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AVIAN CHOLERA EXPOSURE AND CARRIERS IN GREATER WHITE-FRONTED GEESE BREEDING IN ALASKA, USA

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ABSTRACT: We conducted a 3-yr study (2001–03) on greater white-fronted geese (*Anser albifrons frontalis*) breeding in Alaska, USA, to determine the exposure of this population to *Pasteurella multocida* and the potential role of these birds as disease carriers. We tested sera from nearly 600 adult geese for antibodies to *P. multocida* serotype 1. We found a low prevalence (<5%) of positive antibodies in adult geese, and based on the short duration of detectable antibodies, these findings indicate recent infection with *P. multocida*. Prevalence was similar to serologic results from both breeding and wintering lesser snow geese. We also collected oral (n=1,035), nasal (n=102), and cloacal (n=90) swab samples to determine the presence of avian cholera carriers in this population. We were unable to isolate *P. multocida* serotype 1 from any of the birds sampled. Based on comparison with other waterfowl species, we concluded that these geese may be exposed to avian cholera during the winter or spring migration but are unlikely to play a significant role as carriers of the bacterium causing avian cholera.

Key words: Anser albifrons frontalis, avian cholera, carriers, greater white-fronted geese, *Pasteurella multocida*, serology.

INTRODUCTION

Avian cholera is the most important infectious disease among North American waterfowl, especially geese, and epizootics often kill thousands of birds (Samuel et al., 2006). The disease is caused by the bacterium *Pasteurella multocida.* Transmission typically occurs via ingestion of the bacterium from contaminated water or food, through inhalation of aerosolized wetland surface water, or by direct bird-to-bird contact. Wetlands are likely to become contaminated with bacteria when large numbers of birds die from the disease or if infected birds shed *P. multocida.*

Although avian cholera has important implications for wildlife management, our knowledge regarding the epizootiology of this disease remains limited. Of primary concern has been the role of carrier birds and wetlands as the reservoir for this disease (Botzler, 1991). In previous studies, we found that lesser snow geese (*Chen caerulescens caerulescens*) had detectable antibodies to *P. multocida* at arctic breeding colonies and wintering areas, indicating that these birds have survived recent infection and that the disease is probably transmitted throughout the annual cycle in these goose populations (Samuel et al., 1999a, b, 2005). We also found that apparently healthy lesser snow and Ross's geese (*Chen rossi*) sampled during the winter were carriers of pathogenic *P. multocida* serotype 1 (Samuel et al., 2005), which typically causes outbreaks of avian cholera throughout most of North America. Currently, little is known about the exposure of other waterfowl species to avian cholera, the ability of these species to function as carriers of *P. multocida,* and therefore, the role of these species in avian cholera epizootics.

The subpopulation of greater white-fronted geese (*Anser albifrons frontalis*) in Alaska, USA, has experienced recent population declines and lower survival rates than subpopulations from the Northwest Territories, Canada (Ely and Schmutz, 1999), and mortality from avian cholera has been suggested as a possible contributor. These geese likely are exposed to avian cholera on wintering grounds and migratory routes in the Central Flyway, which are shared with large numbers of other waterfowl, including lesser snow

geese. In particular, the interior Alaskan subpopulation migrates through the Rainwater Basin of Nebraska, USA, where frequent spring epizootics of avian cholera occur. In some years, >10,000 white-fronted geese have died during these outbreaks (Ely and Schmutz, 1999). Although lesser snow geese can be *P. multocida* carriers, to our knowledge no studies to determine whether other waterfowl species can survive infection with avian cholera or become carriers have been conducted.

The objectives of the present study were to determine the frequency of breeding interior white-fronted geese with exposure to *P. multocida* as measured by antibody response, to evaluate the occurrence of avian cholera carriers in the population by collecting swab samples from live birds, and to compare our findings with those of concurrent studies involving lesser snow and Ross's geese.

MATERIALS AND METHODS

We collected samples from adult greater white-fronted geese banded by the Division of Migratory Bird Management, U.S. Fish and Wildlife Service, from breeding areas at Koyukuk (65°22′N, 156°27′W), Innoko (63°08′N, 158°38′W), and Selawick (66°28′N, 159°13′W) national wildlife refuges in Alaska; from Noatak (67°40′N, 162°39′W) and Kanuti (66°09′N, 152°57′W) in the interior of Alaska; and from the North Slope (70°34′N, 151°18′W) of Alaska during the summers of 2001–03 (Fig. 1). Birds were captured using float planes to drive molting flocks into portable nets. Each bird was banded with an aluminum leg band, and sex was determined by cloacal examination. We used sample collection and diagnostic methods developed for detection of *P. multocida* in laboratory studies of carrier waterfowl (Samuel et al., 2003), which also were applied to snow geese (Samuel et al., 2005). Two swab samples were taken from the oral cavity of each bird and stored in 2-ml polypropylene cryogenic vials (catalog no. 430488, Corning Costar Corporation, Cambridge, Massachusetts, USA) containing 1.25 ml of either brain heart infusion broth (BHI) or 10% dimethyl sulfoxide. Nasal and cloacal swabs also were collected from a subset of the geese in 2002 and 2003 and stored in cryogenic vials containing the two media types. All swabs were stored frozen at −196 C in a dry-shipper liquid nitrogen tank

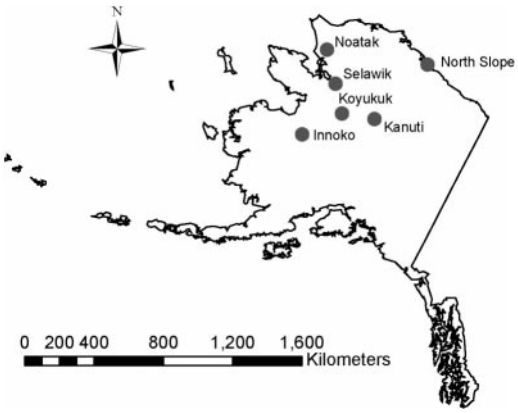

FIGURE 1. Greater white-fronted geese collection areas from the interior and northern slope of Alaska, USA, during the summers of 2001–03.

(model SC 4/2v, Minnesota Valley Engineering, Inc., Bloomington, Minnesota, USA) for transport to the National Wildlife Health Center (NWHC, Madison, Wisconsin, USA).

In the laboratory, frozen swab samples were processed in BHI broth and *Pasteurella multocida*–selective media (Moore et al., 1994) following the procedures described by Samuel et al. (2003, 2005). Following a second incubation, the tubes were thoroughly mixed, and one fifth of a blood agar plate was swabbed and the plate streaked for bacterial isolation. *Pasteurella multocida*–like, gram-negative isolates were identified using the analytical profile index 20E identification system (bioMerieux, St. Louis, Missouri, USA). Confirmed *P. multocida* isolates were serotyped using the agarose gel precipitin test (Heddleston et al., 1972).

Blood samples were collected from each goose by jugular venipuncture using an S-Monovette® Z syringe apparatus with a 20-gauge, 1.5-inch needle (catalog nos. 05.1104.100 and 85.1160, Sarstedt, Inc., Newton, North Carolina, USA). Samples were then centrifuged for 10 min at 1,500 × G, and the serum was dispensed into a sterile, 2-ml polypropylene vial, which was stored chilled until shipment to the NWHC for testing. Serum samples were tested for antibodies against *P. multocida* serotype 1 using the enzyme-linked immunosorbent assay (ELISA) (El Tayeb, 1993) enhanced as described by Samuel et al. (1999, 2003, 2005). The ELISA value percentages were measured for each serum sample based on pooled negative and positive reference sera and were considered to be positive to *P. multocida* at levels >17.4% (Samuel et al., 1999b). Serologic prevalence of *P. multocida* was evaluated for differences related to sex, breeding areas, and year

TABLE 1. *Pasteurella multocida* serologic results for greater white-fronted geese sampled at banding areas in Alaska, USA, during the summers of 2001–03.

	2001		2002		2003		Total	
Banding site	No. tested	No. positive	No. tested	No. positive	No. tested	No. positive	No. tested	No. positive
Innoko	71	6[a] (8.5)[b]	91	2 (2.2)	90	5 (5.6)	252	13 (5.2)
Koyukuk	37[c]	0	27	0	—	—	64	0
Noatak	—	—	58	4 (6.9)	32	0	90	4 (4.4)
Selawik	29	1 (3.5)	12	0	—	—	41	1 (2.4)
North Slope	—	—	—	—	132	4 (3.0)	132	4 (3.0)
Kanuti	—	—	—	—	12	0	12	0
Total	137	7 (5.1)	188	6 (3.2)	266	9 (3.4)	591	22 (3.7)

[a] One female had an enzyme-linked immunosorbent assay value of 17.4% and was considered to be suspect positive.
[b] Values in parentheses represent the percentage positive.
[c] Includes one gosling of unknown sex.

of banding using logistic regression (SAS Institute, 1989). We used χ^2 analysis (Fisher's exact test) to compare *P. multocida* exposure and carrier rates between lesser snow and greater white-fronted geese.

RESULTS

We collected serum samples from 136, 188, and 266 adult white-fronted geese from six separate banding areas in Alaska during the years 2001–03, respectively (Table 1). During 2001, seven of the 136 geese tested (5.1%) had positive serum antibody levels, indicating previous infection with *P. multocida.* The prevalence of seropositive geese was 8.5% (six of 71) at Innoko and 3.4% (one of 29) from Selawick. The prevalence of antibody-positive males was 3.8% (three of 80), compared to a prevalence of 7.1% (four of 56) in females. One of the seropositive geese at Innoko was identified as a Tule white-fronted goose (*Anser albifrons gambelli*), an uncommon subspecies that winters in the Pacific Flyway. During 2002, six of the 188 geese (3.2%) were seropositive, including two of the 91 geese (2.2%) from Innoko and four of the 58 geese (6.9%) from Noatak. No antibody-positive birds were found at Koyukuk and Selawik. During the year 2002, the prevalence of antibody positive males was 4.5% (five of 112), compared to 1.3% (one of 75) in females. During 2003, nine of 266 adult geese (3.4%) had positive serum antibody levels, with five (5.6%) from Innoko and four (3.0%) from the North Slope. No antibody-positive geese were found at Noatak or Kanuti, but samples were limited from those two banding locations. When considered over all 3 yr, 3.7% (22 of 590) of adult geese were seropositive from the six different banding sites, with most of serologic samples being obtained from the Innoko (42.6%), North Slope (22.3%), and Noatak (15.2%) sites. No significant differences ($P>0.40$) in serum antibody prevalence were found for males vs. females, among breeding areas, or between years; however, sample sizes, especially at some banding sites and among years, were limited.

Oral swab samples were collected from 131, 188, and 196 adult white-fronted geese banded in Alaska during the years 2001–03. When considered over all years, most of the geese we tested were from Innoko ($n=226$), followed by Noatak ($n=87$), North Slope ($n=86$), Koyukuk ($n=63$), Selewik ($n=43$), and Kanuti ($n=12$). In 2002, nasal swabs were obtained from 53 geese at the Innoko breeding area. Cloacal swabs also were collected from 47 geese at Innoko during 2003. All the swab samples were negative for *P. multocida*. Prevalence rates of avian cholera carriers in greater white-fronted geese in Alaska (0 of 515 birds) were lower ($P<0.01$) than prevalence rates for lesser snow geese (five of 266 birds) sampled from wintering areas

in the Playa Lakes region of the Central Flyway (Samuel et al., 2005).

DISCUSSION

Approximately 4% of the adult greater white-fronted geese sampled during the years 2001–03 on breeding areas in Alaska had levels of antibody to *P. multocida* indicating that birds had survived a previous infection with *P. multocida* serotype 1. Prevalence of seropositive white-fronted geese was similar to that reported in studies on breeding lesser snow geese at Wrangel Island, Russia and at Banks Island, Northwest Territories, Canada (3%) when no avian cholera outbreaks occurred on the breeding grounds (Samuel et al., 1999a) and for wintering lesser snow geese in the Playa Lakes region (3%) (Samuel et al., 2005). In previous studies, we found that birds maintain detectable levels of antibody to *P. multocida* serotype 1 for only 3–4 mo postinfection (Samuel et al., 1999b, 2003); thus, we believe the occurrence of seropositive birds likely indicates recent disease transmission. We note, however, that El Tayeb (1993) reported differences in antibody responses among species of waterfowl vaccinated for avian cholera.

We expected to isolate *P. multocida* from white-fronted geese, especially considering the serologic evidence that indicated this population of birds had been recently infected. In previous studies, we isolated the bacterium at low prevalence (<5%) from lesser snow and Ross's geese populations with seropositive birds (Samuel et al., 2005). Because we collected swab samples from >500 adult white-fronted geese, our sampling was sufficient (>99% probability) to detect avian cholera carriers in <1% of the population of greater white-fronted geese. Negative results suggest this population of greater white-fronted geese does not harbor significant carriers of avian cholera, which implies they are likely being infected by other waterfowl species. It is interesting to note the absence of significant avian cholera outbreaks in the Rainwater Basin and other areas in the Central Flyway during the period of our study. As a result, we expect that disease exposure, prevalence of seropositive geese, and perhaps, frequency of disease carriers would increase following spring avian cholera epizootics in the Rainwater Basin.

Recent studies support the hypothesis that waterfowl (Samuel et al., 1997, 1999b, 2003, 2005) and not wetlands (Samuel et al., 2004) are the reservoir for avian cholera. Although our knowledge regarding the epizootiology of avian cholera is increasing, the role of various waterfowl species in maintaining and distributing the disease remains uncertain. Other researchers have hypothesized that some species, such as lesser snow geese, may play an important role in maintaining avian cholera throughout the year because of their highly gregarious behavior (Samuel et al., 1999a, b; Samuel and Mensik, 2000). Based on these assumptions, management actions that attempt to reduce waterfowl densities and to separate carrier from noncarrier species have been suggested (Samuel et al., 2004). Specific recommendations about these strategies, however, will require research to determine which species of waterfowl, in addition to lesser snow and Ross's geese, likely are carriers of avian cholera and, therefore, play an important role in the epizootiology and distribution of the disease.

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