Asian J. Med. Biol. Res. 2015, 1 (3), 442-448; doi: 10.3329/ajmbr.v1i3.26451

Asian Journal of Medical and Biological Research
ISSN 2411-4472 (Print) 2412-5571 (Online)
www.ebupress.com/journal/ajmbr

Article

Prevalence of haemoprotozoa in fruit bats (*Pteropus giganteus*), small bats (*Megaderma lyra*) and rodents in Bangladesh

Md Rakib uddin[1], Hossain Kabir[2], Khurshida afrin[3], Nusrat sultana[4] and Sharmin Akter[5*]

[1]Department of Microbiology and Veterinary Public health, Chittagong Veterinary & Animal Sciences University, Bangladesh
[2] Faculty of Veterinary Medicine, Chittagong Veterinary & Animal Sciences University, Bangladesh
[3]Department of Animal Science and Nutrition, Chittagong Veterinary & Animal Sciences University, Bangladesh
[4]Faculty of Food science and Technology, Chittagong Veterinary & Animal Sciences University, Bangladesh
[5]Department of Medicine and Surgery, Chittagong Veterinary & Animal Sciences University, Bangladesh

[*]Corresponding author: Sharmin Akter, Chittagong Veterinary & Animal Sciences University, Bangladesh. E-mail: sharmin_rhima@yahoo.com

Received: 03 November 2015/Accepted: 10 December 2015/ Published: 30 December 2015

Abstract: The field study was undertaken between 2010 to 2013 by joint research team of Ecohealth Alliances and ICDDR'B and laboratory work was done during the period of June, 2013 to February, 2014 in Pharmacology laboratory of Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong, Bangladesh to find out the prevalence of haemo-protoza in blood smears of fruit and small bats. Giemsa was used to stain the pre-prepared slides and emulsion oil was used to visualize the protozoa under microscope. A total of 350 blood smears were included in the study and the prevalence of *Babesia* and *Plasmodium* were 2.65%, 2.32%, and 6.25%, 0%, respectively in fruit bats (*Pteropus giganteus*) and small bats (*Megaderma lyra*). The highest prevelence of *Babesia* was found in Khagrachari (6.90%) and lowest in Chakaria (0), Dinajpur (0) and Ramnagar (0) but the highest prevelence of *Plasmodium* was found in Faridpur (4.76%) and lowest in Khagrachari (0), Rangpur (0), Chakaria (0) and Dinajpur (0). The prevalence in both fruit and small bat were found no significant (*p>0.05*) variation. It is necessary to conduct more research on haemoparasties of bats due to public health concern.

Keywords: fruit bats; small bats; haemoprotozoa; prevelence; *Babesia; Plasmodium*

1. Introduction

Bangladesh is a moderately hot and humid country with short winter and prolonged rainy season. The geo-climatic condition of Bangladesh is suitable for the development and survival of various parasites within the wild mammal. Bats in tropical lowlands are excellent indicators of ecosystem health and ecosystem change due to their species richness, differential reactions to fragmentation and crucial ecological functions. Bats also provide value to ecosystems as primary, secondary, and tertiary consumers that support and sustain both natural and human dominated ecosystems ranging from the simple to the complex. Bats have long been postulated to play important roles in arthropod suppression, seed dispersal and pollination; as well as maintain the ecosystem. The rich diversity of dietary habits of bats, ranges from species that feed on insects and other arthropods to those that feed on fruit, nectar, and flowers, thus provide valuable ecosystem services (Khan, 1985).

Bangladesh as a deltaic flood plain of recent origin has a variety of habitats suitable for diverse fauna. As many as 125 species of mammals has been reported from the country (IUCN Bangladesh, 2000). This is inclusive of 31 species of bats (Khan, 1985). Sarker, 1988 reported only 17 species while IUCN Bangladesh, 2000 largely based on checklist of Khan, 1985 listed 29 species of bats from Bangladesh. However, they are also associated with diseases deadly to humans. Several highly fatal diseases have been linked to bats. Rabies is perhaps the most well-known disease associated with bats. Along with animals such as dogs, foxes, raccoons, and skunks, bats are one of the primary animals that transmit rabies. In addition, Histoplasmosis is caused by a fungus that grows in soil and material contaminated with droppings from animals, including bats. It is widespread in certain areas of the U.S.A (CDC, 2013).

Fruit bats, also known as 'flying foxes' of the genus *Pteropus* are natural reservoir hosts of the Nipah and Hendra viruses. The virus is present in bat urine and potentially bat feces, saliva, and birthing fluids. Nipah and related viruses are also associated with the same group of bats in Southeast Asia and parts of Africa, although outbreaks of disease in humans have so far been limited to Malaysia, Singapore, India, and Bangladesh (OIE, 2008). Ever since bats have been identified as possible reservoirs of *Trypanosoma cruzi*, *Babesia* & *Plasmodium* they have been included in medical epidemiological surveys (Tsuji M et al., 2001). This interest has been fuelled by the presence of bat-specific blood protozoa. In previous decade large efforts given upon regarding the bats could be reservoirs for human pathogenic haemoparasites have been undertaken. So the knowledge is essentially need for adequate risk assessments about chances of disease transmission, either it is transmittable between bats and humans. The incidence of infectious diseases, especially general vector-borne parasitism, often increases as species diversity decreases.

Haemoprotozoa are protozoan parasites living within blood cells or free in the blood of their hosts. These blood stages are usually part of complex life cycles, which may include both sexual and asexual reproduction in a wide range of cells and tissues of the same host or in a second, vector, host required for the parasite's transmission. The haemoprotozoa of bats were first reported at the end of the nineteenth century in Italy; Dionisi, 1899 who examined bats as possible hosts of human malaria. Previous records of haemoparastie of bats have been limited to localized studies involving a small number of bats, representing only four of the 15 British species. Several species of protozoa was identified in bat, among them *Babesia, Trypanosoma and Plasmodium* of several species are pronounced (Coles, 1914). In the genus of *Babesia* the *B. vesperuginis* is found in bats (Baker *et al.*, 1963).

The results demonstrate that, *B. vesperuginis* can be pathogenic in its natural host because of splenomegaly, depressed blood hemoglobin, debilitation and possibly haemoglobinuria. Congestion of the spleen resulting from *B. vesperuginis* infection could interfere with vascular changes. During these periods the spleen acts as an erythrocyte reservoir, permitting alterations to the blood composition, and probably easing the workload of the heart; release of erythrocytes from the spleen reverses this condition as soon as arousal begins (Worth, 1932). The genus *Polychromophilus* (one of the genus of the *Hemosporidia*) was first described by Dionisi, 1899 who named two species *P. murinus* and *P. melanipherus. Polychromophilus* a malaria parasite of Chiropteran was first reported in England (Gardner *et al.,* 1987). As far known, no study was conducted to evaluate the prevalence of haemoprotozoal infection in bats of Bangladesh. So the present study was undertaken with regarding the following objectives**:**

a) To know the prevalence of haemo-parasite in fruits and small bats
b) To know the prevalence of bat haemo-parasite at different location in Bangladesh

2. Materials and methods

2.1. Study area

The study was carried to measure the prevalence of blood parasitic diseases in fruit and small bats of Khagrachari, Faridpur, Rangpur, Dinajpur districts and Chakaria, and Ramnagar upazila of Bangladesh.

2.2. Study period

The field study was undertaken between 2010 to 2013 by joint research team of Ecohealth Alliances and ICDDRB and laboratory work was done during the period of June, 2013 to February, 2014 in Pharmacology laboratory of Chittagong Veterinary and Animal Sciences University (CVASU), Chittagong, Bangladesh.

2.3. Source population

A total of 350 blood smears were collected randomly from fruit bats and small bats from Khagrachari, Faridpur, Chakaria, Rangpur, Dinajpur and Ramnagar during the study period.

2.4. Processing of bats

a. At first live bats were placed in a cotton bag and kept in a cool dry place until sampling time. Then bat weight was taken in bags using a Pesola hanging scale. After that the bat was removed from the bag for sampling and bag weight taken and subtracted from previous total
b. Then manually restrain the bats during collection of blood. Three peoples were used for these manipulations, one person used for safely restrain the bat, one person for to take samples and third one to manage the tubes and record samples
c. Ketamine (5mg/kg) was used for anesthesia of bat. At that time the person was restraining the bat were monitored respiration. At that time one of the handler also monitor any type of severe stress
d. Bat that had not any disease or abnormality in health was selected for sample collection. The animal's health and safety is always more important than collecting samples. It can be mentioned that bats can die from stress at any time
e. In live bats caution was taken to maintain a ratio not greater than 10 µl of blood to 1 gm of bat body weight which is equivalent to 1% of bodyweight
f. During the time of blood collection pressure was applied to the site of bleeding using a cotton ball until bleeding ceased (approximately 1 minute). Then the collected blood was placed into conical eppendorf tubes
g. Blood smears from each bat were collected from wing vein by puncturing with sterile needle. The slides were touched to the coming out blood and then spread by another slide. The slides were air dried and fixed by 100% methyl alcohol for 2 min.

2.5. Staining and examination of blood smears

The prepared blood smears were stained with the Giemsa stain (working solution) for 25 to 30 minutes. After rinsing with water of the stained blood smears, they were air dried and examined under microscope (10x, 100x) with immersion oil for the identification of blood parasites as described previously by Soulsby, 1986.

2.6. Identification of parasites

Identification of following haemo-parasites was done based on the salient characteristics found in microscopic examination:

***Babesia* spp.**

The parasites in the erythrocytes were ring shaped, oval bizarre amoeboid shapes (Kahn C.M., 2005), frequently appearing vacuolated (Figure 3a). Ring or oval-shaped parasites usually had a single nucleus on their periphery, and larger parasites had 2 or 3 nuclei (Figure 3b).

***Plasmodium* spp.**

Numerous inclusion bodies were seen. They were circular or oval in section (Figure 4), oval forms, bounded by discrete walls and containing fibrous material (Beers et al., 2004). The bodies were found lying singly or in aggregations within the cell cytoplasm. Both single bodies and the aggregations appeared to be enclosed in a membrane of host origin (Beers et al., 2004).

2.7. Statistical analysis

The data obtained were imported in the Excel-2007 and transferred to the STATA/IC-11.0 software. Descriptive study was done to find out the percentages and Chi-square test was done to compare the prevalence of haemo-parasites within species and location. The significant level was anticipated when the value of $p<0.05$.

3. Results

Table 1 shows analysis among the positive case where the prevalence of haemo-parasites in the fruit bat (86%) is comparatively greater than the small bats (14%). In terms of locations highest prevalence was found in Faridpur district (36%) followed by Ramnagar (22.85%), Dinajpur (17.14%), Chakaria (10.28%), Khagrachari (8.28%) and Rangpur (5.42%) respectively. From the total blood smears 94.85% (N=350) of the sample is found as a negative and rest of them are positives in relation to the presence of haemo-protozoa.

Table 1. Descriptive analysis of species, location and haemo-parasites of Bats (n=350).

Variable	Category	Percentage (%)	SEM	95% CI
Species	Fruit Bats	86	1.84	82.66-89.90
	Small Bats	14	1.84	10.09-17.33
Location	Khagrachari	8.28	1.47	5.38-11.18
	Rangpur	5.42	1.21	3.04-7.8
	Chakaria	10.28	1.62	7.08-13.48
	Dinajpur	17.14	2.01	13.17-21.11
	Faridpur	36	2.56	30.94-41.05
	Ramnagar	22.85	2.24	18.43-27.27
Parasities	Negative	94.85	1.18	92.53-97.18
	Babesia spp.	3.14	0.93	1.30-4.97
	Plasmodium spp.	2.0	0.74	0.52-3.47

CI=confidence interval

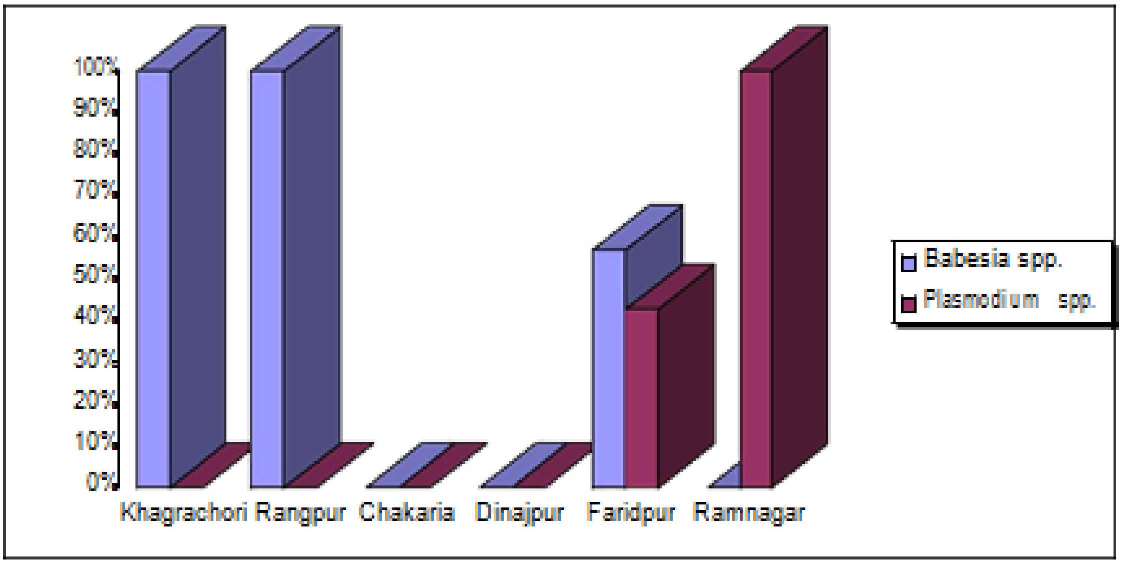


Figure 1. Distribution of haemo-parasitic infection in bats of different areas.

Table 2. Comparative scenario of parasitic infection on different species and locations (n=350).

Variable	Category	Percentage (Positive)		P-value
		***Babesia* spp.**	***Plasmodium* spp.**	
Species	Fruit Bats	2.65(8)	2.32(7)	0.24
	Small Bats	6.25(3)	0	
Location	Khagrachari	6.90(2)	0	0.02
	Rangpur	5.26(1)	0	
	Chakaria	0	0	
	Dinajpur	0	0	
	Faridpur	6.35(8)	4.76(6)	
	Ramnagar	0	1.25(1)	

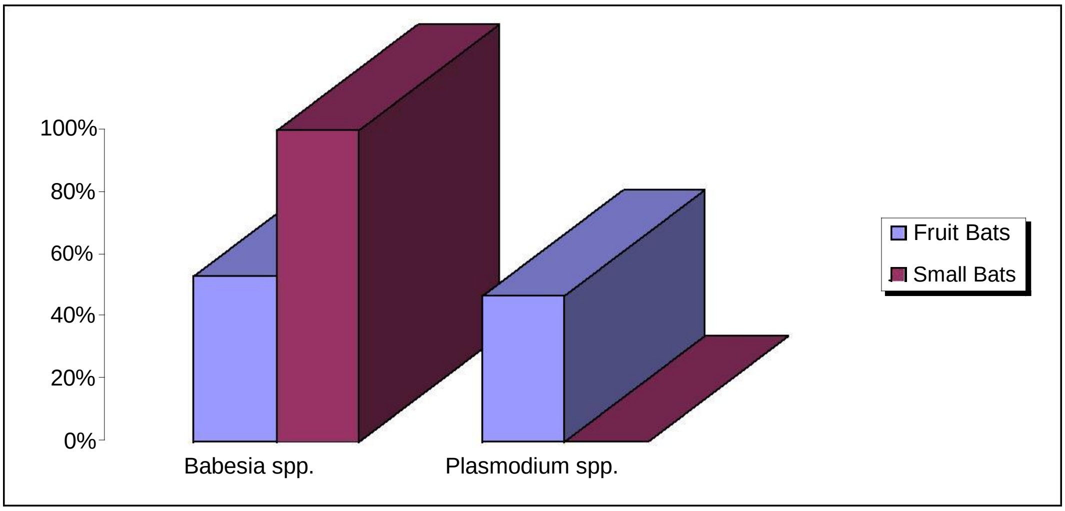
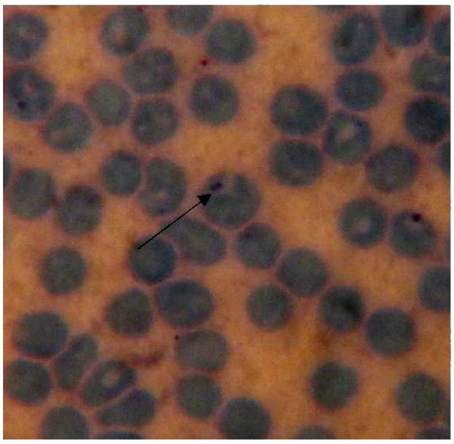
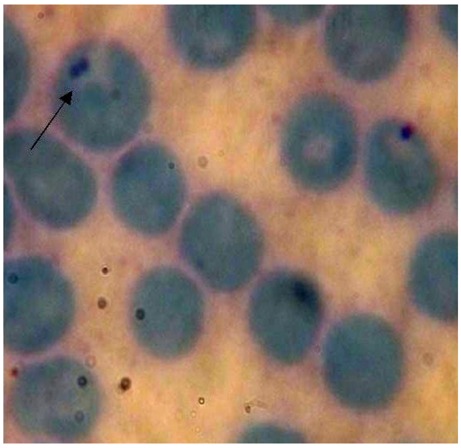


Figure 2. Haemo-parasitic infection in different bat species.



3(a)



3(b)

Figure 3. *Babesia* spp.

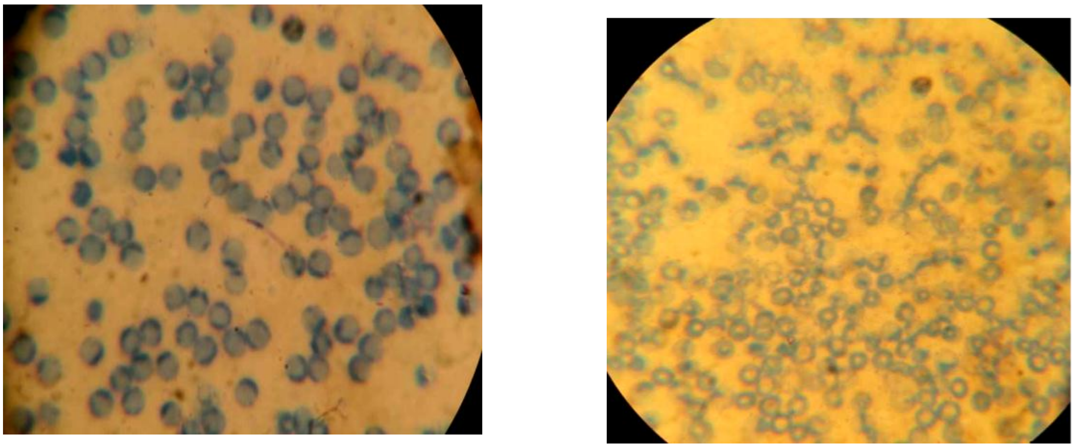


Figure 4. *Plasmodium* spp.

The overall prevalence of *Babesia* within the positive samples were bit higher in small bats (6.25%) than fruit bats (2.65%) and there were significant variation in prevalence of *Babesia*, on the other hand prevalence of *Plasmodium* was higher in fruit bats (2.32%) than small bats but the differences were not significant ($p > 0.05$). On the basis of location, the highest prevalence of *Babesia* was found in Khagrachari (6.90%) and lowest in Chakaria, Dinajpur and Ramnagar but the highest prevalence of *Plasmodium* was found in Faridpur (4.76%)

and lowest in Khagrachari, Rangpur, Chakaria and Dinajpur. The overall prevalence of *Babesia* and *Plasmodium* within location varies significantly (*p< 0.05*) (Table 2).
In case of graphical distribution of haemo-parasite, *Babesia* was found much higher in the Khagrachori and Ranpgur district comparatively to other locations of Bangladesh. But in case of *Plasmodium,* Ramnagar represents the highest percentages of positive samples (Figure 1). The figure represents the percentages of haemoprotozoa only within the positive samples.
Figure 2 represents the percentage of haemo-protozoa fruit and small bat species. Small bats was found as a highest percentages of prevalence of *Babesia* was higher in small bats in comparison to fruit bats. Other side both bats are infected with *Plasmodium.*

4. Discussion

Prevalence of *Babesia* was found in fruits and small bats of current study. Gardener, 1987 also reported 50% prevalence of *Babesia* within the bats in Cambridgeshire. Monaska *et al*., 2004 reported that the proportional prevalence of *Babesia* infection of bats was 14.94% in Canada which is more than the present study result.
Worth, 1932 reported that 63.73% bats were infected with *Babesia* spp. in Mexico which is more than the study result in Bangladesh. This variation may be varying due to area, duration of study and resistance of the bats.
Mer and Goldblum, 1947 found that the prevalence of *Plasmodium* in fruit bats were 47% which is greater than our present study. Gardener, 1987 found a high proportion (25%) of *P. murinus* in fruit bat. Siemers *et al.*, 2004 reported that after rodents, bats are more susceptible to *Plasmodium* which is nearest about 58%.
In current report the sample is much higher (n=350) including fruit bats and small bats. The overall prevalence of *Babesia* within the positive samples were 6.25% in small bats than fruit bats 2.65% and the infected rate of fruit bats within the positive sample is 86% and in case of small bats it is 14%.
The prevalence of *Plasmodium* in current study is more or less similar with the finding of Garnham, 1966 who found (3-18%), (6-16%) and (3%) in August, September & October respectively. In regards it is not possible to ensure the presence of haemoparasties within the bat without any molecular characterization. This molecular characterization would help to give a significant result.

5. Conclusions

In conclusion, these results appear to be compatible with assumption that the small bats (*Megaderma lyra*) which are the most part, younger than the fruit bats (*Pteropus giganteus*), harbor with parasites of the *Plasmodium* spp., which we consider to be the most archaic, while small bats harbor parasites of the *Babesia* is comparatively more.

Acknowledgements
The author would like to acknowledge the stuffs of the Department of Physiology, Biochemistry & Pharmacology and Department of Pathology and Parasitology, CVASU for their cordial supports during the work.

Conflict of interest
None to declare.

References

Baker JR, D Chitty and E Phipps, 1963. Blood parasites of wild voles, Microtusagrestis, in England. Parasitology, 53: 297-301.
Beers, Mark H., MD, and Robert Berkow, MD, editors. "Extraintestinal Protozoa: Malaria." Section 13, Chapter 161. In *The Merck Manual of Diagnosis and Therapy*. Whitehouse Station, NJ: Merck Research Laboratories, 2004.
Centers for Disease Control and Prevention, 2013. Diseases Spread by Bats*: http://www.cdc.gov/features/bats/.* Accessed on 15-03-2014.
Coles AC, 1914. Blood parasites found in mammals, birds and fishes in England. Parasitology, 7: 17-61.
Dionisi A, 1898. Les parasites endoglobulaires des chauves-souris. AttidellaReale Academia deiLincei, 7: 153-156.
Dionisi A, 1899. La malaria di alcuni specie di pipistrelli. Atti dell Societa per glistudi Malaria, 1133-1173.
Gardner RA and DH Molyneux, 1987. *Babesia vesperuginis*: natural and experimental infections in British bats

(Microchiroptera). Parasitology, 95: 461-469.

Gardner RA, DH Molyneux and RE Stebbings, 1987. Studies on the prevalence of Haematozoa of British Bats. Mammal Review, 17:75-80.

Garnham PCC, 1966. Malaria Parasites and other Haemosporidia.*Oxford: Blackwell Scientific Publications*.

IUCN Bangladesh, 2000. *Red book of threatened mammals of Bangladesh.* Dhaka: IUCN country office, Bangladesh 12-71 pp

Khan MAR, 1985. Wildlife of Bangladesh, a checklist. Dhaka University Journal, 164-174.

Kahn CM, 2005. Merck Veterinary Manual. Merck & Co. Inc. and Merial Ltd.

Mer GG and N Goldblum, 1947. A haemosporidian of bats.Nature, 159: 444.

Monaska HC, HS Gunger and JL Zaugg, 2004. Babesiosis. In: Smith, B. P. (Eds): *Wild Animal Internal Medicine. Mosby*, *Elsevier*, *St. Louis*, pp. 1157.

OIE Technical Disease Card, 2008. www.oie.int/en/animal-health-in-the-world/technical-disease-cards. Accessed on 15-03-2014.

Sarker SU, 1988. Wlidlife of Bangladesh- A Systematic List. The Rico Printers, 21-59.

Siemers BM and HU Schnitzler, 2004. Echolocation signals reflect niche differentiation in five sympatric congeneric bat species. Nature, 429:657-661.

Soulsby EJL, 1986. *Helminths*, *Arthropods and Protozoa of Domesticated Animals*,7th edn. Baillere Tindall, London. pp. 707-717, 729-735.

Tsuji M, Q Wei, A Zamoto, C Morita, S Arai, T Shiota, M Fujimagari, A Itagaki, H Fujita and C Ishihara, 2001. Human babesiosis in Japan: epizootiologic survey of rodent reservoir and isolation of new type of Babesia microti-like parasite. J. Clin. Microbiol., 39: 4316-22.

Worth R, 1932. Observations on the blood and blood-forming organs of certain local Chiroptera. Folia Haematologia, 48: 337-345.