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EXPOSURE OF ALASKA BROWN BEARS (*URSUS ARCTOS*) TO BACTERIAL, VIRAL, AND PARASITIC AGENTS VARIES SPATIOTEMPORALLY AND MAY BE INFLUENCED BY AGE

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ABSTRACT: We collected blood and serum from 155 brown bears (*Ursus arctos*) inhabiting five locations in Alaska, US during 2013–16 and tested samples for evidence of prior exposure to a suite of bacterial, viral, and parasitic agents. Antibody seroprevalence among Alaska brown bears was estimated to be 15% for *Brucella* spp., 10% for *Francisella tularensis*, 7% for *Leptospira* spp., 18% for canine adenovirus type 1 (CAV-1), 5% for canine distemper virus (CDV), 5% for canine parvovirus, 5% for influenza A virus (IAV), and 44% for *Toxoplasma gondii*. No samples were seropositive for antibodies to *Trichinella* spp. Point estimates of prior exposure to pathogens among brown bears at previously unsampled locations generally fell within the range of estimates for previously or contemporaneously sampled bears in Alaska. Statistical support was found for variation in antibody seroprevalence among bears by location or age cohort for CAV-1, CDV, IAV, and *T. gondii*. There was limited concordance in comparisons between our results and previous serosurveys regarding spatial and age-related trends in antibody seroprevalence among Alaska brown bears suggestive of temporal variation. However, we found evidence that the seroprevalence of CAV-1 antibodies is consistently high in bears inhabiting southwest Alaska and the cumulative probability of exposure may increase with age. We found evidence for seroconversion or seroreversion to six different infectious agents in one or more bears. Results of this study increase our collective understanding of disease risk to both Alaska brown bear populations and humans that utilize this resource.

Key words: Gates of the Arctic, Katmai, Kodiak Island, Lake Clark, pathogen, serology, *Ursus arctos*, Yakutat Forelands.

INTRODUCTION

Brown bears (*Ursus arctos*) are among the largest extant terrestrial carnivores and exhibit a circumpolar distribution. In North America, this species occurs at highest population densities in Alaska, US, specifically at coastal areas with seasonally abundant runs of Pacific salmon (*Oncorhynchus* spp.; Miller et al. 1997). Although Pacific salmon represent a calorie-rich food source that supports high densities of animals, increased body mass, and

improved reproductive success of pregnant females (Hilderbrand et al. 1999), brown bears often utilize diverse prey (Mowat and Heard 2006; Mangipane et al. 2018) and exhibit seasonal or sustained omnivory (Hilderbrand et al. 1996). Given high population densities and dietary plasticity, Alaska brown bears may be exposed to a diversity of bacterial, viral, and parasitic agents via intraspecific contact and through consumption of varied prey. Thus, assessments of exposure

of Alaska brown bears to a variety of pathogens may be informative for understanding potential population-level impacts of disease and for assessing risk of human exposure to zoonotic pathogens through harvest (Maynard and Pauls 1962). As such, information on exposure of bears to pathogens may be useful for making informed management decisions for declining bear populations or those of conservation concern and in developing recommendations for the public regarding safe processing, handling, and consumption of harvested animals.

Previous investigations explored the exposure of Alaska brown bears to various pathogens (Zarnke 1983; Zarnke and Evans 1989; Chomel et al. 1995, 1998; Zarnke et al. 1997a, b); however, these studies assessed exposure to a single or few infectious agents or were conducted using samples collected >20 yr before present. For example, serosurveys conducted using samples collected during 1973–91 found evidence for exposure of Alaska brown bears to bacterial agents including *Brucella* spp. and *Leptospira* spp. (Zarnke 1983; Chomel et al. 1998); viruses including canine adenovirus type 1 (CAV-1), canine distemper virus (CDV), and canine parvovirus (CPV; Zarnke and Evans 1989; Chomel et al. 1998); and parasites such as *Toxoplasma gondii* and *Trichinella* spp. (Chomel et al. 1995; Zarnke et al. 1997a, b). Several observations regarding spatial and age-related trends in seroprevalence were noted as part of these investigations. Specifically, several serosurveys reported antibody seroprevalence to bacterial (Zarnke 1983; Chomel et al. 1998) and parasitic agents (Chomel et al. 1995, 1998; Zarnke et al. 1997a, b) to be higher in bears sampled at northern areas of Alaska as compared with areas farther south. Additionally, Chomel et al. (1998) found highest seroprevalence of antibodies to CAV-1 and CDV in bears sampled on Kodiak Island. Whether potential differences among sampling locations were driven by variation in local population demographics, epidemiological disparities, or were instead a function of typical spatiotemporal variation among bears within Alaska remains unknown. Additionally,

observations by Chomel et al. (1998) of increased seroprevalence of antibodies to *Brucella* spp. and *Francisella tularensis* in bears >2.5 yr old compared with cubs (<2 yr old) and a general positive correlation between detection of antibodies to CAV-1 and CDV with increasing age suggest that population demography may influence seroprevalence among Alaska brown bears.

In this study, we opportunistically collected and analyzed blood and serum samples from brown bears captured by state and federal agency personnel in Alaska during 2013–16 as part of previously planned research and management activities to: 1) gain preliminary inference into the exposure of bears from three previously unsampled areas (i.e., Gates of the Arctic National Park and Preserve [NPP], Lake Clark NPP, and the Yakutat Forelands) to numerous pathogens, 2) evaluate possible changes in exposure over time for bears inhabiting previously sampled areas (Katmai National Park [NP] and Kodiak Island), 3) assess if previous observations regarding spatial and age-related trends in seroprevalence are supported through analysis of contemporary samples, and 4) investigate seroconversion and seroreversion for bears sampled multiple times throughout our study. By addressing these four objectives, we aimed to increase information for assessing the health of brown bear populations in Alaska, refine inference relative to spatiotemporal variation in seroprevalence for this taxon, and gain insight into potential unrecognized epizootics in Alaska.

MATERIALS AND METHODS

A total of 155 brown bears was captured and sampled, and in 44 cases recaptured and resampled (after approximately 1.5–25 mo) by personnel from the Alaska Department of Fish and Game, National Park Service (NPS), US Fish and Wildlife Service (USFWS), and US Geological Survey (USGS) from July 2013 to July 2016 as part of ongoing research or management activities at five general locations in Alaska: Gates of the Arctic NPP, Katmai NP, Kodiak Island, Lake Clark NPP, and the Yakutat Forelands (Fig. 1). Bears were captured and handled as previously reported (Crupi et al. 2017; Hilderbrand et al.

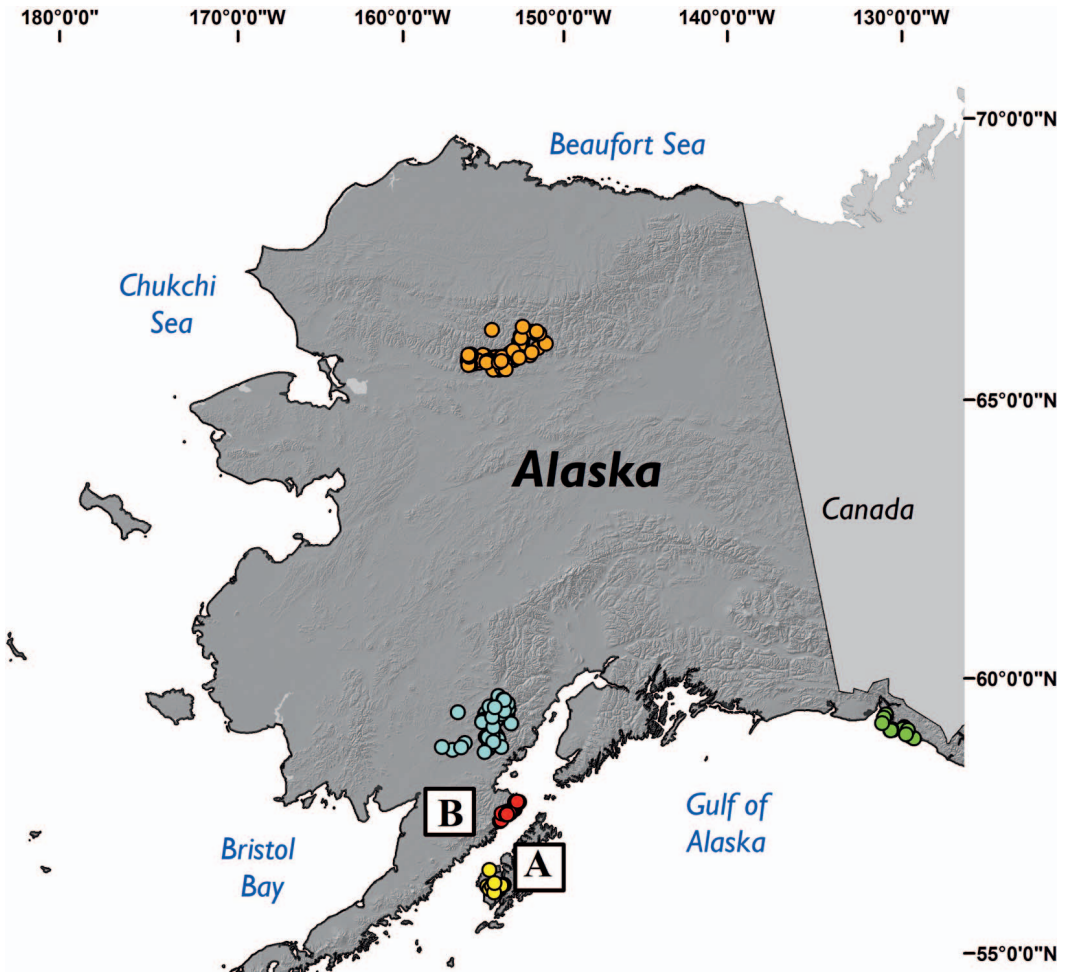


FIGURE 1. Approximate capture locations for Alaska brown bears (*Ursus arctos*) at five general locations from which samples were taken for testing for antibodies to bacterial, viral, and parasitic pathogens, July 2013–July 2016: Gates of the Arctic National Park and Preserve (orange circles), Katmai National Park (red circles), Kodiak Island (yellow circles), Lake Clark National Park and Preserve (blue circles), and Yakutat Forelands (green circles). Boxes labeled A and B represent the approximate locations from which samples collected from Kodiak Island and the Alaska Peninsula originated in the previous serosurveys (e.g., Chomel et al. 1998) with which comparisons are made.

2018). Blood (<60 mL) was collected through cephalic venipuncture into 10-mL tubes with no additive, stored at ambient temperatures in the field, and processed the evening of collection. Blood samples were spun for 15 min at $3,000 \times G$ and serum decanted into cryovials. All capture, handling, and sampling procedures were approved by Animal and Care Use Committees for Alaska Department of Fish and Game (2013-028), NPS (2014.A2, 2014.A3), USFWS (2012-14), and USGS (2014-1, 2014-10, 2015-4, 2015-6).

Serum samples were screened for evidence of exposure to bacterial, viral, and parasitic agents including: *Brucella* spp., *F. tularensis*, *Leptospira*

spp., CAV-1, CDV, CPV, influenza A virus (IAV), *T. gondii*, and *Trichinella* spp. Diagnostic assays were not specifically designed for, nor validated in, Alaska brown bears, although previous investigations (Zarnke 1983; Zarnke and Evans 1989; Chomel et al. 1995, 1998; Zarnke et al. 1997a) have applied assays used in this study to sera collected from this species and geographically proximate sampling locations, facilitating direct comparisons. Serologic assays and laboratories conducting procedures are summarized in Table 1. Briefly, for *Brucella* spp., sera were tested using the brucella antibody card test using standard protocols (US Department of Agriculture Nation-

TABLE 1. Summary of diagnostic assays, laboratories conducting diagnostic procedures, criteria for assessing antibody seroprevalence, and sources for complete methodological procedures for assessing exposure of Alaska brown bears (*Ursus arctos*) sampled during July 2013–July 2016 to bacterial, viral, and parasitic agents.^a

Agent type	Agent	Assay	Diagnostic lab	Seropositive criteria	Sources for methodology
Bacteria	<i>Brucella</i> spp.	Card test	UGAVDL	Agglutination	USDA NVSL, Ames, Iowa, USA
	<i>Francisella tularensis</i>	FAAT	SCWDS	Titer ≥ 20	Becton, Dickinson and Company, Sparks, Mississippi, USA
	<i>Leptospira</i> spp.	SNAP test	SCWDS	Formation of reaction products	IDEXX Laboratories, Westbrook, Maine, USA
Virus	Canine adenovirus type 1	SN	UGAVDL	Titer ≥ 4	Appel et al. 1973
	Canine distemper virus	SN	SCWDS	Titer ≥ 4	Appel and Robson 1973
	Canine parvovirus	HI	UGAVDL	Titer ≥ 10	Carmichael et al. 1980
	Influenza A virus	bELISA	SCWDS	S/N < 0.5	IDEXX Laboratories, Westbrook, Maine, USA
Parasite	<i>Toxoplasma gondii</i>	MAT	USDA APDL	Titer ≥ 25	Dubey and Desmonts 1987
	<i>Trichinella</i> spp.	ELISA	USDA APDL	Optical density $< 0.30^b$	SafePath Laboratories, Carlsbad, California, USA

^a FAAT = febrile antigen agglutination test; SN = serum neutralization; HI = hemagglutination inhibition; bELISA = blocking enzyme-linked immunosorbent assay; MAT = modified agglutination test; UGAVDL = University of Georgia Veterinary Diagnostic Laboratory; SCWDS = Southeastern Cooperative Wildlife Disease Study, University of Georgia; USDA APDL = US Department of Agriculture Animal Parasitic Diseases Laboratory; S/N = signal-to-noise ratio (i.e., ratio of the optical density of the test sample/optical density of the negative control); USDA NVSL = US Department of Agriculture National Veterinary Services Laboratory.

^b After subtracting value for negative control.

al Veterinary Services Laboratory, Ames, Iowa, USA) and visualization of agglutination was interpreted as a positive assay result. For *F. tularensis*, we screened sera using a febrile antigen agglutination test with positive and negative controls per the manufacturer's recommended protocol (Becton, Dickinson and Company, Sparks, Mississippi, US). Sera were diluted twofold in 0.85% sterile saline from 1:20 to 1:320 and titers (the reciprocals of the final serial dilutions) > 20 were interpreted as antibody positive. Cross-reactions are reported by the manufacturer to sometimes occur between *Brucella* and *Francisella* antigens and antisera. To test for prior exposure to *Leptospira* spp., we used a commercially available assay developed to detect antibodies to four *Leptospira* serovars in canine sera (IDEXX Laboratories, Westbrook, Maine, USA). This assay provided a qualitative (positive or negative) result. Using dog (*Canis lupus familiaris*) sera, agreement between this assay and those obtained using a modified agglutination test (MAT) increased with MAT titer (Curtis et al. 2015) and therefore this assay may not efficiently detect low levels of antibodies reactive to leptospires. For CAV-1, we tested brown bear sera using serum neutralization as described by

Appel et al. (1973). Sera were each diluted twofold in high-glucose Dulbecco's modified Eagle's medium (Sigma-Aldrich, St. Louis, Missouri, USA) with 0.1% 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and 0.01% gentamicin from 1:4 to 1:512. A titer > 4 was interpreted as a positive assay result. For CDV, we screened sera for neutralizing antibodies using the protocol described by Appel and Robson (1973). Sera were diluted twofold in minimal essential medium from 1:4 to 1:256. Samples exhibiting titers > 4 were interpreted as antibody positive. To test for antibodies to CPV, we used hemagglutinin inhibition as reported by Carmichael et al. (1980). Sera were diluted in Sorenson's phosphate-buffered saline twofold from 1:10 to 1:512. Samples with titers > 10 were interpreted as antibody positive. For IAV, we used a commercially available blocking enzyme-linked immunosorbent assay (ELISA) developed to detect antibodies to the IAV nucleoprotein (IDEXX Laboratories). A ratio of the optical density of the test sample to the optical density of negative control < 0.5 was interpreted as a positive assay result. For *T. gondii*, sera were tested using the MAT, as described by Dubey and Desmonts (1987). Sera were diluted twofold in

phosphate-buffered saline from 1:25 to 1:200 and sera providing titers >25 were considered positive on the basis of extensive testing of animal and human samples (Dubey 2009). Finally, to test for antibodies to *Trichinella* spp., we used a commercially available ELISA (SafePath Laboratories, Carlsbad, California, USA) modified for use with bear sera. Sera were tested at a 1:200 dilution, and positive and negative black bear (*Ursus americanus*) sera collected during sanctioned black bear hunts in Maryland were included on each ELISA plate (Dubey et al. 2013). Samples with an optical density <0.30 after subtracting the values for negative controls were interpreted as antibody positive.

Most bears were screened for all pathogens listed; however, because of agreements with collaborating agencies, limited sample volume, and funding constraints, some individuals were tested for exposure only to selected pathogens. For example, no bears from the Yakutat Forelands were screened for antibodies to *Brucella* spp. or *Leptospira* spp. All data used in this study were archived (Reeves et al. 2018).

To evaluate potential differences in seroprevalence of antibodies to pathogens by sampling location and age, we considered results from the screening of sera collected only at the initial sampling event for a given individual. We estimated age at initial sampling by evaluation of tooth wear by experienced observers (Gates of the Arctic NPP, Katmai NP, Kodiak Island, and Lake Clark NPP; Hilderbrand et al. 2018) or by extracting a premolar tooth for cementum analysis (Yakutat Forelands; Matson 1993) and used this estimate to define age of resampled individuals at time of subsequent recapture. Age was not estimated upon capture for a single bear from Gates of the Arctic NPP and therefore this individual was omitted from summaries and statistical analyses involving age. Bears were assigned to age cohorts equivalent to those reported by Chomel et al. (1998) to facilitate direct comparison. We assessed statistical differences in seropositivity among bears by sampling location and age cohort using Fisher's exact tests in R (R Core Team 2017). Sparse data (i.e., detections) relative to the number of parameters of interest (i.e., five sampling locations and four age cohorts) precluded other statistical approaches, such as evaluation of competing models within an information theoretic analytical framework. These same considerations limited the power of our analyses and led us to decide not to apply a Bonferroni correction despite conducting numerous statistical tests. Therefore, we interpreted $P < 0.05$ to be statistically significant.

To explore seroconversion and seroreversion (defined here as an observed change from non-detectable antibodies to detectable antibodies or

vice versa), we compared assay results for samples collected from bears at initial sampling and subsequent resampling events. We considered a bear to have seroconverted if an individual was seronegative for antibodies to a given infectious agent at initial sampling and an antibody titer for a given infectious agent exceeded the positive threshold value at any subsequent sampling event. Similarly, we considered a bear to have seroreverted if an individual was identified as seropositive for antibodies to a given infectious agent at the initial sampling event and the antibody titer failed to exceed the positive threshold value for the same infectious agent upon resampling.

RESULTS

Using 121 serum samples collected from brown bears captured at previously unsampled locations, specifically Gates of the Arctic NPP, Lake Clark NPP, and the Yakutat Forelands, we found point estimates for antibody seroprevalence to bacterial, viral, and parasitic agents to range between 0% and 59%. Antibodies to *F. tularensis*, CAV-1, CPV, and *T. gondii* were detected from bears at each of these three sampling locations (Table 2). Antibodies reactive to *Brucella* spp. and *Leptospira* spp. were identified in bear sera from Gates of the Arctic NPP and Lake Clark NPP, but samples from the Yakutat Forelands were not tested for seroreactivity to these bacterial agents (Table 2). Among these three sampling locations, CDV antibodies were only identified in sera from bears sampled at Gates of the Arctic NPP; this was in contrast to IAV antibodies, which were only detected in bear sera from Lake Clark NPP and the Yakutat Forelands (Table 2). Antibodies reactive to *Trichinella* spp. were not identified in brown bear sera from any of these locations (Table 2).

Thirty-four serum samples collected from brown bears captured at Katmai NP and Kodiak Island, locations sampled as part of previous serosurveys, provided point estimates for antibody seroprevalence to bacterial, viral, and parasitic agents ranging between 0 and 93%. Antibodies to *Brucella* spp., CAV-1, and *T. gondii* were detected from bears at both sampling locations (Table 2). Antibodies

TABLE 2. Summary of seroprevalence of brown bears (*Ursus arctos*) sampled in Alaska, USA during July 2013–July 2016 for antibodies to bacterial, viral, and parasitic agents by location. Summary includes samples obtained from only the initial sampling event for individuals sampled more than once during this study.

Sampling location	n	Percent seropositive (positive samples/total samples)									
		<i>Brucella</i> spp.	<i>Francisella tularensis</i>	<i>Leptospira</i> spp.	Canine adenovirus type 1	Canine distemper virus	Canine parvovirus	Influenza A virus	<i>Toxoplasma gondii</i>	<i>Trichinella</i> spp.	
Gates of the Arctic National Park and Preserve	50	16 (8/50)	12 (6/50)	2 (1/42)	10 (5/50)	14 (7/50)	4 (2/50)	0 (0/50)	40 (20/50)	0 (0/50)	
Katmai National Park	19	11 (2/19)	0 (0/19)	0 (0/9)	26 (5/19)	0 (0/19)	0 (0/19)	0 (0/19)	16 (3/19)	0 (0/19)	
Kodiak Island	15	7 (1/15)	13 (2/15)	7 (1/15)	93 (14/15)	0 (0/15)	13 (2/15)	13 (2/15)	53 (8/15)	0 (0/15)	
Lake Clark National Park and Preserve	46	17 (8/46)	9 (4/46)	13 (5/38)	4 (2/46)	0 (0/46)	2 (1/46)	2 (1/46)	59 (27/46)	0 (0/46)	
Yakutat Forelands	25	Not tested	12 (3/25)	Not tested	8 (2/25)	0 (0/25)	12 (3/25)	20 (5/25)	41 (9/22)	0 (0/25)	
Total	155	15 (19/130)	10 (15/155)	7 (7/104)	18 (28/155)	5 (7/155)	5 (8/155)	5 (8/155)	44 (67/152)	0 (0/155)	

reactive to *F. tularensis*, *Leptospira* spp., CPV, and IAV were detected in bear sera from Kodiak Island but not Katmai NP (Table 2). No evidence for CDV or *Trichinella* spp. antibodies were identified in brown bear sera from either of these previously sampled locations (Table 2).

When characterizing results for 154 serum samples by age cohort, we detected antibodies to *F. tularensis*, CPV, IAV, and *T. gondii* in samples from bears assigned to each of four cohorts (Table 3). Antibodies to *Brucella* spp., *Leptospira* spp., CAV-1, and CDV were detected in three age cohorts each, but not the youngest age cohort (2.5 to 4 yr old) for which we had samples (Table 3). *Trichinella* spp. antibodies were not detected in sera from bears of any age.

We found statistical support for differences in point estimates of antibody seropositivity among brown bears by location using Fisher’s exact tests for CAV-1, CDV, IAV, and *T. gondii* (Table 4). We also found statistical support for differences in seroprevalence of CAV-1 antibodies among bears by age cohort (Table 4). We did not, however, find support for statistical differences in seropositivity among bears by either sample location or age for any bacterial agents (i.e., *Brucella* spp., *F. tularensis*, and *Leptospira*) or for CPV (Table 4).

Using sera from 44 resampled brown bears from three sampling locations, we identified evidence of seroconversion to *Brucella* spp., *F. tularensis*, CAV-1, CPV, IAV, and *T. gondii* (Table 5). However, only for IAV was there evidence for antibody seroconversion by more than one individual from any given location (Table 5). We did not find any evidence for seroconversion of antibodies to *Leptospira* spp. or CDV among resampled brown bears from Gates of the Arctic NPP, Katmai NP, or Lake Clark NPP (Table 5). Seroreversion of antibodies to six infectious agents (*Brucella* spp., *F. tularensis*, CAV-1, CDV, CPV, and *T. gondii*) was evidenced among our collection of sera from resampled brown bears from three sample locations in Alaska (Table 5). Upon resampling, antibodies to these six infectious agents were

TABLE 3. Summary of antibody seroprevalence to bacterial, viral, and parasitic agents among Alaska brown bears (*Ursus arctos*) sampled during July 2013–July 2016 by age cohort. Summary includes samples obtained from only the initial sampling event for individuals sampled more than once during this study.

Age cohort (yr)	n	Percent seropositive (positive samples/total samples)							
		<i>Brucella</i> spp.	<i>Francisella tularensis</i>	<i>Leptospira</i> spp.	Canine adenovirus type 1	Canine distemper virus	Canine parvovirus	Influenza A virus	<i>Toxoplasma gondii</i>
2.5–4	13	0 (0/1)	15 (2/13)	0 (0/1)	0 (0/13)	0 (0/13)	15 (2/13)	15 (2/13)	50 (6/12)
4.5–8	31	12 (3/25)	13 (4/31)	8 (2/24)	6 (2/31)	6 (2/31)	6 (2/31)	3 (1/31)	29 (9/31)
8.5–12	38	18 (6/34)	5 (2/38)	8 (2/26)	18 (7/38)	3 (1/38)	3 (1/38)	5 (2/38)	38 (14/37)
>12	72	14 (10/69)	10 (7/72)	6 (3/53)	26 (19/72)	6 (4/72)	4 (3/72)	4 (3/72)	54 (38/71)
Total	154	15 (19/129)	10 (15/154)	7 (7/104)	18 (28/154)	5 (7/154)	5 (8/154)	5 (8/154)	44 (67/151)

detected in <80% of previously seropositive bears (Table 5).

DISCUSSION

We assessed the exposure of Alaska brown bears to bacterial, viral, and parasitic agents to address four objectives. First, we used samples collected from Gates of the Arctic NPP, Lake Clark NPP, and the Yakutat Forelands to provide information on the exposure of brown bears to pathogens at previously unsampled locations. Generally, point estimates for anti-

body seroprevalence at these locations were relatively low and fell within the range of estimates for contemporaneously sampled bears at other areas (i.e., Katmai NP and Kodiak Island) or bears previously sampled in Alaska (Zarnke et al. 1983, 1997a, b; Chomel et al. 1995, 1998). Exceptions included relatively high point estimates for antibodies to *Leptospira* spp. (13%) and *T. gondii* (59%) for bears sampled at Lake Clark NPP and for antibodies to IAV for bears sampled at the Yakutat Forelands. Although *Leptospira* spp. antibody seroprevalence was not statistically

TABLE 4. Results of Fisher's exact tests to assess statistical support for differences in seropositivity among brown bears (*Ursus arctos*) sampled in Alaska, USA during July 2013–July 2016 by location and age cohort. Summary includes samples obtained from only the initial sampling event for individuals sampled more than once during this study. Bold text indicates statistically significant differences ($P < 0.05$).

Fisher's exact test	<i>P</i> values for Fisher's exact tests to assess differences in seropositivity							
	<i>Brucella</i> spp.	<i>Francisella tularensis</i>	<i>Leptospira</i> spp.	Canine adenovirus type 1	Canine distemper virus	Canine parvovirus	Influenza A virus	<i>Toxoplasma gondii</i>
Location: Gates of the Arctic National Park and Preserve vs. Katmai National Park vs. Kodiak Island vs. Lake Clark National Park and Preserve vs. Yakutat Forelands ^a	0.8104	0.5554	0.2380	<0.0001	0.0072	0.1598	0.0012	0.0218
Age: 2.5–4 yr vs. 4.5–8 yr vs. 8.5–12 yr vs. >12 yr	0.8678	0.5778	0.8926	0.0246	0.8828	0.2960	0.3691	0.1037

^a Yakutat Forelands was omitted from statistical tests for *Brucella* spp. and *Leptospira* spp. as samples from this location were not screened for these bacterial agents.

TABLE 5. Summary of evidence for seroconversion and seroreversion among brown bears (*Ursus arctos*) sampled and subsequently resampled in Alaska, USA during July 2013–July 2016 and tested for antibodies to bacterial, viral, and parasitic agents. Summary includes only samples from bears that were seronegative or seropositive for antibodies to an infectious agent at time of initial sampling for seroconversion and seroreversion summaries, respectively.

Summary	Sampling location	Resampled bears (n)	Percent seropositive (positive samples/total samples) ^a									
			<i>Brucella</i> spp.	<i>Francisella tularensis</i>	<i>Leptospira</i> spp.	Canine adenovirus type 1	Canine distemper virus	Canine parvovirus	Influenza A virus	<i>Toxoplasma gondii</i>		
Seroconversion ^b	Gates of the Arctic National Park and Preserve	22	6 (1/17)	6 (1/18)	0 (0/15)	0 (0/21)	0 (0/17)	0 (0/22)	0 (0/22)	7 (1/15)		
	Katmai National Park	11	0 (0/10)	0 (0/11)	0 (0/6)	14 (1/7)	0 (0/11)	0 (0/11)	45 (5/11)	0 (0/10)		
	Lake Clark National Park and Preserve	11	13 (1/8)	0 (0/7)	0 (0/1)	0 (0/11)	0 (0/11)	10 (1/10)	9 (1/11)	0 (0/4)		
Total		44	6 (2/35)	3 (1/36)	0 (0/22)	3 (1/39)	0 (0/39)	2 (1/43)	14 (6/44)	3 (1/29)		
Seroreversion ^c	Gates of the Arctic National Park and Preserve	22	40 (2/5)	75 (3/4)	Not tested	0 (0/1)	100 (5/5)	Not tested	Not tested	71 (5/7)		
	Katmai National Park	11	100 (1/1)	Not tested	Not tested	25 (1/4)	Not tested	Not tested	Not tested	100 (1/1)		
	Lake Clark National Park and Preserve	11	0 (0/3)	100 (4/4)	Not tested	Not tested	Not tested	100 (1/1)	Not tested	57 (4/7)		
Total		44	33 (3/9)	88 (7/8)	Not tested	20 (1/5)	100 (5/5)	100 (1/1)	Not tested	67 (10/15)		

^a Total number of resampled bears from each location; subsequent columns summarize only those individuals that were seronegative (for seroconversion summary) or seropositive (for seroreversion summary) for antibodies to an infectious agent at time of initial sampling.

^b Previously negative resampled individuals seroconverting/total number of recaptured individuals testing negative at initial capture (percent seroconverted); individuals resampled on one to three occasions.

^c Previously positive resampled individuals seroreverting (i.e., previously seropositive bears without detectable antibodies upon recapture/total number of recaptured individuals testing positive at initial capture; percent seroreverted); individuals resampled on one to three occasions.

different among locations in this study ($P=0.238$), the relatively high point estimate for *T. gondii* antibodies in bears at Lake Clark NPP influenced the finding of differences in antibody seroprevalence among sampling sites ($P=0.022$). It was unclear if the relatively high point estimate for *T. gondii* antibodies for brown bears sampled at Lake Clark NPP was a function of differential epidemiology at this location or other sources of variation. A relatively high point estimate for IAV antibody seroprevalence among bears at the Yakutat Forelands also influenced the finding of significant differences in exposure among bears from different sites for this virus ($P=0.001$), which may be explained by a past localized epizootic at this location. The plausibility of this scenario was supported by nondetection of antibodies at several other locations and by a high rate of seroconversion among recaptured bears at Katmai NP during May–July 2016.

To address our second objective, we compared our results for bears sampled at Katmai NP and Kodiak Island with data obtained from these general areas in previous serosurveys to derive inference regarding possible changes in pathogen exposure among bears inhabiting these specific locations through time. In making our comparisons, we inferred that previously sampled “Alaska Peninsula(r)” bears were captured in, or adjacent to, Katmai NP on the basis of the information regarding study locations provided in previously published reports (Zarnke et al. 1989, 1997; Chomel et al. 1995, 1998). Our point estimates for seropositivity of antibodies to *Brucella* spp. (7–11%) and *F. tularensis* (0–13%) for bears inhabiting Katmai NP and Kodiak Island during 2013–16 were comparable with those provided by Chomel et al. (1998) for bears sampled during 1988–91 on the Alaska Peninsula and Kodiak Island (10–13% seroprevalence for *Brucella* spp. antibodies and 4–14% for *F. tularensis*). For CAV-1, Zarnke et al. (1989) found antibody prevalence in bears sampled from 1973–87 to be 29% and 16% at Kodiak Island and the Alaska Peninsula, respectively, comparable with 34% and 9% seropositive results for

bears sampled at these same areas during 1988–91 (Chomel et al. 1998). However, we found much higher point estimates for seroprevalence of CAV-1 antibodies at these two areas: 93% for bears sampled at Kodiak Island and 26% for individuals sampled at Katmai NP. Although these collective results provide evidence that CAV-1 is endemic in southwest Alaska and may have been so for 40 yr or more, our results indicated that the occurrence of this pathogen may have increased over time. Given the high value of brown bears on Kodiak Island and in Katmai NP for hunting and wildlife viewing, respectively, and the potential pathogenicity of CAV-1 virus to bears in general (Pursell et al. 1983; Collins et al. 1984; Schonbauer 1984; García Marín et al. 2018), and specifically to Alaska brown bear cubs (Knowles et al. 2018), CAV-1 could have relevance to future management efforts in southwest Alaska.

Previous assessments of *T. gondii* antibodies in brown bears sampled on the Alaska Peninsula and Kodiak Island from 1973 to 1987 (Zarnke et al. 1997) and 1988 to 1991 (Chomel et al. 1995) reported point estimates of seroprevalence to be 19–28% and 7–9% for these respective locations. Although our point estimate for *T. gondii* antibodies in bears sampled at Katmai NP (16%) are comparable with previous reports for the Alaska Peninsula, our estimated antibody seroprevalence for bears sampled at Kodiak Island (53%) was higher than values previously reported. An explanation for this apparent difference is not clear and the implications of *T. gondii* exposure in brown bears are unknown. However, this apparent increase in *T. gondii* antibody seroprevalence among bears on Kodiak Island should be considered in future investigations of the health status of bears in this region and in efforts to communicate safe handling practices of bear carcasses to hunters to minimize human exposure risk. Finally, a previous investigation of *Trichinella* spp. antibodies in brown bears sampled in Alaska during 1973–87 reported seroprevalence of 22% and 1% for individuals sampled on the Alaska Peninsula and Kodiak Island, respectively (Zarnke et al. 1997b). Although we did

not detect antibodies to *Trichinella* spp. in bears sampled at either of these locations in our study, differences may be a function of differential methodology and the associated sensitivity and specificity of assays used among investigations.

To address our third objective, we explored potential differences in exposure of bears to pathogens relative to location and age cohort and compared our results with observations reported as part of previous serosurveys of Alaska brown bears. We found statistical support for variation in antibody seroprevalence among bears for CAV-1, CDV, IAV, and *T. gondii* and limited concordance with previous observations suggestive of temporal variation. For example, previous serosurveys reported generally higher seroprevalence of antibodies to *Brucella* spp., *T. gondii*, and *Trichinella* spp. in brown bears sampled at northern locations in Alaska as compared with those sampled within the state farther south; however, no such trends were observed in this study. *Brucella* spp. antibody seropositivity was not statistically different ($P=0.810$) among brown bears from four sampling locations in this study, and the point estimate for antibody seroprevalence was highest at Lake Clark NPP, a study area located approximately 800 km south of Gates of the Arctic NPP.

Seroprevalence of *T. gondii* antibodies was statistically different ($P=0.022$) among bears sampled at five locations in Alaska; however, similar to our results for *Brucella* sp., the point estimate for antibody seroprevalence was highest at Lake Clark NPP and there was no clear geographic gradient in seropositivity. Findings of serosurveys therefore collectively suggest that unidentified spatiotemporal variables (e.g., the presence of cyclically abundant lynx populations; felids are the only previously identified definitive host for *T. gondii*) may drive location-specific epidemiological differences in exposure among Alaska brown bears or that our finding of statistically significant spatial differences in *T. gondii* antibody seroprevalence may reflect other sources of variation. Our lack of detection of antibodies to *Trichinella* spp. precluded us from statistically assessing spatial variation

and our differential methodology compared with prior investigations made direct comparisons uninformative. Given the discrepancy in detection of *Trichinella* spp. antibodies in Alaska brown bears in our study as compared with previous serosurveys, we encourage future investigations designed to test and validate serologic assays for these parasites in bears that combine serologic approaches for antibody detection with the testing of tissues for parasite infection.

Previous serosurveys also reported observations regarding increased seroprevalence of antibodies to *Brucella* spp., *F. tularensis*, *Leptospira* spp., CAV-1, and CDV in bears relative to age (Zarnke et al 1983; Zarnke and Evans 1989; Chomel et al. 1998). However, of these five infectious agents, we found support for differences in seropositivity among bears relative to age only for CAV-1. Our finding of statistically different seroprevalence of antibodies to CAV-1 among sampling locations ($P<0.001$) and age cohorts ($P=0.025$) was consistent with previous observations by Zarnke et al. (1989) and Chomel et al. (1998), who noted an apparent increase in seroprevalence with age in Alaska brown bears and highest seroprevalence among bears inhabiting Kodiak Island. The consistent finding of highest seroprevalence of CAV-1 antibodies in older bears and at Kodiak Island suggests increasing cumulative exposure probability with age and that Kodiak Island may represent an area where this viral agent is endemic.

For CDV, antibody seroprevalence was also significantly different among locations in our study ($P=0.007$), a function of all seven seropositive bears having been sampled at Gates of the Arctic NPP (five of which seroreverted before resampling). Considering that seroprevalence of CDV antibodies appeared to be spatially variable in a previous serosurvey and was as high as 30% in brown bears sampled at Kodiak Island (Chomel et al. 1998), a location at which we did not identify seropositive individuals in this study, collective results of research suggest that sporadic, geographically limited outbreaks may play a role in the epidemiology of CDV in Alaska

brown bears. For IAV, antibody seroprevalence was also found to statistically vary among sampling locations ($P=0.001$); however, previous serosurveys did not test for antibodies to this virus and therefore comparisons through time were not possible. Considering that we found evidence for seroconversion of IAV antibodies in a relatively high proportion of resampled bears at Katmai NP, it is plausible that sporadic outbreaks may also play a role in the epidemiology of this virus among Alaska brown bears. Overall, a general lack of concordance regarding spatial and age-related trends in seroprevalence between our study and prior investigations suggested that infectious agents may circulate among bears in a dynamic system, requiring periodic assessments to identify and interpret trends in exposure. However, differences in sampling across space (e.g., we sampled bears from three previously unsampled locations) and population demographics (e.g., we sampled no bears <3 yr-old) may have influenced the general lack of concordance among serosurveys.

Resampling of brown bears from three sampling locations allowed us to explore seroconversion and seroreversion, the fourth and final objective of our study. Evidence for only a single inferred instance of seroconversion at Gates of the Arctic NPP, Katmai NP, and Lake Clark NPP for five infectious agents suggests that exposure of bears to *Brucella* spp., *F. tularensis*, CAV-1, CPV, and *T. gondii* may be relatively infrequent at these locations, or alternatively, that antibody responses may be relatively short lived. In contrast, the apparent seroconversion of IAV antibodies in 45% (5/11) of brown bears sampled in Katmai NP between late May and early July 2016 suggested that a localized and previously unrecognized outbreak may have affected this population of bears. Seroreversion was also evidenced in at least one individual for six infectious agents; however, seroreversion was identified in more than one individual only for *Brucella* spp., *F. tularensis*, CDV, and *T. gondii*. These findings provided evidence that bears may not exhibit lifelong antibody responses to these infectious agents. Seror-

eversion of detectable antibodies to *F. tularensis* and CDV in all bears that were seropositive upon initial capture at Lake Clark NPP and Gates of the Arctic NPP, respectively, may have been evidence of past localized epizootics at these specific locations. Furthermore, seroreversion may, at least partially, explain the finding of discordant trends in antibody seroprevalence among Alaska brown bears as inferred through the comparison of our results and previous serosurveys. That is, for at least several pathogens, it appeared that the duration of the opportunity for antibody detection may be relatively short.

Through our serosurvey of Alaska brown bears, we provided support for prior exposure of animals to a diversity of bacterial, viral, and parasitic agents, including pathogens previously linked to bear mortality in Alaska and other regions (Pursell et al. 1983; Collins et al. 1984; Schonbauer 1984; Cottrell et al. 2013; García Marín et al. 2018; Knowles et al. 2018). Thus, our data may be useful to wildlife managers and veterinary professionals for informing investigations of bear morbidity and mortality in Alaska or those assessing changes in brown bear population age structure (e.g., poor recruitment) that may be influenced by disease. For example, biologists and managers working in southwestern Alaska might consider CAV-1 as an important pathogen to rule out in the investigation of bear mortality events or if seeking to understand the unexpected poor survival of cubs to subadult age classes. Additionally, information provided in this study may be used to assist in decisions regarding the translocation of Alaska brown bears to other regions, including the transfer of orphaned or wild-caught animals to zoological collections. Testing of orphaned bear cubs from Katmai NP and Kodiak Island for CAV-1 may, for example, be prudent before transferring to a captive setting where other potentially susceptible animals may be kept. Finally, our study provided information regarding spatiotemporal variability in pathogen exposure and demographic trends in seroprevalence that served to increase our collective understanding of disease risk to

both Alaska brown bears and humans that utilize this public resource. More specifically, this study provided inference regarding where certain pathogens may be endemic, how epizootics vary in time and space, and the relative risk that zoonotic pathogens such as *F. tularensis* and *Trichinella* spp. may pose to humans harvesting brown bears in Alaska.

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