**Behavioral Ecology and Sociobiology**

**Supplementary materials for “Multi-scale predictors of parasite risk in wild male savanna baboons (*Papio cynocephalus*)”**

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**Table of Contents**

Supplementary materials and methods ……………………………………………………………………………………..2

Supplementary Tables ……………………………………………………………………………………………………………….4

References………………………………………………………………………………………………………………………………….6

**Supplementary materials and methods**

Fecal flotation protocol. To count parasite eggs in each fecal sample, samples were homogenized to ensure equal distribution of ova. We then mixed 12 mL of distilled water with the 4g of fecal sample, and used a strainer to filter debris. We filled a 15 mL centrifugal tube with a mixture of both fecal sample and distilled water, centrifuged the sample for 10 min at 1500 rpm, and then discarded the supernatant. The centrifuge tube with the remaining precipitate was mixed with 7 mL of Sheather’s solution (specific gravity ~1.27), homogenized using a vortexer, and additional Sheather’s solution was added to form a bulging meniscus at the top of the tube. A coverslip was placed on the meniscus, and the sample was centrifuged a second time for 10 min at 1500 rpm. Following the second centrifuge, the coverslip was removed and placed on a microscope slide for identification of parasite ova. The procedure was repeated using the same sample for preparation of a second coverslip. The two slides were examined under 10x magnification starting from the corner of the coverslip and moving systematically column by column until we examined the entire field of view. For each parasite taxon, we counted the total number of eggs and recorded this information onto a database.

Sedimentation protocol. For the sedimentation protocol, 2g of fecal sample was combined with 12 mL of water and strained to filter debris. We transferred the filtrate to a 15mL centrifuge tube, capped the tube, centrifuged the sample for 10 min at 1500 rpm, and then discarded the supernatant. We used a mechanical pipette to add 750 uL of distilled water and 500 uL of Sheather’s solution to the precipitate (to prevent slides from drying out after being placed under the microscope), and then vortexed the solution. We extracted five, 50 uL drops from the centrifuge tube using a pipette; each drop was added onto microscope slides and covered with a coverslip. The five preparations were examined under 10x magnification, and we quantified the total number of eggs per taxon and then recorded this information onto a database.

Hormone extraction. Fecal samples for hormone analyses were collected in the field opportunistically and stored in 95% ethanol. Prior to transport to the laboratory, the samples were placed in an evaporative cooling structure, which maintained the samples at approximately 25°C. In the laboratory, the samples were freeze dried, sifted for removal of vegetation, and weighed in preparation for extraction. To extract glucocorticoid and testosterone metabolites from fecal samples, fecal powder (0.2 g) first underwent extraction in 90% methanol (2 mL) in a multipulse vortexter for 30 min followed by a solid phase extraction using a prepped Oasis cartridge (Waterford, Milford, MA) for further purification. Samples were assayed for glucocorticoids and testosterone by radioimmunoassay (RIA) using well-established protocols (Gesquiere et al. 2008; Gesquiere et al. 2011a; Gesquiere et al. 2011b; full laboratory protocols: <http://amboselibaboons.nd.edu/>). We used a corticosterone kit for rats and mice (ICN Diagnostics, Costa Mesa, CA) previously validated for this population (Khan et al. 2002; Lynch et al. 2003; Gesquiere et al. 2005; Gesquiere et al. 2011b). Inter-assay coefficients of variation were 13.6% and 10.7% (n=49), respectively for a low and high control. Intra-assay coefficients of variation were below 6% for both the low and high control (any duplicate above 15% was re-assayed). For testosterone extraction, we used a testosterone specific antibody from the commercially available Pantex Testosterone Kit (Pantex, Santa Monica, CA) (Gesquiere et al. 2014). The intra-assay coefficients of variation (CV) were 6.8% and 2.3%, respectively for a 50 pg/tube and a 100 pg/tube of male–female fecal pool (N= 10). The inter-assay CVs were 9.7% and 8.9%, respectively for the 50 pg/tube and 100 pg/tube male–female fecal pool (N= 90). Results were reported as ng/g dry fecal matter.

Table S1. Life cycles and transmission modes of common gastrointestinal parasites of savanna baboons (ordered by prevalence).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Parasite | Life cycle | Transmission mode | Location of larval stage | Location of adult stage/adult lifespan | Length of time to hatch | Pathology | References |
| *Trichuris trichiura* | Direct life cycle; ingestion of embryonated egg; L1 larva hatches in small intestine and penetrates the epithelium; following four molts, the adult emerges and is passively transported to the large intestine  | Environmental; ingestion of embryonated egg that may live on food, surfaces, or other hosts in the host’s environment | Epithelium of small intestine | Cecum; large Intestine (mainly transverse and descending colon); lifespan: 1-2 years | Eggs hatch in 10 days (require 10 days in soil) | Heavy infection associated with severe enteritis, anorexia, mucoid diarrhea, possible mortality | (Graham 1960; Cogswell 2012; Strait et al. 2012; Despommier et al. 2016)  |
| Strongyles | Direct life cycle; ingestion of infective L3 larvae; larvae passes to colon where a nodule is formed; nodule ruptures in 5-8 days and worm matures in the lumen | Environmental; ingestion of infective L3 stage larvae that may live on food or surfaces in the host’s environment | After direct ingestion, larvae pass directly to the large intestine; induces development of a large, encapsulated nodule | Adult worms typically reside in small and large intestines; produce 5,000 eggs/day in humans; lifespan: 8-15 years for human infections | Eggs hatch in 24-48 hours | Possible weight loss, diarrhea; ulceration; calcified nodules; debilitation; increased mortality | (Cogswell 2012; Strait et al. 2012; Despommier et al. 2016; Akinyi 2017) |
| *Abbreviata caucasica* | Indirect life cycle; arthropod intermediate host required; second intermediate or paratenic host may also be required | Environmental: Ingestion of intermediate arthropod host | Orthoptera or Coleoptora species | Adult worms are located in the esophagus, stomach, and duodenum; lifespan unknown | Unknown | Esophagitis, gastritis, enteritis; stomach ulcerations | (Brede and Burger 1977; Pettifer 1984; Cogswell 2012; Strait et al. 2012) |
| *Streptopharagus pigmentatus* | Indirect life cycle; arthropod intermediate host required | Environmental: Ingestion of intermediate arthropod host | Coleoptera species including dung beetles | Adult worms are located in the esophagus, stomach and duodenum; lifespan unknown | Unknown | Gastritis; stomach ulcerations | (Machida et al. 1978; Pettifer 1984; Müller Graf et al. 1996 Cogswell 2012) |

**Table S2.** When age was a significant predictor of male parasitism (Table 2; *T. trichiura* intensity and presence or absence of *A. caucasica*),the addition of rank did not improve the model and rank was not significant in models that included male age.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **helminth taxa** | **fixed effects** | **process level** | **estimate** | **SE** | **t value** | ***P*** | **direction** |
| ***T. trichiura*** | social connectedness | Host | 0.108 | 0.031 | 3.467 | 0.001 | ↑SCI, ↑*T. trichiura* |
|  | age | Host | 0.075 | 0.015 | 4.979 | <0.001 | ↑age, ↑*T. trichiura* |
|  | glucocorticoids (fGC) | Host | -0.002 | 0.001 | -2.164 | 0.031 | ↑glucocorticoids, ↓*T. trichiura* |
|  | rank | Host | -0.006 | 0.013 | -0.503 | 0.615 | not significant |
|  | strongyles | Host /Population | 0.224 | 0.061 | 3.670 | <0.001 | ↑strongyles, ↑*T. trichiura* |
|  | *A. caucasica* | Host /Population | 0.150 | 0.055 | 2.740 | 0.006 | ↑*A. caucasica*, ↑*T. trichiura* |
|  | sex ratio | Group | -0.570 | 0.111 | -5.137 | <0.001 | ↑F:M sex ratio, ↓*T. trichiura* |
|  | rainfall (cm) | Population | -0.030 | 0.004 | -7.122 | <0.001 | ↑rainfall, ↓*T. trichiura* |
| ***A. caucasica*** | age | Host | -0.073 | 0.039 | -1.861 | 0.063 | ↑age, ↓*A. caucasica* |
|  | rank | Host | 0.000 | 0.034 | 0.003 | 0.997 | not significant |
|  | *T. trichiura* | Host/Population | 0.506 | 0.137 | 3.700 | <0.001 | ↑*T. trichiura*, ↑*A. caucasica* |
|  | S. pigmentatus | Host/Population | 0.531 | 0.219 | 2.431 | 0.015 | ↑*S. pigmentatus*, ↑*A. caucasica* |
|  | rainfall (cm) | Population | -0.047 | 0.015 | -3.030 | 0.002 | ↑rainfall, ↓*A. caucasica* |

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