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Parasite survey on a captive wolf population using classical techniques and ELISA coproantigen detection, USA

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ABSTRACT

As laws change around the United States, wildlife that were once kept as companion animals are now often confiscated by local authorities. They are then euthanized unless a home is found for them at a sanctuary. Wolf sanctuaries are, therefore, becoming increasingly important for their conservation and management. However, little data is available on best practices for the health management of captive wolves, including data on parasitic diseases. Our objective was to assess the prevalence of parasites of captive wolves combining classical coprological techniques and immunoassays based on the detection of coproantigen of selected canid parasites. Fecal samples of 39 animals were collected upon observation of individual animals defecating. All samples were processed using the Fecal Dx® tests, a suite of coproantigen ELISAs for detection of ascarid, hookworm, whipworm, and *Giardia* (IDEXX Laboratories Inc.). Out of the 39 samples, 38 were processed using the double-centrifugation sugar flotation (DCSF) and 34 using a modification of the Baermann technique. Twenty-eight samples (71.8%) were positive for hookworm, and none positive for the other parasites tested using coproantigen ELISA. *Ancylostoma* sp. (26, 68.4%), *Eucoleus boehmi* (13, 34.2%), and *Trichuris* sp. (2; 5.3%), and *Sarcocystis* sp. (13, 34.2%) were detected using DCSF. No metastrongyloid lungworm larvae were found. The Cohen's kappa index (0.97) showed excellent agreement between the hookworm coproantigen ELISA and the DCSF using feces preserved in ethanol for a short period of time. This study provides a baseline on the parasites of captive wolves, and shows that recent innovative diagnostics in veterinary parasitology, developed and optimized for dogs, may be used for assessing the health of wolves.

1. Introduction

The Endangered Species Act (7 U.S.C. § 136, 16 U.S.C. § 1531 et seq.) prohibits a person from owning any endangered species of fish or wildlife in the United States. However, there are no federal laws that regulate or prohibit keeping exotic animals as pets. This policy gap has created a patchwork of state and local legislation. Authorities confiscate and often euthanize animals found in violation of local laws. Therefore sanctuaries, such as the Wolf Sanctuary of Pennsylvania, are increasingly important for conservation and management. Unfortunately, little data is available on best practices for the health management of captive wolves, including data on parasitic diseases.

As conspecific animals, wolves and dogs share common parasites, including ascarid nematodes, *Toxocara canis* and *Toxascaris leonina*, hookworms such as *Ancylostoma caninum* and *Uncinaria stenocephala*, and *Trichuris vulpis*, among other nematodes; as well as a variety of cestodes and trematodes, and protozoans (Craig and Craig, 2005). Captive wolf populations, are expected to have a lower diversity of parasites compared to wild population due to the low numbers of animals in a given captive populations and limited opportunities for the introduction of parasites by either arrival of new animals or their diets.

To our knowledge, there is scarce information on the parasite fauna of captive wolves in North America. Our objective was to assess the prevalence of parasites of captive wolves combining classical coprological techniques and immunoassays based on the detection of coproantigen of selected canid parasites.

2. Materials and methods

2.1. Sample collection

The Wolf Sanctuary of Pennsylvania provides a home for 47 wolves

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and wolf dogs divided into 17 packs of 1 to 8 animals each, on approximately 40 acres in Lancaster County, Pennsylvania, Unites States. Wolves and wolf dogs managed at the sanctuary originate from various US states. Animals are fed 1 to 3 kg of raw meat three days a week, which may include cattle, sheep, goat, poultry and road killed deer. They also hunt wildlife within their enclosures, mainly consisting of small mammals and birds, but the amount and frequency are unknown. The animals are not on a parasite control program.

Overall, 39 fresh fecal samples were collected from 15 of the 17 packs managed in the sanctuary, with 1 to 4 samples per pack (average = 2.6 samples/pack). Only the upper portion of the sample was collected to void potential contamination. All samples were collected over one week during the last week of March (early Spring). Fecal samples were collected from the ground upon observation of individual animals defecating. All samples were sub-samples for the different diagnostic techniques described below.

The use of diagnostic samples for research is covered under the institutional AUP # A2017 05–014-Y2-A0.

2.2. Coproantigen ELISA

All 39 samples were processed using the Fecal Dx® tests which detect coproantigens from ascarid, hookworm, whipworm, and *Giardia* (IDEXX Laboratories Inc.). A 1-gram fecal samplewas kept refrigerated up to 5 days and then kept at −20 °C until processing. Briefly, 0.1 g of feces was mixed with sample buffer and 100 μL of fecal slurry was tested for each antigen test. Soluble antigen, if present in the sample, is then available to bind to the antibody components of the assays. A positive and negative control were run for each antigen test. The microplates were read at a wavelength of 650 nm, with an optical density (OD) of 0.100 or greater for the nematode assays and the 0.150 or greater for the *Giardia* test to be considered positive. A plate test was considered valid if the positive control was greater than 0.500 and the negative control OD value was less than 0.100.

2.3. Flotation

Thirty-eight samples were processed using the double-centrifugation sugar flotation (DCSF) technique, using Sheather's sucrose solution (specific gravity = 1.25) (Zajac and Conboy, 2012). Samples for flotation were preserved in 70% ethanol and kept refrigerated until processed to avoid degradation of parasite diagnostic stages during shipping for processing in the laboratory. Processing was performed within a week following collection. Three grams of feces from each individual were used for the DCSF. For both centrifugation steps, samples were spun for 10 min at 95 ×*g*. Following the second centrifugation, an additional 1 mL of flotation solution was added to form the reverse meniscus and then a 22 × 22 mm coverslip was placed on top. The sample sat for a additional 10 min. on the bench top allowing for the flotation of parasite diagnostic stages. Slides were read at 100× magnification, and 400× was used to confirm identification and visualize morphologic details such as the pattern of the egg shell of capillarid eggs (Zajac and Conboy, 2012).

Parasite diagnostic stages were enumerated by genus or species by counting all stages under the coverslip area, and categorized according to the shedding level using a scale in which: 1 = 10 or less, 2 = between 11 and 25, 3 = between 26 and 50, and 4 = above 51 eggs/sporocysts (Table 1).

2.4. Modified Baermann

Thirty-four samples were processed by a modification of the Baermann technique. This adaptation of the Baermann consisted of using a 50 mL conical tube for sedimentation of larvae in feces (Hoggard et al., Submitted). Briefly, 2 g of fresh feces were wrapped in a pouch made of one layer of Kim wipe and one layer of cheesecloth, and submerged at the top of the conical tube filled with lukewarm water for up to 24 h. The sample pouch was discarded, and the supernatant was removed with the aid of a 25 mL plastic pipette. The bottom 2.5 mL was transferred into 15 mL test tubes at the sanctuary, shipped to the laboratory, and kept refrigerated until reading. The total volume of each tube was read on glass slides using a compound microscope under 40–100× magnification, and assessed for the presence of larvae of parasitic nematodes and protozoa.

Table 1
Double-centrifugation Sugar Flotation (DCSF) of captive wolf/wolf dogs relative to egg/sporocysts shedding categories.

Parasite	Negative	Shedding categories (n)				Total positive % (n)
	0	1	2	3	4	
Ancylostoma sp.	12	13	7	3	3	68.4 (26)
Eucoleus boehmi	25	2	3	2	5	34.2 (13)
Sarcocystis sp.	25	9	3	0	1	34.2 (13)
Trichuris sp.	34	1	0	1	0	5.3 (2)

Shedding categories: 1 = 10 or fewer, 2 = between 11 and 25, 3 = between 26 and 50, and 4 = more than 51 eggs/sporocysts.

2.5. Technique comparison

Results of the hookworm coproantigen ELISA were compared with the DCSF using feces preserved in ethanol for a short period of time, and the sensitivity, specificity and the Kappa coefficient of agreement were calculated (Thrusfield, 2007).

3. Results

Hookworm coproantigen was detected in 71.8% (28/39) of samples. All samples tested negative for antigens of *Toxocara* sp., *Trichuris* sp., and *Giardia* sp.

Overall on DCSF, 31 samples of 15 of the 16 packs were positive for at least one parasite (81.6%), 18 at least two parasites (47.4%), and 4 had three parasites detected (12.9%). Among nematodes, eggs of *Ancylostoma* sp., *Eucoleus boehmi*, and *T. vulpis* were found in 68.4% (n = 26/38) in 12 packs, 34.2% (n = 13) in 6 packs, and 5.3% (n = 2) in 2 packs, respectively. Among protozoans, only sporocysts of *Sarcocystis* sp. were found in 34.2% (n = 13) of the samples from seven packs (Table 1). No metastrongyloid nematode larvae were found in the 34 samples processed by the modified Baermann technique.

The sensitivity and specificity of the DCSF for detection of *Ancylostoma* sp. eggs when compared to the hookworm coproantigen ELISA were 96.3% (81.0–99.9%, CI 95%) and 100% (71.5–100%, CI 95%). The Kappa coefficient of agreement was 0.97, which is considered very good to excellent, depending in the scale used (Thrusfield, 2007).

4. Discussion

The captive wolves studied were infected with parasites commonly found in wild wolves and domestic dogs in North America, with coinfections by two or more parasites being common. Knowledge on the specific parasite fauna affecting this population should guide strategies for management and control, including anthelmintic treatment.

Ancylostoma sp. was the most prevalent parasite (71.8%) considering the combined coproantigen-ELISA and flotation results in this study, consistent with previous reports in captive wolves from New York State (Kreeger et al., 1990) and dogs across North America (Blagburn et al., 1996; Little et al., 2009). This parasite has a direct life cycle, which may facilitate its establishment and perpetuation in an environment such as the enclosures, in which larvae may find ideal conditions in the soil for developing into the infective stage, depending on the season of the year (Anderson, 2000). High intensity infections of hookworms may cause anemia, and diarrhea, impacting the general health of the animal (Bowman, 2014).

To our knowledge there are no published reports on *E. boehmi* infecting wolves in North America (Craig and Craig, 2005), but it has been reported in wild wolves in Sweden (Al-Sabi et al., 2018). *Eucoleus boehmi* is a capillarid nematode found in the nasal turbinates and frontal and paranasal sinuses of carnivores, causing respiratory disease that involves and sneezing and nasal discharge (Conboy, 2009). This parasite is found in domestic dogs in North America at low prevalence (Blagburn et al., 1996), and may have been introduced into the sanctuary premises by wolves or wolf dogs that were kept along domestic dogs. The life-cycle of this nematode is not fully understood, but it is believed that earthworms may serve as intermediate hosts (Anderson, 2000; Conboy, 2009).

Sarcocystis are protozoan parasites with an indirect life-cycle, in which the definitive host must ingest the infective stages found in skeletal muscles of various animal species (Dubey, 1976). It most likely circulates among the animals in the sanctuary through either the ingestion of fresh meat they are fed, and/or hunting small prey within their enclosures. Wolves have been reported as definitive host for several *Sarcosystis* species (Dubey, 1976; Emnett, 1986; Lindsay et al., 1988), and unequivocal species-level identification of the isolates would require molecular genetic techniques, which could point to the potential food source/ its intermediate hosts. *Sarcocystis* spp. are, however, non-pathogenic to the carnivore definitive hosts (Dubey, 1976), and should not pose a health problem in this captive wolf population.

No nematode larvae were found using Baermann technique, however various metastrongyloid species have been reported in wild wolves, including *Oslerus osleri* in the US and Canada (Byman et al., 1977; Erickson, 1944; Samuel et al., 1978; Verocai et al., 2013), and *Angiostrongylus vasorum* and *Crenosoma vulpis* in Eurasia (Craig and Craig, 2005). Fecal examination is not the most appropriate diagnostic technique for detection of *O. osleri* larvae, as only a portion are shed in feces, and these are often degenerated (Yao et al., 2011).

4.1. Technique comparisons

There was almost perfect agreement between the hookworm coproantigen ELISA test and the DCSF in the detection of hookworm infections. Only a single positive sample at the coproantigen ELISA tested negative in the DCSF, which may be attributable to a pre-patent infection or low fecal egg shedding. In addition, while ethanol preservation of feces may impact the detection of diagnostic stages of parasites due to ion binding or morphological damage (Foreyt, 1986), the authors did not perceive it as an issue since the eggs and sporocysts detected kept their characteristics and remained diagnosable. This may have been because of the short-term preservation period (up to a week), and the fact that preserved samples were kept cooled or refrigerated from collection until processing in the laboratory. Nevertheless, ethanol preservation may have impacted the false-negative diagnosis of hookworm found in the DCSF, especially if egg shedding was low, as well as lead to underestimating the prevalence of other parasites. Elsemore et al. (2017) has shown that the hookworm ELISA and flotation have high percent agreement, but the former shows higher sensitivity and specificity. Similarly, these authors have shown higher sensitivity and specificity for the ascarid ELISA. All samples from the present study tested negative using both techniques, and because all studied animals were adults, absence of *T. canis* may be related to their age (Bowman, 2014).

The *Trichuris* coproantigen ELISA failed to detect the two samples found positive by the DCSF. The morphometry of the eggs overlapped to *T. vulpis* and *Trichuris muris*, but not that of *Trichuris* species infecting ungulates, which tend to be smaller and more globose (Koyama, 2013; Zajac and Conboy, 2012). Nevertheless, we cannot state unequivocally if these *Trichuris* eggs were product of a true infection or spuriously shed. It is noteworthy that no cross-reactivity was observed with the closely related *E. boehmi*, in accordance to the findings of Elsemore et al. (2014). Moreover, the same authors demonstrated that the whipworm ELISA is more sensitive and specific than classical flotation methods, however there were a few instances in which only the flotation tested positive. Discrepancies between results may be attributable to low intensity of infection with secreted/excreted antigen below a detectable level or the detection of spurious eggs.

All samples were negative for *Giardia* in both DCSF and antigen-detection test. While true absence is likely due to the high sensitivity and specificity of the latter test, the potential for false negative results cannot be ruled out because of the lack of a gold standard test. This is the first time these novel coproantigen-detection ELISA tests were used in wolves, and these may be useful diagnostic tools for parasitological screening or surveys in captive or wild wolves, and other wild carnivores that serve as hosts for Ancylostomatidae hookworms, ascarids, trichurids, and *Giardia* sp. In addition to target species, the coproantigen-detection ELISA has been shown to detect infection by other species of hookworms in dogs and cats, *Uncinaria stenocephala*, *Ancylostoma tubaeforme,* and *A. braziliense*; ascarids in dogs and raccoons, *Baylisascaris procyonis*; and whipworms in cats, *Trichuris* sp. (Elsemore et al., 2017; Elsemore and Hanna, 2018; Geng et al., 2018a; Geng et al., 2018b; Sapp et al., 2018).

It is recommended to use the coproantigen tests in combination with fecal flotation techniques because the only validated coproantigen-detection tests are for hookworms, ascarids, *Trichuris*, and *Giardia*, the potential discrepant results exists (Elsemore et al., 2017; Elsemore et al., 2014), and classical techniques may detect other helminths and protozoans.

Conflict of interest

R. Hanna and D. A. Elsemore are employees of IDEXX Laboratories Inc. The other authors declare no conflict of interest.

Ethical statement

No animal experimentation was performed for this case report. The use of a diagnostic sample for research is covered by the UGA IACUC (AUP # A2017 05–014-Y1-A0).

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