The Presence of Protozoa in Oklahoma City Parks: *Theileria cervi* and *Babesia sp. Coco*

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Abstract

Babesia and *Theileria spp.* are tick transmitted protozoa parasites affecting various animals worldwide. *Theileria cervi* affects the white tailed deer populations of the United States, particularly fawns, and *Babesia sp. coco* was recently described and affects dogs in the United States. Ticks were collected from 15 sites in the Oklahoma City area and screened using polymerase chain reaction (PCR) assays. *T. cervi, B. sp. coco,* and a possible *Babesia sp.* were detected in the ticks, and a notable difference in prevalence rates was present in 3 sites. The presence of these pathogens suggests that their natural hosts are also present in the area, though more investigation is required to determine the source of these populations.

Introduction

Both babesiosis and theileriosis are diseases arising from protozoal infections commonly transmitted by ticks. Theileriosis specifically is caused by intraerythrocytic *Theileria* parasites (Cauvin et al. 2019, Dantas-Torres and Otranto 2016, Haus et al. 2018, Laird et al. 1988, Wood et al. 2013, and Yabsley et al. 2005). The species *Theileria cervi* was first described in the United States in 1962, and affects ungulates specifically. The white tailed deer is a major host in its life cycle, though mule deer, and elk can also become infected. The Lone Star tick, *Amblyomma americanum*, is the definitive host for *T. cervi,* as well as its vector (Cauvin et al. 2019, Haus et al. 2018, Hazen-Karr et al. 1987, Laird et al. 1988, Wood et al. 2013, and Yabsley et al. 2005). It is currently unknown if *A. americanum* nymphs can transmit *T. cervi*, so it is

believed to overwinter in nymphs and reach its infective stage when nymphs develop into adults (Laird et al. 1988). Though there does not appear to be any vertical transmission from mother deer to fawn, it was found that animals infected with *T. cervi* remain infective to ticks for at least 6 months (Cauvin et al. 2019).

T. cervi is considered less significant than other ungulate diseases due to its clinical rarity in adult individuals, but it can affect adults if certain conditions are met, such as high deer population, malnutrition, or presence of other diseases (Cauvin et al. 2019, Haus et al. 2018, Wood et al. 2013, and Yabsley et al. 2005). Though adult clinical signs are rare, fawn clinical signs are more common and can lead to mortality. Fawns experience more cases because they are younger, less mobile than adults, and hide in underbrush, leading to higher tick infestation rates and therefore higher *T. cervi* infection rates (Cauvin et al. 2019, Haus et al. 2018, Hazen-Karr et al. 1987, Laird et al. 1988, Wood et al. 2013, and Yabsley et al. 2005). As demonstrated by Wood et al. (2013), though it is rare for adults to be symptomatic, relocation from a nonendemic to an endemic area in tandem with heavy tick infestations can result in illness and death. Symptoms of general theileriosis include fever, labored breathing, pale mucous membranes, jaundice, and hemoglobinuria (Dantas-Torres and Otranto 2016). Documented cases of *T. cervi* also reported autolyzed cells, anemia, lung membrane inflammation, abomasum inflammation, enlarged spleen, liver lesions, leukocytosis, lethargy, weight loss, and/or death (Haus et al, 2018, Wood et al. 2013, and Yabsley et al. 2005).

Babesiosis is caused by *Babesia spp.*, another blood parasite transmitted primarily through ticks (Baneth 2017, Fujita et al. 2015, Lehtinen et al. 2007, Qurollo et al. 2017, and Shock et al. 2013). The newly recognized *Babesia sp. coco* affects dogs, was first identified in 2004 in a Labrador Retriever from North Carolina, and has since been identified in several cases

in North Carolina, Texas, and Oklahoma (Baneth 2017, Fujita et al. 2015, Lehtinen et al. 2007, and Shock et al. 2013). There was also a trend recorded between the infected dogs and travel to Mid-Atlantic states (Shock et al. 2013). Historically, any large *Babesia spp.* found in dogs was identified as *B. canis* until recently (Baneth 2017, Fujita et al. 2015, and Lehtinen et al. 2007). Though *B. sp. coco* is morphologically identical to *B. canis*, it is genetically distinct, and is more closely related to the horse parasite *B. caballi* and cattle parasite *B. bigemina* (Lehtinen et al. 2007). Though a protozoa suspected to be a *B. sp. coco* variant was identified in bobcats in Georgia, dogs are suspected to be the main host (Shock et al. 2013).

Classic Babesiosis signs include anemia, multiple inflammatory responses that can contribute to tissue hypoxia and organ malfunction, an enlarged spleen, fever, and death. However, babesiosis symptoms can differ wildly based on the species present (Baneth 2017, and Qurollo et al. 2017). The recorded cases of *B. sp. coco* reported anemia, bloody and mucoid diarrhea, severely low platelet counts, and abnormally low neutrophils, or white blood cell counts (Fujita et al. 2015, and Lehtinen et al. 2007). All the dogs infected were also immunosuppressed, including several dogs that underwent splenectomies and one that was receiving treatment for lymphoma, suggesting that immunosuppression plays an important role in contraction of *B. sp. coco* (Fujita et al. 2015, Lehtinen et al. 2007, and Shock et al. 2013).

Though multiple dogs have been identified with *B. sp. coco* in Oklahoma, there are currently no published papers with information regarding its presence in the natural tick population. Additionally, there has been little to no investigation of protozoan prevalence in the Oklahoma City area in general. This report attempts to address these issues by collecting ticks from this area and screening them using PCR assays to obtain data regarding protozoan prevalence.

Methods

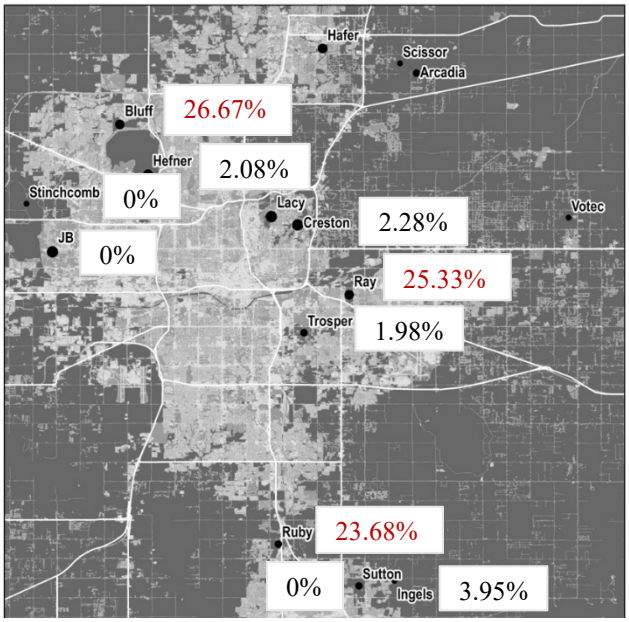
2155 ticks were sampled from 15 sites surrounding the Oklahoma City area between May and August of 2018. Each site had 4 sampling events. The 2155 ticks were organized into pools containing 1 to 10 ticks based upon species (*Amblyomma americanum, Amblyomma maculatum,* and *Dermacenter variabilis*), which site, and which round they were collected from, resulting in 353 pools. 1µl was taken from each pool and placed in 25µl total solutions containing 12.5µl 12.5 ml GoTaq® Green Master Mix, 10.5µl DNAse/RNAse free water, and 0.5µl each of primers BJ1 and BN2. These pools were then screened for protozoans using polymerase chain reaction (PCR) assays along with a negative control and DNA positive banding solution. The products were next separated by electrophoresis in agar and ethidium bromide gels in 1XTBE solution, and illuminated using ultraviolet light. The presence of bands was recorded, and pictures were taken of each gel for documentation. This particular PCR assay had not been used at Oklahoma State University before, and therefore did not have a positive control available. The first band detected was extracted and sent for sequencing to identify it and determine if the PCR primers were functioning properly, and was used as the basis for a positive sample. This process was repeated with individual tick solutions from each positive pool recorded. Once all individual ticks were screened and documented, 22 individuals were chosen based on suspected protozoan present and sample location to be screened again and extracted for DNA sequencing at a separate lab. The resulting DNA sequences were searched through the NCBI Blast database and identified.

Results

Of the 22 individuals sent to the lab for sequencing, 19 contained DNA that was a 100% match for *T. cervi*. The main strain of *T. cervi* was identified as isolate WTD 181 clone from

Oklahoma, though 1 individual was infected with a separate strain identified as a North Texas white-tailed deer clone. The remaining 3 individuals contained DNA that was 100% matched to *B. sp. coco,* and was identified as a 185 ribosomal RNA gene. Another strain of DNA that was 14% related to *Babesia spp.* in the system was detected, as well.

Figure 1: Map of sample sites with park prevalence rates for 3 notable and surrounding sites



Of the 2155 ticks screened, the PCR produced at least one band in a total of 130 individuals. 103 of these individuals displayed a upper band associated with *T. cervi* and *B. sp. coco.* 99 of these infected ticks were *A. americanum*, 3 were *A. maculatum,* and 1 was *D. variabilis* (Table 1). The overall statistically predicted prevalence of this specific band in *A. americanum* was 4.90%, the prevalence in *A. maculatum* was 5.56%, and the prevalence in *D. variabilis* was 1.32%. The other 27 individuals produced a different band that was thought to be associated with another *Babesia sp.*, and were found only in *A. americanum* ticks. The overall prevalence of this band in *A. americanum* was 1.34%.

Out of the 15 sites sampled, 3 sites contained a notable difference in prevalence rates of *T. cervi* and *B. sp. coco* in *A. americanum* ticks. The prevalence rate for Ruby was 23.68%, Ray was 25.33%, and Bluff was 26.67%. The remaining rates for this category ranged from 0% to 20%. The prevalence rates for the unnamed *Babesia sp.* ranged from 0% to 50% (Table 2)..

Table 1: Number of ticks with protozoa present and total numbers tested by species.

Species	*T. cervi*	*B. sp. coco*	# ticks tested
A.americanum	99	27	2005
A. maculatum	3	0	54
D. variablilis	1	0	76
Grand Total	103	27	2155

Table 2: Developed land (%), species caught, protozoa positive, number of ticks caught, and site prevalence rates for *T. cervi* and *B. sp. coco* (%)

Site	Surrounding developed land (%)	Species	*T. cervi*	*B. sp. coco*	# ticks tested		Park prev *T. cervi/B. sp. coco* (%)	Park prev *Babesia* (%)
		aam						
jb	100		0	1	2		0.00	50.00
creston	92.5		5	3	219		2.28	1.37
lacy	84.9		7	2	128		5.47	1.56
hafer	76.8		0	1	147		0.00	0.68
ray	73.5		19	1	75		25.33	1.33
hefner	71.1		1	3	48		2.08	6.25
bluff	66		8	0	30		26.67	0.00
ruby	54.4		18	0	76		23.68	0.00
sutton	53.3		0	0	1		0.00	0.00
trosper	52.8		6	2	306		1.96	0.65
arcadia	52		6	6	176		3.41	3.41
stinchcomb	36.5		0	0	1		0.00	0.00
scissor	33		10	1	147		6.80	0.68
ingels	24.6		7	6	177		3.95	3.39
votec	16.9		12	1	472		2.54	0.21
		amac						
bluff	66		1		5		20.00	0.00
ruby	54.4		2		21		9.52	0.00
		dv						

ruby	54.4		1		10		10.00	0.00

Discussion

Since only the 22 selected individuals were sent for sequencing, it is impossible to identify the exact numbers of ticks infected with which protozoa. However, we can statistically surmise that out of the 2155 ticks sampled and screened, a total of 130 were possibly infected with *T. cervi, B. sp. coco,* or an unnamed *Babesia sp.* at the prevalence rates listed in the results and Tables 1 and 2..

This statistical analysis extends to the site prevalence rates. It should be noted that the three sites with larger prevalence rates than their neighbors (Table 2 and Figure 1), Ray, Bluff, and Ruby, also have larger percents of developed land around them than a majority of the other sites. This suggests that populations of hosts for the two respective protozoa are nearby and are accessible to the local tick population despite the land development. This could include possible deer farms, and wild deer that are somehow present for *T. cervi*. Laird et al. (1988) found that ticks can only be infected by deer with parasitemia above 1%, and a 100% infective rate was found at 6%. This data suggests that at least some the host population present in the Oklahoma City area for this study must also be above 1% parasitemia to infect the ticks. The presence of *B. sp. coco* may be due to the presence of pet or stray dogs in the area. Shock et al. (2013) found that bobcats can contain possible *B. sp. coco* variants, so investigation regarding whether bobcats or other animals can support *B. sp. coco* infection or transmission may assist in understanding prevalence rates. The definite cause of high prevalence rates would need to be further investigated in the future.

Additionally, the unnamed *Babesia sp.* requires further study. The sequence was revealed to be a 14% match to *Babesia spp.* present in the NCBI Blast database, suggesting that the sequence is either a new species or strain to the system, or a sequence that is merely similar. Regardless, it requires further investigation.

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