Prevalence of Ovine theileriosis in Northern Karnataka

S. Shantaveer Biradar, Placid E. D'souza and G. S. Mamatha

Department of Veterinary Parasitology,
Centre of Advanced Faculty Training Veterinary College,
Karnataka Veterinary, Animal and Fisheries Sciences University
Bengaluru-560 024, Karnataka.

ABSTRACT

An epidemiological survey on ovine theileriosis was conducted in six districts of northern Karnataka, India. On examination of 525 (209 clinical and 316 healthy/tick infested) Giemsa stained thin blood smears of sheep, 107 (51.20%) and 129 (40.82%) sheep smear samples from clinical and healthy/tick infested flocks were found positive for *Theileria* organisms respectively, with an overall prevalence of 44.95% (236/525). The parasitemia ranged between 0.8 to 1.2 and 0.2 to 0.4 per cent in clinical and apparently healthy/tick infested sheep, respectively. The haemogram of *Theileria* infected sheep revealed severe anaemia with haemoglobin levels ranging between 2.3 to 4.6 g / dl. However, no significant ($P< 0.05$) difference was found between the age, gender, breed and district wise prevalence. The present study indicated that ovine theileriosis is an endemic disease in this study area and aids in understanding and implementation of measures to control ovine theileriosis in northern Karnataka.

Key Words: Stained blood smear, Prevalence, Theileriosis, Sheep, North Karnataka.

INTRODUCTION

Small ruminants especially sheep contribute to the livelihood of the rural population in most of the developing countries. As per 19th Livestock Census (2012), India accounts for 65.06 million sheep. Karnataka stands second position in sheep population with 9.58 million (Shiva Kumara *et al*., 2017). The global loss due to ticks and tick borne diseases (TTBDs) was estimated to be between US$ 13.9 and 18.7 billion annually (de Castro, 1997), whereas in India, the estimated loss due to tick borne disease was around US$ 498.7 million per annum (Minjauw and Mcleod, 2003).

Ovine theileriosis is an important hemoprotozoan disease of sheep in tropical and subtropical regions (Altay *et al*., 2007) that leads to economic losses in these animals. The disease is also important due

Corresponding author: Dr. Shantaveer S. Biradar, PhD Scholar, Center of Advanced Faculty Training, Department of Parasitology, Veterinary College, KVAFSU Regional Campus, Hebbal, Bengaluru-560024 Karnataka, India. Email ID: shantuvet@gmail.com, Mob: 8722415201

to its significance in the international trade of animals and animal products (Uilenberg, 2001). In India *T. hirci, T. ovis* and *T. luwenshuni* are the most prevalent species reported in small ruminants (Sisodia, 1981; Kaufmann, 1996; Mamatha *et al*., 2017).

Diagnosis of theileriosis is based on the clinical findings and microscopic observation (MO) of thin blood smears stained with Giemsa in acute cases (Aktas *et al*., 2005). In the present study, an epidemiologic survey of *Theileria* infection in sheep in six districts of northern part of Karnataka by blood smear study was undertaken.

MATERIALS AND METHODS

In the present study, a total of 525 (209 clinical and 316 healthy/tick infested) sheep blood samples were collected in EDTA vaccutainers from both apparently healthy/ tick infested sheep and clinically suspected (pyrexia 105–107°F, tick infestation, inappetance, weakness and anaemia) sheep from flocks located in six districts covering five different agro climatic zones of northern part of Karnataka during the period from August 2017 to October 2018 (Table 1). Thin blood smears were prepared immediately after the blood collection, air-dried, fixed in methanol for one minute and stained with 10 per cent Giemsa staining solution (1:10) for 20 minutes and subsequently examined microscopically under oil immersion. The *Theileria* organisms were identified as per Yin *et al.* (2007), Lefevre *et al.* (2010) and Soulsby (2012). Parasitemia was assessed by counting the number of infected red blood cells by examination of 200 microscopic fields (approximately 1,00,000 RBCs) (Jalali *et al.,* 2014) and expressed as percentage. Haemoglobin level was determined in an automated haematology analyzer (Erba, Germany). Age, gender, breed and district wise prevalence of theileriosis in sheep was recorded and statistical analysis of data were carried out by Chi-square test using graph pad prism software, version 5.01.

Table1: Details of blood samples collected from sheep from different Agro climatic Zones of Northern Karnataka

Sl. no.	Districts	Agro climatic zones	No. of samples collected	Latitude	Longitude	Elevation (m)	Average annual temperature (C^0)	Average annual precipitation (mm)	Mean relative humidity (%)
1	Yadgir	North eastern dry zone	45	16.76 N	77.14 E	389	27.8	711	16 to >99
2	Kalaburgi	North eastern transition zone, North eastern dry zone	105	17.40 N	76.64 E	454	27.2	777	19 to >99
3	Raichur	Northern dry zone, North eastern dry zone	75	16.21 N	77.34 E	407	27.7	713	21.5 to >90
4	Belagavi	Northern dry zone, Northern transition zone and Hilly zone	150	15.84 N	74.51 E	753	24.2	1200	18.3 to 86.2
5	Vijayapur	Northern dry zone	75	16.82 N	75.72 E	601	26.6	553	17.7 to >99
6	Bagalkot	Northern dry zone	75	16.17 N	75.65 E	537	25.8	683	17.3 to >99

RESULTS AND DISCUSSION

On examination of 525 blood smears 107 (51.20%) and 129 (40.82%) sheep samples from clinical and healthy/tick infested flocks were found positive for *Theileria* organisms respectively, with an overall prevalence of 44.95 (236/525) per cent (Table 2). The highest rate of infection was observed in Raichur (58.66%) followed by Yadgir (48.88%), Kalburgi (46.66%), Vijayapur (44%), Bagalkot (40%) and Belagavi (38.66%). Based on statistical analysis, there was no significant difference between different districts in sheep ($P< 0.05$) (Table 2).

Table 2: Prevalence of theileriosis in sheep from six districts of north Karnataka

Sl. No.	Districts	No. of samples collected			No. positive (% positive)		
		Clinical cases	Healthy/Tick infested flocks	Total	Clinical cases	Healthy/Tick infested flocks	Total
1	Yadagir	15	30	45	9 (60)	13 (43.33)	22 (48.88)
2	Kalaburgi	50	55	105	28 (56)	21 (38.18)	49 (46.66)
3	Raichur	30	45	75	17 (56.66)	27 (60)	44 (58.66)
4	Belagavi	48	102	150	23 (47.92)	35 (34.31)	58 (38.66)
5	Vijayapur	31	44	75	14 (45.16)	19 (43.18)	33 (44)
6	Bagalkot	35	40	75	16 (45.71)	14 (35)	30 (40)
Total		**209**	**316**	**525**	**107 (51.20)**	**129 (40.82)**	**236 (44.95)**

The statistical difference between district wise prevalence in sheep was found to be non significant ($p<0.05$).

In the present study, the highest prevalence of *Theileria* spp., (44.95%) were recorded in all six districts of north Karnataka, however 56.1 per cent (799/1424) infection was reported in sheep from Mandya district from six sheep farms (Prabhakar and Hiregoudar, 1977); 69.7 and 46 per cent in sheep from Iran (Jalali *et al.,* 2014; Yaghfoori *et al.,* 2013); 58.59% in small ruminants from China (Li *et al.,* 2014) and these findings are in contrast to the lower prevalence rates reported elsewhere viz., 19.35% of prevalence was recorded in sheep from Turkey (Altay *et al.,* 2005); 11.9, 18.6 and 22.27% in sheep from Iran (Razmi *et al.,* 2006; Razmi and Yaghfoori, 2013; Bami *et al.,* 2009). The differences in the prevalence of theileriosis in different geographical areas could be due to varied climatic conditions that affect both intensity of tick infestation and parasitemia ratio and severity of disease (Zaeemi *et al.,* 2011).

The climatic conditions that affect the intensity of ticks that feed on hosts are effective on parasitemia ratio and severity of disease. In this study, dry zones (Yadgir, Kalaburgi, Raichur, Vijayapur and Bagalkot) with mean annual temperature (25.8 to 27.8 C^0) and latitude (16.17 to 17.40 N) have relatively suitable conditions for development of ixodid ticks that lead to high prevalence of theileriosis in sheep (Table 1) rather than cold regions such as Belagavi with mean annual temperature (24.2 C^0) and latitude (15.84 N). These findings were in accordance with the findings of Zaeemi *et al.* (2011). The variability in the prevalence

among districts was due to close relationship between incidence of theileriosis, activity period and differences in climate which probably had an effect on distribution of vector especially hard ticks.

Table 3: Age, gender and breed wise prevalence of theileriosis in sheep

Age (months to years)	Total No. of animals examined	Total No. of animals positive	Per cent Positive
6 months to 1 year	104	44	42.30
1 year to 3.5 years	421	195	46.31
Total	525	239	45.52
Gender			
Male	228	95	41.66
Female	297	144	48.48
Total	525	239	45.52
Breed			
Kenguri	124	58	46.77
Shahapuri	64	25	39.06
Deccani	125	59	47.2
Bellary	83	43	51.80
Non-descript (ND)	129	54	41.86
Total	525	239	45.52

The statistical difference between age, gender and breed wise prevalence in sheep was found to be non significant ($p<0.05$).

The *Theileria* organisms observed in the red blood cells of sheep during this study were highly pleomorphic. The nail forms (28%) were predominant followed by rod forms (16%), comma forms (14%), round forms (11%), dot forms (10%), parachute forms (8%) and other forms (13%) (Plate 1). Different forms of *Theileria* organisms observed in the present study were also reported in sheep and goats from China (Guo *et al.,* 2002; Yin *et al.,* 2007); sheep from Turkey (Aktas *et al.,* 2005); sheep from Iran (Razmi *et al.,* 2006).

A higher prevalence of theileriosis was noticed in the age group of one to 3.5 years of age (46.31%) followed by six months to one year of age (42.30%); Females were showing more positive (48.48%) when compared to males (41.66%) and Bellary sheep breeds were showing more positive percentage (51.80%) followed by other breeds (Table 3). However, no significant ($p< 0.05$) difference was found between age, gender, breed and district wise prevalence.

During this study, symptoms like high fever (105–107 °F), inappetance, weakness and anaemia with heavy tick infestation were noticed in clinically affected sheep.

The level of parasitemia in the present study ranged between 0.8 to 1.2 and 0.2 to 0.4 per cent in clinical and apparently healthy/tick infested animals respectively, which were in contrast to the lower parasitemia levels reported elsewhere viz., parasitemia of 0.01 to 0.1 and 0.00001%

recorded in sheep from Turkey (Aktas *et al.,* 2005; Altay *et al.,* 2005). This could be attributed to the stage of the disease at which the blood smears were made because, high parasitemia will be seen in acute/ clinical stage whereas, low parasitemia is a characteristic feature of carrier or chronic stage of the disease (Yin *et al.,* 2008).

The haemogram of *Theileria* infected animals revealed severe anaemia with haemoglobin levels ranging between 2.3 to 4.6 g / dl. The change in the haematological values could probably be due to development of intravascular hemolysis or destruction of red cell by intra-erythrocytic stages of *Theileria* spp. (Barnett, 1978).

The present study indicated that ovine theileriosis is an endemic disease in six districts of northern part of Karnataka.

ACKNOWLEDGEMENTS

The paper is a part of research work conducted by the first author for Ph.D. degree programme under KVAFSU, Bidar. The facilities provided by the Indian Council of Agricultural Research (ICAR), New Delhi through the Centre of Advanced Faculty Training is acknowledged.

REFERENCES

Aktas, M., Altay, K. and Dumanli, N. (2005). Development of a polymerase chain reaction method for diagnosis of *Babesia ovis* infection in sheep and goats. Veterinary Parasitology 133: 277–281.

Altay, K., Aktas, M. and Dumanli, N. (2007). *Theileria* infection in small ruminants in the East and Southeast Anatolia. Turkish Journal of Parasitology 31: 268-271.

Altay, K., Dumanli, N., Holman, P.J. and AKTAS, M. (2005). Detection of *Theileria ovis* infected sheep by nested PCR. Veterinary Parasitology 127: 99-104.

Bami, M.H., Haddadzadeh, H.R., Kazemi, B., Khazraiinia, P., Bandehpour, M. and Aktas, M. (2009). Molecular identification of ovine *Theileria* species by a new PCR–RFLP Method. Veterinary Parasitology 161: 171–177.

Barnett, S.F. (1978).*Theileria*. In parasitic protozoa, Edn. Kreier, J.P. Academic press (New York), Vol 1; pp 77-113.

De Castro, J.J. (1997). Sustainable tick and tick-borne disease control in livestock improvement in developing countries. Veterinary Parasitology 71: 77–9.

Guo, S., Yuan, Z., Wu, G., Wang, W., Ma, D. and Du, H. (2002). Epidemiology of ovine theileriosis in Ganan region, Gansu Province, China. Parasitology Research 88: 36–37.

Jalali, S.M., Khaki, Z., Kazemi, B., Rahbari, S., Shayan, P., Bandehpour, M. and Yasini, S.P. (2014). Molecular detection and identification of *Theileria* species by PCR-RFLP method in sheep from Ahvaz, Southern Iran. Iranian Journal of Parasitology 9: 99-106.

Kaufmann, J. (1996). Text book of parasitic infections of domestic animals.

Birkhauser Verlag, Postfach 133, CH-4010 Basel, Schweiz, pp 169-170.

Lefevre, P.C., Blancou, J., Chermette, R. and Uilenberg, G. (2010). Infectious and Parasitic Diseases of Livestock. Vol 2., World Organisation for Animal Health (OIE), Alfort National Veterinary School, French Ministry of Culture, France, pp 1860-1861.

Li, Y., Zhang, X., Liu, Z., Chen, Z., Yang, J., He, H., Guan, G., Liu, A., Ren, Q., Niu, Q., Liu, J., Luo, J. and Yin, H. (2014). An epidemiological survey of *Theileria* infections in small ruminants in central China. Journal of Veterinary Parasitology 200: 198– 20.

Mamatha, G.S., Shruthi, R., Chandranaik, B.M., Placid, E. D'Souza., Thimmareddy, P.M., Shivashankar, B.P. and Puttalakshmamma, G.C. (2017). Molecular epidemiology and phylogenetic characterisation of *Theileria luwenshuni* in India: A first Report. Small Ruminant Research 154: 52-57.

Minjauw, B. and Mcleod, A. (2003). Tick-borne diseases and poverty. The impact of ticks and tick-borne diseases on the livelihood of small scale and marginal livestock owners in India and eastern and southern Africa. Research report, DFID Animal Health Programme, Centre for Tropical Veterinary Medicine, University of Edinburgh, UK, pp 124.

Prabhakar, K.S. and Hiregoudar, L.S. (1977). Prevalence of theileriosis among sheep in Karnataka. Indian Journal of Animal Sciences 47: 848-857.

Razmi, G. and Yaghfoori, S. (2013). Molecular surveillance of *Theileria ovis*, *Theileria lestoquardi* and *Theileria annulata* infection in sheep and ixodid ticks in Iran. Onderstepoort Journal of Veterinary Research 80: 1-5.

Razmi, G.R., Eshrati, H. and Rashtibaf, M. (2006). Prevalence of *Theileria* spp., infection in sheep in South Khorasan province, Iran. Veterinary Parasitology 140: 239-243.

Shiva Kumara, C., Reddy, B.S. and Patil, S.S. (2017). Small ruminant production in Karnataka state of India-An Overview. European Journal of Zoological Research 5: 28-35.

Sisodia, R. S. (1981). Present status of sheep Theileriosis in India–A review. Tropical Animal Health and Production 5: 97-102.

Soulsby, E.J.L. (2012). Helminths, Arthropods and Protozoa of domesticated animals. 7th ed., Affiliated East-West Press Private Limited, New Delhi, pp 736.

Uilenberg, G. (2001). Babesiosis. In: Service, M.W., (Ed.), Encyclopedia of arthropod-transmitted infections of man and domesticated animals. CABI, Wallingford, pp 53–60.

Yaghfoori, S., Razmi, G. and Heidarpour, M. (2013). Molecular detection of

Theileria spp., in sheep and vector ticks in Fasa and Kazeroun areas, Fars Province, Iran. Archives of Razi Institute, 68: 159-164.

Yin, H., Leonhard, S., Luo, J., Seitzer, U. and Ahmed, J.S. (2007). Ovine theilerioisis in China: a new look at an old story. Parasitology Research 101:191– 195.

Yin, H., Liu, Z., Guan, G., Liu, A., Ma, M., Ren, Q. and Luo, J. (2008). Detection and differentiation of *T. luwenshuni* and *T. uilenbergi* infection in small ruminants by PCR. Transboundary and Emerging Diseases 55: 233–237.

Zaeemi, M., Haddadzadeh, H., Khazraiinia, P., Kazemi, B. and Bandehpour, M. (2011). Identification of different Theileria species (*Theileria lestoquardi*, *Theileria ovis*, and *Theileria annulata*) in naturally infected sheep using nested PCR-RFLP. Parasitology Research 108: 837-843.

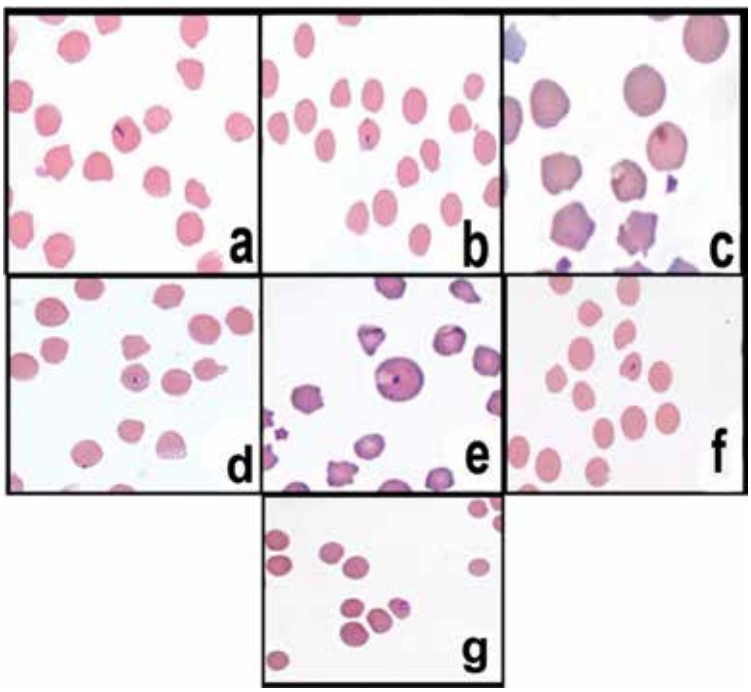

Plate 1: Different morphological forms of *Theileria* organisms in Giemsa stained blood smears

a: Rod form b: Nail form c: Dot form d: Round form

e: Parachute form f: Comma form g : Other forms