Pathogen acquisition in people and primates in Nigeria

by

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ABSTRACT

Environmental change coupled with increased rates of human-wildlife contact can have numerous health related consequences. The work presented here examines the differential susceptibility of individuals to pathogens, the impact of pathogens on host behavior and physiology, and the potential for zoonotic disease transmission – all critical issues impacting the well-being of wildlife and humans in changing environments. The first three chapters present results from a field experiment, in which 49 semi-free-ranging red-capped mangabeys, *Cercocebus torquatus*, in Nigeria were treated for parasites. Data from simultaneous observations of infection, stress, behavior, and clinical symptoms showed: 1) that individual position in the social network was more important than the immunosuppressive effects of physiological stress or host traits in determining risk of reinfection, 2) a coordinated behavioral and physiological response to infection that promoted energy balance, reduced risk of transmission, and increased defense when in vulnerable states, and 3) that individuals infected with lung flukes, *Paragonimus africanus*, exhibited associated respiratory pathology, and may serve as sylvatic hosts for re-emerging paragonimiasis in Nigeria. Wildlife health is particularly relevant in areas of high geographic overlap between humans and wildlife, where emerging zoonotic diseases continue to be a problem. Bushmeat hunting clearly increases the risk of novel and zoonotic pathogen transmission. Chapter 5 reports responses from 327 men from Nigerian hunting communities on the nature a frequency of human-wildlife contact, perceptions of risk, and drivers of hunting behavior. Results showed that participants contacted a diversity of prey in "risky" ways, that hunting was considered to be an undesirable livelihood, and that the decision to become a hunter was deeply rooted in family tradition, modified by economic necessity. Together these results help identify root drivers of exposure and susceptibility to novel pathogens in populations of people and primates living in altered environments. More broadly, they advance our understanding of how health is differentially experienced in human and wildlife populations as a result of individual and population-level factors. This combined work is especially relevant to populations experiencing dramatic changes in their environment and new exposures to zoonotic pathogens, such as wildlife experiencing rapid habitat loss or people experiencing sociopolitical displacement.

DEDICATION

To my parents, Chris and Rod,
for listening.

I heard my parents once say, that if you listen to your children, they will tell you want they want to do at a very young age. Mom and Dad, if everyone listened as well as you, I think we might live in a very different world. I am so excited to share this accomplishment with you, as the origins of this work can be traced back further than my own memory. Not only did you listen, but you recognized my dreams as possible, and nurtured them as they evolved. You are always with me. Dad, wherever I am in the world, I carry with me your insatiable curiosity. Mom, the personal attributes that allow me to live in the most distant and unforgiving environments, all come from you. Together your constant love and support have made this journey possible. Although, I can't help but wonder, if you had it to do all over again, if you would have listened so closely to that young girl, knowing that someday she would end up living in hunting sheds in the depths of a Nigeria rainforest…

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PROLOGUE

The Snout I Was Given

I had already lived in Nigeria for 2 years – I thought I had it down. I had learned firsthand some pretty important lessons. Some were relatively simple. Look through the garden egg before biting right through it, for most contain sizable worms. Don't run from a swarm of black flies, they are attracted to movement. Always ignore sweat bees. Although they may be slow and tempting to swat, they exude a smell that attracts more sweat bees.

Some lessons were much harder to learn. Don't ever forget to list dog as an animal you will not eat, even though it is not on your endangered species list. A polite decline of the duiker lung at dinner is likely to result in day old lung and tea for breakfast the next morning. Other important lessons came from the stomach. Don't eat cold meat. Never rush a shawarma man. Don't eat meat that has "plenty plenty peppe", as it is frequently used to cover up the taste of spoiling meat.

Some lessons seemed inevitable. When pronounced incorrectly, Ebe, the local name for Garri made from cassava, also means a woman's breast. Bathe at off times, and not in popular bathing areas. *But* make sure they are not unpopular for good reason, for example, downstream from the carcass cleaning area. If you *do* see an intestine float by you in the river, find a new bathing spot, or stick to the bucket bath. When in a pinch, and used strategically, one Nalgene full of water is suitable for a bath.

And possibly the most important lessons involved kai kai, the locally distilled palm wine offered to guests. Only take one shot of kai kai to show your appreciation. You will surely be drinking kai kai again at the next house, and then the next, and you will be more than appreciative by the end of the day. Never gift someone kai kai, if you can help it. This will surely turn into one more occasion in which you are expected to drink kai kai. *Always* travel with kai kai though. When you are stopped unexpectedly and asked to show your belongings, one sip of it by the *oyinbo* (white [wo]man) can get you out of most hairy situations. Finally, whenever possible, dance. "Dancing diplomacy" as I have come to call it, turns you, the scary weird *oyinbo*, into a friendly woman who people want to talk to.

Despite all of this *well*-earned knowledge, I was more hesitant and nervous than ever to enter an enclave village in the middle of the national park. I had spent the past years skirting around the edges, visiting communities that may be, but were not necessarily, hunting within the national park. Now, I was trekking straight up the middle. I had been warned about the inhospitable nature of the community, who lived under the constant threat of displacement (or promise of relocation?) under the formation of the national park. But, those folks who entered previously and left hurriedly with stories of tears, may have had very different agendas? Or perhaps they were not acutely aware of the importance of dancing diplomacy? I was soon to find out. Now, lost to the middle of nowhere, it was a 30km trek into this village from the nearest road. A few things seemed to be sure, I should not expect to be welcomed with open arms, and the facilities would be, well, limited.

The Oban Hills lift the forest above the horizon so that it emerges all around you. Driving down the Oban corridor to the foot of the trail, I could see, for the first time, the immensity of the forest. Adding to my sense of the surreal, several kilometers into the trek, I caught sight of a man crossing a stream with a rock on his head, and was told that it was to keep him from getting swept away. And then it hit me. Everything going in and out of this village has to be carried on someone's head.

After a day of trekking, I approached the village tired, muddy, and with blistered feet. I advanced with a familiar sense of caution. I had grown bold in recent months, and the last time I remembered feeling this wary was over a year ago, when I first entered a hunting shed. So I slowed down, and hung back, allowing my guides to take the lead.

As we emerged from the forest, my mind drifted to the last hunting shed I had been in, where I happened upon four hunters with whom I would share the shed for the night. I had heard them through the forest as we approached, but it wasn't until I entered the small clearing that I realized exactly what all the clamor was all about - they were singing in unison along with Celine Dion blasting from a cell phone. They sang happily while they went about their butchering and prepping of meat. They seemed less disturbed by my presence than normal, until the phone ran out of battery. The four men, looking distressed, chatted for a moment in their local language before turning their attention to me, also distressed from being the target of the four hunters gaze. My guide relayed their shared message, "Auntie, they say that now you should sing". And that is how, in the heart of the Nigerian forest, I sat together with bushmeat hunters singing "Neaaaaaayah, faaaaaaaah, wheeeeereva you are..." Smiling, and laughing to myself (to which I had grown pretty accustomed), I entered the village tucked away in the middle of the national park.

I don't know if it was my goofy smile, or complete misconceptions about what I was walking into, but I was greeted right away, with unexpected enthusiasm by an excited and chatty group of women. "Auntie, you get powa!", "You go trek all dis way to see us!?", "Come come, we go baf." Seemingly moments later, there I was, naked in a stream, bathing with 15 giggling women; my reservations about this place washing away with all the dirt from the trail. I gazed around at the expansive forest, still hesitant about the type of reaction I would get from the hunters I had come to interview.

Almost everything about this village was unexpected. First, the chief's son gave me his room, and it had the nicest foam mattress I had ever slept on. I asked him where I could go to "ease" myself. He escorted me to what seemed like a mirage after a long day's journey: the first pit latrine I had ever seen in a village, and sitting on top of it was a sparkling white porcelain toilet. That evening, I sat on the stoop of the house dazed, hungry, still concerned about the hunters, wondering what I would find to eat, and imagining people carrying that mattress and toilet 30km on their heads to get them here. Eventually the neighbors came up to me with a tray of food. I opened the lid and let out a sigh of relief that was quickly replaced with nervous apprehension. There it was sitting in bowl of broth, an indication that the hunters would be welcoming and open with me – the snout of a bush big. Unfortunately, how to eat a snout, was a lesson I had not yet learned.

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CHAPTER 1: Introduction

Global change and infectious disease

The global environment is changing at an unprecedented rate, due to such factors as climate change, population growth, human migration, and political unrest. Environmental change coupled with increased rates of human-wildlife contact can have numerous health related consequences. Ecological and social changes can alter the dynamics of endemic diseases, while increased contact between humans and wildlife promotes exposure to novel pathogens and presents opportunities for disease emergence. Differential exposure and susceptibility of individuals to pathogens, their impact on populations, and the potential for zoonotic disease transfer are critical issues affecting the well-being of wildlife and humans as they struggle to co-exist in shared environments.

The introduction of novel pathogens, including those originating in other species, have occurred throughout human history. Epidemic neglected tropical diseases, such as Ebola, Zika, and Lassa Fever, and pandemics such as HIV/AIDS, severe acute respiratory syndrome (SARS), and H1N1 swine influenza, are modern instances of an ecological phenomenon (microbes invading and adapting to new niches) that has afflicted humans for millennia [1]. However, the frequency of disease emergence (disease causing agents that appear in new hosts, new geographic areas, or demonstrate a marked increase in prevalence) is on the rise, due in part to anthropogenic changes to our planet [2–4]. Although rare compared to endemic pathogens, emerging infectious diseases can have grave impacts on global health and economies [3,5]. Understanding the key factors that drive the transmission, emergence, and re-emergence of infectious diseases will be critical to predicting and managing current and future threats.

Infectious disease transmission results from a complex interaction of biological, ecological, and social processes [6–8]. Although the identification of biological factors (i.e.

characteristics of the pathogen and interactions with hosts on the cellular level) are a critical predictive tactics in emerging infectious disease research [9–11], social, ecological, and behavioral host factors are the fundamental precursors to host-pathogen interactions. For example, neglected tropical diseases are associated with and perpetuate poverty [12,13]. Political instability and conflict can proliferate epidemics, such as Ebola and Lassa hemorrhagic fevers in West Africa, and cutaneous leishmaniasis in the Middle East [14–17]. Ecological factors promote spillover of infection from wildlife (e.g. deforestation effects on bat spread paramyxoviruses, SARS coronavirus, and possibly Ebola virus [4,18]) and enable the expansion of vectors for increased transmission of vector borne diseases (e.g. leishmaniasis [19,20], dengue [21], and Chagas disease [22]). Human behaviors promoting the exchange of bodily fluids between and humans and chimpanzees, presumably through hunting, were necessary precursors to the transmission of simian immunodeficiency virus from chimpanzees (SIVcpz) to humans, ultimately leading to the global AIDS pandemic[23]. Social and ecological approaches will therefore be critical in understanding the human activities that drive transmission and emergence, and how risks are altered by social, behavioral, and environmental factors [24–27].

Primate models of health and infectious disease

In human societies, health responses to environmental change are difficult to predict [28]. Animal health and well-being are threatened by habitat destruction, population displacement, and consequent altered infectious disease dynamics. This challenge tends to be viewed as a conservation concern, but can also serve as a model for human societies, offering an opportunity to study exposure and response to infection in model systems. In light of the complex nature of human societies, simpler but relevant animal models should yield more interpretable (and comparative) outcomes than could be achieved through the study of humans alone.

The use of primates as models for human health is common in biomedical research because of the close evolutionary relationship between humans and other primates, as well as other similarities including high degrees of sociality and pathogen sharing. In human societies, social factors, including stress, socioeconomic status, and social behavior can affect susceptibility to, and severity of, infectious disease [29–32]. Similar processes in social animals can serve as models for humans, and help us understand how sociality affects health [33], including socioeconomic-status-related health disparities [34]. Traditionally, however, primate models for human health have been restricted to drug and vaccine development and psychological well-being. The application of primate models of human health to afflictions of populations and society, has not been fully realized.

Additionally, the health status of primates and other wildlife can have both direct and indirect impacts on nearby human populations. Phylogenetic similarity is an important predictor of pathogen sharing [35], and indeed humans and primates have a long list of shared infectious diseases [36]. Infectious disease dynamics in primate populations can provide important clues to the effects of natural infections on hosts and how they change in altered environments and new hosts [31,37]. Given the difficulties associated with eradicating pathogens capable of infecting multiple hosts, coupled with their high potential for emergence and re-emergence in human populations [38], monitoring potential wildlife hosts is important for the control of zoonotic infections [39,40].

Human-wildlife contact and risk of zoonoses

As people encroach into new environments, pathogens from other species repeatedly spill over. Zoonotic diseases of public health significance can come from bacterial (e.g. leptospirosis, anthrax, brucellosis, Q-fever), parasitic (e.g. cysticercosis, echinococcosis, toxoplasmosis, Chagas), and viral pathogens (e.g. rabies, type A influenzas, rift valley fever, severe acute respiratory syndrome (SARS), Ebola hemorrhagic fever, SIV precursors to HIV) [25]. Endemic zoonoses are persistent as global health problems, amounting to roughly a billion cases and millions of deaths annually [41]. Zoonoses are additionally responsible for a majority of emerging infectious diseases, with wildlife playing an increasingly important role [3,28,42].

Perhaps the most pervasive form of human-animal contact is through animal based food systems. Globally, people rely on domestic and wild animal food sources capable of harboring zoonotic pathogens. Our long history of close association with livestock has resulted in many associated zoonoses becoming endemic to humans [43]. Zoonotic food borne parasites, including trematodes (liver, lung, and intestinal flukes) and cestodes (tapeworms), are part of the neglected tropical diseases, and contribute to substantial disease burden throughout the tropics [44,45]. Although wild animals are consumed far less frequently than domestic livestock [46], contact associated with wildlife consumption is associated with the emergence of diseases with high-case fatality rates and global mortalities such as HIV/AIDS [23], SARS [47], and Ebola [48], as well as localized outbreaks of Japanese Encephalitis virus [49], paramyxoviruses (Hendra and Nipah) [50,51], anthrax [52], monkeypox virus [53], and Lassa virus [54] among others.

Increasing zoonotic disease emergence is coupled with a marked increase in wildlife harvest over the past few decades [55,56]. Harvest rates are particularly high in tropical forests, where millions of tons of wild animal meat are extracted annually [57]. Although bushmeat consumption has long been an integral nutritional, economic, and cultural component of rural livelihoods in West and Central Africa [58], increased population growth, extractive resource industries, and advances in hunting practices have led to dramatic changes in the scale of the trade [59,60]. Bushmeat, and associated microbes, are now traded internationally [61,62].

Still, the risk of transmission is harbored disproportionately by bushmeat hunters and handlers at the first point of contact [63–65]. In regions where reliance on bushmeat is linked to income and/or education level [66,67], socio-economic status may put certain individuals at higher risk through increased rates of contact with wild animals. The emergence of zoonotic infectious diseases therefore becomes an event influenced by complex interactions between key ecological, behavioral and sociological drivers.

Emerging health threats in Nigeria

Nigeria has high biodiversity and high relative risk of infectious disease emergence [3,68–70]. It is the most populous country in Africa (est. 182 million people) [70] and is expected to outgrow the U.S by 2050 and become the world's third most populous country by the end of this century [71]. Currently, over 10 million people are living undernourished; there is an annual 2.5% (2015) population growth rate and 70% of the population are living under the poverty line [70,72]. Lagos, the largest city in Africa (est. 13 million people; 18,000/km^2) [70], is increasingly connected to rural areas and wildlife habitats. Particularly, the Cross-Sanaga-Bioko ecoregion, situated between the eastern bank of Cross River in Nigeria and the Sanaga River in Cameroon, is characterized by some of the highest species richness of any African forest and high levels of endemicity [73]. In the southeast corner of Nigeria, Cross River National Park makes up a total area of about 4,000 km^2 and contains the largest closed-canopy rainforest in Nigeria. There are two separate sections, the northern Okwangwo (established 1991) and the southern Oban (1988) divisions, which consist of lowland tropical rainforests. Illegal logging, slash and burn farming, and poaching threaten biodiversity in both divisions of the park. With a lack of purchasing power and growing populations, people in rural forested areas turn to bushmeat for food and income. Bushmeat from the park supplies rural and urban markets throughout southeastern Nigeria and into Cameroon [74]. Given its high biodiversity, high human population density and growth (and associated changes in behavior and ecology), Nigeria provides an extreme example of environmental degradation that raises public health concerns.

Thesis synopsis

Understanding the ecology of infectious diseases, and the underlying processes that drive transmission is a complex challenge, requiring interdisciplinary approaches. Through the dissertation research described herein, I combine ecological and sociological methods to investigate emerging environmental health threats in humans and wildlife in shared environments in Southeast Nigeria.

In **Chapters 2 – 4,** I use the reinfection of primates with gastrointestinal parasites following veterinary intervention, as a model for exposures to new pathogens in the context of environmental change. Understanding interactions between social and health-related variables in human populations remains a challenge. By studying primate populations as they become exposed to pathogens, these data help identify the social parameters that influence health and well-being. In **Chapter 5**, I use interview responses from people in nearby hunting communities to identify risk factors for transmission of wildlife pathogens to human populations.

Individuals vary in their exposure and susceptibility to infectious agents. In fact, one of the few predictable patterns in parasite ecology is that parasites aggregate within hosts, with few individuals harboring the majority of infections [75,76]. In **Chapter 2**, I identify host factors that determine patterns of parasite aggregation following experimental treatment of a group of red-capped mangabeys (*Cercocebus torquatus*). Host traits along with behavioral and physiological predictor variables were incorporated into marginal Cox proportional hazards models to estimate the effects of classic measures (sex, age, dominance) and mechanistic factors (stress, centrality) on time to reinfection. Results show that position in the social network, and in particular space sharing, were more important than the immunosuppressive effects of physiological stress or host traits in determining risk of infection. These findings suggest that future studies of disease ecology should focus on measures of network association, in addition to individual host traits.

The effects of parasitism on natural hosts are often elusive and difficult to detect. In **Chapter 3**, I describe the behavioral and physiological responses of red-capped mangabeys to parasite infections, including the effects of parasites on energy balance, social behavior, and body condition. Mean cortisol levels, percent time allocated to different activities (i.e. feeding, foraging, socializing, traveling, and resting), and vigilance behavior were incorporated into a series of mixed-effects linear models to measure the effect of infection status on behavioral and physiological changes. Increased cortisol, behavioral time re-allocation toward low energy behaviors, reduced group cohesion, and increased vigilance appear to be part of a coordinated host response to parasite infection that maximizes energy balance, reduces risk of transmission, and increases defense when in vulnerable states. These results show that parasitism, not only virulent pathogens, have the potential to regulate host behavior, health, and fitness.

The health of primates is relevant to human health, especially given the zoonotic potential of many primate pathogens. In **Chapter 4**, I describe infections from a lung fluke (*Paragonimus* sp.) recovered from red-capped mangabeys, including molecular identification of species, population prevalence and intensity, and clinical signals of infection. Prevalence and intensity of infection were positively associated with clinical signs of infection, and these were cleared when infections were treated. Sequencing of DNA extracted from eggs in feces and amplified via PCR showed that the parasite was 100% identical at one genetic locus to *Paragonimus africanus* recovered from humans in Cameroon. Results offer a method for surveillance re-emerging human paragonimiasis in sylvatic hosts.

Bushmeat hunting is associated with the transmission of numerous zoonotic agents [77]. The disruption of transmission requires improved understanding of the interactions between key biological, behavioral and sociological drivers of human-animal contact in such settings. In **Chapter 5,** I describe risk factors for zoonotic transmission in people from Nigerian hunting communities. I used interview responses to define transmission risk by the nature and frequency of human-wildlife interactions, describe perceptions of zoonotic disease risk, determine individual views on the merits of hunting as a livelihood, and identify the socio-economic risk factors of hunting behavior and infection risk. Results from this chapter highlight important drivers of human-wildlife contact. This information is used to suggest strategies for modifying the impact of hunting and preventing zoonotic disease transmission.

In **Chapter 6**, I briefly summarize the results from this research, and identify avenues for future work. Specifically, I outline ideas for: 1) further use of antiparasitic treatment in field experiments to test key hypotheses in parasite ecology, 2) investigation of parasite-host assemblages as models of health in changing global environments, and 3) the study of factors explaining zoonotic disease transmission in wild animal food systems.

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CHAPTER 2: Primate reinfection with gastrointestinal parasites: behavioural and physiological predictors of parasite acquisition

Friant, S., Ziegler, T. E. & Goldberg, T. L. 2016 Primate reinfection with gastrointestinal parasites: behavioural and physiological predictors of parasite acquisition. *Anim. Behav.* **117**, 105–113. (doi:10.1016/j.anbehav.2016.04.006)

Abstract

Infectious disease transmission is a cost of sociality in humans and other animals. Nevertheless, the mechanisms linking social behaviour to infection risk are poorly known. We conducted a field experiment to examine how host intrinsic traits, behaviour and physiology affect infection of nonhuman primates with gastrointestinal parasites. We measured rate to reinfection in a social group of red-capped mangabeys, *Cercocebus torquatus*, following chemotherapeutic treatment for parasite infections. By measuring behaviour, infection and glucocorticoid levels, we compared the relative effects of space sharing, directional contact and physiological stress on risk of acquiring new infections. We found that, within proximity networks, individuals that were central and well connected and that had a tendency to switch groups were at increased risk of infection with helminths. Protozoan infections, however, were acquired more uniformly across the population. In general, position in the social network and, in particular, space sharing appears to be more important than the immunosuppressive effects of physiological stress or host traits in determining risk of infection. Our results suggest that future studies of disease ecology within wildlife populations should focus on measures of network association in addition to individual host traits.

Introduction

In humans and other social animals, variation in behaviour and physiology can alter the risk of exposure to, and infection by, pathogens, ultimately affecting host fitness (Kappeler, Cremer, & Nunn, 2015; Nunn, Craft, Gillespie, Schaller, & Kappeler, 2015; Silk, 2014). Variation in parasitism often has direct links to host social behaviour, such that infection-related costs of sociality are considered important selective forces in human and animal evolution (Altizer et al., 2003; Kappeler et al., 2015; Møller, Dufval, & Allander, 1993). Clarifying the mechanisms whereby sociality translates to infection is important for our understanding of disease ecology and host–parasite coevolution. For example, it is currently unclear whether close proximity and high levels of contact or increased physiological stress resulting from within-group social dynamics is more important for infection in primates.

Macroparasites are generally aggregated within populations, with few hosts harbouring the majority of infections (Crofton, 1971; Poulin, 2007; Shaw & Dobson, 1995). Classic measures typically associated with infection include age, sex and dominance status (Nunn & Altizer, 2006). Behavioural and physiological mechanisms that influence encounter rates and immune status can vary with these measures, further explaining why certain individuals are at increased risk of infection. Focus on classic measures alone may therefore obscure important contributions of social connectivity and/or physiological stress (Cavigelli & Caruso, 2015; Kappeler et al., 2015).

In primates, trade-offs between sociality, encounter rates and immune function result in conflicting predictions for disease risk of individuals (Nunn & Altizer, 2006). For example, age can increase parasitism if larger-bodied individuals occupy more space, require more resources and contact contaminated foods, conspecifics, and substrates disproportionately. Conversely, lack of acquired immunity in younger individuals may increase risk of parasitism in juveniles (Hudson &

Dobson, 1997). Parasitism tends to be more common in males than in females across vertebrate; however, male-biased parasitism is confounded by body size, such that the immunosuppressive effects of stress and testosterone are unclear (Zuk & McKean, 1996). In primate societies with dominance hierarchies, greater access to resources and rank-mediated social contact should increase risk for high-ranking individuals (MacIntosh et al., 2012; Rushmore et al., 2013). Meanwhile, immunosuppressive effects of stress hormones can increase susceptibility in either dominant or subordinate individuals depending on species-typical dynamics and hierarchical stability (Cavigelli & Caruso, 2015; Habig & Archie, 2015; Sapolsky, 2005).

Empirically, intraspecific differences in physical contact, proximity (González-Hernández et al., 2014; MacIntosh et al., 2012; Rimbach et al., 2015) and physiological stress (Chapman, Saj, & Snaith, 2007; Clough, Heistermann, & Kappeler, 2010; Muehlenbein, 2006) are associated with transmission of parasites within primate groups. In Japanese macaques, *Macaca fuscata*, for example, socially mediated exposure seems to be more important than the immunosuppressive effects of stress in explaining why dominant females have more infections from directly transmitted parasites (MacIntosh et al., 2012). Nevertheless, the relative importance of network connectivity versus physiological stress as mechanisms for facilitating pathogen spread is not well understood.

In this study, we investigated how social connectivity and physiological stress compare to host intrinsic factors with respect to explaining patterns of parasite aggregation in primates. To overcome confounding heterogeneities in exposure, susceptibility and resulting infection levels over time, we experimentally removed parasites and measured rate to reinfection. To date, experimental manipulations of parasite infections in wild animals have focused primarily on behavioural, immune and fitness responses to parasitism (Coster, Neve, Martín-Gálvez, Therry, & Lens, 2010; Hillegass, Waterman, & Roth, 2010; Raveh, Neuhaus, & Dobson, 2015). Here, we investigated patterns of parasite reacquisition following chemotherapeutic treatment of red-capped mangabeys, *Cercocebus torquatus*, for gastrointestinal helminth and protozoan parasites. Specifically, we examined how centrality within social networks and individual stress varied within the population according to sex, age and dominance. We then compared how these mechanistic explanations (e.g. contact, proximity and/or stress) performed against classic measures in predicting rate to reinfection. We compared reinfection from helminths and protozoans separately, given their inherent differences in time to infection and aggregation within hosts (Shaw & Dobson, 1995). By focusing on gastrointestinal parasites, which can be collected noninvasively and can be treated with oral medications, we were able to compare results from our field experiment to other observational studies that also investigated gastrointestinal parasites. We predicted that following experimental manipulation of infection, centrality would augment classic measures to more powerfully explain differences in infection rates.

Methods

Study Site and Population

The study took place at Rhoko Research and Conservation Education Centre (41.21º N, 16.16º E), the forest site of the Centre for Education, Research and Conservation of Primates and Nature (CERCOPAN). Rhoko is located in the transition zone surrounding the Oban division of Cross River National Park in Cross River State, Nigeria. The vegetation is characteristic of lowland rainforest, forming a mosaic of disturbed and relatively undisturbed forest patches. Climate includes a long wet season from April to November and a short dry season from November to March.

We studied 49 red-capped mangabeys living in a multimale–multifemale social group that either had been rescued from the bushmeat and pet trades as young juveniles, or were first- to third-generation captive born. The group was housed in a 1 ha open-topped forest enclosure with full canopy cover and within the natural home range of the species. The population was provisioned daily but also ate wild foods opportunistically and drank from a stream running through the enclosure. Animals were vulnerable to natural predators (e.g. snakes and birds of prey) and parasites. All animals were well habituated and individually recognizable to the trained observer through individual differences in size, pelage and facial characteristics; all data were collected from animals where observers had achieved 100% agreement on identification. The age of each individual at the start of data collection (range 1.08–18.5 years) was known from birth records or estimated from tooth wear, pelage characteristics and sexual maturity at date of rescue. We categorized males as adult (≥6 years, *N* = 9), subadult (≥3 year, *N* = 9) and juvenile (<3 years, *N* = 7), and females as adult (≥4 years, *N* = 19) or juvenile (<4 years, *N* = 5). *Cercocebus torquatus* is currently listed as vulnerable by IUCN (Oates, Gippoliti, & Groves, 2008).

Chemotherapeutic Treatment

In June of 2012, the entire population was treated for gastrointestinal parasites via simultaneous administration of metronidazole (50 mg/kg for 7 days) for protozoans, mebendazole (50 mg/kg for 3 days) for nematodes, and praziquantel (20 mg/kg for 3 days) for cestodes and trematodes. For all drugs, a single dose was delivered in maize cereal to identified individuals to ensure that each animal received at least one dose. The remaining doses were dissolved in fruit and administered via group feeds following standard practice for the population-level treatment at CERCOPAN. The treatment period lasted 10 days in total.

Study Design

The study took advantage of a planned treatment event, providing a unique opportunity to measure patterns of infection following an intervention and minimizing additional risk. The treatment regimen was developed from standard treatment practices at CERCOPAN and in consultation with two wildlife veterinarians. Oral administration of drugs and noninvasive assessment of parasites were used to minimize adverse risks and enhance welfare. The Institutional Animal Care and Use Committee at University of Wisconsin-Madison approved all research activities (protocol V1490).

We collected faecal samples and behavioural and health data between May and September 2012. We conducted faecal sampling 1 month prior to treatment and parallelbehavioural sampling for 3 months immediately following treatment. We collected pre-treatment faecal samples in triplicate from each individual to increase detection of parasites shed intermittently. To assess variation in protozoan infection, which are infectious upon shedding and have short prepatent periods, we collected post-treatment faecal samples at the highest frequency for the month immediately following treatment (ca. every 3 days/individual). We measured time to infection from protozoans during the period of high sampling intensity only (30 days post treatment). We gradually decreased sampling intensity in the second month (ca. every 5 days) and third month (ca. every 10 days) to detect new infections from helminths, which develop in the external environment and have longer prepatent periods, such that we measured helminth reinfection over this longer time period (80 days post treatment). We extracted hormones from triplicate samples directly following treatment (ca. every 10 days), with sampling intensity reduced to twice a month (ca. every 15 days) for the second and third months.

Behavioural Data Collection

We collected associational data by recording all grooming partners, direction of grooming and nearest neighbours (within 2 m) of focal individuals during three observation periods daily: early morning (0700–1000 hours), mid-morning (1100–1500 hours) and evening (1600–1900 hours). The mangabeys were often dispersed in dense undergrowth throughout their enclosure, making it difficult to collect data on a predetermined schedule or in a specific order. We therefore selected individuals opportunistically. We allowed a minimum of 1.5 min to elapse between observations to reduce interdependence of data. We did not sample an individual if it was an associate or nearest neighbour in the preceding observation. We collected dominance data by recording all observed agonistic interactions as well as the directionality of submissions and supplants using structured ad libitum sampling, as conducted for similar species (Range & Noë, 2002). Three observers collected all data for 81 days over 3 months post treatment. We tested and accepted interobserver reliability by calculating Fleiss's Kappa test for categorical agreements between multiple observers (Kappa = 0.89, $P < 0.001$ for observers 1 and 2; Kappa = 0.92, $P < 0.001$ for observers 1 and 3).

Faecal Collection and Preservation

We collected faecal samples immediately following defecation and fixed within 2 h of collection. We used separate aliquots of each sample for preservation of gastrointestinal parasites and hormones. First, we took a 2 g aliquot from within the faecal mass and stored it in a 3:1 ratio of 10% formalin to faeces for preservation of gastrointestinal parasites (Greiner & McIntosh, 2009). We then separated a second aliquot, mixed it thoroughly, and placed 0.50–0.55 g in a 15 ml tube. We mixed equal parts (2.5 ml) of distilled water and ethanol with the sample by shaking it vigorously for 5 min for preservation of hormones (Ziegler & Wittwer, 2005).

We extracted hormones in the field via solid-phase extraction (SPE) (Ziegler & Wittwer, 2005). Samples were stored for over 24 h until faecal material naturally sedimented at the bottom of the tube and a clear supernatant was evident. We decanted supernatant into a small weighing dish, removed and pushed it through the SPE columns (Prevail C18, Alltech, Deerfield, IL, U.S.A.) using a 5 ml disposable syringe at a flowrate of 1 ml/min. We then washed solid-phase extraction columns by pushing 1 ml of distilled water through the cartridges at the same rate to eliminate contaminants. We capped cartridges and stored them at room temperature out of direct sunlight until transport. We transported formalin-preserved samples and SPE cartridges to the University of Wisconsin, Madison following all applicable import, export and International Air Transport Association regulations.

Behavioural Data Analysis

We constructed dominance matrices from post-treatment dyadic supplants and aggressive and avoidance interactions between adults of the same sex using SOCPROG 2.6 (Whitehead, 2009). We used David's scores (DS) to dichotomize individuals as 'usually dominant' (DS >0) or 'usually subordinate' (DS <0) (de Vries, Stevens, & Vervaecke, 2006). We assessed linearity of male and female hierarchies using de Vries' test and *h*' with 1000 permutations for dominance hierarchies containing unknown or tied relationships (de Vries 1995).

We constructed adjacency matrices for social network analyses directly from observed post-treatment pairwise associations ('proximity' network) and directional grooming interactions ('contact' networks) using SOCPROG 2.6 (Whitehead, 2009). We imported matrices with attribute information into R v.3.2.2 (R Core Team, 2014), where we conducted all further analyses unless otherwise specified. We performed calculations of symmetric network metrics from proximity matrices and calculations of asymmetric metrics from directional contact matrices using the 'sna' package (Farine & Whitehead, 2015). For each individual in each network, we calculated node-based measures commonly used for modelling transmission of infectious diseases: (1) degree centrality: the number of associates or interactants (hereafter referred to as 'centrality'); (2) strength: the number of associations or interactions; (3) closeness: the shortest number of paths needed to reach all other individuals; and (4) betweenness: the number of shortest paths going through an individual (Drewe & Perkins, 2015). We calculated in degree and out degree for directional networks, representing groom-receive and groom-give, respectively.

Given the nonindependence of data in network analyses, we compared network metrics to host characteristics using permutation tests. We built null models were built from data stream-based randomizations and measured significance by comparing the test statistic of the models fitted to the observed data with the test statistic calculated from 1000 permutations of the network using the ‘asnipe’ package (Farine, 2013; Farine & Whitehead, 2015). We calculated Cohen’s *d* effect size for all comparisons using the ‘compute.es’ package (Farine & Whitehead, 2015; Re, 2015). We constructed network diagrams using UCINET software’s NetDraw program (Borgatti, Everett, & Freeman, 2002), with node size representing individual centrality, weighted edges representing strength, and without filters.

Parasitology

We concentrated 1 g of formalin-preserved faeces via faecal sedimentation following the protocol of Greiner and McIntosh 2009 for assessment of gastrointestinal parasites of primates (Greiner & McIntosh, 2009). Briefly, we suspended 1 g of faeces in 40 ml of sedimentation solution (soapy water), mixed it gently to avoid formation of bubbles and filtered it twice through cheesecloth to remove large debris. We allowed the mixture to sediment for 10 min, after which we decanted the supernatant. We resuspended the remaining pellet in distilled water and allowed it to sediment for another 10 min. We then removed the supernatant with a transfer pipette and preserved the sediment in formalin until examination. We systematically examined the entirety of the sediment at 10× objective light magnification for helminth eggs and larvae. We then examined one drop of sediment from each sample at 40× for identification of protozoan cysts. We measured representative parasites with a calibrated ocular micrometer and photographed them at 40× magnification. All helminth eggs, larvae and protozoan cysts were assigned to taxa based on their size, shape, colour and contents. Parasite richness (number of different parasite taxa within a host) and prevalence (percentage of individuals infected with a particular parasite taxon) were calculated (Bush, Lafferty, Lotz, & Shostak, 1997). We evaluated the efficacy of treatment by examining changes in parasite richness over time (before and after treatment) using one-way ANOVA and Tukey HSD post hoc tests. Prior to this study, parasite communities had not been reported for this species.

Faecal Cortisol Analysis

We measured faecal cortisol levels via enzyme immunoassay at the Wisconsin National Primate Research Center Assay Services Unit. We eluted steroid hormones were using 2 ml of 100% methanol after washing cartridges with 1 ml of 5% methanol. We then evaporated eluted and rehydrated hormones in 1 ml of 100% ethanol and stored them at 4 °C. Prior to EIA, we removed and evaporated 25 μl from each sample. We performed all assays using R4866 (anti-cortisol-bovine serum albumin) developed by Stabenfeldt and Munro at the University of California, Davis, with 60% cross-reactivity to corticosterone (Ziegler, Scheffler, & Snowdon, 1995). We read plates using SpectraMax 340PC microplate reader. Recovery was 105.68 ± 3.15%. We demonstrated parallelism using serial dilution curves derived from high-value faecal extracts with no significant difference from the slope of the standard curve ($t_{24} = 0.93$, $P > 0.05$). Interassay variation was 18.3% for the high pool and 22.2% for the low pool, whereas intra-assay variation was 3.8% for the high pool and 7.9% for the low pool. We compared average post-treatment faecal cortisol levels (ng/g) across sex, age and dominance status using Spearman rank correlations and Mann–Whitney *U* tests. Prior to this study, cortisol had not been reported for this species.

Statistical Analysis

We used marginal Cox proportional hazards models for multiple events data to examine host traits, behaviour and physiology as predictors of time to reinfection (Kleinbaum & Klein, 2012; Wei, Lin, & Weissfeld, 1989). The marginal approach focuses on the total time from study entry to occurrence of each event, thereby combining time to infection and number of infections (i.e. richness) within a single model. In these models, we defined the baseline hazard function (i.e. dependent variable) as time to infection, stratified by parasite type. Stratification by parasite within models allowed the baseline hazard function to vary for each parasite taxon. We defined new infections as shedding of cysts/eggs in the post-treatment sampling period after testing negative for the entirety of the parasite-specific prepatent period. Because drug administration typically reduces burden but does not typically clear infections entirely (Pedersen & Fenton, 2015), we included only parasites that showed more than 50% reduction in prevalence and contributed to subsequent reinfections. Individuals that did not experience an event by the end of the study were right-censored (e.g. time to infection for these individuals was considered to be at least as long as the duration of the study).

We incorporated individual characteristics, including sex, age class, dominance status, faecal cortisol level and centrality, as covariates in maximal models. We assigned robust variance estimates to adjust for the likely correlation among multiple events on the same subject (Lin & Wei, 1989). To control for the increased opportunity for infection in individuals successfully treated for all parasites (compared to those that retained some infections following treatment), we forced a covariate into each model to represent the maximum number of events possible per subject.

We built separate maximal models for protozoan and helminth infections. Because juveniles and subadults were not assigned dominance values, we ran two models under each category: (1) all ages (dominance excluded) and (2) adults only (dominance included). We incorporated centrality measures into maximal models one at a time (i.e. each model only contained one centrality metric from one network) to avoid multicollinearity. Model selection was then carried out independently for each model using the likelihood ratio test selection criterion (Kleinbaum & Klein, 2012). We used backwards elimination of predictor variables to select models that retained only significant covariates (at the alpha < 0.05 level) and first-order interactions. We did not use permutation-based methods, which are often used in the statistical analysis of network data (Croft, Madden, Franks, & James, 2011), because the response variable was not based on relational data (VanderWaal, Atwill, Hooper, Buckle, & McCowan, 2013). We accepted if they had generalized variance inflation factors (GVIF) within reason (<4) (O'Brien, 2007), satisfied the proportional hazards assumption (i.e. residuals were not significantly correlated with time, ZPH: Pearson's r: $P > 0.05$), and they explained significantly more variance than the null model (likelihood ratio test: $P < 0.05$) (Kleinbaum & Klein, 2012)

We report hazard ratios (HR), the ratio of the chance of parasite acquisition in one level of an explanatory variable relative to the other, for all significant predictors. We produced survival curves for significant predictors using the Kaplan–Meier method. We dichotomized entrality scores at the median because hazard ratios and Kaplan–Meier curves are more interpretable when comparing groups, and because in general, models with dichotomized variables outperformed those with continuous variables (i.e. produced higher log likelihoods and fewer ZPH violations). We performed all analyses with the ‘surv’, ‘coxph’ and ‘cox.zph’ functions in the ‘survival’ package using R v.3.2.2 (R Core Team, 2014; Therneau, 2015).

Results

Dominance

We constructed dominance matrices were from 628 female (μ + SD = 66.11 + 30.94 per individual; μ + SD = 3.48 + 1.63 per dyad) and 368 male (μ + SD = 81.78 + 32.33 per individual, μ + SD = 9 + 3.59 per dyad) dyadic dominance interactions. For both male and female hierarchies, deVries test of linearity indicated that dominance was moderately linear and non-random for female (h’= 0.73, P<0.001) and male (h’= 0.84, P<0.01) hierarchies.

Centrality

We constructed proximity networks from 2374 observed pairwise associations (μ + SD = 96.50 + 48.54 per individual; μ + SD = 1.97 + 0.98 per dyad) and contact networks from 555 dyadic grooming interactions (μ + SD = 11.32 + 9.37 per individual; μ + SD = 0.23 + 0.19 per dyad). Directed contact networks were more heterogeneous (varied across sex, age and dominance status) compared to proximity networks (Fig. 1). Females had higher closeness ($t_{47} = 4.64$, $P < 0.001$, $d = 1.33$) and betweenness ($t_{47} = 3.64$, $P < 0.001$, $d = 1.04$) in the contact networks compared to males, and higher centrality ($t_{47} = 5.83$, $P < 0.001$, $d = 1.66$) and strength ($t_{47} = 6.17$, $P < 0.05$, $d = 1.76$) measures in the groom-give network. Adults had significantly higher closeness ($t_{47} = 3.63$, $P < 0.001$, $d = 1.05$) in the contact networks and higher centrality ($t_{47} = 6.15$, $P < 0.001$, $d = 1.78$) and strength ($t_{47} = 4.08$, $P < 0.05$, $d = 1.18$) in groom-receive network. High-ranking males were more central in the groom-give network ($t_7 = 2.97$, $P < 0.001$, $d = 1.99$) and had stronger connections in both proximity ($t_7 = 2.19$, $P < 0.05$, $d = 1.47$) and groom-receive networks ($t_7 = 2.61$, $P < 0.01$, $d = 1.75$). Dominant females had higher closeness

($t_{17} = 2.58$, $P < 0.01$, $d = 1.2$) and betweenness ($t_{17} = 2.02$, $P < 0.001$, $d = 0.94$) in contact networks and higher centrality ($t_{17} = 2.14$, $P < 0.01$, $d = 0.99$) and stronger connections in groom-give network ($t_{17} = 3.05$, $P < 0.001$, $d = 1.42$).

Faecal Cortisol

We collected a total of 343 post-treatment faecal samples (7 per individual) for hormone analyses. Faecal cortisol levels ranged from 0.57 ng/g to 49.39 ng/g among individuals. Cortisol levels were positively related to age ($r_S = 0.55$, $P < 0.001$), and low-ranking individuals had higher levels (mean = 12.77 ng/g) than high-ranking individuals (mean = 8.77 ng/g) ($U = 50$, $N_1 = 16$, $N_2 = 12$, $P < 0.05$). Females (mean = 9.95 ng/g) had marginally higher cortisol values than males (mean = 8.07 ng/g) ($U = 215$, $N_1 = 24$, $N_2 = 25$, $P < 0.1$). There was no relationship between centrality within social networks and average faecal cortisol levels.

Parasitism

We collected a total of 982 faecal samples, for an average of 20 samples per individual. Mangabeys were infected with six protozoan and nine helminths prior to treatment (Table 1). Average protozoan ($F_{5,287} = 39.22$, $P < 0.01$) and helminth ($F_{7,383} = 13.18$, $P < 0.01$) richness differed significantly over time. Post hoc comparisons showed significant reductions in parasite richness between pre-treatment (helminth: $\mu + SD = 1.55 + 1.29$, $P < 0.001$; protozoa: $\mu + SD = 2.77 + 1.36$, $P < 0.001$) and post-treatment samples (helminth: $\mu + SD =.67 + .87$; protozoa: $\mu + SD = 0.27 + 0.86$). Protozoan infections were acquired more quickly than helminth infections. There was no significant difference between pre-treatment richness and richness at the final sample for helminths or protozoans, demonstrating return to baseline levels (Fig. 2a, b). Prevalence of five protozoans and four helminths was reduced by at least 50% and contributed to subsequent reinfections (Table 1). These nine parasites were therefore included in Cox proportional hazard models. Protozoans and helminths showed different distributions at the final time points, further supporting our decision to model infection separately for each group of parasites (Fig. 2c, d). A right-skewed distribution of helminth richness showed that a small number of individuals harboured many parasite taxa whereas most individuals were infected with only a few taxa (Fig. 2d).

Following treatment (and prior to the end of the study), 81 new helminth infections (48 in adults) and 189 new protozoan infections (111 in adults) were detected. High centrality, closeness and betweenness in proximity networks were associated with rate to infection with helminth parasites in adult mangabeys (Fig. 3, Table 2). When all age classes were included, these patterns were in the same direction but marginal. No centrality measures explained time to reinfection with protozoans. Neither host traits (e.g. sex, age or dominance) nor average post-treatment cortisol levels were associated with rate to infection under either model.

Discussion

Measures of social centrality were more important than individual host traits (e.g. sex, age and dominance) or physiological stress for explaining rates of infection with helminth parasites in mangabeys. Specifically, we found that individuals with more associates (centrality) that were well connected (closeness) and that had a tendency to switch groups (betweenness) were at higher risk of acquiring helminth parasites. Number of associations (strength) and measures of connectivity within contact networks were not associated with infection risk. Acquisition of protozoan infections, which do not tend to aggregate in populations, did not vary according to centrality, stress or host traits. Together, our results show that parasite aggregation was determined primarily by host associations and that social connectivity, rather than the immunosuppressive effect of stress, may explain enhanced infection risk.

The probability of acquiring new helminth infections was higher in animals that were central in the proximity network. This finding demonstrates a potential cost associated with social connectivity. A growing body of evidence suggests that social centrality increases risk of infection from macroparasites. In primates, centrality within grooming networks has been indirectly associated with nematode infections in female Japanese macaques, *Macaca fuscata yuki*, and directly associated with parasite species richness in brown spider monkeys, *Ateles hybridus* (MacIntosh et al., 2012; Rimbach et al., 2015). Our findings differ from studies of brown spider monkeys, however, in which centrality in contact networks was more important than proximity for explaining elevated infection risk (Rimbach et al., 2015).

Helminths must develop in the external environment for days to months before they become infective. Risk associated with connectivity in proximity networks, rather than contact networks, may therefore reflect increased exposure to infectious life stages persisting on fomites and food items (Freeland, 1980). We cannot rule out the possibility that spatial associations serve as proxies for contact (Farine, 2015). However, this is unlikely given that grooming networks showed a more heterogeneous structure (Fig. 1) despite being based on fewer observations. Indeed, space sharing has been identified as an important predictor of risk for infection from macroparasites in other taxonomic groups (Fenner, Godfrey, & Bull, 2011; Godfrey, Moore, Nelson, & Bull, 2010; Perkins, Cagnacci, Stradiotto, Arnoldi, & Hudson, 2009).

We found that protozoans, which are immediately infective once shed into the environment, were acquired uniformly across the population. This result aligns with our current understanding of the transmission biology of protozoan parasites, which tend not to aggregate in hosts like macroparasites (Shaw & Dobson, 1995) (Fig. 2). Similarly, centrality was associated with parasite richness in brown spider monkeys, but these results were not significant for a protozoan (*Entamoeba* spp.) alone (Rimbach et al., 2015). In other taxa, however, contact appears to be important for predicting the spread of directly transmitted and immediately infectious pathogens such as *Mycobacterium bovis* in meerkats, *Suricata suricatta* (Drewe, 2009), and fungi in garden ant, *Lasius neglectus*, colonies (Theis, Ugelvig, Marr, & Cremer, 2015).

Unfortunately, low prevalence often reduces the power of transmission mode-specific models. Nevertheless, systematic studies using aggregate models are important, since cumulative effects of multiple infections can have marked impacts on host health and fitness (Bordes & Morand, 2011). Identifying the hosts with high rates of infection from multiple parasites can therefore help identify fitness costs associated with social behaviour. Such models will be critical in improving our understanding of the role of social contact in pathogen spread (Craft, 2015; Grear, Luong, & Hudson, 2013).

We did not find an effect of glucocorticoid level on acquisition of parasites. Similarly, in female Japanese macaques, social contact was determined to be more important than the immunosuppressive effects of stress in mediating the relationship between dominance and parasite infection (MacIntosh et al., 2012). Parasitism in white-handed gibbons, *Hylobates lar*, and black howler monkeys, *Alouatta pigra*, is also not affected by faecal cortisol (Gillespie, Barelli, & Heistermann, 2013; Martinez-Mota, 2015), although positive associations between parasitism and faecal cortisol have been documented in other primate species (Arlet et al., 2015; Foerster, Kithome, Cords, & Monfort, 2015; Muehlenbein, 2006). The lack of predictable outcomes may be due, in part, to the dynamics of the stress response (Cavigelli & Caruso, 2015) and competing effects of acute and chronic cortisol elevations on immunity (Dhabhar & McEwen, 1999).

Our results show that the nature of connections within a network affect infection risk. In our study, dominant males had stronger relationships in proximity networks, and females, dominant animals and adults tended to be have higher connectivity in grooming networks. However, none of these centrality measures was associated with parasite acquisition. Attempts to link transmission of pathogens to certain host traits based on centrality metrics alone should therefore be interpreted with caution. Furthermore, risk associated with social connectivity is not likely to be static, but rather can vary over time (Rushmore et al., 2013). Heterogeneities in social connectivity, and resulting changes in infection over time, may therefore obscure the relationship between social network position and individual infection risk in cross-sectional studies.

Conclusion

In our study population of Nigerian red-capped mangabeys, animals central in the social network had a higher probability of acquiring macroparasites than did peripheral individuals. Individuals with high centrality may facilitate transmission throughout the population, perhaps acting as 'super spreaders' (Lloyd-Smith, Schreiber, Kopp, & Getz, 2005). We found no direct associations between intrinsic host traits and time to infection, which concords with the result of previous studies. In fact, individual predictors of parasite aggregation (e.g. sex, age and dominance) documented in previous studies may be confounders of the direct relationship between social connectivity and infection risk. Overall, our results suggest that being central in a social network confers costs in terms of infection risk. Variation in social networks structure and dynamics should be considered in studies of infection risk in social species.

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Tables

Table 1 Biological classifications of parasites recovered from faeces of red-capped mangabeys showing prevalence prior to and immediately following deworming, and at the final time point for protozoa and helminths

Classification	Genus	Species	Life cycle	Mode of transmission	Prevalence[a]		
					Pre	Post	Final
Protozoa[b]	*Entamoeba**	*hartmanni*	Direct	Ingestion of trophozoites and cysts	0.80	0.02	0.51
	*Entamoeba**	*histolytica/dispar*	Direct	Ingestion of trophozoites and cysts	0.98	0.02	0.78
	*Entamoeba**	*coli+*	Direct	Ingestion of trophozoites and cysts	0.69	0.02	0.67
	*Iodamoeba**	*bütschlii*	Direct	Ingestion of trophozoites and cysts	0.69	0.02	0.61
	*Chilomastix**	*mesnili*	Direct	Ingestion of trophozoites and cysts	0.47	0.00	0.55
	Balantidium	*coli*	Direct	Ingestion of trophozoites and cysts	0.88	0.02	0.00
Nematoda	*Trichuris*	*trichiura*	Direct	Ingestion of eggs	0.02	0.00	0.00
	Capillaria	sp.	Unknown	Unknown	0.06	0.00	0.04
	*Strongyloides**	sp.	Direct	Ingestion of eggs/penetration of skin by larvae	0.22	0.06	0.35
	Enterobius	sp.	Direct	Ingestion of eggs	0.16	0.02	0.00
	Hookworm*		Direct	Penetration of skin by larvae	0.12	0.02	0.12
	Unknown*	sp.	Unknown	Unknown	0.06	0.00	0.12
	*Abbreviata**	sp.	Indirect1	Ingestion of arthropod	0.87	0.28	0.59
Trematoda	*Paragonimus*	*africanus*	Indirect2	Ingestion of crab	0.43	0.35	0.43
Cestoda	*Bertiella*	sp.	Indirect1	Ingestion of mite	0.04	0.00	0.00

* Indicates that the parasite met the criteria for incorporation into Cox proportional hazards models.

[a] Prevalence was calculated from triplicate samples collected prior to treatment, a single post-treatment sample (protozoan; *N* = 45) and triplicate samples collected at the end of the study.

[b] Species identifications are putative based on size and morphological characteristics of cysts.

Table 2 Marginal Cox proportional hazard models associating host characteristics with rate to parasite infection

Response variable	Network type	Predictors[a, b]	*P*>|*z*|	HR (95% CI)	LRT	ZPH	GVIF[c]
Helminth reinfection	Proximity	Degree	<0.01	2.33 (1.39, 3.91)	<0.01	0.87	<4
		Strength	NS				
		Betweenness	<0.05	1.91 (1.02, 3.57)	<0.05	0.96	<4
		Closeness	<0.01	2.20 (1.33, 3.63)	<0.01	0.94	<4
	Contact[d]		NS				
Protozoan reinfection	Proximity		NS				
	Contact		NS				

HR: hazard ratio; LRT: likelihood ratio test; ZPH: proportional hazard assumption (i.e. residuals not significantly correlated with time); GVIF: generalized variance inflation factor.

[a] Sex, age, dominance status and faecal cortisol were not significant under any model.

[b] Reference level is central.

[c] Generalized variance inflation factors met the rule GVIF <4 for all maximal models.

[d] In degree/out degree (groom-receive) and in strength/out strength (groom-give) were incorporated into models as measures of centrality within contact networks.

Figures

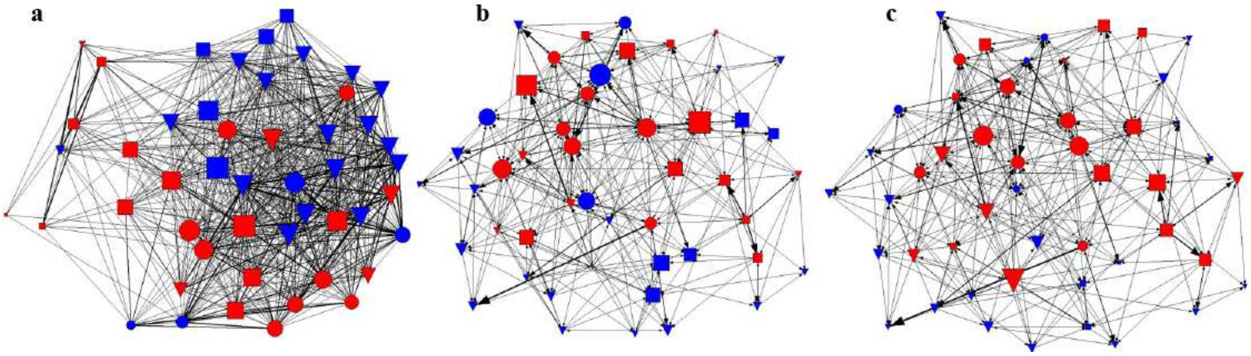

Figure 1. Mangabey social networks showing the position of individuals according to host traits. Nodes represent individuals; edges (lines connecting nodes) represent (a) proximity, (b) directed grooming received (groom-receive) and (c) directed grooming given (groom-give). Node size and colour represent host characteristics, including sex (female = red; males = blue), age (juveniles and subadults = triangles) and dominance status (high ranking = circle; low raking = square). Individuals with more contacts (high degree centrality) are represented by larger nodes. Directed relationships are indicated by edges with arrows. Thickness of lines represents strength of the relationship (strength). No filters were applied.

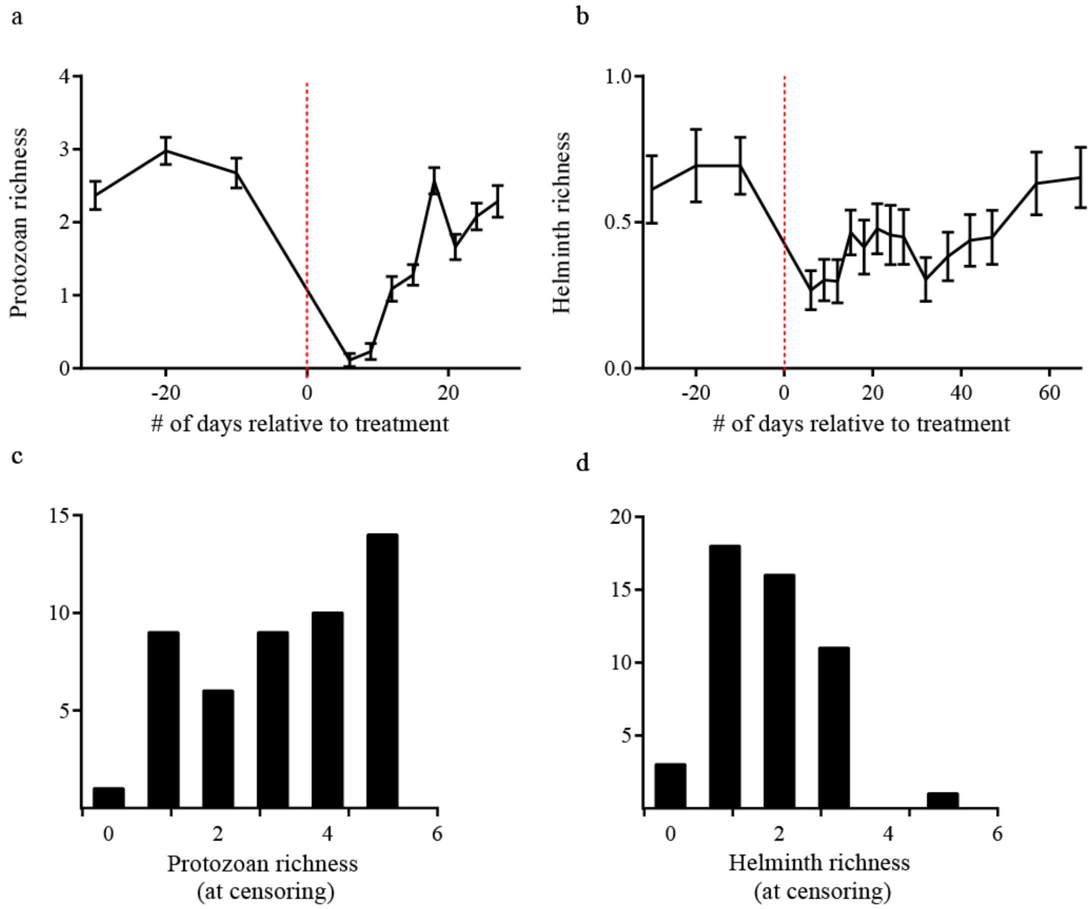

Figure 2. Average parasite richness at each sample point relative to treatment (vertical dashed line) for (a) protozoans and (b) helminths. Error bars show standard error of the mean. Histograms show different distributions of (c) protozoan and (d) helminth parasites within the host population at censoring.

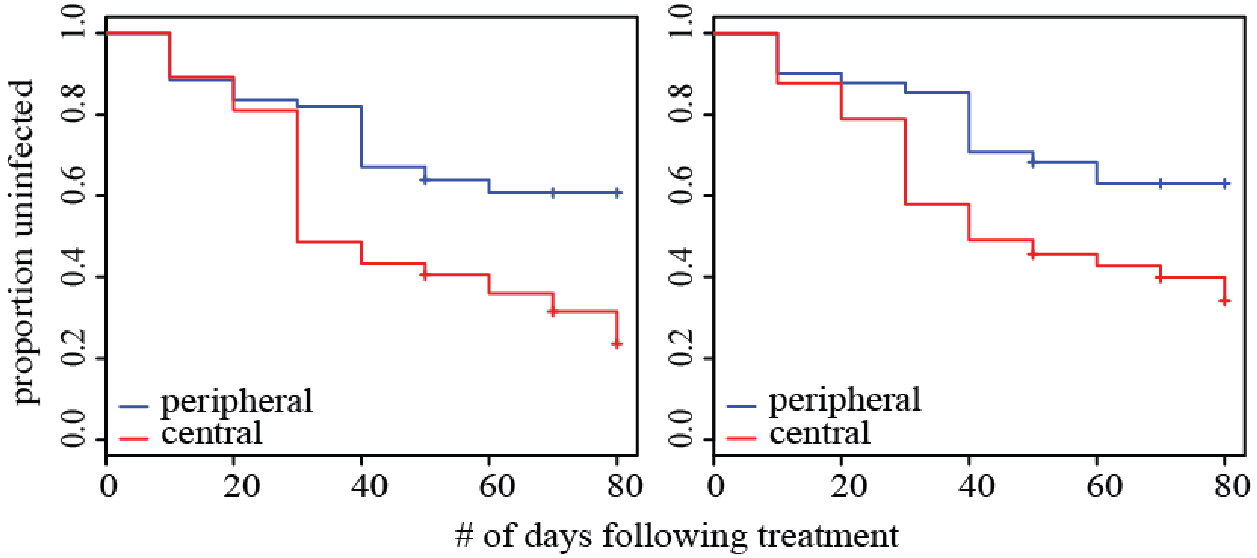

Figure 3. Kaplan–Meier curves showing rate to reinfection for peripheral and central individuals following treatment for parasites based on individuals' (a) degree and closeness centrality and (b) betweenness in proximity networks.

CHAPTER 3: Changes in physiological stress and behaviour in semi-free-ranging red-capped mangabeys (*Cercocebus torquatus*) following antiparasitic treatment

Friant, S., Ziegler, T. E. & Goldberg, T. L. (2016) . Changes in physiological stress and behaviour in semi-free-ranging red-capped mangabeys (Cercocebus torquatus) following antiparasitic treatment. *Proc. R. Soc. B Biol. Sci.*

Abstract

Parasites are ubiquitous in wildlife populations, but physiological and behavioural responses of hosts to infection are difficult to measure. We experimentally treated semi-free-ranging red-capped mangabeys (*Cercocebus torquatus*) in Nigeria with antiparasitic drugs and examined subsequent changes in glucocorticoid production and behaviour. Because both parasites and stress impact energy balance and health, we measured: 1) behavioural time re-allocation via activity budgets, 2) social relationships (e.g. social connectivity and dominance hierarchy stability), and 3) body condition. We collected triplicate faecal samples (n=441) from 49 individuals prior to and following treatment. Cortisol levels fluctuated in parallel with parasite abundance. Elevations in cortisol, but not parasitism, were related to reduced body condition. Behaviour also shifted according to infection status, with uninfected individuals spending more time foraging and less time resting and vigilant compared to when they were infected. Time spent feeding, traveling, or socializing did not differ between pre- and post-treatment time periods. Group cohesion, but not dominance stability, changed following treatment, suggesting parasite induced social avoidance. Together, these findings show a coordinated response to infection that promotes tolerance through stress and energy conservation, reduces transmission risk, and increases protection when infected hosts are vulnerable.

Introduction

Infectious disease, like predation and resource limitation, can constrain population growth [1]. Pathogens with high case-fatality rates (e.g. epidemic viruses) can cause dramatic population declines and can even contribute to local and species extinctions [2–5]. Parasitic infections (e.g. protozoa and helminths) on the other hand, tend to cause only mild or subclinical effects, and their impact on populations are often less dramatic and more protracted [6,7]. As a result, we know less about the effects of "benign" parasitism in wildlife populations than we do about the effects of more virulent pathogens.

Host behavioural and physiological responses to parasitic infection provide important clues into host defences as well as pathogenicity. Defence strategies against parasitic diseases include avoidance (behavioural mechanisms that reduce risk of exposure), resistance (immune response that reduces pathogen burden), and tolerance (multiple mechanisms that reduce susceptibility to fitness costs by limiting damage caused by the pathogen) [8]. Investment in avoidance and tolerance strategies, compared to resistance, has important implications for host infectiousness [9]. Tolerance-promoting behavioural and physiological responses can arise when immunoregulatory cytokines interact with the endocrine system and cause downstream release of glucocorticoids and sickness behaviours [8,10–12]. Indeed, enhanced glucocorticoid production in response to experimental infection from parasites has been documented in domestic animals [13], laboratory amphibians [14], and fish [15,16]. Despite a growing understanding of such processes in controlled settings, little is known about host endocrine response to parasites in naturally infected populations, including the pathophysiological and behavioural consequences of parasite infection and stress.

Behavioral indicators that are expected to decline in frequency or magnitude with illness include social activity, exploratory behaviour, and feeding [17–20]. Indeed, sickness behaviours of whipworm (*Trichuris* spp.) infected red colobus monkeys (*Procolobus rufomitratus tephrosceles*) included a shift to less energetically expensive behaviours [21]. In social animals, time constraints imposed by energetic responses to infection may also impair social relationships [22]. Parasitic infection may inhibit an individual's ability to engage in energetically costly behaviours, such as challenging dominant individuals [23,24]. Furthermore, social contact and proximity can facilitate parasite transmission [25–27], such that animals may modify their behaviour directly to avoid infected conspecifics and reduce infection risk [28].

Parasitism, hormonal changes, and behavioural time reallocation can alter body condition and lead to reductions in fitness [29–32]. For example, low body condition in parasitized Iberian hares (*Lepus granatensis*) contributes to reduced antipredator defence and higher host mortality [33]. In male fence lizards (*Sceloporus occidentalis*), hormonal changes resulting from parasitism can reach levels capable of inhibiting reproduction [34]. In addition, time reallocation toward behaviours that conserve energy and facilitate recovery can impair survival and reproduction [22,35].

Research Aims

We investigated whether gastrointestinal and pulmonary parasites influenced the behaviour and physiology of hosts living in a complex social environment. We followed a group of semi-free-ranging red-capped mangabeys (*Cercocebus torquatus*) before and after chemotherapeutic treatment for protozoan and helminth parasites and measured ensuing changes in: 1) glucocorticoid production, 2) activity budgets, 3) social relationships (e.g. social connectivity and dominance hierarchy stability), and 4) body condition. We tested the hypothesis that antiparasite treatment would reduce tolerance and avoidance strategies. Specifically, we predicted that a reduction in parasitic infections would result in a corresponding reduction in cortisol levels, time reallocation toward energetically expensive behaviours with potential positive fitness consequences (e.g. reduced stress, and enhanced resource acquisition and predator detection), increased social connectivity, decreased hierarchical stability, and a subsequent reversion in behavioural and hormonal changes as animals became reinfected.

Methods

Study site & population

All study activities took place at Rhoko Research and Conservation Education Centre (41.21º N, 16.16º E), the forest site of the Centre for Education, Research and Conservation of Primates and Nature (CERCOPAN) in Nigeria. We studied 49 individually recognizable red-capped mangabeys living as a multi-male, multi-female social group in a one-hectare open topped forest enclosure within the natural home range of the species. Animals were vulnerable to natural predators (e.g. snakes and birds of prey) and parasites. No immigration or emigration events took place, thereby limiting external changes in the social environment throughout the duration of the study. The population was provisioned three times daily, which lessened the effects of temporal variation in resource availability, but the animals still ate wild foods opportunistically and drank from a stream running through the enclosure [25]. Climate included a long wet season from April to November and a short dry season from November to March.

Study design

Faecal sampling and behavioural and health data were collected during the rainy season (May - August 2012) to reduce effects of seasonality. In June, the population was treated for gastrointestinal parasites using orally administered metronidazole for protozoans, mebendazole for nematodes, and praziquantel for cestodes and trematodes [25]. Faecal and behavioural sampling was conducted over three sampling periods: 30 days prior to treatment ("pre-treatment"), and two 30-day post-treatment periods ("post-treatment 1" and "post-treatment 2"). Triplicate faecal samples from each period were analysed to maximize detection of infections from parasites with variable shedding rates and generate mean glucocorticoid values for each individual. Pre-treatment sampling was conducted prior to any experimental procedures, and a 10-day gap without any behavioural observations was included between pre-treatment and post-treatment 1 periods to minimize influence of any behavioural changes that may have occurred during the drug administration process.

Parasitological analyses

One gram of faeces was taken from formalin preserved samples and concentrated via faecal sedimentation for assessment of gastrointestinal parasites [25,36]. The entirety of the sediment was systematically examined at X10 objective light magnification, and all helminth eggs and larvae and large protozoan trophozoites and cysts were counted. One drop of sediment from each sample was examined at X40 for identification of small protozoan cysts. Protozoan densities were scored as many (4), moderate (3), few (2), rare (1), or none (0) [37]. Population infection status at each time point was calculated by taking the average number of diagnostic stages (e.g. eggs, larvae, cysts or trophozoites; hereafter referred to as eggs per gram [epg] for simplicity) of triplicate samples. Mean abundance of infection (epg in any host) was calculated with bootstrap confidence limits using Quantitative Parasitology software [38,39].

Faecal cortisol analyses

Faecal cortisol levels were measured via enzyme immunoassay [40]. Methodological details and assay validation are described in detail in Friant et al. [25]. Interassay variation for the high pool was 18.3% and for the low pool was 22.2%, whereas intra-assay variation was 3.8% for the high pool and 7.9% for the low pool. Individual average faecal cortisol levels (ng/g) were calculated from triplicate samples collected during each time period.

Behavioural observations

Behaviour was measured via 1 min focal observations with combined continuous and instantaneous point sampling, and structured *ad libitum* sampling methods [41,42]. The number of seconds an animal was vigilant (defined herein as any visual search or directed gaze beyond arm's reach) was recorded during 1 min continuous follows [42]. A single instantaneous point sample was taken at the end of each focal observation period to record activity (e.g. feed [bringing food to mouth, biting, or chewing], forage [actively searching for or externally processing foods, including nut cracking], travel, social, or rest) and identifications of all nearest neighbours within 2 meters [42]. Focal individuals were selected opportunistically, and at least

30 seconds were allowed to elapse between observations to reduce interdependence of data [25]. Focal observations for each individual in the group were conducted three times daily: early morning (7:00-10:00), mid-morning (11:00-15:00), and evening (16:00-19:00).

All observed agonistic interactions and directionality of submissions and supplants were recorded using structured *ad libitum* sampling as conducted for similar species [43]. All data were collected by three observers and inter-observer reliability was tested and accepted by calculating Fleiss's Kappa test for categorical agreements between multiple observers (Kappa= 0.89, $p < 0.001$ for observers 1 and 2; Kappa = 0.92, $p < 0.001$ for observers 1 and 3), and Pearson's correlation coefficients for continuous measures of vigilance ($r = 0.97$, $p < 0.001$ for observers 1 and 2, and 1 and 3).

Social variable construction

Dominance ranks at multiple time periods were calculated using the Elo-rating procedure based on progressive evaluation of dyadic supplants, and aggressive and avoidance interactions between adults of the same sex throughout the study period. Elo-ratings were used because they allow for rank assignment at multiple time points without constructing new matrices and are therefore preferred for comparisons across short time periods [44,45]. Hierarchical stability was calculated from Elo-ratings over each sampling period, allowing a 10-day burn-in period during the pre-treatment period. The stability measure (S) represents the ratio of rank changes per individuals present at a given point in time, and ranges between 0 (unstable) and 1 (stable) [44]. Elo-ratings and stability scores were calculated using the *EloRating* and *zoo* packages in R 3.2.2 [46].

Weighted and unweighted proximity networks were constructed based on observed pairwise associations between focal individuals and all their nearest neighbours within 2 m using SOCPROG 2.6 [47]. Weights were calculated from the total number of associations between dyads within each study period. Symmetric matrices with attribute information were imported into UCINET software for calculation of group cohesion during pre-treatment, post-treatment 1 and post-treatment 2 periods [48].

Visual health assessments

Ordinal indices of health along five dimensions representing the major organ systems and clinical syndromes were recorded for each individual during a veterinary visual health assessment conducted between the pre-treatment and post-treatment 1 period (modified from Fig. S1). Indices were scored as unaffected (0) to 100 percent affected (4), and included: pelage condition (color, sheen, roughness), body condition (prominence of ilium, scapula, ribs, vertebrate, and cheek bones), and mobility (arms, legs, tail). Respiration was scored as the number of sneezes or coughs per min and faecal consistency as firm (0), soft (1), runny (2), or mucoid (3) averaged over 3 samples. Individual monkeys were scored independently by each of the three observers, then collectively to reach consensus where scores differed.

Statistical analyses

The efficacy of chemotherapeutic treatment and occurrence of subsequent reinfection events were measured by comparing mean abundance between paired pre-treatment/post-treatment 1, under the directional hypothesis of reduced infection and post-treatment 1/post-treatment 2 samples, under the directional hypothesis of increased epg following reinfection respectively. Because of the skewed distributions characteristic of parasites, comparisons were made with permutation tests using the *coin* package in R 3.2.2 [46,49].

We incorporated mean cortisol levels, percent time allocated to different activities (*i.e.* feeding, foraging, socializing, traveling, and resting), and average number of seconds per minute spent vigilant, into a series of mixed-effects linear models with an autocorrelation structure of 1. Response variables with non-normal distributions were transformed to meet assumptions of normality. We incorporated sample period, sex, and age-class as main effects in each model, and included individual identification as a random effect. We set the first post-treatment period as the reference period to examine changes associated with removal of parasites (pre-treatment vs. post-treatment 1) and subsequent reinfection (post-treatment 1 vs. post-treatment 2). We initially included all variables in the models and used backwards elimination and Akaike information criterion (AIC) to select the best models. Analysis of variance (ANOVA) was run on final models to test significance. We retained only significant variables and first-order interactions (at the alpha < .05 level) in final models where AIC of the model was lower than the null (difference ≥ 2). Age and sex were only included as main effects in the final model if there was a significant interaction with sampling period, thus allowing us to test only for significant differences in physiological and behavioural responses to infection among individuals. We performed analyses with the *nlme* package in R 3.2.2 [46].

We quantified network density of weighted and unweighted networks as measures of group cohesion [50]. We compared network densities at different time points using a paired (same nodes) bootstrap technique in UCINET (analogous to the classical paired sample t-test) for comparing networks with the same actors [51]. Network diagrams were constructed using UCINET software's NetDraw program, with node size representing individual degree centrality (number of associates), weighted edges representing strength (number of associations between nodes), and without filters.

We used principal components analyses (PCA) to generate uncorrelated health indices from visual health assessments that retained much of the original variation. We compared principal components representing health indices to measures of parasitism and cortisol generated from samples collected prior to the date of the health assessment. The principal components representing at minimum 80% of the variance were plotted following standardization around zero on each axis. Measures of parasitism and cortisol were then colour coded and visualized using convex hulls. Hulls were constructed to represent cortisol, parasite richness (total number of species), and individual infection status from three parasites with high prevalence and intensities: a protozoan (*Balantidium coli*), a nematode (*Abbreviata* sp.), and a trematode (*Paragonimus africanus*). Individuals were assigned "high" or "low" richness and cortisol values by dichotomizing log-normal continuous variables at the median. Continuous measures were retained by scaling individuals according to parasite intensity, richness, and cortisol level (ng/g) within plots.

Results

Treatment effect on parasites

Parasites were recovered from triplicate faecal samples from every individual during each sampling period (n = 441). Mangabeys were infected with six protozoan and nine helminth taxa prior to treatment [25]. Chemotherapeutic treatment significantly reduced the abundance of protozoan ($p < 0.001$ [95% CI: 0.000 - 0.001]) and helminth ($p < 0.001$ [95% CI: 0.000 - 0.001]) infections from pre-treatment to post-treatment 1 samples. Mean parasite abundance significantly increased indicating reinfection between post-treatment 1 and post-treatment 2 samples (protozoans ($p <.001$ [95% CI: 0.000 - 0.001]); helminths ($p <.05$ [95% CI: 0.008 - 0.013]) (Table S1; Fig. 1 a,b).

Treatment effect on faecal cortisol

Individual cortisol levels were calculated from triplicate faecal samples during three 30-day time periods ($n = 441$). Cortisol levels changed significantly over time (log transformed: $F_{2,96} = 19.54$, $p < 0.0001$) (Fig. 1c). Mean cortisol decreased significantly following treatment ($t_{96}= 6.23$, $p < 0.0001$) and then increased in post-treatment 2 ($t_{96}= 2.64$, $p < 0.01$). Cortisol change did not differ significantly based on an individual's sex or age.

Treatment effect on behavioural time allocation

Activity budgets were calculated from 11,019 instantaneous point samples ($M \pm SD = 225 \pm 6.66$ per individual) over three 30-day time periods (pre-treatment n = 3,661; post-treatment 1 n = 4,063; post-treatment 2 n = 3,295). Time spent foraging ($F_{2,96} = 41.16$, $p < 0.0001$) and resting ($F_{2,96} = 44.51$, $p < 0.0001$) changed significantly between treatment periods (Fig. 2a). Foraging behaviour increased significantly (9%) following treatment ($t_{96}=31.5$, $p < 0.0001$), and corresponded to a significant decrease (7%) in resting behaviour ($t_{96}=6.52$, $p < 0.0001$). Feeding, traveling, and social behaviour did not change significantly between pre-treatment and post-treatment 1 periods. Resting behaviour continued to decrease (3%) between post-treatment 1 and post-treatment 2 periods (t_{96}= 2.64, $p < 0.01$). Other behaviours did not change significantly between post-treatment 1 and post-treatment 2. Behavioural changes did not differ significantly between sex and age classes.

Vigilance levels were calculated from 178 hours (M ± SD = 3.63 hrs ± 14 min per individual) of focal observations over three 30-day time periods (pre-treatment n = 58 hrs; post-treatment 1 n = 66 hrs; post-treatment 2 n = 54 hrs). Vigilance levels changed significantly between time periods ($F_{2,96}$ = 180.61; $p < 0.0001$; Fig 2b). Specifically, vigilance reduced significantly following treatment (t_{96} = 15.80, $p< 0.0001$), but did not change significantly in the post-treatment 2 period. Change in vigilance did not differ significantly based on an individual's sex or age.

Treatment effect on social relationships

Dominance ranks at multiple time periods were calculated from Elo-ratings based on 888 dyadic supplants and aggressive and avoidance interactions between adults of the same sex (male n = 367; female n = 521) throughout the study period. Rank changes occurred throughout the study, and dominance hierarchy stability varied by 1.38% in female hierarchies (pre-treatment $S = 98.35$; post-treatment 1 $S = 98.39$; post-treatment 2 $S = 99.73$) and 0.89% for male hierarchies (pre-treatment $S = 98.32$; post-treatment 1 $S = 99.21$; post-treatment 2 $S = 98.85$) (Figure S2).

Proximity networks were constructed from 4,042 observed pairwise associations (pre-treatment n = 970, post-treatment 1 n = 1112, post-treatment 2 n = 1117). The number of associates increased marginally between pre-treatment (binary density = 0.38) and post-treatment 1 sampling (binary density = 0.42) (t_{48}= -1.92, p = 0.05, d = -0.39), and continued to increase between post-treatment 1 and post-treatment 2 (binary density = 0.45) (t_{48}= -1.98, $p < 0.05$, d = -0.40). Number of associations increased significantly between pre-treatment (valued density = 0.82) and post-treatment 1 sampling (valued density = 0.94) (t_{48} = -2.30, $p < 0.05$, d = -0.46), but did not change between post-treatment 1 and post-treatment 2 (valued density = 0.95).

Relationship among body condition, infection, and stress

Eighty-six percent of the variation in visual health indices was explained by PC 1 (60%) and PC2 (26%) together. Principal component loadings were pelage colour (PC1: - 0.42; PC2: - 0.45), pelage roughness (PC1: - 0.40; PC2: - 0.22), pelage sheen (PC1: - 0.48; PC2: - 0.43), ilium prominence (PC1: - 0.50; PC2: 0.53), and scapula prominence (PC1: - 0.42; PC2: 0.53). Faecal consistency contributed only to PC4 and PC5, which together accounted for only 3% of the overall variance, and was therefore omitted from further analyses. Health measures with no observable variation were omitted from PCA. Individuals with higher cortisol levels occupied PCA values associated with more affected body condition (Fig. 3). Individuals infected with *B. coli* occupied PCA values associated with more variable body condition compared to uninfected individuals, which tended to be centred around the mean (Fig. S2 b). Body condition did not appear to be affected by *Abbreviata* sp. or *P. africanus* infection or overall parasite richness (Fig. S2 a,c,d).

Discussion

Our results show that reduced stress levels and altered behaviour accompanied treatment of parasitic infections in red-capped mangabeys. Average population cortisol levels co-varied with parasite abundance, and high cortisol levels were associated with decreased body condition. When parasites were removed, individual activity patterns changed from resting and vigilance to active foraging. Although time spent engaging in social behaviour and hierarchical stability were not affected by parasite infection, the number and frequency of spatial associations increased following parasite treatment. Interestingly, behavioural changes did not revert to baseline levels by the end of the study, suggesting that parasite-induced behavioural change only occurs above a certain threshold of infection, or alternatively, that there is a delay in behavioural responses to immune defence and signalling. Behavioural and physiological responses to parasite treatment did not differ between sex and age-classes, showing that the effects of parasitism were distributed equally across these subpopulations. Together, these results suggest that parasite-associated alterations in host physiology and behaviour have negative consequences for host fitness. Whether observed changes occurred as a coordinated host response to the effects of parasitism (e.g. tolerance), or as a result of parasite exploitation of the host [52,53], remains to be determined.

Parasite infections appear to have induced stress in naturally infected red-capped mangabeys. To date, it has remained unclear whether positive associations between cortisol and parasitism observed in wild primates was the result of increased susceptibility due to immunosuppressive effects, or if the parasites themselves induced a host stress response [54–56]. Indeed, explanations for patterns of parasite aggregation in certain hosts (e.g. males or dominant individuals) typically invoke hormonal regulation of the immune response as an important mechanism underlying susceptibility [57–60]. However, variation in cortisol levels did not explain time-to-reinfection [25]. Our results suggest that parasites themselves elicited a stress response, which to our knowledge had previously been demonstrated only in domestic and laboratory animals [13–16]. In addition, glucocorticoid production helped explain variation in physical estimators of health, suggesting that elevated stress, including contributions of parasitic infection to allostatic load, negatively influences body condition.

Experimental parasite reduction and associated reductions in glucocorticoid production corresponded with time reallocation away from resting and vigilance and toward increased foraging activity. Movement around the enclosure was primarily foraging for food, as opposed to simply "travel", which was observed far less frequently (Fig. 2). Energetic trade-offs between resting and foraging are consistent with sickness behaviour, in that parasitized animals favoured low energy states when infected with parasites. Similarly, red-colobus monkeys increased resting behaviour when infected with whipworm [21], and experimentally treated Grant's gazelles (*Nanger granti*) increased foraging behaviour and decreased vigilance compared to parasitized controls [61].

Interestingly, we found that parasite removal lead to a significant increase in foraging but not feeding, despite feeding suppression being common during parasitic infections [17]. Our results lend support to the notion that parasite induced feeding suppression results from a motivational state to conserve energy (*i.e.* reduced food consumption is a result of energetic trade-offs that decrease foraging) [62]. For example, early experiments in rodents found that operantly conditioned and experimentally infected rats stopped pressing a lever to receive water, but would drink it when readily available [63]. In this study, we may not have seen reductions in feeding because provisioned foods were readily available. Alternatively, infected individuals may have been less selective in their diets, thereby leading to reduced time spent foraging, yet equal time spent feeding. Further investigations combining information on food availability, feeding and foraging frequency, and dietary composition in naturally infected wild animals will be useful in determining if food quality, as opposed to only food quantity, changes in infected versus uninfected individuals. In red colobus monkeys, for example, feeding frequency did not vary with infection status, but whipworm-infected animals shifted dietary composition to include more plants with medicinal properties [21].

Vigilance, which is protective against both predators and conspecific competition [64], decreased following treatment for parasites. Decreased vigilance (and increased foraging) was also observed in Grant's gazelles following experimental reduction in parasitism [61]. These findings suggest that parasite infected individuals allocate more time proportionally to vigilance to compensate for greater vulnerability [65]. Similar trade-offs between foraging and vigilance behaviour in inherently vulnerable animals were documented in pregnant European rabbits with poor physical condition (*Oryctlagus cuniculus*) [66], and vulnerable marmots (*Marmota flaviventris*) to enhance over-winter survival [67]. Reduced vigilance in our study population may indicate reduced vulnerability to predation and conspecific competition, resulting from parasite removal.

Where social structure impacts transmission dynamics, social isolation of infected individuals should reduce transmission through avoidance [68]. For example, guppies (*Poecilia reticulata*) actively avoided experimentally infected individuals and reduced network clustering in the presence of infected conspecifics [28]. Indeed, risk of parasite transmission is increasingly attributed to social connectivity in primate populations [25–27]. In this study, individual connectivity through spatial associations, a known risk factor for acquisition of new infections in this population [25], increased when population levels of parasitism were reduced, despite no observed changes in time spent engaging in social behaviour. Determining whether reduced cohesion resulted from active avoidance of infected conspecifics or was a by-product of highly variable activity budgets will require further investigation.

Conclusion

In a population of semi-free-ranging red-capped mangabeys, parasitism was associated with avoidance and tolerance responses. Specifically, cortisol levels, activity budgets, vigilance, and spatiotemporal associations co-varied with levels of parasitism following treatment to remove parasites. This response to infection appears to maximize energy balance, reduce risk of transmission from infected conspecifics, and increase defence against competition and predation when animals are vulnerable. These findings suggest that fitness advantages of parasite-induced sickness behaviour may be mediated by neuroimmunoendocrine mechanisms that facilitate host tolerance.

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Figures

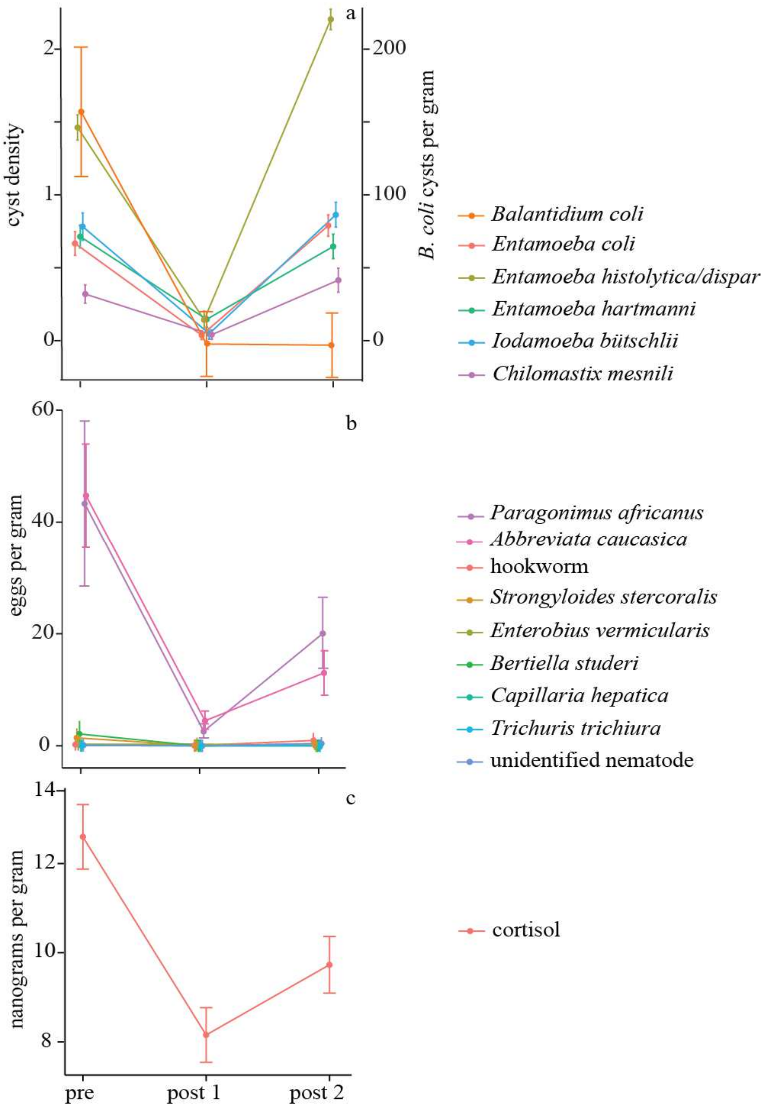


Figure 1. Parasite abundance and faecal cortisol relative to chemotherapeutic treatment for parasitic infections. Protozoan densities were scored as many (4), moderate (3), few (2), rare (1), or none (0). *Balantidium coli* cysts and all helminth eggs/larvae were counted as cysts or eggs per gram, respectively.

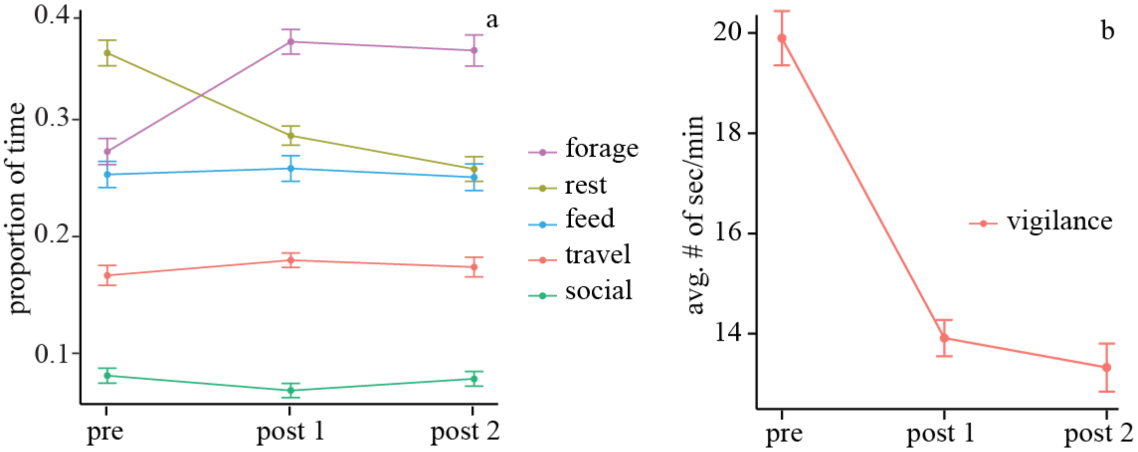


Figure 2. Mean ± SE proportion of time that red-capped mangabeys spent foraging, resting, feeding, traveling, and socializing (a) and vigilant (b) prior to and following chemotherapeutic treatment for parasites.

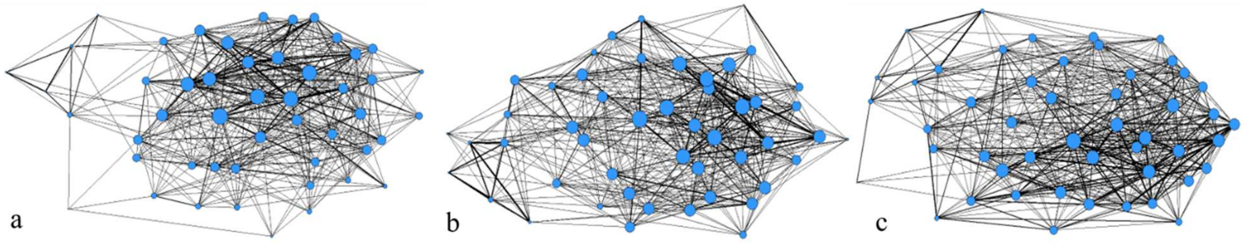


Figure 3. Social networks during pre-treatment (a), post-treatment 1 (b), and post-treatment 2 (c) sampling periods. Nodes represent individuals and edges (lines connecting nodes) show interactions defined by proximity. Individuals with more associates (high degree centrality) are represented by larger nodes. Thickness of lines represents the number of associations between individuals (strength).

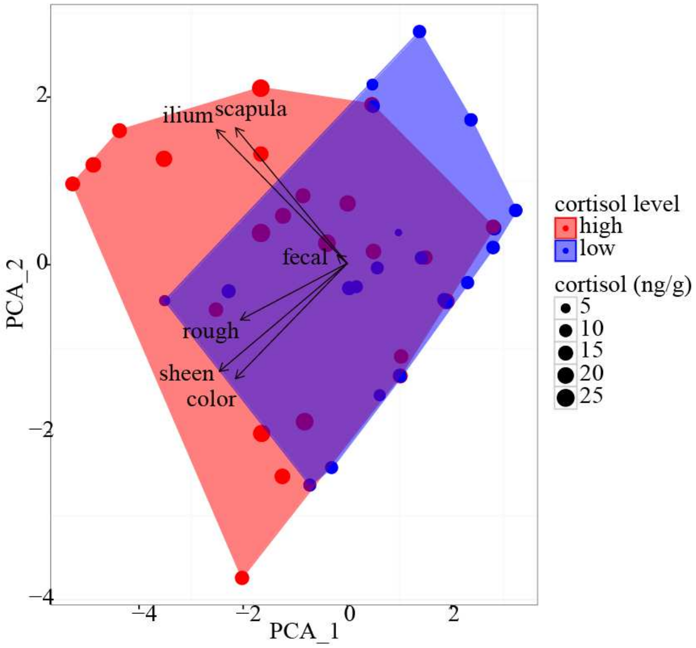


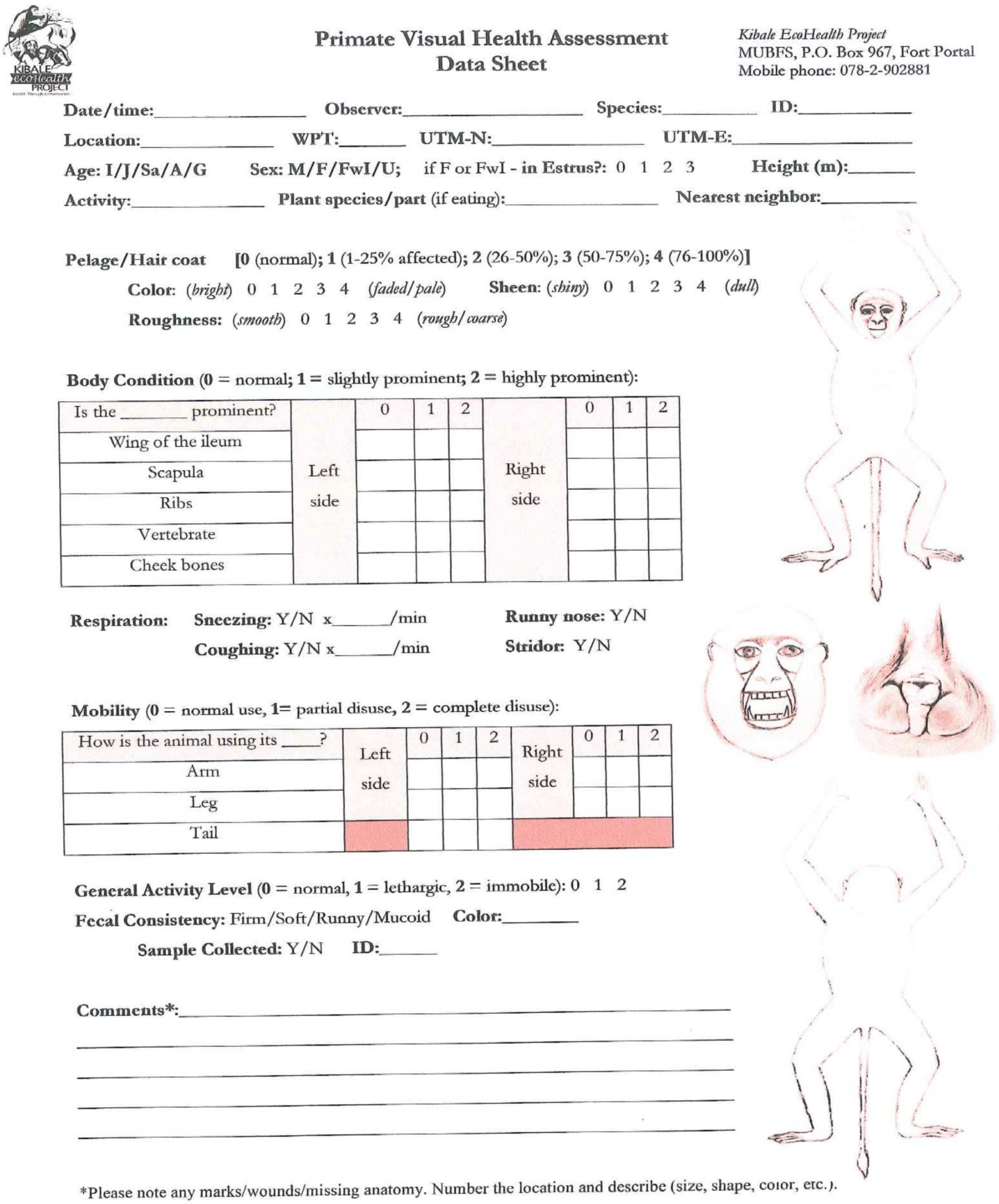
Figure 4. Visualization of principal component analysis (PCA) of five visual health indices (ilium and scapula prominence, faecal consistency, and fur roughness, sheen, and colour), with first two components (PC1 and PC 2) shown. Data points represent individuals, and are scaled by faecal cortisol level (ng/g). Convex hulls are occupied by individuals with high (red) or low (blue) cortisol. The individual points projecting furthest in the direction of a given vector are the individuals with most affected body condition relevant to that variable, while those projecting opposite of variable vectors are least affected.

Supplementary information

Table S1. Parasite abundance prior to and following parasite treatment.

Parasite	Abundance			Bootstrap comparisons[a]	
	pre-treatment	post-treatment 1	post-treatment 1	pre-treatment vs. post-treatment 1	post-treatment 1 vs. post-treatment 2
Entamoeba coli	0.67 (0.51-0.84)	0.04 (0.00-0.12)	0.78 (0.64-0.95)	<0.01 (0.00-0.00)	<0.01 (0.00-0.00)
Entamoeba histolytica/ dispar	1.46 (1.29-1.63)	0.14 (0.06-0.40)	2.20 (2.07-2.34)	<0.01 (0.00-0.00)	<0.01 (0.00-0.00)
Entamoeba hartmanni	0.71 (0.57-0.87)	0.14 (0.07-0.31)	0.64 (0.49-0.82)	<0.01 (0.00-0.00)	<0.01 (0.00-0.00)
Iodamoeba bütschlii	0.78 (0.59-0.99)	0.05 (0.00-0.17)	0.86 (0.70-1.06)	<0.01 (0.00-0.00)	<0.01 (0.00-0.00)
Chilomastix mesnili	0.32 (0.22-0.45)	0.04 (0.00-0.10)	0.42 (0.29-0.61)	<0.01 (0.00-0.00)	<0.01 (0.00-0.00)
Balantidium. coli	164 (91.1-348)	0.86 (0.00-2.57)	0.00 (NA)	<0.01 (0.00-0.00)	NS
hookworm	0.25 (0.06-0.49)	0.04 (0.00-0.10)	0.96 (0.12-5.10)	NS	NS
Strongyloides stercoralis	1.43 (0.24-6.12)	0.06 (0.00-0.12)	0.35 (0.20-0.49)	NS	NS
Enterobius vermicularis	0.29 (0.12-0.65)	0.31 (0.00-1.22)	0.00 (NA)	NS	--
Bertiella studeri	2.12 (0.00-9.49)	0.00 (NA)	0.00 (NA)	--	--
Capillaria. hepatica	0.08 (0.02-0.19)	0.02 (0.00-0.06)	0.06 (0.00-0.18)	NS	NS
Trichuris trichiura	0.05 (0.00-0.15)	0.00 (NA)	0.00 (NA)	--	--
unidentified sp.	0.11 (0.20-0.31)	0.00 (NA)	0.42 (1.22-1.33)	--	--
Abbreviata sp.	44.70 (28.7-68.4)	4.48 (2.41-9.18)	13.0 (7.37-25.80)	<0.01 (0.00-0.00)	<0.05 (0.04-0.05)
Paragonimus africanus	43.30 (22.5-88.1)	2.60 (1.22-6.17)	20.0 (9.86-38.10)	<0.01 (0.00-0.00)	<0.01 (0.00-0.01)

[a] Permutation tests unable to compute p-values for periods containing only one level. P-values omitted for comparisons between samples containing only zeros.



Kibale EcoHealth Project
MUBFS, P.O. Box 967, Fort Portal
Mobile phone: 078-2-902881

Primate Visual Health Assessment
Data Sheet

Date/time:__________ **Observer:**__________ **Species:**__________ **ID:**__________

Location:__________ **WPT:**_______ **UTM-N:**__________ **UTM-E:**__________

Age: I/J/Sa/A/G **Sex: M/F/FwI/U;** if F or FwI - **in Estrus?:** 0 1 2 3 **Height (m):**_______

Activity:__________ **Plant species/part** (if eating):__________ **Nearest neighbor:**__________

Pelage/Hair coat **[0** (normal); **1** (1-25% affected); **2** (26-50%); **3** (50-75%); **4** (76-100%)**]**

Color: (*bright*) 0 1 2 3 4 (*faded/pale*) **Sheen:** (*shiny*) 0 1 2 3 4 (*dull*)

Roughness: (*smooth*) 0 1 2 3 4 (*rough/coarse*)

Body Condition (**0** = normal; **1** = slightly prominent; **2** = highly prominent):

Is the _______ prominent?		0	1	2		0	1	2
Wing of the ileum	Left side				Right side			
Scapula								
Ribs								
Vertebrate								
Cheek bones								

Respiration: **Sneezing:** Y/N x______/min **Runny nose:** Y/N

Coughing: Y/N x______/min **Stridor:** Y/N

Mobility (**0** = normal use, **1**= partial disuse, **2** = complete disuse):

How is the animal using its ____?		0	1	2		0	1	2
Arm	Left side				Right side			
Leg								
Tail								

General Activity Level (**0** = normal, **1** = lethargic, **2** = immobile): 0 1 2

Fecal Consistency: Firm/Soft/Runny/Mucoid **Color:**________

Sample Collected: Y/N **ID:**______

Comments*:____

*Please note any marks/wounds/missing anatomy. Number the location and describe (size, shape, color, etc.).

Figure S1. Primate visual health assessment data sheet

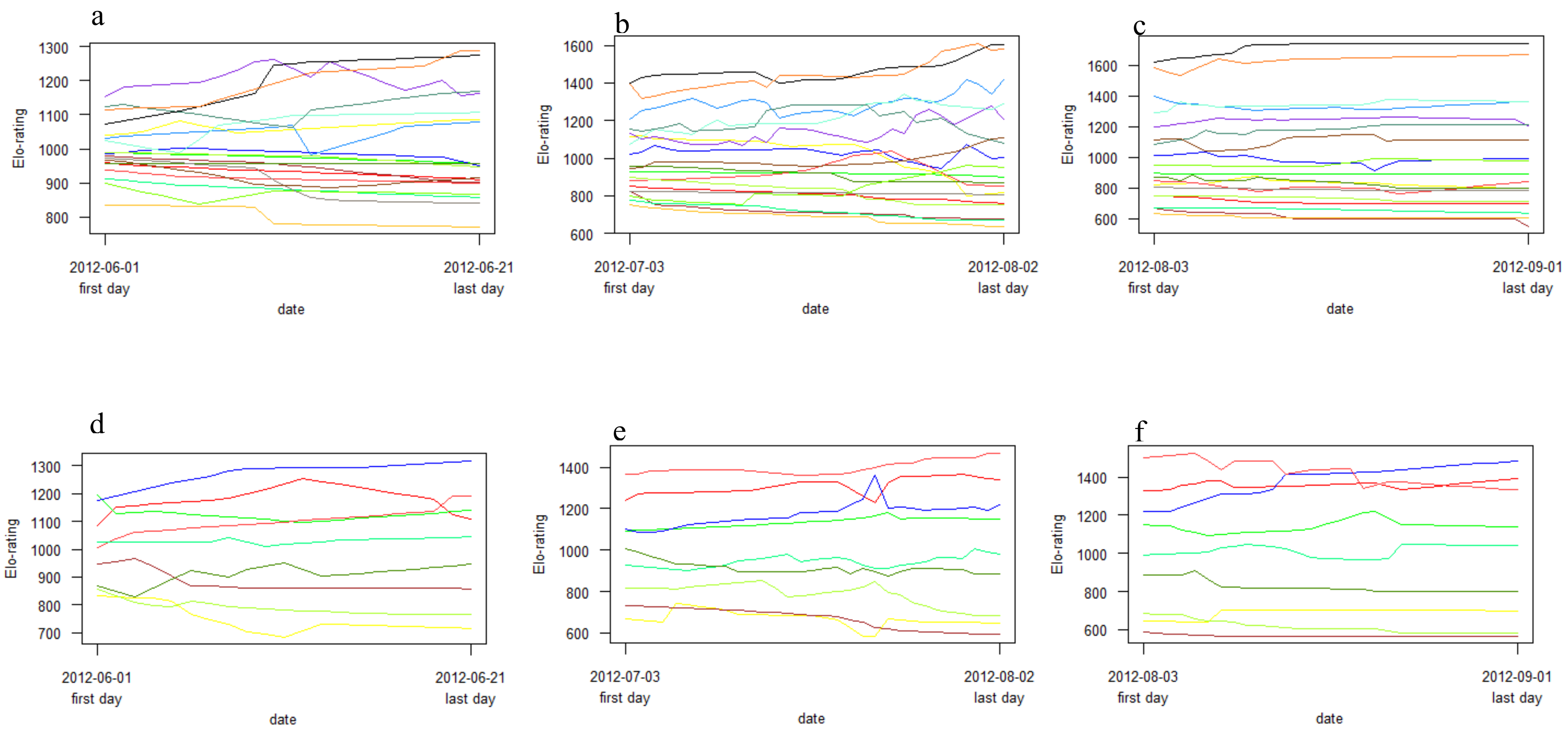


Figure S2. Elo-ratings of 19 female (a-c) and 9 male adult mangabeys (d-f). Each line represents one individual and intersecting lines show interactions resulting in rank changes. Stability scores were 98.35% (a; pre-treatment), 98.39% (b; post-treatment 1), and 99.73% (c; post-treatment 2) for female hierarchies and 98.32% (c; pre-treatment), 99.21% (d; post-treatment 1), and 98.85% (e; post-treatment 2) for male hierarchies.

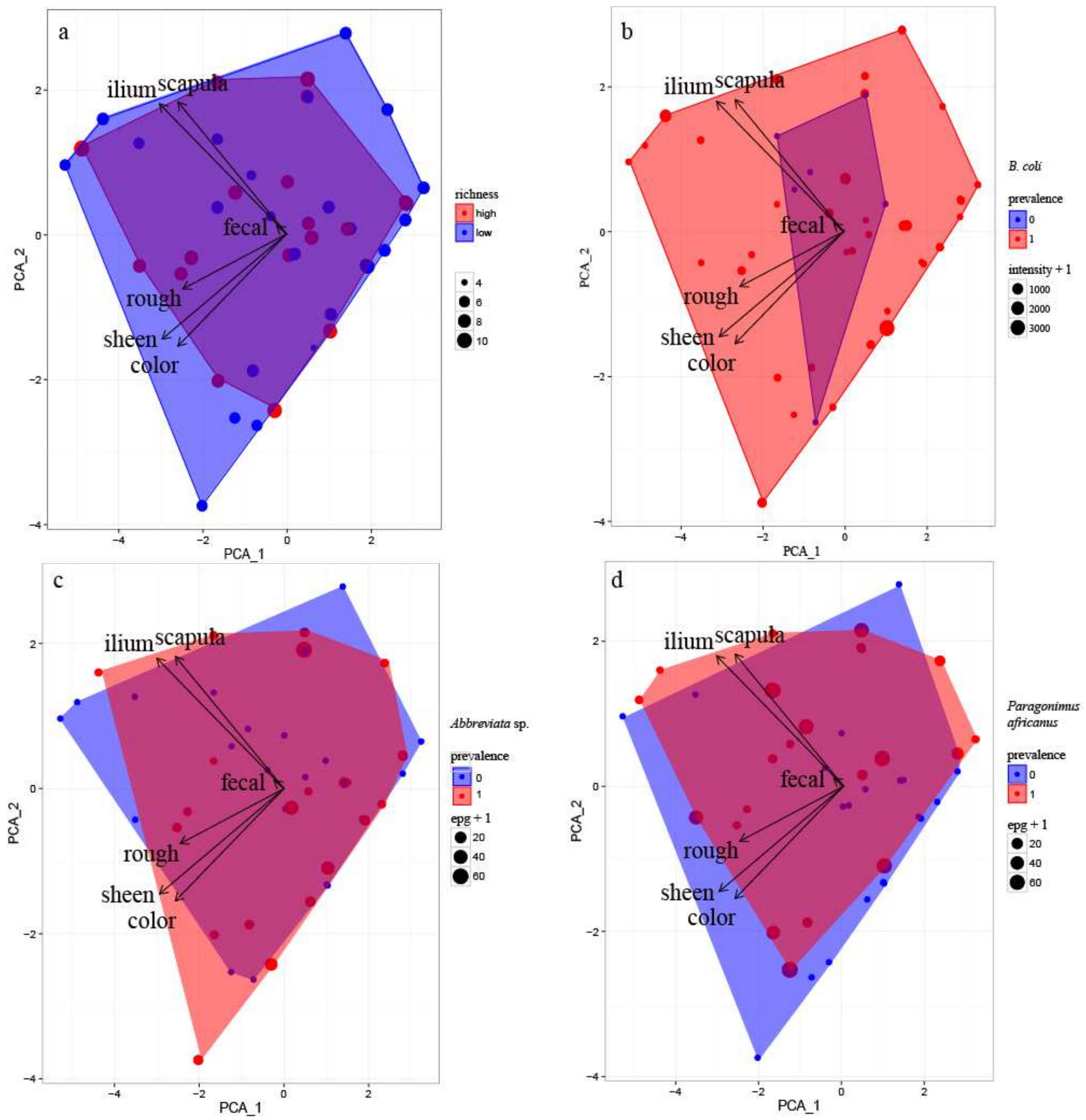


Figure S3. Visualization of principal component analysis (PCA) of five visual health indices (ilium and scapula prominence, fecal consistency, and fur roughness, sheen, and color), with first two components (PC1 and PC 2) shown. Data points represent individuals, and are scaled by parasite richness (a), and *B. coli* (b) *Abbreviata* sp. (c) and *Paragonimus africanus* (d) intensity. Convex hulls are occupied by infected (red) or uninfected (blue) with each parasite. The individual points projecting furthest in the direction of a given vector are the individuals with most affected body condition relevant to that variable, while those projecting opposite of variable vectors are least affected.

CHAPTER 4: Lung fluke (*Paragonimus africanus*) infects Nigerian red-capped mangabeys and causes respiratory disease

Friant, S., Brown, K., Saari, M. T., Segel, N. H., Slezak, J. & Goldberg, T. L. 2015 Lung fluke (*Paragonimus africanus*) infects Nigerian red-capped mangabeys and causes respiratory disease. *Int. J. Parasitol. Parasites Wildl.* 4, 329–332. (doi:10.1016/j.ijppaw.2015.08.003)

Abstract

Eggs of the lung fluke genus *Paragonimus* were detected in red-capped mangabeys (*Cercocebus torquatus*) in Nigeria. We assess the role of these primates as potential sylvatic hosts and the clinical effects of the parasite on monkeys. DNA sequenced from eggs in feces were 100% identical in the ITS2 region to *P. africanus* sequences from humans in Cameroon. *Paragonimus*-positive monkeys coughed more than uninfected monkeys. Experimental de-worming led to reduction in parasite intensity and a corresponding reduction of coughing to baseline levels in infected monkeys. This report provides the first evidence of *Paragonimus* sp. in *C. torquatus*, of *P. africanus* in Nigerian wildlife, and the first molecular evidence of the parasite in African wildlife. Coughing, sometimes interpreted as a communication behavior in primates, can actually indicate infection with lung parasites. Observations of coughing in primates may, in turn, provide a useful mechanism for surveillance of *Paragonimus* spp, which are re-emerging human pathogens, in wildlife reservoirs.

Introduction

Paragonimiasis is a food-borne illness of the lung caused by trematodes of the genus *Paragonimus.* Humans can become infected with this lung fluke after consuming raw or undercooked freshwater crustaceans. Before infecting mammalian hosts, *Paragonimus* species require a snail as the first intermediate host, and a freshwater crab or crayfish as the second intermediate host. In mammalian definitive hosts, the infective metacercariae excyst in the duodenum and migrate to the lungs, causing pulmonary paragonimiasis, with respiratory symptoms (e.g. coughing) that mimic tuberculosis (Toscano et al., 1995). Ectopic paragonimiasis occurs when flukes migrate internally, causing damage to muscles and organs, including the brain (cerebral paragonimiasis) (Blair, 2014). *Paragonimus* spp. infect more people globally than any other foodborne trematode, and infections cause an estimated 196,710 disability adjusted life-years (Fürst et al., 2012). These estimates do not account for infections in Africa.

Paragonimus spp. is best known from Asia and Latin America (Fürst et al., 2012). However, two known species, *P. africanus* and *P. uterobilaterlis*, infect humans in Africa (Blair, 2014). *Paragonimus uterobilateralis* is considered the principal causative agent of paragonimiasis in Nigeria (Aka et al., 2008). The epidemiology of *Paragonimus* sp. infections in Nigeria is tightly bound to post-colonial history. Prior to the Biafran war in Nigeria (1967-1970), paragonimiasis was known only from a handful of cases (Nnochiri, 1968; Nwokolo, 1964). During the war, food shortages and limited access to cooking facilities led to increased consumption of inadequately cooked or raw crab, and cases of paragonimiasis increased dramatically (Nwokolo, 1972). Human infections nearly disappeared again after the war, until recent surveys revealed unexpected high prevalence (up to 13.2%) in communities in the

Southeast part of Nigeria (Aka et al., 2008). The epidemiology of *Paragonimus* sp. in Nigeria differs from that in Cameroon, where the disease has a longer history of endemicity due to the cultural practice of eating raw crabs in some areas (World Health Organization, 1995).

The African civet (*Viverra civetta*) is considered the natural host of *P. uterobilateralis* in Nigeria (Voelker and Sachs, 1974), with the swamp mongoose (*Atilax paludinosus*) and domestic dog (*Canis familiaris*) harboring the parasite in Cameroon and Liberia, respectively (Voelker and Vogel, 1965). *Paragonimus africanus* has a broader host range, infecting the mongoose (*Crossarchus obscurus*), palm civet (*Nandinia binotata*), drill monkey (*Mandrillus leucophaeus*), potto (*Perodicticus potto*), and domestic dog (*Canis familiaris*) in Cameroon (Voelker and Vogel, 1965; Sachs and Voelker, 1975). The intermediate and definitive hosts of *P. africanus* range throughout the contiguous forest of southeastern Nigeria bordering Cameroon (Kingdon, 2005; Abraham and Akpan, 2011), suggesting that *P. africanus* could be more widely distributed than is currently appreciated.

We report the discovery of *Paragonimus* sp. eggs in red-capped mangabeys (*Cercocebus torquatus*) in Nigeria. We sequenced *Paragonimus* sp. DNA directly from eggs in feces to identify it to species and compare it to parasites reported in human populations. We also made observations of primate hosts for clinical signs of infection. Finally, we examined clinical observational data prior to and following treatment of the study population with anthelminthic drugs. We use this information to assess the presence of *Paragonimus* sp. in red-capped mangabeys in Nigeria, the role of these primates as potential hosts, and the clinical effects of the parasite on monkeys.

Methods

Between May and August 2012, we collected fecal samples and recorded coughing opportunistically from a group of 49 [23 adults/sub-adults (≥ 3 yo) and 12 juveniles (< 3 yo)] individually identifiable red-capped mangabeys that lived in a one-hectare open topped forest enclosure within the natural home range of the species (Fig. 1). The population was provisioned daily, but also had access to wild foods within the enclosure. The animals had access to water *ad libitum*, from a natural stream that ran through the enclosure. They were exposed to natural predators (e.g. snakes and birds of prey) and parasites. All animals were rescued from the bushmeat and pet trades in Nigeria as young juveniles, or were captive-born. Thirteen individuals were moved from a sancutary to the open-topped enclosure in 2004 as part of the rehabilitation and release program of the Centre for Education, Research and Conservation of Primates and Nature (CERCOPAN), and the remaining 36 individuals were born in the enclosure.

For 30 days in late May and June 2012, we collected triplicate fecal samples from each individual. Then, in late June 2012, the population was treated for *Paragonimus* sp. via orally administered praziquantel (approximately 20mg/kg for three consecutive days). We collected subsequent triplicate fecal samples from each individual over 30 days post-treatment, for a total of 294 samples (147 pre-treatment and 147 post-treatment). Over the same time periods (30 days pre-treatment and 30 days post-treatment), we recorded all observed instances of coughing between 6:00 and 17:00 daily. The Institutional Animal Care and Use Committee at University of Wisconsin, Madison approved all research activities (protocol v1490).

Fecal samples were collected from known individuals immediately following defecation, stored temporarily in plastic bags, and fixed within two hours of collection. We removed two aliquots from each sample for preservation of gastrointestinal parasite eggs and DNA separately. One aliquot was fixed in 10% formalin for microscopic analysis, and the other in RNA*later*® nucleic acid stabilizing solution for genetic analysis. Samples were transported to the University of Wisconsin, Madison following all applicable import, export, and International Air Transport Association regulations. One gram of formalin-preserved feces was concentrated by sedimentation and examined microscopically at X10 and X40 magnification (Greiner and McIntosh, 2009). We calculated prevalence (percent of individuals infected) as number of individuals shedding eggs divided by the total number of individuals examined, and we approximated mean and median intensity of infection (number of eggs per gram (epg) of a particular parasite species in the feces of a single infected host) (Bush et al., 1997; Greiner and McIntosh, 2009). We calculated pre- and post-treatment intensity by taking the average epg of triplicate samples for each individual. We compared *Paragonimus* sp. prevalence and intensity to host characteristics and rates of coughing using Fisher's exact test, Mann-Whitney test, and Spearman rank correlation. We then compared parasite intensity and coughing rates pre- and post-treatment using paired Wilcoxon rank sum test. For all pre- and post- treatment comparisons, we used one-tailed tests under the directional hypotheses that coughing frequency would be positively associated with parasite infection.

We extracted DNA from 150mg of the fecal sample with the highest egg count (1,067epg) using the Zymo ZR Fecal DNA MiniPrep Kit (Zymo Research Corporation, Irvine, CA, USA), following the manufacturer's protocols. PCR and nucleotide sequencing were performed on the internal transcribed spacer 2 region (ITS2) using primers 3S (5’-CGGTGGATCACTCGGCTCGT-3’) and A28 (5’-CCTGGTTAGTTTC TTTTCCTCCGC-3’), previously used to amplify *P. africanus* (Nkouawa et al., 2009). PCR was performed using Phusion High-Fidelity PCR mastermix (New England BioLabs, Ipswich, MA), and cycled in a BioRad CFX96 platform (Bio-Rad Laboratories, Hercules, CA,USA) with the following cycling parameters: 98ºC for 30 min; 40 cycles of 98ºC for 10 sec, 55ºC for 30 sec, 72ºC for 90 sec; and a final extension at 72ºC for 10 min. Amplicons were electrophoresed on an agarose gel stained with ethidium bromide and then purified using the Zymoclean Gel DNA Recovery Kit (Zymo Research Corporation, Irvine, CA, USA). Amplicons were sequenced on ABI 3730xl DNA Analyzers (Applied Biosystems, Grand Island, NY, USA) at the University of Wisconsin-Madison Biotechnology Center DNA Sequencing Facility. Sequences were aligned to published *Paragonimus* sequences using CLUSTAL W (Thompson et al., 1994).

Results

We recovered parasite eggs that were consistent with *P. africanus* (87.3±7.9 X 45.4±4.5) in 43% of the population. (Fig. 2). Our DNA sequence (Gen Bank accession number KR780065) was 100% identical to a *P. africanus* sequence from a human in Kumba, Cameroon and 99% identical to another from Bulutu, Cameroon (Nkouawa et al., 2009). Mean intensity prior to treatment was 96.70 epg (95% CI: 29.73-163.67; median= 32.67; range= 1-1,067).

We found no association between parasitism (prevalence or intensity) and host sex or age. We recovered *Paragonimus* sp. from animals transferred to the enclosure and those born in the enclosure. We observed 516 coughs in 88% (n=43) of individuals. Frequency of coughing was over two times higher in *Paragonimus*-positive individuals (μ=11.42) than in *Paragonimus*-negative individuals (μ=4.03; W=192; p <.02; Fig.3a). Coughing frequency also increased with the number of *Paragonimus* sp. eggs shed in feces (r_s=.64, p< .001; Fig.3b). De-worming led to a significant reduction in epg (μ=6.13;p <.000; Fig. 3c) and a corresponding reduction of coughing to baseline levels (μ=4.95; p <.01; Fig. 3d) in infected monkeys.

Discussion

We provide the first molecular evidence of *Paragonimus* sp. in African wildlife, and the first report of *Paragonimus* sp. infection in *C. torquatus* and *P. africanus* in Nigerian wildlife. Recent surveys revealing re-emergence of *Paragonimus* sp. in human populations in southeast Nigeria suggest a sylvatic cycle in which the parasite is maintained in crab-eating wildlife reservoirs (Blair, 2014). Indeed, *C. torquatus* in Gabon eat crabs as a normal part of the diet (Cooke, 2014). Our results demonstrate that *C. torquatus* can be a host for *P. africanus*, and infection is associated with respiratory illness. However, given limited sampling and DNA sequencing, we cannot exclude the possibility that this population hosts other species within the genus *Paragonimus*. Together with observations from Voelker and Sachs (1977) and Sachs and Voelker (1980), these results suggest that wild primates in Nigeria may help maintain *Paragonimus* sp. perhaps contributing to human disease.

Currently, limited genetic data exist for African *Paragonimus* spp. For example, there was no sequence information available for *P. uterobilateralis* on GenBank as of August 7, 2015, and only two sequences from *P. africanus* were available (Nkouawa et al., 2009). Our *P. africanus* sequences were between 99% and 100% identical to sequences from humans in

Cameroon, demonstrating only limited intraspecific variation in the ITS2 region of the parasite. *Paragonimus africanus* therefore appears to be genetically homogeneous across Nigeria and Cameroon, albeit based on a limited number of samples and only one genetic locus. *Paragonimus mexicanus,* previously considered the sole etiological agent of paragonimiasis in the Americas, is now believed to include cryptic species (López-Caballero et al., 2013). Further sampling may indicate similar genetic diversity in *P. africanus*.

Significantly, we found that coughing was more frequent in infected individuals, and that animals with more intense infections coughed more frequently. Furthermore, treatment with praziquantel in our study population led to reduced parasite burden and a corresponding reduction of coughing to baseline levels in infected monkeys. Determining whether coughing is communicative or physiological is problematic in studies of primate behavior (Hauser, 2000). Our results suggest that coughing may actually indicate respiratory disease, such as infection with lung flukes. This observation not only complicates interpretations of primate behavior, but it also suggests a method for clinical assessment of wild primates for paragonimiasis and similar respiratory pathogens. Interestingly, hunters in this area report using primate skulls and feces to treat cough (Friant et al., 2015). In interviews, hunters made reference to seeing monkeys in the area cough as justification for these traditional remedies (S.Friant, unpub.data), indicating an intriguing link between parasitism, clinical disease, local beliefs, and primate conservation.

Knowledge of sylvatic reservoirs will be critical for improved understanding and control of re-emerging paragonimiasis in Nigeria. Given the difficulties associated with eradicating multi-host pathogens, as well as their high potential for emergence and re-emergence, control of paragonimiasis will require not only sustained behavior change away from raw or undercooked crab consumption and improved education, but also surveillance of potential wildlife hosts. In the case of primates, observations of coughing should be considered suspicious for paragonimiasis.

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Figures

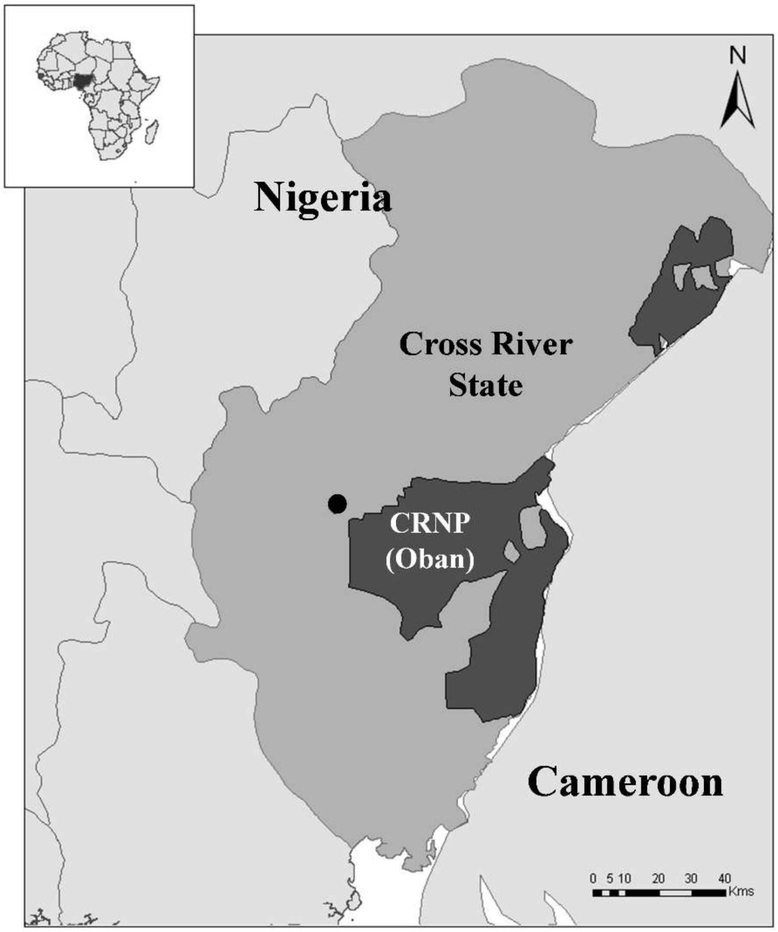

Figure 1. Map of collection site. Map shows the location of the study population (*black circle*) relative to the Oban Division of Cross River National Park (CRNP), in Cross River State, Nigeria.

Figure 2. Microscopic image of *Paragonimus africanus* egg.

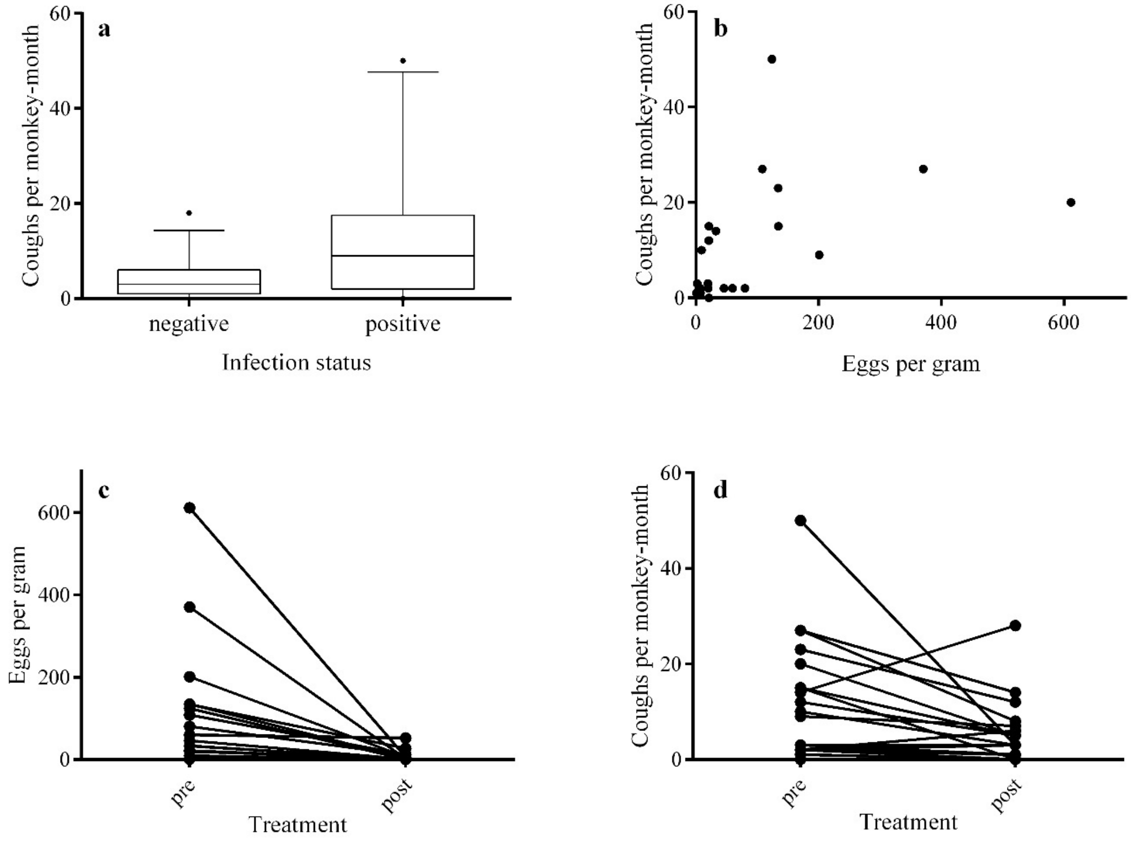

Figure 3. Coughing and *Paragonimus* infection. a) *Paragonimus* infection status and coughs per monkey-month (CPMM) (W=192; p<.02), b) intensity of *Paragonimus* infection [eggs per gram of feces (epg)] and CPMM (r_s=.64, p < .001), c) de-worming and epg (μ=6.13; W=0; p <.0001), and d) de-worming and CPMM (μ=4.95; W=26.5; p <.01).

CHAPTER 5: Drivers of bushmeat hunting and perceptions of zoonoses in Nigerian hunting communities

Friant, S., Paige, S. B. & Goldberg, T. L. 2015 Drivers of bushmeat hunting and perceptions of zoonoses in Nigerian hunting communities. *PLoS Negl. Trop. Dis.* **9**. (doi:10.1371/journal.pntd.0003792)

Abstract

Bushmeat hunting threatens biodiversity and increases the risk of zoonotic pathogen transmission. Nevertheless, limited information exists on patterns of contact with wildlife in communities that practice bushmeat hunting, especially with respect to social drivers of hunting behavior. We used interview responses from hunters and non-hunters in rural hunting communities in Nigeria to: 1) quantify contact rates with wildlife, 2) identify specific hunting behaviors that increase frequency of contact, 3) identify socioeconomic factors that predispose individuals to hunt, and 4) measure perceptions of risk. Participants engaged in a variety of behaviors that increased contact with wild animals, including: butchering to sell (37%), being injured (14%), using body parts for traditional medicine (19%), collecting carcasses found in forests and/or farms (18%), and keeping as pets (16%). Hunters came into contact with wildlife significantly more than non-hunters, even through non-hunting exposure pathways. Participants reported hunting rodents (95%), ungulates (93%), carnivores (93%), primates (87%), and bats (42%), among other prey. Reported hunting frequencies within taxonomic groups of prey were different for different hunting behaviors. Young age, lower education level, larger household size, having a father who hunts, and cultural group were all associated with becoming a hunter. Fifty-five percent of respondents were aware that they could contract diseases from wild animals, but only 26% of these individuals reported taking protective measures. Overall, hunters in this setting frequently contact a diversity of prey in risky ways, and the decision to become a hunter stems from family tradition, modified by economic necessity. Conservation and public health interventions in such settings may be most efficient when they capitalize on local knowledge and target root socio-economic and cultural drivers that lead to

hunting behavior. Importantly, interventions that target consumption alone will not be sufficient; other drivers and modes of interaction with wildlife must also be considered.

Introduction

An estimated 282 grams of bushmeat are consumed per person per day in the Congo Basin, with over three million tons harvested in Central Africa annually [1,2] . Hunting of wild animals on this scale threatens wildlife conservation and increases risk of zoonotic disease transmission [3,4]. Rural communities across the tropical forests of West and Central Africa rely heavily on bushmeat as a nutritional, economic and cultural component of their livelihoods [5,6]. However, increasingly intense extraction is unsustainable and results in enhanced opportunities for zoonotic disease transmission [7]. A general shift towards cash economies, increased access to previously remote areas for natural resource extraction, and widespread use of guns have altered traditional hunting behavior and increased dependency on the sale of bushmeat to meet urban demands [8–12]. Market surveys in Nigeria estimate that over 900,000 kilograms of bushmeat are sold annually [13]. Large profit margins create incentives for the bushmeat trade across all levels of the supply chain, allowing bushmeat to reach national and international markets [13]. In the Ivory Coast, for example, the bushmeat trade is valued at 150 million USD [2]. An estimated five tons of bushmeat are smuggled from Africa to Europe per week [14]. Worldwide, wildlife is second only to narcotics among black market trades [15].

Frequent contact with wildlife through the bushmeat trade puts people at risk of infection with zoonotic pathogens. Pathogens transmissible to humans through bushmeat include: simian immunodeficiency virus, human T-cell lymphotrophic virus, simian foamy virus, monkeypox virus, Ebola and Marburg filoviruses, anthrax, herpes viruses, hepatitis viruses, paramyxoviruses and various parasites [16]. Among prey taxa, bats, rodents and primates consistently stand out as important sources of zoonoses. Bats and rodents have high zoonotic viral richness, and the close genetic similarity between humans and non-human primates makes exposure particularly risky[17–20]. For example, pandemic HIV originated from viruses of Central African chimpanzees, providing a striking example of the global consequences of zoonoses resulting from contact with primates [21]; and other simian retroviruses appear to "jump" between primates and people with regularity (for review see: [22]). Compared to primates, rodents are a far more abundant and geographically widespread taxon[23,24]. Forest dwelling and peridomestic rodents in West Africa host viruses such as Lassa virus and monkeypox virus, as well as a range of vector-borne pathogens [18]. Bats harbor the highest number of zoonotic viruses per host species and have received a great deal of recent attention because of outbreaks of zoonotic corona-, filo-, and paramyxoviruses [25,26]. The nature and frequency of human interaction with these and other wildlife taxa determine the pathways by which zoonotic diseases emerge.

The disruption of transmission pathways requires improved understanding of the interactions between key biological, behavioral and sociological drivers of human–animal contact. In places where reliance on wild foods and income are linked, certain individuals may be at particular risk of infection. Conventional wisdom holds that the poorest households in rural communities rely most heavily on wild foods [27–31], but this paradigm is not universal [32–34]. Still, little information exists on social and economic factors that influence whether individuals hunt.

In this study, we conducted interviews in remote Nigerian hunting communities to identify: 1) transmission pathways by nature and frequency of interactions between humans and wildlife; and 2) socioeconomic factors that may put individuals at increased risk of zoonotic infections from wild animals. Because perceptions of risk are known to vary among hunters in

West and Central Africa [30,35], we also used closed- and open-ended interviews to measure zoonotic disease awareness, perceived risk and self-protective behavior.

Study Site

We conducted interviews in five rural hunting communities near the Oban Division of Cross River National Park in Cross River State, Nigeria (Fig 1). The park was created in 1991 and has two non-contiguous divisions. The southern Oban division is about 3,000 km^2 of lowland rainforest, making it the largest closed-canopy rainforest in Nigeria. It is ecologically contiguous to Korup National Park in Cameroon and is recognized as a biodiversity and infectious disease hotspot, where pathogen transmission from wildlife to humans is most likely [19,36,37]. Illegal logging, agricultural expansion and hunting threaten biodiversity in the park. The forest surrounding the Oban division is characteristic of lowland rainforest, forming a mosaic of disturbed and relatively undisturbed forest patches. To increase the generality of our results, we selected communities that varied in proximity to the national park (outside, support zone, or enclave) and cultural group (primarily Efik or Ejagham).

Methods

Study Design

We interviewed 327 participants between August and December 2012. All interviews were conducted in Nigerian Pidgin English, a language spoken as *lingua franca* across Nigeria, by the first author with the assistance of local translators when necessary. Administrative visits to each village preceded interviews to meet with clan heads, chief hunters and hunter groups, hold informational sessions and request permission for research activities.

We enrolled participants to obtain responses from an approximately equal number of hunters and non-hunters. Enrollment was restricted to men because women in this area do not hunt. We first enrolled self-identified hunters and then identified non-hunters through random-selection of households. If household members chose not to participate, or were not home after three visits, we replaced the household with its nearest neighbor. During non-hunter interviews, we frequently discovered individuals who were, in fact, actively hunting or had hunted previously in their lives, and in several villages, we were unable to find a sufficient number of men who had never hunted. Because we were interested in whether or not a participant's current social and economic situation influenced hunting behavior, we re-defined "hunter" as any individual who reported killing an animal in the past year, excluding one individual who reported killing a single snake on his farm.

Ethics Statement

Nigeria National Parks Service and the University of Wisconsin-Madison Institutional Review Board (protocol #SE-2011-0859) approved all research activities. With the help of two Nigerian assistants, we translated all documents (site visit script, consent form, and questionnaire) into Nigerian Pidgin English. All participants provided informed oral consent. We did not obtain written consent because of low literacy rates, and because of concerns about confidentiality. We documented oral consent with the signature of the individual responsible for obtaining consent.

Questionnaire

We designed and administered a four-part questionnaire to obtain basic demographic information, information on exposure to animals, views on the merits of hunting as a livelihood, and perceptions of zoonotic risk. Questionnaires were informed by similar studies [30,35,38], which provided the basis for establishing categories of contact modes . Local translators back-translated documents to validate the survey instrument for each village. We collected information to identify socioeconomic factors that may put individuals at risk of zoonotic infections from wild animals through hunting. These data included: age (*years*); marital status (*number of wives*); children (*number*); religion (*open*); ethnic group (*open*); education [*(0) none, (1) primary school, (2) secondary school, (3) beyond secondary school*]; primary occupations (*top 3; open*); father is/ was a hunter (*y/n*); house roof type [*(0) vegetation , (1) zinc without ceiling, (2) zinc with ceiling , (3) aluminum without ceiling, (4) aluminum with ceiling*], house material [*(0) mud, (1) mud with plaster, (2) cement, (3) cement with plaster*], domestic animals [*animal type; (0) none, (1) 1-5, (2) 6-10 , (3) >10*]; other possessions (*generator/ television/ DVD player/ CD player/ motor bike/ cell phone*).

To assess contact frequency by species, we showed each participant published drawings of local wildlife [39], and referred to their local or English names (S1 Table). For each animal, we asked participants how often they consumed, hunted, sold, received an injury from, collected if they found dead, or kept it as a pet. These behaviors are termed "risky" throughout, as they result in direct contact between humans and wildlife species. Frequency data, unless otherwise indicated, were collected on a six-level ordinal scale (*never, 1-5 times in their lifetime, 1-2 times/year, 1-2 times/ month, 1-2 times/week or daily*). The following additional data were collected from each participant: meat preference (*bushmeat/domestic meat/ top 3 preferred wild animals*), domestic and bushmeat consumption (*frequency*), whether they: butchered bushmeat to sell (*y/n/average price*); accidently cut themselves while butchering (*y/n*); received an injury from a wild animal (y/n) ; used bushmeat for medicinal purposes (*y/n; animal type; description of use*); adhered to local taboos or laws against killing and/or consuming wild animals (*y/n; examples*); and used bushmeat for cultural purposes (*y/n; examples*). The following information was collected from hunters only: easiest animals to hunt (*top 3; open*), most desirable prey (*top 3; open*); hunting technique (*gun/ trap/ machete/dog*), hunting location (*forest/farm/both*), hunting time (*night/day/both*), hunting season (*occasional/wet/dry/all year*), hunting frequency, and whether they slept in the forest while on hunting excursions (*frequency*).

To characterize individual views of participants on the merits of hunting as a livelihood, we asked hunters whether they would still hunt if they had alternatives (*y/n/sometimes),* and if they wanted their children to hunt (*y/n; why or why not*). Finally, to measure zoonotic disease awareness, perceived risk, and precautions taken to mitigate exposure, we collected data on knowledge of wildlife zoonosis (*y/n; types of diseases; source animals*); source of information (*open*); perceived threat (*y/n*); and precautionary measures taken (*y/n; explain*).

Analyses

We used data on roofing material, housing material and household assets to create an index of household wealth. This index was based on published results of participatory and small-scale survey research comparing livelihood data for a range of households relying on non-timber forest goods in West and Central Africa [40]. Specifically, we assigned points based on roofing material (0-4), housing material (0-3), number of livestock (0-3), and non-essential household items (0-6). Thus the maximum possible score was sixteen; the higher the score, the wealthier the household.

For certain analyses we converted hunting and consumption frequencies from ordinal indices to conservative numeric estimates of minimum yearly off-take, in units of numbers of animals (never = 0, rarely = 0, yearly = 1, monthly = 12, weekly = 52 and daily = 104) for incorporation into generalized linear models. For less frequent behaviors (collect dead, injured by, kept as pet), we used the total number of animals contacted over the participant's lifetime. We omitted cases with missing values from our analyses.

For hunters who reported hunting daily, weekly, or monthly but only during one season, we corrected hunting frequency estimates by one-half (seasonal hunters) or one-third (occasional hunters). To determine whether there were significant differences in reported contact between hunters and non-hunters, we used chi-square tests. For modes of contact where most participants engaged in the specific behavior, we compared ordinal frequencies of contact using Mann-Whitney U tests. We constructed generalized linear mixed models to examine behavioral and socioeconomic predictors of individual hunting activity and frequency of contact with wildlife. Since modes of contact are not mutually exclusive (e.g. animals can be hunted and sold or hunted and consumed), and covariance among these factors makes it difficult to separate the effect of any single behavior on overall risk, we limited our analyses of behavioral and socio-economic predictors of risk to hunting behavior alone.

To identify hunting behaviors significantly associated with high frequency of contact with all wild animals, and with specific taxa, we used mixed effects linear regression models with backwards elimination of behavioral predictor variables. We then used the same selection method in a mixed effects logistic regression model to determine which socioeconomic variables were significantly associated with being a hunter. We incorporated village as a random effect in all models. We performed analyses with *nlme* and *glmer* functions in RGui (3.0.2)[41]. We initially included all variables in the models; however, we retained only significant variables (at the alpha=.05 level) and first-order interactions among significant main effects in the final model.

Results

Demographic Information

Demographic information was collected from 327 individuals, representing 188 hunters and 139 non-hunters. The median age of all participants was 31.5 (range = 15-93) years. Fifty percent (n=163/323) of individuals had the equivalent of a primary school education or lower, 32% had finished secondary school, and 18% had at least one year of higher education. Sixty-nine percent (n=223/325) of individuals were married, and 7% had multiple wives. The average number of children was four (range = 0-26). The study populations were predominantly Christian (93%, n=302/324), with the remainder practicing traditional religions (6%) or Islam (1%). Participants identified their tribal affiliations primarily as Efik (73%), Ejagham (14%), and a variety of other cultural groups (primarily Ibibio from neighboring Akwa Ibom state) (13%). Farming (subsistence agriculture and selling of crops) was the most common occupation (69%). Hunting (33%) and trapping (19%) were the second and third most common occupations, followed by salaried work (15%), being in school (12%), having a skilled trade (10%), selling goods (7%), driving a motorbike taxi (5%), being a village leader, being unemployed, collecting forest goods, being a member of the clergy, collecting palm wine, fishing, and livestock farming (each less than 5%).

Contact with wildlife

Over 99% of participants reported consuming bushmeat at some time in their lives. The study population, in aggregate, reported hunting and/or consuming all animals included in the survey (S2 Table). Brush-tailed porcupine (*Atherurus africanus)* was listed as the most preferred animal (45% of participants), followed by pangolin (*Manis* spp.; 16%), and monkey (*Cercocebus* *torquatus, Mandrillus leucophaeus,* and *Cercopithecus* spp.; 14%). Participants reported consuming monkeys more than once per week, and porcupine and blue duiker (*Cephalophus monticola*) slightly less than once per week. Participants also contacted wild animals through: butchering to sell (37%, n=121), being injured (14%, n=81), using body parts for traditional medicine (19%, n=62), collecting carcasses found in forests and/or farms (18%, n=60) and keeping as pets (16%, n=53) (Fig 2a). Sixteen percent of participants reported accidently cutting themselves while butchering meat. Monkeys were reported as most frequently used for medicinal purposes (24%), followed by water chevrotain (*Hyemoschus aquaticus*; 16%) and rock python (*Python sebae;* 14%) (S3 Table). Putty-nosed guenons (*Cercopithecus nictitans*) were reported as most frequently kept as pets (23%), followed by pangolin (15%) and mona monkey (*Cercopithecus mona*) (13%). Overall, participants reported contacting primates more frequently than any other taxon (19% of "yes" responses across all species and contact modes), followed by ungulates (17%), rodents and carnivores (14% each) (Fig 2b).

Hunters were more likely than non-hunters to have reported contacting wildlife through butchering (χ^2=43.67, df=1, p<.0001), injury (χ^2=46.7, df=1, p<.0001)**,** traditional medicine use (χ^2=7.78, df=1, p<.01), collecting carcasses (χ^2=9.83, df=1, p<.01) and keeping as pets (χ^2=7.76, df=1, p<.01) (Fig 2a). Although 99% of participants reported consuming wildlife, hunters did so more frequently (multiple times a week) than non-hunters (weekly) (U=9287.5, p<.0001).

Seventy-five percent of participants reported consuming wildlife for cultural purposes, including festivals, holidays and special occasions. Eight percent of participants reported a taboo that prevented them from killing or consuming a certain wild animal. Taboos were typically due to family traditions (72%) or views that animals contain the spirits of ancestors (28%). Twelve percent of individuals reported that laws of the Nigerian government or local communities prevented them from killing certain animals (primates and /or endangered species) or in restricted areas.

Hunting behaviors

Participants reported having hunted rodents (95%), ungulates (93%), carnivores (93%), primates (87%), and bats (42%), among other prey (S2 Table). Hunters reported hunting monkeys on average more than once a week, porcupine approximately once a week, and blue duiker less than once a week over the past year. Porcupine was mentioned 47% of the time as being easiest to kill, followed by blue duiker (19%), cane rat (*Thryonomys swinderianus*) (8%), monkey (8%), and giant pouched rat (*Cricetomys emini*) (6%). Porcupine was also the most frequently mentioned (26%) as a very desirable animal, followed by red river hog (*Potamochoerus porcus*) (22%), blue duiker (17%), bay duiker (*Cephalophus dorsalis*) (8%) and monkey (7%).

Hunters used a variety of techniques, including: traps (75%), guns (71%), machetes (71%) and dogs (18%). A majority of hunters hunted only in the forest (56%), while others hunted in both the forest and on their farms (22%) or strictly on their farms (16%). Most hunted equally during the night and day (58%), but some hunted solely by day (24%) or by night (16%). The mean experience level of hunters was 11 years (range = 1-50). Seventy-five percent of hunters hunted year-round, 21% hunted in the wet season only, and 5% hunted occasionally throughout the year. On average, hunters hunted on a weekly basis, and 73% reported having slept in the forest during hunting trips.

High rates of contact with wildlife through hunting were statistically significantly associated with hunting during night and day, high hunting frequency, and hunting with a gun and dog. The frequency of primate hunting was positively associated with frequency of sleeping in the forest and time of day of hunting (night and both day and night). The frequency of hunting rodents was associated with using a gun, hunting during both the day and night, and high hunting frequency. The frequency of hunting ungulates was associated with hunting in the forest, using a machete, using a trap, and high hunting frequency. Hunting with a dog was associated with high contact with all taxa, except rodents (Table 1; S4 Table).

Reasons for Hunting

Overall, participants reported a strong preference (84%) for bushmeat over domestic meat. Participants hunted to both sell and eat meat (73%), although some hunted exclusively for household consumption (22%) or exclusively to sell (5%). The top five preferred animals and average market price (in USD per animal, converted from 2012 exchange rates from Nigerian Naira to US Dollars), were: 1) porcupine (42%; $16); 2) pangolin (15%; $8); 3) monkey (11%; $15); 4) red-river hog (8%; $106); and 5) blue duiker (4%; $17). Seventy-five percent (n=111/156) of hunters had fathers who were also hunters.

Eighty-four percent (n=145/173) of participants reported that they would choose not to hunt if they had an alternative source of income. Ninety-seven percent of participants reported not wanting their children to hunt. The most common reason that people gave for not wanting their children to hunt was that hunting was too difficult (49%; n=159/324). Thirty-eight percent of respondents used the word “suffer” or “stress” to explain why they did not want their children to hunt. Other common reasons were that hunting was too dangerous (15%), was not a real job (10%) or that it was no longer as profitable due to declining wildlife numbers (7%). Education and age were negatively associated with becoming a hunter, whereas household size, having a father who hunts, and being of the resident cultural group were significantly associated with becoming a hunter (Table 2).

Zoonotic Disease Awareness

Fifty-five percent of participants reported awareness of wildlife zoonoses, with information spread primarily through broadcast news outlets, forestry/ conservation workers, or word of mouth. Of the individuals reporting awareness of zoonoses, 89% said that they perceived an actual risk and 26% reported taking measures to protect themselves from infection (Fig 3). Participants described 21 diseases that they believed came from wild animals: HIV (55%), cough (11%), malaria (5%), poison (5%), tumbu flies (*Cordylobia anthropophaga,* a parasitic fly; 4%), flu, gonorrhea, body pain, sleeping sickness (2% each), typhoid, fever, rash, cholera, scabies, worms, SARS, rickets, boil, typhus, syphilis, rabies (each 1%). Wild animals believed to be responsible to zoonotic infections included: monkey (55%), python (12%), red-river hog (10%), chimpanzee (7%), leopard (5%) and duiker (4%).

Discussion

We found that younger age, lower education level, larger household size, having a father who hunts, and being of the resident cultural group were all significantly associated with becoming a hunter. Hunters had more frequent contact with wildlife through both hunting and non-hunting behaviors, likely experiencing higher exposure risk to zoonosis than non-hunters. Specific hunting behaviors, namely high hunting frequency, hunting during both day and night, hunting specifically at night, and hunting with a gun and with a dog were all associated with high rates of contact with wildlife. Other behaviors were associated with higher rates of contact with specific taxa, namely:

sleeping in the forest (primates), and using a machete and trap (ungulates). Carnivore and rodent hunting frequency was not uniquely associated with any specific hunting behaviors

Our results shed new light on the social-cultural contexts of wildlife contact in this region and have implications for conservation and public health. We found a negative association between education level and hunting, and no effect of wealth. These results differ from those of Le Breton and colleagues (2006) who found that hunting in Cameroon was more common among poorer households (as measured by roof type) with no effect of education level. Similarly, in Tanzania, participation in illegal hunting decreased with increasing wealth, as measured by ownership of sheep and goats [28]. Negative results in our study may reflect low variation in economic status, in that all participants were almost uniformly materially disadvantaged. This conclusion is supported by our observation that larger family sizes appeared to generate a greater need for income, which may be most accessible through hunting, particularly for individuals from families with experienced hunters. Individuals with higher education levels, a factor associated with lower probability of hunting in our study, do not necessarily have higher income, but may engage in activities that generate extra income or in other commitments that keep them out of the forest.

Our data also show that resident cultural groups were more likely to hunt than other cultural groups, which tend to be migrants from nearby states. Our study sites varied in numbers of migrants from neighboring states, but when present, they commonly resided in the periphery of villages as farmers and were not permitted to hunt by order of village chiefs. Our results contrast those of other studies that found that migrants hunted a majority of bushmeat (Congo), had higher rates of primate contact (Uganda), and were more likely to be involved in butchering (Cameroon) than resident groups [12,30,42]. Our results may reflect cultural differences among migrant populations, or a unique local response from resident cultural groups who fear loss of livelihoods to migrant populations.

Although hunting is illegal and considered an undesirable livelihood, strong incentives to hunt still persist. Indeed, we struggled to identify men who had never hunted or trapped wildlife. Nevertheless, almost all participants claimed that if given an alternative, they would choose not to hunt. Virtually all said they did not want their children to become hunters because it is too difficult, dangerous, stressful, not a legitimate occupation, and is no longer profitable. This contrasts directly with historical accounts of hunting in this region, in which hunters were described as being "economically independent" and "far too important a person" to employ [43; pg.152] . We suggest that declines in wildlife numbers coupled with increasing distances between wildlife habitat and villages have decreased incentives to hunt. With lower returns per hunt, those who are able turn to alternatives. Many who continue to hunt do so out of necessity, and in turn, hunting is viewed as a low-merit livelihood, even among hunters themselves. While preference for bushmeat will inevitably drive the trade to a certain degree, our data suggest that provision of alternative livelihoods would reduce hunting behavior by restricting hunting frequency and providing supplemental income. However, individuals in need of extra income would remain free to hunt at night and set traps, which were predictors of primate and ungulate hunting frequency, respectively.

Nearly all participants reported consuming bushmeat, and there was a strong preference for bushmeat over domestic meat. A majority of participants had strong cultural ties to the consumption of bushmeat, and very few recognized laws protecting wildlife. A majority of hunters reported selling bushmeat, indicating that demand from rural and urban markets continue to provide incentives for bushmeat hunters, who often lack alternative ways to generate income. These incentives may be modified by hunting taboos. For example, in neighboring states in Nigeria, certain guenons are held as deities and are protected within particular villages [44]. In this region however, such taboos were uncommon. As a result, hunting in and near protected areas remains common in Nigeria [13,45].

Although previous studies reported frequencies of carcasses in bushmeat markets in this region [13], we are aware of none examining hunting preferences and cultural uses that may be driving human-wildlife contact at the local level. Significantly, we found that participants reported having had contact with primates more than with any other wildlife taxon. This remains true whether keeping wildlife as pets is included in our analyses, since it was reported far less frequently than hunting. However, we note that keeping animals as pets presents a different kind of risk, in which people come into frequent contact with animals over a prolonged period. This behavior, unlike others, leads to opportunities for repeated injury and exposure to animals that may be persistently stressed.

Eighty-seven percent of individuals reported consuming primates, and monkeys were listed among the most desirable animals to eat and were most frequently mentioned as useful for medicinal purposes or kept as a pet. These data parallel high primate consumption rates [30], and preferences for primates [35] documented for other regions. Porcupine and blue duiker were consumed by over 90% of individuals, with porcupine most frequently mentioned as a preferred meat. Ebola epidemics have been previously associated with handling duiker carcasses [46], and though we are unaware of zoonotic viruses transmitted directly through contact with porcupines, rodents in general host more than 60 known zoonotic viruses[17]. Bats, along with other small prey, were anecdotally referred to as “children’s meat”, in that they are small and thus given to children to play with and eat, thereby potentially putting children at greater risk. The link between bushmeat hunting and zoonotic disease risk through such pathways has been discussed extensively[17,18,30]; our data expand these risks to a new region and a new cultural setting.

Of the 55% of participants who reported awareness of zoonotic diseases from wildlife, a majority reported believing that there was an actual risk associated with contact. Awareness of wildlife zoonoses was considerably higher than reported in hunting communities in Sierra Leone ([35]; 55% *versus* 24%), but overall perceived risk was lower than in Cameroon ([30]; 46% *versus* 74%). Differences across study sites may be due to educational campaigns in the respective areas. We are unaware of public health outreach campaigns related to wildlife and disease in this region. However, given the proximity to the national park, participants may have previously received information of risks associated with hunting and consumption of wildlife species of conservation concern, particularly primates. In our study, information about risk came primarily through broadcast news outlets, forestry/conservation personnel, and word of mouth. Only one individual reported a public health official as a source of information about zoonotic diseases, despite the fact that such individuals are in strong positions to enhance knowledge of risks associated with bushmeat, especially near protect areas where wildlife contact rates are high.

Fifty-five percent of participants who reported awareness of wildlife zoonoses gave monkeys and HIV as an example. However, many other examples were of unconfirmed hosts or non-zoonotic pathogens. Despite such knowledge, very few individuals reported protecting themselves from infection. Avoidance was the most frequently cited protective measure, including avoidance of eating bushmeat, touching blood, sexual contact, or eating fruit from trees where monkeys had been feeding. Of those who protected themselves, 31% reported taking traditional and/or commercial medicine as a treatment or prophylaxis. Many potential zoonoses are viral and therefore locally available treatments such as saline injections, antibiotics and acetaminophen would be ineffective. Additionally, the effectiveness of traditional treatments such as consumption of wild herbs or bitter cola (*Garcinia afzelii*) against zoonotic pathogens is as yet unproven. Only five percent of participants reported using safe meat handling practices, such as cleaning or cooking meat well prior to consumption, as a protective measure. We recorded differences in consumption patterns among locations (e.g. consumption of partially smoked innards at the hunting sheds *versus* well-smoked meat sold in markets), suggesting that risk of contact and zoonoses varies across space and time. Participants also reported wearing clothes and/or boots for protection, for example when hunters carry carcasses over long distances (wearing clothing) or restrain animals with their feet (wearing boots). One participant reported wearing protective gloves while butchering. The efficacy of these measures for protecting against exposure to infectious material is unknown, but is likely to be higher than using no protection at all. Education programs implemented through conservation programs and/or news outlets should therefore include information on avoidance strategies, with specific attention to dispelling misconceptions about routes of transmission and promoting effective and accessible strategies for mitigating exposure.

Our findings highlight the value of understanding socio-cultural drivers of bushmeat hunting for reducing contact with wildlife in high-risk groups. Hunting wildlife for meat is widespread in West and Central Africa, and effective public health solutions are unlikely to emerge from conservation and regulatory agencies alone. Our data suggest that effective solutions will include implementation of alternative livelihood programs specifically targeting hunters and aimed at providing alternative protein sources that would satisfy local taste preferences (e.g. raising desirable species in captivity [47,48]). Conservation rules that limit hunting, or prohibit hunting with dogs, and are implemented with the help of local chiefs may be most effective in reducing hunting pressure, such as in the case of effectively restricting hunting privileges to resident groups. However, given the cultural and economic contexts of the bushmeat trade, a complete shift to alternative protein sources may be impractical at present.

Novel self-protective strategies should be developed through consultation with individuals who currently protect themselves, make use of locally available goods, and be tested locally for cultural acceptability. Our data suggest that conservation and public health initiatives that tap into existing outlets for transmitting information, such as word of mouth and radio broadcasting, are likely to be most effective in reaching and influencing people in high risk areas. Although our study focuses on drivers of hunting, a behavior practiced only by men in this region, women are at risk from butchering and trading the animals brought back by hunters [35,49], and should also be targeted during educational programs and interventions.

Results from Nigeria demonstrate that hunters in this setting frequently contact a diversity of prey in “risky” ways, and that the decision to become a hunter is rooted in family tradition, modified by economic necessity. Improved education, reduced family sizes, and provision of alternative livelihoods may result in reduced contact with wildlife and lower zoonotic disease risk in rural hunting communities in Nigeria and similar locations. We acknowledge that such solutions require the mobilization of significant resources toward development and conservation jointly. We also advocate targeting neglected transmission pathways, such as distinct cultural uses of wildlife that motivate off-take and provide novel routes for pathogen exchange. These potential routes of transmission have received less attention than those associated with hunting of bushmeat for consumption, but they may in aggregate confer equal or greater risk.

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Tables

Table 1. Percentage of animals reported hunted with associated hunter behaviors

Behavior	Primates %		Rodents %		Ungulates %		Carnivores %		All taxa %	
Hunt often (≥ than once per week)	51.0		39.8	*	44.4	*	39.9		43.6	*
Sleep in forest often (≥ than once per week)	30.3	*	24.4		22.4		20.6		24.9	
Hunting location										
Forest	59.8		63.5		70.5	*	65.3		66.5	
Farm	17.0		13.7		10.3		13.7		11.8	
Both	23.3		22.7		19.2		21.1		21.7	
Time of day										
Day only	8.6		20.6		20		19.6		17.9	
Night only	10.8	*	13.2		10.3		13		11.8	*
Both	80.6	*	66.2	*	69.6		67.4		70.3	*
Hunts with machete	85.2		80.0		83.7	*	83.3		82	
Hunts with trap	90.5		83.7		86	*	85.5		85.2	
Hunts with gun	73.6		78.2	*	74		75.8		76.2	*
Hunts with dog	35.7	*	33.0		40.6	*	34.7	*	36.3	*

* p-value with significance at ≤ .05 based on multiple linear mixed effects regression models predicting reported hunting frequencies (S4 Table).

Table 2. Factors associated with hunting

Factor*	OR (95% CI)	*p-value*
Age (years)	0.96 (0.93-0.99)	<.01
Education		
none (reference)	--	--
primary school	0.61 (0.20-1.82)	ns
secondary school	0.30 (0.10-0.93)	<.05
post-secondary school	0.19 (0.06-0.62)	<.01
Household size (# of individuals)	1.20 (1.08-1.32)	<.001
Father hunts (yes versus no)	2.86 (1.57-5.26)	<.001
Resident cultural group (yes versus no)	4.14 (1.50-11.50)	<.01

* Village was incorporated as a random effect into a logistic regression model. There was no effect of wealth on whether or not an individual hunted.

Figures

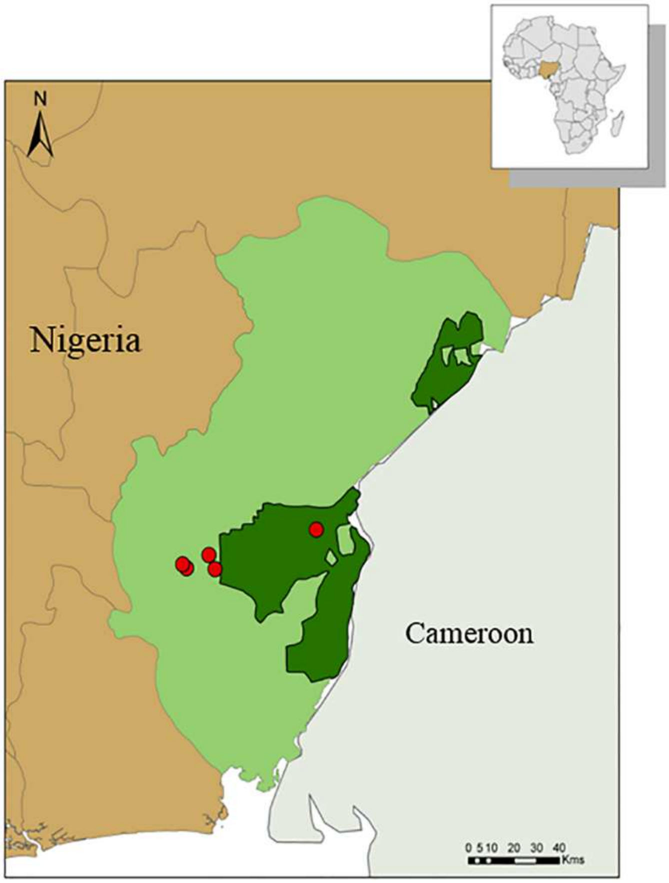

Figure 1. Study Sites. Map showing location of study communities relative to the Oban Division of Cross River National Park (dark green) in Cross River State, Nigeria (light green).

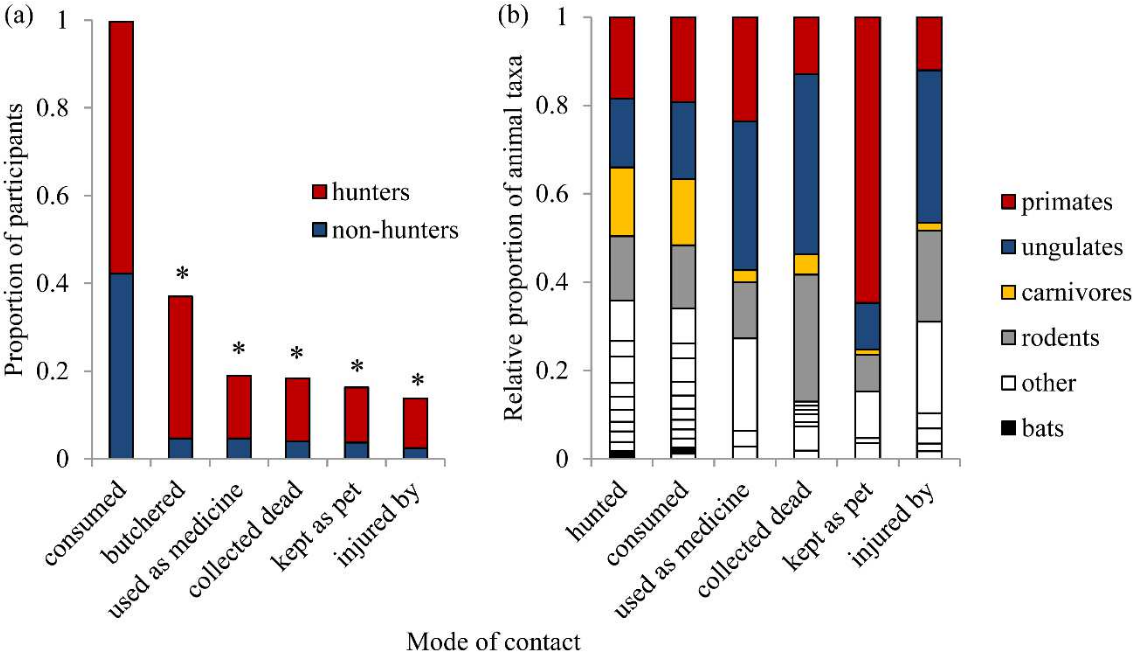

Figure 2. Human-wildlife contact. The proportion of participants who reported animal contact, comparing hunters (n=188) and non-hunters (n=137) (a), and the relative proportion of animals they reported contacted with through multiple modes (b). Asterisk indicates statistical significance at p<.05.

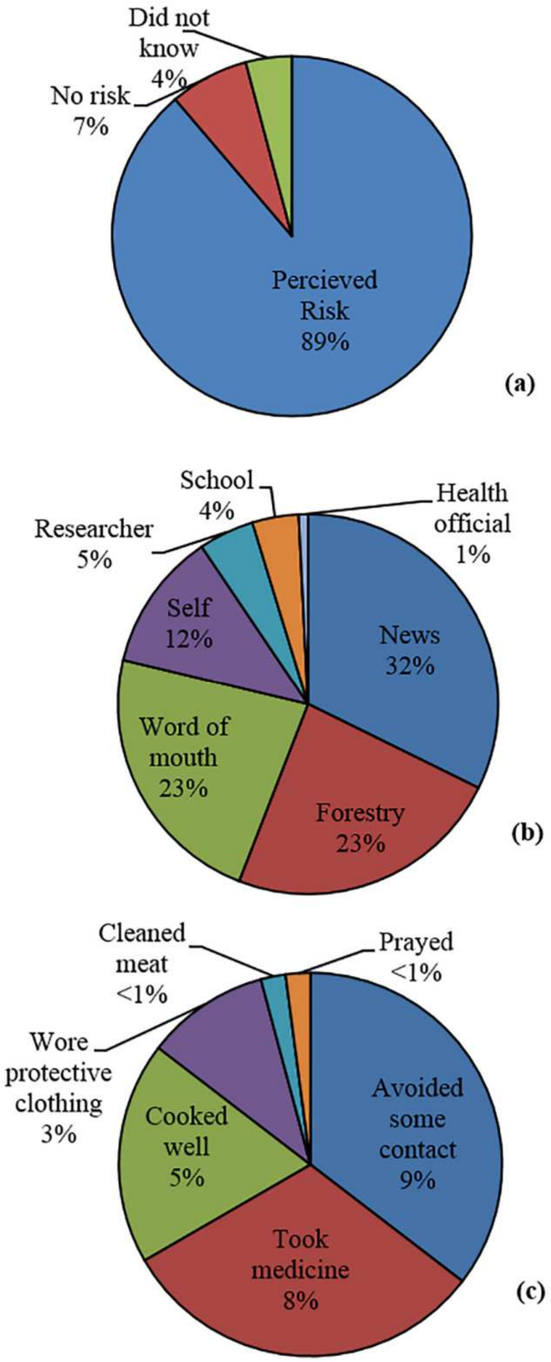

Figure 3. Perceptions of zoonotic disease risk. Level of perceived risk (a), sources of information (b), and protective behaviors (c), of the 55% of participants who reported awareness of wildlife zoonoses.

Supplementary information

Table S1. Classifications of contacted animals

English name	Scientific Name	Family	Order	Class	Local English names*	Efik dialects		Ejagham dialect
Fruit bat	several genera	Pteropodidae	Chiroptera	Mammalia	Bat	Iyumamonc	Igegemi	Egangang
Squirrel	several genera	Sciuridae	Rodentia	Mammalia	Squirrel	Epang	Kwakuru	Ikomey
Flying squirrel	*Anomalurus* sp.	Anomaluridae	Rodentia	Mammalia	Flying rabbit	Erret	Erre	Ebaghi
Giant-pouched Rat	*Cricetomys emini*	Nesomyidae	Rodentia	Mammalia	Rabbit	Eboi	Ewe	Nku
Marsh cane-rat	*Thryonomys swinderianus*	Thryonomyidae	Rodentia	Mammalia	Cutting Grass	Ephip	Ebeck	Kobiya
Tree hyrax	*Dendrohyrax dorsalis*	Procaviidae	Hyracoidea	Mammalia	--	Bjururum	Ekorurum	Ekpim
Cusimanse	*Crossarchus obscurus*	Herpestidae	Carnivora	Mammalia	--	Boyok	Bidam	Ifet
Mongoose	*Herpestes sanguinea*	Herpestidae	Carnivora	Mammalia	Fox/ Bush dog	Epetam	Gowut	Ebi
Brush-tailed porcupine	*Atherurus africanus*	Hystricidae	Rodentia	Mammalia	Chucuchucu	Iyup	Ikup	Nyop
Tree pangoline	*Phataginus tricuspus*	Manidae	Pholidota	Mammalia	Pangoline/ Catta beef	Iyan	Gegang	Ika
Giant otter shrew	*Potamogale velox*	Tenerecidae	Afrosoricida	Mammalia	Water rabbit	Betek	Ebochabugai	Esimsor
African clawless otter	*Aonyx capensis*	Mustelidae	Carnivora	Mammalia	--	Ebuboia	--	Ikiyork
Common genet	*Genetta gentta*	Viverridae	Carnivora	Mammalia	Bushbaby/ Bush cat	Akokanta	Birun	Nsim
Palm civet	*Nandinia bibotata*	Nandiniidae	Carnivora	Mammalia	Stone beef	Udi	Inie	Mbai

African civet	*Civettictis civetta*	Viverridae	Carnivora	Mammalia	Bush Dog/ Hyena	Sup	Chup	Ejor
Golden cat	*Felis aurata*	Felidae	Carnivora	Mammalia	Lion	Ekbaiba	--	Ekparim mgbe
Leopard	*Panthera pardus*	Felidae	Carnivora	Mammalia	Tiger	Ekpe	Gewaiwai	Mgbe
Chimpanzee	*Pan troglodytes*	Hominidae	Primates	Mammalia	Chimpanzee	Idubatam	Inumadam	Nyork
Drill	*Mandrillus leucophaeus*	Cercopithecidae	Primates	Mammalia	Drill	Iyum	Iyum	Nsum
Red-capped mangabey	*Cercocebus torquatus*	Cercopithecidae	Primates	Mammalia	Red Head	Ekpo	Iku	Mbi
Red colobus	*Procolobus pennatii preussi*	Cercopithecidae	Primates	Mammalia	--	Udim	Iku	Ekabok
Red-eared Monkey	*Cercopithecus erythrotis*	Cercopithecidae	Primates	Mammalia	Monkey- red tail	Iona	Iku	Mbi Mbuk
Mona monkey	*Cercopithecus mona*	Cercopithecidae	Primates	Mammalia	Monkey	Epem	Iku	Mbarambuk
Putty-nosed Monkey	*Cercopithecus nictitans*	Cercopithecidae	Primates	Mammalia	Monkey- white nose	Upena	Iku	Numyak/ Nyakambuk (male/female)
Bushbaby	*Galago spp.*	Galagidae	Primates	Mammalia	--	Bikboon	Gecacari	Ebop
Potto/ calabar Angwantibo	*Perodicticus potto/ Arctocebus calabarensis*	Lorisidae	Primates	Mammalia	Fox	Dechai	Gecat Kagon	Efe
Blue duiker	*Cephalophus monticola*	Bovidae	Artiodactyla	Mammalia	Frutumbo	Bituna	Biduna	Iseh
Red river hog	*Potamochoerus porcus*	Suidae	Artiodactyla	Mammalia	Bush Pig	Iyrre	Genibagai	Mgumi
Bay duiker	*Cephalophus dorsalis*	Bovidae	Artiodactyla	Mammalia	Red Deer	Ebin	Inumadam	Nsun

Yellow-backed duiker	*Cephalophus sivicultor*	Bovidae	Artiodactyla	Mammalia	Bush Cow	Ajima	Gemem	Ngugu
Water chevrotain	*Hyemoschus aquaticus*	Tragulidae	Artiodactyla	Mammalia	Water Beef	Bejuy	Eget	Iku
Sitatunga	*Tragelaphus spekei*	Bovidae	Artiodactyla	Mammalia	Antelope	Idup	Ochup	Ngongum
African buffalo	*Syncerus caffer*	Bovidae	Artiodactyla	Mammalia	Buffalo	Ebongai	Etua	Mfumg
African forest elephant	*Loxodonta africana cyclotis*	Elephantidae	Proboscidea	Mammalia	Elephant	Idi	Ini	Njok
Crocodile	*Osteolaemus tetraspis*	Crocodylidae	Crocodilia	Reptilia	Crocodile	Etararam	Gegum	Nyip
Tortoise	*Kinixys sp.*	Testudinidae	Testudines	Reptilia	Tortoise	Bejin	Gegen	Nkui
Monitor lizard	*Varanus sp.*	Varanidae	Squamata	Reptilia	Iguna	Uti	Gowen	Ebak
Black cobra	*Naja melanoleuca*	Elapidae	Squamata	Reptilia	cobra	Ugwema	Ugema	Nyor Nyak
Rock python	*Python sebae*	Boidae	Squamata	Reptilia	Snake	Etan	Edang	Nkum
Great blue turaco	*Corythaeola cristata*	Musophagidae	Musophagiformes	Aves	--	Okonoc	Kundok	Nkurak
Hornbill	several genera	Bucerotidae	Bucerotiformes	Aves	--	Etogi	Gedobi	Mgon
Black guinea fowl	Agelastes *niger*	Numididae	Galliformes	Aves	--	Etamphana odone	Genon bana	Eviechi
Guinea fowl	*Guttera plumifera*	Numididae	Galliformes	Aves	--	Otamma	Gedami	Enyeng

* Blanks indicate that there was no name for the animal in English, Efik or Ejagham

Table S2. Summary of wild animals hunted and consumed

Order	Species	Hunted[a]	Consumed[b]
Squamata		**0.97**	**0.86**
	Monitor lizard (*Varanus sp.*)	0.83	0.76
	Rock python (*Python sebae*)	0.74	0.71
	Black cobra (*Naja melanoleuca*)	0.65	0.50
Rodentia		**0.95**	**0.99**
	Brush-tailed porcupine (*Atherurus africanus*)	0.93	0.98
	Marsh cane-rat (*Thryonomys swinderianus*)	0.84	0.86
	Giant-pouched rat (*Cricetomys emini*)	0.84	0.81
	Squirrel (Family: Sciuridae)	0.64	0.58
	Flying squirrel (*Anomalurus* sp.)	0.30	0.28
Artiodactyla		**0.93**	**0.96**
	Blue duiker (*Cephalophus monticola*)	0.92	0.94
	Bay duiker (*Cephalophus dorsalis*)	0.78	0.83
	Red-river hog (*Potamochoerus porcus*)	0.67	0.83
	Sitatunga (*Tragelaphus spekei*)	0.57	0.63
	Water chevrotain (*Hyemoschus aquaticus*)	0.55	0.55
	Yellow-backed duiker (*Cephalophus sivicultor*)	0.23	0.36
	African buffalo (*Syncerus caffer*)	0.04	0.14
Carnivora		**0.93**	**0.91**
	Cusimanse (*Crossarchus obscurus*)	0.81	0.78
	Common genet (*Genetta gentta*)	0.80	0.71
	Palm civet (*Nandinia bibotata*)	0.77	0.73
	Mongoose (*Herpestes sanguinea*)	0.69	0.61
	African civet (*Civettictis civetta*)	0.44	0.50
	African clawless otter (*Aonyx capensis*)	0.26	0.25
	Golden cat (*Felis aurata*)	0.03	0.03
	Leopard (*Panthera pardus*)	0.01	0.06
Primates		**0.87**	**0.87**
	Potto/ Angwantibo (*Perodicticus sp./ Arctocebus sp.*)	0.80	0.68
	Putty-nosed monkey (*Cercopithecus nictitans*)	0.66	0.70
	Red-eared monkey (*Cercopithecus erythrotis*)	0.56	0.56
	Drill (*Mandrillus leucophaeus*)	0.55	0.65
	Red-capped mangabey (*Cercocebus torquatus*)	0.53	0.57
	Mona monkey (*Cercopithecus mona*)	0.51	0.55
	Bushbaby (*Galago* spp.)	0.51	0.47
	Red colobus (*Procolobus pennatii preussi*)	0.23	0.24
	Chimpanzee (*Pan troglodytes*)	0.15	0.33
Testudines	Tortoise (*Kinixys sp.*)	0.86	0.81
Galliformes	Guinea fowl (*Guttera plumifera, Agelastes niger*)	0.83	0.81
Pholidota	Pangolin (*Manis* sp.)	0.77	0.78
Crocodilia	Crocodile (*Osteolaemus tetraspis*)	0.72	0.72
Bucerotiformes	Hornbill (Family: Bucerotidae)	0.64	0.63
Afrosoricida	Giant otter shrew (*Potamogale velox*)	0.58	0.51
Hyracoidea	Tree hyrax (*Dendrohyrax dorsalis*)	0.54	0.53
Musophagiformes	Great blue turaco (*Corythaeola cristata*)	0.49	0.50
Chiroptera	Fruit bat (Family: Pteropodidae)	0.42	0.35
Proboscidea	African forest elephant (*Loxodonta africana cyclotis*)	0.03	0.29

[a] Proportion of hunters who report hunting each animal/ taxa

[b] Proportion of participants who reported consuming each animal/taxa

Table S3. Summary of wild animals used as traditional medicine.

Animal*	Body Part	Use	# of reports
Monkey	skull	used as cup to drink boiled water from	45
	feces	drank with water/ local liquor	13
	intestine	boiled and drank broth	2
	hair	mixed with local liquor, drank	1
	ground bone	mixed with water, used as enema	1
Water	leg	roasted, mixed ash with water or local liquor, drank	40
chevrotain	bone	roasted, mixed ash with water, used as enema	1
Rock python	fat	Used as rub; drank with liquor; dried in sun to use as lozenge	21
	bile	drank with liquor	9
	kidney	put in local liquor and drank	2
	teeth	used to lacerate boil or breast	2
	flesh	ate	1
Blue duiker	bone	ground, mixed with water and used as rub	26
	skull	kept palm oil in skull for rub; used as cup	3
	flesh/ intestine	mixed with herbs, ate	2
	roasted skin	mixed with medicine and oil, used as lozenge	1
Flying squirrel	hair or skin	used as rub	21
Red-river hog	ground bone/skull	mixed with water, used as rub	14
Tortoise	bone	tied to body	5
	intestine/heart/skin	mixed with palm oil, drank	3
	ground bone/shell	mixed with water, drank or used as enema; used as rub in scarifications	3
Brush-tailed	burned spines	used as rub	3
porcupine	spine	used to lacerate boil	1
	intestine	boiled, drank broth; ate	2
	heart	mixed with alcohol, drank	1
Monitor lizard	flesh	boiled, drank broth	5
	skin	dried in sun, used lozenge; wore on necklace	2
African forest	feces	mixed with water, drank; used as rub	4
elephant	bile	mixed with local liquor, drank	1
	fat	melted, drank	1
	stomach	use for pillow, drink water from	1
Bay duiker	roasted skin	mixed with water or alcohol, drank	4
	thigh flesh	ate	1
Potto/	flesh/ roasted leg	ate	3
angwantibo	boiled meat	drank broth	2
Giant-pouched rat	meat/ gall bladder/ intestine	boiled, ate	4
Common genet	roasted skin	mixed with water, drank	2
African civet	scent gland	used as	1
	roasted leg	mixed with ash and oil, drank	1
Black cobra	flesh	ate	1
Palm civet	feces	mixed with water, used as enema	1
Chimpanzee	dried finger bone	mixed with native chalk, used as rub	1
Leopard	flesh	ate	1

*Animals are listed in order of times they were mentioned as used to cure a sickness.

Table S4. Behavioral predictors of contact with wildlife taxa

									Estimated #[a] of											
	animals hunted				primates hunted				rodents hunted				ungulates hunted				carnivores hunted			
Predictors[b]	β	*df*	*t*	*p*	β	*df*	*t*	*p*	β	*df*	*t*	*p*	β	*df*	*t*	*p*	β	*df*	*t*	*p*
Constant	12.96	1	3.44	--	1.42	1	0.7	--	6.89	1	5.88	--	-0.5	1	-0.3	--	8.36	1	6.42	--
Hunting frequency	0.05	1	2.08	<.05	--	--	--	--	0.03	1	3	<.01	0.02	1	2.17	<.05	--	--	--	--
Sleep in forest (*frequency*)	--	--	--	--	0	1	2.43	<.05	--	--	--	--	--	--	--	--	--	--	--	--
Hunting location	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Forest	--	--	--	--	--	--	--	--	--	--	--	--	1.97	1	2.27	<.05	--	--	--	--
Farm	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Both	*ref*	--	--	--	*ref*	--	--	--	*ref*	--	--	--	*ref*	--	--	--	*ref*	--	--	--
Time of day	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Day only	*ref*	--	--	--	*ref*	--	--	--	*ref*	--	--	--	*ref*	--	--	--	*ref*	--	--	--
Night only	2.27	1	0.76	*ns*	4.97	1	2.16	<.05	--	--	--	--	--	--	--	--	--	--	--	--
Both	5.57	1	2.07	<.05	7.22	1	3.63	<.001	1.94	1	2.49	<.05	--	--	--	--	--	--	--	--
Machete (*yes/ no*)	--	--	--	--	--	--	--	--	--	--	--	--	2.17	1	2.26	<.05	--	--	--	--
Trap (*yes/ no*)	--	--	--	--	--	--	--	--	--	--	--	--	3.92	1	2.21	<.05	--	--	--	--
Gun (*yes/ no*)	3.99	1	2.31	<.05	--	--	--	--	2.16	1	2.9	<.01	--	--	--	--	--	--	--	--
Dog (*yes/ no*)	3.75	1	2	0.05	2.71	1	2.11	<.05	--	--	--	--	2.54	1	3.13	<.01	2.20	1	2.39	<.05

[a] Numerical response variables were transformed by square root to satisfy assumptions of normality.

[b] Village was incorporated as a random effect.

CHAPTER 6: Summary and future directions

The overarching purpose of this dissertation research is to improve our understanding of the ecology and epidemiology of neglected and zoonotic diseases. These combined studies integrate perspectives and methodologies from the biological and social sciences, and demonstrate the early applications of a One Health approach that spans animal health, public health, and conservation communities in Nigeria. This dissertation therein provides the basis of a research program centered in Nigeria with a long-term goal of improving human, animal and environmental health in areas of conservation concern throughout Sub-Saharan Africa. Ongoing and future research will focus on the health-related consequences of resource extirpation and environmental change. Below I outline avenues for future research using animal models to inform public health, and investigating health consequences of human wildlife contact, including potential strategies for interventions.

Primate models of health and infectious disease

Knowledge of the relationships among sociality, health, and fitness in wildlife populations advances our understanding of human health [1–3], as evidenced by a multitude of studies in the field of disease ecology [4,5]. Field experiments, which remain relatively uncommon, can be used to investigate risk in a more controlled manner. Antiparasitic treatment, in the case of this research, reduces heterogeneities in individual exposure and susceptibility that are accumulated over time, thereby reducing confounding variables inherent in cross-sectional approaches. However, field experiments, by their very nature are limited in application, as veterinary interventions cannot, and arguably should not, be used in most wild populations as removal of endemic parasites opens niche space that may lead to increased susceptibility to novel infectious agents. There are, however, circumstances that may lend themselves particularly well to such studies, including treatment of: semi-free ranging populations (as described herein), populations in highly disturbed or urban settings, or populations prior to or after reintroduction/translocation for conservation purposes.

Experimental studies of wildlife health further our understanding of how environmentally associated health disparities differentially affect individuals and vulnerable populations. For example, **Chapter 2** describes a field experiment designed to better understand how host behavior and physiology affect exposure and susceptibility to environmentally transmitted pathogens. Through simultaneous observations of behavior, stress, and infection, these data showed how host traits, stress, and social behavior interact during periods of host exposure to new pathogens to influence rate to infection. By further expanding this model, we can produce novel insight into drivers of infection risk in humans and wildlife populations alike.

Sociality and health

Group size does not fully explain variation in parasitism across species [6–9]. An improved understanding of the effects of social structure and social dynamics may provide further insight into the nature of risk. Experimental and comparative approaches will be particularly informative in understanding shared risk factors, and the unique behavioral, physiological, and ecological forces that alter exposure and susceptibility to different infectious agents.

Primates, in particular, are highly variable in their diet, social structure, and behavior, all of which can affect the transmission of infectious diseases [10,11]. Furthermore, parasites vary in their biology and transmission modes, having either direct or indirect lifecycles, and sometimes involving multiple intermediate hosts. Exposure to vectors, intermediate hosts, and contaminated environments may vary with nature and degree of sociality. For instance, there is a growing consensus that risks from increased exposure (e.g. centrality within the social network) are generally more pervasive than enhanced susceptibility (e.g. immune-response) in determining patterns of parasite aggregation in primates [12–16]. But, which individuals experience disproportional risk under different social systems or social dynamics? Does this vary with different parasite transmission modes? Given the variability in the organisms that we group together as "parasites," where possible, future statistical models should stratify by species, or at least transmission mode, to reveal important variation in risk. Furthermore, identification of the exceptions to this pattern may prove particularly valuable; for example, in understanding the effects of ecology and sociality on risk, and also the health consequences of environmental change events that reduce habitat, and increase population density and physiological stressors.

Interestingly, there was no observed effect of mean glucocorticoid production, an important component of physiological stress, in acquisition of new infections in red-capped mangabeys [12]. It is unclear whether high mean fecal cortisol levels in this study reflect chronically high levels of circulating hormones or frequent high elevations. This distinction is particularly important because these two patterns of hormone secretion can produce different physiological host responses [17–19]. Specifically, acute glucocorticoid elevations can activate the adaptive immune response, while chronic elevations can have immunosuppressive effects [20]. Species typical stress dynamics and hierarchical stability can further affect the production of stress hormones, increasing susceptibility in either dominant or subordinate individuals [19,21]. Furthermore, the endocrine system may be particularly well adapted to low-level glucocorticoid responses to social competition. Glucocorticoid recovery rate, which has particular functional and health significance, may be more important than mean concentrations over time [19,22].

Future studies that incorporate the temporal dynamics of glucocorticoid production, including recovery rate, may be better equipped to investigate the specific role of stress in infection. For instance, with higher sampling intensity, future studies could compare average minimum and maximum fecal cortisol levels of dominants and subordinates to define whether stress dynamics are determined by dominance (accumulation of acute stressors via greater peak production) or from subordination (chronic stress via greater trough production) [19]. Field experiments that capitalize on stressful events (e.g. dominance takeovers, immigration events, introduction/removal of individuals from semi-free ranging populations, or translocation/re-introduction events) may find that stress plays an important role in reinfection when levels rise above low-level responses to social competition. Because stress also interacts with nutrition [23,24], future studies should investigate the relative roles of nutritional and social stressors in susceptibility.

Parasite community stability

Wildlife hosts are commonly infected with multiple parasitic taxa [25,26]. However, the interspecific parasite interactions that regulate community stability and determine how communities respond to environmental and social perturbations are not well understood [27], primarily due to the observational nature of many wildlife studies. Stability and structure of parasite communities could be altered by internal (e.g. coinfection) and/or external (e.g. ecological or social stressors) processes. For instance, co-infecting parasites may compete directly for resources or interact indirectly via effect on the host immune system, resulting in both positive and negative associations between parasites [28–31]. Experimental perturbations that treat only a single group of parasites (e.g. protozoan, nematode, or playhelminthes) and investigate the response of non-target species could help us detect competition or facilitation among taxa. Such studies could be further expanded to investigate the responses of bacterial and viral infections to perturbations of parasite communities. Furthermore, characterization of parasite assemblages in populations undergoing dramatic changes to their physical and/or social environments can improve our understanding of how external forces impact parasite communities. Results will be important in understanding how dramatic environmental change and social disruption affect the structure and stability of communities of infectious agents in individuals and populations, and subsequent health related consequences.

Parasite colonization dynamics

Antiparasitic interventions in animal populations can also provide important information on colonization and priority effects. Understanding the relative roles of interspecific parasite interactions versus inherent differences in hosts is key to understanding parasite colonization dynamics [32]. By considering multiple pathogens with known infection times, future studies can evaluate the relative importance of host factors against the sequence in which parasites infect the host to determine patterns of parasite aggregation [33,34]. Importantly, such studies should focus on parasite-host associations with known prepatencies, such that the margin of error in patency estimates will not obscure the relative times of infection.

Furthermore, treated and released animals may serve as important yet underused tools for surveillance of infectious agents. Sentinel surveillance, which focuses on a subset of a population (often with high risk), is used to monitor changes in prevalence or incidence and identify outbreaks and epidemics caused by infectious agents [35,36]. Zika virus, for example, was discovered through a sentinel rhesus monkey program for studying yellow fever in Uganda [37]. Following antiparasitic treatment, hosts cleared of infection may be more susceptible to novel pathogens, in part because infectious agents don’t have to compete during establishment, making them useful sentinels for infection. The use of treated animals as sentinels for infection would be particularly interesting (and feasible) in areas where domestic animals serve as possible bridge hosts for diseases thought to be important to both humans and wildlife [38–42]. For example, health surveys of dogs in Canada have recovered parasites of health significance to both humans and wildlife, implicating them as both sources and sentinels of infectious disease [43,44]. Future studies that follow livestock and companion animals after veterinary interventions, and measure colonization from new infections, may reveal increased susceptibility to novel pathogens and unrealized capacity for host switching in endemic parasites. This paradigm could be further extended to wildlife populations during reintroduction or translocation programs, as well as displaced human populations.

Fitness effects and behavioral counter strategies

It remains unclear whether parasite-induced changes to individual behavior and physiology affect host fitness or population dynamics. Long-term drug treatment experiments could reveal the true impact of parasites at the population level, for example, by comparing host abundance or population dynamics (e.g. survival, fecundity) between periodically drug-treated and untreated populations. Indeed, few studies have managed to detect fitness and population effects, likely due to logistical constraints [45–48]. Long-term effects of parasites are particularly difficult to detect in long-lived species, such as primates. Fitness effects of parasitism may therefore be best understood through related effects (e.g. body condition, activity levels, vigilance, etc. as described herein), long-term behavioral and demographic studies that integrate parasitology, or combined experimental, observational, and modeling approaches.

Field experiments can be useful tools for investigating how infection affects individual behavior and fitness. For example, **Chapter 3** describes the behavioral and physiological responses of red-capped mangabeys to parasitic infection. Results suggest a coordinated defense strategy that conserves energy, limits transmission risk, and reduces vulnerability to predation and competition. Similarly, **Chapter 4** describes signals of respiratory disease associated with zoonotic lung fluke (*Paragonimus africanus*) infections, providing a potential surveillance tool for detecting this re-emerging human pathogen in sylvatic hosts. Future investigations into how wildlife experience infection and health can provide new insights into interactions between clinical pathology and animal behaviour to identify individual and population-level impacts of infectious agents, host tolerance and avoidance strategies, and opportunities for syndromic surveillance.

The behavioral and physiological responses to antiparasite treatment described in **Chapter 3** suggest that increased vulnerability to predation (e.g. increased vigilance) and negative physiological consequences (e.g. increased cortisol and altered activity budgets) were associated with parasitic infection. Future studies of feeding ecology surrounding antiparasite treatment would be well equipped to investigate dietary responses to infection, including self-medication. Furthermore, studies with different treatment groups that target specific parasitic taxa will be better equipped to detect whether associated host changes are due to the cumulative effects of multiple infections, or specific parasites.

Increased group cohesion following antiparasitic treatment suggests active avoidance of parasitized conspecifics. While sickness behavior is associated with decreased sociality over all, the nature of relationships may vary with different individuals, in some cases with increased social tendencies toward potential caregivers. For example, experimentally induced cytokine-mediated inflammation in rats lead to increased contact time with familiar conspecifics [49]. Directed egocentric networks constructed from focal observations of a subset of treated animals (or conversely, animals with persistent infections) could be used to gain a more nuanced understanding of the frequency, duration, and nature of interactions with specific individuals. Similar techniques could also be used to investigate whether parasitism results in active avoidance of infected conspecifics (i.e. infected individual receives less contacts/ approaches), reduced population-level clustering (as described in **Chapter 3**), or both. In guppies, for example, experimental introduction of fish into populations showed that population fish actively avoided experimentally infected fish compared to uninfected controls, and that presence of an infected individual resulted in declined social network clustering in the population as a whole [50]. Such information could help to identify mechanisms of behavioral flexibility in response to parasite pressure in increasingly large groups, as proposed by long-term observational studies of primates [51].

Red-capped mangabeys infected with lung flukes were found to cough more frequently than uninfected individuals, and coughing decreased to baseline levels following treatment for parasites. These results demonstrate clinical signs associated with parasite infections, an association that has been difficult to demonstrate in non-laboratory conditions. Routine collection of visual and behavioral health assessments during parasitological studies could provide further information on the clinical effects of parasites. In the case of lung flukes, it would be interesting to see if similar trematodes infect other primates that consume crustaceans (e.g. macaques, capuchins, or other mangabeys species [52–55]), and if coughing is a ubiquitous signal of infection in these primates or other known wildlife hosts (e.g. otters, Bengal tigers [56]). In addition, there are numerous zoonotic protozoan and helminth parasites with pulmonary involvement [57], and future studies should investigate whether coughing is associated with these infections in primates and other mammalian hosts. In circumstances where animals are captured, such as reintroduction and translocation programs, more detailed health assessments could provide better information on parasite associated clinical pathologies.

Multi-host pathogens

The health of wildlife can directly impact the health of nearby human populations. For example, in **Chapter 4**, the lung fluke (*Paragonimus africanus*) recovered from red-capped mangabeys was found to be identical at the ITS2 region to *P. africanus* infecting humans in Cameroon. Currently, the prevalence of *Paragonimus* sp. is at unexpected high levels in people living in rural southeastern Nigeria, raising concern for the re-emergence of this pathogen in human populations [58]. The presence of crustacean intermediate hosts with infective metacercariae in areas of very low human prevalence, suggests that non-human mammalian hosts are serving as reservoirs for this parasite [59–63]. Re-emergence in Nigeria, after a negligible number of infections since the Biafran war, may indicate a true sylvatic cycle, which can be very hard to break.

The lung fluke recovered from red-capped mangabeys was identified as *P. africanus*, whereas *P. uterobilateralis* is considered the principle causative agent of re-emerging paragonimiasis in Nigeria. However, we do not currently know whether all mangabey infections were indeed from to *P. africanus* (and all human infections in the region from *P. uterobilateralis*), or if they in fact represent mixed infections between these two species and possible other cryptic species and/or lineages. For example, recent molecular and morphological analyses of *Paragonimus mexicanus,* previously considered the sole species present in the Americas, is strongly suggestive of multiple cryptic species raising concerns regarding diagnosis, treatment, and control [64]. Furthermore, other parasites once thought to be regularly transmitted between humans and primates reveals that they are in some cases genetically distinct, and differ in host range and cross-species transmission potential [65–67]. Genetic characterization of African *Paragonimus* spp., as well as other potential zoonotic parasites, will be important not only for definitively identifying taxa, but will also for providing information about evolutionary relationships and thus epidemiological linkages [68].

Parasite assemblages and environmental change

Host - parasite relationships forged throughout species evolutionary history can also reveal novel insight into the patterns and processes of environmental change. For example, parasite-host dynamics can serve as indicators of: biodiversity [69]; pollution [70]; species introductions [71,72]; climate change [73–75]; habitat fragmentation and expanding human-wildlife interfaces [76]; human population growth, range expansion and global travel [77,78], historical cultural influences [79], and even extinct hominin ecology [80]. Unfortunately, the utility of parasitology to global change biology is currently constrained by technological barriers that limit our ability to detect or even estimate parasite diversity within ecosystems, populations, or individuals.

Information on parasitic fauna comes from a wide array of methods which must be pieced together to fully characterize host-parasite assemblages. Morphological identification of adult forms for taxonomic assessment requires invasive or often destructive sampling of hosts. As a consequence, many parasites are putatively characterized on the basis of morphological characteristics of infectious stages shed in feces [81,82]. Even when adult forms are available, parasitology faces a “taxonomic impediment” – only a small fraction of species have been identified globally due to a shortage of professional taxonomists and systematists [83,84]. In such cases, morphology can confound our understanding of parasite diversity and distribution.

Genetic characterization of parasites can help overcome this obstacle. Molecular methods are most informative, but they are historically expensive and difficult to apply to the characterization of entire parasite communities. Conventional sequencing relies on amplification of a small number of target genes or regions, the selection of which is dependent on *a priori* identification of parasites of interest, thus leading to a biased approach. Because parasitological studies tend to focus predominately on human pathogens, and specifically those with notable economic and health consequences (e.g. malaria and schistosomiasis), there is a dearth of genetic information from the vast majority of parasitic taxa, including those that threaten animal health and that might emerge as human pathogens. Furthermore, when these different methods are applied to the same dataset, they can produce highly divergent results [66].

We need a "universal" method for parasitology that will be specific and informative for a broad range of taxonomic groups. Next-generation sequencing and bioinformatic technologies can overcome many of the challenges faced by conventional sequencing, and provide new and exciting opportunities for a unified approach for unbiased genetic characterization of parasite assemblages [68]. Future research objectives should be aimed at developing and applying a novel method for unbiased molecular characterization of parasite communities, or the "parasitome", using next-generation sequencing technology. Similar techniques developed for bacteria (the "microbiome"), have dramatically improved our understanding of their role in shaping human, animal, and environmental health. Once the tool is developed, it can then be used for species identification, tracking cross-species transmission, and monitoring responses of parasite-host assemblages to environmental perturbations.

Human-wildlife contact and risk of zoonoses

Cross-species transmission is constrained not only by the biology of the pathogen and host, but also by human behaviors and ecological processes that facilitate transmission. **Chapter 5** provides information from interview responses about behaviors in Nigerian hunting communities that put individuals at risk of zoonotic infections from wildlife, including the drivers of human-wildlife contact. Participants reported consuming bushmeat more often than domestic meat, and there was a strong preference for the former over the latter. A majority of participants reported consuming wildlife for cultural purposes, including festivals, holidays and special occasions. Family and cultural tradition increased the likelihood that an individual was a hunter, even when it was considered an undesirable livelihood. A majority of participants also reported selling meat that they hunted, but the most common reason people gave for hunting, was to feed their children. Indicators of need, including large family size and low education (but not household wealth) also influenced whether individuals hunted as part of their livelihood. These results suggest an underlying role of culture and preference, alongside nutritional and economic need, in driving hunting and consumption patterns. Future research coupling quantitative and qualitative interview approaches with biological sampling from humans and wildlife populations will achieve a more in-depth understanding of the epidemiology and transmissibility of zoonoses, as well as the cultural, nutritional, and economic drivers of "risky" contact.

Food safety

Humans can become infected with infectious agents of wildlife origin, of which novel forms are continually being discovered [85–88]. Broad viral screening techniques for pathogen discovery often requires invasive sampling that is not typically feasible for studies of wild and often endangered animals, thereby inhibiting our ability to collect samples from most wild populations. Wildlife hunted for bushmeat can provide an opportunity for collection of biological specimens for detection of novel and/or zoonotic pathogens. Broad viral screening for zoonotic and/or novel pathogens in both humans and wildlife should be coupled with in-depth interviews to determine risk factors for transmission of different pathogens, as opposed to using contact as a proxy for “risk”. Such information will be important for developing strategies to block transmission pathways.

Food security

Despite the risks to human health and well-being, the issue of bushmeat hunting remains a problem for conservation, but it is rarely considered in public health and development strategies. However, bushmeat provides important benefits to nearby communities, including livelihood strategies for the poor and food security (i.e. improved access and dietary diversity). Thus, unsustainable hunting threatens food security and livelihoods long-term, as well as threatening wildlife populations while increasing zoonotic transmission risk. The nature of the relationship between bushmeat and human health, especially to poor and disadvantaged groups, is not well understood. Future work should focus on the role of bushmeat as a driver of health disparities in areas of diminishing natural resources, and help bring the issue of bushmeat into the scope of development and public health policy [89,90].

There is a growing body of evidence demonstrating that forest cover is directly related to increased dietary diversity in Africa [91]. A strong link between biodiversity and food security has been demonstrated in agricultural systems [92–95]. It is clear, however, that wild food use extends well beyond the use of wild plants for consumption and production of new cultivars, and that wild animals contribute greatly to local diets across Sub-Saharan Africa [96–98]. Still, the contribution of diverse wild animals to food security is routinely undervalued. Future studies that couple dietary intake data (e.g. food diversity and access) with measures of malnutrition (e.g. anemia, stunting, weight loss, kwashiorkor) will improve our understanding of nutrition related health benefits from bushmeat, and monitor and project change associated with declined access to wildlife resources.

Malnutrition extends well beyond issues of food quantity. Localized studies are needed to gain a better understanding of the role of wildlife in meeting the specific nutritional needs of local people, and to explain heterogeneities in food security within regions. Analyses that lump nutritional data based on general food types can obscure the individual and often significant variation found between species. For example, information from farmed plants and animals indicate statistical and nutritionally significant differences [99,100]. In West Africa, data are available for a variety of domestic foods, wild plants, and fish, but remain remarkably limited for other wild animals. In fact, the Food and Agriculture Organization of the United Nations (FAO) food composition table for West Africa has no information on bushmeat [101,102], despite its importance to local diets. Future studies that generate nutritional compositional data for critical wild foods, including bushmeat, will improve our understanding of the link between bushmeat and human nutrition, and eventually of the role of bushmeat in food security and development strategies.

Given the diversity of animals hunted for the bushmeat trade, we should expect high levels of variation in nutritional composition. However, protein estimates for bushmeat are typically based on livestock, or a limited number of wild animals, the majority of which are rodents [103,104]. Nevertheless, for those species which data are available, protein values differ more than two-fold [103]. For all species, including those with known macronutrient values, there is a paucity of information on other nutrients, including vitamin and mineral profiles

[101,103,105–107]. Data are particularly limited for bats and primates, which are commonly consumed, highly threatened by the bushmeat trade [108–111], and present a disproportionate risk for zoonotic disease transmission [112–115]. However, because most bushmeat species are consumed by impoverished populations and through informal and often illegal trade networks, nutritional quality of this critical food resource is not well understood.

Food culture

In regions where bushmeat features prominently in human diets, conservation initiatives commonly operate under the paradigm that alternative livelihoods and protein sources will quell the bushmeat trade. This paradigm ignores not only the potentially unique nutritional contributions of bushmeat in maintaining balanced local diets, but also the strong cultural ties to hunting and consumption, and how these factors interact to shape dietary choices and drive off-take. Reliance on bushmeat extends well-beyond nutrition and economics, and future work should focus on the important role of cultural drivers, including food preferences, and medicinal, social, political and symbolic use.

The degree to which nutritional quality shapes dietary choices is dependent on available foods and food perceptions, as well as a variety of cultural factors that are poorly understood [116]. For example, many cultures believe wild animals confer special powers when consumed. In Nigeria, the consumption of tortoise and potto meat is believed to give strength to a pregnant woman and her unborn child [108,117]. In Cameroon, wild cats are believed to make consumers more agile and protect them against car accidents [118]. Preferences are not static, as is the case in Equatorial Guinea, where a shift away from consumption of mandrills corresponded with a number of respondents calling it “dirty meat” [119]. On the contrary, the emerging view of wild meat in Ethiopia is that it is an organic and a healthier alternative to other meat [120]. Similarly, in Ghana bat meat has a reputation as an especially healthy food (and low in cholesterol) [110]. Zoonotic disease risk may also play a role in shaping preferences and hunting behavior, but is poorly understood [121–126]. An improved understanding of human dietary choices will ultimately require integrative models that incorporate multiple facets (e.g. nutrition, preference, and culture) and how they interact [127,128].

In West and Central Africa, people still hunt, even when alternatives are available, suggesting strong cultural ties to wildlife and hunting [129]. Explorations of human-animal relationships are rampant in the anthropological literature [130–136]. In fact, hunting behavior is used in ethnographic works as a lens by which to glimpse gender relations, social organization, and symbolic meaning further demonstrating its deep cultural roots [137–142]. Still, these meanings are often dismissed in research and policy. Understanding the socio-cultural contexts in which hunting and consumption occur will be critical for developing culturally relevant and effective conservation and/or health interventions related to bushmeat extraction. Future studies should work to identify the individual and interactive roles of need-based and cultural factors that drive bushmeat hunting and alter human and ecosystem health.

Intervention strategies

Population expansion and demand for food makes our global population inherently vulnerable to food-borne zoonoses [143]. In rural communities with inadequate access to food, bushmeat simultaneously improves health and well-being while threatening livelihoods long-term and increasing disease risk. Effective and culturally relevant solutions to these problems will require engagement from development, environmental, health, and economic sectors where bushmeat hunting occurs. Alternative livelihood interventions should take into account the local drivers of bushmeat hunting and work to identify alternatives that provide adequate nutrition, are marketable, and satisfy local cultural food preferences. For example, increased livestock production, and raising of preferred wildlife species [144,145], should be coupled with disease management to help minimize health risks and improve sustainability.

Alongside veterinary care, simple behavioral precautions have the capacity to greatly reduce transmission risk in occupational-exposed individuals (e.g. livestock owners, hunters, and butcherers). For example, reducing contact with bodily fluids, conducting routine sanitation, and improving waste management for preventing emergence of H5N1 in poultry farms [146] could be adapted for diverse settings, including bushmeat handling in forests. Interventions that make use of locally available goods will be most promising, since access to materials for management of infectious disease is a major barrier in the success of intervention strategies [147]. Risk perceptions can also be a barrier, as they are important for ensuring individuals adopt and adhere to best practices [108,146]. Culturally appropriate communication campaigns and public health responses to outbreaks may help improve perceptions of risk. Finally, because disease emergence and re-emergence in human and wildlife populations is often coupled with environmental change (i.e. by altering the dynamics of pre-existing diseases or facilitating the introduction of novel pathogens) [148–153], development projects should include the integration of health impact assessments and prevention and response strategies into evaluation of deforestation and land use policies.

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