B A N I S T E R I A

A JOURNAL DEVOTED TO THE NATURAL HISTORY OF VIRGINIA

ISSN 1066-0712

Published by the Virginia Natural History Society

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Banisteria 54: N14–22

SHORTER CONTRIBUTIONS

PARASITE LOADS AND AGING TECHNIQUES ASSESS THE CONDITION OF A BOBCAT (*LYNX RUFUS*) KITTEN IN VIRGINIA

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Editor: T. Fredericksen | Received 3 November 2020 | Accepted 5 November 2020 | Published 1 December 2020

https://virginianaturalhistorysociety.com/banisteria/banisteria.htm#ban54

Citation: Powers, K.E., T. H. D. Marshall, L. M. Van Meter, R. R. Sheeshy, and S. Garvin. 2020. Parasite loads and aging techniques assess the condition of a Bobcat (*Lynx rufus*) kitten in Virginia. Banisteria 54: N14–22.

ABSTRACT

On 7 September 2020, the Southwest Virginia Wildlife Center of Roanoke admitted a Bobcat (*Lynx rufus*) kitten from a patron in Bedford County, Virginia. Despite the best efforts of the staff, the 1030-g kitten succumbed to its maladies on 10 September 2020. We discuss the Center's attempts to remove the parasites, and our subsequent collective efforts to quantify and identify them through DNA barcoding. We identified a number of internal (*Toxocara cati* and *Giardia*) and external (Lone Star Tick, *Amblyomma americanum*) parasites, and quantified the external parasite load (644 ticks across all 3 life stages). In comparing the kitten's intake weight to multiple aging techniques, we determined that this Bobcat was approximately 12 weeks old and showed multiple signs of severe malnourishment. Because Bobcats in the wild are expected to be capable of withstanding a high parasite load, we believe other unidentified ailments led to this kitten's lethargy. Its demise was likely hastened when the hard ticks overwhelmed and anemiated the kitten. We present this case study not only to document illnesses affecting a young Bobcat kitten (to our knowledge, the youngest kitten to be examined in depth for maladies) but also to alert other wildlife rehabilitation specialists about roadblocks to treating Lone Star Tick infestations.

Keywords: *Amblyomma americanum*, dental eruption, DNA barcoding, ectoparasite, endoparasite, *Giardia*, Lone Star Tick, roundworm, serous fat atrophy, *Toxocara cati*.

BACKGROUND

On 7 September 2020, the Southwest Virginia Wildlife Center of Roanoke (hereafter, SWVA Wildlife Center) admitted a Bobcat (*Lynx rufus*) kitten from a patron in the city of Thaxton in Bedford County, Virginia. The kitten was on their back porch on 6 September, and was assumed to have been attracted by cat food.

When the kitten arrived at the SWVA Wildlife Center, it was sexed as a female, described as emaciated, weak, and infested with a "tremendous" number of ticks (Fig. 1A). Its recorded weight was 1030g. It had loose stool, a low body temperature, and was listed as anemic. Immediate efforts included warming it with a heating pad. Co-author Garvin described larval or nymph-stage ticks on or around the live Bobcat while it was in the Center's care - with ticks visibly crawling on the carrier, on the towels, and more. On this first day, staff applied Adams Plus topical flea and tick spray for dogs (Adams Corp., Phoenix, AZ), but this treatment did not appear to encourage the ticks' detachment from the Bobcat kitten.

A standard fecal analysis on 8 September 2020 determined that the Bobcat kitten was infected with roundworms and *Giardia* (cysts discovered). The kitten was offered canned cat food, given 40 cc (subcutaneous) and then 100 cc (bolus) of Lactated Ringers Solution (LRS). At the direction of advising veterinarian, Dr. E. Dominguez (formerly of The Wildlife Center of Virginia, Waynesboro, VA), it was administered pyrantel pamoate (prescribed 6.5 ml [Q every 24 h] x 5 days) to treat *Giardia* and roundworms. Beef liver paste (pureed and diluted with water) was fed to the kitten during evening hours. Staff also topically applied Revolution Plus for cats (Zoetis, Kalamazoo, MI) as a second attempt to remove the ticks. This tick medication appeared to have no immediate effect.

By 9 September 2020, the kitten was less responsive, but treatments continued. This Bobcat was administered 40 cc (bolus) of LRS. At the direction of advising veterinarian Dr. K. Thomason (retired, Blue Ridge Veterinary Hospital, Floyd, VA), staff administered 1 cc of injectable Iron Dextran (100 mL iron; Vedco Inc., Saint Joseph, MO) for anemia, 1 cc of B-12 complex, and an additional 100 cc of LRS (bolus). The kitten also was force-fed 35 cc of pureed chicken liver. By the end of the day, the kitten was unresponsive.

On 10 September 2020, the Bobcat kitten was catheterized, and an LRS IV was started. Unfortunately, it succumbed to its multiple illnesses on this date.

PARASITE EXTRACTION

The deceased Bobcat was frozen and transferred to co-author Powers on 12 September 2020. Over the next several weeks, co-authors Marshall and Van Meter removed ticks from the carcass and sorted by stage: larva (<1 mm, 6 legs), nymph (1.5-2.5 mm, 8 legs), and adult (3-4 mm, 8 legs; Holderman & Kaufman, 2013). Ticks were preserved and remained separated by life stage: larva catalogued as RU 14554, nymphs catalogued as RU 14555, adults catalogued as RU 14556 (Fig. 1B). Nearly every tick was fully engorged; multiple exoskeletons also were recovered, but not counted in the totals.

Following the extraction of ticks from the carcass, co-author Powers prepared the Bobcat as a museum specimen, RU 14525. Once the internal organs were accessible post-taxidermy, co-author Sheehy investigated intestinal parasites. Six ascarid worms were located in the large intestine, and none in the small intestine. This location suggests that the pyrantel pamoate was effective, and the roundworms were exiting the kitten. Two of the worms were preserved (RU 14553; Fig. 1C).

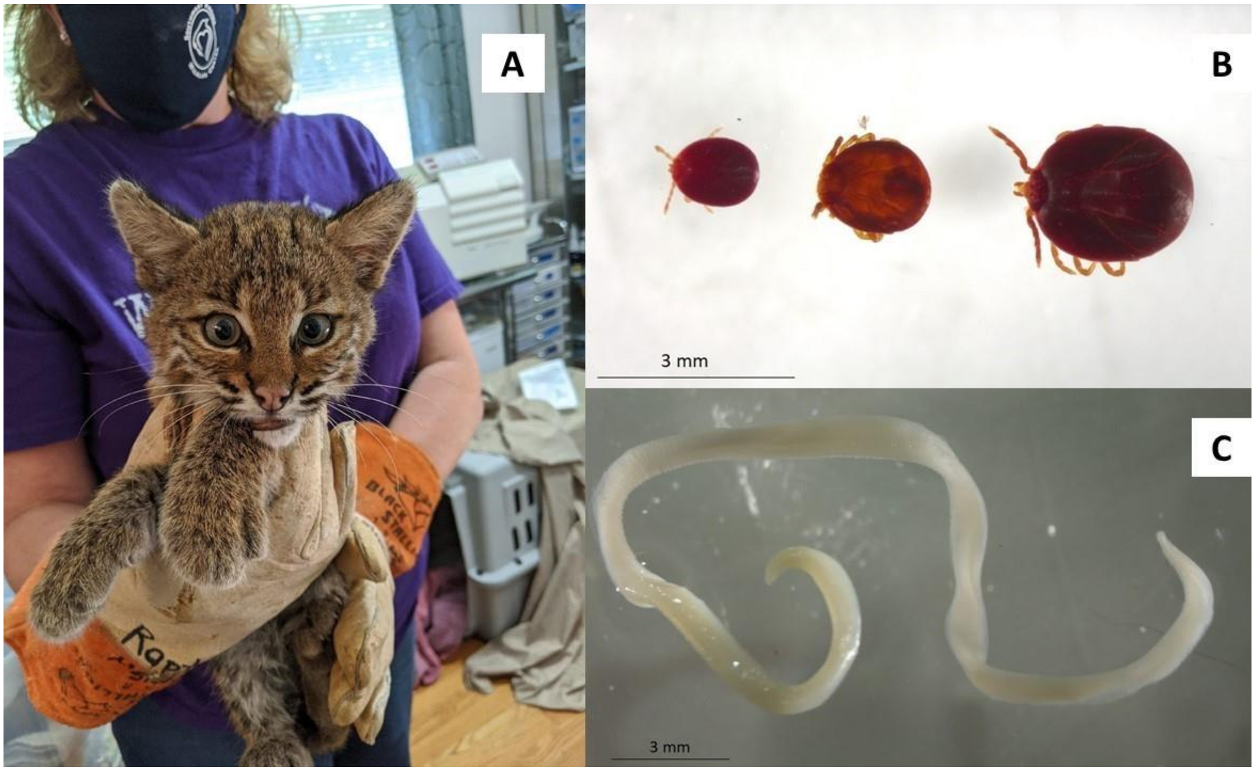

Figure 1. Images of a Bobcat (*Lynx rufus*) kitten and its parasites: A) Bobcat at time of admission to the Southwestern Virginia Wildlife Center of Roanoke on 7 September 2020; B) Lone Star Ticks (*Amblyomma americanum*; L-to-R: larva, nymph, adult) found on the pelage, and C) roundworm (*Toxocara cati*) found in the large intestine.

SPECIES IDENTIFICATION

We used DNA barcode analysis to identify the Bobcat's parasites to the species level. We collected tissue from four tick specimens (two adults and two nymphs; legs only, to avoid contamination with Bobcat blood), one roundworm specimen and the Bobcat itself (liver sample). Roundworm sections, tick legs and Bobcat liver samples (ca. 5-25 mg) were homogenized using disposable micropestles.

We extracted DNA from homogenized tissue using *Qiagen* DNeasy® Blood and Tissue kit (Qiagen Inc., Valencia, CA). Extracted DNAs served as templates for polymerase chain reaction (PCR) amplification of a 709-bp fragment of the mitochondrially-encoded COI gene. We included both positive and negative controls for each set of amplifications.

We amplified this fragment of the COI gene using M13 tailed primers. We used primers M13F-LCO 1490 and M13R-HCO 2198 modified from Folmer et al. (1994) to amplify DNA extracted from roundworm and tick samples. The Bobcat DNA template was amplified using primers VF1d_t1 and VR1d_t1 (Ivanova et al., 2006). Both the invertebrate primers and the vertebrate primers amplify the Folmer region of the COI gene - the standard mitochondrial region used in DNA barcoding. Both strands of PCR products were sequenced by Sanger Dideoxy sequencing performed by GeneWiz Inc. (www.genewiz.com). Assembly of forward and reverse sequences and manual trimming of primer sequences were performed using Codon Code Aligner (CodonCode Corporation, www.codoncode.com).

Consensus of forward and reverse COI sequences were compared with sequences in the nucleotide sequence database at NCBI using MegaBLAST (Zhang et al., 2000; Morgulis et al., 2008) with default settings. We also used the consensus sequence as a search query using the identification tool provided by *Barcode of Life Data Systems* (Ratnasingham & Hebert, 2007; www.boldsystems.org). We searched both databases using data available on 3 November 2020.

DNA barcoding identified each of the four ticks as Lone Star Ticks, *Amblyomma americanum*, with a 100% match to other members of this species in the database. Barcoding of the roundworm identified it as *Toxocara cati* with its DNA sequencing matching other sequences in the database with 99.5% similarity. The closest alternate taxon in the database, *Toxocara canis*, demonstrated 92.6% similarity and solidified our confidence in the identification. The DNA barcode sequence of the Bobcat matched other Bobcat entries in the database showing 99.85% sequence similarity; it does, however, contribute to the database by representing only the second sample from the United States.

ECTOPARASITE ANALYSES

Lone Star Ticks from the Bobcat carcass totaled 644 individuals: 342 larvae, 294 nymphs, and 8 adults. Co-author Garvin estimated an equal number removed from or recovered from around the kitten while in the Center's care. Ticks were especially concentrated inside the ear (e.g., 37 larvae were removed from an area of approximately 0.5 x 0.5 cm), all fully engorged. Lone Star Ticks are recognized as generalist ticks, able to jump among mammalian and avian hosts (Stafford, 2007). Indeed, when the Bobcat was kept in semi-isolation, ticks jumped from the Bobcat to a Red-tailed Hawk (*Buteo jamiacensis*) in the same triage room.

Lone Star Ticks typically are three-host ticks, feeding on a different host during each stage. They are aggressive pursuit ticks and will actively travel relatively long distances to find a host and will release pheromones to attract other Lone Star Ticks to the host (White & Gaff, 2018), and could likely explain the multiple life stages attached to the kitten. Once attached, larvae will blood-feed for 7-10 days. When engorged, the larvae drop off and digest the blood meal away from a host. They molt and reach the nymph stage. Nymphs will find and attach to a new host (via the same methods as larvae), feed for 10-18 days, disassociate from the second host, and molt into an adult. Adults attach to a third host to feed and mate. Adults may remain on the hosts up to 18 days (Holderman & Kaufman, 2013). Despite finding tick exoskeletons on the Bobcat kitten, there are no reports of Lone Star Ticks staying on the same host across life stages. The exoskeletons recovered were likely those that died (via failure to latch or hyperparasitism) on the Bobcat rather than actual molts (H. Gaff, Old Dominion University, personal communication).

This hard tick species will cause anemia but not exsanguination of the host (Goddard & Varela-Stokes, 2009). This Bobcat kitten's anemia may have been partly a result of the initial tick infestation, making it lethargic; this lethargy enabled the parasitism by additional ticks of multiple stages. Further, the presumed failure of tick treatments may have been an artifact of this hard tick species' behavior; Lone Star Ticks are more likely to die attached than to drop off the host (H. Gaff, personal communication).

Tick parasitism of Bobcats has been reported, but only quantified for adults. Wehinger et al. (1995) reported three tick species (99% in the adult stage) in 85 Bobcats in Florida: *Ixodes scapularis* (Deer Tick; prevalence rate: 71.7%), *Ixodes affinis* (no common name; prevalence rate: 12.9%), and *Dermacentor variabilis* (American Dog Tick; prevalence rate: 65.9%). Surprisingly, *Amblyomma americanum* and *Amblyomma maculatum* (Gulf Coast Tick) made up less than 1% of all ticks collected (Wehinger et al., 1995), despite being ranked as a high-density state for *A. americanum* (Shock et al., 2011). Although not quantified, *A. americanum* has been documented on adult and juvenile Bobcats in multiple studies (e.g., Shock et al., 2011; Zieman et al., 2017) often associated with the tracking of a protozoan parasite, *Cytauxzoon felis*. Lone Star Ticks are the primary tick to transmit this protozoan parasite that causes cytauxzoonosis, otherwise known as Bobcat fever (Glenn et al., 1983). If this Bobcat was infected by this protozoan, paralysis (Persky et al., 2020) and death (Nietfeld & Pollock, 2002) could have resulted within 24 h of infection. However, other publications suggest higher rates of infection in the wild (e.g., Zieman et al., 2017: 70.6% of 125 Bobcats in Illinois; Shock et al., 2011: 79% of 39 Bobcats in Missouri) with no apparent symptoms exhibited by the adult or juvenile Bobcats.

Gaff (personal communication) did suggest infection with *Rickettsia* (documented in Bobcats by Guzmán-Cornejo et al., 2019) as a possible underlying cause for the mortality of this kitten. Tissue samples have been preserved for testing at a later date.

ENDOPARASITE ANALYSES

Past endoparasitic studies of adult Bobcats included the presence of several roundworm species, including *Toxocara cati* and *Toxascaris leonina*. Of 146 Bobcats examined in the western United States, Carver et al. (2012) reported that 14 were parasitized by these roundworms. In a study of 50 Bobcats, Rollings (1945) reported a 36% prevalence rate of *Toxocara cati*. One adult Bobcat in the study was infested with 44 individual worms. Despite this high intensity parasite load, Rollings (1945) reported no obvious health effects. Hiestand et al. (2014) examined 67 Bobcats (52 adults, 15 juveniles) and found no significant difference in endoparasitic infection rates between age groups. Within this study, Bobcats in both age groups were infected by *Taenia releyi* (70% of the individuals), *Alaria marcianae* (42%), and *Toxocara cati* (25%).

The endoparasites we documented are Bobcat or generic felid specialists (Hiestand et al., 2014). Further, none of these publications suggest compromised health from the roundworms. It is unlikely that roundworms were the ultimate cause of the kitten's death.

Giardia is a protozoan parasite that causes infections not expected to be lethal. Carver et al. (2012) found that in some survey locations in Colorado, more than half the Bobcat fecal samples contained *Giardia* cysts or trophozoites. They discovered a greater number of cysts or trophozoites (in fecal matter) of *Giardia* at locations closer to areas of high densities of humans. Therefore, it is not surprising that this kitten (picked up in a suburban neighborhood in Bedford County) was infected. Although adult Bobcats are slightly more likely than juveniles to be infected with *Giardia*, the differences in prevalence were not significant (Carver et al., 2012).

VISUAL METRICS TO ESTIMATE BOBCAT AGE AND FURTHER ASSESS CONDITION

At the time of taxidermy, standard body measures were taken: total length = 430 cm, tail length = 70 cm, hind foot length = 103 cm, ear length = 45 cm, weight = 1250g. The weight suggested that efforts to rehydrate and feed the Bobcat added ca. 21% to its intake weight.

In order to determine if the Bobcat was medically underweight, we used several features to estimate its age, including tooth eruption patterns and comparison to published growth rate charts for kittens. First, an examination of the teeth revealed that its full complement of baby teeth had erupted, and it was missing one upper incisor (I1) and one lower incisor (I2). Using Jackson et al.'s (1988) chronological description of eruption and tooth replacement in young Bobcats, eruption patterns suggest the Bobcat was markedly older than 9 weeks old (when its full complement of baby teeth have erupted) but less than 16-18 weeks old (when the first and second adult incisors erupt; Jackson et al., 1988). After sharing dental photos with this lead author, Miller (née Jackson) estimated that the kitten was ca. 12 weeks old (D. Miller, University of Tennessee-Knoxville, personal communication). Second, we extrapolated growth rates from a kitten growth chart by Stys & Leopold (1993), presuming the published linear growth rates from Bobcats aged 0-7 weeks held true in subsequent weeks. Our total body and ear lengths suggested this kitten would be 13 weeks old, while the hind foot length suggested 12 weeks, and the tail length suggested 14 weeks. Were assumed that if Bobcats followed an asymptotic growth pattern, our age estimate would have been skewed higher.

The intake weight corresponded to a wild kitten weight of about 9 weeks (Stys & Leopold, 1993). Furthermore, Miller (personal communication) remarked that the skull presented visible serous fat atrophy. Such atrophy is a measure of the nutritional state of wild animals; muscle and bone marrow in severely malnourished individuals would appear gelatinous (Hooser et al., 2006). Although several vet schools can quantify bone marrow atrophy for this malady, the effort currently is cost-prohibitive; the long bones of this kitten will remain frozen, if future analyses are possible. This combination of metric evidence and visible serous fat atrophy supports our theory that the kitten was severely malnourished.

CONCLUSION

Despite the documentation of a number of parasites and a number of maladies, we cannot point to one ultimate cause of death. Although the combination of ticks, *Giardia*, and roundworms could have debilitated the animal, we presume that a still-undefined underlying condition may have been present. Once the kitten was lethargic, it was likely susceptible to increased tick infestation. The anemia was a result of or was enabled by the engorged Lone Star Ticks. The roundworm parasitism and *Giardia* infections were not unexpected or presumed life-threatening.

We present this case study as a way to document the numerous parasitic threats to a Bobcat kitten in the wild. To our knowledge, this kitten is the youngest to be examined in detail for parasites in published literature. We document the efforts to address myriad problems with said parasites, so that other wildlife rehabilitation professionals may learn what was successful and, more importantly, what was not. When wildlife rehabilitation centers receive these "worst" cases, it is sometimes too late for the animal to recover; this was certainly the case for the severely malnourished Bobcat kitten. Our research to determine the ultimate cause of death will continue, and the SWVA Wildlife Center will continue to collaborate with knowledgeable veterinarians on cases like this in the future.

ACKNOWLEDGEMENTS

We thank veterinarians Dr. E. Domiguez and Dr. K. Thomason for assistance with treating the Bobcat. We thank the staff at the Southwest Virginia Wildlife Center of Roanoke for their extraordinary efforts to help the kitten. Two LVTs, A. Crosswhite Miller and J. Spangler of Veterinarians to Cats (Roanoke, VA), catheterized the kitten. Veterinarian Dr. D. Miller (University of Tennessee-Knoxville, Knoxville, TN) provided insight into aging the Bobcat by tooth eruption, and diagnosed the condition of serous fat atrophy. Dr. H. Gaff (Old Dominion University, Norfolk, VA) provided information about Lone Star Tick life cycles, infection rates, and general behaviors. We thank the Biology Department at Radford University for providing supplies for this project. Dr. J. Lau (Radford University) assisted with preparation of parasite images.

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