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Hemoprotozoa and *Anaplasma* spp. in rodents and shrews of Bangladesh

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Abstract. Hemoprotozoans are important pathogens of animals and humans, among which some species have zoonotic significance. The prevalence of different hemoprotozoa and *Anaplasma* spp. in larger mammals have been reported from different regions of the world. But, very few studies have been conducted to estimate the prevalence of hemoprotozoa in rodents and shrews of South-East Asia. The study assessed the prevalence of hemoprotozoa and *Anaplasma* spp. in rodents and shrews of Bangladesh. Blood samples (n=451) were collected from rodents and shrews between June 2011 and June 2013 and July-December 2015 from 4 land gradients of Bangladesh. Giemsa-stained blood smears revealed that 13% of animals were harboring hemoprotozoa (4.7% *Babesia* spp., 0.67% *Plasmodium* spp.), and *Anaplasma* spp. (7.5%). The study may serve as a guide for future hemoparasitic research of rodents and shrews.

INTRODUCTION

Small mammals are the key mammalian group to survive in diverse environments in the world (Polacikova, 2013). Rodents are the largest group among the small mammals, with extensive habitat range from sub-terrestrial to terrestrial. Two rodent species, *Mus musculus* (house mouse) and *Rattus rattus* (black rat/house rat) and one shrew species, Asian house shrew (*Suncus murinus*) are commonly found in Bangladesh. They are commensal with humans and found near human habitation and other synanthropic habitats such as rice fields and grain warehouses (Veciana *et al.*, 2012).

Rodents and shrews play an important role as a reservoir host and final host of many infectious zoonotic organisms (Gratz, 1994). More than 20 well-known diseases are directly transmitted from rodents to humans, usually through the blood-sucking parasites such as fleas, ticks, and mites (Ellis *et al.*, 1999; Favorov *et al.*, 2000; Wang *et al.*, 2000; Singla *et al.*, 2008; Raj *et al.*, 2009). Urban rats carry many zoonotic pathogens, which can be spread through increased contact with people due to urbanization (Sumangali *et al.*, 2007). Rodents serve as a reservoir

host for hemoprotozoa and rickettsia, such as *Babesia* and *Anaplasma*, respectively (Kallio *et al*., 2014). *Anaplasma* spp. was reported in small mammals of Thailand (Thanee *et al*., 2009), whereas filariae, piroplasms, and trypanosomes were found in rodents of Colombia (Ayala *et al*., 1973). Moreover, rats, and mice have been found to be infected with *Trypanosoma* spp. (Sebek, 1975; De Lima *et al*., 2003; Laakkonen *et al*., 2003). In a study on 34 Australian bush rats, 32% of samples were positive for hemoprotozoa with a specific prevalence of 10% *Trypanosoma* spp. (McDonogh *et al*., 2015). *Plasmodium* spp. (43.3%) and *Trypanosoma* spp. (25.2%) were found in *Rattus* spp. in Malaysia, and the presence of the hemoprotozoa was influenced by host, age and sex (Alias *et al*., 2014; McDonogh *et al*., 2015). Although *Babesia* spp. was common in field vole/short-tailed vole (*Microtus agrestis*), it was rarely found in other rodents. *Trypanosoma* spp., *Babesia* spp., *Hepatozoon* spp. and *Grahamella* spp. were all found in *Myodes glareolus* (bank vole) and *M. agrestis* (Wiger, 1979). *Hepatozoon* spp. was isolated previously from *M. glareolus* with 4.44% prevalence (Criado-Fornelio *et al*., 2006). According to a previous study in Nigeria, hemoparasitic prevalence was 75% in *R. rattus* and 72.22% in *M. musculus*; 51.67% of male and 72.86% of female rodents were found to be parasitized, and this difference in prevalence was not significant (Ajayi *et al*., 2006).

Like rats and mice, house shrews can also transmit important pathogens such as Thottapalayam virus (Yadav *et al*., 2007), Hantavirus (Arai *et al*., 2007), *Toxoplasma gondii* (Kijlstra *et al*., 2008) and *Yersinia pestis* (plague) (Renapurkar, 1988). They can also harbor hemoprotozoa and can be the source of infection to humans. In one report, hemoprotozoa were less prevalent and less diverse in shrews than in rodents (Karbowiak *et al*., 2005). Prevalence of hemoprotozoa in the common shrew (*Sorexa raneus*) and Eurasian water shrew (*Neomys fodiens*) were reported as 32.5 and 41.2%, respectively (Karbowiak *et al*., 2005).

Species of hemoprotozoa are related to the habitat selection by host (Thanee *et al*., 2009). When habitats are disturbed and the composition of the small mammals' community changes, hemoprotozoa may encounter different hosts near their nest (Nava *et al*., 2003; Thanee *et al*., 2009). Humans can be considered as accidental hosts of some hemoprotozoa, acquiring an infection during interaction with the natural enzootic cycle. To date, most of the human cases reported were infected by a tick carrying the rodent hemoprotozoa (Gorenflot *et al*., 1998). The transmission of parasites is influenced by the close association of rodents and house shrew with humans and livestock and their exposure to blood-sucking arthropods, beetles, cockroaches, and other invertebrates.

There are few published studies concerning animal reservoirs of hemoprotozoa in Asia. The parasitic infections that rodents and shrew harbor and convey to human or animal populations have not been as thoroughly investigated as the microbial infections, especially in Bangladesh. Therefore, the aim of the study was to screen the rodents and shrews of Bangladesh for hemoprotozoa and *Anaplasma* spp.

MATERIALS AND METHODS

Study sites and duration

The study was carried out between June 2011 to June 2013 and July-December 2015 to determine the prevalence of blood protozoa in *R. rattus*, *M. musculus* and *S. murinus* from four land gradients of Bangladesh, namely Urban (Chattogram metro and Khulshi); Peri-urban (Faridpur and Sitakunda); Rural (Dinajpur, Rajbari, Moulvibazar, Rangpur, Mirsharai, Lalmonirhat, Chakaria, Rangpur and Joypurhat) and Hilly (Rangamati, Khagrachari and Bandarban) (Figure 1).

Data recording, animal capture, and sample collection

The live rodents and shrews were captured using locally made steel wire traps (27cm × 13cm × 13cm) that have proven efficacy in sampling of medium-size and large-size rodents and shrews (Shafiyyah *et al*., 2012).

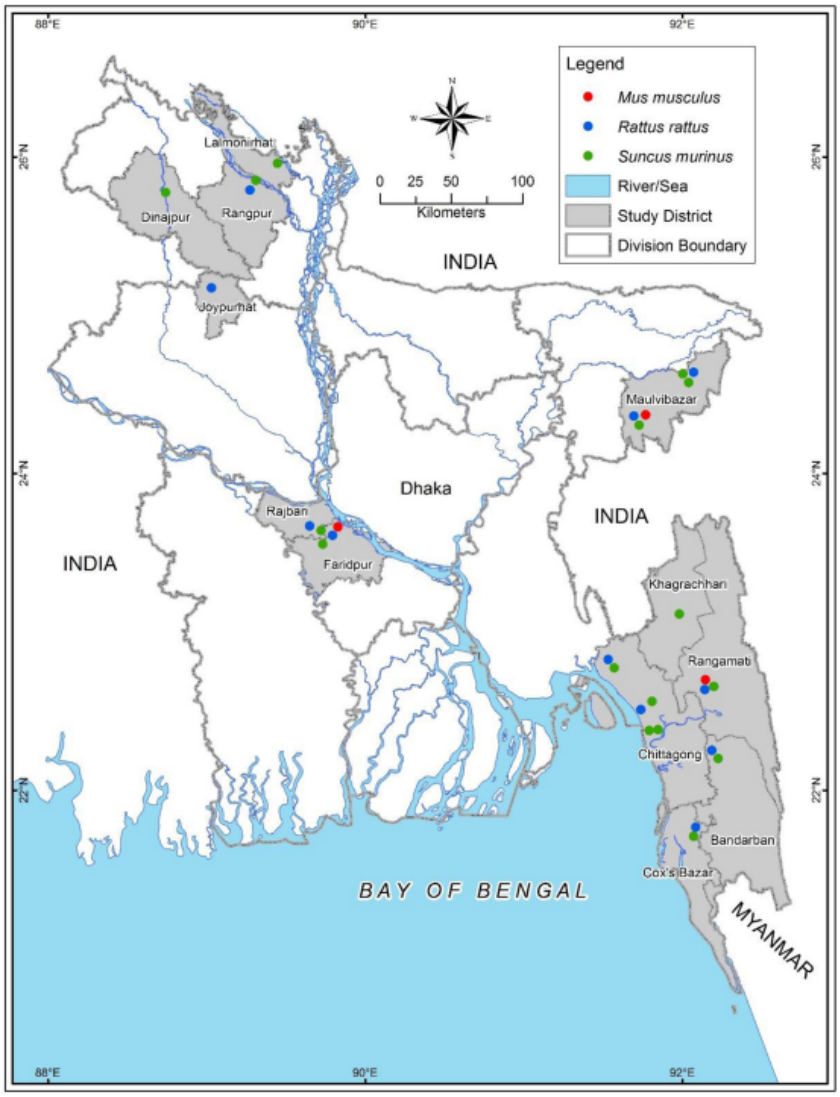

Figure 1. Rodents and shrew capturing sites in Bangladesh.

The traps were baited with ghee-smeared biscuit and dried fish. The traps were set at dusk and collected at the next dawn (Rahman *et al.*, 2018). Trapped rodents and shrews were anesthetized by isoflurane. All the rodents and shrews were identified based on their morphological characters (Aplin *et al.*, 2003). Blood was collected from the suborbital sinus, tail vein, or saphenous vein based on the size of each animal. A total of 451 rodents and shrews consisting of 299 *S. murinus*, 125 *R. rattus*, and 27 *M. musculus* were captured. The geographic area, the land gradient, and the animals' species, age, and gender were recorded. Male (n=83; 55%) and adult (n=131; 86%) rodents were prevalent among the captured animals. In contrast, females (n=166; 56%) and adult (n=278; 93%) were predominant in captured shrews. Maximum number (n=61; 40%) of rodents and shrews were captured from the rural area.

Laboratory examination

All blood analyses were conducted at the clinical pathology laboratory, Chattogram Veterinary and Animal Sciences University (CVASU), Bangladesh. A thin blood smear was prepared with a small drop of blood. The slides were air-dried and fixed by absolute

methanol for 3-5 minutes and stained with Giemsa at pH 7.1 (Thanee *et al.*, 2009). The stained blood smears were examined under 1000X magnification using immersion oil, and approximately 200 fields of vision were inspected for the identification of blood parasites (Siñski *et al.*, 2006; Thanee *et al.*, 2009). Blood parasite identification was done based on the morphology of different stages of the parasites (Thanee *et al.*, 2009).

Ethical declaration

The study protocol was approved by the Animal Experimentation Ethics Committee of CVASU (approval no. CVASU/Dir (R and E) AEEC/2015/07) and International Centre for Diarrheal Disease Research, Bangladesh (icddr,b; protocol: 2008-074) and IACUC of the University of California, Davis (protocol: 16048).

Statistical analysis

All the collected data regarding age, sex, species, and location were entered into MS excel-2007 (Microsoft Corporation, Redmond, WA 98052-6399 USA). Data analysis were done by STATA/IC-13 (StataCorp, 4905, Lakeway Drive, College station, Texas 77845, USA). Descriptive statistics were performed based on species of mammals, age, sex and land gradient. The results were expressed in percentage and 95% Confidence Interval (CI).

RESULTS

The study identified *Anaplasma* spp., *Babesia* spp. and *Plasmodium* spp. in tested rodents and shrews (Figure 2). Altogether, 13% (58/451) of captured mammals were harboring hemoprotozoa in their blood, among which 7.5% (n=34) were *Anaplasma* spp., 4.7% (n=21) were *Babesia* spp. and rest 0.67% (n=3) were *Plasmodium* spp.

Higher prevalence of hemoprotozoa were found in shrews (14.38%; n=299) than in rodents (9.87%; n=152). *S. murinus* showed a higher percentage of blood parasitism (n=43; 14.4%; 95%CI: 11-19) than *R. rattus* (n=14; 11.2%) and *M. musculus* (n=1; 3,7%). Among three parasites, *Anaplasma* spp. was most prevalent in shrews (10.03%; 95 CI: 7-14), whereas *Babesia* spp. was most prevalent in *R. rattus* (5.6%; 95% CI: 2-11). *Plasmodium* spp. (2.4%; 95% CI: 1-7) was found only in *R. rattus* (Table 1). The only positive *M. musculus* was a male adult animal from the peri-urban area, which harbored *Babesia* spp. (Table 1).

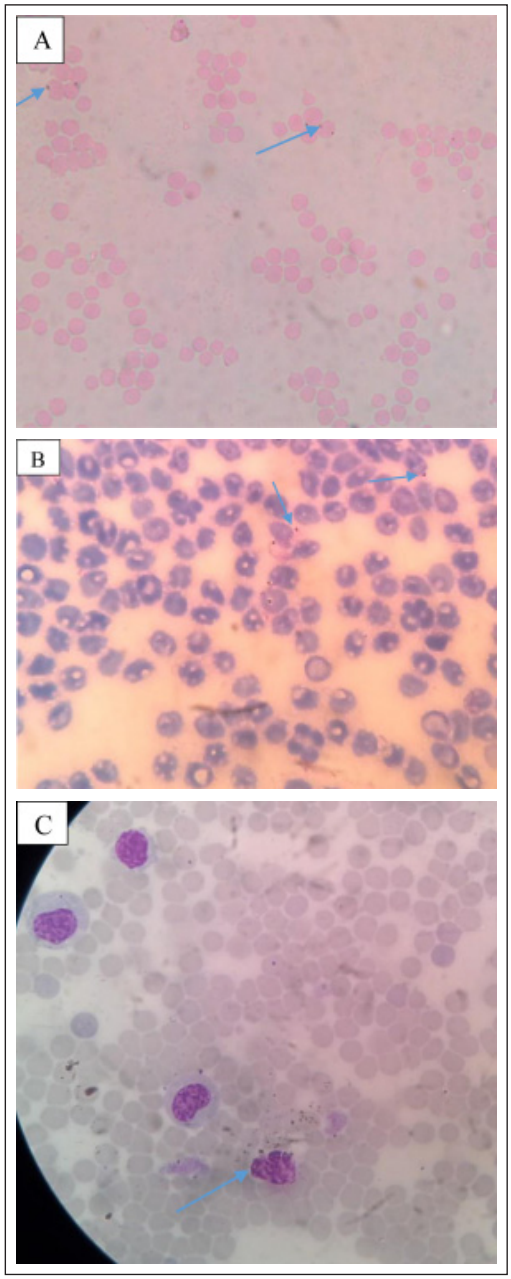

Figure 2. (A) *Anaplasma* spp., (B) *Babesia* spp. and (C) *Plasmodium* spp. found in microscopic examination of blood smear from rodents and shrews from Bangladesh.

Table 1. Frequency distribution of *Anaplasma* spp. and hemoprotozoa of rodents and shrews captured from Bangladesh (N=451)

Variable	Category	*M. musculus*		*R. rattus*				*S. murinus* % (n)		
		N	*Babesia* % (n)	*N*	*Anaplasma* % (n)	*Babesia* % (n)	*Plasmodium* % (n)	*N*	*Anaplasma* % (n)	*Babesia* % (n)
Prevalence		27	3.7 (1)	125	3.2 (4)	5.6 (7)	2.4 (3)	299	10 (30)	4.3 (13)
Sex	Male	20	5 (1)	63	3.2 (2)	4.8 (3)	3.2 (2)	133	11.2 (15)	2.3 (3)
	Female	7	–	62	3.2 (2)	6.5 (4)	1.6 (1)	166	9.0 (15)	6.0 (10)
Age	Adult	23	4.3 (1)	108	3.7 (4)	4.6 (5)	2.8 (3)	278	9.7 (27)	4.7 (13)
	Juvenile	4	–	17	–	11.8 (2)	–	21	14.3 (3)	–
Land gradient	Forest	8	–	10	–	–	–	47	10.6 (5)	2.1 (1)
	Urban	–	–	–	–	–	–	51	17.6 (9)	7.8 (4)
	Peri-urban	18	5.5 (1)	36	–	2.8 (1)	2.8 (1)	100	7 (7)	4 (4)
	Rural	1	–	79	5.1 (4)	7.6 (6)	2.5 (2)	99	9.1 (9)	4.04 (4)

All three parasites were found in *R. rattus*. *Babesia* spp. and *Plasmodium* spp. were found in a greater percentage among females (6.5%) and male (3.2%) animals, respectively. Positive animals were mostly of adult age. In peri-urban areas, *Babesia* spp. (2.8%, n=1, 95% 0.07-15) and *Plasmodium* spp. (2.8%, n=1, 95% 0.07-15) were found at equal percentages, whereas in a rural area, *Babesia* spp. (7.6%, n=6, 95% 3-16) was more prevalent than the other two parasites (Table 1).

No *plasmodium* spp. was detected in house shrews. Males (11.2%, n=15, 95% 6-18) were more infected by *Anaplasma* spp. whereas females (6.0%, n=10, 95% 3-11) were more infected with *Babesia* spp. Shrews were captured from all landscape gradients. But the detected two hemoprotozoa were higher in urban areas (Table 1).

DISCUSSION

The diversity of blood parasites found in rodents and shrews of this study could not be neglected. Blood parasites of rodents and shrews have not been previously reported from Bangladesh, and this information is important for human health, as well. Altogether, 13% of captured mammals were harboring hemoprotozoa, which includes 14.38% of shrews, and 9.87% of rodents. A previous study conducted on domestic rats in Nigeria found 16% prevalence of hemoprotozoa (Dada, 2016). Besides, 22.9% prevalence of hemoprotozoa in rodents has been reported from Tanzania (Katakweba, 2018). Another study conducted on small mammals on the borderland of boreal and temperate forest zones found 32.5% of common shrews, and 41.2% of Eurasian water shrews were infected with blood parasites (Karbowiak *et al.*, 2005). Moreover, rodents and shrews from selected localities in Tanzania and Swaziland reported 31.3% and 1.33% prevalence of blood parasites, respectively (Katakweba *et al.*, 2012). The variation in the percent positive is likely due to geographical difference, species of parasites identified, availability and distribution of tick vector in the study area, methods used to detect and identify the parasites (Katakweba, 2018).

Among different hemoprotozoa identified, 7.5% were *Anaplasma* spp., 4.7% were *Babesia* spp. and 0.67% were *Plasmodium* spp. The prevalence of hemoprotozoa in rats (11.2%) is lower than the findings by Ajayi *et al.* (2006) from Nigeria (63.08%). This is also true for mice, in which 3.7% prevalence was found, comparatively lower than the previous report (Ajayi *et al.*, 2006; McDonogh *et al.*, 2015). Rats can be infected by *Plasmodium* spp. following the infective bite of *Anopheles* mosquito. The study presented here found a higher *Babesia* spp. prevalence in rats compared to mice, in contrast to a previous study in Lahore, which found greater prevalence and higher in mice than rats (Ahmad *et al.*, 2011). *Anaplasma* spp. was found in a higher percentage (10.03%) in shrew, which is similar to previous reports (Bray *et al.*, 2007), whereas *Babesia* spp. was found higher in the rat in this study. Some investigators reported *Babesia* spp. as a rare parasite in rodents (Wiger, 1979).

Anaplasma spp. was found only in adult rats, which may be due to their diverse feeding habits, making them more prone to parasitic infections. Male and juvenile shrews were more infected by *Anaplasma* spp. in this study. Multiple factors like abundance and population structure of the tick vector, the climatic, and ecological features, including the sampling period, might be considered for the variation in parasitism (Matei *et al.*, 2018). *Anaplasma* spp. is transmitted by multiple tick species, including genus *Ixodes* which are available in Bangladesh (Roy *et al.*, 2018). Further detailed epidemiological investigations are required to determine the vector tick species and pathogen carried by ticks in Bangladesh.

Male and adult mice were parasitized more with *Babesia* spp., which corresponds to a prior study (Habicht *et al.*, 1983). Hemoprotozoa were observed only in mice captured from peri-urban areas. The presence of *Babesia* spp. in rats was higher in females, in the juvenile animals, and rural areas. Similarly, *Babesia* spp. was also found in female shrews from urban areas. Higher

percentage of adult animals (7.6%) were parasitized by *Babesia* spp. in this study. A study conducted in the Indo-Chinese peninsula found a similar pattern for helminth infection on Murid rodents (Chaisiri *et al*., 2015). Older hosts get more expose and acquire the parasite infections than juveniles. Moreover, for foraging and breeding purpose, adult animals exposed in the large area that gives more opportunity to come in contact with infective stages of parasites (Rossin *et al*., 2009; Kataranovski *et al*., 2011).

Plasmodium spp. infection was higher in male rats and only found in adults. The percentage of *Plasmodium* spp. was similar in both peri-urban and rural areas, and no infection was found in the shrew. *Plasmodium* spp. infection was also recorded in the urban rat population in Peninsular Malaysia (43.3%) (Alias *et al.*, 2014). The present study reported the prevalence of *Plasmodium* as 0.67%, though there is no significant variation in relation to sex and age, but a higher prevalence found among males.

In this study, *Plasmodium*, *Babesia*, and *Anaplasma* species were identified based on morphological characters under a compound microscope using a blood smear. But there are some inherent limitations. It was sometimes challenging to distinguish *Babesia* from the early stage of trophozoite (ring form) of *Plasmodium* parasites (Teal *et al*., 2012). Usually, less than 1% of erythrocytes become parasitized in the early course of infection that may cause missing of parasites in the blood smear. Molecular characterization of the organisms should be done for confirmation. Although *Babesia* spp. is zoonotic, but only limited species are responsible for this. However, the findings of this study will enrich the literature and can be used as a baseline for future research on hemoprotozoa in house shrews and rodents. Further research with larger sample size and molecular characterization is highly recommended for a better understanding of the ecology, epidemiology, and risk factors of hemoprotozoal infection in rodents and shrews.

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Conflict of Interests

The authors declare that they have no conflict of interests.

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