhead forms; Group III the comma forms and Group IV representing the dot forms. The ring-shaped measured 0.5-1.5µ in diameter, oval forms 1.5-2.0µ, rods 1.0-2.00 x 0.5-0.75µ, comet-shaped and pinhead forms 2.0-2.5µ, comma forms 1.5-2.5µ and dot forms 0.5µ in diameter. The percentage of the ring and oval forms noted in clinical cases, splenectomized calves, exotic-, cross-bred and indigenous- carriers was 70.29%, 72.90%, 38.25 to 43.36%, 33.18% and 29.65% respectively, showing the predominance these forms in the first two categories. Similar morphological observations were also noticed by Ramananda (1996).

Incidence of *T. annulata*

More information on the incidence and the epidemiology of theileriosis and other haemoparasitic diseases was built up after the commencement of the research project on parasite-borne diseases at Veterinary College, Bengaluru and the establishment of four veterinary diagnostic laboratories (VDL) at Bengaluru, Mysuru, Hassan and Tumukuru under University of Agricultural Sciences and Karnataka Dairy Development Corporation (UAS-KDDC, 1977-1984). Additionally, VDL, Bengaluru served the needs of Kolar district, VDL, Mysuru covered Mandya and Kudugu district, Hassan served Chikkamagaluru district. Other institutions in Karnataka were also provided information on the incidence.

The information collected on the incidence of *T. annulata* in bovines in different parts of Karnataka between 1969 and 2014 has been furnished in Table 1. Overall data indicated an undulating pattern of incidence with a maximum incidence of 58.91% (1977) and minimum of 0.75% (2011). Mixed infection of *T. annulata and Babesia bigemina* (0.50%) as well as *T. annulata* and *Anaplasma marginale* (0.88%) was recorded at VDL, Mysuru (Seshadri *et al*. 1985; Muraleedharan *et al*., 1994b).

Season-wise

VDL, Bengaluru had recorded the highest incidence of Theileria infection in cold weather (January to February) while VDL, Hassan it was in north-east monsoon (October to December). VDL, Mysuru and Tumukuru had noted the highest incidence in south-east monsoon (June to September). However, the incidence of infection was recorded in all months in the eight southern districts of Karnataka (Seshadri *et al*, 1985). Venugopal (1983) observed maximum incidence during May-July followed by minimum during August-October at Bengaluru.

Age-wise

Analysis of data of VDL, Mysuru showed that the maximum age incidence of 22.57% was observed in 6 month-1 year age group followed by 19.23% in 1-4 year and 18.92% in 4-8 years age groups and the least incidence of 5.32% in cattle of above 8 years indicated that older animals was appeared to be less susceptible. Very young calves of 1-6 months of age showed 17.35% of infection and the infection was noticed in a two month old CB calf (Muraleedharan *et al*., 1994b). Setty *et al*. (1985) reported 33.8% infection in cross-bred calves less than one year old in KDDC areas. Naik *et al*. (2010) noticed infection in five calves below one month which was the minimum age for getting infection.

Breed-wise

Setty (1969) reported 27.27%, 57.58% and 15.15% infection in exotic breeds (EB) cross breeds (CB) and indigenous (IG) cattle respectively. Setty *et al*. (1985) observed 41.64% of infection in CB cattle whereas 5.82% infection in IG cattle. According to Muraleedharan *et al*. (1994b), the highest incidence of 26.31% of infection was noted in EB followed by 19.47% in CB and the least incidence of 12.19% in IG cattle of Mysuru-Mandya districts. Venugopal (1983) reported that 40.00% of EB, 30.80% of CB and 45.00% of Hallikar cattle were found infected.

Pathogenesis

Muraleedharan *et al*. (2008) categorized the common clinical symptoms exhibited by CB cattle (n=798) ailing from theileriosis into four groups in order of priority - very frequent, frequent, occasional and rare. They are: pyrexia, anorexia, enlarged lymph glands (very frequent), excessive salivation, bronchitis, dullness, pale mucus membrane, red conjunctiva, recumbency, diarrhea, swelling of dependant parts, debility, drop in milk yield (frequent), shivering, pneumonia, nasal discharge, impaction, coffee-coloured urine, constipation (occasional), nervousness, rolling of eye balls, colic, erection of hair, rough body coat, fits, circling moments, staggering gait and mucus-tinged faeces (rare). Naik *et al*. (2010) observed typical clinical signs in CB calves below one month of age.The mortality based on necropsy findings among bovines in Hassan, Mysuru, Mandya and Tumukuru district was to 1.39%, 8.04% and 11.90% respectively (Seshadri *et al*., 1985; Ziauddeen *et al.*, 1987).

Haematology

Variations in parasitaemia and haematological norms were observed depending on the stages of the disease. Macrocytic hypochromic anemia was observed due to lowered TEC (Total erythrocyte counts) to 3.27-4.37million/µl and Hb (Haemoglobin) value to 5.5-6.9 g/dl. TLC (Total leucocyte counts) was below normal, 3000-4790/µl and DLC (differential leucocyte counts) showed lymphocytes 38-49%, neutrophils 16-24%, monocytes 27-46%, eosnophils 0.3-2% and basophils 0.3-0.5%. Preponderance of monocytes was seen in 97.0% of the cases studied (Setty, 1969). Seshadri *et al*. (1985) presented the haemogram of bovines of Mysore-Mandya district: TEC 4.44-6.49million/µl, Hb 7.44-9.42 g/dl, TLC 7209-10,852/µl, DLC-lymphocytes 56.14-68.10%, neutrophils 24.27-37.08%, monocytes 2.30-3.13% and eosinophils 2.53-3.56%. Muraleedharan *et al.* (2009) tried to co-relate nine combinations of various haematological parameters of infected cattle of Mysore Union *viz.* Hb levels, TEC, TLC, DLC, with grades of parasitaemia and temperature ranges. Appreciable reduction was noted in Hb level in 31.0% and TEC in 35.0% of the infected cattle. Studies on TLC on positive cattle showed leucopenia in 6.88% while 47.71% in the border line, and 19.72% exhibited leucocytosis and DLC indicated that 44.94% had lymphocytosis, 1.71% monocytosis, 16.69% neutrophilia, 4.21% eosinophilia. Ananda *et al.* (2009) recorded

Table 1. Incidence of *Theileria* infection in bovines of Karnataka state based on stained blood smear examinations

District / Locality	No. exam	% Positive	Main breeds	Reference
A. Cattle				
Bengaluru city & suburbs	247	29.15	CB, JY, RD, IG	Setty (1969)
Bengaluru city & suburbs	129	58.91	CB,RD,HF, IG	Renukaprasad (1978) ; Renukaprasad and Hiregoudar (1987)
Jersey Farm, Dharwad	55	37.70	JY, JYx	Sastry *et al*. (1981)
Red Dane Farm, Hessarghatta, Bengaluru	45	20.00	RD, RDx, IG	-do-
Bengaluru city	78	38.5	CB,IGL	Krishna Murthy *et al.* (1982)
Bengaluru city & suburbs	452	39.60	EB, CB, IG	Venugopal (1983)
Mysuru & Mandya dt.	2750	20.29	EB, CB, IG	Muraleedharan *et al.* (1983)
10 farms in Bengaluru & one in Hassan dt.	739	2.4-23.1	EB, CB	Setty *et al*. (1985)
Bengaluru KDDC Union	2331	38.86	Dairy herd	-do-
Mysuru KDDC Union	931	31.69	-do-	-do-
Tumukuru KDDC Union	297	40.74	-do-	-do-
Hasan KDDC Union	2331	19.51	-do-	-do-
Karnataka KDDC unions	2973	41.64	CB adults	-do-
Karnataka KDDC unions	468	33.76	CB calves	-do-
Karnataka KDDC unions	206	5.82	IG	-do-
Bengaluru dt.	*1375	54.47	EB, CB, IG	Seshadri *et al.* (1985)
Kolar dt.	*1234	56.81	CB, IG	-do-
Hassan dt.	*2726	9.10	-do-	-do-
Chikkamagaluru dt.	*145	14.48	-do-	-do-
Tumukuru dt. & Maddur tk. (Mandya dt).	*3154	19.62	-do-	-do-
Somavarpet tk. (Kodagu dt.) Virajpet tk.(Kodagu dt.)	*96 *29	4.17 13.79	-do- -do-	-do- -do-
Mysuru dt.	3318	18.56	-do-	Seshadri *et al*. (1985); Muraleedharan *et al.* (1994)
Mandya dt.	1174	15.16	-do-	-do-
Bengaluru city & suburbs	129	58.91	CB,RD,HF	Renukaprasad and Hiregoudar (1987)
Mysuru dt.	3318	18.56	-do-	Muraleedharan *et al.* (1994)
Mandya dt.	1174	15.16	-do-	-do-
Bengaluru city & suburbs	375	15.20	HFx, JYx ,RDx, IG	Ramesh (1995)
Karnataka (different localities)	11,755	16.31	EB, CB, IG	Harish *et al*. (2006)
Bengaluru north	132	31.06	CB	Ananda *et al.* (2009)
Doddaballapura tk. (Bengaluru Rural dt.)	372	9.14	-	Chetan Kumar and Sunita (2011)
Bengaluru city	1730	0.75	-	Sunita *et al.* (2011)
Shivamogga town & suburbs	443	8.58	CB, IG	Ananda *et al.* (2014)
Shivamogga region	215	28.80	-	Krishna Murthy *et al.* (2014)
B. Buffaloes				
Mysuru & Mandya dt.	213	8.91	IG	Muraleedharan *et al.* (1983)
Karnataka state	265	36.22	-do-	Setty *et al.* (1985)
Bengaluru city & suburbs	152	23.63	-do-	Venugopal (1983)
Mysuru dt.	334	8.43	-do-	Muraleedharan *et al*. (1991)
Mandya dt.	177	5.08	-do-	-do-
Shivamogga town & suburbs	123	20.32	-do-	Ananda *et al.* (2014)
Shivamogga region	85	12.90	-	Krishna Murthy *et al.* (2014)

JY-Jersey; HF-Holstein Friesian, RD-Red Dane; CB-Cross of exotic breeds (EB) and indigenous (IG) cattle; x= crosses; *bovines (include almost 80% cattle and 20% buffaloes); dt.- district; tk.-taluk.

reduction of Hb, TEC and packed cell volume (PCV) in severely infected animals.

Setty (1969) expressed that the severity of anaemia was directly proportional to the number of erythrocytes infected. But according to Muraleedharan *et al*. (2005) in two-third cases the infected erythrocytes appeared to be removed rapidly from the circulation especially in the advanced stage of the disease by erythrophagocytosis. Mild anaemia and leucocytosis with pyrexia was indicative of early phase while severe anaemia with mild leucopenia was found in advanced stage (Muraleedharan *et al*., 2009).

Experimental infection with *T. annulata*

Setty *et al.* (1985) tried to infect infection-free splenectomized calves with ground-up tick supernate (GUTS) of *Hyalomma anatolicum* ticks collected from field cases. Schizonts were detected in the enlarged lymph nodes. Pyrexia of 40.6-40.9^{0}C was noticed with the appearance of parasites on 11th- 13th day and parasitaemia increased to 12%-15% on the 15th- 18th day respectively (Setty *et al*., 1985). Tachycardia was evident. The calves were dull and showed pale mucus membrane. A large macula developed in the left eye of one of the calves. Calves died following experimental infection exhibited typical lesions of *T. annulata*. The lymph glands nearest to the site of inoculation were hyperaemic with petechiae, congestion and oedema. The abomasal mucus membrane showed small, circular and shallow ulcers. Renukaprasad and Hiregoudar (1987) observed that the appearance of *T. annulata* in circulation on 16th day after experimental infection in splenectomized carrier calf and the infection reached the height of parasitaemia on 27th day with fall in Hb and PCV values. Inoculation of GUTS was resorted due to the lack of schizonts in blood to produce infection in calves.

Sero-diagnosis

Various serological tests were conducted to detect infection using antigen prepared from local stains of *T. annulata*. They were capillary tube agglutination test (CTAT), tube agglutination test (TAT), complement fixation test (CFT), rapid slide agglutination test (RSAT), modified slide agglutination test (MSAT), agar gel diffusion test (AGDT), A-B ELISA, A-B micro ELISA and SDS-PAGE analysis.

Renukaprasad (1978) screened 129 sera by CTAT and 37.98% of CB and EB cattle were positive while of IG carriers were negative. Of the 104 sera subjected to RSAT and MSAT, 50.00% and 47.11% were positive respectively. But AGDT was not efficient. Sastry *et al.* (1981) conducted CFT in cattle of three organized dairy farms at Dharwad, Munirabad and Hessarghatta with antigen prepared from Bengaluru isolate. Out of 194 sera screened, 53.6% revealed positive titers. Breed-wise prevalence of 56.7%, 45.2% and 25.0% was noted in EB, CB and IG cattle respectively. Animals above one year of age showed higher percentage of infection (58%) compared to those below one year (35.9%). For conducting TAT, the antigen was prepared from the spleen of infected cattle with high parasitaemia and the test provided higher efficacy of 49.9% while GSBS examinations showed 38.5% positive result (Krishna Murthy *et al*., 1982). Nagaraja (1986) was conducted a detailed study on plasma pepsinogen levels in bovine theileriosis.

A-B Elisa technique was applied to measure antibody levels in bovine theileriosis (Thimma Reddy, 1991; Thimma Reddy *et al*., 1994). The sero-conversion of Rakshavac-T vaccinated cattle was observed and adequate antibody levels persisted till 11 months. The antibody response was higher in CB (0.67) compared to pure-breds (0.37) and in animals of 2-3 years of age (0.38) as measured by optical density (OD). Prashanth (1992) estimated the transfer of *T. annulata* schizont maternal antibodies to calves born to dams vaccinated with Rakshavac-T vaccine by A-B ELISA test which revealed significant higher antibody levels than those born to unvaccinated dams as indicated by OD values, highest in Red Sindhi, followed by HF and JY cattle. The antibody titer of calves showed a progressive increase till 30 days of birth attaining a peak at 60 days and a gradual decline from 90 days onwards. Renukaprasad (2009) reiterated that SDS-PAGE with stage specific antigen of *T. annulata* revealed that piroplasm antigens had a molecular weight between 18 and 72kd and schizont antigens between 36 and 80kd. Two piroplasm-specific antigens free from contaminating erythrocyte antigens were

obtained by DEAC-Sephacel chromatography. Piroplasm and schizont antigens extracted with triton X-100 and lauryl sarcosin respectively were used for detection of *T. annulata* carrier animals and also for measuring *T. annulata*-specific antibodies in vaccinated animals by highly sensitive AB-micro ELISA. Schizont antigens appeared to be more suitable than piroplasm antigen for routine sero-monitoring of disease.

Tick vectors and culturing of schizonts of *T. annulata*

Jagannath *et al.* (1979) observed that the known tick vector of *T. annulata*, *Hyalomma anatolicum anatolicum* occurred in cattle throughout the year, abundantly during south-west monsoon in Bengaluru. Setty *et al.* (1985) detected infection in *H. anatolicum* ticks collected from positive cases. Thimma Reddy *et al.* (1993) described a simple and rapid procedure for the isolation and release of *T. annulata* schizonts from infected lymphoblastoid cells. Renukaprasad (2009) reported that *T. annulata* infection was maintained *in H.* (*a*) *anatolicum* and the bovine lymphocytes infected with schizonts were cultured *in vitro.*

***T. annulata* infection in buffaloes**

Panduranga *et al.* (1978) reported an isolated incidence of *T. annulata* infection with 2.0% parasitaemia in a 7 years old non-descript buffalo from Bengaluru. Reports on the incidence of infection in buffaloes based on various surveys are noted in Table 1. Muraleedharan *et al.* (1991) recorded higher incidence in age group 6-12 month and low in 4-8 year age group. Infection was mostly recorded in the month of March. Symptoms exhibited were high temperature (39.5-43.3^{0}C), anorexia, enlargement of superficial lymph nodes, bronchitis, conjunctivitis and occasional swelling of joints.

Theileria orientalis

Morphology

Studies of Ramesh (1995) and Ramesh *et al*. (2003) confirmed the previous findings of Setty *et al*. (1985) on the morphological identification of *T. orientalis* by WMT. According to them, GBSE revealed the intra-erythrocytic forms, but the bar-like structure could be faintly seen as an appendage to the parasite slightly darker than the Hb background. Ramananda (1996) opined that the organisms appeared as elongated rod-shaped bacillary forms associated with bar-like structures in most of the cells and veils were noticed in some cells. There were instances where both structures were seen in the same cell.

Pathogenesis of *T. orientalis*

Clinical symptoms exhibited were less severe with mild pyrexia and anaemia compared to those of *T. annulata*. Gowda (1993) had studied the hematological and the biochemical observations in 33 infected calves with parasitaemia ranging from 0.4-3.0%. TEC reduced to 1.60-9.60million/µl. Hb ranged from 4.00 to18.20g/dl and PCV from 14.50 to 40.60%. The estimation of total bilurubin levels in infected cattle was 0.25-3.12mg/dl, total serum protein 6.18-9.99g/dl and alkaline phosphatase 32.57-249.7i.u/litre. No significant difference was found in the value of serum calcium, sodium and potassium.

Experimental infection with *T. orientalis*

Setty *et al*. (1985) could readily produce clinical form of *T. orientalis* (Type II Theileria) infection 5-31 days post-splenectomy in carrier cross-bred male calves aged 4-8 months with initial parasitaemia below 1%. Temperature of calves rose to 39.1 to 39.6^{0}C though they were apparently normal and active. The lymph nodes had not enlarged and schizonts were not detected in any of the cases. PCV ranged between 22-29% and Hb was 7.5-10.5g/dl at the peak parasitaemia. Ramesh (1995) reported initial 1% parasitaemia and 11g/dl Hb of two carrier calves. Parasitaemia rose to a maximum of 25% and 32% while Hb level reduced to 7.0 and 7.4g/dl in calves on day 40 and 42 post-splenectomy. The parasitaemia dropped to 2% on day 49 and 52. The experimental calves those died, on necropsy revealed that superficial lymph glands were normal and abomasum showed slight hyperaemia (Setty *et al*., 1985).

Diagnosis and incidence of *T. orientalis*

T. orientalis infection in cattle occurred in many districts of Karnataka. Renukaprasad (2009) reported high incidence of *T. orientalis* in calves on blood smear examinations during period 1984-87. Gowda (1993) and Gowda and Setty (1994) subjected 164 blood samples of

calves of 3-6 months age from seven different endemic areas and reported that 34.76% of them was positive by WMT and 21.95% by GBSE. Ramesh (1995) observed *T. orientalis* infection in the proportion of 80.0%, 49.4% and 45.9% in RD, HF and JY crosses respectively and 37.8% in Zebu breeds in 10 farms in Bengaluru.The adult cattle showed higher infection than calves. An overall prevalence of *T. orientalis* infection was 48.0% by WMT and 15.73% by GBSE.

Serological and molecular studies

SDS-PAGE analysis of Hb (haemolysate) of parasitized and non-parasitized erythrocytes showed no difference in protein bands indicating that 'bar' or 'veil' like structure in Hb of parasitized erythrocytes did not resolve into a separate or unique peptide band by 8% gel SDS-PAGE (Ramesh,1995). Stationary culture of *T. orientalis* infected erythrocytes from cattle showed division of parasites *in vitro*. Further a sensitive AB-micro ELISA was developed and standardized to measure *T. orientalis* specific antibodies in cattle. Serological cross-reactivity between two isolates of this parasite was established using indirect fluorescent antibody test, AB-micro ELISA and immunoblot experiments. The piroplasm-specific antigen of *T. orientalis* in cattle was between 18 and 95 kd proteins as analysed by Western blotting whereas 39 kd protein was absent in buffalo isolate. Gowda (1993) and Gowda and Setty (1994) reported 11.58% *T. orientalis* infection in calves by CTAT.

Vector and cross transmission

The ixodid tick, *Haemaphysalis bispinosa*, a known vector of *T orientalis* of cattle, was prevalent among cattle of Bengaluru (Jagannath *et al*., 1979) and the parasite was found transmissible to cattle through this tick (Renukaprasad, 2009).

Theileria in sheep

Examination of blood smears of sheep of six sheep-breeding farms in Karnataka revealed the presence of 56.1% theilerial parasites, the highest incidence in exotic sheep (70.1%) followed by Bandur crosses (64.28%) and indigenous sheep (52.30%). Mixed infection of Theileria with *Anaplasma* organisms in 22.0% of sheep was observed in all the farms while 3 sheep of one of the farms had mixed infection with *Babesia* (Prabhakar, 1976; Prabhakar and Hiregoudar, 1977).

Experimental studies

Prabhakar and Hiregoudar (1979) studied the effect of splenectomy on blood values, on the multiplication rate and morphological characters of the parasites in *T. hirci* infected carrier Bandur sheep. Before splenectomy they had a parasitic count of 0.39-0.59% which on splenectomy slowly increased from 4th to 8th day and thereafter increase was rapid till parasitaemia attained 50.0% between 16th and 24th day (Prabhakar and Hiregoudar, 1979). Increased in number of signet-ring forms was noted after 8th day. Rod and comma forms of parasites were also observed. The minimum Hb and PCV in the splenectomized theilerial carrier sheep were 2.3g/dl and 10% respectively with rapid fall in TEC ranging from 2.45 to 4.00million/µl. These changes were in relation to the extent of parasitaemia. Increase in leucocyte counts ranged from 19.98 to 26.8 thousand/µl. Relative monocytosis was evident.

Setty *et al*. (1985) noticed the prevalence rate of 37.2% of *Theileria* sp. in sheep population of Karnataka. An outbreak of theileriosis due to *T. hirci* was reported in a flock of 80 Merino cross-bred sheep in three villages of Tumukuru district with typical symptoms such as pyrexia (40.0-41.67^{0}C), anorexia, depression, icteric mucus membrane, haemoglobinuria and enlargement of superficial lymph nodes with parasitaemia ranged from 2 to 7% (Srinivas *et al*., 1985). Muraleedharan *et al*. (1994a) reported *T. hirci* infection in 31.1% of sheep which exhibited pyrexia (41.1^{0}C), dyspnoea, bronchitis, rhinitis, edema of the throat, and diarrhea. However, no appreciable reduction of Hb (10%) was noticed. Mortality percentage was 4.44%. Concurrent infection with *Anaplasma ovis* was recorded in two. *T. ovis* infection was rarely seen in sheep. The most predominant ticks found in *Theileria* carrier sheep in farms were *Haemaphysalis bispinosa* followed by *H. marginatum issaci* (Hiregoudar and Prabhakar, 1977).

Treatment and control measures

Hegde *et al*. (1971) treated *T. annulata* infection in cows with chloroquine phosphate

(Nivaquine) Panduranga *et al*. (1978) treated in a non-descript buffalo with Malaquin and oxytetracycline followed by chloroquine phosphate in subsequent days. Ananda *et al*. (2009) treated theileriosis with single dose of buparvaquone along with oxytetracycline. Buparvaquone along with haematinics cured theileriosis of calves below one month old (Naik *et al*., 2010) and CB cattle aged 2-3 years (Sumathi and Veena, 2012). Chetan Kumar and Nagaraju (2011) assessed the comparative efficacy of oxytetracycline and buparvaquone as 83.33% and 100% respectively. Experimentally infected splenectomized calves with *T. annulata* and *T. orientalis* and natural infection *of T. annulata* in EB and CB cows were treated with single dose of halofuginone (Setty *et al*., 1985). Srinivas *et al.* (1985) treated sheep ailing with natural theilerial infection with Berenil. Tick control measures were practiced using different acaricides.

Conclusion

The pattern of disease occurrence and the epidemiological information on theileriosis of Karnataka State could identify the priority areas where timely effective preventive measures should be implemented. The incidence of clinical cases and mortality from theileriosis in livestock had been drastically reduced by the prompt diagnosis, treatment and control measures. Along with routine GBSE, WMT has to be made mandatory for haemoparasites, so that the presence of *T. orientalis* does not escape from detection. Therefore submission of whole blood to the laboratory is highly essential. Prime importance has to be given for incorporating molecular tools for accurate, easy and early identification of parasites concerned. Phytotherapeutic agents having higher efficacy against theilerial parasites and their vectors should be explored for effective control of infection.

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