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Trichuris Burdens in Zoo-Housed *Colobus guereza*

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Abstract *Trichuris* spp. infect the majority of captive primate species along with an estimated 1049 million people worldwide, making it an important zoonosis [Stephenson, L. S., Holland, C. V., & Cooper, E. S. *Parasitology, 121*(Suppl.), S73–S95, 2000]. We investigated the efficacy of methods used to evaluate the prevalence of *Trichuris* spp. in 2 groups ($n=12$) of socially housed Abyssinian colobus (*Colobus guereza kikuyensis*) at Paignton Zoo Environmental Park and the factors that may affect density. We collected individual and group fecal samples over 6 mo and estimated burden (egg counts/g of feces) of *Trichuris* spp. via the McMaster technique. Shedding was significantly higher in the afternoon than in the morning (matched-pairs *t*-test: $t_{[5]}=-4.46$, $p<0.01$) and in dominant adult male colobus (Spearman rank: $r_{[5]}=-0.94$, $p<0.01$; age: $r_{[5]}=0.89$, $p<0.05$). Parasitological studies of zoo-housed primates can be a useful tool to explore factors that may affect burdens of *Trichuris* spp. in them.

Keywords colobus monkey · dominance · hierarchy · parasite · *Trichuris* spp.

Introduction

Trichuris spp. are parasitic nematodes that infect the ceca and colons of a variety of mammalian species. The species that infects simians is believed to be *Trichuris trichiura*, which researchers have identified in *Macaca fascicularis* (Dinh Son 2002), *Macaca mullatta* (Taylor *et al*. 1994), and other Old and New World primate species. *Trichuris trichiura* is easily transmitted between humans and

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nonhuman primates and infects an estimated 1049 million people worldwide, making it an important zoonosis (Stephenson *et al*. 2000). Wild-living primates infected with *Trichuris* spp. are a threat to human well-being, directly and indirectly (Muriuki *et al*. 1998).

Trichuris trichiura has a simple, direct life cycle. After copulation, each female lays 2000–10,000 eggs/d (Urquhart *et al*. 1988), which are passed in the host's feces and can be counted to give a relative burden of *Trichuris*. Reinfection occurs by ingestion of embryonated eggs, after which the larvae migrate to the intestine. Once mature, the anterior of the worm burrows into the intestinal mucosa, where it ingests cellular secretions. Blood loss due to damage of the intestinal epithelium may cause a variety of symptoms including anaemia, colitis, and growth retardation (Schmidt and Roberts 1985).

Researchers have studied the relationship between *Trichuris* spp. and a range of nonhuman primate (NHP) host species (*Pan paniscus*, Hasegawa *et al*. 1983; *Papio anubis*, Emikpe *et al*. 2002; *Macaca mulatta*, Phillippi and Clarke 1992; *Macaca fascicularis*, Janagi 1981). Fatal burdens of *Trichuris* spp. also occur (*Colobus guereza*, Loomis and Wright 1986; *Papio anubis*, Emikpe *et al*. 2002).

The study of NHP parasitology in zoos is both interesting and important, in terms of human and NHP health and welfare and also to broaden our understanding of the parasite-host relationship, which can affect nutrition and behavior. As a source of prolific zoonoses, we need to monitor incidence and density of *Trichuris* spp. to limit human health risks and ensure good health in our animals. Consequently, we require knowledge of individual differences in burdens of *Trichuris* spp. and the factors that may affect them, which we can achieve only with an accurate and reliable sampling technique.

We evaluated the parasite burden of *Colobus guereza* at Paignton Zoo via the McMaster technique 3 times/yr, in-house, and had it validated by an outside laboratory. The method that zoos commonly use involves collecting a group fecal sample from several feces in different areas in the enclosure (Goossens *et al*. 2004). We report on the periodic and individual variation in burden of *Trichuris* spp. and compare estimations of burden of *Trichuris* spp. via 2 fecal sampling methods (group vs. individual).

Methods and Results

Subjects, Housing, and Husbandry

Two groups of *Colobus guereza kikuyuensis* live at Paignton Zoo Environmental Park, Devon, UK. There is no physical contact between the groups. Group A consists of 8 individuals: 3 adult males (>4 yr at start of study), 3 juvenile males (<4 yr), and 2 adult females (>4 yr), one of which was lactating and pregnant. Group B comprises 4 females: 2 adults and 2 juveniles.

Both groups have 24-h access between a sheltered indoor area (A, 10.0×5.0×2.5 m; B, 5.0×3.0×2.5 m) and an enclosed outside area (A, 10.0×5.0×5.0 m; group B, 5.0×10.0×4.0 m). Both inside areas have wooden perches, Perspex feeding tables, and cement floors with a thin layer of shavings; outdoor floors consist of a deep litter system of bark for A and grass for B.

Keepers clean inside areas daily (0830 h), replace floor shavings, and wipe fixtures with Annihilate A.C.R.® (Hydra International, Milton Keynes), using the same cleaning tools in both enclosures. Outside enclosures are spot cleaned. Keepers feed all subjects a mixed diet of leafy vegetables, tubers, and occasional nuts and fruit.

Sample Collection and Analyses

We collected data between October 2002 and March 2003 opportunistically when feces were voided; hence the number of samples available varied. We marked feces voided inside that had not touched other feces on a map of the enclosure and collected them in labeled (individual's name and date) plastic pots before storage at −5°C. We estimated mean egg count/g feces (epg) values of *Trichuris* spp. via the McMaster technique, using 2 slides prepared from each fecal sample (Urquhart *et al.* 1988). All statistical analyses are 2-tailed and non-normal data are log transformed.

Fecal Collection 1: Time of Day Effects

We collected feces from group A at 1100–1200 h and 1400–1500 h. Estimated mean epg are significantly lower in feces collected in the morning compared to the samples collected in the afternoon (matched-pairs *t*-test: $t_{[5]}=-4.46$, $p<0.01$; Fig. 1). To ensure consistency, we collected all future fecal samples during the afternoon.

Fecal Collection 2: Seasonal Effects

We collected feces in autumn (October–November; group A, $n=7$ collected over 28 d, group B, $n=8$ collected over 14 d) and spring (February–March; $n=17$ collected over 50 d, $n=10$ collected over 46 d).

There is no significant difference between the estimated mean epg in autumn and spring (matched pairs *t*-test: $t_{[11]}=0.74$, $p>0.05$), so we pooled autumn and spring data sets for all further analyses.

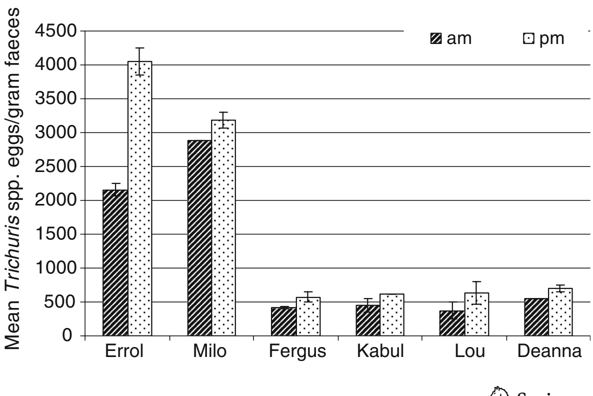

Fig. 1 Comparison of the mean (± SEM) eggs/g of feces of *Trichuris* spp. estimated from fecal samples collected in the morning and afternoon.

Fecal Collection 3: Group versus Individual Sampling Technique

We collected feces from the individuals, per the method above, and from the group, per the method the zoo used. We collated the estimated daily mean epg values for all individuals to provide a collective individual mean (CI) and compared it with the mean epg estimated from the group samples.

There is no significant difference between the mean epg estimated from the CI or the group (A matched-pairs *t*-test: $t_{[17]}=0.001$, $p>0.05$; B matched-pairs *t*-test: $t_{[23]}=0.091$, $p>0.05$; Fig. 2). There is also no significant correlation between the 2 estimated mean epg values (A Pearson: $r_{[17]}=0.36$, $p>0.05$; B Pearson: $r_{[23]}=-0.029$, $p>0.05$).

Individual Variation in Parasite Burden, Including Behavioral Data

There is considerable individual variation in epg for the 12 colobus. Beattie's (adult female, group B) burden was ≥5 times greater (11,329 mean epg) than that of the other females (2780 mean epg; Fig. 2). Because Beattie's epg was greatly different and more variable than that of the other females, we considered her an outlier.

We established that an interaction between age and sex significantly influenced mean epg (2-way ANOVA: age, $F_{[1,10]}=12.01$, $p<0.01$; age*sex, $F_{[1,10]}=7.4$, $p<0.05$, Fig. 3). Adult males have a significantly higher estimated mean epg, compared to adult females and juveniles. We observed the males in group A via continuous focal sampling (Martin and Bateson 1994) for 15 min ($n=6$ subjects, 12 sessions/subject). All displacement behaviors are noted, where displacement is "movement of one animal (displacer) towards another stationary animal, causing the stationary animal (displacee) to move away." Females are excluded because published literature suggests they do not form hierarchies (Grunau and Kuester 2001). We created a dominance hierarchy for the male colobus, by ordering them according to how many other males they supplant. There are significant correlations between male estimated mean epg and dominance rank, mass in kg, and age in yr (Spearman rank: $r_{[5]}=-0.94$, $p<0.01$; mass: $r_{[5]}=0.93$, $p<0.01$; age: $r_{[5]}=0.89$, $p<0.05$; Fig. 3).

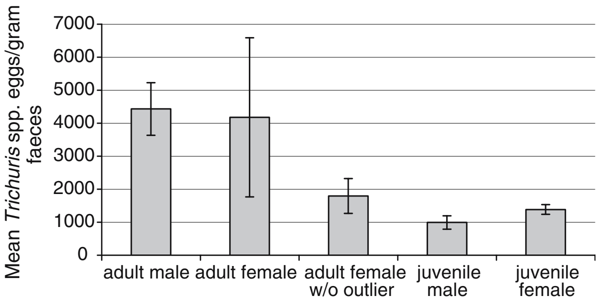

Fig. 2 Mean (± SEM) eggs/g of feces of *Trichuris* spp. estimated for different sex/age groups of colobus.

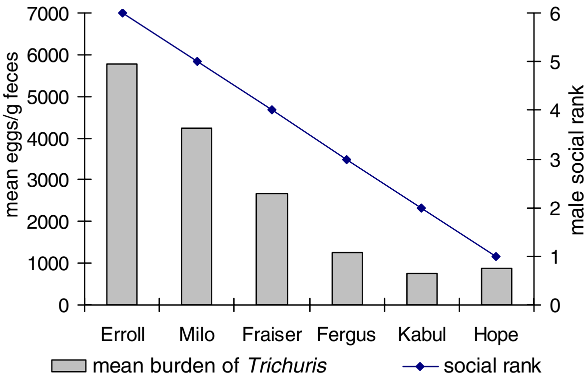

Fig. 3 Mean (± SEM) estimations of eggs/g of feces of *Trichuris* spp. for male colobus correlate significantly with male dominance rank.

Discussion

We outline some of the factors that affect the burden of *Trichuris* spp. in zoo-housed colobus. Our data indicate that time of day and individual differences are associated with significant variations in epg of *Trichuris* spp.

Previous researchers have noted periodicity in helminth transmission, e.g., with *Schistosoma* a circadian pattern occurs when cercariae are voided from their snail hosts and again when the matured eggs of *Schistosoma* are voided from their human hosts (Bogea *et al*. 1996; Doehring *et al*. 1983). For some helminths the periodicity enhances transmission under favorable conditions (Fingerut *et al*. 2003). For example, microfilarial numbers of *Brugia malayi* and *Wuchereria bancrofti* increase in the peripheral blood in synchrony with the biting behavior of local mosquitoes (Maizels and Kurniawan-Atmadja 2002; Shriram *et al*. 2005). Because *Trichuris* spp. require time in soil to become infective, it is unclear why shedding more eggs in the afternoon compared to the morning enhances transmission.

Equally unclear is the stimulus (zietgeber) that triggers the circadian pattern, which could be naturally occurring, including light/dark cycles or temperature (Sharma and Chandrashekaren 2005), or an artefact of the zoo environment, e.g., interactions with keepers, cleaning, or feeding times. It is unlikely that one can attribute feeding times or food composition to the pattern. Edwards and Ullrey (2001) noted that the passage rate for liquids and solids through the gut for *Colobus guereza kikyuensis* is >30 h and relatively unaffected by fiber content. Researchers have reported similar findings from other tripartite foregut fermenters (NRC 2003).

We noted no seasonal variation in burden of *Trichuris* spp., which favor wet conditions (Maipanich *et al*. 1998). Changes in climate may not be sufficiently different across seasons— southwest England is mild throughout the year— or captive husbandry negated any seasonal change in climate, e.g., daily cleaning.

Mean epg estimations calculated from individually identified fecal samples do not correlate with or are significantly different from group samples from around the enclosure. Both sampling methods provide comparable information about the colobus group's mean burden of *Trichuris* spp., probably attributable to large intra- and interindividual epg variation. Practically, collecting group fecal samples provided adequate information about the colobus group's burden of *Trichuris* spp., but information about the variability of the burden was missing.

Individual differences significantly affected the burden of *Trichuris* spp. Adult male colobus have significantly higher worm burdens than those of juvenile males and females. We located no comparable datum for wild *Colobus guereza*; however, Gillespie *et al*. (2005) noted that the prevalence of *Trichuris* spp. in wild *Colobus guereza* was not related to age or sex classes. Researchers in previous studies documented that burdens of *Trichuris* spp. are significantly higher in children than in adults, which they suggest is a consequence of age-dependent immunity response rather than decreased exposure with age (*Macaca mulatta*, Knezevich 1998; *Homo sapiens*, Bundy *et al*. 1991).

The burdens of *Trichuris* spp. we estimated may be due to social rank; male social dominance correlates strongly positively with burden of *Trichuris* spp. Unfortunately, male age, dominance rank, and weight are all confounding variables; older males were heavier and of higher rank. Hausfater and Watson (1976) also noted that dominant male free-ranging olive baboons (*Papio anubis*) and mid-ranking females shed more ova than low-ranking individuals did. Hence it is possible that in our study, burden of *Trichuris* spp. was not age-dependent but due to dominance.

Dominance hierarchies are associated with social stress: physiological consequences of social interactions (Sapolsky 2005). Low- and high-ranking individuals have experienced the deleterious ramifications of social stress, though their manifestations are likely to vary according to species (social system) or individual differences (Abbott *et al*. 2006). Researchers have associated high parasite burdens with impaired immune system function and other indications of poor health (Bradley and Jackson 2004). We suggest that worm burden provides an indication of social stress because females and juvenile males that would not be vying for dominance had low burdens of *Trichuris* spp. relative to those of adult males. Ecologically, one would expect dominant males to sire more offspring, which may compensate for impaired health status due to a higher worm burden (Krebs and Davis 1997).

In summary, this study outlines factors that affect *Trichuris* spp. burden in zoo-housed colobus. Our data suggests that time of day affected the level of eggs shed, which is likely to be a consequence of husbandry. Individual differences are also apparent in *Trichuris* spp. burdens, which the authors believe reflects underlying social stress; male rank is positively correlated with *Trichuris* spp. burden. Finally, estimations of *Trichuris* spp. epg from individually identified or group collected faecal samples provided comparable information for managing a preventive health programme.

Conclusion

We outlined factors that affect the burden of *Trichuris* spp. in zoo-housed colobus. Our data suggest that time of day affects the level of eggs shed, which is likely a consequence of husbandry. Individual differences are also apparent in burdens of *Trichuris* spp., which we believe reflects underlying social stress; male rank correlates positively with burdens of *Trichuris* spp. Finally, estimation of epg of *Trichuris* spp. from individually identified or group-collected fecal samples provides comparable information for managing preventative health programs.

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