

Original Contribution

# A Legacy of Low-Impact Logging does not Elevate Prevalence of Potentially Pathogenic Protozoa in Free-Ranging Gorillas and Chimpanzees in the Republic of Congo: Logging and Parasitism in African Apes

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**Abstract:** Many studies have examined the long-term effects of selective logging on the abundance and diversity of free-ranging primates. Logging is known to reduce the abundance of some primate species through associated hunting and the loss of food trees for frugivores; however, the potential role of pathogens in such primate population declines is largely unexplored. Selective logging results in a suite of alterations in host ecology and forest structure that may alter pathogen dynamics in resident wildlife populations. In addition, environmental pollution with human fecal material may present a risk for wildlife infections with zoonotic protozoa, such as *Cryptosporidium* and *Giardia*. To better understand this interplay, we compared patterns of infection with these potentially pathogenic protozoa in sympatric western lowland gorillas (*Gorilla gorilla gorilla*) and chimpanzees (*Pan troglodytes troglodytes*) in the undisturbed Goulougo Triangle of Nouabalé-Ndoki National Park and the adjacent previously logged Kabo Concession in northern Republic of Congo. No *Cryptosporidium* infections were detected in any of the apes examined and prevalence of infection with *Giardia* was low (3.73% overall) and did not differ between logged and undisturbed forest for chimpanzees or gorillas. These results provide a baseline for prevalence of these protozoa in forest-dwelling African apes and suggest that low-intensity logging may not result in long-term elevated prevalence of potentially pathogenic protozoa.

**Keywords:** *Cryptosporidium*, disease ecology, disturbance ecology, *Giardia*, zoonoses

## INTRODUCTION

Selective logging is the dominant commercial activity in forested areas of the tropics with strong conservation potential (Frumhoff, 1995; Struhsaker, 1997). This potential

is arguably strongest in central Africa, where a combination of weak communications and infrastructure, lack of logistics systems, and high transportation costs result in highly selective timber extraction, focusing on a few economically valuable species occurring at low densities (Boardman et al., 2007; Clark et al., 2009).

During the last decade, the timber industry has thrived in central Africa and many of the remaining forests have been allocated to timber concessions. With extraction rates of 1–4 trees harvested per hectare, damage to remaining forest is relatively limited and disrupts only 7–20% of the canopy (Hall et al., 2003; Van Gemerden, 2003). This is compared to extraction rates of 5–10 trees per hectare and associated disruption of 25–50% of the canopy typical of other forested regions of the tropics (Skorupa, 1987; Chapman et al., 2000). Whereas some species of wildlife thrive in such low-intensity operations, even low-level extraction appears to have a negative effect on some primate species, including chimpanzees (White, 1992; Barnes and Lahm, 1997; White and Tutin, 2001; Matthews, 2004).

Although logging is known to reduce the abundance of some primate species due to associated hunting and the loss of food sources for frugivores (Wilkie and Carpenter, 1998; Chapman et al., 2000), the potential role of parasite infections in such primate population declines remains largely unexplored. Logging results in a suite of alterations in host ecology forest structure and human-wildlife overlap that may alter infection prevalence and infection risk in resident populations. Patterns of parasitism in wildlife populations are influenced by host ranging patterns, density, intraspecific and interspecific contact rates, diet, and immune function (Hudson et al., 2001; Nunn et al., 2003; Gillespie et al., 2005, 2008; Beldomenico et al., 2008). Studies on a variety of species have demonstrated that these characteristics can be affected by changes in forest structure (Olupot et al., 1994; Heydon and Bulloh, 1997; Patriquin and Barclay, 2003).

*Giardia* and *Cryptosporidium* are protozoal pathogens that infect humans, domestic animals, and wildlife worldwide (Appelbee et al., 2005). In humans and livestock, these parasites cause diarrhea and other enteric disorders that can contribute to nutritional deficiencies and impaired weight gain (Savioli et al., 2006; Thompson, 2004). Little information exists about the clinical affects of these parasites on their wildlife hosts; however, environmental pollution with human fecal material is recognized as a potential pathogen pathway for wildlife infections with zoonotic protozoal parasites, such as *Giardia* and *Cryptos-*

*poridium* (Appelbee et al., 2005). Considering the capacity of such infections to put wildlife populations at risk, it is important to monitor the health status of wildlife in general and endangered species in particular. It is equally important to identify potential pathogen transmission pathways from humans and domestic animals to wildlife.

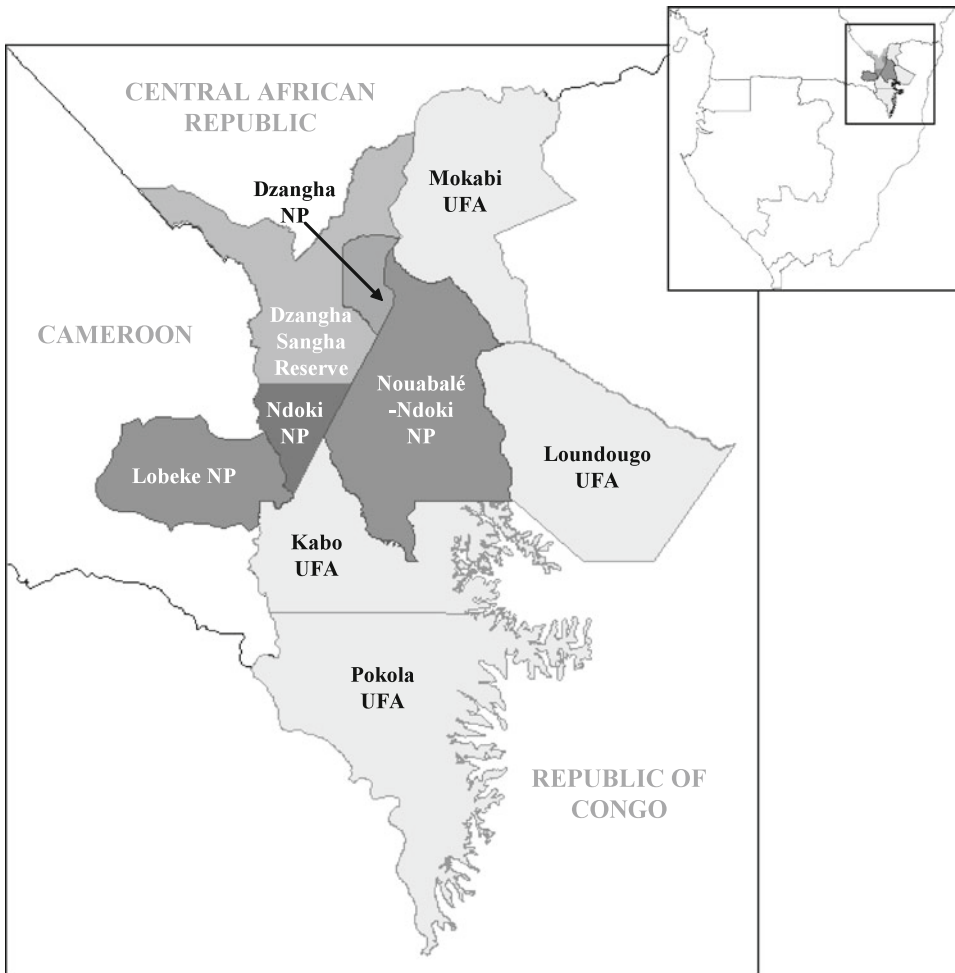
To better understand the affect of selective logging on the transmission and persistence of these protozoal pathogens, we examined noninvasively collected fecal samples from 134 sympatric western lowland gorillas (*Gorilla gorilla gorilla*) and chimpanzees (*Pan troglodytes troglodytes*) in the northern Republic of Congo. We compared patterns of infection between ape samples from the Kabo Logging Concession with those sampled from the adjoining undisturbed forest of the Nouabalé-Ndoki National Park.

## METHODS

### Study Site

The Sangha River Tri-National Conservation Area (36,236 km<sup>2</sup>) is notable for its extensive tracks of forest and transboundary protected area network: Nouabalé-Ndoki National Park in Republic of Congo, Lobéké National Park in Cameroon, and Dzanga-Ndoki National Park and Dzanga-Sangha Special Reserve in Central African Republic (Fig. 1). This region is largely unpopulated with human densities estimated at 0.7 to 0.8 people per km<sup>2</sup> (Auzel and Wilkie, 2000). Semi-nomadic Ba'Aka pygmies and Bantu agriculturalist-fishermen live in complex, interdependent economic and social relationships (Eves and Ruggiero, 2000). Most villages are along major waterways leaving the remote interior forest with little human development and associated pressures. However, commercial logging is rapidly expanding along the periphery of the Tri-National Conservation Area. Timber exploitation in these remote areas is relatively labor-intensive and can result in the transient relocation of skilled workers and their families, introducing the risk of novel opportunities for pathogen transmission from people to resident wildlife.

The Kabo Logging Concession (2800 km<sup>2</sup>), operated by the *Congolaise Industrielle des Bois*, has been logged to varying degrees for the past 40 years. The pristine forests of the adjoining Goulougo Triangle (310 km<sup>2</sup>) were initially part of the Kabo Concession until biological surveys by the Wildlife Conservation Society revealed the conservation significance of this region's great ape populations and their habitat. In June 2003, the Congolese government



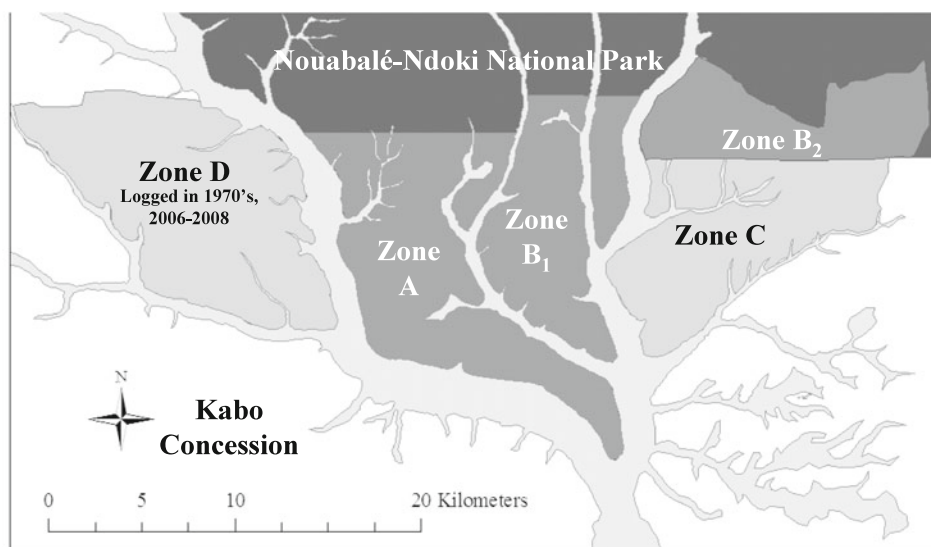
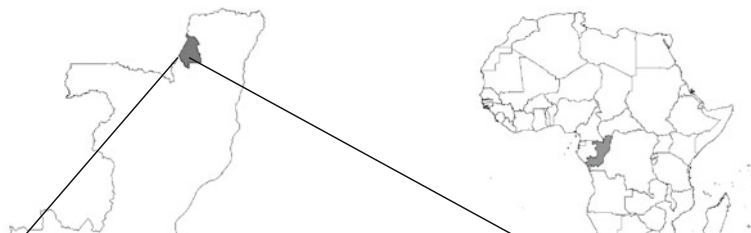
**Figure 1.** Land use in the Sangha Tri-National Conservation Area at the boundary of Republic of Congo, Cameroon, and the Central African Republic. The Goulougo Triangle is located in the southern portion of the Nouabalé-Ndoki National Park. The Kabo Logging Concession is contiguous to the national park's southern boundary.

announced that it would annex the majority of the Goulougo Triangle to the Nouabalé-Ndoki National Park (4400 km<sup>2</sup>, 2.05'–3.03'N, 16.51'–16.56'E). However, the adjacent forest remains in an active logging concession. As part of the Goulougo Triangle Ape Project's mandate to evaluate the impacts of logging on wild apes, this area was stratified into study zones (A, B<sub>1</sub>, B<sub>2</sub>, C, and D) with relation to the national park boundaries and logging activities (Fig. 2). Zones A, B<sub>1</sub>, and B<sub>2</sub> are undisturbed forest within the national park. Zone D was logged in the early 1970s, and zone C is slated for logging in the near future. All study zones are characterized by similar terra firma forest dominated by mixed species interspersed with monodominant *Gilbertiodendron dewevrei*, seasonally flooded forest, and open swamp forests (Morgan et al., 2006).

### Sample Collection and Analysis

Between 2004 and 2009, we conducted repeated reconnaissance and line transect surveys for ape signs in zone D

of the Kabo Logging Concession and zones A and B<sub>1</sub> within the Goulougo Triangle (Morgan et al., 2006, 2009). Our first surveys of zone D were conducted after forestry activities had been dormant for more than 30 years. Ape fecal samples were collected opportunistically in the field and preserved in 10% neutral buffered formalin (Gillespie, 2006). Fecal samples were characterized as chimpanzee or gorilla based on morphological features. At similar sites using similar methods, samples characterized in this fashion were verified to the correct species more than 94% of the time by genetic methods, with field researchers able to correctly identify gorilla dung (318/324 = 98%) more often than chimpanzee fecal remains (200/211 = 94%) (Arandjelovic, 2009, personal communication). When collected, fecal material was characterized as soft or firm depending on the consistency, assuming that sick apes will evacuate soft stools (Gillespie et al., 2008). A Merifluor *Cryptosporidium/Giardia* Direct Immunofluorescent Detection Kit (Meridian Bioscience, Inc., Cincinnati, OH) was used to detect both parasites (Johnston et al., 2003). Fecal samples



**Figure 2.** Location of study zones within the Goulougo Triangle (A, B<sub>1</sub>, and B<sub>2</sub>) and adjacent logging concession (C and D) in Republic of Congo.

were scored both for presence or absence of the pathogens as well as quantification of *Cryptosporidium* sp. oocysts and *Giardia* sp. cysts in feces. Fecal samples were concentrated and then resuspended in 1 g/1 ml suspensions. Counts were calculated by analyzing 10  $\mu$ l of the 1 g/ml solution of concentrated feces from each sample. Oocysts or cysts were then quantified by counting total numbers in 150 microscope fields (400 $\times$  magnification) and extrapolating results to the entire sample. This method was validated by spiking negative fecal samples with known numbers of *Cryptosporidium* sp oocysts and *Giardia* sp. cysts. Results were analyzed using EpiInfo, Version 3.3.2 (Centers for Disease Control, Atlanta, GA). A power analysis was conducted using the Finite Population Correction for Proportions (Israel 1992). The corrected sample sizes are calculated using the original sample size results calculated using the equation for a large population. These numbers are based on a confidence level of 95% and level of precision of 0.05.

## RESULTS

All 134 samples were negative for *Cryptosporidium* sp. (Table 1). Three of 34 chimpanzee samples (8.82%) from the logged site, 1 of 33 chimpanzee samples from the undisturbed site (3.03%), 0 of 31 gorilla samples from the logged site (0%), and 1 of 36 gorilla samples from the undisturbed sites (2.78%) were infected with *Giardia* sp. (Table 1). Prevalence of infection with *Giardia* sp. was not significantly different between logged and undisturbed forest for chimpanzees ( $\chi^2 = 1.00$ ,  $P = 0.32$ , degrees of freedom ( $df$ ) = 1) or gorillas ( $\chi^2 = 0.87$ ,  $P = 0.35$ ,  $df = 1$ ).

Of all 67 chimpanzee samples, one was characterized as soft (1.5%) and the remainder as firm (98.5%). Of all 67 gorilla samples, 3 were characterized as soft (4.5%) and the remainder as firm (95.5%). There was no relationship between fecal consistency and *Giardia* infection status for chimpanzees ( $\chi^2 = 0.00$ ,  $P = 0.78$ ,  $df = 1$ ) or gorillas ( $\chi^2 = 0.07$ ,  $P = 0.78$ ,  $df = 1$ ).

**Table 1.** Prevalence and intensity of *Cryptosporidium* and *Giardia* spp. in chimpanzees and gorillas in the Goulougo Triangle of Nouabalé-Ndoki National Park and Kabo Logging Concession, Republic of Congo

Species	Population	Samples	<i>Cryptosporidium</i>		<i>Giardia</i>	
			Prevalence (%)	Mean intensity	Prevalence (%)	Mean intensity
Chimpanzee	Goulougo	33	0	0	3.03	1000
	Kabo	34	0	0	8.82	1833 ± 573.49
Gorilla	Goulougo	36	0	0	9.78	900
	Kabo	31	0	0	0	0

Mean intensities are expressed as oocysts (*Cryptosporidium*) or cysts (*Giardia*) per gram, standard error of the mean; negative samples are not included in this calculation.

Our power analysis demonstrated that additional zone D chimpanzee sampling would be required to ensure that the lack of significance observed in intersite comparisons of chimpanzees *Giardia* sp. prevalence is indeed valid (47 zone D compared with the 34 collected thus far). Samples were collected during repeated line transect surveys during a period of 5 years. Collection of fresh ape feces in this system requires a tremendous amount of effort. Three to five samples can be collected in a day by a team of five trackers. Samples are collected by backtracking sighted apes to find feces voided within the past hour. Aged feces, such as those from night nests are not collected. Due to these limitations, we regard the lack of significant differences in *Giardia* sp. prevalence between sites for chimpanzees to be preliminary. Additional sampling will be required to definitively examine this comparison.

We also compared the intensity of infection for each ape species between logged and undisturbed sites. The mean intensity of infection was highest in chimpanzees from the disturbed site (1833 ± 573.49 cysts/g), followed by chimpanzees in the undisturbed site (1000 cysts/g). Mean intensity of infection was slightly lower for gorillas from the undisturbed site (900 cysts/g), and no infections were found in gorillas from the disturbed site, resulting in an intensity of zero (Table 1); mean intensities of infection could not be compared statistically across sites due to rarity of infection.

## DISCUSSION

Our results demonstrate that *Giardia* sp. is present at low prevalence in chimpanzees and lowland gorillas in undisturbed forests and forests with a history of selective logging. In these same populations, there is no evidence of infection

with *Cryptosporidium* sp. A lack of *Cryptosporidium* sp. in all samples examined suggests that *Cryptosporidium* sp. is not a natural component of ape parasite communities at this site and that historical land-use patterns are not exposing apes to zoonotic sources of this pathogen. Low prevalence of *Giardia* sp. in the study area regardless of historical land-use patterns suggest that a low level of circulating *Giardia* is a natural component of Congolese ape parasite communities and that historical land-use patterns are not exposing apes to zoonotic sources of this pathogen. Our study area is located only 32 km from the nearest village and the large river that serves as a major transportation source for the region. However, the forest is continuous and the encounter rate of human signs in the study zones was low, which confirms that these forests have remained relatively undisturbed despite past timber exploitation. This post-logging scenario is much different than the high human densities and forest fragmentation typical in east and west Africa.

*Giardia* and *Cryptosporidium* are notable for cross-species transmission (Thompson, 2004). The propensity of these protozoans to cross species barriers results from the host nonspecificity of trophozoites of either genus, and the high infectivity and environmental stability of *Giardia* cyst and *Cryptosporidium* oocysts (Smith and Nichols, 2006). Previous field-based studies confirm this pattern, demonstrating that high levels of primate–human overlap enhance transmission of potentially pathogenic protozoa, including *Giardia* sp. and *Cryptosporidium* sp. (Nizeyi et al., 2002; Graczyk et al., 2001a, b; Graczyk et al., 2002; Salzer et al., 2007). The results of the current study are the first to examine the long-term effects of historical primate–human overlap on contemporary patterns of infection with these pathogens.

Considered among the most common human intestinal protozoa, *Giardia* ranges in clinical presentation from



asymptomatic to highly pathogenic, causing chronic mal-absorptive diarrhea in some patients (Thompson, 2004). Both host factors (e.g., nutrition, immunity, coinfection with other agents) and pathogen factors (e.g., strain, infectious dose) are thought to contribute to the severity of clinical disease (Thompson, 2000). Giardiasis is now considered by the World Health Organization to be a “neglected disease,” due to its ubiquity in equatorial latitudes, its chronic detrimental health effects (especially in children), its propensity to infect economically disadvantaged populations, and the paradoxical availability of relatively inexpensive and effective pharmaceuticals for its treatment (Savioli et al., 2006).

In our study, we found no relationship between infection status and fecal consistency (soft vs. firm). The lack of a strong measurable association between infection and acute gastrointestinal symptoms in our study population was surprising, but it is concordant with recent studies of humans. Although *Giardia* is associated with clinical diseases in developed economies, evidence is nevertheless mounting that it may be commensal in rural settings where people may normally be exposed to diverse parasite communities from an early age. For example, Pérez Cordon et al. (2008) found *Giardia* at 28.1% prevalence in diarrheic Peruvian children, as well as in 19.5% of nondiarrheic children, emphasizing the importance of asymptomatic patients in *Giardia* transmission where hygiene and sanitation are poor (Pérez Cordon et al., 2008). Other studies in India (Traub et al., 2003), Ethiopia (Ayalew et al., 2008), Bangladesh (Dib et al., 2008), and Peru (Hollm-Delgado et al., 2008) have found similarly weak or nonexistent associations between *G. duodenalis* infection status and gastrointestinal symptoms, suggesting that *G. duodenalis* may coexist benignly with human hosts under conditions where acquired immunity to the pathogen can develop. Lack of early exposure and acquired immunity to *G. duodenalis* may, in fact, account for the role of the pathogen as a cause of sporadic diarrheal outbreaks in developed countries (Istre et al., 1984), as well as a major cause of traveler’s diarrhea for people from developed countries who visit endemic areas (Reinthal et al., 1998; Shlim et al., 1999). The interplay between clinical symptoms and *Giardia* infections in wildlife are far less known. We hope to explore in detail the association between symptom and infection in apes in more detail in future work.

Our results from the Goulougo Triangle provide a rare opportunity to observe baseline patterns of infection in undisturbed ape populations. Baseline patterns of parasitism are necessary to understand both individual animal

health status and to assess what would constitute an “outbreak”: a level of disease or parasitism in excess of what is normally observed. Thus, these results provide a first step toward an index of population health and disease risk assessment for conservation and management plans of these endangered African ape populations.

Our results from the Kabo Concession suggest that a legacy of low-intensity selective logging did not result in the persistence of elevated prevalence of *Cryptosporidium* sp. and *Giardia* sp. in these ape populations. It is encouraging that the resident ape populations are not suffering from infections of these protozoa, because this suggests such potential health threats may be mitigated. However, if these protozoa are exceptionally pathogenic in wild ape populations, infected individuals may have been removed from the population long ago due to high associated mortality.

We have yet to examine how patterns and risk of parasitism may differ during the various stages of the active logging process (i.e., exploration, road construction, and timber harvesting) from those that are the product of a legacy of selective logging. The expansion of timber activities in the neighboring undisturbed forests of the Kabo concession will enable us to address these questions through an ongoing progressive monitoring program that involves documenting ape populations, human disturbance, and the health implications during their interactions.

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