

*Article*

**Emerging status of anaplasmosis in cattle in Sirajganj district with therapeutic evaluation of traditional treatments**

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**Abstract:** The present study was carried out to detect the prevalence of anaplasmosis in cattle at Rayganj and Ullahpara upazila in Sirajganj district of Bangladesh and to evaluate the therapeutic efficacy of traditional drugs like Oxetetracycline (Renamycine®), Imidocarb dipropionate (Babenil®) and Diminazine aceturate (Berenil®) against anaplasmosis in cattle. The study was conducted from July 2016 to December 2016. During six months of study period a total of 150 blood samples were collected from clinically ill and suspected cattle, among which 28 samples were positive for anaplasmosis by Geimsa stained blood smear method. It was observed that the overall prevalence of anaplasmosis in cattle was 18.67%, where 16.25% was Geimsa stained blood smear at Rayganj and 21.43% at Ullahpara upazila respectively and the variation was not statistically significant ( $p > 0.05$ ). In respect of age the prevalence of anaplasmosis was significantly ( $p < 0.05$ ) higher (31.7%) in 2-3 years of age group cattle than above 3 years (16.67%) and 6 months to 2 years age (10.20%) group. On the basis of sex, it was observed that the variation in prevalence in male (15.94%) and female (20.99%) was not statistically significant ( $p > 0.05$ ). Breed-wise prevalence was higher in crossbred cattle (19.5%) than local cattle (17.8%), was not statistically significant ( $p > 0.05$ ). It was observed that among three drugs used in this study, the best effectiveness of drugs was seen by Oxytetracycline (Renamycine LA®) @ 10 mg/kg body weight followed by Imidocarb dipropionate (Babenil®) @ 3.5 mg/kg body weight moderately and less by Diminazine aceturate (Berenil®) @ 3.5 mg/kg body weight. From the study it was evident that cattle were infected with the organisms and caused a heavy economic loss which recommended taking necessary preventive measurements.

**Keywords:** anaplasmosis; oxytetracycline; imidocarb dipropionate; diminazine aceturate; cattle

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## 1. Introduction

Animal diseases are perhaps the most important limitation to animal production in developing countries. Major epizootic diseases of livestock that threaten production have either been eradicated or are under control in the developed world. Among these, the parasitic diseases have gained special significance due to adverse effects on production (Drummond *et al.*, 1978). Anaplasmosis occupy major place because of its fatality and adverse effects on animal productivity. In Bangladesh anaplasmosis in cattle is an economically important and endemic in the tropics, subtropics and sporadic in temperate region. Bangladesh is usually hot and humid climatic condition and very conducive to a wide variety of parasites as well as ticks which have been recognized as the notorious threat due to severe irritation, allergy and toxicosis (Niyonzema and Kiltz, 1986). Tick-borne diseases cause substantial losses to the livestock industry throughout the world (Ananda *et al.*, 2009; Kakarsulemankhel, 2011) as these have got a serious economic impact due to obvious reason of death, decreased productivity,

lowered working efficiency (Uilenberg, 1995), increased cost for control measures (Makala *et al.*, 2003) and limited introduction of genetically improved cattle in an area (Radostits *et al.*, 2000). There are many *Anaplasma* species but, *Anaplasma marginale* is the most important one (Kumar *et al.*, 2010). Prevalence of blood rickettsia like *Anaplasma marginale*, *Anaplasma centrale* have been reported in animals of Bangladesh (Samad and Gautam, 1984). Anaplasmosis are the more prevalent in different areas of Bangladesh where Chowdhury *et al.*, 2006 recorded 70% anaplasmosis in Sirajganj district. Talukdar and Karim (2001) also documented higher prevalence (33%) of anaplasmosis in Baghabari Milk Shed Area, Sirajganj. Kamani (2010) and Kocan *et al.*, 2003 was observed that the prevalence of anaplasmosis was higher in adult than young cattle. Breed and seasonal variation on anaplasma infection was reported by Ananda *et al.*, 2009 and Radostits *et al.*, 2000. The climatic condition and geographical location of the areas might favor the growth and multiplication of ticks which act as natural vectors of anaplasmosis and that causes one of the major veterinary problems affecting livestock industries. There are some antirickettsial and antiprotozoal drugs available in the local market and are being used by the field veterinarians. Tetracyclines (Chlortetracycline, Tetracycline and Oxytetracycline) are used for the treatment of anaplasmosis. Other compounds such as Imidocarb eliminate parasites from carrier animals (Urquhart *et al.*, 1988). Oxytetracycline (Renamycine®), Imidocarb dipropionate (Babnil®) and Diminazine aceturate (Berenil®) have been marketed locally for animal use in Bangladesh. Therefore, present study was designed to know the overall prevalence of anaplasmosis and to evaluate the therapeutic efficacy of different commercially available drugs against anaplasmosis in cattle at Rayganj and Ullahpara upazila of Sirajganj district.

## 2. Materials and Methods

On the basis of animal population, Rayganj and Ullahpara upazila under the district of Sirajganj was selected for study and study was conducted from July 2016 to December 2016. During six months of study a total of 150 blood samples were collected from clinically ill and suspected cattle and brought at parasitology laboratory of Bangladesh Livestock Reserch Institute (BLRI), Savar, Dhaka for laboratory diagnosis in ice bags for microscopic examination following the method of Adam, Paul and Zaman (1971). Then 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 (100x) with immersion oil for the identification of blood parasites as described by Soulsby (1982). To fine out the therapeutic efficacy of different types of drugs on anaplasmosis apply oxytetracycline 10mg/kg body weight, imidocarb dipropionate 3.5mg/kg body weight and Diminazine aceturate 3.5mg/kg body weight respectively. After that again the sample was collected and observed under 100 x objective microscopic. All data were analyzed by SPSS version-20 software. The Multivariate analysis was done following the strategy described by Hosmer and Lemeshow (1989). Later, a backward elimination procedure was applied and statistical significance of individual variable was tested using likelihood-ratio test with a p value  $\leq 0.05$ . The prevalence of anaplasmosis infections in cattle was estimated through using formula denoted by Thrusfield, 1995.

## 3. Results

### 3.1. Overall prevalence of anaplasmosis in cattle

The prevalence of anaplasmosis in cattle at Rayganj and Ullahpara upazila of Sirajganj district was shown in Table 1. The overall prevalence of anaplasmosis in Sirajganj district was 18.67% and among two upazila the highest prevalence of anaplasmosis were found in Ullahpara (21.43%) than raygonj upazila which was not statistically significant.

### 3.2. Microscopic examination of blood smears

Microscopic examination of blood smears containing high amount of *Anaplasma spp.* in Figure 1, less amount of *Anaplasma spp.* in Figure 2 and moderate amount of *Anaplasma spp.* in Figure 3 (100 x objective).

### 3.3. Age-wise prevalence of anaplasmosis in cattle

Age-wise prevalence of anaplasmosis in cattle was shown in Table 2. The highest prevalence rate (31.7%) was significantly found in the 2-3 years age group followed by (16.67%) in above 3 years and lowest (10.20%) in 6 months – 2 years of age group.

### 3.4. Sex-wise prevalence of anaplasmosis in cattle

Sex-wise prevalence of anaplasmosis in cattle was shown in Table 3. The highest rate of prevalence was reported in female (20.99%) than male (15.94%) and was not statistically significant.

### 3.5. Breed-wise prevalence of anaplasmosis in cattle

Breed-wise prevalence of anaplasmosis in cattle was shown in Table 4. The highest prevalence was recorded in cross breed (19.5%) followed in local (17.8%) and the variation was not statistically significant.

### 3.6. Therapeutic efficacy of oxyteracycline

During the study period of 6 months the sample collection procedure was divided into 3 periods. In first period on June to July 2016 a total of 50 samples from 50 suspected animals were collected and tested. Among them, 8 animals were positive for *Anaplasma* spp. of which 5 animals were selected for therapeutic efficacy test. The selected animals were treated with Oxyteracycline (Renamycine® LA 10 mg/kg bwt). After 7 days of treatment results showed that four animals negative for *Anaplasma* spp. by 100 × objective microscopic examination (Table 5).

### 3.7. Therapeutic efficacy of imidocarb dipropionate

In second period on September to October in 2016 a total of 50 samples from 50 suspected animals were collected and tested. Among them, 10 animals were positive for *Anaplasma* spp. of which 5 animals were selected for therapeutic efficacy test. The selected animals treated with Imidocarb dipropionate (Babenil® @ 3.5 mg/kg bwt). After 7 days of treatment results showed that two animals negative for *Anaplasma* spp. by 100× objective microscopic examination (Table 6).

### 3.8. Therapeutic efficacy of diminazine aceturate

In third period on November to December in 2016 a total of 50 samples from 50 suspected animals were collected and tested. Among them, 10 animals were positive for *Anaplasma* spp. of which 5 animals were selected for therapeutic efficacy test. The selected animals treated with Diminazine aceturate (Berenil® 3.5mg/kg bwt). After 7 days of treatment results showed that only one animal negative for *Anaplasma* spp. by 100× objective microscopic examination (Table 7).

**Table 1. Overall prevalence of anaplasmosis in 150 cattle in Sirajganj district and their proportional prevalence rate in Raygonj and Ullahpara Upazila.**

Upazilla	No. of cattle tested	Positive case	Prevalence (%)
Rayganj	80	13	16.25
Ullahpara	70	15	21.43
Overall	150	28	18.67
P-value (Chi-square test)			0.417
Level of significance			NS

NS means ( $p > 0.05$ )

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

Age group	No. of cattle tested	Positive case	Prevalence (%)
6 months-2 years	49	5	10.20
2-3 years	41	13	31.7*
Above 3 years	60	10	16.67
Total	150	28	18.67
P-value (Chi-square test)			0.029
Level of significance			*

\* Significant at 5% level of significance

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

Sex group	No. of cattle tested	Positive case	Prevalence (%)
Male	69	11	15.94
Female	81	17	20.99
Total	150	28	18.67
P-value (Chi-square test)			0.43
Level of significance			NS

NS means ( $p > 0.05$ )**Table 4. Breed-wise prevalence of anaplasmosis in cattle.**

Breed	No. of cattle tested	Positive case	Prevalence (%)
Local/Indigenous	73	13	17.8
Cross	77	15	19.5
Total	150	28	18.67
P-value (Chi-square test)			0.79
Level of significance			NS

NS means ( $p > 0.05$ )**Table 5. Efficacy of oxyteracycline against anaplasmosis in cattle.**

Drug used	Animal number	Treatment Period			
		Day 0 (intensity before treatment)		Day 7 (intensity after treatment)	
Oxyteracycline (Renamycine® LA @10 mg / kg bwt)	A1	1	+	1	-
	A3	2	+++	2	+
	A12	3	++	3	-
	A20	4	++	4	-
	A46	5	+	5	-

**Table 6. Efficacy of imidocarb dipropionate against anaplasmosis in cattle.**

Drug used	Animal number	Treatment Period			
		Day 0 (intensity before treatment)		Day 7 (intensity after treatment)	
Imidocarb dipropionate (Babenil® @ 3.5 mg/kg bwt)	A61	1	+	1	-
	A63	2	++	2	+
	A71	3	+	3	-
	A83	4	+++	4	+
	A90	5	+	5	+

**Table 7. Efficacy of diminazine aceturate against anaplasmosis in cattle.**

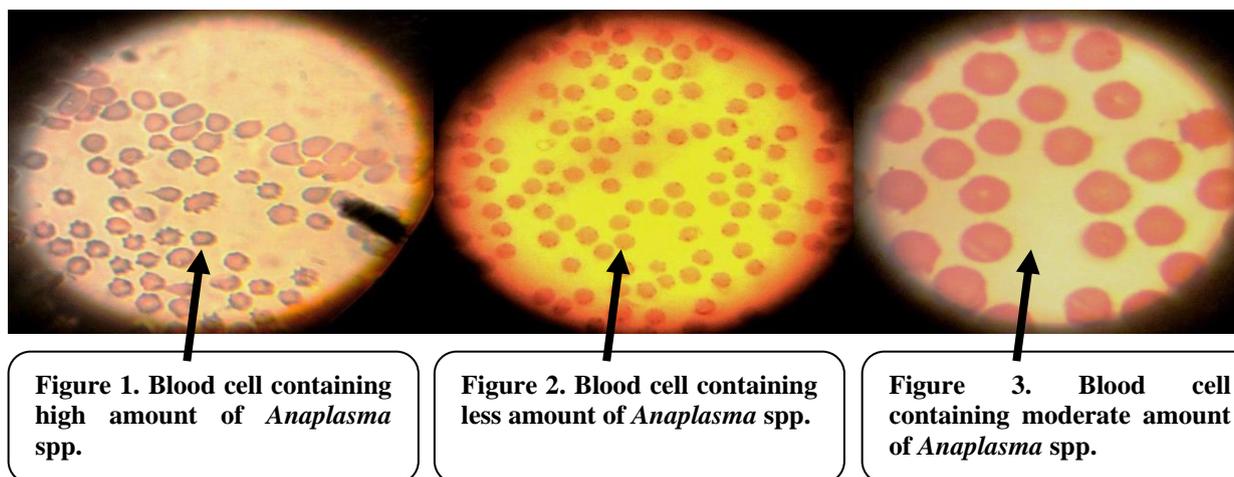
Drug used	Animal number	Treatment Period			
		Day 0 (intensity before treatment)		Day 7 (intensity after treatment)	
Diminazine aceturate (Berenil® @ 3.5mg/kg bwt)	A103	1	+	1	+
	A124	2	++	2	+
	A129	3	+	3	+
	A137	4	+	4	-
	A144	5	+++	5	++

**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

+++ ( Heavy) 1-10 parasites per thick blood film field.



#### 4. Discussion

The prevalence obtained in this study was found relating to the stock density in different upazila (Report of DLS and statistical pocketbook, 2011). High number of cattle population with tick, high number of lowland/ flood plain based area/ ponds and veterinary practitioners in Ullaparas upazila favored the transmission of organism among animals probably causing high prevalence. The overall prevalence of anaplasmosis in this study 18.67 % supports the earlier report of *Anaplasma* infection in Bangladesh (Talukdar *et al.*, 2001) who was observed higher prevalence of anaplasmosis in cattle that was 33 % in Baghabari (Shahjadpur) Milk Shed Area. The occurrence of subclinical *Anaplasma* infection in 5.93 % cattle has been reported from Bangladesh (Samad *et al.*, 1989). The present study also support with the report of Arunkumar *et al.* (2013) who observed that the overall prevalence rate of *A. marginale* infection was 19.3 % in Chennai district of Tamil Nadu in India. *Anaplasma* spp. can cause infections in bovine of all age categories where severity and mortality rate increases with augmentation of animal age (Richey 1984). In divergence with the current study, Khan *et al.* (2004) and Atif *et al.* (2013) found adult population more prone to bovine anaplasmosis. Age-wise occurrence of anaplasmosis recorded in this study support the report of Chakraborti (2002) who recorded animals over 3 years of age are highly affected by anaplasmosis. Observation of this study also supported by the findings of Kamani *et al.* (2010) who observed higher prevalence in adult than young cattle. Endemic instability of the study areas might responsible for frequent infections in adult cattle where newborn calves were protected by colostral immunity (Cynthia *et al.*, 2011). It is hypothesized that the strong innate immunity and age resistance of young cattle are responsible for their less vulnerability to tick infestation (Sarkar, 2007) and in such way, leads to less ectoparasitic burden.

In the present study, higher prevalence of *Anaplasma* infections in crossbreed cattle as compared to local cattle was found in agreement with the reports of Radostits *et al.* (2000). 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). The report of Sarkar (2007) who reported the prevalence of ectoparasites was significantly higher in female than male. The prevalence of hemoprotozoan diseases in female cattle of this investigation showed uniformity with the report of Kamani *et al.* (2010). Higher prevalence in female cattle possibly due the fact that they were kept longer for breeding and milk production purpose, supplied insufficient feed against their high demand (Kamani *et al.*, 2010).

During therapeutic efficacy test, 1st group was treated with a single dose of oxytetracycline LA (10 mg / kg body weight). In 1st group four animals recovered completely, one moderately. This was related to (Urquhart *et al.*, 1988) who used oxytetracycline for the treatment of anaplasmosis. In case of 2nd group complete recovery was recorded in two animal, moderate recoveries in one animal. 2nd Group was treated with Iimidocarb dipropionate (Babenil® @ 3.5 mg/kg bwt) administered as a single dose. This study is in line with Chakrabarti, Production. India (1996), McDougald and Edward, (1988) and Robertson, (1976), who also used Imidocarb at the rate of 5 mg/kg body weight. In case of 3rd group one animal recovered completely. One animal recovered moderately and three animals not response. 3rd group was treated with a single dose of Diminazene acceturate

(3.5 mg / kg body weight). The findings are in accordance with Nasir (2000), who reported 55 percent efficacy. In contrast Muhammad *et al.*, (1999) reported 93 percent efficacy of Buparvaquone and Oxytetracycline in Faisalabad, the difference may be due to the use of Oxytetracycline with Buparvaquone, While in India, Anonymous, (1993) reported 98.8 percent cure rate and Patil *et al.* (1995) recorded 80 percent cure rate.

## 5. Conclusions

Based on the result of present study it may be concluded that blood samples collected from cattle reveals the highest prevalence of anaplasmosis were found at Ullahpara Upazila than Rayganj Upazila varies in all age groups and the highest prevalence was observed above 2 to 3 years age group followed by moderate in above 3 years and less in 6 months to 2 years age group. According to sex the prevalence of anaplasmosis was higher in female than male. Cross breed animals were more prone to anaplasmosis infection than the local cattle. Treatment with Oxytetracycline was most effective against anaplasmosis in cattle followed by moderate with imidocarb dipropionate and less effective by diminazene aceturate treatment. Further investigation using modern serological and molecular techniques with large number of samples for the identification of carriers, tick vectors and particularly hematophagic flies are needed. Further study can be done for molecular characterization.

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## Conflict of interest

None to declare.

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