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Survey for Selected Parasites in Alaska Brown Bears (*Ursus arctos*)

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ABSTRACT: To assess infection with or exposure to endo- and ectoparasites in Alaska brown bears (*Ursus arctos*), blood and fecal samples were collected during 2013–17 from five locations: Gates of the Arctic National Park and Preserve; Katmai National Park; Lake Clark National Park and Preserve; Yakutat Forelands; and Kodiak Island. Standard fecal centrifugal flotation was used to screen for gastrointestinal parasites, molecular techniques were used to test blood for the presence of *Bartonella* and *Babesia* spp., and an ELISA was used to detect antibodies reactive to *Sarcoptes scabiei*, a species of mite recently associated with mange in American black bears (*Ursus americanus*). From fecal flotations (n=160), we identified the following helminth eggs: *Uncinaria* sp. (n=16, 10.0%), *Baylisascaris* sp. (n=5, 3.1%), *Dibothriocephalus* sp. (n=2, 1.2%), and taeniid-type eggs (n=1, 0.6%). Molecular screening for intraerythrocytic parasites (*Babesia* spp.) and intracellular bacteria (*Bartonella* spp.) was negative for all bears tested. We detected antibodies to *S. scabiei* in six of 59 (10.2%) individuals. The relatively low level of parasite detection in this study meets expectations for brown bear populations living in large, relatively undisturbed habitats near the northern edge of the range. These results provide a contemporary understanding of parasites in Alaska brown bears and establish baseline levels of parasite presence to monitor for changes over time and relative to ecologic alterations.

Key words: *Babesia*, *Bartonella*, grizzly bear, helminths, mange, *Sarcoptes*.

There is considerable research on conservation, population dynamics, and management of brown bears (*Ursus arctos*), but limited current data exist on parasites of Alaska brown bears because the most recent parasite surveys were conducted in Canada (Gau et al. 1999; Catalano, Lejeune, Tizzani et al. 2015; Catalano, Lejeune, van Paridon et al. 2015). Therefore, our goal was to conduct a contemporary survey of brown bears from Alaska, US, to investigate 1) presence of infection or exposure to selected parasites and 2) associations with age, sex, and location of sampled bears.

As part of ongoing interagency research, personnel from the Alaska Department of Fish and Game, National Park Service (NPS), U.S. Fish and Wildlife Service (USFWS), and U.S. Geological Survey (USGS) sampled 166 brown bears from July 2013 to July 2017 at five locations: Gates of the Arctic National Park and Preserve (GAAR); Katmai National Park (KATM); Kodiak Island (KOD); Lake Clark National Park and Preserve (LACL); and the Yakutat Forelands. Bears were captured and handled as reported by Ramey et al. (2019), with all capture, handling, and sampling procedures approved by Animal and Care Use Committees for Alaska Department of Fish and Game (2013-028), NPS (2014.A2

TABLE 1. Results of fecal flotation assays from samples opportunistically collected from brown bears (*Ursus arctos*) in 2013–17 from four sites in Alaska, USA: Gates of the Arctic National Park and Preserve (GAAR), Katmai National Park (KATM), Kodiak Island (KOD), and Lake Clark National Park and Preserve (LACL). Multiple samples collected from the same animal are included to fully represent the data collected in this study. Age was not estimated for two individuals.

Parasite	Site				Sex		Age (yr)[a]				Total
	GAAR	KATM	KOD	LACL	Female	Male	5–9.5	10–14.5	15–19.5	20–24.5	
n	73	38	9	40	123	37	47	55	36	10	160
Nematodes											
Baylisascaris sp.	0	5 (13.2)[b]	0	0	5 (4.1)	0	4 (8.5)	1 (1.8)	0	0	5 (3.1)
Uncinaria spp.	10 (13.7)	2 (5.3)	0	4 (10.0)	13 (10.6)	3 (8.1)	5 (10.6)	6 (10.9)	4 (11.1)	1 (10.0)	16 (10.0)
Cestodes											
Dibothriocephalus sp.	1 (1.4)	1 (2.6)	0	0	2 (1.6)	0	1 (2.1)	0	0	1 (10.0)	2 (1.2)
Taeniid-type	1 (1.4)	0	0	0	1 (0.8)	0	0	0	1 (2.8)	0	1 (0.6)
Positive for any parasite	12 (16.4)	7 (18.4)	0	4 (10.0)	20 (16.3)	3 (8.1)	9 (19.1)	7 (12.7)	5 (13.9)	2 (20.0)	23 (14.4)

[a] Five bears <5 yr and five bears 25–30 yr were sampled and were negative for all parasites.

[b] Percentage of samples with each parasite detected is shown in parentheses.

and 2014.A3), USFWS (2012-14), and USGS (2014-1, 2014-10, 2015-4, and 2015-6). Feces were collected opportunistically from the rectums of 114 anesthetized bears from GAAR, KATM, KOD, and LACL one to five times during the study period, stored in 70% ethanol, processed using double centrifugal flotation with Sheather sucrose solution (specific gravity 1.25), and examined under microscopy at 100–400× magnification. Parasite egg detection was analyzed across years, months, and sites, as well as by bear sex and age group. Generalized linear models were created predicting detection of each parasite by year, month, and location of sampling and by bear sex and age (function glm, R package lme4, Bates et al. 2015) using RStudio (version 1.4.1717, R Development Core Team 2020). Statistical significance was assessed at α=0.05.

Blood was collected as described by Ramey et al. (2019) from 156 bears from all sites and tested by PCR for *Bartonella* and *Babesia* species, with 44 bears screened twice. Genomic DNA was extracted using a DNeasy® Blood and Tissue Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol. Nested PCR was performed using GoTaq® Flexi DNA Polymerase (Promega, Madison, Wisconsin, USA) and primers and cycling conditions as previously described (Supplementary Material Table S1). Amplicons were purified using the QIAquick Gel Extraction Kit (Qiagen) and submitted for bidirectional sequencing at the Georgia Genomics and Bioinformatics Core (Athens, Georgia, USA).

Serum samples (*n*=59) were collected from 53 individual bears in 2016–17 from GAAR, LACL, and KATM and tested for antibodies to *Sarcoptes scabiei* using a commercial indirect ELISA kit designed for domestic dogs (*Sarcoptes*-ELISA 2001, AFOSA GmbH, Blankenfelde-Mahlow, Germany). Modifications for use in black bears (*Ursus americanus*) were implemented as described (Niedringhaus et al. 2020).

Eggs of gastrointestinal parasites were found in 23 (14.4%) of 160 fecal samples (Table 1). Two species of nematodes (*Uncinaria* sp., *n*=16, 10.0%; and *Baylisascaris* sp. *n*=5, 3.1%) and two species of cestodes (*Dibothriocephalus* sp., *n*=2, 1.2%; and taeniid-type, *n*=1, 0.6%) were detected. One female from KATM was coinfected with *Dibothriocephalus* sp. and *Baylisascaris* sp. Of the 35 bears with multiple fecal examinations, 22 were repeatedly negative, and 13 were positive once and negative on other

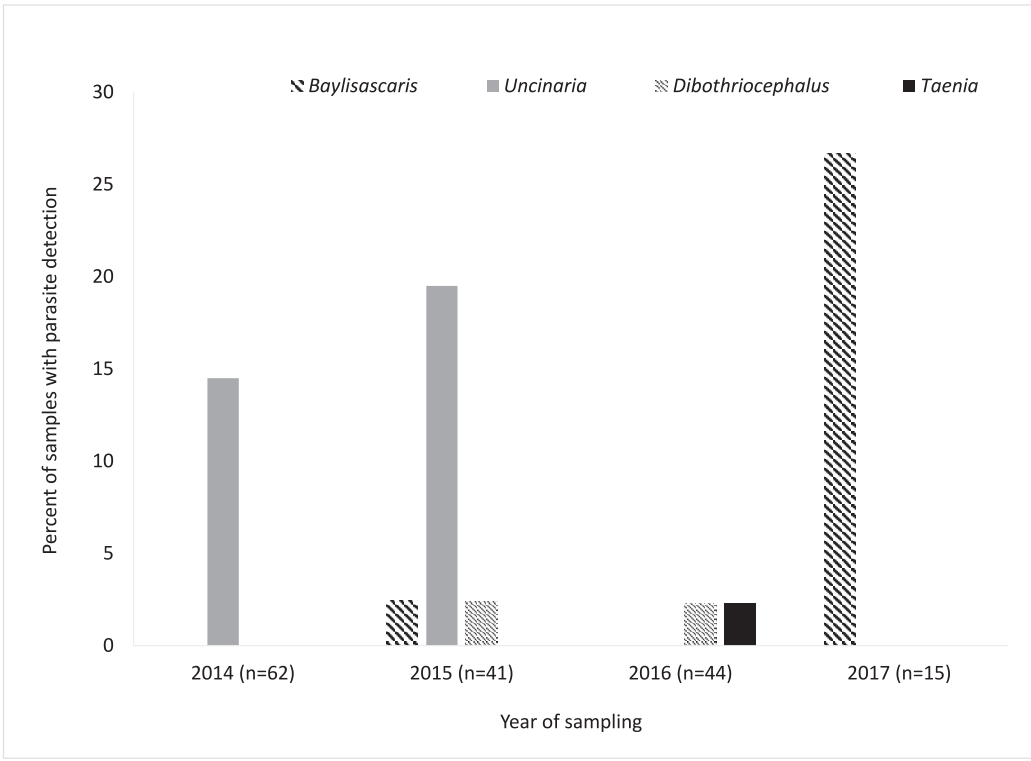

FIGURE 1. Percentage of samples with parasite detection by year of four genera of gastrointestinal parasites detected via fecal floatation assays from samples collected from brown bears (*Ursus arctos*) in 2013–17 from four sites in Alaska, USA. Sample size for each year is shown in parentheses. Multiple samples collected from the same animal are included to fully represent the data collected in this study.

examinations, suggesting periodic shedding. Detection of each parasite varied by year and month of collection (Figs. 1, 2), but the generalized linear models did not indicate that any of the predictors were significantly associated with parasite detection ($P>0.05$).

All 156 animals were PCR negative for *Bartonella* and *Babesia* spp. Antibodies to *S. scabiei* were detected in six (10%) of 59 blood samples (Table 2), with all positive samples originating from LACL and KATM in May 2016.

Overall, we found a low level of intestinal parasite detection relative to previous studies on brown bears in North America (Choquette et al. 1969; Gau et al. 1999). This may have been due to differences in detection methods, as flotation techniques using feces collected from live bears may underdiagnose infections compared with necropsy-based studies. Potential explanations include intermittent egg shedding patterns for some parasites, egg shedding below the detection threshold of the technique, and lack of egg shedding in prepatent and single-sex infections. Specifically, *Baylisascaris transfuga* detection is generally higher in necropsy-based studies (Choquette et al. 1969; Worley et al. 1976; Catalano, Lejeune, Tizzani et al. 2015) compared with studies that used fecal flotation techniques (Gau et al. 1999; Schaul 2006). However, sampling from live animals is less invasive and enables a larger sample size when dealing with wildlife with protected conservation status in which euthanasia is undesirable.

In our study, *Uncinaria* hookworms were the most commonly detected parasites. Two species of *Uncinaria* have been reported in brown and black bears from North America: *Uncinaria rauschi* and *Uncinaria yukonensis* (Olsen 1968; Catalano, Lejeune, Tizzani et al. 2015; Catalano, Lejeune, van Paridon et al.

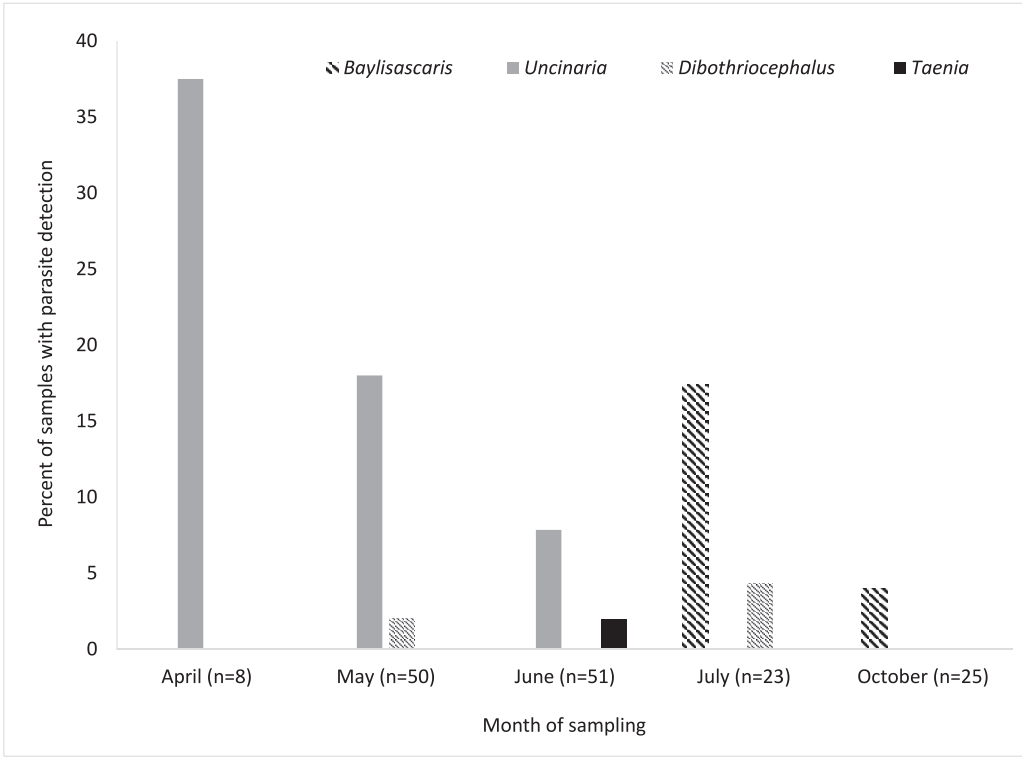

FIGURE 2. Percentage of samples with parasite detection by month of four genera of gastrointestinal parasites detected via fecal floatation assays from samples collected from brown bears (*Ursus arctos*) in 2013–17 from four sites in Alaska, USA. Sample size for each month is shown in parentheses. Multiple samples collected from the same animal are included to fully represent the data collected in this study.

2015). Both may be present in Alaska, and it was not possible to distinguish between species using our methods. *Baylisascaris*, probably *B. transfuga*, has been reported from *Ursus* spp. across the range (Catalano, Lejeune, Tizzani et al. 2015; Sapp et al. 2017). A seasonal effect on *B. transfuga* prevalence has been previously reported (Catalano, Lejeune, Tizzani et al. 2015) but was not noted in this study, probably due to the low level of detection.

We found eggs of the pseudophyllidean cestode *Dibothriocephalus* (=*Diphyllobothrium*) in two samples from female bears: one from GAAR and one from KATM. These zoonotic cestodes are acquired by the definitive hosts,

TABLE 2. Results of ELISA testing for antibodies against *Sarcoptes scabiei* in brown bears (*Ursus arctos*) sampled in 2016–17 from three sites in Alaska, USA: Lake Clark National Park and Preserve (LACL), Gates of the Arctic National Park and Preserve (GAAR), and Katmai National Park (KATM).

	Sex		Location			Year		
Result	Male	Female	LACL	GAAR	KATM	2016	2017	Total
n	14	45	18	22	19	56	3	59
Positive	4 (28.6)[a]	2 (4.4)	5 (27.8)	0	1 (5.3)	6 (10.7)	0	6 (10.2)
Negative	10 (71.4)	43 (95.6)	13 (72.2)	22 (100.0)	18 (94.7)	50 (89.3)	3 (100)	53 (89.8)

[a] Percentage of samples testing positive is shown in parentheses.

including bears and humans, by ingestion of infected salmonid fish (*Oncorhynchus* spp.; Scholz et al. 2019). To date, two species of *Dibothriocephalus* have been genetically identified from brown bears in North America: *D. dendriticum* and *D. nihonkaiense* (Catalano, Lejeune, Tizzani et al. 2015).

The taeniid-type eggs were probably *Taenia arctos*. This recently described species has been reported from brown bears in Eurasia and North America and black bears in North America, with moose (*Alces alces*) as the intermediate host (Haukisalmi et al. 2011; Lavikainen et al. 2011; Catalano et al. 2014). The low level of detection of both cestode species found in our study could be an underestimation due to the methods used or intermittent shedding of gravid proglottids and eggs (Adolph et al. 2017).

We did not detect *Bartonella* spp. in sampled Alaska brown bears, which is consistent with previous studies in black bears (Bai et al. 2016; Chern et al. 2016). We also did not detect *Babesia* spp., although these parasites have been detected in black bears from New Jersey, North Carolina, and Oklahoma, US (Shaw et al. 2015; Skinner et al. 2017; Westmoreland et al. 2019). Established populations of ixodid ticks, some of which may be vectors for *Babesia* spp., have been detected in Alaska (Hahn et al. 2020), but the absence of an appropriate vector for pathogen transmission among bears may explain why we did not detect *Babesia* spp.

None of the sampled bears had clinical evidence of mange, but a low percentage were positive for antibodies reactive to *S. scabiei*. Although the assay used has not been validated for brown bears, it has been validated for black bears (Niedringhaus et al. 2020). Notably, there have not been any reported cases of sarcoptic mange in any wildlife species in Alaska, and it is possible that this assay may cross-react with other mites, such as *Ursicoptes*; therefore, additional work is needed to validate these serologic results, as well as continuing to monitor susceptible wildlife species in Alaska for cases of mange.

Our study provides contemporary baseline data about the diversity of parasites in Alaska brown bears, complementing previously published information on bacteria and parasites (Ramey et al. 2019). Brown bears in Alaska largely exist in expansive and undeveloped ecosystems but may still be subject to accelerated ecologic change due to climate shifts. Therefore, understanding baseline conditions, such as parasite diversity, will be critical to detect change over time.

Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. government. Data are publicly accessible through the Dryad Digital Repository at https://doi.org/10.5061/dryad.xd2547dm3.

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