

## **Noninvasive Assessment of Gastrointestinal Parasite Infections in Free-Ranging Primates**

**Thomas R. Gillespie<sup>1</sup>**

*Received December 10, 2004; revision March 24, 2005; accepted June 24, 2005; Published Online August 11, 2006*

---

*Recent evidence of emerging human diseases with origins or likely transmission to humans, or both, that involve primates and a greater recognition of the risk of human pathogen transmission to free-ranging primates have raised awareness of the potential impact of zoonotic pathogen transmission on primate conservation and nonhuman primate and human health. As human population density continues to increase exponentially, speeding the reduction and fragmentation of primate habitats, greater human-primate contact is inevitable and even higher rates of pathogen transmission are likely. Thus interest has grown in collecting baseline data on patterns of parasitic infections in wild primate populations to provide an index of population health and to begin to assess and, to manage disease risks. Primatologists traditionally have been involved with such surveys through noninvasive assessment of gastrointestinal parasites. Unfortunately, previous studies have tended toward divergent methodologies, compromising the potential for longitudinal and comparative work. Here, I provide practical guidelines and standardized methodologies for the noninvasive assessment of gastrointestinal parasites of primates.*

---

**KEY WORDS:** field protocols; intensity; parasitology guidelines; sample size; standardized methodologies.

### **INTRODUCTION**

Parasites play a central role in ecosystems, affecting the ecology and evolution of specific interactions (Esch and Fernandez, 1993), host

<sup>1</sup>To whom correspondence should be addressed at Program in Ecology and Evolutionary Biology and Departments of Anthropology and Veterinary Pathobiology, University of Illinois, Urbana, IL 61802; e-mail: trg@uiuc.edu.

population growth and regulation (Hochachka and Dhondt, 2000; Hudson *et al.*, 1998), and community biodiversity (Hudson *et al.*, 2002). Parasites can impact host survival and reproduction directly through pathologic effects and indirectly by reducing host condition (Boyce, 1990; Chandra and Newberne, 1977; Coop and Holmes, 1996; Dobson and Hudson, 1992; Hudson *et al.*, 1992). Severe parasitosis can lead to blood loss, tissue damage, spontaneous abortion, congenital malformations, and death (Chandra and Newberne, 1977; Despommier *et al.*, 1995). However, less severe infections are more common and may impair nutrition, travel, feeding, predator escape, and competition for resources or mates, or increase energy expenditure (Coop and Holmes, 1996; Dobson and Hudson, 1992; Hudson *et al.*, 1992; Packer *et al.*, 2003).

The close phylogenetic relationship between humans and nonhuman primates results in high potential for pathogen exchange (Ott-Joslin, 1993; Wolfe *et al.*, 1998). Recent studies have highlighted emerging human diseases, e.g., HIV/AIDS, Ebola, with origins or likely transmission to humans that involves nonhuman primates (Gao *et al.*, 1999; Leroy *et al.*, 2004). Likewise, evidence from well studied ape populations suggests that epidemics of polio, respiratory diseases, and scabies originated from humans (Hill *et al.*, 2001; Kalema-Zikusoka *et al.*, 2002). As human population density continues to increase exponentially, speeding the reduction and fragmentation of primate habitat, greater human-primate contact is inevitable and higher rates of pathogen transmission are likely. Baseline data on patterns of parasitic infections in wild primate populations are critical to provide an index of population health and to begin to assess and manage disease risks. In addition, considering the evolutionary and ecological linkages between primates and their parasites (Stuart and Strier, 1995), one can view parasites as indicator species, potentially alerting us to imminent threats to primate conservation.

Though many studies have documented the gastrointestinal parasites of wild populations of African apes (Ashford *et al.*, 1990, 2000; Lilly *et al.*, 2002; McGrew *et al.*, 1989), baboons (Appleton *et al.*, 1986; Eley *et al.*, 1989; Hahn *et al.*, 2003; Müller-Graf *et al.*, 1997), and howlers (Stoner, 1996; Stuart *et al.*, 1990, 1998), the gastrointestinal parasites of other primate taxa remain poorly known (*cf.* Gillespie *et al.*, 2004, 2005a; Stuart *et al.*, 1993). In addition, though researchers have accomplished much admirable work, primate parasite studies have often made use of divergent methodologies, compromising the potential for longitudinal or comparative work. Here, I provide practical guidelines and standardized methodologies for the non-invasive assessment of gastrointestinal parasite infections in free-living primate populations.

## PRACTICAL GUIDELINES FOR THE STUDY OF PRIMATE PARASITES

### Use Standard Terminology

The American Society of Parasitologists has standardized parasitological terminology (Margolis *et al.*, 1982), which Stuart and Strier (1995) and Bush *et al.* (1997) have reviewed. However, studies of primate parasites frequently perpetuate the use of inappropriate terms. Using vague language such as parasite load or alternatives to standardized terms may lead to misinterpretation of results or compromise the value of findings to the broader scientific and conservation communities. Noninvasive studies of primate parasites can readily provide data on presence or absence, richness, and prevalence of parasitic infections.

### Avoid Use of Egg Counts to Provide a Measure of Infection Intensity

The aforementioned invalid assumption is pervasive in the primate parasite literature. Intensity is the number of adult individuals of a particular taxon infecting an individual host. Many factors affect the number of parasite eggs contained in host fecal material and they cannot reliably provide an index of adult worm burden, i.e., intensity. Studies of livestock; laboratory animals, including primates; and humans demonstrate that host immunity, density-dependent factors, and environmental cues can depress worm ovulation (Christensen *et al.*, 1995; Roepstorff *et al.*, 1996; Stear *et al.*, 1995) and inherent differences in parasite fecundity, size, age, and sex ratio also affect egg output (Coadwell and Ward, 1982; Dineen *et al.*, 1965; Stear *et al.*, 1995). Even in parasite taxa in which a linear relationship exists between egg production and adult worm burden—the case for a limited number of taxa—variation in fecal condition, e.g., moisture content and consistency, and inherent temporal and spatial sampling heterogeneity, compromise intersample comparisons (Anderson and Schad, 1985; Eberhard *et al.*, 2001). Studies of domesticated animals suggest that egg counts may provide a qualitative measure of intensity, i.e., low, moderate, high (Anderson and Schad, 1985; Tarazona, 1986); however, the generality of the relationship requires testing. Hence, noninvasive studies of primate parasites should not report intensity.

### Avoid Collecting Anonymous Samples

Knowing the identity or even the age and sex of individual primates sampled improves data quality exponentially. Unless samples are from

known individuals without duplication, one cannot treat them as independent data points (OIE, 2004), which limits conclusions that one can draw regarding prevalence and specific interactions for both the primates and the parasites. In addition, samples not collected immediately after defecation may be contaminated, potentially leading to misinterpretations regarding host specificity for a given parasite taxa (MAFF, 1979). Anonymous samples can be of value for providing basic presence or absence information, and researchers should seek them for long-term monitoring of unhabituated populations.

### Ensure Adequate Sample Size

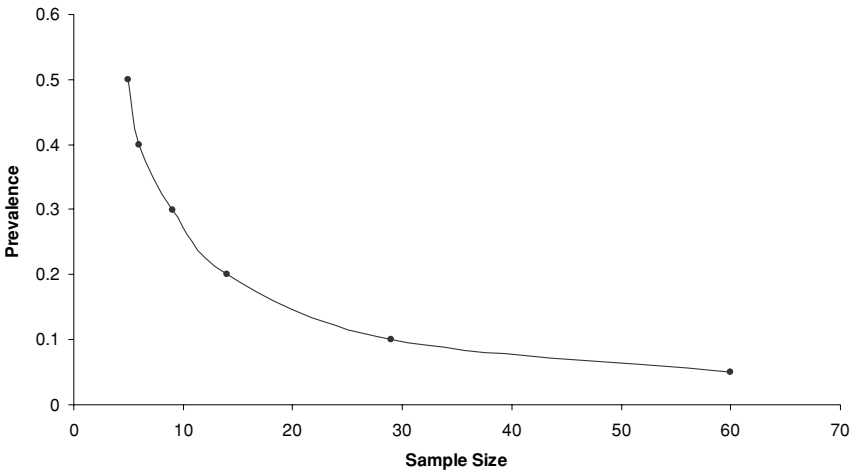
Though the number of independent fecal samples required for a study will depend on the questions asked, samples required to determine whether a parasite is present in a primate population are the minimum. The minimum sample size  $n$  required to detect at least 1 infection is calculated via the following formula, where  $\alpha$  is the significance level and  $p$  is the prevalence in the population:

$$n = \ln(\alpha) / \ln(1 - p)$$

Accepting a 0.05 level of significance and recognizing the broad range of values for gastrointestinal parasite prevalence in free-ranging primates, minimum required sample size depends greatly on expected prevalence of the parasite of interest (Fig. 1). For general surveys of primate parasites, one should apply an assumed prevalence of 5% (Leech and Sellers, 1979; OIE, 2004; Putt *et al.*, 1988), resulting in a minimum requirement of 60 independent fecal samples (Fig. 1). Studies incorporating comparisons over space and time, among age and sex categories, or among species should aim for substantially more samples.

### Present Details of Parasite Identification

Gastrointestinal parasite classification by fecal analyses is weak by its very nature. Consequently, it is critical that researchers present details of the characteristics used to identify a given parasite taxa, e.g., color, size, defining features, and provide conservative identifications as warranted by data collected. Researchers should measure eggs, larvae, and cysts using a calibrated micrometer ( $n = 10/\text{species/sample}$ ) and photograph representatives (MAFF, 1979). Trichostrongyloid, strongyloid, and rhabditoid nematode eggs are similar in size and appearance, making differentiation



**Fig. 1.** Minimum sample size required to be 95% confident of finding a parasite in a population of free-ranging primates in relation to prevalence.

extremely difficult (OIE, 2004), and one should culture larvae to contribute information not available from egg traits alone. Opportunistic necropsies of primates dying of natural causes allow for critical validation of egg identifications. Presentation of details of parasite identification will facilitate longitudinal and comparative studies.

### Provide Supportive Data

Details regarding the environment, density, behavior, and health status of primates sampled can provide critical information that may inform patterns of parasitism observed. The following is not intended as a comprehensive overview of the topic, but instead a synopsis to stimulate future discussion of the issues.

Habitat attributes can strongly influence patterns of parasitism in free-ranging primates. For example, studies have demonstrated infection prevalence to be higher in primates ranging in humid compared to more arid habitats (Hausfater and Meade, 1982; Stuart *et al.*, 1990, 1993). Evidence of general patterns of seasonal infection in primates is equivocal, with clear seasonal patterns of infection for some primate species (Freeland, 1977; Huffman, 1997), and no clear pattern for others (Gillespie *et al.*, 2004, 2005a). Lastly, recent studies demonstrate that patterns of habitat disturbance including selective logging and forest fragmentation can affect

primate-parasite dynamics in dramatic ways (Gillespie and Chapman, 2005; Gillespie *et al.*, 2005b). Consequently, researchers should collect data, or cite published data, on climate, habitat, patterns of disturbance, and history of the environment in which they sample primates for parasites to improve our understanding of the interplay.

Density estimates for the primate of interest, as well as sympatric primates and other potential hosts of generalist parasites, also provide critical information. Host density is of central importance to infection rates in directly transmitted parasites (Poulin, 1998), and intraspecific studies have demonstrated that host density positively correlates with parasite prevalence and diversity (Morand and Poulin, 1998; Packer *et al.*, 1999). However, studies that have concurrently examined primate density and habitat characteristics in relation to primate parasite prevalence reveal that habitat characteristics may be a better predictor of parasitic infection than primate density is (Gillespie, 2004; Gillespie and Chapman, 2005; Stuart *et al.*, 1993). Future studies are needed to distinguish more systematically the effects of host density and habitat characteristics.

A wide variety of primate behaviors including patterns of ranging, grooming, interindividual and intergroup associations, and foraging may influence patterns of parasitic infection. For example, yellow baboons (*Papio cynocephalus*) appear to avoid potential infection by regularly rotating their sleeping sites (Hausfater and Meade, 1982). Likewise, mangabeys (*Lophocebus albigena*) presumably reduce the risk of contact with fecal contamination and consequent infection by traveling further on days of heavy rainfall and avoiding foraging in the same areas on consecutive days (Freeland, 1980). Similarly, Gilbert (1997) suggests that red howlers (*Alouatta seniculus*) reduce contact with parasites by consistently defecating above gaps in the forest vegetation.

Grooming likely affects patterns of parasitism in complex ways. Though grooming appears to be an important mechanism for removing potentially damaging ectoparasites (Freeland, 1981; Gilbert, 1997), there is no published study on how grooming behavior may alter the risk of gastrointestinal parasite infection. Grooming brings individuals into close contact, increasing the risk of transmission of parasites with direct life cycles. In addition, ectoparasites often act as intermediate hosts for gastrointestinal parasites (Despommier *et al.*, 1995; Muller and Baker, 1990; Poulin, 1998). Consequently, primates may unintentionally infect themselves with parasites with intermediate hosts by ingesting ectoparasites while grooming.

Group size may also affect primate parasitic infections. Freeland (1979) found a significant correlation between the size of groups of mangabeys and blue monkeys (*Cercopithecus mitis*) and the number of

protozoan infections each group maintained, and Freeland (1980) demonstrated that the prevalence of protozoan infections increased with group size for mangabeys. A meta-analysis of a wide variety of host-parasite relationships showed a positive correlation between intensity of infection by parasites and host group size (Côté and Poulin, 1995). It is expected that for generalist parasites, the frequency and duration of mixed-species associations affect patterns of parasitism in similar ways. Thus, by limiting group size and the frequency of multispecies associations, primates may be able to limit their risk of parasitic infection.

Patterns of primate foraging may also affect patterns of parasitism. Researchers have systematically documented dietary self-medication of parasitic infections in the great apes (Huffman, 1997; Huffman and Wrangham, 1994), and anecdotal evidence supports the potential generality of this behavior among primates and other taxa (Garber and Kitron, 1997; Glander, 1994; Janzen, 1978; Phillips-Conroy, 1986). Consequently, primatologists should be aware of the potential role self-medication may play in the patterns of parasitism they observe.

Though parasites are a normal component of a functioning ecosystem and low-intensity infections are often asymptomatic (Anderson and May, 1979), anthropogenic change may result in altered transmission rates, parasite host range, and parasite virulence (Daszak *et al.*, 2000; Patz *et al.*, 2000). Resultant changes in host susceptibility may result in elevated morbidity and mortality, and ultimately, population declines. By evaluating fecal samples for symptoms of illness, i.e., diarrhea, blood, etc., before collection, researchers provide data that one can analyze in relation to patterns of infection to examine potential relationships between parasitic infections and pathology or disease in free-ranging primates.

## STANDARDIZED METHODOLOGIES

The following protocols are intended to provide primatologists with simple yet effective methods to evaluate primate gastrointestinal parasitic infections based on the collection and analysis of fecal samples.

### Collection of Fecal Samples for Gastrointestinal Parasite Analysis

1. Prepare collection tubes containing 10% buffered formalin (preferred for helminths) or polyvinyl alcohol (preferred for protozoa).

2. Before collecting feces, examine macroscopically for, and note, consistency, presence of blood, mucus, tapeworm proglottids, and adult or larval nematodes.
3. With gloved hands, use a wooden applicator or spatula to scoop a *ca.* 2-g sample from within the fecal mass into the collection tube. By taking the sample from within the fecal mass, you reduce risks of contamination by free-living nematodes in the immediate environment.
4. Close tube and label with identification no., date, time, initials of collector, primate species, site (ideally Global Positioning System [GPS] coordinates), and age/sex/identity of individual sampled if possible.
5. Shake the tubes vigorously to maximize contact between sample and storage solution.
6. Collect replicate sample in similar fashion using RNAlater (Ambion) as preservative if planning molecular confirmation of parasite identities.

## **Techniques for Recovery and Examination of Gastrointestinal Parasites**

### *Direct Smear*

This method involves examining a thin smear of fecal material with normal saline on a microscope slide. Though direct smear can demonstrate the presence of helminths and protozoa, it is not ideal because it is effective only when egg, larvae, or cyst, or all, concentrations are high. In addition, large amounts of detritus in feces can interfere with identifications and quantitative assessment of egg production is not possible (MAFF, 1979). Consequently, I recommend the method only as a supplemental procedure. A combined recovery using fecal flotation and sedimentation techniques provides the best results.

### *Fecal Flotation*

The method is optimal for separating many helminth eggs and protozoan oocysts and cysts from fecal debris. Occasionally, parasitic mites groomed from the skin will float as well. Solutions of saturated NaCl or sugar can be effective, but NaNO<sub>3</sub> is optimal. ZnSO<sub>4</sub> and MgSO<sub>4</sub> are unsuitable for general analyses because they will not isolate many of the nematodes commonly infecting wild primates. The following protocol is

appropriate for the analysis of primate fecal samples preserved in formalin or polyvinyl alcohol.

1. Add 1 g of feces to centrifuge tube.
2. Fill centrifuge tube 2/3 with distilled water and homogenize fecal pellet with a wooden applicator.
3. Centrifuge samples at 1800 rpm for 10 min.
4. Pour off supernatant.
5. Resuspend fecal material in  $\text{NaNO}_3$  solution.
6. Fill tube to meniscus with  $\text{NaNO}_3$  solution, and place microscope cover slip on lip of tube.
7. Centrifuge samples at 1800 rpm for 10 min.
8. Remove cover slip from centrifuge tube and place on a slide labeled with the sample number.
9. Scan slide using the  $\times 10$  objective lens of a compound microscope and identify and count all parasite eggs, larvae, and cysts. Use the  $\times 40$  objective lens for measurement and confirmation of identifications.
10. Scan slide thoroughly under  $\times 40$  objective lens to confirm presence or absence of protozoan cysts (add a drop of iodine to facilitate identification).
11. Measure the length and width of individual eggs, cysts, and larvae using a calibrated ocular micrometer.
12. Photograph representatives.

If examining undiluted fresh samples, omit steps 2–4.

### *Fecal Sedimentation*

The method allows for the isolation and identification of trematodes (flukes) which, unlike other helminths, are too heavy to float up in  $\text{NaNO}_3$  solution. The following protocol is appropriate for the analysis of primate fecal samples preserved in formalin or polyvinyl alcohol and can use the fecal pellet remaining after the previously described flotation methodology.

1. Suspend fecal pellet in 40 ml of sedimentation solution (dilute soapy water) in a 50-ml beaker.
2. Filter the suspension through cheesecloth held over the lip of the beaker into a 50-ml centrifuge tube. Rinse cheesecloth with sedimentation solution and refilter through cheesecloth. Dispose of cheesecloth and remaining fecal pellet.

3. Allow filtered suspension to settle until sediment is apparent (5 min).<sup>2</sup>
4. Remove supernatant by pipette and rinse remaining material into disposable beaker with sedimentation solution.
5. Repeat until supernatant is clear.
6. Transfer 5 drops of sediment to a slide labeled with the sample number and cover with 2 cover slips placed side by side.
7. Scan slide under  $\times 10$  objective lens and identify and count all parasite eggs, larvae, and cysts. Use the  $\times 40$  objective lens for measurement and confirmation of identifications.
8. Scan slide thoroughly under the  $\times 40$  objective lens to confirm presence or absence of protozoan cysts (add a drop of iodine to facilitate identification).
9. Measure the length and width of individual eggs, cysts, and larvae using a calibrated ocular micrometer.
10. Photograph representatives.

### *Fecal Cultures*

The similarities in size and appearance of the eggs of different species of gastrointestinal nematodes are such that their differentiation is extremely difficult. Their third-stage larvae, however, are sufficiently different and it is possible to distinguish between different genera, and species in some cases. The method below is suitable for the culture of trichostrongyloid, strongyloid, and rhabditoid larvae.

1. Transfer 5 g of fresh feces to a beaker and thoroughly mix with an equivalent volume of vermiculite, using distilled water as needed (*ca.* 10 ml).
2. Tie off mixture inside  $20 \times 20$  cm cheesecloth using a *ca.* 50-cm fishing line or string.
3. Suspend mixture in a 100-ml container with lid.
4. Add distilled water to container until bottom of cheesecloth makes contact with surface of water.
5. Allow culture to incubate at room temperature ( $20\text{--}21^\circ\text{C}$ ) for 2 wk, checking culture daily for fungal growth and water level.
6. Spray mixture with a fine mist of water every few days to deter fungal growth and add water as needed.

<sup>2</sup>For samples from primates consuming large amounts of insect or vertebrate material or both, an ethyl acetate concentration step added at this point may improve results (OIE, 2004).

7. After incubation, collect larvae by pipette and transfer onto a microscope slide for examination and identification. A drop of Lugol's iodine solution (1 g of iodine, 2 g of potassium chloride, 300 ml of distilled water) can facilitate identification.

### *Opportunistic Necropsy*

The following protocol will allow for the isolation of adult and immature gastrointestinal parasites from primates found dead.

1. Procure a fecal sample from the individual for later analysis.
2. Ligate and remove stomach and intestines from the individual.
3. Tie off stomach, small intestine, large intestine, and colon with fishing line or strong string.
4. Wash each section of gastrointestinal tract separately as described below.
5. Cut open stomach and collect contents in a container.
6. Above same container, wash stomach wall thoroughly with water, carefully rubbing mucous membrane to remove any worms.
7. Slowly pour small amounts of wash onto wire-mesh stackable sieves (top screen aperture of 0.15 mm [for recovering adult worms] and bottom screen aperture of 0.038 mm [for recovering immature worms]).
8. Wash material on screen until clear water passes through.
9. After washing all material in this way, invert each screen and wash adhering material into separate containers.
10. Carefully examine the surface of the stomach with dissecting microscope for parasites that remain attached.
11. Treat each section of intestine as stomach.

### **Preservation of Recovered Helminths**

Helminths preserved in the following manner can be delivered to a qualified parasitologist for identification.

#### *Nematodes*

Because of the thick cuticle of the worms, optimal fixation requires use of a hot solution. Thoroughly wash nematodes in 2 or 3 changes of water or

1% saline, then transfer them to hot (70–80°C) 70% alcohol or 5% formalin. After cooling, store in clean fluid of the same kind.

### *Cestodes*

Carefully wash tapeworms in 1% saline. Fix in 5–10% formalin between 2 pieces of glass or by dipping repeatedly while suspending posterior with forceps. The techniques will facilitate subsequent identification by minimizing contraction by the parasite.

### *Trematodes*

Vigorously shake flukes in 1% saline, replace saline with 5–10% formalin while continuing to shake. Vigorous shaking throughout this process prevents contraction by the parasite.

## CONCLUSIONS

Primatologists are situated to provide high-quality and needed data on gastrointestinal parasite infections in wild primate populations. The guidelines and methodologies presented here will help to achieve the goal.

## ACKNOWLEDGMENTS

I thank Ellis Greiner of the Department of Pathobiology, College of Veterinary Medicine, University of Florida; Tony Goldberg of the Department of Pathobiology, College of Veterinary Medicine, University of Illinois; Patricia Reed of the Wildlife Conservation Society Field Veterinary Program; and 2 anonymous reviewers for valuable comments on the manuscript.

## REFERENCES

- Anderson, R. M., and May, R. M. (1979). Population biology of infectious-diseases, vol. 1. *Nature* 280: 361–367.
- Anderson, R. M., and Schad, G. A. (1985). Hookworm burdens and faecal egg counts: An analysis of the biological basis of variation. *Trans. Roy. Soc. Trop. Med. Hyg.* 79: 812–825.
- Appleton, C. C., Henzi, S. P., Whitten, A., and Byrne, R. (1986). The gastro-intestinal parasites of *Papio ursinus* from the Drakensberg Mountains, Republic of South Africa. *Int. J. Primatol.* 7: 449–456.

- Ashford, R. W., Reid, G. D. F., and Butynski, T. M. (1990). The intestinal faunas of man and mountain gorillas in a shared habitat. *Ann. Trop. Med. Parasitol.* 84: 337–340.
- Ashford, R. W., Reid, G. D. F., and Wrangham, R. W. (2000). Intestinal parasites of the chimpanzee *Pan troglodytes*, in Kibale Forest, Uganda. *Ann. Trop. Med. Parasitol.* 94: 173–179.
- Boyce, M. S. (1990). The red queen visits sage grouse leks. *Am. Zool.* 30: 263–270.
- Bush, A. O., Lafferty, K. D., Lotz, J. M., and Shostak, A. W. (1997). Parasitology meets ecology on its own terms: Margolis *et al.* revisited. *J. Parasitol.* 83(4): 575–583.
- Chandra, R. K., and Newberne, P. M. (1977). *Nutrition, Immunity, and Infection*, Plenum Press, New York.
- Christensen, C. M., Barnes, E. H., Nansen, P., Roepstorff, A., and Slotved, H. C. (1995). Experimental *Oesophagostomum dentatum* infection in the pig: Worm populations resulting from single infections with three doses of larvae. *Int. J. Parasitol.* 25: 1491–1498.
- Coadwell, W. J., and Ward, P. F. (1982). The use of faecal egg counts for estimating worm burdens in sheep infected with *Haemonchus contortus*. *Parasitology* 85: 251–256.
- Coop, R. L., and Holmes, P. H. (1996). Nutrition and parasite interaction. *Int. J. Parasitol.* 26: 951–962.
- Côté, I. M., and Poulin, R. (1995). Parasitism and group size in social animals: A meta-analysis. *Behav. Ecol.* 6: 159–165.
- Daszak, P., Cunningham, A. A., and Hyatt, A. D. (2000). Wildlife ecology—emerging infectious diseases of wildlife—threats to biodiversity and human health. *Science* 287: 443–449.
- Despommier, D. D., Gwazda, R. W., and Hotez, P. J. (1995). *Parasitic Diseases*, Springer-Verlag, New York.
- Dineen, J. K., Donald, A. D., Wagland, B. M., and Offner, J. (1965). The dynamics of the host-parasite relationship: The response of sheep to primary infection with *Haemonchus contortus*. *Parasitology* 55: 515–525.
- Dobson, A. P., and Hudson, P. J. (1992). Regulation and stability of a free-living host-parasite system: *Trichostrongylus tenuis* in red grouse: 2 population models. *J. Anim. Ecol.* 61: 487–498.
- Eberhard, M. L., Kovacs-Nace, E., Blotkamp, J., Verwij, J. J., Asigri, V. A., Polderman, A. M. (2001). Experimental *Oesophagostomum bifurcum* in monkeys. *J. Helminthol.* 75(1): 51–56.
- Eley, R. M., Strum, S. C., Muchemi, G., and Reid, G. D. F. (1989). Nutrition, body condition, activity patterns and parasitism of free-ranging baboons (*Papio anubis*) in Kenya. *Am. J. Primatol.* 18: 209–219.
- Esch, G., and Fernandez, J. C. (1993). *A Functional Biology of Parasitism: Ecological and Evolutionary Implications*. Chapman and Hall, London.
- Freeland, W. J. (1977). *Dynamics of Primate Parasites*. Ph.D. Dissertation. University of Michigan, Ann Arbor.
- Freeland, W. J. (1979). Primate social groups as biological islands. *Ecology* 60: 719–728.
- Freeland, W. J. (1980). Mangabey (*Cercocebus albigena*) movement patterns in relation to food availability and fecal contamination. *Ecology* 61: 1297–1303.
- Freeland, W. J. (1981). Parasitism and behavioral dominance among male mice. *Science* 213: 461–462.
- Gao, F., Bailes, E., Robertson, D. L., Chen, Y., Rodenburg, C. M., Michael, S. F., Cummins, L. B., Arthur, L. O., Peeters, M., Shaw, G. M., Sharp, P. M., and Hahn, B. H. (1999). Origin of HIV-1 in the chimpanzee *Pan troglodytes troglodytes*. *Nature* 397: 436–441.
- Garber, P. A., and Kitron, U. (1997). Seed swallowing in tamarins: Evidence of a curative function or enhanced foraging efficiency? *Int. J. Primatol.* 18: 523–538.
- Gilbert, K. A. (1997). Red howling monkey use of specific defecation sites as a parasite avoidance strategy. *Anim. Behav.* 54: 451–455.
- Gillespie, T. R. (2004). *Effects of Human Disturbance on Primate-Parasite Dynamics*. Ph.D. Dissertation. University of Florida, Gainesville.
- Gillespie, T. R., and Chapman, C. A. (2006). Forest fragment attributes predict parasite infection dynamics in primate metapopulations. *Cons. Biol.* 20:441–448.
- Gillespie, T. R., Greiner, E. C., and Chapman, C. A. (2004). Gastrointestinal parasites of the guenons of western Uganda. *J. Parasitol.* 90: 1356–1360.

- Gillespie, T. R., Greiner, E. C., and Chapman, C. A. (2005a). Gastrointestinal parasites of the colobus monkeys of Uganda. *J. Parasitol.* 91: 569–573.
- Gillespie, T. R., Chapman, C. A., and Greiner, E. C. (2005). Effects of logging on gastrointestinal parasite infections and infection risk in African primate populations. *J. Appl. Ecol.* 42:699–707.
- Glander, K. E. (1994). Nonhuman primate self-medication with wild plant foods. In Etkin, N. L. (ed.), *Eating on the Wild Side: The Pharmacological, Ecologic, and Social Implications of Using Noncultigens*. University of Arizona Press, Tuscon, pp. 227–239.
- Hahn, N. E., Proulx, D., Muruthi, P. M., Alberts, S., and Altmann, J. (2003). Gastrointestinal parasites in free-ranging Kenyan baboons (*Papio cynocephalus* and *P. anubis*). *Int. J. Primatol.* 24: 271–279.
- Hausfater, G., and Meade, B. J. (1982). Alteration in sleeping groves by yellow baboons *Papio cynocephalus* as a strategy for parasite avoidance. *Primates* 23: 287–297.
- Hill, K., Boesch, C., Goodall, J., Pusey, A., Williams, J., and Wrangham, R. (2001). Mortality rates among wild chimpanzees. *J. Hum. Evol.* 40: 437–450.
- Hochachka, V. W., and Dhondt, A. A. (2000). Density-dependent decline of host abundance resulting from a new infectious disease. *Proc. Natl. Acad. Sci. USA* 97: 5303–5306.
- Hudson, P. J., Dobson, A. P., and Newborn, D. (1992). Do parasites make prey vulnerable to predation: Red grouse and parasites. *J. Anim. Ecol.* 61: 681–692.
- Hudson, P. J., Dobson, A. P., and Newborn, D. (1998). Prevention of population cycles by parasite removal. *Science* 282: 2256–2258.
- Hudson, P. J., Rizzoli, A., Grenfell, B. T., Heesterbeek, H., and Dobson, A. P. (2002). *The Ecology of Wildlife Diseases*, Oxford University Press, Oxford, UK.
- Huffman, M. A. (1997). Current evidence for self-medication in primates: A multidisciplinary perspective. *Yrbk. Phys. Anthropol.* 40: 171–200.
- Huffman, M. A., and Wrangham, R. W. (1994). Diversity of medicinal plant use by chimpanzees in the wild. In Wrangham, R. W., McGrew, W. C., deWaal, F. B. M., and Heltne, P. G. (eds.), *Chimpanzee Cultures*. Harvard University Press, Cambridge, pp. 129–148.
- Janzen, D. H. (1978). Complications in interpreting the chemical defenses of trees against tropical arboreal plant-eating vertebrates. In Montgomery, G. G. (ed), *The Ecology of Arboreal Folivores*. Smithsonian Institution Press, Washington, DC, pp. 73–84.
- Kalema-Zikusoka, G., Kock, R. A., and Macfie, E. J. (2002). Scabies in free-ranging mountain gorillas (*Gorilla beringe beringe*) in Bwindi Impenetrable National Park, Uganda. *Vet. Rec.* 150: 12–15.
- Leech, F. B., and Sellers, K. C. (1979). *Statistical Epidemiology in Veterinary Science*. Charles Griffen, London.
- Leroy, E. M., Rouquet, P., Formenty, P., Souquiere, S., Kilbourne, A., Froment, J. M., Bermejo, M., Smit, S., Karesh, W., Swanepoel, R., Zaki, S. R., and Rollin, P. E. (2004). Multiple Ebola virus transmission events and rapid decline of central African wildlife. *Science* 303: 387–390.
- Lilly, A. A., Mehlman, P. T., and Doran, D. (2002). Intestinal parasites in gorillas, chimpanzees, and humans at Mondika Research Site, Dzanga-Ndoki National Park, Central African Republic. *Int. J. Primatol.* 23: 555–573.
- Margolis, L., Esch, G. W., Holmes, J. C., Kuris, A. M., and Schad, G. A. (1982). The use of ecological terms in parasitology (report of an ad hoc committee of the American Society of Parasitologists). *J. Parasitol.* 68(1): 131–133.
- McGrew, W. C., Tutin, C. E. G., Collins, D. A., and File, S. K. (1989). Intestinal parasites of sympatric *Pan troglodytes* and *Papio* spp. at two sites: Gombe (Tanzania) and Mt. Assirik (Senegal). *Am. J. Primatol.* 17: 147–155.
- MAFF (Ministry of Agriculture, Fisheries, and Food). (1979). *Manual of Veterinary Parasitology Laboratory Techniques*. Her Majesty's Stationary Office, London, U.K.
- Morand, S., and Poulin, R. (1998). Density, body mass and parasite species richness of terrestrial mammals. *Evol. Ecol.* 12: 717–727.
- Muller, R., and Baker, J. R. (1990). *Medical Parasitology*. Gower, London.

- Müller-Graf, C. D., Collins, D. A., Packer, C., and Woolhouse, M. E. (1997). *Schistosoma mansoni* infection in a natural population of olive baboons (*Papio cynocephalus anubis*) in Gombe Stream National Park, Tanzania. *Parasitology* 15: 621–627.
- OIE (Office International des Epizooties). (2004). *Manual of Diagnostic Tests and Vaccines for Terrestrial Animals*, Office International des Epizooties, Paris, France.
- Ott-Joslin, J. E. (1993). Zoonotic diseases of nonhuman primates. In Fowler, M. E. (ed.), *Zoo and Wild Animal Medicine*. W. B. Saunders, Philadelphia, pp. 358–373.
- Packer, C., Altizer, S., Appel, M., Brown, E., Martenson, J., O'Brien, S. J., Roelk-Parker, M., Hofmann-Lehmann, R., and Lutz, H. (1999). Viruses of the Serengeti: Patterns of infection and mortality in African lions. *J. Anim. Ecol.* 68: 1161–1178.
- Packer, C., Holt, R. D., Hudson, P. J., Lafferty, K. D., and Dobson, A. P. (2003). Keeping the herds healthy and alert: implications of predator control for infectious disease. *Ecol. Lett.* 6: 1–6.
- Patz, J. A., Graczyk, T. K., Geller, N., and Vittor, A. Y. (2000). Effects of environmental change on emerging parasitic diseases. *Int. J. Parasitol.* 30: 1395–1405.
- Phillips-Conroy, J. E. (1986). Baboons, diet, and disease: Food plant selection and schistosomiasis. In Taub, D. M., and King, F. A. (eds.), *Current Perspectives in Primate Social Dynamics*. Van Nostrand Reinhold, New York, pp. 287–304.
- Poulin, R. (1998). *Evolutionary Ecology of Parasites: From Individuals to Communities*, Chapman and Hall, London.
- Putt, S. N. H., Shaw, A. P. M., Woods, A. J., Tyler, L., and James, A. D. (1988). *Veterinary Epidemiology and Economics in Africa—A Manual for Use in the Design and Appraisal of Livestock Health Policy*, FAO, Rome.
- Roepstorff, A., Bjørn, H., Nansen, P., Barnes, E. H., and Christensen, C. M. (1996). Experimental *Oesophagostomum dentatum* infections in the pig: Worm populations resulting from trickle infections with three dose levels of larvae. *Int. J. Parasitol.* 26: 399–408.
- Stear, M. J., Bishop, S. C., Doligalska, M., Duncan, J. L., Holmes, P. H., Irvine, J., McCririe, L., McKellar, Q. A., Sinski, E., and Murria, M. (1995). Regulation of egg production, worm burden, worm length and worm fecundity by host responses in sheep infected with *Ostertagia circumcincta*. *Parasit. Immunol.* 17(12): 643–652.
- Stoner, K. E. (1996). Prevalence and intensity of intestinal parasites in mantled howling monkeys (*Alouatta palliata*) in northeastern Costa Rica: Implications for conservation biology. *Cons. Biol.* 10: 539–546.
- Stuart, M. D., Greenspan, L. L., Glander, K. E., and Clarke, M. R. (1990). A coprological survey of parasites of wild mantled howling monkeys, *Alouatta palliata palliata*. *J. Wildlife Dis.* 26: 547–549.
- Stuart, M. D., Pendergast, V., Rumpf, S., Greenspan, L., Glander, K., and Clarke, M. (1998). Patterns of parasitism in wild howler monkeys, *Alouatta* spp., with observations from a long-term study of *Alouatta palliata* in Costa Rica. *Int. J. Primatol.* 19: 493–512.
- Stuart, M. D., and Strier, K. B. (1995). Primates and parasites: A case for a multidisciplinary approach. *Int. J. Primatol.* 16: 577–593.
- Stuart, M. D., Strier, K. B., and Pierberg, S. M. (1993). A coprological survey of parasites of wild muriquis, *Brachyteles arachnoides*, and brown howling monkeys, *Alouatta fusca*. *J. Helminthol. Soc. Wash.* 60: 111–115.
- Tarazona, J. M. (1986). A method for the interpretation of parasite egg counts in faeces of sheep. *Vet. Parasitol.* 22(1–2): 113–119.
- Wolfe, N. D., Escalante, A. A., Karesh, W. B., Kilbourn, A., Spielman, A., and Lal, A. A. (1998). Wild primate populations in emerging infectious disease research: The missing link? *Emerg. Infect. Dis.* 4: 149–158.