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Stress and parasitism in translocated vervet monkeys (*Chlorocebus pygerythrus*) in Malawi

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Abstract

Wildlife translocations are a contentious practice that is on the rise. Wildlife rescue and release centers are one of the largest practitioners of translocation, but are often overlooked by the scientific community. In order to increase the success rates and efficiency of translocation projects it is necessary to highlight the reasons for project successes as well as project failures. This study aims to explore the relationship between two of the most commonly cited reasons for translocation failure, stress and illness, in a troop of translocated vervet monkeys (*Chlorocebus pygerythrus*) in Malawi. To do this, level of stress was determined through behavioral observations while binomial infection status and infection densities of parasitic helminth groups was determined through collection of fecal samples and a variety of diagnostic techniques. These translocated individuals showed higher helminth eggs per gram (EPG) than their wild counterparts. Individuals that displayed more stress related behavior had a higher chance of being positive for helminth infection, strongly suggesting that immunological impact of chronic stress incurred from being held in captivity. Juveniles tended to be more stressed than adults even though they tended to have lower EPG's. This discrepancy may be explained by the fact that juveniles alter social interaction with infected individuals, possibly to reduce the chances of being infected themselves. These results show that there is a significant relationship between stress and helminth infection. This conclusion has wide-ranging management implication both in the translocation field as well as more general wildlife management.

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Introduction

Conservation and wildlife management are diverse fields that span multiple disciplines and have nearly endless applicable management techniques. One such technique that is both highly contested as well as widely practiced is translocation. In a world where habitat is being reduced, populations are being fragmented and humans are increasingly globally mobile, translocation events are going to become more frequent (Germaino & Clulow, 2015; Streicker et al., 2013). There are on average 700 recorded intentional translocation events every year encompassing both conservation and sport stocking purposes (Griffith et al., 1989, Kock et al., 2010). There is also a third, highly prevalent translocation field known as mitigation driven translocation that are not included in annual counts. These projects occur frequently and are relatively undocumented. Mitigation driven translocations are explicitly performed to reduce the impact on wildlife due to human activities and are predominantly performed by wildlife rescue and release centers (Germano & Clulow, 2015). However, despite the high number of translocation events, there is still relatively low success rate of these projects (Griffith et al., 1989; Guy et al., 2015). The lack of a universal definition of what a successful mitigation translocation means, as well as the diverse species, ecosystems and project procedures, are all reasons why it can be difficult to uncover what factors can increase translocation success.

Alternatively, reasons for translocation failures can be difficult to identify, but they can primarily be attributed to lack of appropriate monitoring as well as failure to publish unsuccessful projects procedures and outcomes (Griffith et al., 1989; Fischer & Lendenmayer, 2000). Where reason for translocation failure has been recorded, disease and stress are two of the most common factors (Kock et al., 2010, Teixeira et al., 2007). Minimal progress in reducing these negative impacts is primarily due to a lack of knowledge of naturally occurring wildlife pathogens within the release ecosystem, improper behavioral compatibility and acclimatization at rescue centers leading to stress, and lack of monitoring procedures (Leighton, 2002).

While no translocation events are simple, mitigation translocations are particularly complicated due to the fact that the animals typically spend a substantial amount of time in captivity. This means that the rehabilitation facilities that undertake the release of wild animals must adhere to strict pre-release guidelines in order to reduce disease spread, assure behavioral adaptation and generally reduce the significant stress associated with reintroduction and thus

increase success of the program and of conservation goals (Guy, 2014; Teixeria et al., 2007). This is complicated and the process is highly species specific, especially in highly social and intelligent animals like primates. The IUCN Best Practices Guidelines for Re-Introduction of Great Apes (2013) notes that it is not only sufficient to know the ecological requirements for any given species, but also what behavioral benchmarks should be looked for and encouraged in order to increase the probability of success in any primate rehabilitation and reintroduction. These behaviors and guidelines include construction of a natural social networks, conspecific socialization, predator avoidance, foraging on natural food, and acclimatization to release site (Fischer & Lindenmayer, 2000; Seddon et al., 2007). In a study conducted by Guy et al., (2013) that analyzed 28 different primate rehabilitation and release programs in South Africa, only 53% operated within the IUCN guidelines, with only 29% utilizing a quarantine period and only 43% had any post release monitoring. Survivorship is notoriously difficult to document, not least because of the lack of monitoring, but also due to missing animals. One translocation event involved eight different troops of vervet monkey's in South Africa and at the end of a 12 month monitoring period only 21% of all released individuals could be accounted for (Guy et al., 2014). While it is difficult to attribute these low survivorship numbers on any single procedural failing due to low sample sizes, programs that utilize enrichment aimed at developing behavioral flexibility particularly in foraging, predator avoidance, and conspecific socialization, tend to have higher survivorship rates (Reading et al., 2013).

Quarantines are a critical component to reintroduction programs in order to reduce introduction of novel pathogens into both the captive environment as well as the release site environment (Griffith et al., 1989; IUCN, 2013). Recently the advent of more accurate diagnostic tools, veterinary practices and the acknowledgement of the importance of quarantine periods have drastically reduced the spill-over of pathogens from translocated individuals into novel ecosystems as long as the appropriate precautions are taken (Sainsbury et al., 2012; Leighton, 2002). The more pressing concern now is naturally occurring pathogens and parasites within the release site that can infect the released individuals. Even if the species of the release animals are endemic to the area, the released individuals may be at higher risk of suffering pathogenicity from novel pathogens or from incurring higher infection densities than naturally occurring populations due to the immunological impacts of stress (Sainsbury et al., 2012; Bordes and Morand, 2008; Tiexeria et al., 2006, Kock et al., 2010). In order to address the risk of novel

infections it is critical to know what parasites and pathogens naturally occur at the release site and in the natural population.

There are many different kinds of pathogens that infect wildlife and these pathogens are typically separated into mircoparasites (found within the blood stream or cells of hosts) and macroparasites (found within gastrointestinal tract [GIT] or externally on hosts). Helminths are a group of macroparasites that consist of varied species across many genus' but that all exist in their reproductive stages within the GIT of primary host species. Three of the most ubiquitous groups of helminths found in non-human African primates are *strongyloids* (threadworm), *trichuris* (whipworm), *enterobius* (pinworm) and hookworms (Ghai et al., 2015; Petrasova et al., 2010; Munene et al., 1998, Wren et al., 2016). Species from these groups are found in nearly all environments globally, however specific species may differ between ecosystems and hosts, as well as pathogenicity within and between hosts (Bordes and Morand, 2008). Transmission of helminths occurs from ingestion of infectious stages that have been expressed in infected individual's feces and can contaminate water sources, soil or food. Some helminths are specialists, but many are generalists that can infect multiple host species and new research suggests that helminths exhibit more host plasticity than previously believed (Fenton et al., 2015). Within host populations, helminth show aggregate population dynamics, where not all individuals within the host population will be infected while some individuals will have moderately high infection densities due to a variety of factors such as social status or immunological variation (Jolles et al., 2008). Pathogenicity of helminth infection is relatively low and mortality due to infection is low. However, helminth infection in isolation is rare in natural environments. When co-infection with other pathogens occurs or a host is naïve or immunologically suppressed, pathogenicity and mortality have been shown to increase (Dazsak et al., 2000; Jolles et al., 2008; Telfer et al., 2010).

There is also strong laboratory evidence showing there are host immunological trade-offs between T-helper 1 and T-helper 2 systems which impacts the effect of helminth infections; making hosts potentially susceptible to infection from other, more virulent, pathogens (Pedersen and Fenton, 2007). These immunological effects are also impacted by increased or prolonged periods of stress; leading to reduced immune system response to pathogens and increasing individual susceptibility to infection and suffering higher pathogenicity (Teixeira et al., 2006).

Despite helminths being one of the better studied parasitic infections, there is still gaps in information. Particularly in relation to immunological and co-infection effects, as well as surveys of helminth types, epidemiology, and population dynamics over time and space within natural environments and wildlife hosts.

In order to further increase the success of translocations and reduce the risk of spreading pathogens, there needs to be further studies done on the endemic parasites within host populations. Only 24% of all animal species have any information regarding their corresponding pathogens populations (Pedersen & Fenton, 2007). While census of naturally occurring pathogens is critical, it is also important to understand how multi-host systems impact pathogen persistence and densities in ecosystems by identifying host species that may act as pathogen amplifiers (Keesing et al., 2013). Within the realm of translocations, one such cause for amplification of a pathogen within any given host population may be chronic stress caused by captivity (Teixeria et al., 2006). In order to increase success of mitigation translocations there needs to be further knowledge of parasites that naturally infect wildlife, particularly parasites associated with the species being released, as well as rehabilitation procedures aimed at reducing spread of disease and reduction of stress (Molony et al., 2006; Teixeria et al., 2006; IUCN, 2013). Mitigation translocations that involve wildlife rescue and release centers offer a unique opportunity to study not only naturally occurring pathogens in wildlife hosts, but also the relationship between stress and pathogen infection within and between wildlife host populations (Geramano & Clulow, 2015).

This study will allow for the improvement of practices at wildlife rescue and release centers as well as setting a baseline for a growing body of data being acquired in order to further study the naturally occurring helminth infections of non-human primates. There is also a need to create a census of the endemic helminth genus' known to infect vervet monkeys within the study regions in order to document potential regional variation in infection as well as to aid in future studies. The purpose of this study is to explore the relationship between stress related behaviors and biological factors influencing helminth infection in a translocated troop of vervet monkeys. In order to do this, the following objectives will be met: 1) what factors influence the frequency of observed stress related behaviors, 2) what factors influence eggs per gram (EPG), 3) if helminth groups observed vary by region, 4) if frequency of stress related behavior differs

between pre and post-release, and finally 5) if there is a relationship between stress and binomial infection status. This project fulfills the IUCN best practices goals for the monitoring section of translocation of non-human primates, while fulfilling the release guidelines set by Lilongwe Wildlife Center (LWC).

Methods and Materials

Study Site and Species

Kasungu National Park was founded in 1970, is located in the central region of Malawi at 13°0′S 33°10′E and is the second largest national park in the country with an area of 2,316 km². The park is surrounded by moderately dense, populated farm land; with communities surrounding the park relying on agriculture, bee keeping and caterpillar collecting as primary sources of subsistence (Mkanda and Munthali, 1994). The primary ecosystem type within the park is Miombo woodland, consisting predominantly of tree genus *Brachystegia, Julbernardia* and *Isoberlina*, with varying degrees of brush and grassland as an understory and along river beds (White et al., 1983). The wildlife within the park is relatively diverse, if not as dense as other East African national parks, and there has been a reduction in high value species due to poaching in recent years (Munthali and Mkanda 2002). The park is known for its population of elephants (*Loxodonta africana*), as well as the presence of sable antelope (*Hippotragus niger*), African wild dogs (*Lycaon pictus*) and leopards (*Panthera pardus*). There are three annual seasons defined by temperature and rainfall; a hot wet season from December to April (average 900 mm of rainfall and average temperature of 31 degrees Celsius), a cool dry season from May to August (virtually no rainfall and an average temperature of 22 degrees Celsius) and a hot dry season from September to November (virtually no rainfall and an average temperature of 31 degrees Celsius)..

Vervet monkeys are native to the park as well as throughout eastern and southern Africa. They live in complex social troops that often show altruistic grooming behavior, with females making up the primary family unit and males typically migrating away to breed (Seyfarth & Cheney, 1984). It has also been shown that there is a strong relationship between social status of individual vervet monkeys and illness driven mortality; with lower ranking individuals, more susceptible to serious consequences of illness particularly in resources limited areas or seasons (Cheney et al., 1981). Vervet monkeys have developed complex predator specific alarm calls due

the fact that they are an important food source for many animals such as snakes, predatory mammals, baboons (*Papio cynocephalus*), and birds of prey (Seyfarth et al., 1980). Vervet monkeys subsist on a wide range of food sources that varies with each troops habitat, but can consist of leaves, buds, insects, and fruit (Teichroeb et al., 2015). Troop territories range considerably in size, and are determined by access to year-round water, food, competing troops and presence of predators. As a species, they are also considered semi-terrestrial, and spend on average 19% of their time on the ground foraging, which may increase individual's exposure to certain soil transmitted helminths (McFarland et al., 2014).

Study troop

In March of 2016 LWC translocated a troop of 20 vervet monkeys that was formed and resided at the center for no less than one year. The troop was formed at LWC over the course of multiple years, with the last individual being integrated into the troop over a year pre-release. At the beginning of March, 2016 the study troop was moved from the captive center at LWC to a transitional enclosure within the park at the release site. The make-up of the study troop at the time of release was, four adult males, eight adult females, one sub adult male, and seven juveniles (two of whom were born at the center). Once released, two adult males went missing within two weeks, assumed to have emigrated, and were excluded from the study. There were also three wild adult males that joined the group throughout the monitoring period.

Individuals came to LWC for a variety of reasons, but many were victims of illegal wildlife trade. Upon entering the center each individual went through the IUCN recommended quarantine period of 30+ days and received any required veterinary care including treatment for any injuries or infections. Individuals were integrated into the troop one by one under close observation of center staff and were chosen for integration based on natural troop demographics and appropriate behavioral characteristics. Once the troop was complete, human interaction was strictly limited, and antipredator training occurred. One week before transport to the release site, all individuals within the troop were treated once again for parasitic infections. This is to assure that no foreign infections were introduced to the national park as well as to assure that all helminth infections observed throughout the study are endemic to the Kasungu National Park ecosystem. Once the troop was deemed to have reached the behavioral benchmarks indicative of wild behavior, which were determined by the Primate Release Manager, the troop was sedated, 8

core members of the group fitted with VHF collars, while every member was fitted with unique colored ID ear tags, and the entire troop was transported to the release site. Core members in this case are the single dominant male, and seven dominant adult females.

The release site within Kasungu National Park was determined by the Primate Release Manager by assuring year-round water, food and lack of immediately competing wild vervet troops. The troop was kept in the temporary enclosure at the release site for a month, and the troop was released using soft release techniques (IUCN, 2013). Food was supplemented for the first 10 days post release and the temporary release enclosure was left open at night in case the troop returned. By end of study (November 15th, 2016) the troop's territory was 5.78 km^2, and crossed multiple wetlands and river beds, as well as crossed the Lingadzi road (Fig. 1) which was utilized to locate the troop every day.

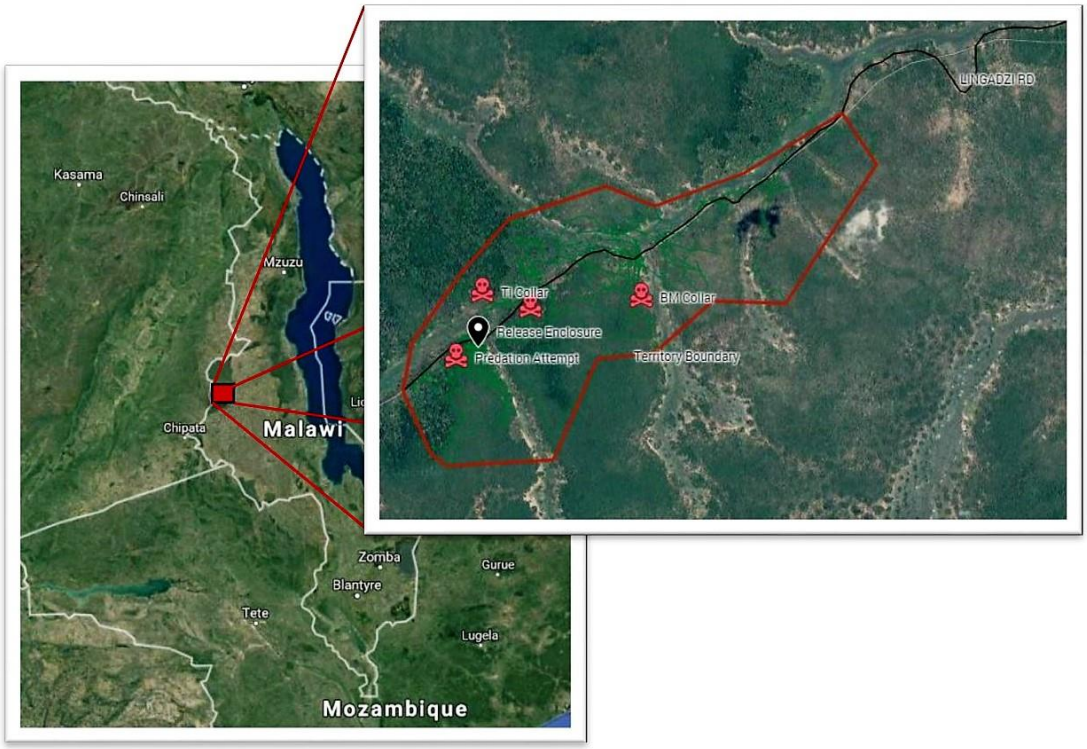

Figure 1: Map of studies troop territory within Kasungu National Park. Black line indicates the Lingadzi access road, black marker shows transitional release enclosure, and red markers indicate where individuals remains/collars were located after confirmed predation events. Map was created using hourly GPS markers acquired throughout all contact hours with the troop.

Data Collection

Behavioral Data

Monitoring and behavioral data commenced on March 1st, 2016; immediately after release. Each study day was broken up into four different study periods; 0600-0900 (M1), 0900-1200 (M2), 1200-1500 (A1), and 1500-1800 (A2). Every week, each individual in the troop was the focal animal in each study period, leading to 4 study periods per individual per week for 34 weeks. At the beginning of each study day the troop was tracked using the VHF signals from the core troop members. If the signals diverged, locating all the signals of the core members became priority. These core troop splits were due to predation events and all VHF collars were accounted for during the study period, either by finding skeletal remains of troop members, or by the single incident where live members were located within calling distance of the rest of the troop and were in the process of re-joining.

Once the troop was located, 10 min was spent taking a census of the troop, looking for injuries and allowing the troop to return to normal behavior after any disturbance possibly caused by researcher arrival. Throughout the study, no members of the troop were directly approached by researchers and researchers never got within 10m of any troop members. When the troop was moving, all efforts were made to not separate or get between troop members while still observing the focal individual. This was all in an attempt to reduce the impact of human presence on the troop's behavior and the only exception to these practices were when troop members passed around the researchers, or when researchers unwittingly approached troop members while attempting to follow the focal individual.

Focal periods of 20 min each started after the 15 min prior to locating the troop, and focal individuals were chosen either by necessity, if they had not been observed yet during the week or during the specific study period for the week, or when there was no priority, an initially readily visible and identifiable individual was chosen. All data collection during the study was collected using the behavioral data sheet utilized by LWC (Annex 1) which was developed based on Altmann et al., (1974). Individual ID, gender, approximate age (adult or juvenile), study period and weather were all recorded before the 20 min focal period started. The initial zero minute of the study period was spent determining the proximity of other individuals in the troop in relation to the focal individual, this proximity scan took place again at minute 10 and min 20 min of the

focal period. Starting on minute one, continuous behavior was recorded every minute during a 20 min focal period. Instantaneous data of social and stress behavior was also recorded and this was count data of every type of behavior, behavior codes can be seen in Annex 2. If stress or social behavior was observed on the minute, it was recorded in both the continuous and instantaneous sections of the data sheet.

Due to logistical constraints, not every individual was observed at all four study periods for all 34 weeks. Due to the time it took to habituate the wild joiners, these individuals had the lowest number of observation periods at 13, while the dominant male had the most observation periods at 64. There were repeated wildfires at the study site, and at certain times of year it was not possible to complete study period A2 due to it being unsafe to be in the field after sunset. Kasungu is a large park with limited staff which periodically made it difficult to go into the field due to a lack of a required scout. Study individuals were also altered throughout the course of the study due to the predation or emigration of individuals, as well as the fact that the wild joiners to the troop were only added to the behavioral collection process once they were habituated to the researchers presence.

Fecal Samples

Fecal samples from the study troop were collected opportunistically throughout the monitoring period. For samples where identifiable individuals were observed defecating, the sample was collected, and individual, age, sex, and date was recorded. Periodically samples were collected where individual ID was unknown. This typically occurred while the troop was resting, and samples were found under the resting tree's after the troop had moved on. In these instances, samples were also collected and date was noted, but these samples were only utilized to compare infection between Kasungu and Lilongwe sites, and not for the analysis of infection and behavior.

Three wild troops that live in the forest preserve surrounding LWC were sampled in order to collect the samples in Lilongwe. These troops are identifiable by dominant male, number of members, and while troop territories are distinct, there is some overlap and each troop would frequent the center to forage and sleep. These troops are quasi habituated as they are accustomed to human presence, but not habituated enough to collect behavioral data or to follow through the property throughout the day to identify fecal samples to age and gender. Due to these constraints,

fecal samples were collected under each troops sleeping tree. The sleeping trees were identified when the troops were observed around the center near dusk and were followed until they moved into the canopy and stopped traveling. The next morning at sunrise, if the troop was found in the same location, the ground was searched for any fresh fecal samples and were labeled with the troop ID (T1,T2,T3) and the date.

Diagnostics were performed on the fecal samples in collaboration with the vet nurses and head veterinarian of LWC both at the center and in a field lab at Lifupa camp site in Kasungu National Park. All samples were analyzed within three days of collection and any samples that had hardened were discarded while any samples that had been found have larvated in the direct smear were still analyzed, but results were not used in any statistical analysis. All samples underwent direct smear, NaCl table top floats, and centrifuge with NaCl solution to determine final diagnoses and eggs per gram (EPG) as based on the study by Ghai et al., (2015). One gram of fecal material from each sample was used for both the table top floatation and the centrifugation. Where less than two grams of feces was collected, only centrifugation was performed since it is a more precise diagnostic tool, and EPG results are more reliable than floatation alone. For all usable samples, infection type (parasite genus) was recorded for all diagnostic techniques and EPG was recorded for all individual parasite genus' as well as overall EPG.

Statistical Analysis

All statistics were completed using the statistical program R version 63.3.3.1. Initial descriptive statistics and initial visualization of data was done following the recommendations of Zuur et. al., 2009. A GLM model was used to determine which factors related to stress behavior. For the purpose of analysis the count data for observed stress related behavior was transformed into percentage chance of observing the behaviors for each study week. This was done by taking the number of observed stress behaviors from the instantaneous observations for each individual for all observation periods during the week and dividing it by the total possible instances of observations (4x20 for each focal period) and then multiplying by 100. This was done due to the fact that some individuals were not observed four times a week so raw count data would have biased more frequently sampled individuals. For the initial GLM model, percentage chance of observing stress behavior was the dependent variable, and the independent variables used were

age (juvenile or adult), sex (male or female), wild or released, and weeks post release (n=366). Interactions between age and sex were initially explored as well, but due to the age and sex make-up of vervet troops, the data was unbalanced and inclusion of these interactions was not possible so they were dropped. A GLM model was also used to explore whether Age (Adult vs. Juvenile) or Sex (Male vs. Female) influenced average EPG for each released individual where fecal samples were collected (n=13). Both final GLM models selected utilized negative binomial distributions and were run using the glm.nb command from the MASS package. Final model selection was done by dropping insignificant terms and each iteration being compared using a least likelihood ratio test (lrtest command) to assure terms were insignificant.

A paired t-test was performed in order to see if there was a significant difference in stress behavior in the study troop between pre and post-release. This was done by calculating the average number of stress related behaviors observations divided by total number of twenty-minute observation periods for both the pre-release monitoring period (n=14 weeks) and the entire post-release monitoring period (n=34 weeks). While the troop was monitored for longer than 14 weeks while at LWC, the data for the other weeks was lost and was not used. Only 14 individuals from the study troop were utilized for this analysis due to the loss of some pre-release data as well as the death or emigration of individuals very quickly post-release. Both averages were tested for normality using a Shapiro Wilkes test and the data were shown to be normally distributed so no data transformation was necessary.

In order to see if there was a significant difference in the EPG of wild and released individuals a Wilcoxon Test as well as a GLM with a negative binomial distribution was used. For the purpose of this analysis only fecal samples that could be attributed to identifiable individuals were used from the Kasungu National Park site in order to be able to assign wild or released status to the sample. While all samples were used from the Lilongwe site due to knowledge that all these individuals were wild, even if age and gender were not known. From the Kasungu site, 51 usable samples were collected, while 50 were collected from the three troops in Lilongwe, for a total sample size of 101. EPG was the dependent variable in both tests, and wild or released was the independent variable. To see if location had an effect on the type of infections observed, a Fischer's Exact Test was used. The number of samples that tested positive for s a genera of parasite at each location was recorded (Table 1). Some individuals were

infected with more than one parasite genera, and in these instances, all identified parasites were recorded and added to the total count for each parasite group. The total number of parasite infections observed was 118, with 101 samples and 4 genera of parasites identified.

In order to explore what variables influence parasitic infection a GLMM with a binomial distribution was used using the glmer command from the lme4 package. The dependent variable was a binomial infection status (infected vs. not infected), with any genera of parasite. EPG was also considered as the dependent variable, but the binomial infection status model had lower dispersion and higher significance. The independent variables in this test were age, sex, wild or released, and average number of stress behavior observations per week. Individual ID was used as the random variable due to individuals being sampled multiple times throughout the study. In order to assure that the stress behavior observations could be attributed to infection status, only observations of the individual that fell three days before and after the fecal sample was collected were used to obtain the average number of stress behavior observations per focal period (Ghai et al., 2015). Only fecal samples that could be attributed to stress related behavior (i.e. yawning, scratching and self-grooming) were used for this analysis. Behavior data from the first two weeks after release was used for negative infection for the released individuals. This was possible because week post release showed to have no impact on stress behavior and all individuals were released without any parasitic infection. Total number of fecal samples and corresponding stress related behavior was 39. Final model selection was done by eliminating insignificant variables and confirming these eliminations using an ANOVA test.

Results

Over the course of the monitoring period of 34 weeks, 766 total observation periods occurred and three helminth types and one protozoa were observed (Table 1). Over the course of the monitoring period, our troop experienced a 52% loss of members, 4 of whom were collard individuals and who's remains were found along with their corresponding VHF collar. Other troop members who went missing are either assumed emigrated or predated. There were three births during the study period, but each infant was a victim of a predation event, and in two out of the three of these predation events the mothers were also killed. All of the known predated individuals, those where VHF collars were found, were predated by leopards.

Age

Age did have an impact on stress (GLM test, $p < 0.05$, n=248), with juveniles being more stressed than the adults of the study troop (Fig. 2). EPG also varied by age within the study troop with adults on average suffered significantly higher EPG's than did juveniles (GLM test, $p< 0.01$, n=12) (Fig.3). Stress didn't differ between sex's, nor did it change over the 34 week study period, so these terms were dropped from the final model.

Figure 2: Stress behavior varies by Age in the study troop with adults (A, n=248) showing lower levels of stress than juveniles (J, n=115). GLM test, P-value= 0.047, df=362.

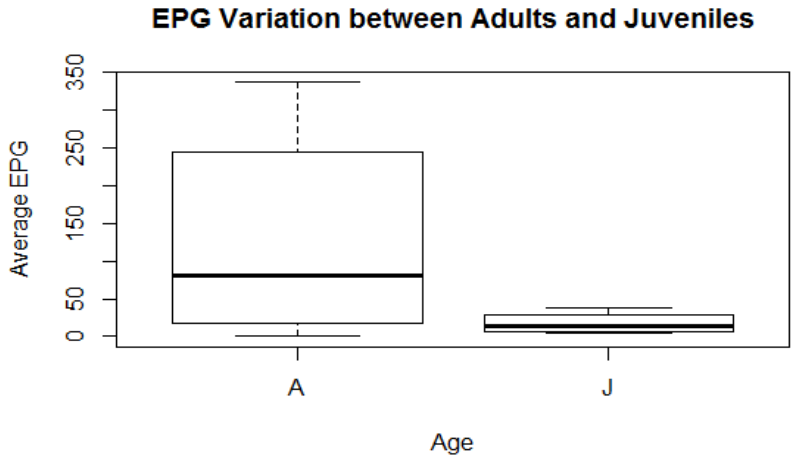

Figure 3: Adults (A, n=8) show higher EPGs than juveniles (J, n=4). GLM test, P-value=0.006, df=10.

Regional and Translocation Status Variation

Parasitic GTI groups observed varied between the two sites significantly, both in types of parasites found as well as total number of samples fond to be positive for infection (Fisher ExacTest, $P < 0.001$, $df = 3$; Table 1). Within study troop in Kasungu National Park, released individuals had higher EPGs than the wild joiners (GLM test, $P < 0.001$, Fig. 4). Wild members of the study troop had lower EPG than their released counterparts (Wilcoxon Rank Sum Test, $P < 0.05$).

Table 1: Percentage of samples from each sub-group of the study troop found positive for the various parasite groups and percentages from each sub-group found to be positive for any parasitic infection.

	Sex		Age		Release Status		Site	
Parasite Type	Male (n=17)	Female (n=25)	Adult (n=31)	Juvenile (n=10)	Wild (n=64)	Released (n=36)	Kasungu (n=51)	Lilongwe (n=50)
Hookworm	41%	32%	32%	50%	3%	36%	29%	0
Whipworm *(Trichurus)*	59%	76%	68%	60%	77%	75%	62%	92%
Strongyloids	6%	0	3%	0	25%	3%	2%	32%
Coccidia	0	0	0	0	11%	0	0	16%
Infection Positive	65%	88%	74%	90%	84%	86%	74%	96%

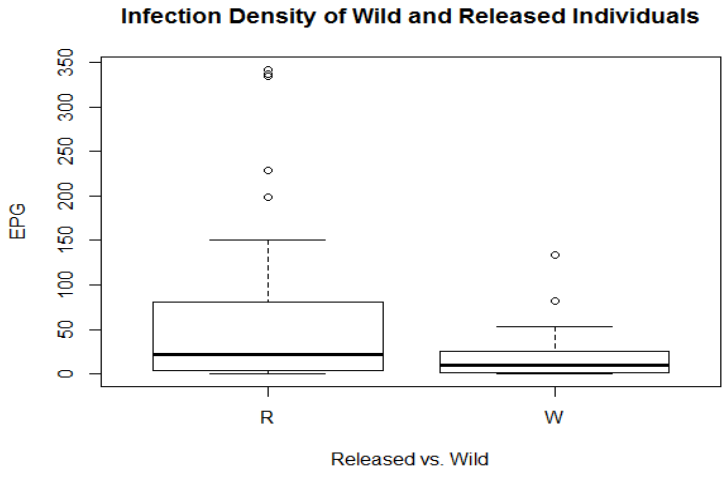

Figure 4: Released individuals suffered higher infection densities (EPG) than their wild counterparts found at both sites (GLM test, P-value<0.001, df=99).

Stress and Helminth Infection

Proportionate stress related behaviors decreased between pre and post release at the 95% confidence interval (t-value=5.24, P <0.001). Every member in the study troop showed decrease frequency of stress related behavior in the post release study period, ranging from 17-70% overall decrease (Fig.5)

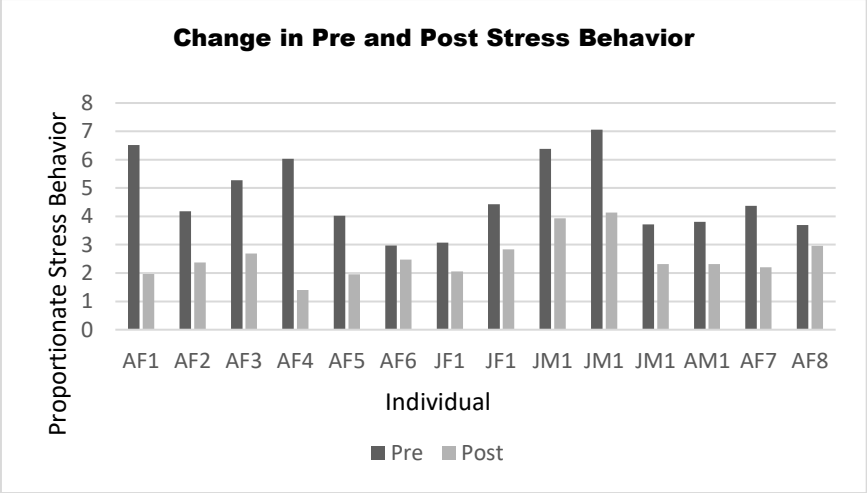

Figure 5: Change in proportionate stress behavior pre and post release for individuals in study troop. First letter in 'Individual' indicates Age (A=Adult, J=Juvenile), second letter indicates Sex (F=female, M=male), number indicates unique individuals of above combinations. All members of troop showed a significant drop in stress post-release Paired t-test, t-value=5.24, P-value <0.001.

The final model selected had individual identity as a random intercept, binary infection status as the dependent variable and average number of stress behavior observation per week as the only significant independent variable. Individuals who were observed performing stress related behavior more frequently were more likely to be positive for helminth infections (GLMM test, $P < 0.05$, Fig. 6).

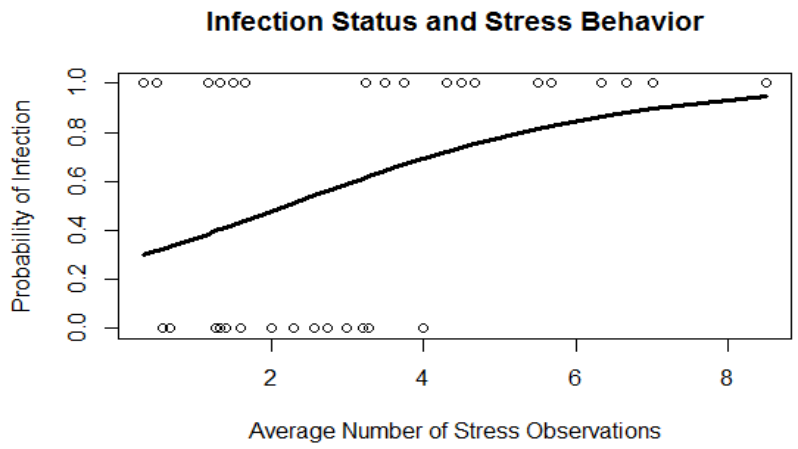

Figure 6: Results from GLMM test showing that as number of stress observation increases, so does the probability of that individual being positive for GTI helminth infection, P-value < 0.05, df= 35.

Discussion

Both stress and EPG vary by Age in the study troop, with juveniles displaying more stress related behaviors while adults suffer higher EPG. Helminth group type varied between the Lilongwe and Kasungu site and translocated individuals had higher EPG's than their wild counterparts. This may possibly be due to the chronic stress of prolonged captivity, which was shown by the significant drop in stress related behaviors once the study troop was released. There was also a positive relationship between infection status and stress related behaviors, with individuals who were more stressed being more likely to be positive for helminth infection. These results support the hypothesis that there is a significant relationship between stress and helminth infection in translocated vervet monkeys. Vervet monkeys are one of the most extensively studied primate species; however, it hasn't been until recent years that there has been any exploration into the parasite communities that coexist with vervet monkey populations (Wren et al., 2015). There is also a lack of information on how parasitism and the relationship between stress and infection may impact the success of translocation attempts (Sainsury et al., 2012).

Helminth Groups Observed and Regional Variation

Three helminth groups and one protozoa were identified between the two study sites. When compared to similar studies of vervet monkey parasites populations, the aggregate percentages of positive samples and the parasite types observed in this study are similar; any

variation may be attributed to naturally occurring variance in parasite populations between climates and regions (Wren et al., 2015). While aggregate percentages of positive fecal samples and helminth genus' found are similar to other studies, it is important to note the difference between Lilongwe and Kasungu National Park. There was no observed presence of infection of hookworm at the Lilongwe site, while there was no presence of *coccidia* and very limited presence of *strongyloids* in Kasungu National Park. There is a significant difference between helminth genus' found between the two study sites which supports the use of quarantine periods and vetting in order to reduce the risk of pathogen spread during translocations even within the same country (Sainsbury et al., 2012; IUCN 2013). One possible explanation of this variation is the fact that one site was urban and one site was within a protected National Park. Human activity and potential for pathogen spill-back to wildlife populations is possible and a is a risk in translocations where wildlife is being housed in close contact to humans or domesticated animals (Kock et al., 2010). The presence of *coccidia* in this study is one such example. This pathogen, while not a helminth, is a GIT parasite that is commonly found in pigs and frequently infects humans and other domesticated mammals (Lindsay et al., 1997). While it is possible that the variation in the presence of multi-host pathogens may be explainable by environmental factors instead of anthropogenic contact, it is still an area of management concern and efforts should be made to keep pathogens that are anthropogenic in origin from being introduced to novel environments (Gaetano et al., 2014).

Stress and Helminth Infection

Helminths can be generalists or specialists, but have typically co-evolved with their available host populations to the point where hosts have developed tolerance to many types of helminth infections (Boots et al., 2009). However, recent studies on various non-human primate species has shown that GIT helminth infections vary in their effect on individuals within the population and that helminth infections do cause behavioral variations. A study done on red colobus monkeys (*Procolobus rufomitratus tephrosceles*) in Uganda showed that individuals who were positive for whipworm (*trichurus*) showed significant variation in sickness behaviors which show hosts are clinically sensitive to whipworm infection (Ghai et al., 2015). While sickness behaviors in this case were labeled as behavioral adaptations to helminth infection, this study shows that there is also a relationship between stress related behaviors and GTI helminth

infection. The one exception to this result was that juveniles in the study troop were found to display more stress related behavior, but had lower infection densities than the adults in the troop. This could possibly be explained by the way vervet monkey social networks change in response to helminth infection. Chapman et al., (2016) found that juvenile vervet monkeys tended to avoid social contact with infected individuals when compared to uninfected individuals; possibly leading to reduced transmission rates of helminth infections. While this is one possible explanation, further study that includes de-worming procedures and a larger or more balanced study population would be needed to elucidate the relationship between stress, age and helminth infection.

It has been shown that higher stress correlate to higher helminth EPG, however it is difficult to say if stress causes increased chance of infection, or if infection causes increased stress (Tiexeria et al., 2006; Cizauskas et al., 2015). However, based on previous studies it is possible to infer a causative relationship. It is important to highlight the difference between acute stress, such as a predation event, and chronic stress, such as continued environmental instability (i.e. being held in captivity). Acute stress and its behavioral reaction, such as expending energy associated with fleeing a predator, is a behavioral adaptation in reaction to an immediate stressor; while chronic stress's effects are typically subclinical, additive and lead to negative impacts on host behavior or other biological functioning's, such as lowered immune response (Mendl 1999; Tiexeria et al., 2006). The study troop had been held in captivity for an extensive period and were shown to have much higher instances of stress behavior pre-release when compared to post-release. The immunological impacts of chronic stress is one possible explanation of why released individuals in the study troop showed much higher EPG's than wild individuals. A larger sample size for both translocated and wild individuals from the same site is needed to further explore this possibility.

The natural make up of vervet monkey troops led to unbalanced data in this study and a larger sample size would be needed to further explore the effect age and sex have on stress and parasitism. There is also the possibility that social rank within the troop alters both infection status as well as stress behaviors, which was not included in this study (Foerster et al., 2015). Individuals who are of lower rank will typically experience more group harassment and have less opportunity to acquire high value resources, both of which could lead to lower fitness and higher

instances of stress and helminth infection (Teichroeb et al., 2015). It would be useful to utilize anti-helminthic drugs and fecal cortical steroids as a measure of stress levels would also be beneficial in future studies (Pederson & Fenton, 2015; Macintosh et al., 2012).

Management Implications

Translocations is a contentious conservation tool at best, and a problematic and detrimental one at worst. There is little scientific documentation of failed translocations which has limited the adaptive management and improvement of the entire field. Many translocations occur without any definition of what constitutes a success, or with any effort to improve practices to increase success rates and thus efficiency and legitimacy of translocations as a conservation tool. In this study I found that released individuals were significantly more stressed for a long period of time in captivity compared to once they were released into Kasungu National Park. These same individuals were found to be suffering much higher infection densities than their wild counterparts. With the already established knowledge that chronic stress can lower immune response, it is possible that the chronic stress from captivity led to a suppressed immune system thus making them more susceptible to GIT helminth infections. While further study is necessary to support this conclusion, it is worth addressing the possibility from a management standpoint. If chronic stress is leading to higher infection densities of naturally occurring parasitic infections, then translocated individuals may be acting as amplifiers of these pathogens and increasing their overall presence within the ecosystem (Streiker et al., 2013). While a lot of time, attention and resources goes into avoiding introduction of novel pathogens into new environments, quarantine periods and intensive vetting, little is known about how translocated individuals may alter the endemic host-pathogen interplay in the environments in which they are released. This potential impact of translocations may require managers and wildlife rescue and release centers to re-evaluate release criteria to focus more on the chronic stress impacts of long term captivity of individuals bound for release in order to reduce the potential for pathogen amplification at the release cite.

There are also acute impacts of this increased parasitism on translocated individuals. Helminth infection has been shown to alter social networks in vervet monkeys, which can impact possible predation, access to resources and overall fitness of individuals (Chapman et al., 2016). Parasitism has also been shown to cause energy costs and behavioral changes in the form of time

allocations in other primate species (Ghai et al., 2015). There is even evidence to suggest that in parasitism and the subsequent energetic and behavioral impacts on hosts have the potential to increase predation rates in certain species (Murray et al., 1997). Many of the documented failed vervet monkey translocations, limited as they are, cite predation as a major cause of failure (Guy et al., 2014). Even within this study troop, individuals suffered high levels of predation (52% of released individuals having gone missing since release, not including births occurring post-release). This is even with efforts made by WLC to train individuals within the study troop to identify predators, which in previous studies has been found to reduce predation post-release (Guy et al., 2014). It would be worth exploring in future studies the possibility that parasitism may be impacting translocation survivorship through energy costs and behavioral changes leading to increased predation; and that stress caused by the prolonged time spent in captivity may be effecting these outcomes.

Chronic stress in wildlife is not just caused by captivity, but can also be caused by prolonged exposure to multiple stressors (Tiexeria et al., 2006). Many of these stressors come in the form of environmental change that can impact not only released individuals of any one species, but all individuals of almost all species (Pedersen et al., 2007; Streicker et al., 2013). Environmental stressors can be natural or anthropogenic in nature and can impact not only host species immunity in the form of chronic stress but can also change the host-pathogen relationship (Chapman et al., 2005). Land use change is possibly the most ubiquitous of the causes of chronic stress that can potentially impact host species as well as pathogens (McKenzie & Townsend, 2007). Land use change can alter water and food availability, amount of cover to hide from predators, and exposure to human presence; all of which over a prolonged period of time can cause chronic stress and lowered immune response at a species population level. Not only can these stressors lead to increased parasitism within stressed hosts, but the increased interaction with human activity can lead to spill-back and spill-over of communicable parasitic infections. This has the potential to put wildlife species, such as vervet monkeys, that live in human impacted areas to be susceptible to not only higher rates of infection due to stress, and thus higher fitness costs, but also novel pathogens that are anthropogenic in origin. This possibility of spill-back of parasites from human or domesticated animal populations is one possible explanation for the difference in the parasitic infections found in the Lilongwe troops and the Kasungu National Park troops in this study. It is not within the scope of this study to flesh out the

complicated relationship between the multitude impacts of anthropogenic environmental change on the host-pathogen relationship; however, these issues are further explored in Chapman et al., (2005); Pedersen et al., (2007); Patz et al., (2004) and de Castro and Bolker, (2005). Understanding the source of chronic stress and how this stress may impact the role of parasitism in wildlife populations is an important management concern and should be studied further.

Conclusion

Translocations are going to continue to increase in frequency and it is critical to implement standardization in practices; from defining success, pre-release procedures, and monitoring (Woodford and Rossiter, 2003). In order to do this translocation managers, such as wildlife rescue and release centers, must be willing to publish findings from both failed and successful translocations that highlight practices that effect success rates. This study attempts to aid in this body of research. Based on the findings of this study there is a clear relationship between GIT helminth infections and stress in translocated individuals of the study troop. Individuals who were chronically stressed in captivity showed much higher infection densities once released compared to their wild, non-chronically stressed counterparts. Not only that but individuals that displayed more stress behaviors once released were also found to have a higher chance of being positive for infection. These results show that wildlife managers and wildlife centers who are performing translocations must be careful when releasing wildlife that may be suffering from chronic stress. While quarantine and other vetting procedures are in place to reduce the spread of pathogens into novel environments, the release of stressed individuals may be leading to an amplification of endemic pathogens and a change of the natural host-pathogen relationship at the release site. The impact of chronic stress on the pathogen host relationship extends beyond translocation science. As more environmental changes are occurring that are leading to chronic stress in wildlife, it is possible that once benign endemic pathogens will alter their epidemiology leading to increased pathogenicity and disease outbreaks. Uncovering the way chronic stress and pathogen infection interact is not only necessary to increase translocation efficiency and success, but is also applicable to wildlife and disease management. It is necessary to fill in the gaps of our knowledge regarding stress and infections in order to increase the effectiveness of management strategies.

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Annexes

Annex 1: Behavioral Data Sheet Provided by LWC

Start Time:	Individual ID:				Weather:			Sex:		Age:		GPS:								
	Instantaneous Recordings																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
Behavior																				
Association																				
Food Type																				
Plant Species																				
Position in Canopy																				
	Continuous Recordings (social, feeding, locomotion, resting, vigilance, predator avoidance, aggression, dominance, stress)																			
	0→1				1→2			2→3				3→4					4→5			
Behavior																				
Association																				
Outcome/Conflict																				
	5→6				6→7			7→8				8→9					9→10			
Behavior																				
Association																				
Outcome/Conflict																				
	10→11				11→12			12→13				13→14					14→15			
Behavior																				
Association																				
Outcome/Conflict																				
	15→16				16→17			17→18				18→19					19→20			
Behavior																				
Association																				
Outcome/Conflict																				

Annex 2: Behavioral Codes Provided Used in Data Collection, Provided by LWC

	CODE	BEHAVIOUR DESCRIPTION	Cat.
1	G-	Grooming – Taking care of the fur of another individual, by pushing aside its fur and inspecting for foreign objects (dirt/insects). Also includes taking care of another animals' teeth or skin	Social
2	G+	Getting groomed – The focal animal is groomed (as described above) by another individual	
3	PR-	Presenting – Presenting itself (either the body or hind quarters) to another primate. Inviting them for social contact, such as grooming or mounting	
4	PR+	Being presented – Being presented to by another individual	
5	C	Contact - Individuals touching in a non-aggressive way, such as nosing or cuddling, but grooming or playing	
6	CL	Clinging – Clinging to another individual while being carried, specifically for infants	
7	N	Nursing young – Mother breast feeding an infant	
8	SU	Suckling – Feeding from the mother, specifically for infants/juveniles	
9	PL	Playing – All types of interactions between two or more animals possibly using the relaxed open mouth play face (mouth is half or wide open, teeth are covered by lips) Interactions such as touch, pull, push, hit, chase, bite	
10	MA	Mating – A male mounting a female, or a female is mounted by a male, with actual penetration. Sometimes accompanied by a copulation call	
11	MO	Mounting – The focal animal mounts another individual or is mounted by another individual., Either male/female without penetration, male/male or female/female	
12	FE	Feeding – The actual act of eating, food is touching the lips or is in the mouth in combination with chewing	Feed
13	FO	Foraging – Looking for food to eat. Includes turning rocks or other objects upside down and pushing away objects on the floor/sand.	
14	L	Locomotion - Any movement to get from one place to another, such as walking running and jumping. In any direction possible on the ground, in the trees or on buildings.	Other
15	R	Resting – Sitting or laying down without any activity, and low levels of awareness of the environment. The eyes may be open or closed, but generally the head is down.	
16	V	Vigilance - Any level of observation of their environment. This includes sitting in a tree or on the ground, with head up eyes open looking. Not only for extreme vigilance	
17	PA	Predator Avoidance – Any form of predator avoidance behaviour, this includes alarm cal or responding to alarms calls and hiding into the trees or running away	
18	A+	Aggression - Physical aggression with a (potentially) damaging action, including biting, slapping, grabbing and hair pulling. Sometimes occurs with mouth-open and teeth exposed	Dominanc e
19	A-	Receive aggression - Receiving physical aggression	

20	**TH+**	Threat: Non-physical aggression towards another individual, such as chasing. Potentially accompanied by vocalizations. Also includes threatening other individuals with staring, raising eyebrows, head-bobbing (short movements with head and/or shoulders) towards other animals whilst staring, lunging, and display behaviours (shaking trees, bushes or other objects)	
21	**TH-**	Receive threat – Receiving threat as described above	
22	**MP+**	Making place - Another animal moves away when the focal animal approaches.	
23	**MP-**	Making place - Focal animal moves away when other animal approaches.	
24	**SC**	Scratching - A single scratch or repetitive movement of scratching the body with hand or feet	**Stress**
25	**SG**	Self-Grooming - The focal animal grooms itself by pushing aside its fur and inspecting for foreign objects (dirt/insects). Includes taking care of its own skin, teeth and fur and masturbating.	
26	**YA**	Yawning - The focal animal yawns, opening mouth	

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