Journal of Threatened Taxa

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ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

COMMUNICATION

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26 July 2020 | Vol. 12 | No. 10 | Pages: 16245–16250
DOI: 10.11609/jott.5466.12.10.16245-16250

Journal of Threatened Taxa | www.threatenedtaxa.org | 26 July 2020 | 12(10): 16245–16250

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

DOI: https://doi.org/10.11609/jott.5466.12.10.16245-16250

#5466 | Received 14 October 2019 | Final received 06 July 2020 | Finally accepted 10 July 2020

Detection of hemoparasites in bats, Bangladesh

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Abstract: A cross sectional study was conducted (2010–2013) to determine the diversity of hemoprotozoa among bats of Bangladesh. Microscopic examination of blood smears (N=533; *Pteropus medius* (377), *Rousettus leschenaultii* (111), *Megaderma lyra* (45)) revealed 9% of bats (95% confidence interval CI: 7–12%) were positive for hemoprotozoa. The overall prevalence of hemoparasites among *P. medius* was 5% (n=20, 95% CI: 3–8%); where *Babesia* sp. was 3% (n=12, 95% CI: 2–5%) and *Hepatocystis* sp. was 2% (n=8, 95% CI: 1–4%). Moreover, 13% of *R. leschenaultii* were positive (n=14, 95% CI: 7–20%) where prevalence of *Babesia* sp. was 10% (n=11, 95% CI: 5–17%) and prevalence of *Hepatocystis* sp. was 3% (n=3, 95% CI: 1–8%). Twenty-nine percent (n=13, 95% CI: 16–44%) of *M. lyra* harbored hemoparasites, among which 20% (n=9, 95% CI: 10–35%) were *Babesia* sp. and 9% (n=4, 95% CI: 2–21%) were *Hepatocystis* sp. The study indicates bats remain important hosts for various zoonotic parasites and suggests further research.

Keywords: *Babesia*, Bangladesh, Bat, Hemoprotozoa, *Hepatocystis*, prevalence.

Editor: Bahar S. Baviskar, Wild-CER Society for Wildlife Conservation, Nagpur, India. **Date of publication:** 26 July 2020 (online & print)

Citation: Islam, S., R.U. Ahmed, M.K. Rahman, J. Ferdous, M.H. Uddin, S. Akter, A.A. Faruq, M.M. Hassan, A. Islam & A. Islam (2020). Detection of hemoparasites in bats, Bangladesh. *Journal of Threatened Taxa* 12(10): 16245–16250. https://doi.org/10.11609/jott.5466.12.10.16245-16250

Funding: The present study was supported by USAID PREDICT project (cooperatve agreement number GHN-A-OO-09-00010-00) and Chattogram Veterinary and Animal Sciences University.

Competing interests: The authors declare no competing interests.

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Author contribution: Conceptualization, validation, project administration, investigation and supervision: AI and AI; Methodology and data curation: RUA, MKR, MHU, SA, AAF, AI & AI; Formal analysis: SI & JF; Writing—Original draft: SI & JF; Writing—Review & editing: SI, JF, MMH & AI. All authors have read and agreed to the published version of the manuscript.

Acknowledgements: This study was made possible by the support of the American people through the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT project (cooperative agreement number GHN-A-OO-09-00010-00). We thank the Bangladesh Forest Department and the Ministry of Environment and Forest for permission to conduct this study. We are also grateful to International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), and its core donor, the Governments of Australia, Bangladesh, Canada, Sweden, and the UK for providing unrestricted support to icddr,b. We thanks to Peter Daszak, Jonathan H. Epstein, Kevin J. Olival, Melinda K. Rostal, Emily S. Gurley, Najmul Haider, Tapan Kumar Dey, Abdul Hai, Pitu Biswas, and Gafur Sheikh for their contributions to this study.

INTRODUCTION

Bats, classified under the order Chiroptera, have long been postulated to play an important role in arthropod suppression, seed dispersal, and pollination. The rich diversity in bat dietary habits assists in maintaining ecosystem health. In Bangladesh, 31 bat species are found, three of which are fruit-eating. Of all frugivorous bats, *Pteropus medius* and *Rousettus leschenaultii* are common and widely distributed in the country. The False Vampire Bat *Megaderma lyra*, largest of insectivorous bats, is also quite common and widespread in Bangladesh (Khan 2001).

Bats are associated with zoonotic transmission of coronaviruses including severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), middle-east respiratory syndrome coronavirus (MERS-CoV), Ebola, Nipah, and Hendra viruses (Calisher et al. 2006; Zhang & Holmes 2020), as well as malaria-causing protozoa like *Plasmodium* sp., *Hepatocystis*, *Nycteria*, and *Polychromophilus* (Schaer et al. 2013). Among nine hemosporidian genera, *Hepatocystis* infects a wide range of hosts including primates, bats, ungulates, and rodents, in addition to *Plasmodium* (Manwell & Kuntz 1966). Parasites of seven other hemosporidian genera, however, have been found exclusively in bats, emphasizing that they might harbor the most diverse set of hemosporidian parasites within the mammalian clade. The prevalence of hemosporidian parasites among fruit and insectivorous bats has been detected previously to be 40% (Schaer et al. 2013). *Hepatocystis* sp. was identified from a species of flying fox, *P. hypomelanus* (Olival et al. 2007), displaying an unusually high diversity and is also prevalent in Epauletted Fruit Bats *Epomophorus wahlbergi* (Schaer et al. 2013).

In light of these findings, bats have been identified as possible reservoirs of hemoprotozoa. They are included in epidemiological surveys, and particularly for the detection of bat-specific blood protozoa. Due to the gross destruction of habitat with rapid urbanization, contact between human and bats is showing an increasing trend. Frugivorous bats usually suck the juice of fruits instead of eating the whole fruits. They may play an important role in the transmission of infectious agents to rural communities, particularly small children, who collect those bat-wasted fruits (Rahman et al. 2012). In addition, ectoparasites which feed on hemoprotozoa-infected bats, could serve as a route of transmission to humans. The potential public health threats posed by bats thus suggests the importance of studying hemoprotozoa towards its proper control and better management of human diseases related to bats. Maximum research has led on emerging viruses in bats; however, bacterial and parasitic agents in bats have been least studied and most neglected. For a better understanding of parasitic pathogens in bats, we conducted this study to identify the hemoparasites of bats in Bangladesh.

MATERIALS AND METHODS

As part of a larger study through the United States Agency for International Development (USAID) Emerging Pandemic Threats PREDICT project and associated Ecology of Nipah virus survey, we captured bats in seven districts within or near human settlements across Bangladesh (Figure 1). A total of 533 (*P. medius* 377, *R. leschenaultii* 111, *M. lyra* 45) blood samples were collected randomly from bats during 2010 and 2013. The methods of bat sampling, species identification; age, weight, sex, physiological, and reproductive status determination were done based on PREDICT One Health Consortium (2017) and Epstein et al. (2008). The bats were released immediately after sample collection.

Blood smears were stained with Romanowsky-Giemsa solution (working solution) for 25–30 minutes, examined by an Olympus BX61 light microscope (Olympus, Shinjuku Monolith, 2-3-1 Nishi-Shinjuku, Shinjuku-ku, Tokyo 163-0914, Japan) equipped with Olympus DP70 digital camera (Olympus, Tokyo, Japan) and imaging software AnalySIS FIVE (Olympus, Tokyo Japan). A skilled parasitologist examined one blood film from each bat. Approximately 100 fields were examined at low magnification (400), and then at least 100 fields were studied at high magnification (1,000). In total, the approximate number of screened red blood cells was 5×10^5 for each blood film. The intensity of infection was estimated as a percentage by counting the number of parasites per 10,000 erythrocytes examined, as recommended (Godfrey et al. 1987). Parasites were identified using previously published works (Marinkelle 1996; Olival et al. 2007). The data were recorded in MS Excel-2007 (Microsoft Corporation, Redmond, WA 98052-6399 USA) and transferred to the STATA/IC-13.0 software (StataCorp, 4905, Lakeway Drive, College Station, Texas 77845, USA).

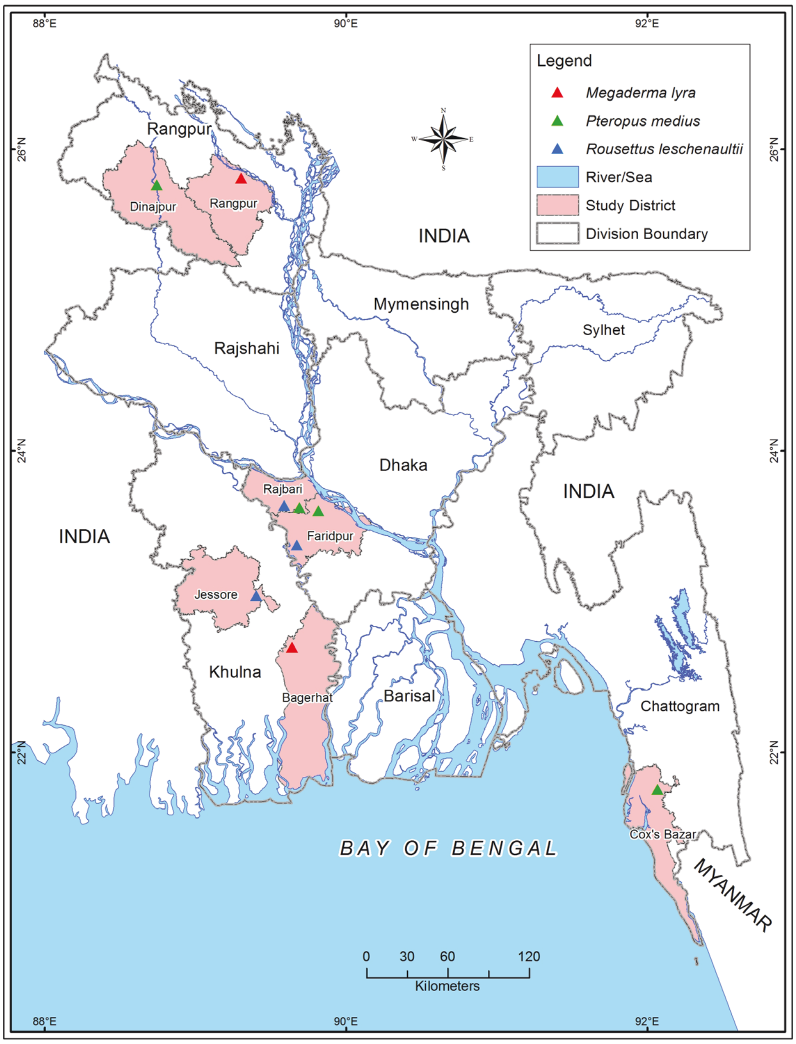

Figure 1. Bat sampling sites in Bangladesh 2010–2013.

RESULTS

Nine percent (n=47; 95% CI: 6.6-11.6%) of the total sample was found to be positive for hemoprotozoa. The overall prevalence of hemoprotozoa was 5%, 13%, and 29%, respectively in *P. medius* (n=20, 95% CI: 3–8), *R. leschenaultii* (n=14, 95%CI: 7–20), and *M. lyra* (n=13, 95%CI: 16–44).

In *P. medius*, *Babesia* sp. was found at the same percentage in both sexes (3%), *Hepatocystis* sp. was found higher in females (3%). The prevalence of *Babesia* sp. was higher in adults (4%) while *Hepatocystis* sp. prevalence was higher in neonates (6%). Both *Babesia* sp. (4%) and *Hepatocystis* sp. (3%) prevalence were higher in peri-urban area compared to rural settings (Table 1). In *M. lyra*, male were more infected (25%) by *Babesia* sp. than females (16%) whereas *Hepatocystis* sp. infection was higher in females (12%) than in males (5%). On the other hand, *Babesia* sp. infection is more prevalent in adult *M. lyra* (20%) and bats of rural areas (20%) than *Hepatocystis* sp. (9%) (Table 1). In case of *R. leschenaultii*, *Babesia* sp. infection was higher in males (13%) than in females (6%) but *Hepatocystis* sp. was found to be at higher percentage in females (4%) than males (2%). Juveniles were more prone to *Babesia* sp. (13%) than adult bats (8%). No *Hepatocystis* sp. infection was found in juveniles. In rural areas, *Babesia* sp. infection was more frequent (10%) than *Hepatocystis* sp. (2.7%). No associations, however, were found to be statistically significant (Table 1).

DISCUSSION

To the authors' knowledge, this is the first study to report the prevalence of hemoprotozoa in bats of Bangladesh. The study identified *Babesia* sp. and *Hepatocystis* sp. in three different bat species (Figure

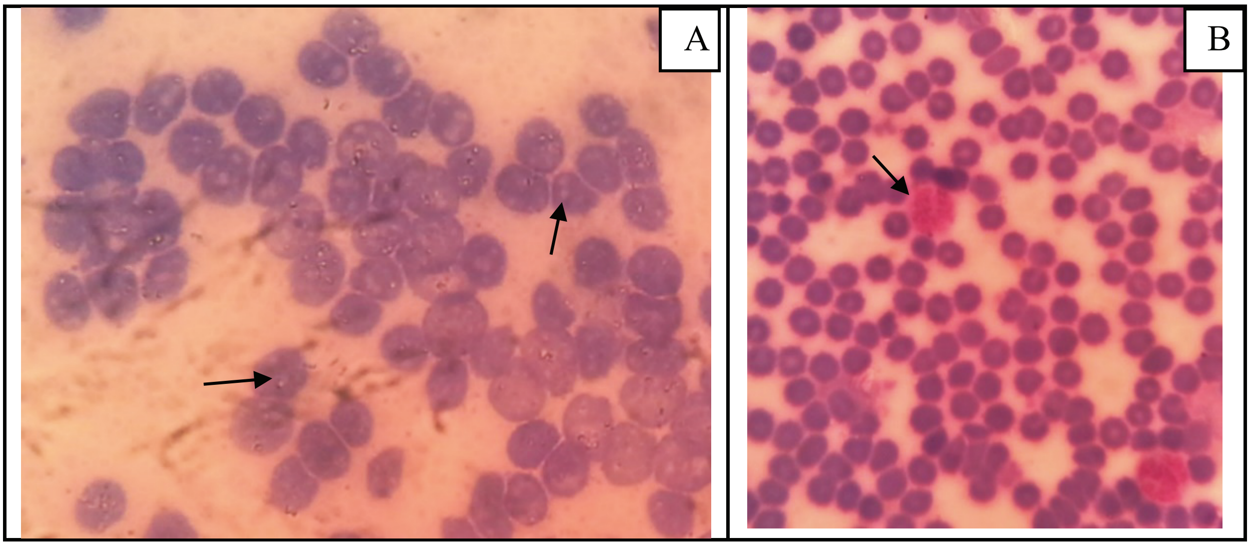

Image 1. A—*Babesia* sp. | B—*Hepatocystis* sp. found in microscopic examination of *Pteropus medius* from Bangladesh. © Rakib Uddin Ahmed, Abdullah Al Faruq & Sazeda Akter.

Table 1. Prevalence of hemoparasites in 03 bat species (N=533) from Bangladesh (2010–2013).

Bat species	Variables (n)	Babesia % (n)	95% CI	Hepatocystis % (n)	95% CI
P. medius	Male (211)	3 (7)	1.3–6.7	1.4 (3)	0.3–4.1
	Female (166)	3 (5)	0.9–6.8	3 (5)	0.99–6.9
	Adult (199)	4 (8)	1.8–7.8	1 (2)	0.1–3.6
	Sub-adult (143)	2 (3)	0.4–6.0	3 (4)	0.8–7.0
	Juvenile (35)	3 (1)	0.07–14.9	6 (2)	0.7–19.2
	Peri-urban (237)	4 (10)	2.0–7.6	3 (6)	0.9–5.4
	Rural (140)	1 (2)	0.2–5.1	1 (2)	0.2–5.1
Sub-total	377	3 (12)	1.7–5.5	2 (8)	0.9–4.1
M. lyra	Male (20)	25 (5)	8.7–49.1	5 (1)	0.1–24.9
	Female (25)	16 (4)	4.5–36.1	12 (3)	2.6–31.2
	Adult (45)	20 (9)	9.6–34.6	9 (4)	2.5–21.2
	Rural (45)	20 (9)	9.6–34.6	9 (4)	2.5–21.2
Sub-total	45	20 (9)	9.6–34.6	9 (4)	2.5–21.2
R. leschenaultii	Male (62)	12.9 (8)	5.7–23.9	2 (1)	0.04–8.7
	Female (49)	6.1 (3)	1.3–16.9	4 (2)	0.5–14.0
	Adult (103)	9.7 (10)	4.8–17.1	3 (3)	0.6–8.3
	Juvenile (8)	12.5 (1)	0.3–52.7	-	-
	Rural (111)	9.9 (11)	5.1–17.0	3 (3)	0.6–7.7
Sub-total	111	9.9 (11)	5.1–17.0	3 (3)	0.6–7.7
Total (N)	533	6.0 (32)	4.1–8.4	3 (15)	1.6–4.6

2). The identified hemoparasites in bats are similar to other reports from bats globally (Hornok et al. 2015; Manwell & Kuntz 1966; Marinkelle 1996; Olival et al. 2007; Schaer et al. 2015, 2017). Bats have harbored a diverse set of hemosporidian species for centuries (Schaer et al. 2013) and *Hepatocystis* was found to be at a high endemic level in Pteropodidae (Schaer et al. 2017). Although the identified parasite species have not been associated with public health implications, there is evidence of co-infection of primates and crossing of the primate barrier by *Hepatocystis* sp. (Thurber et al. 2013). Furthermore, some of the hemosporidian species from bats resemble rodent mammalian parasites (Schaer et al. 2013). The potential for bat-human, bat-rodent-human, and bat-arthropod-human cross-species transmission of hemoprotozoa is not known but warrants further investigation, particularly as the bat species included in the study are native to Bangladesh and share habitat as well as food and water sources with humans, suggesting potential plausible routes of accidental transmission.

The overall prevalence of blood protozoa (9%) was lower than that of earlier reports from various countries (Nartey 2015; Schaer et al. 2013). Hemoparasites in bats can be found as a result of feeding habits (e.g., feeding on insect vectors from which they may acquire the hemoprotozoa). The prevalence of *B. canis* in bats was reported as 2.7% by Hornok et al. (2015) which is much lower than the present study. Other studies reported 50% (Gardner & Molyneux 1987) and 23% (Lord 2010) prevalence of *Babesia* sp. in bats. Most of the previous studies identified *B. vesperuginis* (Gardner & Molyneux 1987; Marinkelle 1996; Lord 2010) in bat species throughout the world. The role of bats in the ecology of *Babesia* sp. and the vectors involved in transmission of *Babesia* sp. among them warrants further investigation. In the present study, the protozoa were identified up to the level of genus. *Hepatocystis* sp. prevalence was lower in this study than in a previous study in Malaysia (Olival et al. 2007). These findings, however, may vary due to the study area, duration of the study, resistance of bats and lack of bat fly vectors in Bangladesh.

Infection with *Babesia* sp. was higher in males (*M. lyra* and *R. leschenaultii*) whereas in case of *Hepatocystis* sp. the prevalence was higher for females. These differences can be attributed to variation in behavior, feed composition, and body mass between sexes (Wilson et al. 2002). Besides, sex hormone, testosterone increase the susceptibility to parasitism (Wilson et al. 2002). Moreover, parasite development and transmission is favored by the colonial habits of females (Christe et al. 2000). Adult *P. medius* had higher *Babesia* percentage than juvenile, may be due to increased growing host age. Young animals are less susceptible to *Babesia* due to inverse age resistance (Christensson 1989). But the same hemoparasite was higher in juvenile *R. leschenaultii* which can be attributable to the ability of the parasite's vertical transmission. *Hepatocystis* was higher in juvenile *P. medius*, because they have low body mass, naive immune system, and nearly no anti-parasite behavior. The pattern of parasitism in bats, however, should be explored in-depth in future studies.

CONCLUSION

We report a survey of hemoparasites in bats undertaken over three consecutive years at habitat fragmented landscape in human settlements areas in Bangladesh, where the prevalence and diversity of bat-infecting hemosporidian parasites have not been studied before. Molecular screening should be undertaken in future to overlay data in the microscopy with those from molecular biology. Molecular characterization is the only way to definitively confirm the species of a hemoparasite. The findings, however, remain of great interest. Further studies are needed to determine the species of parasites harbored in bats of Bangladesh.

REFERENCES

Calisher, C.H., J.E. Childs, H.E. Field, K.V. Holmes & T. Schountz (2006). Bats: important reservoir hosts of emerging viruses. *Clinical Microbiology Review* 19(3): 531–545.

Christe, P., R. Arlettaz & P. Vogel (2000). Variation in intensity of a parasitic mite (*Spinturnix myoti*) in relation to the reproductive cycle and immunocompetence of its bat host (*Myotis myotis*). *Ecology Letters* 3(3): 207–212.

Christensson, D.A. (1989). Inverse age resistance to experimental *Babesia divergens* infection in cattle. *Acta Veterinaria Scandinavica* 30(4): 453–464.

Gardner, R.A. & D.H. Molyneux (1987). *Babesia vesperuginis*: natural and experimental infections in British bats (Microchiroptera). *Parasitology* 95(3): 461–469.

Godfrey, Jr. R.D., A.M. Fedynich & D.B. Pence (1987). Quantification of hematozoa in blood smears. *Journal of Wildlife Disease* 23(4): 558–565.

Epstein, J.H., V. Prakash, C.S. Smith, P. Daszak, A.B. McLaughlin, G. Meehan, H.E. Field & A.A. Cunningham (2008). Henipavirus infection in fruit bats (*Pteropus giganteus*), India. *Emerging Infectious Diseases* 14: 1309.

Hornok, S., P. Estok, D. Kovats, B. Flaisz, N. Takacs & K. Szoke (2015). Screening of bat feces for arthropod-borne apicomplexan protozoa: *Babesia canis* and *Besnoitia besnoiti*-like sequences from Chiroptera. *Parasites & Vectors* 8(1): 441.

Khan, M.A.R. (2001). Status and distribution of bats in Bangladesh with notes on their ecology. *Zoos' Print Journal* 16(5): 479–483. https://doi.org/10.11609/JoTT.ZPJ.16.5.479-83

Lord, J.S. (2010). Micro and macroparasites of bats (Chiroptera). PhD Thesis, University of Salford, Manchester. http://usir.salford.ac.uk/id/eprint/9737/

Manwell, R.D. & R.E. Kuntz (1966). *Hepatocystis* in Formosan mammals with a description of a new species. *Journal of Protozoology* 13(4): 670–672.

Marinkelle, C.J. (1996). *Babesia* sp. in Colombian bats (Microchiroptera). *Journal of Wildlife Disease* 32(3): 534–535.

Nartey, N.A.N. (2015). Common parasites of fruit-eating bats in Southern Ghana: MPhil Thesis. Department of Animal Biology and Conservation Science, University of Ghana, XV+144pp. http://ugspace.ug.edu.gh/handle/123456789/8892

Olival, K.J., E.O. Stiner & S.L. Perkins (2007). Detection of *Hepatocystis* sp. in Southeast Asian flying foxes (Pteropodidae) using microscopic and molecular methods. *Journal of Parasitology* 93(6): 1538–1540.

PREDICT One Health Consortium (2017). PREDICT operating procedures: Bat sampling methods, 1–13pp. https://ohi.sf.ucdavis.edu/sites/g/files/dgvnsk5251/files/files/page/predict-sop-bat-sampling-2017.pdf

Rahman, M.A., M.J. Hossain, S. Sultana, N. Homaira, S.U. Khan, M. Rahman, E.S. Gurley, P.E. Rollin, M.K. Lo, J.A. Comer & L. Lowe. (2012). Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector-Borne and Zoonotic Diseases* 12(1): 65–72.

Schaer. J., D.M. Reeder, M.E. Vodzak, K.J. Olival, N. Weber & F. Mayer (2015). Nycteria parasites of Afrotropical insectivorous bats. *International Journal of Parasitology* 45(6): 375–384.

Schaer, J., S.L. Perkins, J. Decher, F.H. Leendertz, J. Fahr & N. Weber (2013). High diversity of West African bat malaria parasites and a tight link with rodent *Plasmodium* taxa. *Proceedings of the National Academy of Sciences* 110(43): 17415–17419.

Schaer, J., S.L. Perkins, I. Ejotre, M.E. Vodzak, K. Matuschewski & D.M. Reeder (2017). Epauletted fruit bats display exceptionally high infections with a *Hepatocystis* species complex in South Sudan. *Scientific Reports* 7(1): 6928.

Thurber, M.I., R.R. Ghai, D. Hyeroba, G. Weny, A. Tumukunde & C.A. Chapman (2013). Co-infection and cross-species transmission of divergent *Hepatocystis* lineages in a wild African primate community. *International Journal of Parasitology* 43(8): 613–619.

Wilson, K.R., O.N. Bjornstad, A.P. Dobson, S. Merler, G. Poglayen, S.E. Randolph, A.F. Read & A. Skorping (2002). Heterogeneities in macro-parasite infections: patterns and processes, 6–44. In: Hudson P.J., A. Rizzoli, B.T. Grenfell, H. Heesterbeek, A.P. Dobson (eds.) *The Ecology of Wildlife Diseases.* Oxford University Press, New York, 216pp.

Zhang, Y.Z. & E.C. Holmes. (2020). A genomic perspective on the origin and emergence of SARS-CoV-2. *Cell* 26.

Journal of Threatened Taxa

www.threatenedtaxa.org

PLATINUM OPEN ACCESS

ISSN 0974-7907 (Online) | ISSN 0974-7893 (Print)

July 2020 | Vol. 12 | No. 10 | Pages: 16195–16406
Date of Publication: 26 July 2020 (Online & Print)
DOI: 10.11609/jott.2020.12.10.16195-16406

Editorial

Communications

Short Communications

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