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SURVEY OF ARCTIC ALASKAN WILDLIFE FOR INFLUENZA A ANTIBODIES: LIMITED EVIDENCE FOR EXPOSURE OF MAMMALS

Caroline Van Hemert,[1,5] Timothy J. Spivey,[1] Brian D. Uher-Koch,[1] Todd C. Atwood,[1] David R. Sinnett,[2] Brandt W. Meixell,[1] Jerry W. Hupp,[1] Kaijun Jiang,[3] Layne G. Adams,[1] David D. Gustine,[1,4] Andrew M. Ramey,[1] and Xiu-Feng Wan[3]

[1] US Geological Survey, Alaska Science Center, 4210 University Drive, Anchorage, Alaska 99508, USA
[2] US Department of Agriculture, Animal and Plant Health Inspection Services Wildlife Services, 9001 E Frontage Road, Palmer, Alaska 99645, USA
[3] Department of Basic Sciences, College of Veterinary Medicine, Mississippi State University, PO Box 6100, Mississippi State, Mississippi 39762-6100, USA
[4] Current affiliation: National Park Service, Grand Teton National Park, PO Box 170, Moose, Wyoming 83012, USA
[5] Corresponding author (email: cvanhemert@usgs.gov)

ABSTRACT: Influenza A viruses (IAVs) are maintained in wild waterbirds and have the potential to infect a broad range of species, including wild mammals. The Arctic Coastal Plain of Alaska supports a diverse suite of species, including waterfowl that are common hosts of IAVs. Mammals co-occur with geese and other migratory waterbirds during the summer breeding season, providing a plausible mechanism for interclass transmission of IAVs. To estimate IAV seroprevalence and identify the subtypes to which geese, loons, Arctic foxes (*Vulpes lagopus*), caribou (*Rangifer tarandus*), and polar bears (*Ursus maritimus*) are potentially exposed, we used a blocking enzyme-linked immunosorbent assay (bELISA) and a hemagglutination inhibition (HI) assay to screen for antibodies to IAVs in samples collected during spring and summer of 2012–16. Apparent IAV seroprevalence using the bELISA was 50.3% in geese (range by species: 46–52.8%), 9% in loons (range by species: 3–20%), and 0.4% in Arctic foxes. We found no evidence for exposure to IAVs in polar bears or caribou by either assay. Among geese, we estimated detection probability from replicate bELISA analyses to be 0.92 and also found good concordance (>85%) between results from bELISA and HI assays, which identified antibodies reactive to H1, H6, and H9 subtype IAVs. In contrast, the HI assay detected antibodies in only one of seven loon samples that were positive by bELISA; that sample had low titers to both H4 and H5 IAV subtypes. Our results provide evidence that a relatively high proportion of waterbirds breeding on the Arctic Coastal Plain are exposed to IAVs, although it is unknown whether such exposure occurs locally or on staging or wintering grounds. In contrast, seroprevalence of IAVs in concomitant Arctic mammals is apparently low.

Key words: Arctic, influenza A virus, seroprevalence, wild bird, wild mammal.

INTRODUCTION

Influenza A viruses (IAVs; family *Orthomyxoviridae*, genus *Orthomyxovirus*) have a worldwide distribution and can infect a diverse suite of taxa, including both avian and mammalian hosts (Fereidouni et al. 2016; US Geological Survey [USGS] National Wildlife Health Center 2018). Wild waterbirds of the orders Anseriformes and Charadriiformes have been identified as the natural reservoir of IAVs (Halvorson 2008), but much less is known about the ecology of IAVs in other species of birds and mammals. Most reports of influenza in mammals have been associated with spillover events from birds, the effects of which range from asymptomatic infection (Hall et al. 2008) to large-scale morbidity and mortality (Zohari et al. 2014). Several studies have also suggested that mammals may contribute to IAV outbreaks among birds (Root et al. 2015; Puryear et al. 2016; Su et al. 2016), but additional research is needed to assess the risk of natural IAV exposure among wild mammals, particularly in areas of concentrated interclass contact.

Influenza A viruses have been isolated or detected serologically in a variety of wild mammals, including some cases in which widespread morbidity or mortality occurred. The first documented IAV-associated mass mortality event in wild mammals occurred in 1979 on the northeast coast of North America, where an H7N7 subtype caused illness and

death in harbor seals (*Phoca vitulina*; Webster et al. 1981). Subsequently, sporadic outbreaks of IAVs have resulted in die-offs of seals in North America and Europe (Zohari et al. 2014; Fereidouni et al. 2016). Antibodies to IAV have been detected in a number of other marine mammal species, including several species of whales, Pacific walruses (*Odobenus rosmarus divergens*; Fereidouni et al. 2016), northern elephant seals (*Mirounga angustirostris*; Li et al. 2014), and sea otters (*Enhydra lutris*; Goldstein et al. 2013). Among wild terrestrial mammals, there is evidence of natural infection in numerous species, including mink (*Mustela vison*; Klingeborn et al. 1985), stone marten (*Martes foina*; World Health Organization 2006), raccoon (*Procyon lotor;* Hall et al. 2008), striped skunks (*Mephitis mephitis*; Britton et al. 2010), house mice (*Mus musculus*; Shriner et al. 2012), plateau pikas (*Ochotona curzoniae*; Zhou et al. 2009), water deer (*Hydropotes inermis*; Kim et al. 2018), leopard cats (*Prionailurus bengalensis*; Kim et al. 2018), and bats (Tong et al. 2013). In many of these instances, birds were identified as a probable source of infection, with presumed exposure occurring via direct consumption of infected birds or through contact with water or other environmental sources contaminated by fecal material (Root et al. 2014a; Zohari et al. 2014; Fereidouni et al. 2016). However, knowledge about community dynamics of IAVs in wildlife is limited, particularly for northern environments.

The Arctic Coastal Plain of Alaska consists of a mosaic of tundra and wetlands adjacent to the Beaufort Sea. This habitat hosts large numbers of migratory waterbirds during the breeding season, including species known to have high rates of exposure to IAVs, although the timing and locations of such exposure are unknown (Wilson et al. 2013). In this region, migratory birds have long co-occurred with terrestrial and marine mammal species, including Arctic foxes (*Vulpes lagopus*), caribou (*Rangifer tarandus*), and polar bears (*Ursus maritimus*), providing considerable opportunity for interclass contact. Arctic foxes are common avian nest predators and regularly encounter eggs and juvenile and adult birds during summer months (Samelius and Alisauskas 2000; Meixell and Flint 2017; Fig. 1a). Caribou graze in close proximity to nesting waterfowl, where infection via consumption of feces or fecal-contaminated vegetation and water is plausible (van der Wal and Loonen 1998; Fig. 1b). Because of the recent reduction in sea ice cover, there may now be more frequent contact among wildlife species (Van Hemert et al. 2015). For example, numbers of geese have increased in recent years on the Arctic Coastal Plain (Flint et al. 2008), and polar bears have been spending increasing amounts of time on land during the peak of the breeding season for migratory birds (Atwood et al. 2016), sometimes depredating goose nests (Iles et al. 2013; Prop et al. 2015) or foraging on subsistence-harvested whale or seal carcasses also frequented by terrestrial species. Additionally, coastal and marine water sources provide shared habitat among known reservoir hosts of IAVs (i.e., waterfowl, gulls, and shorebirds; Olsen et al. 2006), other aquatic birds for which there are limited data on IAV exposure (e.g., loons), and a diverse assemblage of terrestrial and marine mammal species. Given the potential for contact among birds and mammals, the Arctic Coastal Plain is a suitable location for investigating patterns of IAV exposure.

Our study examined the prevalence of IAV antibodies among migratory waterbirds (geese, loons) and wild mammals (Arctic foxes, caribou, and polar bears) that co-occur on the Arctic Coastal Plain of northern Alaska during summer. Our specific objectives were to 1) determine seroprevalence of IAVs in sympatric-nesting geese and loons, 2) evaluate whether mammals in the same region are exposed to IAVs, and 3) identify potential sources of exposure based on subtype-specific IAV antibodies detected in birds and mammals. Results from this study provide insight about the ecology of IAVs in northern Alaska.

MATERIALS AND METHODS

Study area and sample collection

The Arctic Coastal Plain of northern Alaska encompasses low tundra and wetland complexes that extend from the coast of the Beaufort Sea to the foothills of the Brooks Range in northern

FIGURE 1. Nest camera images from the Arctic Coastal Plain of northern Alaska showing interactions between White-fronted Geese (*Anser albifrons frontalis*) and (a) Arctic fox (*Vulpes lagopus*), and (b) caribou (*Rangifer tarandus*). Frequent interclass contact has the potential to facilitate transmission of pathogens, including influenza A viruses, between birds and mammals.

Alaska (Fig. 2). To assess IAV seroprevalence among seasonally abundant birds and mammals, we collected sera from Greater White-fronted Geese (*Anser albifrons frontalis*), Lesser Snow Geese (*Chen caerulescens caerulescens*), Black Brant (*Branta bernicla nigricans*), Pacific Loons (*Gavia pacifica*), Red-throated Loons (*Gavia stellata*), Yellow-billed Loons (*Gavia adamsii*), Arctic foxes, caribou, and polar bears.

We captured nesting geese during June at the Colville River Delta (70°26′N, 150°40′W) and molting geese during July and August at the

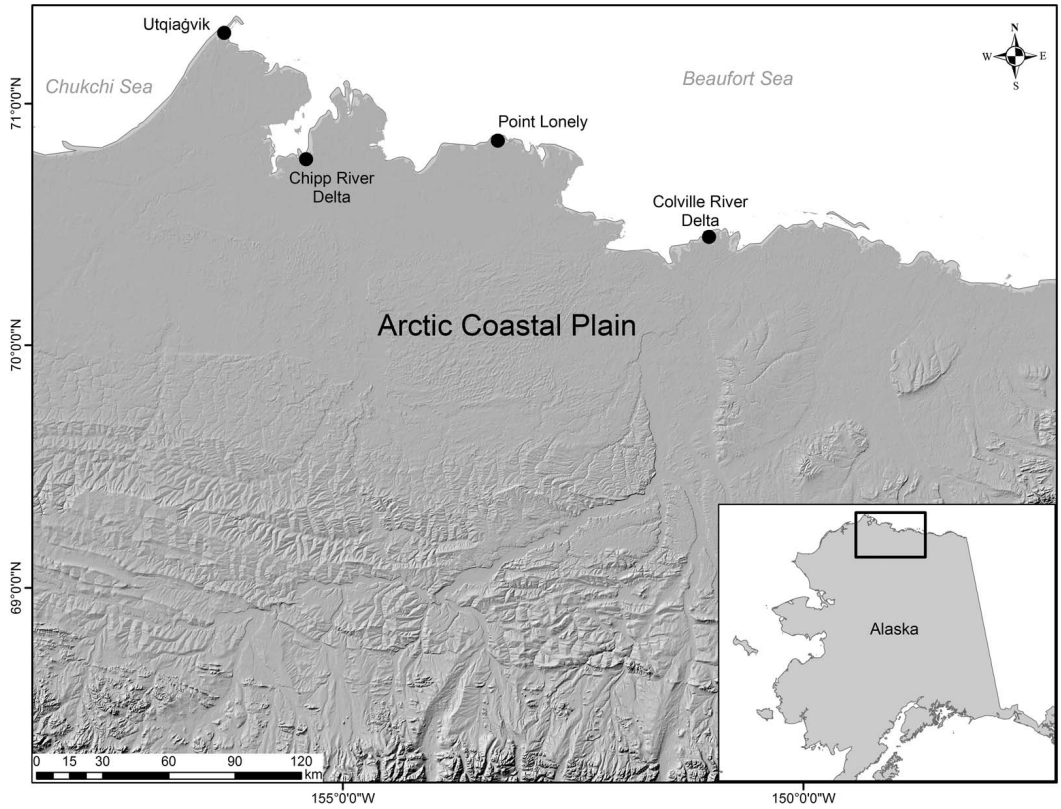

FIGURE 2. Map showing Arctic Coastal Plain and adjacent Beaufort Sea of Alaska, including specific locations where samples from geese, loons, Arctic foxes (*Vulpes lagopus*), caribou (*Rangifer tarandus*), and polar bears (*Ursus maritimus*) were collected during 2012–16.

Colville River Delta, along the Chipp River (70°41′N, 155°18′W), and near Point Lonely (70°54′N, 153°14′W) in 2014–16. We captured loons on the Colville River Delta and Chipp River Delta during July and August 2012–16. From each bird, we collected a 3.0–5.0 mL sample of whole blood from the jugular vein (USGS Animal Care and Use Committee [ACUC] permits 2010-04, 2014-12, 2009-13, and 2013-05).

Foxes were shot or trapped near Utqiaġvik (formerly Barrow), Alaska (71°17′N, 156°47′W) during May and June 2014–16, and blood was collected from the heart using a syringe, after euthanasia. We captured caribou on their winter range in the east-central Brooks Range (67°1′–67°50′N, 147°45′–149°0′W) in April 2015 and 2017, and blood was collected from the jugular or cephalic vein (USGS ACUC permit 2015-5). We captured polar bears during March and April 2013–16 on the sea ice of the Southern Beaufort Sea between Utqiaġvik and the US-Canada border as described in Atwood et al. (2016), and collected blood by venipuncture of either the femoral or jugular veins (USGS ACUC permit 2010-14). Goose and loon samples were collected from after hatch-year (≥1 yr old) birds; fox, caribou, and polar bear samples were collected from adult or subadult animals. Blood samples from all species were drawn into additive-free and ethylenediaminetetraacetic acid–treated evacuated tubes, centrifuged at 1,200–1,600 × G for 5–15 min on the day of collection to derive sera, aliquoted into cryovials, stored at −20 C in the field and then at −80 C upon receipt in the laboratory. All work was conducted under authority granted by state and federal permits.

Blocking enzyme-linked immunosorbent assay

Serum samples were screened for antibodies to IAV using a commercially available blocking enzyme-linked immunosorbent assay (bELISA; AI MultiS-Screen Avian Influenza Virus Antibody Test Kit, IDEXX Laboratories, Westbrook, Maine, USA) following the manufacturer's instructions, with a single well for each sample. We used a sample to negative control (S/N) ratio of 0.5 as a cutoff for interpretation of results, with

any value <0.5 considered positive for IAV antibodies, per the manufacturer's recommendation. For each species, we present apparent mean (SE) seroprevalence. We analyzed 15% of samples (n=117 total) from all species in duplicate by bELISA for quality assurance and quality control; results from the first replicates were used for estimates of seroprevalence. For geese, the only group with an adequate number of positive samples for occupancy modeling (Lachish et al. 2012), we also estimated detection probability (P). When P remains unaccounted for, imperfect detection of serologic or other disease state (i.e., occurrence of false-negatives) may result in biased estimates of prevalence (McClintock et al. 2010). Therefore, we used occupancy modeling (Program MARK; White and Burnham 1999) with replicate bELISA assays to estimate the probability of detecting antibodies within a goose sample, given that antibodies were present ($\hat{P}$). Because of limited sample sizes of geese, replicates from all species were combined for that analysis.

Hemagglutination inhibition assays

To provide inference regarding assay sensitivity and specificity relative to the bELISA assay and further to identify hemagglutinin (HA) subtype-specific antibodies for comparison among taxa, a subset of serum samples from each taxonomic group was also analyzed by hemagglutination inhibition (HI) assay against 15 HA subtypes (H1–H14 and H16) of IAVs. Because of limited serum volume, we tested seroreactivity to only one avian IAV for each HA subtype under the assumption that HI antigens were representative of the antigenic diversity of low-pathogenic avian IAVs to which animals would potentially be exposed (Bailey et al. 2016; Xu et al. 2016). We selected and analyzed 63 serum samples as part of our subset, which comprised sera with a range of S/N values, including both those deemed positive (<0.5; n=13) and those deemed negative (≥0.5; n=50) by bELISA. Among the negative samples, we included 21 with S/N values of 0.5–0.7 because 0.7 has been previously proposed as a more sensitive threshold for determining presence of antibodies to IAVs in wild birds (Brown et al. 2009; Shriner et al. 2016). Our selections were restricted by available sera volume, but included all taxa. Among geese, only samples from Greater White-fronted Geese had adequate volume for testing by HI.

To minimize nonspecific agglutination, the serum sample was mixed with 10% turkey red blood cells (RBCs) in a volume ratio of 1:1 (Kistler et al. 2015) and incubated at 37 C for 30 min. After centrifugation, the serum was collected without disturbing packed turkey RBCs. In the HI assay, 0.5% turkey RBCs were used; serum samples were determined to be positive against a specific virus if the HI titer was ≥1:40 (Hobson et al. 1972) or suspected to be positive if the HI titer was 1:10 or 1:20.

All raw data that support the findings of this publication can be found in the accompanying USGS data release (Van Hemert et al. 2018).

RESULTS

Serological results from bELISA

Overall IAV seroprevalence for geese was 50.3±2.9% (n=294) by bELISA (S/N range, mean±SE for positive samples: 0.08–0.49, 0.24±0.02). We detected antibodies in 52.8% (±4.8%; n=108) of Lesser Snow Geese, 50.9% (±4.8%; n=110) of Black Brant, and 46% (±6%; n=76) of Greater White-fronted Geese (Table 1). Overall IAV seroprevalence for loons was 9±3% (n=87; S/N range, mean±SE for positive samples: 0.14–0.49, 0.31±0.2). We detected antibodies in 20% (±13%; n=10) of Red-throated Loons, 10% (±4%; n=48) of Pacific Loons, and 3% (±3%; n=29) of Yellow-billed Loons (Table 1). We also detected IAV antibodies in one Arctic fox (0.4%±0.4%; n=231; S/N=0.49; Table 1), although two subsequent bELISA assays of that sample had S/N ratios >0.5 (0.65 and 0.78), and serum volume was inadequate for HI testing. We detected no IAV antibodies in caribou (n=46) or polar bears (n=100; Table 1).

Replicate samples by bELISA

Geese were the only taxa for which we had sufficient positive samples to estimate P using occupancy modeling. Of the 46 goose samples analyzed in duplicate by bELISA, 27 were positive one or more times, and four showed inconsistencies among runs (P=0.92±0.04). Sample sizes were not adequate to calculate species-specific P values, so that value reflected a combined estimate across the three species we sampled (Greater White-fronted Goose, Lesser Snow Goose, and Black Brant). All goose samples with discordant results had S/N values close to the 0.5 cutoff (range: 0.44–0.64). Among duplicate samples from loons (n=14), foxes (n=37), caribou (n=7), and polar bears (n=15), there was only a single discor-

TABLE 1. Seroprevalence of influenza A viruses in birds and mammals sampled on the Arctic Coastal Plain of northern Alaska in 2012–16. A positive result was determined by blocking enzyme-linked immunosorbent assay using a sample-to-negative control ratio cutoff of <0.5.

Species				
Common name	Scientific name	No. sampled	No. positive	Percent (±SE)
Geese		294	148	50.3 (±2.9)
Black Brant	*Branta bernicla nigricans*	110	56	50.9 (±4.8)
Greater White-fronted Goose	*Anser albifrons frontalis*	76	35	46 (±6)
Lesser Snow Goose	*Chen caerulescens caerulescens*	108	57	52.8 (±4.8)
Loons		87	8	9 (±3)
Pacific Loon	*Gavia pacifica*	48	5	10 (±4)
Red-throated Loon	*Gavia stellata*	10	2	20 (±13)
Yellow-billed Loon	*Gavia adamsii*	29	1	3 (±3)
Mammals		377	1	0.3 (±0.3)
Arctic fox	*Vulpes lagopus*	231	1	0.4 (±0.4)
Polar bear	*Ursus maritimus*	100	0	0
Caribou	*Rangifer tarandus*	46	0	0

dant sample from an Arctic fox (see earlier details).

Serological results from HI assay

Using HI assays for 15 HA subtypes of IAVs, we identified antibodies reactive to H1, H6, and H9 among samples from White-fronted Geese (n=15; Table 2) and reactive to H4 and H5 among samples from Red-throated and Pacific loons (n=20; Table 2). No samples from Arctic foxes (n=15), caribou (n=5), or polar bears (n=8) were positive by HI against any IAV tested.

Comparison between results from bELISA and HI assays

Results from HI in geese were generally concordant (86.7%) with those from bELISA, and supported the use of 0.5 as a highly specific S/N threshold value. All goose samples tested by HI that previously tested positive by bELISA (n=6) had antibody titers >1:40 for one or more IAV HA subtypes. Two goose serum samples with S/N values of 0.69 and 0.82 had low (1:20) suspect positive titers for H6 and/or H9 antibodies. For loon samples, however, bELISA and HI results differed. Of seven samples positive by bELISA, only one (14%) was suspect positive by HI; all others were negative for subtype-specific antibodies.

DISCUSSION

Transmission of IAV from avian to mammalian hosts has been demonstrated both experimentally and via natural exposure (Vandalen et al. 2009; Fereidouni et al. 2016), and wild mammals have occasionally been identified as important bridge or spillover hosts (Root et al. 2015; Puryear et al. 2016; Su et al. 2016). However, the exposure of wild mammals to IAVs is not well understood. In this study, we sampled waterbirds and mammals that occur contemporaneously on the Arctic Coastal Plain of northern Alaska. This region is unique in its abundance of potential IAV waterfowl hosts and the frequent occurrence of interclass contact among wild birds and a diversity of wild mammal species. We hypothesized that Arctic foxes, caribou, and polar bears could be exposed to IAV through consumption of infected birds or via environmental contamination and, further, that shared water bodies between geese and loons could promote interspecies transmission. We detected relatively high IAV seroprevalence in waterbirds, although we were not able to assess timing or sources of exposure. In

Table 2. Summary of subtype-specific antibodies detected by hemagglutination inhibition to H1–H14 and H16 influenza A viruses (IAVs) subtypes among Greater White-fronted Geese (*Anser albifrons frontalis*) and Pacific (*Gavia pacifica*), Red-throated (*Gavia stellata*), and Yellow-billed (*Gavia adamsii*) loons collected on the Arctic Coastal Plain of northern Alaska, 2012-16.

Virus	Geese (n=15)		Loons (n=20)	
	No. positive[a,b]	Titer range[c]	No. positive[a,b]	Titer range[c]
A/mallard/Ohio/11OS2073/2011(H1N2)	4	1:20–1:320	0	—
A/mallard/Iowa/10OS2721/2010(H2N2)	0	—	0	—
A/mallard/Wisconsin/A00661712/2009(H3N2)	0	—	0	—
A/herring gull/Iceland/3831/2014(H4N2)	0	—	1	1:10
A/mallard/Illinois/3974/2009(H5N2)	0	—	1	1:20
A/mallard/Wisconsin/10OS3066/2010(H6N2)	5	1:20–1:80	0	—
A/mallard/RI/A00449368/2009(H7N3)	0	—	0	—
A/American black duck/Nova Scotia/02043/2007(H8N4)	0	—	0	—
A/mallard/Ohio/13OS3856/2013(H9N2)	6	1:20–1:40	0	—
A/mallard/Missouri/14OS4111/2014(H10N2)	0	—	0	—
A/mallard/Ohio/11OS2213/2011(H11N2)	0	—	0	—
A/mallard/Ohio/09OS2904/2009(H12N2)	0	—	0	—
A/black-legged kittiwake/Quebec/02838-1/2009(H13N6)	0	—	0	—
A/northern shoveler/Mississippi/12OS456/2012(H14N2)	0	—	0	—
A/herring gull/Iceland/4549/2014(H16N3)	0	—	0	—

[a] Includes suspect positive samples with titers 1:10 and 1:20.

[b] These data should not be used to infer subtype-specific or overall IAV seroprevalence. Only a subset of samples was tested, including some that were seropositive (geese: n=6, loons: n=7) and some that were seronegative (geese: n=9, loons: n=13) by a blocking enzyme-linked immunosorbent assay. Some samples yielded titers to multiple antigens.

[c] — = no antibodies were detected, and thus, titer information is not applicable.

contrast, we found minimal support for exposure of mammals to IAVs in this Arctic environment.

We detected antibodies in more than one half of the geese sampled, which is in accordance with previous studies of IAV antibody seroprevalence in waterfowl sampled in Alaska (Ely et al. 2013; Wilson et al. 2013). The relatively high seroprevalence for multiple goose species (46%–52.8%; Table 1) supports the premise that migratory waterfowl could be a source of IAV infection to other birds and mammals in the Arctic if transmission of IAVs occurs on the breeding grounds. Unfortunately, surveillance for active virus shedding among waterbirds in the Arctic has been limited. Detection probability of the bELISA assay based on replicate analyses of goose samples was 0.92, suggesting that our estimates of seroprevalence had only minor bias from incomplete detection. Subtype-specific HI analyses demonstrated the presence of antibodies reactive to H1, H6, and H9 IAV HA subtypes in samples from geese. Those viral subtypes have previously been documented in waterfowl (Latorre-Margalef et al. 2014), including those sampled contemporaneously elsewhere in Alaska (Spivey et al. 2017; Reeves et al. 2018). These were also the most prevalent IAV subtype-specific antibodies detected in a recent study of wintering Snow Geese (Wong et al. 2016). Two of these subtypes (H1 and H6) have been shown to have the potential to infect wild terrestrial (Hall et al. 2008; Shriner et al. 2012) and marine (Li et al. 2014; Fereidouni et al. 2016) mammals, as well as domestic mammals and humans (Sun and Liu 2015). This supporting evidence suggests the potential for interclass transmission of H1 and H6 IAV subtypes, provided exposure to an adequate infective dose; however, the only evidence for mammalian exposure on the Arctic Coastal Plain in our study was from a single Arctic fox sample that tested positive for IAV exposure via bELISA.

Loons, which often share water bodies with waterfowl, had lower overall seroprevalence than the geese had. Seroprevalence varied by loon species (3–20%; Table 1), with the highest apparent exposure in Red-throated Loons, although the sample size for that species was small. Surprisingly, only one sample from a Pacific Loon yielded a suspect positive result by HI, with a 1:10 antibody titer to an H4 virus and a 1:20 antibody titer to an H5 virus. Subtypes such as H4 and H5 are typically maintained in waterfowl hosts; however, we cannot extend our inference about potential sources of exposure in loons based on a single sample yielding weak seroreactivity. Limited information is available about IAV infection in loons, but an H1N1 virus was isolated from an Arctic Loon (*Gavia arctica*) in Romania (Iftimovici et al. 1980), antibodies reactive to an H3 IAV were detected in a Red-throated Loon in Russia (Zakstelskaya et al. 1975), and antibodies reactive to an H2 IAV were detected in an unidentified loon from Maine, US (Senne 2007). The relatively common detection of IAV antibodies among loon samples in our study suggests that loons should be considered in future assessments of IAV ecology. Additionally, given the discrepancy between bELISA and HI results, which typically show relatively good concordance, at least during the acute postexposure period (Spackman et al. 2009), further investigation of potential differences in the sensitivity and specificity of IAV antibody assays using samples from loons is warranted.

Despite the detection of IAV antibodies in a variety of waterbird species sampled on the Arctic Coastal Plain and the potential for frequent interclass contact, we detected limited or no evidence for IAV exposure in foxes, caribou, or polar bears. We detected IAV antibodies by bELISA in only a single adult, male Arctic fox collected in 2015. The initial S/N ratio of that sample was near the threshold of 0.5, and two subsequent analyses were negative (S/N>0.5), suggesting a low IAV antibody titer or an equivocal result. Serum volume was inadequate for HI subtype-specific analysis of that sample. All other fox samples tested by HI, including those with S/N ratios near 0.5, were negative. A previous study demonstrated experimental infection of H5N1 in red foxes (*Vulpes vulpes*) through both oral injection and consumption of infected birds, suggesting that wild foxes could be infected with avian-derived IAV (Reperant et al. 2008). Antibodies to H5 viruses were also detected among samples from wild carnivores (leopard cat) collected in Korea near major migratory bird habitats (Kim et al. 2018). However, our results indicate that exposure to IAVs among foxes may only occur rarely, if at all, in Arctic Alaska.

Caribou often occur in close proximity to nesting waterfowl and could be exposed to IAVs via environmental contamination or direct consumption of feces or eggs (Bousfield and Syroechkovskiy 1985; van der Wal and Loonen 1998). Infection with IAVs has not been previously reported in caribou or reindeer, and infections among ungulates are apparently rare (USGS National Wildlife Health Center 2018), although high titers to H5 IAVs were detected in wild water deer in Korea (Kim et al. 2018). Susceptibility of polar bears to infection with IAVs is also unknown, although a single captive polar bear that died of encephalitis was seropositive for IAV (Szentiks et al. 2014). It is plausible that free-ranging polar bears could encounter IAVs through environmental sources or consumption of infected birds, seals, or whales. Previous studies have identified antibodies to avian-origin IAV in several marine mammals from Alaska and northern Canada: ringed seal (*Phoca hispida*), Pacific walrus, and beluga whale (*Delphinapterus leucas*; Danner et al. 1998; Nielsen et al. 2001; Calle et al. 2008).

The near-absence of antibodies to IAVs in Arctic mammals may be explained by a number of different factors. It is probable that only a low proportion of waterfowl are actively shedding influenza viruses during summer when they are on the breeding grounds (Ip et al. 2008; Ely et al. 2013), thus reducing the probability of infection for Arctic mammals. Although other studies have isolated IAVs from spring staging geese (Ely et al. 2013; Reeves et al. 2013; Ramey et al. 2016)

and autumn staging ducks (Ramey et al. 2010; Reeves et al. 2013; Reeves et al. 2018), there is evidence that epidemiologic peaks of infection occur outside the nesting period or in subarctic regions of Alaska (Ip et al. 2008). Furthermore, we cannot rule out the possibility that ducks, rather than geese, are the primary reservoir of IAVs on the Arctic Coastal Plain and that interactions between ducks and mammals are comparably fewer than between mammals and colonial nesting geese.

Alternatively, exposure of mammals to infected waterfowl, including via direct consumption, may occur but only at subinfective doses or concentrations that elicit a weak or short duration immune response. An experimental study of raccoons determined that the infective dose for that species was relatively high and that water appeared to be a more effective transmission route than scavenging was (Root et al. 2014a). In contrast, experimental infection of cottontail rabbits (*Sylvilagus* sp.) required only minimal doses that are routinely detected in waterfowl-contaminated environments (Root et al. 2017). Besides differential susceptibility among species, there are also site-specific factors to consider. For example, viral degradation of IAVs may occur more readily under Arctic climatic conditions that include periodic freeze-thaw cycles and near-constant ultraviolet exposure during summer (Zou et al. 2013). However, there is also evidence to suggest that persistence of IAVs is negatively correlated with temperature (Brown et al. 2007; Dalziel et al. 2016), and the ability of these viruses to overwinter in local water bodies has been proposed based on the results of field studies (Hill et al. 2016).

Finally, we cannot rule out the possibility that prior IAV infection occurred among mammals but was not detected in our study. There are three primary considerations in assessing that possibility: test efficacy, duration of detectable antibodies using bELISA and HI assays, and sampling design. The IAV bELISA has been used successfully to detect antibodies in a diverse suite of other mammals (Sullivan et al. 2009; Vandalen et al. 2009; Root et al. 2010), although it was apparently ineffective for testing previously infected cottontail rabbits (Root et al. 2014b), suggesting that there are some limitations to its efficacy across all species. Similarly, it is possible that birds or mammals in our study were exposed to IAV subtypes with antigenic variability that was not captured among the IAV strains we used for the HI assay, thus explaining some of the discrepancies we observed between HI and bELISA among loons. Because of logistical constraints, our sampling design did not allow for perfect temporal or spatial concordance across species. We sampled caribou and polar bears in the spring before the arrival of most migratory birds. Thus, it is plausible that infections occurred the previous summer, but antibodies did not persist long enough to be detected at the time of sampling (7–10 mo), particularly in cases of occasional, rather than regular, exposure to IAVs. However, previous evidence suggests that limited antibody duration is unlikely to fully explain our lack of detections. A study of captive raccoons found that IAV antibodies for that species remained detectable in blood >9 mo after exposure (Root et al. 2010). Additionally, antibodies in marine mammals were detected in all seasons in Arctic Canada and Alaska (Nielsen et al. 2001; Calle et al. 2008), and outbreaks of IAV among seals in New England have been documented during most months of the year (Fereidouni et al. 2016). There may also be interannual variation in IAV prevalence among species, although other studies of high-latitude–nesting waterfowl have reported similarly high seroprevalence across years (Ely et al. 2013; Wilson et al. 2013). Thus, it is reasonable to assume relatively low variation in exposure rates among geese on the Arctic Coastal Plain.

Our results suggest that foxes, caribou, and polar bears in Arctic Alaska are not commonly exposed to IAVs and thus are unlikely to have an important role in their circulation in this region. However, we cannot exclude the possibility that, because of the timing of our sampling or methodo-

logic limitations (serologic assays have not been optimized for wild mammals), exposure occurred but was not detectable in our study. Additional sampling of mammals during the summer or fall to coincide with the presence of migratory birds would help to confirm the apparent absence of infection. Investigation of IAV persistence in the environment and swab-based sampling to assess evidence for active shedding by waterbirds in the Arctic would also provide useful insights about local risk of infection to birds and mammals. The detection of antibodies in loons suggests that IAVs circulate among sympatric waterbirds, but expanded sampling and additional subtype-specific analyses are needed to assess patterns and potential sources of exposure.

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