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Ticks and tick-borne microbes identified through passive and active surveillance in Alaska

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Rapid environmental change in Alaska and other regions of the Arctic and sub-Arctic has raised concerns about increasing human exposure to ticks and the pathogens they carry. We tested a sample of ticks collected through a combination of passive and active surveillance from humans, domestic animals, and wildlife hosts in Alaska for a panel of the most common tick-borne pathogens in the contiguous United States to characterize the diversity of microbes present in this region. We tested 189 pooled tick samples collected in 2019-2020 for *Borrelia* spp., *Anaplasma* spp., *Ehrlichia* spp., and *Babesia* spp. using a multiplex PCR amplicon sequencing assay. We found established populations of *Ixodes angustus* Neumann *(Acari: Ixodidae)*, *Ixodes uriae* White *(Acari: Ixodidae)*, and *Haemaphysalis leporispalustris* Packard (Acari: Ixodidae) in Alaska, with *I. angustus* found on a variety of hosts including domestic companion animals (dogs and cats), small wild mammals, and humans. *Ixodes angustus* were active from April through October with peaks in adult and nymphal activity observed in summer months (mainly July). Although no known human pathogens were detected, *Babesia microti*-like parasites and *candidatus* Ehrlichia khabarensis were identified in ticks and small mammals. The only human pathogen detected (*B. burgdorferi* s.s.) was found in a tick associated with a dog that had recently traveled to New York, where Lyme disease is endemic. This study highlights the value of a combined passive and active tick surveillance system to detect introduced tick species and pathogens and to assess which tick species and microbes are locally established.

Key words: surveillance, Alaska, tick, pathogen, phenology

Introduction

Rapid environmental change in Alaska and other regions of the Arctic and sub-Arctic has raised concerns about increasing human exposure to ticks and the pathogens they carry (Hvidsten et al. 2014, Khasnatinov et al. 2016, Larsson et al. 2018, Soleng et al. 2018, Hahn et al. 2020). Climatic changes may make these regions more suitable habitat for medically important tick species due to the major role of climatic conditions on tick viability (Eisen et al. 2015). In particular, more mild winters and an increase in cumulative growing degree days in Alaska's more temperate areas may make these regions more suitable for *Ixodes pacificus* (Acari: Ixodidae) in the coming decades (Witmer et al. 2022). Fifteen species of ticks have been documented in Alaska, with *I. angustus* Neumann (Acari: Ixodidae) being the most common human-biting tick, and representing 58% of tick records between 1909 and 2019 (Hahn et al. 2020). Despite the risks that ticks pose to humans and wildlife, there has been limited pathogen testing in ticks or tick-infested wildlife collected in Alaska. Olsen et al. (1995) examined 32 *Ixodes uriae* White (Acari: Ixodidae) ticks from Alaskan tufted puffins (*Fratercula cirrhata*) and fork-tailed storm petrels (*Oceanodroma furcata*) and found *Borrelia*

spp. spirochetes that they identified as *Borrelia garinii* Baranton (Spirochaetales: Spirochaetaceae) in 6% of the ticks (range 0–8% by species). Deardorff et al. (2013) found serologic evidence of a Powassan-like virus in 6% of voles tested from central and southeastern Alaska; however, they found no viral RNA in the *I. angustus* ticks collected from the mammals. The most comprehensive assessment to date found that nearly half of the rodents (voles, mice, and shrews) from coastal areas (Gulf of Alaska, Southeast, and the Seward Peninsula) were positive for parasites in the *Babesia microti* species complex (Goethert et al. 2006). Globally, the *Ba. microti* complex is comprised of at least 5 phylogenetic clades, of which only 1 has been associated with human babesiosis (Goethert et al. 2021). Although no ticks were tested in the Goethert et al. (2006) study, they noted *I. angustus* on the rodents from southern Alaska. Finally, serologic surveys over the last several decades have found evidence of infection with *Francisella tularensis* McCoy and Chapin (Thiotrichales: Francisellaceae) in a wide variety of wildlife species across the state (Hansen et al. 2011).

In this study, we examined ticks collected from wildlife, companion animals, or people in 2019 and 2020 through a passive surveillance system in Alaska, as well as tick samples collected through systematic small mammal trapping in Anchorage, Alaska paired with blood and tissue samples from their small mammal hosts. We used a multiplex PCR amplicon sequencing assay to assess the diversity of tick-borne microbes present in ticks collected in the state.

Methods

Passive Surveillance for Ticks

Most tick samples were collected through a statewide passive surveillance program (Alaska Submit-A-Tick Program) (Hahn et al. 2020). Through this program, the public, veterinarians, clinicians, and biologists can voluntarily submit ticks that they find on themselves, a family member, a pet, in the environment, or on wildlife to the Office of the State Veterinarian. With each tick submission, we requested information on the date of tick collection, tick host, probable geographic location of tick encounter, and history of travel inside or outside of Alaska of anyone or any pet within a submitter's household within the 2 wk prior to submission. Contact information for the submitter is voluntary. Data are deidentified by the Office of the State Veterinarian before they are shared with collaborators at the University of Alaska Anchorage. Empty vials are available upon request, but submitters generally submit ticks in their own container, without any preservative. The program is advertised through posters at veterinary clinics and Fish and Game Offices, public presentations at local events (e.g., summer festivals and conferences), and through the Alaska Submit-A-Tick website.

Active Surveillance for Ticks

Following up on drag sampling for ticks conducted in Summer 2019 (Hahn et al. 2020), we used the same protocol to re-sample 9 recreational sites in southcentral Alaska including parks and campgrounds with trails, off-leash dog parks, and forested areas in order to target locations with overlap between human, dog, and wildlife activity. Briefly, we sampled for ticks by dragging a 1-m^2 cloth made of rubber-bonded cotton fabric with a rope attached to a 1.2 m dowel inside the top edge. Weighted "fingers" were sewn to the bottom half of the drag in order to sample near the ground. The sites in Anchorage were Far North Bicentennial Park (61.1559°N, 149.7515°W), University Lake Park (61.1848°N, 149.8077°W), Ruth Arcand Park (61.1374°N, 149.8123°W), Connors Lake Park (61.1723°N, 149.9409°W), and Kincaid Park (61.1536°N, 150.0554°W). On the Kenai Peninsula, we sampled Centennial Park in Soldotna 60.4799°N, 151.0928°W, Hidden Lake Campground in the Kenai National Wildlife Refuge (60.4666°N, 150.2051°W), Slidehole Campground in Anchor Point (59.7700°N, 151.8556°W), and Jack Gist Park in Homer (59.6591°N, 151.4819°W). All sites were characterized by deciduous or mixed wood forest with dense vegetation in the understory. We drag sampled 1,000 m^2 per occasion at the Anchorage sites 4 times between 4 June and 25 July 2020, and we drag sampled 1,000 m^2 per occasion at the Kenai Peninsula sites twice, once in mid-June and once at the beginning of July.

We also conducted small mammal trapping for 3 nights in July 2020 in 2 of the public recreational sites in Anchorage (Far North Bicentennial Park and Kincaid Park). We set 100 Sherman traps along 4 linear transects (25 traps on each transect) and 10 Tomahawk traps, interspersed within the trapping grid. All traps were spaced 10 m apart. Sherman traps were covered with waterproof paper and placed in the shade or under tall grass where possible. Traps were baited with a mixture of peanut butter and oats as well as ~4 g of freeze-dried mealworms to reduce shrew mortality. A small cotton square was placed in each trap to provide animals with bedding.

Upon capture, animals were transferred to a Ziploc bag and anesthetized by placing a few drops of isoflurane on a cotton ball and holding it inside the closed plastic bag. Following anesthesia, animals were weighed and measured, and the species and sex were recorded. All animals were checked for ticks, and all ticks from an animal were placed in a vial with 1 ml of ethanol. Ear biopsies and blood samples were attempted on all unstressed animals (as determined by visual cues such as rapid breathing with lack of response). Ears were disinfected with an alcohol prep pad and a biopsy was taken using a 2-mm punch tool. Samples were stored in a vial with 1 ml of ethanol. Blood samples were taken from ear punch sites if possible, or from the base of the tail using a Goldenrod lancet (Braintree Scientific, Braintree, MA). We collected between 10–20 µl of blood on a Whatman filter paper strip and then placed the dried paper strip in a 1.5 ml vial for storage. After sampling, animals were placed in a plastic bin with holes until they fully recovered, at which time they were released at their capture site. The animal handling protocol was approved by the University of Alaska IACUC (Protocol # 1624005).

Tick Identification and DNA Extraction

All ticks were morphologically identified to species and life stage at Georgia Southern University using published taxonomic keys (Cooley and Kohls 1944, Cooley 1946a, 1946b, Brinton et al. 1965, Yamaguti et al. 1971, Keirans and Clifford 1978, Robbins and Keirans 1992, Durden and Keirans 1996, Estrada-Peña et al. 2017). Ticks were stored in vials of 80–100% ethanol at –20 °C prior to DNA extraction. If more than 1 tick was collected from a single host, ticks were pooled by species and life stage for pathogen testing because our goal was to assess pathogen presence rather than prevalence (e.g., If 5 *I. angustus* nymphs were collected from a single vole, all ticks were pooled for pathogen testing. If 3 *I. angustus* nymphs and 2 *I. angustus* adults were found on a single vole, the ticks were separated into 2 pools – 1 for the nymphs and 1 for the adults. If 2 *I. uriae* nymphs were found in the same tufted puffin colony at the same time, the ticks were pooled for testing). There was no limit on pool size, and in cases where only 1 tick was found on a host, this tick was tested individually (i.e., a pool size = 1 tick).

Table 1. Pooled tick samples/number of ticks per pool collected in Alaska in 2019 and 2020 through the Alaska Submit-A-Tick Program and tick drags and included in this study by species, host, and life stage

Host	No. pools (% pools by host)	Amblyomma americanum			Dermacentor andersoni			Dermacentor variabilis			Haemaphysalis leporispalustris			Ixodes angustus			Ixodes auritulus			Ixodes pacificus			Ixodes scapularis			Ixodes signatus			Ixodes uriae			Rhipicephalus sanguineus		
		A[a]	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L	A	N	L
Domestic animal																																		
Canis lupis (dog)	57 (30.8)				3/3			5/6						42/48	2/2					1/1			1/4									3/3		
Felis catus (cat)	31 (16.8)													28/35	3/3																			
Gallus gallus domesticus (chicken)	2 (1.1)											1/1					1/1																	
Wildlife (birds)																																		
Hydrobates furcatus (fork-tailed storm petrel)	5 (2.7)																													5/10				
Hydrobates leucorhous (Leach's storm petrel)	4 (2.2)																													4/7				
Uria lomvia (thick-billed murre)	2 (1.1)																												2/2					
Aethia pusilla (least auklet)	1 (0.5)																													1/1				
Turdus migratorius (American robin)	1 (0.5)												1/1																					
Urile urile (red-faced cormorant)	1 (0.5)																									1/2								
Junco hyemali (slate-colored junco)	1 (0.5)												1/12																					
Wildlife (mammals)																																		
Clethrionomys rutilus (northern red-backed vole)	18 (9.7)													16/26	2/8																			
Unidentified vole spp.	7 (3.8)													7/18																				
Unidentified squirrel spp.	11 (5.9)													10/25	1/8																			
Unidentified mouse spp.	11 (5.9)													10/16	1/2																			
Unidentified hare spp.	10 (5.4)										4/86			4/10	2/7																			
Erethizon dorsatum (porcupine)	1 (0.5)													1/3																				
Human	13 (7.0)		1/1					4/4						4/4	1/1														1/1	1/1		1/1		
Environment	9 (4.9)													5/5									1/1						3/19					
TOTAL POOLS	185	0	1	0	3	0	0	9	0	0	4	1	2	127	12	0	1	0	0	1	0	0	2	0	0	1	0	0	6	11	0	4	0	0
TOTAL TICKS	389	0	1	0	3	0	0	10	0	0	86	1	13	190	31	0	1	0	0	1	0	0	5	0	0	2	0	0	22	19	0	4	0	0

[a]A = Adult, N = Nymph, L = Larva.

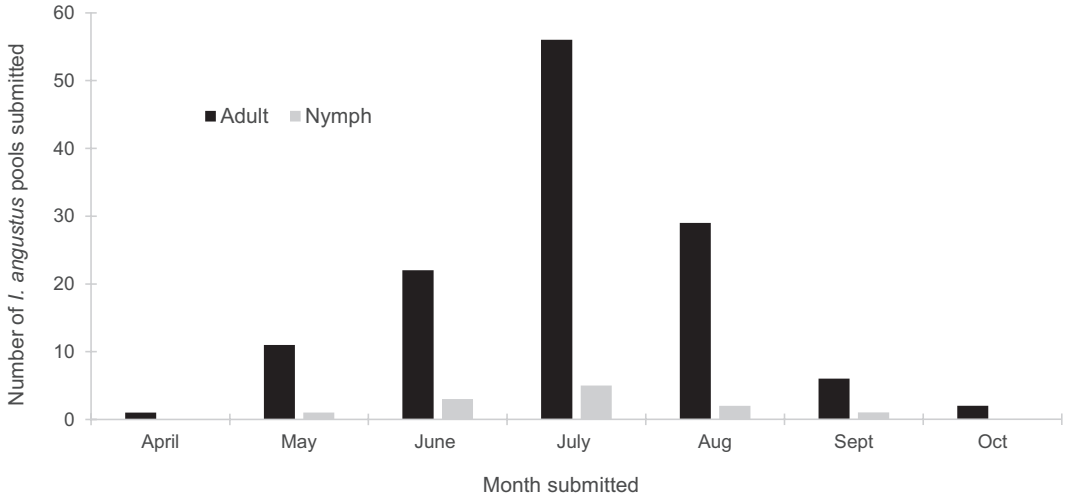

Fig. 1. Seasonality of *Ixodes angustus* adults and nymphs submitted to the Alaska Submit-A-Tick Program in 2019 and 2020 (shown as number of pools, or independent submissions to the surveillance program).

Table 2. Bacteria and parasite species identified in ticks found in Alaska in 2019 and 2020 collected through the Alaska Submit-A-Tick Program and tick drags

Pathogen species	Total tick pools (n = 185) that tested positive[a]	Total tick samples that tested positive by species (%)			
		I. angustus (n = 139)	*H. leporispalustris* (n = 7)	*I. uriae* (n = 17)	*I. scapularis* (n = 2)
Babesia species					
Ba. microti-Clethrionomys	19 (9.8%)	19 (13.7%)	–	–	–
Ba. microti-Sorex	8 (4.2%)	8 (5.8%)	–	–	–
uncharacterized *Babesia* species	1 (0.5%)	–	1 (14.3%)	–	–
Ehrlichia species					
candidatus E. khabarensis	24 (12.4%)	23 (16.5%)	1 (14.3%)	–	–
Ehrlichia spp. (clone 10b)	1 (0.5%)	–	–	1 (5.9%)	–
Borrelia species					
B. burgdorferi B31	1 (0.5%)	–	–	–	1 (50.0%)[b]

[a]Nine tick samples were infected with more than one organism.
[b]This *I. scapularis* was found on a dog in Alaska with recent travel to New York.

After identification, ethanol was aspirated off and samples were air-dried for 5 min. Samples were placed on wax paper and bisected using a sterile scalpel and put into 2 separate microcentrifuge tubes for homogenization in DNA extraction and RNA extraction. Liquid nitrogen was used to flash freeze samples and a sterile pestle was used to crush and homogenize the sample until it was mostly powder. Phosphate buffered saline solution (PBS, 200 µl) was added to suspend the homogenate. GeneJET DNA Purification Kit was used following ThermoFisher Scientific B protocol with the following changes: Samples were incubated at 56 °C and vortexed at 1,400 rpm for 1 h with lysis buffer and proteinase K. Samples were eluted in 45ul of DNase free dH_2O and stored at –20 °C.

Pathogen Testing

We tested for genera of parasites or bacteria previously described as pathogenic in humans and spread by tick vectors (Eisen et al. 2017, Hojgaard et al. 2020). We used a previously described multiplex PCR amplicon sequencing assay where PCR primers were designed to amplify genera of both parasite and bacterial DNA (Hojgaard et al. 2020). In addition to the PCR primers detecting the microbes, the PCR multiplex also has PCR primers that will amplify tick DNA (actin) and therefore serve as a positive control for both the presence and quality of DNA. The genera specific multiplex PCR reactions were performed in 25 µl, which included 12.5 µl 2× Sso Advanced (BioRad, Hercules, CA, USA), 10 µl tick nucleic acids extract, and 2.5 µl PCR primers resuspended in PCR grade water. Following the multiplex PCR reaction NGS sequencing libraries were generated as previously described (Hojgaard et al. 2020, 2021), and sequencing was performed using the MiSeq system (Illumina, San Diego, CA) with the MiSeq reagent kit Nano 500V2 according to the manufacturer's protocol (Illumina). All sequences were analyzed using CLC Genomic Workbench (Qiagen) software, and reference sequences as previously described (Hojgaard et al. 2020).

Table 3. Wildlife species and number of pooled *Ixodes angustus* samples collected and total number of ticks collected through small mammal trapping in Anchorage, Alaska, 2020

Species	Total animals	Number animals with ticks (%)	Number of pooled tick samples collected/ total number of ticks collected		
			Adult	Nymph	Larvae
Clethrionomys rutilus (northern red-backed vole)	34	8 (2.4)	4/4	5/6	4/6
Sorex cinereus (cinerus shrew)	17	2 (11.8)	–	1/1	1/11
Microtus pennsylvanicus (meadow vole)	3	2 (66.7)	–	2/2	–
Tamiasciurus hudsonicus (red squirrel)	1	1 (100)	–	1/2	–
TOTAL POOLS (*n* = 18)			4	9	5
TOTAL TICKS (*n*= 32)			4	11	17

Results

Ticks Submitted Through the Alaska Submit-A-Tick Program

From 1 January 2019 to 31 December 2020, a total of 491 individual ticks were collected through the Alaska Submit-A-Tick Program. These were aggregated by tick host, tick species, and life stage into 227 pools. Forty-two individual ticks or pools of ticks (ticks of the same life stage collected from an individual host) were excluded from subsequent analysis if they could not be definitively identified to species or if DNA was not of suitable quality for testing. Subsequent results refer to the final sample of 185 pooled samples, representing 389 individual ticks (Table 1). For example, 4 pools of adult *Haemaphysalis leporispalustris* Packard (Acari: Ixodidae) were tested for this study. These ticks were collected from 4 hares found at different dates and locations. There were 86 total adult ticks in the 4 pools, and the pool sizes varied depending on the number of ticks collected from each animal (e.g., The 4 pools were 3 ticks, 5 ticks, 29 ticks, and 49 ticks).

The 185 tick pools included twelve species, the majority of which were *I. Angustus* (75.1%, *n* = 139 pools and 221 individual ticks). The majority of *I. angustus* were submitted in summer months with a peak in July, but this species was found in Alaska between April and October (Fig. 1). Other species collected include *I. uriae* (9.3%, *n* = 17 pools and 41 individual ticks), *Dermacentor variabilis* Say (Acari: Ixodidae) (4.9%, *n* = 9 pools and 10 individual ticks), and *H. leporispalustris* (3.8%, *n* = 7 pools and 100 individual ticks). More than 85% (*n* = 158) of the tick samples were adults, 13.5% (*n* = 25) were nymphs, and only 1.0% (*n* = 2) were larvae. Of the adult tick samples, 93.7% (*n* = 148) were females. The mean infestation for *I. angustus* on wildlife was 2.3 ticks per animal (range: 1–10) with 76% (41/54) of wildlife having 1 or 2 ticks collected from them. In contrast, the mean infestation for *H. leporispalustris* in wildlife was 14.3 ticks per animal (range: 1–49).

Nearly half (48.7%, *n* = 90 pools) of the tick pools were collected from domestic animals (*Canis familiaris*: 30.8%, *n* = 57 pools; *Felis catus*: 16.8%, *n* = 31 pools; *Gallus gallus domesticus*: 1.0%, *n* = 2 pool), and 39.5% of the pools (*n* = 73) were collected from wildlife. Wildlife hosts included several bird species, including 5 seabirds and 2 songbirds: fork-tailed storm petrel (*Hydrobates furcatus*) (5 pooled samples containing 10 individuals), Leach's storm petrel (*Hydrobates leucorhous*) (4 pooled samples containing 7 individuals), thick-billed murre (*Uria lomvia*) (2 pooled samples containing 2 individuals), least auklet (*Aethia pusilla*) (1 pooled sample containing 1 individual), American robin (*Turdus migratorius*) (1 pooled sample containing 1 individual), red-faced cormorant (*Urile urile*) (1 pooled sample containing 2 individuals), and slate-colored junco (*Junco hyemalis*) (1 pooled sample containing 12 individuals). Several species of small mammalian wildlife were reported as hosts for submitted ticks, most commonly, the northern red-backed vole (*Clethrionomys rutilus*) (18 pooled samples containing 34 individual) and unidentified squirrel species, likely *Tamiasciurus hudsonicus* (11 pooled samples containing 33 individuals). Thirteen (7.0%) of the tick pools were found on humans, and 9 pools (4.9%) were found in the environment off of a host, including inside buildings (4 pooled samples containing 4 individuals), in a tufted puffin (*Fratercula cirrhata*) colony habitat (3 pools containing 19 individuals), on a small mammal trap (1 pooled sample containing 1 individual), and on a tick drag (1 pooled sample containing 1 individual).

Of the 103 tick pools from domestic animals or humans, we had information on travel history for 87 samples. Of these, 17.2% (*n* = 15) are considered non-native tick species to Alaska and therefore travel history of their hosts is of interest (Hahn et al. 2020). Of the 9 *D. variabilis* found in 2019–2020 in Alaska, 6 were from hosts with no reported recent travel outside of the state (2 from dogs and 4 from humans), and of the 4 *R. sanguineus* Latreille (Acari: Ixodidae), 3 were from hosts without reported recent travel outside the state (2 from dogs and 1 from a human). All other non-native ticks with information on travel history (*n* = 6) were found on hosts that had traveled outside of Alaska within the 2 wk prior to finding the tick.

Bacteria and Parasites Detected in Ticks Submitted Through the Alaska Submit-A-Tick Program

Bacteria or parasites were detected in 25.9% (*n* = 48) of tick pools (Table 2). *Babesia* was the most common genus identified, with 15% of pools (*n* = 28) testing positive, followed by *Ehrlichia* species (identified in 13.5% of pools, *n* = 25). One tick pool (0.5%) tested positive for *Borrelia* species.

Babesia species. Two *Babesia* types that were described previously as *Ba. microti* (GenBank locus AY144687 and AY918952) were identified from *I. angustus* infesting small mammals. The Babesia type described in GenBank locus AY144687 was derived from a *Clethrionomys* spp. red-backed vole (*Ba. microti-Clethrionomys*) and produced a PCR amplicon that is 280 bp long (post primer trimming). *Babesia microti-Clethrionomys* is 93% (261 bp/280 bp) similar to the human pathogenic *Ba. microti* isolate RI. The other Babesia type described in GenBank locus AY918952 was derived from a *Sorex* spp. shrew (*Ba. microti-Sorex*) and produced a PCR amplicon that was 279 bp long (post primer trimming). *Babesia*

Table 4. Bacteria and parasite species identified in blood samples from small mammals collected in Anchorage, Alaska, 2020

Small mammal and bacteria/ pathogen species	Total number of animals tested	Prevalence (no. positive)[a]
Clethrionomys rutilus	17	
candidatus Ehrlichia khabarensis		5.9% (1)
Ba. microti-Clethrionomys		41.2% (7)
Ba. microti-Sorex		41.2% (7)
Microtus pennsylvanicus	3	
Ba. microti-Clethrionomys		33.3% (1)
Tamiasciurus hudsonicus	1	0% (0)

[a]Ear samples were initially tested for *Borrelia spp.* and none were positive so only blood sample results are shown. All blood samples were screened for *Anaplasma spp.*, *Ehrlichia spp.*, and *Babesia spp.* If the organism is not listed under the mammal, then the prevalence was 0%.

Table 5. Bacteria and parasite species identified in *Ixodes angustus* from small mammals collected in Anchorage, Alaska, 2020[a]

Pathogen species	Total tick samples (n = 20) that tested positive	Prevalence in *I. angustus* samples by host (No. positive)			
		Clethrionomys rutilus (northern red-backed vole) (n = 15)	*Sorex cinereus* (cinerus shrew) (n = 2)	*Microtus pennsylvanicus* (meadow vole) (n = 2)	*Tamiasciurus hudsonicus* (red squirrel) (n = 1)
Babesia species[b]					
Ba. microti-Clethrionomys	11 (55.0%)	66.7% (10)	0% (0)	50.0% (1)	0% (0)
Ba. microti-Sorex	5 (25.0%)	33.3% (5)	0% (0)	0% (0)	0% (0)
Ehrlichia species *candidatus* E. khabarensis	4 (20.0%)	26.7% (4)	0% (0)	0% (0)	0% (0)

[a]All ticks found on mammals in the trapping effort were *Ixodes angustus*.
[b]Three tick samples tested positive for *Ba. Microti-Clethrionomys* and *Ba. Microti-Sorex*.

microti-Sorex is also 93% (261 bp/279 bp) similar to the human pathogenic *Ba. microti* isolate RI. The 2 *Babesia microti*-like types (*Ba. microti-Clethrionomys* and *Ba. microti-Sorex*), are nearly identical to each other with *Ba. microti-Clethrionomys* containing 1 extra bp. Neither is identical to the human disease-causing variant; we subsequently refer to these as *Ba. microti*-like organisms.

Of the 28 tick pools that screened positive for *Babesia* species, 19 (67.9%) were infected with a strain 100% identical to *Ba. microti-Clethrionomys*, 7 (25%) were infected with a strain that is 100% identical to *Ba. microti-Sorex*, and 1 (3.6%) was infected with a strain that was 99.7% identical to *Ba. microti-Sorex*. Nine of the *I. angustus* infected with *Ba. microti-Clethrionomys* were found on voles (either *C. rutilus* or unidentified species), 5 on other small wildlife, 4 on domestic animals, and 1 on a human (no information provided about whether the tick was attached or crawling on the human). Two of the domestic animal hosts and the human had no recent travel history outside the state, and we did not have travel history for the other 2 domestic animals. Four of the *I. angustus* infected with *Ba. microti-Sorex* were found on voles (*C. rutilus* or unidentified species), 1 was found on a mouse (unidentified species), and 3 were found on domestic animals. Two of the domestic animal hosts had no recent travel history outside the state, and we did not have travel history for the other domestic animal. All *Ba. microti*-like samples were from hosts in Southcentral Alaska (Anchorage, Cook Inlet, the Matanuska-Susitna Valley, Kenai Peninsula, Kodiak Island) or Southeast Alaska. One pooled sample of 29 *H. leporispalustris* found on a hare (unidentified species, likely *Lepus americanus*) in Fairbanks, was infected with a *Babesia* species most similar (95%, 225 bp/238 bp) to an isolate previously isolated from *Macropus giganteus* (GenBank locus KM206783).

Ehrlichia species. All but 1 of the 25 tick pools that screened positive for *Ehrlichia* species were infected with *candidatus* E. khabarensis. The majority of the tick pools that tested positive for *candidatus* E. khabarensis were *I. angustus* (n = 23) found on wild rodents and hares (n = 12) including mice, voles, hares, and squirrels. Nine *I. angustus* tick samples that tested positive for *candidatus* E. khabarensis were found on domestic animals, 8 of which had no recent travel outside the state (we did not have travel history for 1 dog). Two *I. angustus* were found on humans with no recent travel outside the state (1 of these ticks was co-infected with a *Ba. microti*-like strain. No information was provided about whether either tick was attached or crawling on the humans). A pool of 3 *H. leporispalustris* found on a snowshoe hare (*Lepus americanus*) in the Matanuska-Susitna Valley was also infected with *candidatus* E. khabarensis. All tick pools that tested positive for *candidatus* E. khabarensis were found on hosts in Southcentral, Southeast, or Southwest Alaska (Anchorage, the Matanuska-Susitna Valley, Kenai Peninsula, Kodiak Island, Southeast Alaska, or communities on Bristol Bay). One *I. uriae* found crawling in the habitat of a tufted puffin (*F. cirrhata*) colony on Aiktak Island in the eastern Aleutian Islands was infected with an *Ehrlichia* sp. that has only been detected in Chile.

Borrelia species. The 1 tick sample that tested positive for *Borrelia* species was infected with a strain 100% identical to *B. burgdorferi* sensu stricto, *B. burgdorferi* B31. This was an *I. scapularis*, collected from a domestic dog in Eagle River, Alaska with recent travel to rural, upstate New York.

Babesia and Ehrlichia coinfection in ticks. Nine tick samples (4.9%), all *I. angustus*, were infected with more than 1 organism. Eight were positive for *candidatus* E. khabarensis and *Ba.*

Microti-Clethrionomys, and 1 was positive for *candidatus* E. khabarensis and *Ba. Microti-Sorex*

Wildlife and Ticks Collected Through Small Mammal Trapping in Anchorage and Tick Drags in Southcentral Alaska

Over a period of 660 trap nights at 2 collection sites in Anchorage (3 nights in each site, 110 traps used each night), we collected 32 ticks from 55 small mammals (Table 3). All ticks collected through this trapping effort were identified as *I. angustus* and subsequently pooled into 18 samples by sex and life stage, as described above. The most commonly trapped animal was *C. rutilus*. Of the 34 *C. rutilus* collected, 12% hosted adult *I. angustus*, 18% hosted nymphs, and 6% hosted larvae. The next most commonly trapped animal was the cinereus shrew (*Sorex cinereus*). None were parasitized by adult *I. angustus*, 6% hosted a nymph, and 12% hosted a larva. Of the 3 meadow voles (*Microtus pennsylvanicus*) collected, 2 (67%) were hosted nymphs and no other ticks. The 1 red squirrel (*Tamiasciurus hudsonicus*) we collected was parasitized by a nymph and no other ticks. Only 1 *I. angustus* male was collected via tick drag.

Bacteria and Parasites Detected in Small Mammals and Ticks Recovered From Small Mammals Collected in Anchorage

We were able to obtain a biological sample from all 55 collected animals including ear punches from 54 animals and blood samples from 21 animals (Table 4). We could not collect blood samples from any of the *S. cinereus*. We screened all ear punch biopsies for *Borrelia* spp., but none were positive. Blood samples were screened for *Anaplasma* spp., *Ehrlichia* spp., and *Babesia* spp.; none were positive for *Anaplasma* spp. Of the 17 *C. rutilus* blood samples tested, 1 (5.9%) was infected with *candidatus* E. khabarensis, 7 (41.2%) were infected with *Ba. Microti-Clethrionomys*, and 7 (41.2%) were infected with *Ba. Microti-Sorex*. Of the 3 *M. pennsylvanicus* blood samples tested, 1 (33.3%) was infected with *Ba. Microti-Clethrionomys*. No pathogenic organisms were detected in the 1 *T. hudsonicus* blood sample tested.

Of the 18 *I. angustus* pools from this sample of small mammals, 72.2% (n = 13) were infected with at least 1 species of bacteria or parasite (Table 5). Of the 15 tick samples obtained from *C. rutilis*, 7 (46.7%) were infected with *Ba. Microti-Clethrionomys*, 2 (13.3%) were infected with *Ba. Microti-Sorex*, and 3 (20.0%) were infected with both bacteria. Four (26.7%) of these *I. angustus* samples were infected with *candidatus* E. khabarensis. One of the 2 (50.0%) tick samples collected from *M. pennsylvanicus* was infected with *Ba. Microti-Clethrionomys*. Neither the 2 *I. angustus* samples collected from *S. cinereus* or the 1 tick sample from *T. hudsonicus* tested positive for *Babesia* spp. or *Ehrlichia* spp. None of the *I. angustus* collected from small mammals trapped in Anchorage tested positive for *Borrelia* spp.

Discussion

We tested ticks collected between 2019 and 2020 from humans, domestic animals, and wildlife hosts in Alaska for a panel of the most common tick-borne pathogens in the United States to characterize the diversity of microbes present in this region. As expected based on previous documentation of these species (Hahn et al. 2020), we found established populations of *I. angustus*, *I. uriae*, and *H. leporispalustris* in Alaska. *I. angustus* was found on a variety of hosts including domestic companion animals (dogs and cats), small wild mammals, and humans. Most *I. angustus* were submitted during the summer months with a peak in July, but this species was found in Alaska between April and October. We also report several *D. variabilis* from dogs and humans. With the recent reclassification of this species into *D. variabilis* and *D. similis*, it is not clear which of these species is present in Alaska (Lado et al. 2021). Although no known human pathogens were detected, we did find *Ba. microti*-like parasites, and *candidatus* E. khabarensis in ticks and small mammals. The only known human pathogen detected (*B. burgdorferi* s.s.) was found in an *I. scapularis* tick associated with a dog that had recently traveled to New York, where Lyme disease is endemic and *I. scapularis* is established. Although there was no evidence of human pathogens circulating in the ticks or animals sampled from Alaska, this study highlights the value of a combined passive and active tick surveillance system to detect introduced tick species and to assess which tick species and microbes are locally established. Particularly in regions where ticks are an emerging concern, a passive surveillance system can be a resource-efficient strategy for monitoring changing tick and tick-borne disease risks (Eisen and Eisen 2021, Eisen and Paddock 2021).

Of the ticks collected in Alaska, *I. angustus* was the most common and most frequent human-biter. Although the geographic range of *I. angustus* in the United States is limited, it is commonly found in Washington state alongside other *Ixodes* spp. including *I. pacificus* and *I. spinipalpis* (Xu et al. 2019, Dykstra et al. 2020). In a recent assessment, Dykstra et al. (2020) detected *Borrelia* species in field-collected *I. pacificus* and *I. spinipalpis* but not *I. angustus*, suggesting that although this species can experimentally transmit *B. burgdorferi* s.s. (Peavey et al. 2000), it may not contribute to the maintenance or transmission of *B. burgdorferi* in that region.

A recent review highlighted the diversity of the *Ba. microti* complex that has been elucidated through the use of molecular identification methods (Goethert et al. 2021). In this study, we found Munich-like, Clade 3 *Ba. microti* circulating in *I. angustus* collected in Southcentral and Southeast Alaska and in a sample of small mammals and ticks collected from these mammals from public parks in Anchorage. We are unable to compare this more specific identification to older studies in Alaska that did not use genetic sequencing; therefore, future studies of tick-borne microbes in Alaska should incorporate sequencing to identify which *Ba. microti*-like parasites are present in order to assess the presence or prevalence of known human pathogens.

'*Candidatus* Ehrlichia khabarensis' was recently detected in rodents and fed ticks collected from rodents in British, Columbia in 2013–2014 (Morshed et al. 2020). Prior to this detection, this microbe had only been definitively recorded in a territory in the Russian Far East. As such, the present study provides additional evidence that this microbe is circulating in an enzootic cycle at northern latitudes in North America. Additionally, in the present study, the microbe was detected in ticks from a wide variety of hosts (mice, voles, hares, squirrels, domestic dogs, and humans) across a large geographic region extending from the Matanuska-Susitna Valley in Southcentral Alaska, west to Bristol Bay and Kodiak Island, and south to Southeast Alaska. Although, it is unknown if *candidatus* E. khabarensis has any negative impacts on wildlife, domestic animals, or humans (Morshed et al. 2020), future studies of tick-borne microbes in Alaska should continue to screen for this microbe and assess potential implications for human and animal health.

The *Ehrlichia* species found in the *I. uriae* from the tufted puffin colony on Aiktak Island was recently detected for the first time in the organs of 3 Magellanic penguins (*Sphenicus magellanicus*) and

in an unfed *I. uriae* found on a penguin carcass on Magdalena Island in southern Chile (Muñoz-Leal et al. 2019). Although the authors of this study could not confirm the role of *I. uriae* in the transmission of this microbe, the identical sequences from the birds and the tick suggest that the seabird tick could be a vector and/or reservoir.

Although no human pathogens were found in ticks or wildlife originating in Alaska, the detection of *B. burgdorferi* sensu stricto in an *I. scapularis* collected from a domestic dog with recent travel to rural, upstate New York highlights the role of travel in the importation of medically-important tick species and tick-borne pathogens into Alaska. Hitchhiking ticks have been reported in a variety of contexts including UK holiday travelers returning from 25 different countries (Gillingham et al. 2020), case reports of exotic ticks in the United States from Africa (Molaei et al. 2018) and Central America (Molaei et al. 2020), movement of ticks from the East Coast to the West Coast of the United States on travelers (Xu et al. 2019), and the introduction and spread of the Asian longhorned tick (*Haemaphysalis longicornis*) into the United States (Egizi et al. 2020). Recent modeling work in Alaska showed that under future climate scenarios, regions in southcentral Alaska, Kodiak Island, and southeast Alaska may be suitable tick habitat for imported *I. pacificus* from Canada or the western United States (Witmer et al. 2022), which may increase the risk for potential *B. burgdorferi* transmission in the state. Public education and outreach to veterinarians can improve public awareness of the risks of tick importation when traveling with pets to tick endemic regions (Disler et al. 2022), and an intact passive and active national surveillance program can aid in early detection of non-endemic tick species (Eisen and Paddock 2021).

Since the majority of tick records in this study originated from passive surveillance and citizen scientist, samples were likely biased towards major population centers and likely overrepresented the adult life stage because it is larger and easier to see than nymphs or larvae. Similarly, it is unlikely that submitters collected all ticks present on pets or wildlife. Although we received a substantial number of ticks found on wildlife, this convenience sample likely underrepresents the prevalence of ticks on wildlife in Alaska. In particular, the introduction of winter tick (*Dermacentor albipictus*) is a particular concern for the impact that it could have on the moose population in the state (Walsh 2017). Additionally, ancillary information about submitted ticks is reported by the submitter so we are unable to verify the accuracy of the location where ticks were found or the travel history of the host.

Despite these limitations, this study represents the first attempt to characterize the diversity of potentially pathogenic microbes in ticks in Alaska and thus can serve as a baseline for future investigations into the potential for tick-borne disease in the region. Accurate information on tick distribution and pathogen presence can support more effective tick-borne disease prevention and diagnosis. Particularly in areas where ticks have not historically been a veterinary or public health concern and climatic changes are increasing potential tick habitat, ongoing surveillance and pathogen screening are important tools for early detection.

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Author Contributions

MBH: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft. AH: Formal analysis, Investigation, Methodology, Resources, Writing – review & editing. GD: Data curation, Investigation. WG: Data curation, Formal analysis, Investigation, Methodology. AD: Investigation, Resources, Methodology, Supervision, Writing – review & editing. RS: Investigation. LD: Formal analysis, Writing – review & editing. SC: Data curation. RG: Data curation. RJE: Resources, Writing – review & editing.

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