PREVALENCE OF COMMON HAEMOPARASITIC DISEASES AMONG CATTLE OF THRISSUR DISTRICT

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ABSTRACT

The present study was undertaken to assess the prevalence of the common haemoparasitic infections among cattle in Thrissur district. The smears from apparently healthy animals as well as from clinical cases were examined. A high prevalence of haemoparasitic infections including anaplasmosis and theileriosis could be observed in the study.

Key words: Haemoparasitic Diseases, Prevalence, Thrissur

INTRODUCTION

In the absence of appropriate control strategies, haemoprotozoan diseases have serious economic implications and adverse impact on the dairy industry through animal mortality and reduced milk yield. This presents a major constraint to bovine production, hindering agricultural and socio-economic development of vast areas in India (Suryanarayanan, 1990). Most haemopro tozoan diseases are tick borne and the hot and humid climate of Kerala is favourable for the development and survival of ticks.

The predominant haemoprotozoan diseases affecting the livestock population of India are theileriosis, babesiosis and trypanosomosis along with the rickettsial disease, anaplasmosis. The important species of *Anaplasma* affecting cattle are *Anaplasma marginale* and *Anaplasma centrale*. Anaplasmosis is usually found either alone or in association with *Theileria* and /or *Babesia* infection in exotic and cross bred animals. Animals, which have recovered from acute phase of anaplasmosis, may become chronically infected carriers (Bram, 1983). The chronically infected carrier may relapse to acute anaplasmosis during conditions of immunosuppression. The diagnosis during acute stage is based on clinical signs and demonstration of the organism in erythrocytes by Geimsa staining.

There are six identified theilerial species affecting cattle, but the two most pathogenic and economically important ones are *Theileria annulata* and *Theileria parva* of which the former mainly causes infection in Indian bovines. Cases of Theileriosis are generally observed during summer or rainy season when the ticks have higher activity, although sporadic outbreaks have been recorded year round. Animals recovering from clinical infection may become carriers. The demonstration of schizonts (Koch's blue bodies) in the lymphocytes and monocytes of the lymph node biopsy smear or presence of theilerial piroplasms in peripheral blood smears are pathognomic for the disease.

Trypanosomiasis, caused by

Table. I. Haemoparasites in blood smears taken from apparently healthy animals

Staining technique	*Anaplasma*	*Theileria*	Trypanosome	*Babesia*
Giemsa	24	64	10	-
Acridine orange	27	68	Not done	-

Trypanosoma evansi, commonly termed as Surra, is an important haemoprotozoan disease of domesticated animals, pets and wild animals. Among the several species of trypanosomes, *Trypanosoma evansi* is the most commonly occurring species in India causing the disease. Surra results in anaemia, lowering down of milk yield and working capacity. Hence besides causing economic losses, it also causes high mortality in valuable animals. The demonstration of the parasites in the blood by microscopy is difficult because of the periodically cryptic nature of parasitaemia. This results in long periods during which host has no detectable parasites in blood. More than 50 to 80 per cent infections are cryptic (Reid *et al*., 2001).

Babesiosis caused by *Babesia bigemina* is a common ailment in cattle and is manifested mainly by pyrexia and haemoglobinuria. The demonstration of characteristic piroplasmic stages in the erythrocytes from peripheral blood smear is used for diagnosis.

Among the various diseases affecting the livestock industry in India, haemoparasites causes severe economic losses, as animals which recover from acute infection become carriers creating a potential source of infection to healthy susceptible population (Callow, 1984). Hence diagnosis of these infections in carrier animals has great epidemiological significance.

MATERIALS AND METHODS

Two hundred peripheral blood smears in duplicate were collected at random from apparently healthy cattle from different areas of Thrissur district. The blood smears were subjected to Giemsa and Acridine orange staining. In addition, peripheral blood smears from animals showing various symptoms like pyrexia, salivation, nasal discharge, off feed/reduced feed intake, enlarged superficial lymph nodes etc. and heart blood smears from dead animals were also examined.

Staining techniques

Of the duplicate smears from each apparently healthy animal, one was subjected to staining with Giemsa and other to Acridine orange (Ravindran *et al*., 2007a). Smears from suspected clinical cases were examined only with Giemsa stain.

Statistical significance of the two tests

Table. II. Haemoparasites in blood smears taken from suspected clinical cases

	Anaplasma	*Theileria*	Trypanosome	*Babesia*
Significant numbers (Clinically infected)	279	60	1	1
Stray numbers (Careers)	7	52	-	-
TOTAL	286	112	-	1

Statistical analysis was done using Kappa statistics to compare the agreement between the two staining techniques (Table III and IV)

Table. III. Comparison of Acridine orange staining in comparison with Giemsa staining for the detection of *Anaplasma marginale*

Acridine orange	Giemsa		
	Positive	Negative	Total
Positive	22	5	27
Negative	2	171	173
Total	24	177	200

κ=0.99

were found out by using Kappa statistics, which denoted the percentage agreement of Acridine orange with Geimsa and is denoted by "κ".

RESULTS

Apparently healthy animals

Microscopic examination of 200 peripheral blood smears from apparently healthy animals with Giemsa staining revealed Anaplasma in 24 smears (12%), Theileria like piroplasms in 64 smears (32%), and Trypanosomes in 10 smears (5 %).

Acridine orange stained blood smears from apparently healthy animals revealed Anaplasma in 27 (13.5%) smears and Theileria like piroplasms in 68 smears (34%).

Suspected Clinical Cases

Giemsa staining technique alone was used to screen the 592 blood smears taken from suspected clinical cases. Screening revealed *Anaplasma* in 279 smears (47%), Theilerial piroplasms in 60 smears (10%), Trypanosomes in 1 smear, Babesia in 1 smear and both Anaplasma and Theilerial piroplasms in 28 smears. Among the remaining 281 cases, *Anaplasma* and various morphological forms of Theilerial piroplasms were observed in few numbers indicative of career status (Table II).

DISCUSSION

The present study was conducted as a screening to find out the prevalence of Haemoparasitic infection among the cattle population of Thrissur district employing two different staining techniques- Acridine orange and Giemsa. Both have their own advantages and disadvantages. Acridine orange staining is an easy and reliable technique, which enables rapid detection of haemoparasites. Even though the technique requires costlier equipment like fluorescent microscope; it is more sensitive compared to Giemsa staining (Ravindran *et. al.,* 2010). The advantage of Giemsa staining technique lies in the fact that it is suitable for field level applications and is economical. But in cases of low parasitaemias and for detection of carrier status, more sensitive tests like Polymerase Chain Reaction based techniques should be employed.

In the present study, the Anaplasma organisms were detected at the margin of the erythrocytes indicating the probability of them being *Anaplasma marginale.* The majority of theilerial piroplasms observed were thin rods, which may be of *Theileria orientalis*, an endemic species in Tamil Nadu. *Theiaria orientalis,* which causes benign theileriosis, can attain the status of an acute infection in case of stress conditions (Shastri *et. al.,* 1988).

Cattle are usually subclinical carriers of trypanosomosis (Gill, 1991). Parasitological diagnosis of chronic or latent trypanosomosis is difficult due to the intermittent or low parasitaemia (Luckins, 1998). The smears that were found to be positive for trypanosomes in

Table. IV. Comparison of Acridine orange staining in comparison with Giemsa staining for the detection of Theilerial piroplasms

Acridine orange	Giemsa		
	Positive	Negative	Total
Positive	61	7	68
Negative	3	129	132
Total	64	136	200

Both the tests agreed statistically as indicated by a Kappa value greater than 0.81.
κ=0.99

the present study were prepared from buffy coat which may have increased the sensitivity of detection. In the study, *Babesia* organisms could not be detected in any of the smears from apparently healthy animals. Negative microscopic examination does not exclude the possibility of the infection as in very early and chronic stage of the disease and in subclinical infection the detection of *Babesia* in blood smears is difficult (Talkhan *et al*., 2010).

Among the smears from apparently healthy animals, a total of 24 smears were found positive for *Anaplasma* with Giemsa stain and 27 with Acridine orange staining technique. Five samples, which were negative with Giemsa stain, were positive with Acridine orange indicating the greater sensitivity of Acridine orange stain. Two smears were negative with Acridine orange, but positive with Giemsa stain, which could be due to staining artifacts, or presence of Heinz/ Howell-Jolly bodies that may have given false positives with Giemsa stain.

Among the blood smears submitted to Clinical laboratory, District veterinary centre, Thrissur, from animals exhibiting varying clinical symptoms, 279 smears were positive for anaplasmosis, 60 for theileriosis, 1 for trypanosomosis and 1 for babesiosis. The reason for low number of babesiosis may be due to the fact that, in majority of cases; the clinical signs of babesiosis in cattle include coffee coloured urine, which is almost confirmative when correlated with clinical signs and hence these cases may not have been referred for laboratory examination. Number of clinical cases of trypanosomosis detected in the study is low, which may indicate either the low prevalence of the disease in Kerala when compared to anaplasmosis and theileriosis or may be because most cases are subclinical hence not detected in peripheral smears.

A large number of clinical cases of anaplasmosis and theileriosis occurred in Thrissur district during a massive Foot and Mouth Disease outbreak during August 2013 to January 2014. Large numbers of cattle deaths were reported during this period. This may be due to the activation of the carrier status of haemoparasites in these animals to an acute stage following the stress due to Foot and mouth disease.

The detection of carrier status of anaplasmosis and haemoprotozoan disease becomes more effective by using highly sensitive and specific molecular level techniques. The detection of large number of clinical cases of anaplasmosis and theileriosis in the study clearly indicates a higher prevalence of these haemoparasitic infections in the cattle population of Thrissur district. Hence, the future of the improved management of haemoparasitic

diseases lies in the understanding of vector, pathogen and host relationship through a pragmatic approach of designing accurate epidemiological tools and diagnostics and introducing proper control measures against such disease. The present study is a pilot step towards the execution of the strategy.

SUMMARY

A high prevalence of haemoparasitic infections were observed among the cattle of Thrissur district in the present study and a preliminary screening of the newly introduced animals from other states with staining techniques and subsequent confirmation of the cases with molecular techniques in cases with low parasitaemias/carrier stage will ultimately help in timely diagnosis and treatment of positive cases. This will in turn help to decrease the incidence of these infections in the district to a great extent. This could considerably reduce the burden on farmers for treatment cost and will aid in the animals achieving their production potential to a great extent.

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