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# Blood Parasites of Frogs From an Equatorial African Montane Forest in Western Uganda

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ABSTRACT: In a survey of blood parasites in Ugandan frogs, 30 (17%) of 180 frogs were infected with at least 1 species of *Hepatozoon* or *Trypanosoma*, or with microfilariae. There were significant differences in the prevalence of parasitism among species, with parasitemia detected in only 3 of 9 species. The intensity of blood parasite infection ranged from 1 to 1,080 infected cells per 5,000 cells examined. Seasonal changes in the prevalence and intensity of parasitemia were not observed, nor was there any association between parasitemia and infection with the pandemic fungus *Batrachochytrium dendrobatidis*.

Frogs have been facing global declines from numerous factors, including disease (IUCN et al., 2008). Parasites can affect host life history and fitness (reviewed by Møller et al., 1990), and frogs are known to host a diversity of parasites (Mitchell, 2007). Although many health and parasite surveys have been conducted on frogs throughout the world (listed in Desser, 2001), few have been conducted in sub-Saharan African forests. One study in the 1960s sampled frogs from Kenya and Tanzania but did not provide any information regarding the actual collection sites (Ball, 1967). Because sub-Saharan Africa contains a diversity of herpetofauna (Channing and Howell, 2006), it is important to understand the parasite community in this region and its potential health effects on frogs. In this report, we present the results of a survey of blood parasites of frogs from Kibale National Park, Uganda. To our knowledge, ours is the first multispecies survey of blood parasites in frogs from this location, and the only recent survey of parasitemia for frogs in an East African montane forest.

Kibale is a 795-km$^2$ park located in western Uganda near the foothills of the Rwenzori Mountains (0°13′–0°41′N, 30°32′E). The park is transitional between lowland rainforest and montane forest (elevation ranging from approximately 1,100 to 1,600 m), consisting of moist semideciduous and evergreen forest that is interspersed with grassland, woodland, lakes and wetlands, colonizing forest, and plantations with exotic trees (Chapman et al., 1997; Chapman and Lambert, 2000). From 1990 to 2001, mean daily minimum and maximum temperatures in Kibale were 14.9 C and 20.2 C, respectively. During the same period, mean rainfall across distinct, bimodal wet and dry seasons was 1,749 mm (Chapman et al., 1999, 2005). Over the last 30 yr, Kibale has experienced discernible climate change, with increased annual rainfall, increased maximum mean monthly temperatures, and decreased minimum mean monthly temperatures (Chapman et al., 2005).

Medium- and high-altitude equatorial African forests such as Kibale hold a great diversity of herpetofauna, including many endemic frog species (Channing and Howell, 2006). Twenty-eight frog species were identified in Kibale from 1995 to 1997 (Vonesh, 2001). The herpetofauna assemblage in Kibale is similar to that of the Guinea–Congolean rainforests to the west (Vonesh, 2001). It is different, however, from that of the coastal mountains of Tanzania, which contain many local endemic frog species. *Batrachochytrium dendrobatidis*, the causative agent of chytridiomycosis, a fungal disease that has been responsible for amphibian die-offs globally, has recently been found in Kibale (Goldberg et al., 2007).

Frogs were collected from locations close to Makerere University Biological Field Station (elevation approximately 1,500 m) in Kibale National Park during the dry seasons in January 2006 and 2007 and June 2006. Collection sites were chosen that had high amphibian densities, based on both local anecdotal reports and previous amphibian surveys (Vonesh, 2001). Frogs were captured by hand or with hand nets and held in disposable plastic bags for processing immediately in the field (rarely) or in the laboratory. All frogs were identified to species and measured for snout vent length, and a drop of blood was obtained via cardiac venipuncture with a heparinized insulin syringe. All frogs were processed within 6 hr of capture and released within 24 hr. All protocols were reviewed and approved by the University of Illinois IACUC and the Uganda Wildlife Authority prior to data collection.

Blood smears were created for each frog, air-dried, and stained using a Wright–Giemsa stain (Hema 3 stain, Fisher Scientific, Pittsburg, Pennsylvania). We viewed smears using oil immersion (×100) by scanning fields until we had examined 5,000 red blood cells. Prevalence and mean intensity of parasitemia were used to describe parasite infections (Margolis et al., 1982; Bush et al., 1997).

To determine differences in blood parasite prevalence among species and sampling periods, and between blood parasite and chytrid fungus prevalence, we used Fisher's exact tests. To examine differences in mean intensity between species and sampling periods, we used Kruskal–Wallis tests. Confidence intervals are better than standard deviation for describing parasite distributions (Rózsa et al., 2000). Confidence intervals for prevalence and intensity were computed using Sterne's exact method, and bootstrapping (with 2,000 repetitions), respectively, using the computer program Quantitative Parasitology 3.0 (J. Reiczigel and L. Rózsa, Budapest, Hungary).

Blood smears were obtained from 180 frogs of 9 species: *Bufo funereus* (n = 5), *Bufo regularis* (n = 2), *Hyperolius kivuensis* (n = 25), *Hyperolius viridiflavus* (n = 6), *Leptopelis christyi* (n = 41), *Leptopelis kivuensis* (n = 55), *Phrynobatrachus graueri* (n = 1), *Ptychadena mascareniensis* (n = 21), *Xenopus wittei* (n = 24). Frogs of all species were adults, with the exception of *L. christyi* and *L. kivuensis*, of which approximately 60% of frogs were post-metamorphic juveniles. All frogs appeared clinically normal at the time of sampling.

Seventeen percent of the frogs examined were parasitemic (Table I). Infections were only found in *L. christyi*, *L. kivuensis*, and *P. mascareniensis* (Table I). From these species, 3 genera of blood parasites were identified, including a hemogregarine that appeared to be *Hepatzoon* sp., microfilariae of undetermined classification, and *Trypanosoma* sp. (Table I). Multiple infections were observed, with all 3 blood parasite types found in a single *L. christyi* individual.

The prevalence of blood parasites (all parasite genera pooled) varied significantly by frog species ($P < 0.001$, Fisher's exact test; only data from the 5 frog species with over 20 individuals were included in the analysis); however, intensity did not vary significantly ($F_{2,30} = 4.96$, $P < 0.084$, Kruskal–Wallis, using only the 3 species infected with parasites). *Hepatozoon* sp. was the most common blood parasite, infecting 14% of all frogs sampled, and was found at widely varying intensities, with over 100 intracellular parasites per 5,000 cells in 5 *L. christyi* (raw individual gamont intensities per 5,000 cells scanned: 123, 232, 315, 348, and 1,080), 1 *L. kivuensis* (230), and 1 *P. mascareniensis* (395). Free gamont forms of *Hepatozoon* sp. were also found in 5 *L. christyi* blood smears (mean intensity [95% CI]: 642.4 [426.0–728.0] free gamonts per 5,000 cells scanned) but were not included in the statistical analyses.

*Hepatozoon* sp. prevalence ($P \leq 0.001$, Fisher's exact test) and *Trypanosoma* sp. prevalence ($P = 0.007$, Fisher's exact test) varied significantly among *L. christyi*, *L. kivuensis*, and *P. mascareniensis*. Only *L. christyi* and *L. kivuensis* were infected with microfilariae. No differences in prevalence or intensity of infection were detected between sampling periods (January 2006, June 2006, January 2007; $P = 0.296$, Fisher's exact test; $F_{2,30} = 0.744$, $P = 0.690$, Kruskal–Wallis test, respectively). It should be noted, however, that 5 of the 7 *Hepatozoon* sp. infections of over 100 intracellular parasites per 5,000 cells, all of the free gamont infections, and all microfilariae were only recovered from frogs collected in January 2007.

Sixty-five frogs that were examined for parasitemia had previously been tested for *B. dendrobatidis* (Goldberg et al., 2007). No parasitemic frog was also infected with chytrid fungus, 12 frogs were infected with chytrid fungus but not blood parasites, and 45 frogs were negative for both

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TABLE I. Prevalence and intensity of blood parasite infections in frogs collected from Kibale National Park, Uganda. Three of 9 species of frogs were infected with at least 1 type of blood parasite. Prevalence estimates are percentages, and mean intensities are expressed as the number of parasites per 5,000 red blood cells. Numbers in parentheses indicate 95% confidence intervals calculated using Sterne's exact method (for prevalence) or bootstrapped with 2,000 replicates (for intensity).

| Frog species | Number examined | Number positive | Prevalence | | | Intensity | | |
|---|---|---|---|---|---|---|---|---|
| | | | *Hepatozoon* sp. | *Trypanosoma* sp. | Microfilariae | *Hepatozoon* sp. | *Trypanosoma* sp. | Microfilariae |
| *Leptopelis christyi* | 41 | 16 | 39.0 (25.4–54.9) | 7.3 (2.0–19.3) | 4.9 (0.9–16.7) | 140 (54.1–343.9) | 2.0 (2.0–2.0) | 4.0 (3.0–4.0) |
| *Leptopelis kivuensis* | 55 | 5 | 1.8 (0.1–9.7) | 3.6 (0.7–12.5) | 3.6 (0.7–12.5) | 230.0 (230.0–230.0) | 1.0 (1.0–1.0) | 1.5 (1.0–1.5) |
| *Ptychadena mascareniensis* | 21 | 9 | 38.1 (19.7–59.7) | 28.6 (13.3–50.6) | 0.0 (0.0–15.9) | 57.8 (7.1–249.3) | 2.0 (1.0–3.0) | 0.0 |
| All species | 180 | 30 | 13.9 (9.4–19.7) | 6.1 (3.2–10.7) | 2.2 (0.8–5.7) | 117.4 (53.1–265.2) | 1.8 (1.2–3.4) | 2.8 (1.5–4.0) |

infections. No association was found between *B. dendrobatidis* infection and parasitemia ($P = 0.333$, Fisher's exact test).

Overall, 17% of all frogs examined were infected with at least 1 blood parasite. This number is lower than previous studies that have documented parasitemia in 40% of East African frogs (Ball, 1967) and 34% of Egyptian frogs (Mohammed and Mansour, 1959). The low prevalence reported in this study may not be unusual, however. Tropical birds, for example, often have a low prevalence of blood parasite infections, possibly due to a low availability of insect vectors in the tropics (discussed in Young et al., 1993).

*Hepatozoon* sp. was the most common blood parasite and was found in 14% of all frogs in the present study. Although the prevalence of *Hepatozoon* sp. in our study was similar to that in frogs from Ontario (18%; Barta and Desser, 1984) and Israel (12%; Desser and Yekutiel, 1986), it was lower than the prevalence of hemogregarine parasites in frogs from Costa Rica (23%; Desser, 2001), East Africa (29%; Ball, 1967), the United States (32%; Levine and Nye, 1977), and Corsica (over 45%; Barta et al., 1989). The results from our study also differed from others in that the prevalence of *Hepatozoon* sp. was higher than that of *Trypanosoma* sp.; many other studies that reported both hemogregarine and *Trypanosoma* sp. infections found that *Trypanosoma* sp. was the most common parasite observed in the frogs (Barta and Desser, 1984; Barta et al., 1989; Desser, 2001). Since the prevalence of hemogregarines and *Trypanosoma* sp. were generally lower in our study compared to others, it reinforces the idea that the overall prevalence of parasitemia in our frogs was comparatively low. Microfilariae have been observed in low numbers in other studies (Levine and Nye, 1977; Barta and Desser, 1984; Desser, 2001).

We found significant differences in the prevalence of parasitemia among frog species, possibly reflecting the different habitat preferences of the host species and consequently different vector abundance and contact rates. Frog *Hepatozoon* spp. are transmitted by ingestion of infected mosquitoes (Desser et al., 1995), and mosquitoes and leeches are vectors for anuran trypanosomes (Desser et al., 1973; Barta and Desser, 1984). Although frogs living in more aquatic environments may have higher blood parasite burdens from higher exposure to insect vectors (Barta and Desser, 1984), our results did not follow that pattern. No *X. wittei* in our study were parasitemic, despite the fact that this species was the only wholly aquatic frog sampled, while *L. christyi*, *L. kivuensis*, and *P. mascareniensis* were parasitemic even though they use aquatic habitats only transiently for breeding (Vonesh, 2001). Differences in sites of capture, however, may have affected parasitemia. For instance, most *L. christyi*, *L. kivuensis*, and *P. mascareniensis* were collected from puddles or bushes alongside a road that bisects previously logged and unlogged sections of Kibale National Park, but all *X. wittei* were collected from drinking wells in forest fragments along the park border, and all *Bufo* and *Hyperolius* spp. were collected from interior unlogged sections the park. Previous studies in this location have found that forest fragmentation and logging have altered parasite prevalence in non-human primates (Gillespie et al., 2005; Gillespie and Chapman, 2006; Salzer et al., 2007). Therefore, it is possible that forest disturbance altered the parasite burdens of frogs as well, possibly through altered vector distributions, or host–vector contact rates.

The intensity of parasitemia varied considerably among individual frogs within species but did not vary significantly among species. Because our study was cross-sectional, it is possible that we captured frogs at different stages of infection. The influence of these parasites on frog health is unknown. All frogs appeared healthy at the time of capture, and no frog declines or large mortality events have been reported in the area despite the presence of *B. dendrobatidis* (Goldberg et al., 2007). Even though some of the frogs in the present study were infected with *B. dendrobatidis*, there was no association between fungal infection and parasitemia. Additionally, a few frogs had very high intensities of *Hepatozoon* sp. parasitemia, but with no visible health effects. The virulence of the blood parasites we identified is, therefore, likely to be low.

The lack of seasonality in parasitemia may have resulted from the fact that all sampling took place during dry seasons. Additional sampling during other seasons of the year as well as across a variety of habitat types could help determine the effects of environmental conditions and habitat on infection dynamics. Experimental studies would likely be needed to assess the virulence of these pathogens for the frogs of Kibale National Park and to determine the possible role of infectious disease as a threat to anuran conservation.

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