

THE PREVALENCE OF TROPICAL THEILERIASIS IN CATTLE OF VISAKHAPATNAM DISTRICT, ANDHRA PRADESH - INDIA.

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ABSTRACT

Theileria is a genus of parasitic protozoan that belongs to the phylum Apicomplexa and is closely related to Plasmodium. Two species, *T.annulata* and *T.parva* are important cattle parasites. *T.annulata* causes tropical theileriosis and *T.parva* causes East coast fever. It is transmitted by the zoonotic ticks like *Hyalomma detritum* and *Hyalomma anatolicum*. Tick-borne diseases (TBDs) hamper the growth of the livestock sector and impose serious constraints on the health and productivity of domesticated cattle in tropical and sub-tropical regions of the world (de Castro, 1997). The Tropical *Theileriosis*, bovine babesiosis and anaplasmosis are among the economically most important diseases of cattle. The *Theileriasis* was caused by an apicomplexan intra cellular parasite *T.aannulata* in cattle. This disease is characterized by leuko-proliferative and leuko - destructive phases with marked anemia (Tait and Hall, 1990). In India, the parasite mainly causes clinical disease in exotic and cross-bred cattle and young indigenous calves (Beniwal et al., 1997). So, It is very essential to have complete knowledge of this parasite prevalent in the area for developing an effective control programmes and mass vaccination strategy.

KEY WORDS: *Theileria annulata*, *Theileriosis*, apicomplexan, *Hyalomma detritum*

INTRODUCTION

Theileriasis is the tickborne disease caused by *Theileria* spp. A large number of *Theileria* spp are found in domestic and wild animals in tick-infested areas of the Old World. The most important species affecting cattle are *T.parva* and *T.annulata*, which cause widespread death in tropical and subtropical areas of the Old World. *T. lestoquardi*, *T. lowenshuni*, and *T. uilenbergi* are important causes of mortality in sheep. Both *Theileria* and *Babesia* are members of the suborder Piroplasmorina.

Although *Babesia* are primarily parasites of RBC's, *Theileria* use, successively, WBC's and RBC's for completion of their life cycle in mammalian hosts. The infective sporozoite stage of the parasite is transmitted in the saliva of infected ticks as they feed. Sporozoites invade leukocytes and, within a few days, develop to schizonts. In the most pathogenic species of *Theileria* (eg, *T.parva* and *T.annulata*), parasite multiplication occurs predominantly within the host WBCs, whereas less pathogenic species multiply mainly

in RBCs. Development of the schizont stage of pathogenic *Theileria* causes the host WBC to divide; at each cell division, the parasite also divides. Thus, the parasitized cell population expands and, through migration becomes disseminated throughout the lymphoid system. Later in the infection, some of the schizonts undergo merogony, releasing merozoites that infect RBCs, giving rise to piroplasms. Uptake of piroplasm-infected RBCs by vector ticks feeding on infected animals is the prelude to a complex cycle of development, culminating in transmission of infection by ticks feeding in their next instar (trans-stadial transmission). There is no transovarial transmission as occurs in *Babesia*. Occurrence of disease is limited to the geographic distribution of the appropriate tick vectors. In some endemic areas, indigenous cattle have a degree of innate resistance. Mortality in such stock is relatively low, but introduced cattle are particularly vulnerable. Unlike in *babesiosis*, in *theileriasis* there is no evidence of increased resistance in calves < 6month old.

MATERIALS AND METHODS

STUDY AREA:

Visakhapatnam is one of the north eastern coastal districts of Andhra Pradesh and it lies between 17°58'N and 82°50'E. It is bounded on the north, partly by the Orissa and partly by Vizianagaram district, On the south by East Godavari district, On the West by Orissa state and on the East by Bay of Bengal. April to June are the warmest months. The temperature gets down with the onset of South West monsoon and tumbles to a mean minimum of 18.8 degrees Celsius by December after which there is a reverse trend till the temperature reaches mean maximum of 37.4 degree Celsius by the end of May. The district receives annual rainfall of 1202 mm, which are contributed to by the South West and North East Monsoon. The rest is shared by summer showers and winter rains.

Study Area:

A total of 665 blood samples were collected from 665 animals collected randomly from the cattle of various mandals in Visakhapatnam district of Andhra Pradesh for a period of a year between October 2014 to September 2015, for the preparation of blood smears in three consecutive seasons and tested against *Theilariaannulata*.

Blood sample was collected in heparin capillary tubes drawn directly from the marginal ear vein after clipping and cleaning with a spirit swab. Positive samples were further processed for thin blood smear. Preparation of thin blood smears of all blood samples was prepared by adopting standard methods that is the slide of the blood smears was stained with the standard Giemsa's staining method. The slides are labeled in pencil and were kept in slide box to examine later on in parasitological laboratory at the Andhra University Zoology laboratory. The smears were air dried, fixed in absolute methanol and stained for 30 minutes in a 5% dilution of Giemsa solution in PBS having pH 7.2. The slides were examined with oil immersion x100. The samples were also examined by Leishmann's stained method. Identification of *Theilariasis* was examined

under the microscope at 10X for the presence of the parasite and then to 40x magnification for morphological description by using light microscope.

Symptoms:

The infected animals shows high body temperature from 104 F to 106F. The lymph glands enlargement, anemia, anorexia, tachycardia, tachypnea, nasal discharge, loss of weight are the another symptoms of the disease.

Vectors: The tick vectors of the *T.annulata* are all members of the genus *Hyalomma*. *H. savigyi* (syn. *H.marginatum*) (Asia Minor, India),

Treatment and control:

There are three types of controlling measures are there for the Tick born infections:

1. Chemo therapy
2. Tick control
3. Vaccination

1. Chemotherapy:

Buparvaquone, tetracycline and butalex and oxytetracycline have all shown to be effective. Formulated as a solution for intramuscular injection, the Buparvaquone, offers a safe and convenient antitheilerial products.

2. Tick Control:

Currently a number of methods including chemical tick control, chemotherapy as well as prophylaxis and vaccination are used worldwide to reduce economic losses resulting from Tick borne diseases, in bovines.

For tick control grooming is the most commonly used strategy, particularly at small holding farms. Grooming involves the manual removal of ticks and burning them on the fire. Another method to treat and control ticks is spraying the animals and its surroundings with cyper-methrine during high risk months of the year. Generally the crows act as the exo-paracites on the cattle, will eat the ticks and saves the animals from the TBDs.

3. Vaccination:

The anti Thelerial vaccine “RAKSHAVAC-T” is utilizing by the farmers of the district, to prevent the spread of this disease. But the ignorance of the illiterate poor farmers, is the main cause of the spreading of the disease.

Data Analysis:

The row data and result obtained from blood examination was recorded in the format and entered in to Microsoft excel spread sheet. Prevalence of affected animals out of total animals examined. Possible risk factors such as breed, locality considered during analysis. The *Theilariasis* was determined by Microscopic examination.

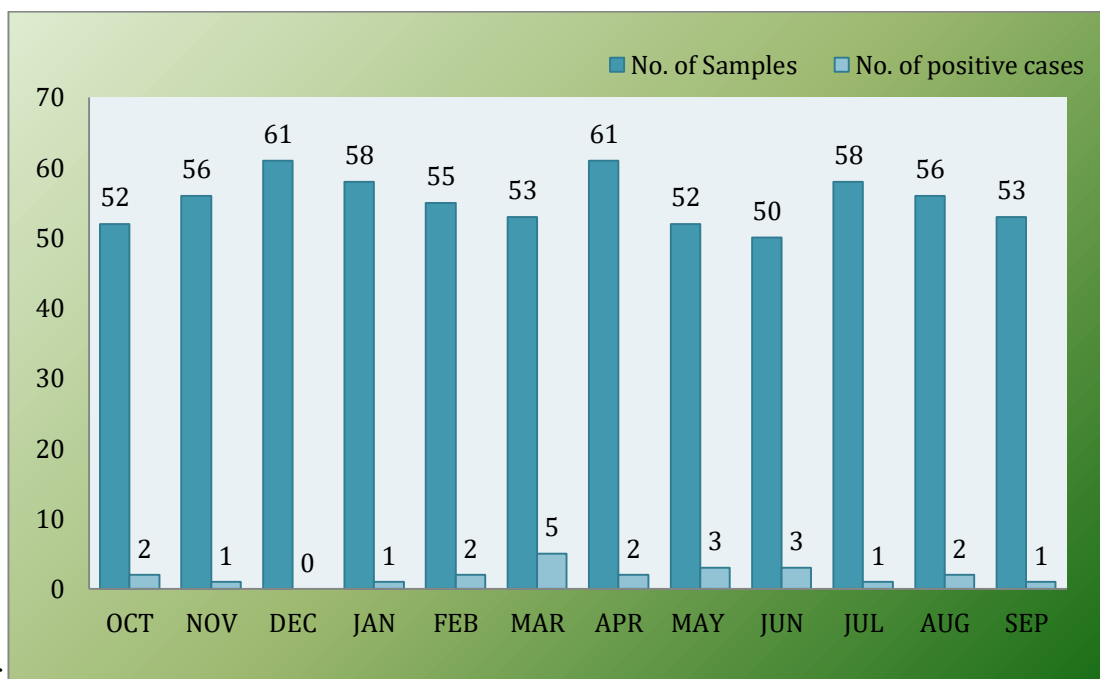
RESULTS AND DISSCUSSION**Indigenous and cross breed cows:**

A total of 665 blood samples were collected and examined and tested by using thin blood smear and an overall prevalence of 3.45 % (23/665) *Theileriaannulata* was recorded in the study area. The highest percentage of Theilaria positive cases were recorded in Natavarammandal(9.43%), followed by Golugonda(6.00%), and Kasimkota(5.76%) while the lowest prevalence was recorded in Sabbavarammandal(0%). Anakapalli(1.72%) and Atchyutapuram (1.72%) mandals are also have lowest prevalence.

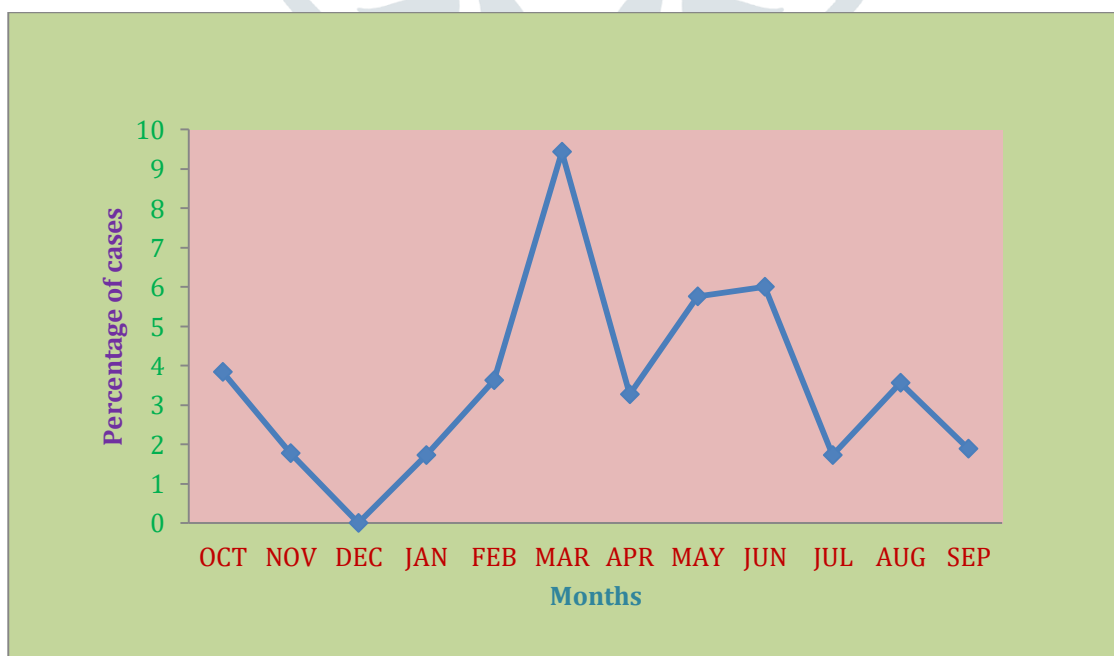
PREVALENCE OF THEILARIASIS IN VARIOUS MANDALS IN VISAKHAPATNAM DISTRICT(Indigenous and cross breed cows)

SL. NO	MONTH	MANDAL	NO. OF BLOOD SAMPLES COLLECTED	NO. OF POSITIVE CASES	PERCENTAGE OF POSITIVE CASES
1	October 2014	Narsipatnam	52	2	3.84
2	November 2014	Makavarapalem	56	1	1.78
3	December 2014	Sabbavaram	61	0	0
4	January 2015	Anakapalli	58	1	1.72
WINTER SEASON			227	4	1.76
5	February 2015	S.Rayavaram	55	2	3.63
6	March 2015	Natavaram	53	5	9.43
7	April 2015	Yellamanchili	61	2	3.27
8	May 2015	Kasimkota	52	3	5.76
SUMMER SEASON			221	12	5.42
9	June 2015	Golugonda	50	3	6.00
10	July 2015	Atchyutapuram	58	1	1.72

11	August 2015	Nakkapalli	56	2	3.57
12	September 2015	Chodavaram	53	1	1.88
RAINY SEASON			217	7	3.22
GRAND TOTAL			665	23	3.45



Bar diagram showing the No. of positive cases and No. of blood samples



Graph showing the month wise *Theileria* positive cases

Estimation of prevalence of *Theileriasis* according the usage of drug “BUPARVAQUONE”

In the Visakhapatnam district the cooperative dairy farm the VijayaVisakha Dairy is providing the Anti Theilarial drug i.e BUPARVAQUONE with subsidy rates for its milk providing farmers. In the study period the information of Anti Theilarialdoses which are distributed to various mandals from the VijayaVisakha dairy in the year 2015, was collected. The table shows the Buparvaquone drug doses in various mandals of Visakhapatnam district.

BUPARVAQUONE doses used by the Visakha diary in 2015

SL.NO	Name of the Mandal	No. of Buparvaquone doses	Prevalence of Theilariasis according to the drug usage.
1	NatavaramMandal	60 doses	1 st
2	GolugondaMandal	50 doses	2 nd
3	KasimkotaMandal	50 doses	3 rd
4	NarsipatnamMandal	44 doses	4 th
5	S.RayavaramMandal	41doses	5 th
6	MakavarapalemMandal	40 doses	6 th
7	NakkapalliMandal	39 doses	7 th
8	YellamanchiliMandal	38 doses	8 th
9	ChodavaramMandal	38 doses	9 th
10	AtchyutapuramMandal	37 doses	10 th
11	AnakapalliMandal	25 doses	11 th
12	SabbavaramMandal	24 doses	12 th
TOTAL DOSES IN 2015 FOR 12 MANDALS		486 Doses	

According to the use of Bupervaquone doses in the above 12 mandals the prevalence rate is high in Natavarammandal and low in Sabbavarammandal. The average drug usage per mandal is $486/12 = 40.5$ doses per year. The prevalence rate per month is $40.5/12 = 3.375\%$. According to the blood smear examination the prevalence rate of the *Theileria* disease is at about 3.45%, and according to the usage of Buparvaquone doses the prevalence rate of the disease is at about 3.37%. As these two results are very near to each other, it is

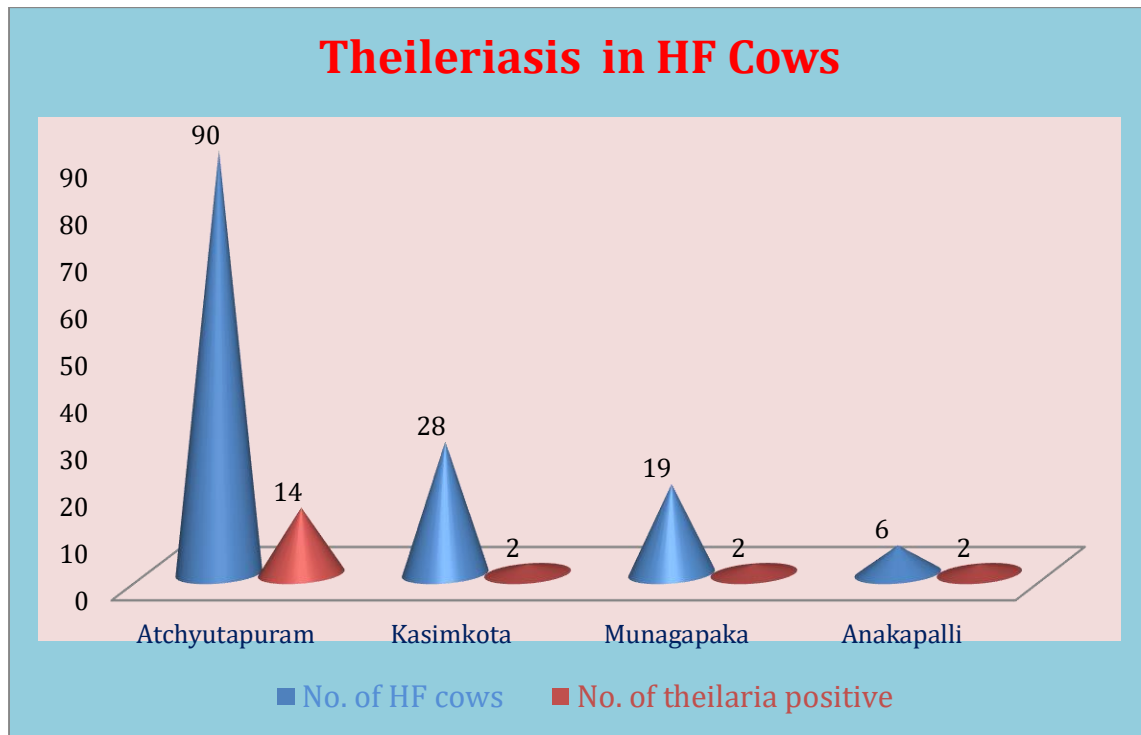
estimated that the prevalence rate of Theilariasis in Visakhapatnam district is at around 3.45% in the study period from October 2014 to September 2015 in the indiginous and cross breed cows.

PREVALENCE OF THEILERIASIS IN HF (Holstein Friesian) BREED COWS:

In this study period the co-operative dairy “Sri VijayaVisakha Milk Producers Company Limited” of Visakhapatnam had imported high milk yielding HF (Holstein Friesian) cows from the Kolar region of Karnataka state and 143 cows are given to the farmers of the 4 mandals of Visakhapatnam district, namely Atchyutapuram (90 Cows), Kasimkota (28 cows), Munagapaka (19 cows), Anakapalli (6 cows), at subsidy rates on 6-11-2015. A survey had been conducted in this area to know the susceptibility of the HF cows to the theileriasis. We have examined blood samples from all these cows and tested for the susceptibility of Thielariaannulata for the period of 6 months from 7-11-2015 to 7-5-2016. Unfortunately, the Theilaria positive cases are high in these animals than that of the indiginous and cross breed cattle. The detailed data of the 143 HF cows is mentioned in the Tables below.

ABSTRACT OF THE SURVEY ON HF (Holstein Friesian) COWS OF FOUR MANDALS IN VSAKHAPATNAM DISTRICT:

SL. NO	NAME OF THE DISTRICT	NAME OF THE MANDAL	NO. OF HF COWS GIVEN TO FARMERS	No. ofTheilaria positive cases	% ofTheilaria positive cases
1	Visakhapatnam	Atchyutapuram	90	14	15.55
2	Visakhapatnam	Kasimkota	28	2	7.14
3	Visakhapatnam	Munagapaka	19	2	10.52
4	Visakhapatnam	Anakapalli	06	2	33.33
	Total		143	20	13.98



Bar diagram showing the No. of HF cows and No. of infected HF cows in the four mandals

From the present study and the data generated it is inferred from the survey that the estimates of percentage of animals which are suffering from *Theileriasis* is 3.45% in the Visakhapatnam District. In summer season 12 blood smears are positive in 221 blood samples. So the prevalence rate in summer of this period is 5.42% (12/221). In rainy season 07 positive cases were obtained in 217 animal blood samples. So the prevalence rate of the disease in the rainy season of the study period is 3.22% (07/217). The same in the case of the winter season the positive cases are low in number i.e. 4 positive cases among the 227 animal blood samples. So the prevalence rate in the winter of the study period is 1.76% (04/227). Highest no. of positive cases were recorded in summer season which corresponds to months between March 2015 to May 2015. Moderate cases were recorded in the rainy season which corresponds the months between June 2015 to September 2015. And low number of cases was identified in the winter season which corresponds the months between October 2014 to January 2015. Microscopic examination of blood smears revealed 3.45% overall prevalence of theileriasis.

From the survey it has been observed that cows of high milk yield were imported from the Kolar region of Karnataka (commonly referred as HF (Holstein Friesian)) are reporting high susceptibility to this theileriasis disease. The highest prevalence of the disease in the HF cows was recorded in the Anakapallimandal. Two HF (Holstein Friesian) cows are identified as theileria positive among the 6 HF cows. The prevalence rate of theileriasis in HF (Holstein Friesian) cows of the Anakapallimandal is 33.33%. And lowest no., of positive cases in the HF cows were recorded in the kasimkotamandal. 2 HF (Holstein Friesian) cows were identified as theileria positive among the 28 HF (Holstein Friesian) cows. The prevalence

rate of theilaria disease in the kasimkotamandal is about 7.14%. At an average in the four mandals (Atchyutapuram, Kasimkota, Munagapaka, Anakapalli) of the study area on the HF (Holstein Friesian) cows which are imported from the kolar region of Karnataka state is 13.98%. 20 HF cows are suffering with theilariasis out of 143 cows in the 6 months study period. It will possibly raise the alarm, for effective and urgent measures against the disease control.

Neena George, VasundhraBhandari, D. Peddi Reddy and Paresh Sharma were reported on 17 September 2015, high prevalence rate of 32.40 % for *T. annulata* infections in cattle at Andhra Pradesh by utilizing 18S rRNA based PCR. In India, there are few reports with less numbers showing the presence of *T. annulata* infection using molecular based assay for e.g., a study from Gujarat where 74 samples were detected positive out of 113 (Kundave VR, Patel AK, Patel PV, Hasnani JJ, Joshi CG 2014), in Bangalore 41 cases were positive out of 132 (Ananda KJ, D'Souza PE, Puttalakshamma GC 2009) while a study from Punjab showed a prevalence rate of 14.65 % using microscopic examination (Singh NK, Jyoti S, Haque M, Rath SS 2012). The current finding will help to shed light on the occurrence of *T.annulata* infection in Visakhapatnam district of Andhra Pradesh and bring it to the forefront of devising control policy to detect and eliminate the infection resulting in reducing the economic losses caused due to the disease.

CONCLUSION

The *Theileriasis* is the important Tick born infection in India. There is a huge loss for the poor farmers of India because of this malign. According to the study it is observed that in summer season it is spreading widely than in winter. So, There is a necessity in the summer season to take some necessary activities to control the problem. There is a difference between the different breed for the susceptibility of the disease and in India the *Theilariasis* has been reported from various regions of the country. So if any government or cooperative body wants to import the high yielding varieties from the other parts of the country it is necessary to follow the quarantine principles before importing that cattle. It is very difficult for the imported animals to maintain in the changing geographical and environmental conditions. So from the present study it is better to import the semen of the high yielding breeds than to import the animals directly. The breed which are born with the semen of the high yielding breed can adopt to the geographical and environmental conditions easily. And it is also recommended that the new born calves should be vaccinate with the *Theileria* vaccine i.e “RAKSHAVAC-T” at the age of 3rd month onwards. To curb the problem there is a necessity to vaccinate all cattle in the endemic areas. Selection of cattle for good ability to acquire immune resistance to ticks is potentially effective. Endemic stability is a state where animals are affected at a low levels or not as susceptible to the disease, and this may be encouraged in endemic areas. There is a need for further investigation using modern serological and molecular techniques for the complete epidemiological picture of *Theileriasis* and other tick borne diseases in Visakhapatnam district.

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