International Journal of Applied Research
Journal HP: www.intjar.com; ISSN: 2411-6610

Molecular detection of *Anaplasma marginale* from cattle and tick in Dinajpur and Sirajganj districts, Bangladesh

Md. Akhtaruzzaman, Anisuzzaman, Md. Shahiduzzaman*

Department of Parasitology, Bangladesh Agricultural University, Mymennsing-2202, Bangladesh

ARTICLE INFO

Article history

Received: 07 December 2022
Accepted: 27 December 2022

Keywords

Anaplasma marginale, cattle, tick, nPCR, Bangladesh

Corresponding Author

M Shahiduzzaman
Email: szaman@bau.edu.bd

ABSTRACT

Detection of *Anaplasma* organism in Bangladesh is mostly performed by microscopic examination which would make poor detection of organism and less specific identification as well. The aim of this study was to detect *Anaplasma marginale* from both tick and cattle reared in Dinajpur and Sirajganj district of Bangladesh by PCR. From February 2017 to February 2018, blood and tick samples were collected from 370 cattle. The blood samples were initially screened for *Anaplasma* spp by staining of blood smear with Giemsa stain and examined under microscope. The DNA was extracted from the microscopically positive blood samples and subjected to Polymerase Chain Reaction (PCR). In the first step, PCR was performed targeting the 16srRNA gene sequence to confirm the presence of *Anaplasma* organism in the samples (PCR product size 781bp). In second step, the presence of *A. marginale* organism was detected in the samples targeting the MSP1β gene of *A. marginale* (PCR product size 95bp). The overall prevalence of tick infestation in cattle was 59.20% (219/370 cattle). *A. marginale* was detected by nPCR in 33.78% blood samples of cattle and 17.35% tick samples. Among the ticks 25 *Rhipicephalus (Boophilus) microplus* and 13 *Haemaphysalis bispinosa*, were positive with *A. marginale*. The study indicates that *A. marginale* is common in cattle of Dinajpur and Sirajganj district of Bangladesh with higher infection rate in Sirajganj district.

Introduction

In Bangladesh 80% rural people rear indigenous cattle (Siddiki *et al.*, 2009) and many people are dependable on dairy farming under the traditional husbandry practices. The growing demand of livestock products emphasizes the necessity to improve the livestock production in Bangladesh. Livestock industry is expanding day by day and plays a vital role in the economy of Bangladesh. While cattle farming play a significant role in rural economy of Bangladesh, the production and productivity of animals are greatly hampered by different diseases including haemoprotozoan diseases (Ngole *et al.*, 2004). Tick-borne diseases especially anaplasmosis, is considered as one of the major obstacle in livestock production and great economic losses (Ahmed, 1976; Samad and Gautam, 1984).

Anaplasma marginale is the most prevalent pathogen transmitted by ticks in the world. It is found worldwide and is responsible for high morbidity and mortality in cattle in temperate, subtropical, and tropical regions (Aubry and Geale, 2011; Kocan *et al.*, 2010). Twenty different tick species are capable of transmitting *A. marginale* and play important roles in maintaining *A. marginale* in cattle (Kocan *et al.*, 2004). The most important biological vector is the tick *Rhipicephalus (Boophilus) microplus*, which is distributed in tropical and subtropical regions (Estrada-Pena *et al.*, 2006). Anaemia, fever, jaundice and sudden death are characteristic signs of anaplasmosis. Other signs include rapid loss of milk production and weight, but the clinical disease can only be confirmed by identifying the organism.

The recorded important ticks in Bangladesh, which infest ruminants, are *Rhipicephalus* microplus, *Haemaphysalis bispinosa*, *Rhipicephalus sanguineus*, and *Hyaloma anatolicum* (Ghosh, *et al.*, 2007). Almost all the ruminants are infested throughout the year with minimum load of these ticks. The load is occasionally very high (several hundred) in young ruminants and alarmingly high in exotic or crossbred animals. All the ticks have a wider distribution, but *H. anatolicum* is only confined to the northwestern dry regions (Rajshahi, Rangpur, and Dinajpur districts) of the country (Ghosh, et al., 2007). These tick-borne blood parasites are prevalent in crossbred cattle rearing in Sirajganj (Islam et al., 2006, Belal *et al.*, 2014). Dinajpur is very close to West Bengal of India where *R. microplus*, *Dermacentor auratus*, *H. bisponosa* and *H. anatolicum anatolicum* are most dominant in population (Sanyal and De, 2001).

Cattle infected with *A.marginale* is difficult to detect because of the low numbers of parasites that

Cite this article: *Akhtaruzzaman, M., Anisuzzaman & Shahiduzzaman, M. (2022). Molecular detection of Anaplasma marginale from cattle and tick in Dinajpur and Sirajganj districts, Bangladesh. International Journal of Applied Research, 8 (2): 42-48. DOI: 10.5281/zenodo.8313951*

occur in peripheral blood and confusion with other tick borne blood protozoa *babesia* and *theileria* spp (Pradeep *et al.,* 2019). However, diagnosis of low-level infections with the parasite is important for epidemiological studies (Fahrimal *et al.,* 1992). PCR has proven to be very sensitive particular in detecting *Anaplasma* spp (Yang *et al.,* 2015). To overcome the economic losses early diagnosis and understanding the molecular epidemiology of anaplasmosis is important in cattle and tick. The present study was aimed at the molecular detection of *A. marginale* by PCR technique in both cattle and the infested ticks in Sirajgonj and Dinajpur districts of Bangladesh targeting MSP1β gene in the PCR.

Materials and Methods

Study location

Sirajganj is located in the north-central region (24° 27'0"N, 89° 45'0"E) of Bangladesh, besides the Jamuna River. The samples were collected from different areas of Sirajganj district. The areas were Belkuchi, Shahjadpur, Sirajganj Sadar and form the "bathan" (a large area of grazing where cattle are housed and maintained) in Shahjadpur upazila (a subunit of a district). 'Baghabari Milk Vita', the largest public-private milk producing company, is located in the same area.

Dinajpur is at north-west part of Bangladesh which is attached to the state of West Bengal, India in the west. This district is located in between 25°10' and 26°04'N and in between 88°23' and 89°18'E. Dinajpur experiences a hot, wet and humid tropical climate. The district has a distinct monsoonal season, with an annual average temperature of 25 °C (77 °F).

Sample collection

In this study, blood and tick samples were collected from a total of 370 cattle of Dinajpur (175) and Sirajganj (195) districts of Bangladesh for a period of 13 months from February 2017 to February 2018. The cattle were both indigenous and crossed bred and kept in semi-intensive or free range system. The blood samples were collected in 4 ml EDTA coated tubes. The collected blood samples were transported to the laboratory of the Department of Parasitology, Bangladesh Agricultural University, Mymensingh by maintaining a cool-chain using ice-box, stained with 10% Giemsa stain and examined for identification of *Anaplasma* spp as described by (Solusby, 1982). The samples were stored at -20°C prior to DNA extraction.

Tick sampling and identification

The tick samples were collected during the blood sample collection by examining the sites of predilection for ticks on the bodies of cattle. Individual ticks were counted on the animals and preserved in separated vials containing 70% ethanol. Adult ticks were identified under a stereomicroscope according to identification keys (Walker et al., 2003; Estrada-Peña et al., 2004). Salivary glands of the ticks were dissected according to Edward et al. (2009). For each tick, sterilized scalpel blades were used to avoid possible contamination.

DNA extraction

Genomic DNA was extracted and purified from 200 µl of the blood of both positive and negative samples using the Purelink®® DNA blood Mini kit (Invitrogen, USA) according to the manufacturer's instructions. The concentration of the extracted DNA was measured using a nanodrop (NanoDrop™, Thermo Fisher, USA).

Polymerase Chain Reaction (PCR)

The presence of parasites of the genus *Anaplasma* is assessed by amplifying the 16srRNA gene as previously reported (Noaman *et al.,* 2009). This primary amplification is performed using primers F1 (5'- AGAGTTTGATCCTGGCTCAG-3') and R1 (5'- AGCACTCATCGTTTACAGCG-3') (PCR product: 781bp). PCR reaction was performed in a 25µl volume containing 12.5µl of PCR master mixture (Go Taq® Green Master, Promega, Madison, WI USA), 2µl of forward primer, 2µl of reverse primer, 2µl DNA sample and 6.5 µl of nuclease free water (Promega, Madison, WI USA). The reaction was run in Applied Biosystems™ 2720 thermal cycler: 95°C for 5 minutes, 35 cycles at 94°C for 45 second, 59°C for 45 second, 72°C for 45 second and a final extension of 72°C for 10 min. The amplified product of PCR assay was analyzed by gel electrophoresis on a 1.5% agarose gel and stained with EZ-Vision® In-Gel (1 - 2 drops of solution per 50 ml gel).

To detect the presence of *A. marginale* organism in the samples the MSP1β gene was amplified using F3 (5´-TTGGCAAGGCAGCAGCTT-3´) and R3 (5´-TTCCGCGAGCATGTGCAT-3´) primers (PCR product: 95bp) (Carelli *et al.*, 2007). PCR reaction was performed in a 25µl volume containing 12.5µl of PCR master mix (Go Taq® Green Master, Promega, Madison, WI USA), 2 µl of forward primer, 2 µl of backward primer, 2µl DNA sample and 6.5µl of nuclease free water (Promega, Madison, WI USA). The reaction condition was: 96°C for 1 min, 35 cycles of denaturation at 96°C for 15 sec, annealing at 53°C for 1 min, extension at 72°C for 20 sec, and a final extension at 72°C for 5 min. The amplified product of PCR assay was analyzed by electrophoresis on a 1.5% agarose gel and stained with ethidium bromide.

Results

The overall prevalence of tick infestation in cattle was 59.20% (219/370). Microscopic examination revealed 34.32% (127/370 blood) positive samples of which 98.43% (125/127) samples were positive for *A. marginale* by nested PCR (Table 1). Overall *A. marginale* was detected in only 38 ticks out 219 ticks species collected of which 25 *Rhipicephalus (Boophilus) microplus* and 13 *Haemaphysalis bispinosa*, were positive with *A. marginale*. Examined animals were classified mainly according to age, gender, species, breed, and the presence of ticks.

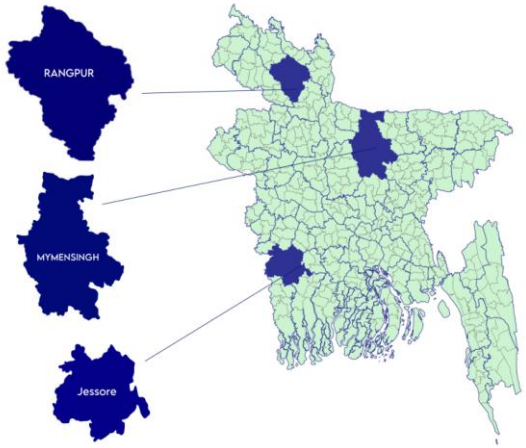

Figure 1: Map of Bangladesh indicating the study areas.

Microscopic Detection of *Anaplasma* spp

All samples (370) were initially processed for microscopic examination of *Anaplasma* organisms. It was observed that 29.92% (38/127) from the Dinajpur and 70.08% (89/127) from Sirajganj samples were positive for *Anaplasma* spp (Figure 2).

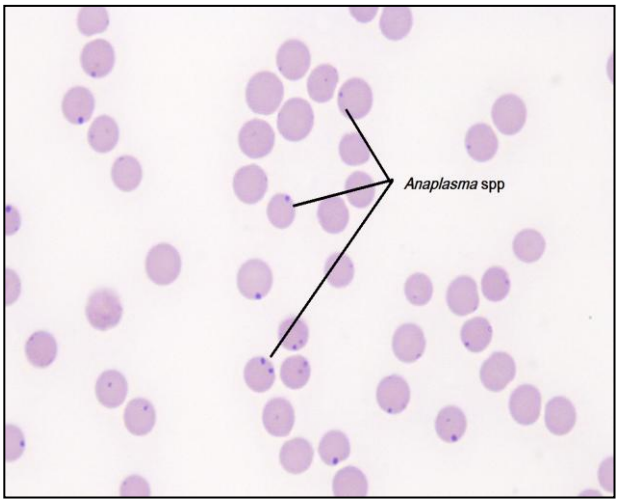

Figure 2: Blood smear showing *A. marginale* inside the red blood cells (arrows) (40X)

Molecular Detection of *Anaplasma* spp. by PCR

The microscopically positive blood samples were screened for confirming *Anaplasma* spp by amplification of 16s rRNA. PCR amplification products size of 781bp was found in gel image (Figure 2).

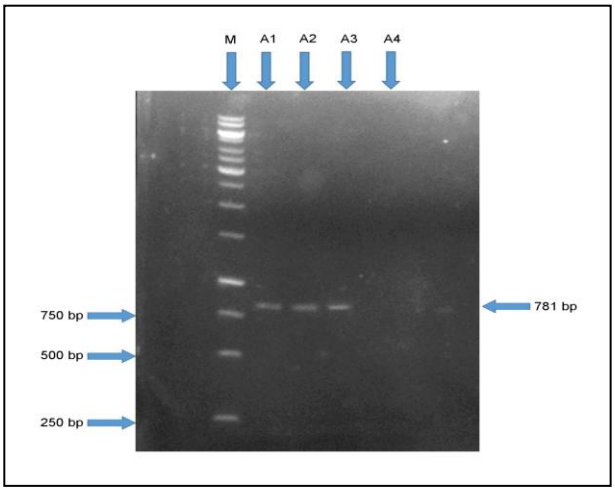

Figure 3: Amplification of 16s rRNA gene of *Anaplasma* spp. by primary PCR (band 781bp). M, Ladder-1kb; A1, A2 are blood samples and A3, A4 are tick samples.

The secondary PCR targeting the MSP1β gene confirmed the presence of *A. marginale* in both blood and tick samples. The amplicon size of 95bp was obtained from the nested PCR of primary PCR product.

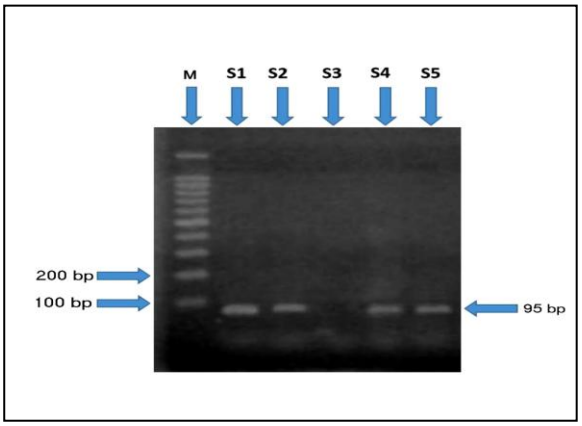

Figure 4: Amplification of MSP1β gene specific to *A. marginale*: Amplicons of 95 bp indicated presence of *A. marginale*. M, Ladder-100bp; S1, S2, S3 blood samples and S4, S5 tick samples.

Molecular prevalence of *Anaplasma marginale* in cattle and tick

During this study, a total of 370 cattle were examined of which 38 (21.71%) cattle were positive for *A. marginale* infection out of 175 samples from Dinajpur and 87 (44.62%) cattle were found positive out of 195 samples from Sirajganj District. A. *marginale* was found in 15 (15.63%) ticks out of 96 ticks collected from Dinajpur and 23 (18.70%) of 123 ticks were positive for *A. marginale* in Sirajganj District. The study indicates that *A. marginale is* common in cattle of Dinajpur and Sirajganj district of Bangladesh with higher infection rate in Sirajganj district (Table 1).

Table 1: Prevalence of *Anaplasma marginale* in cattle and ticks in Dinajpur and Sirajganj District of Bangladesh by nested PCR

Samples	Dinajpur N (positive) % positive	Sirajganj N (positive) % positive	Overall prevalence N (positive) % positive
Cattle (blood)	175 (38) 21.71%	195 (87) 44.62%	370 (125), 33.78%
Tick (Salivary gland)	96 (15) 15.63%	123 (23) 18.70%	219 (38) 17.35%

Table 2: Epidemiological parameters associated with *Anaplasma marginale* infection in cattle detected by *msp1β* qPCR (Chi-square test) (N=370)

Parameter		**p/N**	**%positive**	**χ2**	**P-value**
Breed	Native	59/161	36.65	1.043	0.3069
	Crossbreed	66/209	31.58		
Sex	Male	53/165	32.12	0.368	0.5441
	Female	72/205	35.12		
Age	≤ 1 year	46/142	32.39	8.4408	0.0377*
	≤ 3 years	25/95	26.32		
	≤ 5 years	48/109	44.04		
	≥ 5 years	6/24	25.0		
Season	Summer	59/157	37.58	3.3672	0.18570
	Winter	26/98	26.53		
	Rainy	40/115	34.78		
Locality	Dinajpur	38/175	21.71	14.3287	0.00015**
	Sirajgonj	87/195	44.62		

* $P < 0.05$, **$P < 0.01$
Summer (March to June), Rainy (July to October), and Winter (November to February)
Abbreviations*:* p= number positive; N= total number

Table 3: Epidemiological parameters associated with *Anaplasma marginale* infection in ticks detected by *msp1β* qPCR (Chi-square test) (N=219)

Parameter		p/N	% positive	χ2	P-value
Breed	Native	11/90	12.22	2.8032	0.09407
	Crossbreed	27/129	20.93		
Sex	Male	13/87	14.94	0.5841	0.44469
	Female	25/132	18.94		
Age	≤ 1 year	12/72	16.67	0.5133	0.91595
	≤ 3 years	8/46	17.39		
	≤ 5 years	15/78	19.23		
	≥ 5 years	3/23	13.04		
Season	Summer	18/83	21.67	2.1541	0.34062
	Winter	7/57	12.28		
	Rainy	13/79	16.46		
Locality	Dinajpur	15/96	15.63	0.3553	0.55111
	Sirajgonj	23/123	18.70		

* $P < 0.05$, ** $P < 0.01$
Summer (March to June), Rainy (July to October), and Winter (November to February)
Abbreviations: p= number positive; N= total number

Among the epidemiological parameters of *A. marginale* infection in cattle significant differences were observed in different age and locality (Table 2). Whereas no significant differences were observed in any parameters investigated for infested ticks (Table 3).

Discussion

In this study, the overall prevalence of tick infestation in cattle was 59.20%. Several other studies have been conducted in different geographical areas of Bangladesh to investigate the prevalence of ticks infesting ruminants, with reports varying from 36.3% in Chittagong (Kabir et al., 2011) to 64.0% in Gazipur district (Rony et al., 2010, Roy et al., 2017). The identified ticks were *R. microplus* and *H. bispinosa* in the study areas which is in accordance with study of Ghosh et al. (2007).

Microscopic examination of Giemsa-stained thin blood smears revealed that 127 slides were positive (34.32%, 127/370) for *Anaplasma* species. The present study supports the earlier report of Anaplasma infection in Bangladesh (Talukdar et al., 2001) who was observed that the prevalence of anaplasmosis in cattle was 33% in Baghabari (Shahjadpur) Milk Shed Area. Form microscopically positive samples 125 samples were positive for *A. marginale* by nPCR. The variation of results might be due to misinterpretation or artifacts during observation.

The present study revealed that *A. marginale* infection was significantly higher in cattle in Sirajganj (44.62%) than Dinajpur (21.71%), conferring to the finding of Sandip *et al.,* (2016) in Dinajpur (18.5%). Talukdar and Karim (2001) who reported that 33% cattle of Baghabari Milk Shed area had *Anaplasma* infection. Chowdhury *et al.* (2006) recorded much higher prevalence (70%) of anaplasmosis in clinically suspected cattle of Sirajganj district than those of other inland reports. Occurrence of anaplasmosis of this study was differ from the reports of Siddiki *et al.,* (2009) and Samad *et al.,* (1989), who recorded 3% and 5.93%in different areas of Bangladesh. Bary *et al.* (2018) recorded highest prevalence of anaplasmosis12.0% in crossbred cattle followed by 6.00% in local cattle in summer. However the differences might be due to the use of sampling and methods.

In this study ticks were collected from the cattle body during the blood collection. The results demonstrated that *A. marginale* infection was higher in ticks of Sirajganj than Dinajpur district.

This result can be due to the increased number of cattle and ticks were investigated. However, Sirajganj is an area of milk production and alternate rearing of cattle in bathan during dry season and in house during wet season may increase the chance the getting infection by ticks.

Conclusion

In conclusion, the nPCR is sensitive and specific for detection of *A. marginale* infection. Infections with *Anaplasma marginale* were common in all the investigated areas from Bangladesh. Infection is less in Dinajpur than Sirajganj. Age of the animal was the significant epidemiological factors for having infection of A. *marginale*. However, further studies are needed to investigate the presence of *A. marginale* in more areas throughout the year in order to better understanding about the status and transmission dynamics of infection in cattle in Bangladesh.

References

Alim, M.A., Das, S., Roy, K., Masuduzzaman, M., Sikder, S., Mahmudul, M., Hassan, A.Z. & Hossain M.A. (2011). Prevalence of hemoprotozoan diseases in cattle population of Chittagong division, Bangladesh. Vet Journal, 32, 221-224.

Aubry, P., Geale, D.W. (2011). A review of bovine anaplasmosis. Transbound Emerg Dis., 58(1),1–30.

Bary, M.A., Ali, M.Z., Chowdhury, S., Mannan, A., Nur e Azam, M., Moula, M.M., Bhuiyan, Z.A., Shaon, M.T.W. & Hossain, M.A. (2018). Prevalence and molecular identification of haemoprotozoan diseases of cattle in Bangladesh. Adv Anim Vet Sci., 6, 176-182.

Belal, S.M., Mahmud M.A. & Jannatul, F. (2014). Prevalence of anaplasmosis in cattle in Sirajganj district of Bangladesh. Res Agric Livest Fish., 1, 97-103.

Carelli, G., Decaro, N., Lorusso, A., Elia, G., Lorusso, E., Mari, V., Ceci, L. & Buonavogli, C. (2007). Detection and quantification of *Anaplasma marginale* DNA in blood samples of cattle by real-time PCR. Vet Microbiol., 4, 36-38.

Chowdhury, S., Hossain, M.A., Barua, S.R. & Islam, S. (2006). Occurrence of common blood parasites of cattle in Sirajganj sadar area of Bangladesh. Bangladesh J Vet Med., 4, 143-145.

Estrada-Peña, A., Bouattour, A., Camicas, J. & Walker A.R. (2004). Ticks of domestic animals in the Mediterranean region: a guide to identification of species -Zaragoza: University of Zaragoza. pp. 131

Fahrimal, Y., Goff, W.L. & Jasmer, D.P., (1992). Detection of *Babesia bovis* carrier cattle by using polymerase chain reaction amplification of parasite DNA. J Clin Microbiol., 30(6), 1374-1379.

Ghosh, S., Bansal, G.C., Gupta, S.C., Ray, D., Khan, M.Q., Irshad, H., Shahiduzzaman, M., Seitzer, U. & Ahmed, J.S. (2007). Status of tick distribution in Bangladesh, India and Pakistan. Parasitol Res., 101 Suppl 2:S207-16. doi: 10.1007/s00436-007-0684-7.

Kabir, M., Mondal, M., Eliyas, M., Mannan, M., Hashem, M., Debnath, N. & Elahi, M. (2011). An epidemiological survey on investigation of tick infestation in cattle at Chittagong District, Bangladesh. Afr. J. Microbiol. Res., 5, 346–352.

Kocan, K.M., la Fuente, J. de, Blouin, E.F., Garcia-Garcia, J.C. (2004). *Anaplasma marginale* (Rickettsiales: Anaplasmataceae): recent advances in defining host-pathogen adaptations of a tick-borne rickettsia. Parasitology, 129. S285-S300

Noaman, V., Shayan, P., Amininia, N. (2009). Molecular Diagnostic of Anaplasma marginale in Carrier Cattle. Iran J Parasitol. 4, 26-33.

Ngole, I.U., Ndamukong, K.J., Mbuh, J.V. (2003). Internal parasites and haematological values in cattle slaughtered in Buea subdivision of Cameroon. Trop. Anim. Hlth. Prod. 35(5), 409-413.

Pradeep, R. K., Nimisha M., Sruthi M. K., Vidya P., Amrutha B. M., Kurbet P. S., et al. (2019). Molecular characterization of South Indian field isolates of bovine *Babesia* spp. and *Anaplasma* spp. Parasitol. Res., 118 617–630.

Rony, S., Mondal, M., Begum, N., Islam, M., & Affroze, S. (2010). Epidemiology of ectoparasitic infestations in cattle at Bhawal Forest Area, Gazipur. Bangladesh J Vet Med., 8, 27–33.

Roy, B.C., Krücken, J., Ahmed, J.S., Majumder, S., Baumann, M.P., Clausen, P.H. & Nijhof, A.M. (2018). Molecular identification of tick-borne pathogens infecting cattle in Mymensingh district of Bangladesh reveals emerging species of *Anaplasma* and *Babesia*. Transbound Emerg Dis. 65, 231-242.

Samad, M.A., Bashar, S.A., Shahidullah, M. & Ahmed, M.U. (1989). Prevalence of haemoprotozoan parasites in cattle of Bangladesh. India Vet Med J., 13, 50-51.

Sanyal, A.K. & DE S.K. (2001). Diversity In Ticks (Acari) Of West Bengal. Rec. zoo I. Surv. India: 99 (Part 1-'4), 65-74.

Siddiki, A.Z., Uddin, M.B., Hasan, M.B., Hossain, M.F., Rahman, M.M., Das, B.C., Sarker, M.S. & Hossain, M.A. (2010) Coproscopic and haematological approaches to determine the prevalence of helminthiasis and protozoan diseases of red chittagong cattle breed in Bangladesh. Pakistan Vet J., 30, 1-6.

Soulsby, E.J.L. (1982). Helminths, Arthropods and Protozoa of Domesticated Animals by Baillere Tindall, London. pp.707-717, 729-735.

Subramanian, B., Vijayalakshmi, P., Das, S.S. & Selvi, D. (2018). Prevalence of Anaplasmosis caused by *Anaplasma marginale* in cattle in and around

Puducherry Region, India. Int J Curr Microbiol App Sci., 7, 189-193.

Tay, S.T., Koh, F.X., Kho, K.L. & Ong B.L. (2014). Molecular survey and sequence analysis of *Anaplasma* spp. in cattle and ticks in a Malaysian farm. Asian Pac. J. Trop. Biomed., 31, 769–776

Uilenberg, G. (1995). International collaborative research: significance of tick-borne hemoparasitic diseases to world animal health. Vet Parasitol., 57, 19-41.

Walker A.R., Bouattour A., Camicas J., Estrada-Pena A., Horak I., Latif A., Pegram R. & Preston, P. (2003). Ticks of domestic animals in Africa: a guide to identification of species -Bioscience reports Edinburgh 221pp.

Yang, J., Li, Y., Liu, Z., Liu, J., Niu, Q., Ren, Q., Chen Z, Guan, G., Luo, J. & Yin, H. (2015). Molecular detection and characterization of *Anaplasma* spp. in sheep and cattle from Xinjiang, northwest China. Parasites Vectors, 8: 108.