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ENDOPARASITISM OF REHABILITATING GREY CROWNED CRANES IN RWANDA

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Abstract: Diseases such as parasitism can limit the effectiveness of conservation translocations depending on host-parasite dynamics at the site of release. The Rwanda Wildlife Conservation Association and the Rwandan government are rehabilitating and repatriating grey crowned cranes (*Balearica regulorum*) from illegal captivity to the wild at Akagera National Park in large numbers. Monitoring of cranes at the fenced soft-release site during 4 time points in 2017 showed 50-67% of fecal samples tested were positive for 1 or more parasites, most commonly nematodes (roundworms) of the Order Ascaridida. The prevalences and species diversity observed in the fecal samples were not dissimilar from preliminary surveys of 2 other populations elsewhere in Rwanda, suggesting no new management considerations are needed to accommodate the number of cranes at the release site or during the preceding quarantine period to prevent disease.

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Key words: *Balearica regulorum*, grey crowned crane, helminths, parasites, rehabilitation, repatriation, Rwanda.

The grey crowned crane (*Balearica regulorum*) is the only species of crane in Rwanda and faces serious threat from domestic live trade. The Rwanda Wildlife Conservation Association (RWCA) has partnered with the Rwandan government since 2014 to undertake public awareness campaigns, formally register cranes held in captivity, and rehabilitate and repatriate a healthy subset of cranes to Akagera National Park (ANP), all to decrease illegal trade of cranes. The large number of cranes processed by this program (147 translocated to ANP since 2015) has raised concerns about high stocking density at release site(s) and increased transmission of endoparasites, and whether parasitic infection could be a negative factor to the successful acclimation of the cranes to a free-ranging existence. The objective of this project was to document endoparasites of cranes at the release site used in 2017 and compare results with available information from natural areas in Rwanda to assess if endoparasite diversity or prevalence was substantially different and in need of further management consideration.

All cranes in the RWCA repatriation program were first identified during the official registration process using 2 initial criteria: 1) the crane showed no overt anatomic abnormalities, and 2) exhibited subjectively appropriate species-specific behavior. Cranes meeting these criteria were then transferred to a central quarantine facility in Kigali for a minimum of 45 days. Each crane was given a physical examination and health assessment (complete blood count, blood

biochemistry panel), as well as tested for reportable infectious diseases or of zoonotic potential (e.g., mycobacteriosis, campylobacteriosis, salmonellosis, chlamydiosis, Newcastle disease virus) that could be accidentally translocated as part of the repatriation process. Fresh fecal samples were analyzed using standard microscopic techniques, including direct examination in saline and sodium nitrate flotation, to determine the presence of parasitic ova and larvae (Bowman 2009). Each crane was given an anthelmintic regimen of 2 doses of fenbendazole (100 mg/kg orally) and ivermectin (0.2 mg/kg subcutaneously) 2 weeks apart to decrease helminth burden. Fecal analysis was repeated 2 weeks following the last doses of anthelmintics; positive tests resulted in a second round of the fenbendazole and ivermectin treatment. Amprolium or sulfadimidine were only given to cranes shedding coccidial oocysts. No specific treatment was prescribed for trematode infections due to uncertainty regarding treatment regimens in cranes with the medication available (praziquantel) and unlikely transmission of the parasite in quarantine (trematodes have an indirect life cycle requiring an invertebrate intermediate host, typically snails, that were absent at the quarantine facility). All cranes had negative fecal parasite exams by the time of transfer to ANP and showed no signs of endoparasitic disease.

Releases at ANP in 2017 were conducted at a locale known as the Pecherie (a lakeside, game-fenced, staff-use area of approximately 4 ha, adjacent

to native crane habitat). Anonymous, fresh crane fecal samples were collected from the Pecherie site on 31 March, 31 July, 7 September, and 31 October. A variable number of cranes were present at the site depending on transfers from the quarantine site in Kigali and cranes coming and going on their own through the soft release process. A minimum of 26 and maximum of 42 cranes were likely regularly present at the site during these sampling dates, with some cranes having been present for 4 or more months and others for only a few weeks. The field team was instructed to attempt to collect up to 15 samples; this was the minimum number calculated to provide 90% confidence that parasites were present within or below 10% of the cranes, if no positive fecal test results were observed (Martin et al. 1987). The samples were collected sequentially and not randomly because many samples were weathered and unsuitable for the laboratory tests. The fresh samples found were analyzed using the laboratory methods described above.

Endoparasitic diversity was relatively low in the rehabilitating cranes (Table 1). Nematode

Table 1. Results of parasitological analysis of fecal samples^a collected anonymously from rehabilitating grey crowned cranes, Pecherie release site, Akagera National Park, Rwanda, 2017.

Sample no.	31 Mar	31 Jul	7 Sep	31 Oct
1	Neg	Neg	A	A
2	Neg	A	Neg	A
3	A	A	A	A
4	A/D	A	A	A
5	A	A	Neg	A
6	A	A	A/E	A
7	A	Neg	Neg	A
8	A/C/P	A	A	A
9	A	Neg	Neg	Neg
10	Neg	A/U	Neg	Neg
11	A	Neg	A	Neg
12	Neg	U	Neg	Neg
13	A	Neg	Neg	A
14	Neg	Neg	A	A
15	nc	Neg	nc	Neg
% Positive	64	53	50	67

^a Neg = negative for parasitic ova, A = *Ascaridia* sp. (Nematoda), C = *Capillaria* sp. (Nematoda), D = *Dicrocoelid* sp. (Trematoda), E = *Eimeria* sp. (Protozoa), P = *Porrocaecum* sp. (Nematoda), U = unspecified nematode larvae, nc = not collected.

(roundworm) parasites of the Order Ascaridida were most commonly diagnosed. Our understanding of endoparasitic diversity among Rwandan grey crowned cranes is rudimentary. Limited numbers of fecal samples obtained from wild cranes near the Akanyaru (southwest of ANP, $n = 20$) and Rugezi (north of ANP, $n = 16$) marshes showed greater taxonomic diversity, but similar parasite prevalence (50% and 70% of samples, respectively), compared to the rehabilitating cranes (O. Kayinamura and N. Uwera, unpublished Rwanda National University student thesis data, 20 Apr 2017). Disease-causing protozoal parasites may be less common in grey crowned cranes compared to North American species (Hartman et al. 2010, Bertram et al. 2015). The single collections and limited number of samples obtained from these larger crane populations, however, would benefit from additional evaluations in the future to improve their value as baseline information for comparison.

Our study suggests that rehabilitating cranes acquired modest infestations of endoparasites in the midst of a prolonged soft-release process of repatriation. The infections that we observed commonly consisted of ascarids, parasites with direct life cycles that would facilitate rapid transmission among a group of semi-captive cranes. None of the cranes at the release site showed clinical signs consistent with endoparasitic disease, nor did we observe infection universally among the cranes. We suspect cranes with longer duration at the Pecherie site likely develop higher worm burdens and are most likely to have positive fecal results, but the methodologies used in this preliminary survey were not truly quantitative, only qualitative, and samples were collected anonymously. The origin of the infections is unknown; parasites may have come from an environmental source at the release site, outside the Pecherie site itself since birds can come and go, or have been translocated inadvertently from previous captivity since prophylactic anthelmintic treatment may not have been 100% effective as observed with other crane releases (Spalding et al. 1996) or gone undetected despite several fecal exams.

Cranes regularly used approximately 1 to 2 ha of this site, generally keeping distance from staff activities or other disturbance and areas of thick vegetation. We estimate the greatest density during this study was 42 cranes/ha, which would provide 238 m² of space per crane. This stocking density is much lower than that recommended for captive cranes

to keep soil pathogens in check (50 m² per crane; Swengel and Carpenter 1996), but the facility at ANP does not allow any kind of seasonal rotation. As long as the cranes progress in the repatriation process and disperse from the release site within months, thereby reducing parasite transmission, we predict this management scheme will be associated with limited disease impacts from high parasite burdens and not require additional management consideration. Tracking the parasite status of known individuals combined with survival estimation at specific points following release may also be warranted.

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