Impact of snare injuries on parasite prevalence in wild chimpanzees (*Pan troglodytes*)

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Abstract

Many primate populations are severely threatened by human activity. Illegal hunting with snares frequently causes fatal injuries and permanent mutilations in wild primates. Traumatic injuries and stressful experiences can reduce the efficacy of the immune system to fight parasitic infections. Snare-related changes in primate behaviour may also influence the probability of exposure to parasites. We hypothesized that primates with permanent snare-related injuries would have higher prevalence of intestinal parasites than control individuals. We tested the relationship between snare injuries and the prevalence of intestinal parasites in wild chimpanzees (*Pan troglodytes*) of Budongo forest, Uganda. We collected 487 faecal samples from known individuals (70 control and 20 snare-injured chimpanzees) and used flotation and sedimentation to isolate helminth eggs and an immune-chromatographic assay to identify protozoan cysts. We found that the prevalence of Strongylida nematodes was significantly higher in snare-injured chimpanzees than control individuals. In contrast, we found no association between snare injuries and three other parasite taxa: *Ascaris*, cestode and *Cryptosporidium parvum*. Our study suggests that snare-injured primates may have higher exposure and/or be more susceptible to developing infections with helminth parasites than control individuals. Future studies should investigate whether snare injuries influence parasite prevalence in other species of wild primates.

Keywords

Conservation, helminths, intestinal parasites, *Pan troglodytes*, protozoa, snare injury

Introduction

Illegal snare setting is common in many parts of subtropical Africa, especially in areas where the local population relies on wild game for their livelihood (National Environment Management Authority, 2010; Tumusiime et al., 2010). In tropical forests in West and Central Africa, more than 70 mammal species are hunted (Fa et al., 2005). Poachers usually target animal species indiscriminately, and terrestrial primates are frequent victims, often with fatal consequences. The use of snares is the preferred technique for many rural population because it is easy to set them in a short period of time (Tumusiime et al., 2010).

When caught in a snare, animals usually react by trying to pull the snare from its substrate, which causes the wire to tighten and to cut deeper into the flesh (Noss, 1998). Although larger primates can separate the snare from the substrate, the wire often remains tightly wrapped around the affected body part, causing obstruction in blood circulation, infections, necrosis, loss of function and mutilation. Although not directly targeted, chimpanzees (*Pan troglodytes*) are sometimes accidentally caught in snares, which can cause severe and permanent mutilations (Waller, 1995). These injuries are a substantial problem in many chimpanzee communities. For example, in Budongo Forest, Uganda, an estimated third of the entire chimpanzee population suffers from some form of snare injury (Reynolds, 2005).

Snare injuries can affect chimpanzee behaviour in various ways. One study found that severely disabled females spent more time in small parties than control females, probably to avoid food competition and because they have more difficulty to follow larger travelling parties (Hermans, 2011). Another study found that female chimpanzees with snare injuries carried their offspring less often than uninjured females, especially once the infants got older and heavier (Munn, 2006). In the same study, the severity of a snare injury was also negatively correlated with the daily travel distance, suggesting that disabled mothers may be disadvantaged in their foraging capacities. In addition, snare-injured mothers spent more time in trees than uninjured mothers, suggesting that snare injuries influence climbing abilities. Snare injuries may also reduce the ability of male chimpanzees to rise in social rank, which will negatively affect their mating opportunities and their ability to compete for food (Smith, 1995). Finally, chimpanzees rely heavily on their hands when processing food (Byrne and Stokes, 2002), suggesting that hand injuries will reduce their ability to access food and require them to use suboptimal techniques, such as using their mouth or feet to manipulate food items.

Despite the obvious physical harm of a snare injury, the long-term fitness consequences for injured chimpanzees remain poorly understood. In Budongo Forest (Uganda), there have been two reports of death due to snares between 1990 and 2005, although the true mortality rate is estimated to be much higher, around 2-3 fatalities per year (Reynolds, 2005). Snare injuries do not seem to have an obvious negative impact on the physical condition of chimpanzees that survive (Smith, 1995). However, one adult male of the Sonso community in Budongo Forest suffered from almost total paralysis of both hands due to snare injuries and developed chronic skin problems, presumably due to a lack of auto-grooming. (Hobaiter and Byrne, 2010).

Chimpanzees harbour numerous intestinal parasites that can affect their immune system (Abee et al., 2012). Chimpanzees can acquire these parasites when they come into contact with contaminated faecal matter. There is some circumstantial evidence to suggest that animals with snare injuries might have higher exposure rates to intestinal parasites than healthy animals. For example, snare-injured chimpanzees reuse old nests more often than control individuals (Plumptre and Reynolds, 1997) and are therefore more likely to encounter parasite-contaminated faecal matter. The ability of chimpanzees to fight intestinal parasites also depends on the quality of their immune system (Coe, 2012). The efficacy of the immune system, in turn, depends on nutrition, stress, and other factors. Snare-injured individuals may have lower quality nutrition and higher stress levels, which will reduce their immune-competence to fight parasitic infections.

We aimed to compare the prevalence of intestinal parasites between snare-injured and control chimpanzees in two communities of Budongo Forest, Uganda. We predicted that increased exposure and/or reduced immune-competence would result in chimpanzees with snare injuries having higher prevalence of intestinal parasites than control chimpanzees.

Materials and methods

Study area and subjects

We conducted this study at the Budongo Conservation Field Station (BCFS), a Ugandan NGO, in the Budongo Forest Reserve (latitude 1°37'N–2° 03'N, longitude 31°22'E–31°46'E), Masindi District, North Western Uganda (Mwavu and Witkowski, 2008). Budongo Forest is the largest natural forest in Uganda with an area of about 825 km^2 (Reynolds, 2005) and a mean altitude of 1,100 m. This semi-deciduous tropical rainforest experiences a mean annual rainfall of 1,600 mm. Peak precipitation occurs between March and May and between September and November with a dry season from December to February. Temperatures vary between 19°C and 32°C (Reynolds, 2005).

We obtained faecal samples from two chimpanzee communities, the Waibira group (partially habituated; observations since 2011) and the Sonso group (fully habituated; observations since 1990) over a period of four years. We collected samples from the Sonso community between 2011 and 2014 and from the Waibira community from 2013 to 2014. We classified the chimpanzees that had a morphological change due to a snare injury as “snare-injured”. These morphological changes included clenched toes, hooked hands, missing hands, or missing legs. We classified the chimpanzees that were never injured by a snare or that had suffered a snare injury earlier in life, which did not result in a morphological disability as “controls”. For both communities, we collected faecal samples opportunistically from known individual chimpanzees using non-invasive sampling methods. Some chimpanzees were not well habituated (particularly those in the Waibira community) so it was difficult to collect many samples from these individuals. For the Sonso community, we were unable to obtain samples when chimpanzees foraged on crops in human settlements adjacent to the forest reserve due to the ethical guidelines of BCFS. As a result, we did not obtain a balanced dataset for the different focal animals. To obtain an estimate of population-wide patterns, we included faecal samples from all identified individuals, even if sample sizes were not balanced. We placed all faecal samples in sterile containers and labelled them with the individual’s name, sex, age, and date of defecation.

Laboratory analysis

We analysed all faecal samples at the BCFS field laboratory for a range of intestinal parasites that are known to infect wild chimpanzees in the area (Barrows, 1996; Mugisha, 2004; Zommers, 2010). We identified the following parasites: intestinal helminth worms, such as Strongylid nematodes (Taylor et al., 2007), *Ascaris* nematodes, *Trichuris* nematodes and cestodes. We lumped all cestodes, although this class includes many genera. We also identified intestinal protozoan parasites, such as *Cryptosporidium parvum*, *Giardia lamblia* and *Entamoeba histolytica*.

We used the RIDA®QUICK Cryptosporidium/ Giardia/ Entamoeba Combi Test (R-biopharm AG, Germany), which is an antigen kit designed to detect protozoan parasites (*Cryptosporidium parvum*, *Giardia lamblia*, *Entamoeba histolytica*). For each faecal sample, we transferred ~ 50 mg of material into a tube without formalin and mixed with extraction buffer, before inserting the test strip into the resulting solution. The analysis is based on an immune-chromatographic assay, with parasite-specific antibodies on the strip indicating parasite presence by specific colours. For all other parasites, we transferred samples into tubes with 10% buffered formalin for later analysis. We used two techniques to isolate helminth eggs: faecal flotation for light eggs using Sheather's solution (128 g of sugar in 100 ml of hot water) and faecal sedimentation for heavy eggs (Gillespie, 2006). We visualized helminth eggs using a light microscope (40x and 10x objective) and identified them using a taxonomic key (Matsubayashi et al., 1965; Skrjabin et al., 1952). Parasite prevalence is typically defined by looking for eggs in 1 gram of faecal matter. In this study, we used 3 grams of faecal matter because the number of eggs was very low for some parasites.

Statistical analysis

We considered four helminth taxa for our study: Strongylid nematodes, *Ascaris* nematodes, *Trichuris* nematodes and cestodes. We also considered three protozoan taxa: *Cryptosporidium parvum*, *Giardia lamblia*, and *Entamoeba histolytica*. We analysed eight different parasite response variables: helminth richness (based on the four helminth taxa) and seven parasite prevalence variables (1 variable per taxon).

We defined the helminth richness as the number of helminth genera found in one faecal sample. We defined the prevalence of a particular parasite taxon as the proportion of faecal samples infected by that parasite taxon (binomial data: 0 = parasite absent, 1 = parasite present). The analyses of the helminth richness and the four helminth prevalence variables were based on the entire dataset (487 faecal samples from 90 individual chimpanzees) whereas the prevalence of the three protozoa prevalence variables were based on a subset of faecal samples that had been processed with respect to these variables (98 faecal samples from 69 individual chimpanzees).

We used generalized linear mixed-effects (GLME) models to analyse these parasite response variables as a function of the fixed and random factors of interest. We modelled helminth richness and parasite prevalence using Poisson and binomial errors, respectively. The fixed factors included: snare status (control, snare-injured), community membership (Sonso, Waibira), sex (female, male), age class (adult, juvenile, subadult), season (dry, wet) and the presence of road maintenance workers (absent, present). We included this last variable because road workers visited the study area from November 2013 to February 2014 as part of a project to reopen an old logging road for ecotourism. For snare status, we initially included the degree of injury in our analysis, but the sample sizes of the different categories were too low to carry out a meaningful analysis. We modelled chimpanzee identity as a random factor to control for pseudo-replication since some individuals were sampled more than once. We conducted the statistical analyses using R (R Core Team, 2014) (see the document titled "S2_Script.docx" for the R code used). We used the glmer() function to run the GLME models. For each parasite variable, we compared the full model (containing all the fixed factors of interest) to the null model (containing none of the fixed factors of interest) to determine the overall significance of the full model. To determine the statistical significance of each fixed factor, we used log-likelihood ratio tests to compare the residual deviance between the full model, which contained all fixed factors, and the reduced model, which was missing the fixed factor of interest. We considered the results significant at an alpha level of

0.05. For three parasite taxa (*Trichuris*, *G. lamblia*, and *E. histolytica*), the number of infected faecal samples was so low (6, 7, and 0, respectively) that the R software was unable to calculate the parameter estimates for the models (see Table S1 in the document titled "S3_table.docx"). We therefore did not present these parasite variables in the results section.

Results

We collected a total of 487 faecal samples from chimpanzees in the Waibira and the Sonso communities. There were 366 and 121 faecal samples from 70 control and 20 snare-injured chimpanzees, respectively. Of the 90 individuals in this study, 36 belonged to the Waibira community (9 with snare injuries) and 54 to the Sonso community (11 with snare injuries).

For each of the five parasite variables for which there were enough infected faecal samples to run the analyses, the full model was statistically significant when compared to the null model (Table 1). There was a significant association between snare status and the prevalence of Strongylida (Tables 2 and 3; Figure 1). If we consider an adult female chimpanzee in the Sonso community in the dry season in the absence of road workers as the reference point, the probability that a faecal sample was infected with Strongylida was 81.7% for a control individual and 91.8% for a snare-injured individual. There were no significant differences between snare-injured and control chimpanzees for the other parasite variables: helminth richness, prevalence of *Ascaris*, prevalence of cestodes, and the prevalence of *Cryptosporidium parvum* (Tables 2 and 3).

Other ecological factors that had effects on the parasitic prevalence variables were the chimpanzee community, the season, and the presence of road workers (Tables 2 and 3). The chimpanzees in the fully habituated Sonso community had a higher prevalence for *Ascaris* and cestodes than the Waibira community. Parasite prevalence was higher in the dry season than the wet season for *C. parvum*. The presence of road workers decreased the following four parasite variables: helminth richness, the prevalence of *Ascaris*, the prevalence of *C. parvum* and the prevalence of Strongylida. There was no effect of sex and the class of age on the parasite variables (Tables 2 and 3).

Discussion

We found that snare-injured chimpanzees had significantly higher prevalence of Strongylida nematodes than control individuals. Snare injuries had no impact on the helminth richness and the prevalence of *Ascaris*, cestodes and *Cryptosporidium parvum*. The most interesting result of this study was that snare-injured chimpanzees had a higher prevalence of Strongylida than control individuals. There are at least two possible explanations for this result. First, snare-injured individuals have higher exposure to Strongylida parasites than control individuals. Second, snare injuries facilitate the proliferation of these parasites because of a weakened immune system.

The first hypothesis suggests that snare-injured chimpanzees have higher exposure to helminth parasites than control individuals. Many helminth parasites (e.g. hookworms) have infective stages that penetrate the feet and hands of their chimpanzee hosts. Exposure rates should therefore be correlated with time spent on the ground (Zommers et al., 2013). However, a previous study found that snare-injured individuals spend less time on the ground than control individuals (Munn, 2006). In addition, snare-injured individuals spent more time in small groups (Hermans, 2011) where exposure to ground-dwelling helminth parasites is presumably lower than in large groups. These observations suggest that snare-injured individuals do not have higher exposure rates to ground-dwelling helminth parasites than not snared-injured individuals. However, snare-injured chimpanzees reuse old nests more often than control individuals (Plumptre and Reynolds, 1997) and this behaviour may enhance their probability to encounter parasite-contaminated faecal matter. Thus, it is currently not clear whether snare-injured chimpanzees are more or less likely to encounter helminth parasites than control individuals.

The second hypothesis suggests that snare injuries directly facilitate intestinal parasite infections. The skin is an important defence against pathogens (Janeway et al., 2001) and snare-induced skin injuries trigger inflammatory responses to heal the wound and to kill invading microbial pathogens (Davis, 2008). Severe snare injuries may thus compromise the immune system and, as a consequence, facilitate intestinal infections due to trade-offs between the different components of the immune response (Sadd and Schmid-Hempel, 2009). The efficacy of the immune system also depends on nutrition, stress, and other factors. Snare-injured individuals may have lower quality nutrition and higher stress levels, which would reduce their immune-competence to fight parasitic infections. In summary, snare-injured individuals may have a higher prevalence of intestinal parasites because their immune systems are less competent.

Chimpanzees are known to carry multiple cestode species (Abee et al., 2012). We did not identify the cestode parasites to subgroups. As a result, the cestode variable contained many genera. The statistical analysis found no effect of snare injuries on the prevalence of cestode parasites. However, this test is limited in value because we lumped all the cestode taxa into a single group.

An intriguing question for the future is whether snare-injured chimpanzees, given their higher prevalence of Strongylida infections, are more likely to use medicinal plants than control individuals. Some plants eaten by chimpanzees are used by humans in traditional medicine as treatment for intestinal parasite infections (Ghai et al., 2015; Krief et al., 2005). However, severely injured chimpanzees, generally suffered from impaired feeding efficiency (Stokes and Byrne, 2001), which may prevent them from harvesting medicinal plants and control their parasite infections. Foraging data would be necessary to address this point.

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Figure legends

Fig.1 Prevalence of Strongylida in control and snare-injured chimpanzees (*Pan troglodytes*) in Budongo Forest, Uganda, 2011-2014. The parameter estimates were taken from the generalized linear model analysis (see Table 3) and were back-calculated to the original scale. The effect of snaring is shown for a reference faecal sample, taken from a female adult in the Sonso community during the dry season and in the absence of road workers. The bars show the standard errors.

Supporting Information

The datasets supporting this article are available as Electronic Supplementary Material.

S1_File. Table containing the analysed data

S2_R Script. Example of the R script used to run the statistical analyses

S3_Table. The means of the 8 parasite variables