

*Article*

## **Study of prevalence of theileriosis in cattle and evaluation of therapeutic efficacy of different commercially available drugs**

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**Abstract:** The present study was carried out to detect the prevalence of theileriosis in cattle of Sadar, Singair and Ghior upazila in Manikganj district of Bangladesh and evaluate the therapeutic efficacy of Imidocarb dipropionate (Babenil®), Oxytetracycline (Renamycine®) and Gentamicin (Acigent®). The study was conducted for six months and during this study period a total of 150 cattle were examined, 10 were found to be infected with *Theileria* spp. On Geimsa stained blood smear examination, it was observed that the overall prevalence of theileriosis in cattle was recorded as 6.67%. Animals were screened on the basis of clinical signs like fever, anorexia, with or without superficial lymph node enlargement and blood smear examination for presence of *Theileria* spp. The prevalence of theileriosis in female was non significantly higher (7.50 %) than male (5.71%). In case of age group, the significantly highest prevalence (13.3%) was in adult cattle above 3 years age, followed by 4.00 % in the age group of 2 to less than 3 years old and 0.00 % in 6 months to 2 years which was not significant ( $p > 0.05$ ). The prevalence of theileriosis in respect of breed was non significantly ( $p > 0.05$ ) maximum in cross breed cattle (7.05 %) than in indigenous cattle (6.15%). After identification of causal agent, therapeutic effect of imidocarb dipropionate, oxytetracycline and gentamicin were tested and where highest recovery was found by administration of imidocarb dipropionate (70 %) can be successfully used in the treatment of theileriosis.

**Keywords:** theileriosis; imidocarb dipropionate; oxytetracycline; gentamycin; prevalence

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### **1. Introduction**

Theileriosis is an economically important vector-borne disease of tropical and subtropical parts of the world including Bangladesh. They are of great economic impact on livestock affecting 80% of the world cattle population and causes economic loss due to morbidity and mortality. Theileriosis is one of the tick-borne blood protozoal diseases found in cattle in different areas of Bangladesh (Samad *et al.*, 1989). Theileriosis caused by

Theileria spp. of the family Theileridae are round, ovoid, rod like or irregular forms found in lymphocytes, histiocytes and red blood cells of the hosts. They are transmitted by ixodid ticks and have complex life cycles in both vertebrate and invertebrate hosts. The protozoan parasite *Theileria annulata* is the causative agent of the tick-borne disease tropical theileriosis, responsible for morbidity and mortality of cattle in many developing countries (Campbel and Spooner, 1999). Direct effects of ticks on their host include tick toxicosis metabolic disturbances anemia and tick worry which can result in production losses or deaths (O’Kelly and Seifert, 1969). Several methodologies are currently available for the control of bovine theileriosis. The most practical and widely used method is the chemical control of ticks with acaricides and vaccinations. Therefore, the present study has been undertaken based on the following objectives to determine the prevalence of theileriosis and to evaluate the therapeutic efficacy of different commercially available drugs against theileriosis in cattle in Sadar, Singair and Ghior upazila under the district of Manikganj.

## 2. Materials and Methods

A multistage random sampling method was applied according to Thrusfield (2005). Among seven upazila (Sadar, Saturaia, Daulatpur, Ghior, Shibalaya, Horirampur and Singair) of Manikgonj district three upazila name Sadar upazila, Singair upazila and Ghior upazila were selected randomly. Manikganj located 50 km north-east from Dhaka division headquarter. The blood samples were collected from the study area and brought at parasitology Laboratory of Bangladesh Livestock Research Institute (BLRI) Savar, Dhaka for laboratory diagnosis. The study was carried out for a period of 6 months from July –December, 2016. Samples were collected and tested in every two months interval. Cattle were selected randomly and both the indigenous and cross-bred animals were selected on the basis of their availability. The cattle were grouped according to age into three groups i.e., calves (6 months to 2 year), young (2-3 years) and adults (>3 years). The history and physical examination of each of the patient was carried out for the cattle for diagnosis of theileriosis. Physical examination was done by visual inspection, pulse and respiration rate and rectal temperature. Examination of the different organs and systems of the body was carried out by using the clinical methods of palpation, percussion and auscultation. The study was carried out on 100 indigenous and cross-bred cattle and blood samples were collected randomly from cattle of different areas of selected regions. Blood samples were collected by puncturing Jugular vein of each cattle using sterile disposable needle. 3-5 ml blood samples were collected from the jugular vein of the clinically suspected animals in EDTA containing vacutainers and transported to parasitology lab. of BLRI, Savar, Dhaka in ice bags for microscopic examination following the method of Adam, Paul and Zaman. Briefly, a thin blood smear was prepared from each blood sample, air dried and fixed in methanol for 2-3 minutes. Staining was done in 10% Giemsa’s stain and rinsing was performed in two changes of distilled water buffered to pH 7.2, then examined under microscope (100 ×) with immersion oil for the identification of blood parasites as described by Soulsby (1982). The microscopic examination of the Giemsa-stained blood smears of infected cattle revealed the presence of free and intracellular forms morphologically compatible with theilerial piroplasms and schizonts. Infected erythrocytes showed morphological disorders represented by round-shaped appearance and irregular thorn-like protrusions. The piroplasms within their cytoplasm were predominantly round or oval in shape (Figure1) but rod and comma forms have also been identified. They were observed as individual (one piroplasm per erythrocyte), double, triple and tetra-forms. The schizonts were observed either as free forms or as intracellular forms in some of the monocytes and lymphocytes within the blood smears. They appeared as circular or irregularly shaped structures with blue cytoplasm and varied numbers of red chromatin granules. For therapeutic procedure selected animal were treated by different types of drugs such as Imidocarb dipropionate, Gentamicin and Oxytetracycline and again blood sample were collected after treatment and observed under 100x objective microscope. The experimental data were recorded and analyzed statistically by using SPSS program (version 20).

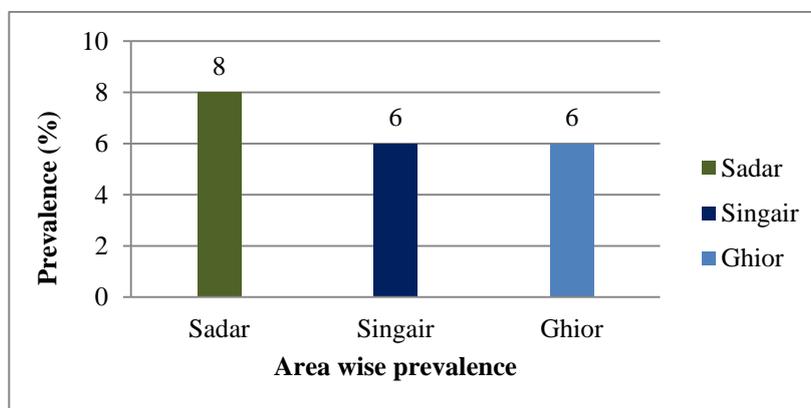
## 3. Results

### 3.1. Overall prevalence of tick infestations in cattle at the selected areas

The prevalence of theileriosis in cattle at Sadar, Singair and Ghior upazila of Manikganj district was shown in Table 1. The overall prevalence of theileriosis in Manikganj district was 6.67% and among three upazila the highest prevalence of theileriosis was found in Sadar (8.00%) than Ghior and Singair upazila which was not statistically significant (Figure 1).

**Table 1. Prevalence of theileriosis in cattle in different areas of Manikganj district.**

Upazila	No. of cattle tested	Positive case	Prevalence (%)	$\chi^2$ value	Level of significance
Sadar	50	04	8	0.214	0.898(NS)
Singair	50	03	6		
Ghior	50	03	6		
Overall	150	10	6.67		



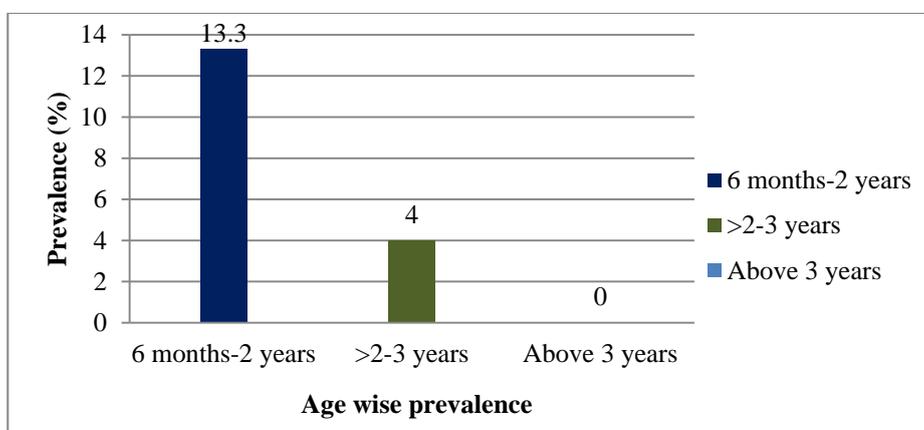
**Figure 1. Area wise prevalence of Theileriosis.**

**3.2. Age related prevalence of theileriosis in cattle**

Age-wise prevalence of theileriosis in cattle was shown in Table 2. The highest prevalence rate (13.3%) was significantly found in above 3 years group followed by (4.00%) in 2-3 years and lowest (0.00%) in 6 months – 2 years of age group (Figure 2).

**Table 2. Age-wise prevalence of theileriosis in cattle.**

Age group	No. of cattle tested	Positive case	Prevalence (%)	$\chi^2$ value	Level of Significance
6 months-2 years	40	0	0.00	7.14	0.021*
>2-3 years	50	2	4.00		
Above 3 years	60	8	13.3		
Total	150	10	6.66		



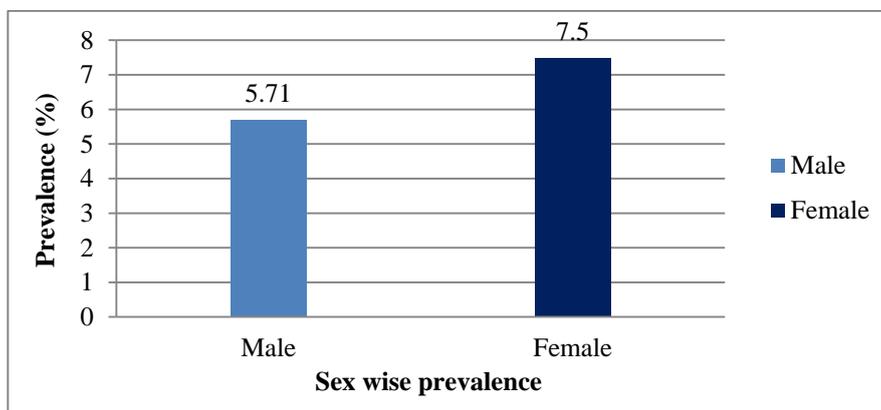
**Figure 2. Age wise prevalence of theileriosis.**

**3.3. Prevalence of theileriosis in relation to the sex of the cattle**

Sex-wise prevalence of theileriosis in cattle was shown in Table 3. The highest rate of prevalence was reported in female (7.5%) than male (5.71%) and was not statistically significant (Figure 3).

**Table 3. Sex-wise prevalence of theileriosis in cattle.**

Sex group	No. of cattle tested	No. of positive case	Prevalence (%)	$\chi^2$ value	Level of significance
Male	70	4	5.71	0.191	0.662(NS)
Female	80	6	7.5		
Total	150	10	6.66		



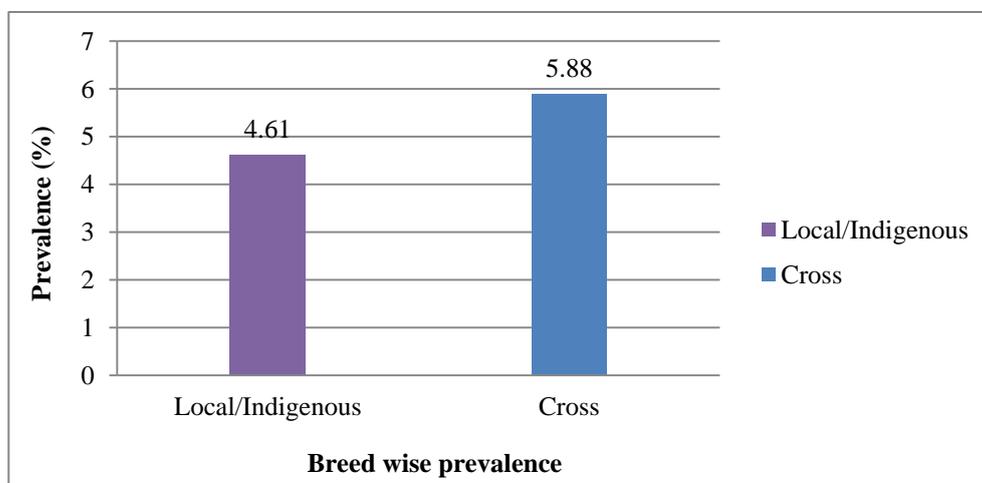
**Figure 3. Sex wise prevalence of theileriosis.**

**3.4. Breed susceptibility of cattle to theileriosis**

Breed-wise prevalence of theileriosis in cattle was shown in Table 4. The highest prevalence was recorded in cross breed (7.05%) followed in local (6.15%) and the variation was not statistically significant (Figure 04).

**Table 4. Breed-wise prevalence of theileriosis in cattle.**

Breed	No. of cattle tested	No. of positive case	Prevalence (%)	$\chi^2$ value	Level of Significance
Local/Indigenous	65	4	6.15	0.117	0.732(NS)
Cross	85	6	7.05		
Total	150	10	5.33		



**Figure 4. Breed wise prevalence of theileriosis.**

**3.5. Therapeutic effect of theileriosis in cattle**

The present study was conducted to check out the efficacy of various antiprotozoal drugs on bovine theileriosis. 10 cattle were found positive for theileriosis among 150 number of cases by microscopic examination. We selected 8 Cattle naturally infected with *Theileria* spp. to study therapeutic efficacy and divided into three

groups. Group A (03), B (03), C (02) infected with *Theileria* spp. In case of group-A, treated with imidocarb dipropionate, complete recovery was recorded in an animal while moderate recovery in one animal in Table 5. Group-B was treated with oxytetracycline (3.5 mg/kg body weight) administered as a single dose where one animal showed moderate recovery and other one animal did not respond in Table 6. In case of group-C, one animal remain non-responsive. Animal of Group-C was treated with a single dose of gentamicin (10 mg/kg body weight) in Table 7.

### 3.5.1. Therapeutic efficacy of imidocarb dipropionate

During the study period of 6 months 50 samples were collected from 50 suspected animals. Among these, 3 animals were positive for *Theileria* spp. Among 3 animals, I selected randomly 3 animals for testing therapeutic efficacy. In this case selected animals treated with imidocarb dipropionate (Babenil® @ 3.5 mg/kg bwt). In day 7 treatment results showed that four animals negative for *Theileria* spp. by 100x objective microscopic examination.

**Table 5. Efficacy of imidocarb dipropionate against theileriosis in cattle.**

Group	Infection	Drug used	Trade name	Animal Number	Intensity (Before treatment)		Intensity (After treatment)	
A	Theileriosis (3 animals)	Imidocarb dipropionate (3.5mg/kg bwt)	Babenil®	M13	1	++	1	-
				M23	2	++	2	+
				M47	3	+	3	-

**Categorization of infection:** Thick blood films were categorized by plus system suggested in Basic Laboratory Methods in Medical Parasitology, by WHO (1991).

+ (Light) 1-10 parasites per 100 thick blood film fields.

++ (Moderate) 11- 100 parasites per 100 thick blood film fields

### 3.5.2. Therapeutic efficacy of oxytetracycline

During the study period of 6 months 50 samples were collected from 50 suspected animals. Among these, 3 animals were positive for *Theileria* spp. Among 3 animals, I selected randomly 3 animals for testing therapeutic efficacy. In this case selected animals treated with oxytetracycline (Renamycin® LA 10 mg/kg bwt). In day 7 treatment results showed that only 1 animal negative *Theileria* spp. by 100x objective microscopic examination.

**Table 6. Efficacy of oxytetracycline against theileriosis in cattle.**

Group	Infection	Drug used	Trade name	Animal Number	Intensity (Before treatment)		Intensity (After treatment)	
B	Theileriosis (3 animals)	Oxytetracycline (10 mg/kg bwt)	Renamycin® LA	S57	1	+	1	+
				S71	2	++	2	+
				S92	3	+	3	-

**Categorization of infection:** Thick blood films were categorized by plus system suggested in Basic Laboratory Methods in Medical Parasitology, by WHO (1991).

+ (Light) 1-10 parasites per 100 thick blood film fields.

++ (Moderate) 11-100 parasites per 100 thick blood film fields

### 3.5.3. Therapeutic efficacy of gentamicin against theileriosis in cattle

During the study period of 6 months 50 samples were collected from 50 suspected animals. Among these, 2 animals were positive for *Theileria* spp. Among 2 animals, I selected randomly 2 animals for testing therapeutic efficacy. In this case selected animals treated with gentamicin (Acigent® 10 mg/kg bwt) In day 7 treatment results showed that only 3 animals negative for *Theileria* spp. by 100x objective microscopic examination.

**Table 7. Efficacy of gentamicin against theileriosis in cattle.**

Group	Infection	Drug used	Trade name	Animal Number	Intensity (Before treatment)		Intensity (After treatment)	
C	Theileriosis (1 animal)	Gentamicin (10 mg/kg bwt)	Acigent®	G117	1	++	1	+
				G145	2	+	2	+

**Categorization of infection:** Thick blood films were categorized by plus system suggested in Basic Laboratory Methods in Medical Parasitology, by WHO (1991).

+ (Light) 1-10 parasites per 100 thick blood film fields.

++ (Moderate) 11- 100 parasites per 100 thick blood film fields

## 4. Discussion

### 4.1. Overall prevalence of theileriosis in cattle

For the incidence of theileriosis, out of 150 animals examined, 10 cattle were found to be infected by theileriosis representing 6.67 %. Similar results were obtained by (Adel, 2007) who reported that, 11.31% of farm animals were infected with *T. annulata* in Gharbia governorate, Also (Abu El-Magd, 1980) reported that, 11.1% of animals were infected with *T. annulata* in Quena governorate and (Salem *et al.*, 1993) reported that, 10% of imported cattle and 8.75% of native cattle in Quena, were infected with *T. annulata* respectively. On other hand these results were disagreed with results of (Gamal EI-Dien, 1993) who recorded the incidence of *T. annulata* was 65.4%.

### 4.2. Age related prevalence of theileriosis in cattle

Analysis of data revealed that, adult animals were more infected by theileriosis as compared with calves. The present findings were in agreement with the findings of (Ruprah, 1985) and (Roy *et al.*, 2004) who reported highest prevalence in animals aged more than 3 years followed by the lowest prevalence in less than one year age group. The lowest prevalence in one year age might due to innate and acquired immunity.

### 4.3. Prevalence of theileriosis in relation to the sex of the cattle

In the study prevalence of theileriosis was higher in female (7.50 %) than male (5.71 %) cattle. The present findings were in agreement with the reports of (Song, 2003), (Atif *et al.*, 2012), (Durrani, 2008) and (Rajput *et al.*, 2005) who also recorded higher prevalence of *T. annulata* infection in female animals. The immune-suppression in advanced pregnancy and lactation in high producing animals are the possible reasons for the higher prevalence of theileriosis in female cattle (Kocan *et al.*, 2010). Moreover, the higher prevalence of theileriosis in female animals may be due to the fact that contaminated needles are commonly used for injecting drugs for milk let down. Calves (11/19) (57.89%) were more infected by Theileriosis as compared with adult animals (8/19) (42.11%) obtained by (Hazem *et al.*, 2014). Further analysis showed that the adult of buffaloes (5/19) (26.32%) were more prone to Theileriosis than their calves (1/19) (5.26%). These results were disagreed with (Abdel- Kader, 1991) who reported that the susceptibility of clinical theileriosis was low among calves of age less than one year old and increased in age of 1-3 years old.

### 4.4. Breed susceptibility of cattle to theileriosis

(Khan *et al.*, 2004) reported higher prevalence of tick-borne disease in cross bred cattle (19.40%) than indigenous cattle i.e., Red Sindhi (17%) and Dhanni (14%) breeds. The European breeds are more susceptible to TBDs (Tick borne disease) due to higher infestation of ticks (Bock *et al.*, 1997; Glass *et al.*, 2003). Variation in geoclimatic condition, breed, exposure of vectors and age of the animals might contribute to variable prevalence of hemoprotozoan diseases in the study areas (Muhanguzi *et al.*, 2010). Constant exposure of infections and development of immunity against such infections might responsible for lower prevalence in indigenous cattle (Siddiki *et al.*, 2010). On the contrary, more attention in the management of HF crossbred cattle gives less chance of pre-exposure of vectors and develop no or less immunity, resulting frequent occurrence of such diseases (Chowdhury *et al.*, 2006; Ananda *et al.*, 2009; Siddiki *et al.*, 2010).

#### 4.5. Therapeutic response of imidocarb dipropionate, oxytetracycline and gentamicin against bovine theileriosis

Our results of treatment with imidocarb dipropionate, oxytetracycline and gentamicin are in accordance with the finding of (Muhammad *et al.*, 1999), who showed 93% curative rate of imidocarb dipropionate in tropical theileriosis. Likewise (Zahid *et al.*, 2005) showed 100% curative rate and 81.73 % recovery rate showed by (Qayyum *et al.*, 2010). In the present study mortality of the cattle could be due to advanced stage of theileriosis because the said drugs usually do not show good result in severe cases of the theileriosis.

#### 5. Conclusions

Therefore, the aim of this study was to identify theileriosis, to know its prevalence and ultimately analysis the therapeutic effect of different drugs on theileriosis in cattle. Therefore, the experiment was conducted at sadar, Singair and Ghior Upazila of Manikgonj district, Bangladesh. Overall prevalence was 6.67 %, where it was highest in adult cattle over 3 years (13.3 %) of female cattle (7.5 %) but prevalence was highest in cattle cross breed cattle (7.05 %). Among 150 cattle, 10 were found positive. Imidocarb dipropionate, oxytetracycline and gentamicin were administered to these 8 cattle to observe the therapeutic effects. Highest recovery rate (70 %) was obtained through administration of imidocarb dipropionate. So imidocarb dipropionate can be successfully used in the treatment of theileriosis. The information generated from this study could be useful as basic information for further advance epidemiological study and formulation of control measures of the tick-borne diseases specially theileriosis. Further investigation using modern serological and molecular techniques with large number of samples for the identification of carriers, tick vectors and particularly hematophagous flies are needed.

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#### Conflict of interest

None to declare.

#### Authors' contribution

Md. Anowarul Haque: conceptualization, methodology, data collection, analysis and manuscript writing; Md. Mehedi Hasan, Md. Ershad Hossain: supervision, reviewing and editing; Md. Rashedul Islam, Tanjina Afrin: data collection, reviewing and editing. All authors have read and approved the final manuscript.

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