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## ANTIBODIES AGAINST *PASTEURELLA MULTOCIDA* IN SNOW GEESE IN THE WESTERN ARCTIC

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**ABSTRACT:** To determine if lesser snow geese (*Chen caerulescens caerulescens*) are a potential reservoir for the *Pasteurella multocida* bacterium that causes avian cholera, serum samples and/or pharyngeal swabs were collected from >3,400 adult geese breeding on Wrangel Island (Russia) and Banks Island (Canada) during 1993–1996. Pharyngeal swab sampling rarely (>0.1%) detected birds that were exposed to *P. multocida* in these populations. Geese with serum antibody levels indicating recent infection with *P. multocida* were found at both breeding colonies. Prevalence of seropositive birds was 3.5% at Wrangel Island, an area that has no recorded history of avian cholera epizootics. Prevalence of seropositive birds was 2.8% at Banks Island in 1994, but increased to 8.2% during 1995 and 1996 when an estimated 40,000–60,000 snow geese were infected. Approximately 50% of the infected birds died during the epizootic and a portion of the surviving birds may have become carriers of the disease. This pattern of prevalence indicated that enzootic levels of infection with *P. multocida* occurred at both breeding colonies. When no avian cholera epizootics occurred (Wrangel Island, Banks Island in 1994), female snow geese (4.7%) had higher antibody prevalence than males (2.0%).

**Key words:** Avian cholera, *Chen caerulescens caerulescens*, disease reservoir, enzyme-linked immunosorbent assay, lesser snow geese, *Pasteurella multocida*, serology.

### INTRODUCTION

Avian cholera, an infectious bacterial disease caused by *Pasteurella multocida*, infects >100 species of wild birds (Botzler, 1991) and typically occurs as epizootics which kill hundreds to thousands of birds. In addition, enzootic mortality occurs that never develops into extensive epizootics (Botzler, 1991; Wobeser, 1992), but the magnitude of these low level losses is unclear. Although avian cholera kills thousands of waterfowl annually in North American wetlands, the reservoir for *P. multocida* remains uncertain (Botzler, 1991). Currently, 16 different serotypes of *P. multocida* are recognized (Rimler et al., 1984) but strains isolated during waterfowl mortality events are usually serotype 1 in the Pacific, Central, and Mississippi flyways, or serotypes 3 and 4 in the Atlantic Flyway (Brogden and Rhoades, 1983; Windingstad et al., 1983; Hirsh et al., 1990; Wilson et al., 1995). Occasionally (<1%), *P. multocida* serotype 3, 4 (Wilson et al., 1995) and serotype 6 (Brogden and

Rhoades, 1983) are isolated from waterfowl in the Pacific Flyway.

Two potential reservoirs have been commonly suggested as a source of *P. multocida* in waterfowl populations: carrier birds and wetland sites. However, neither of these hypotheses have been thoroughly investigated. *Pasteurella multocida* also occurs in many mammals (Botzler, 1991) and the occurrence of virulent and non-virulent carriers in domestic poultry has been known for many years (Pritchett et al., 1930a, b; Wobeser, 1992). Although *P. multocida* has occasionally been isolated from healthy waterfowl (Vaught et al., 1967; Donahue and Olson, 1969; Korschgen et al., 1978; Titcher, 1979), the isolates usually have not been serotyped or tested for virulence (Wobeser, 1992; Samuel et al., 1997). Nevertheless, some authors have speculated that waterfowl carry the bacteria north to the breeding grounds, that birds sustain mortality there (Rosen, 1972), and the disease is thus maintained through carrier birds (Botzler, 1991; Wobeser, 1992). Wobeser et al. (1979) and

Wobeser (1992) reported low level avian cholera mortality in northward migrating white geese in Saskatchewan each spring. Mortality was confined largely to lesser snow geese (*Chen caerulescens caerulescens*) and Ross' geese (*C. rossii*), although other waterfowl share wetlands with these geese. This suggests that white geese may serve as carriers of the disease, which is spread among individuals by direct contact (Wobeser, 1992). Brand (1984) observed that avian cholera mortality patterns in the Central and Mississippi flyways closely followed the migration of snow geese.

This study reports our investigation to document avian cholera infection and antibodies in adult lesser snow geese nesting at Wrangel and Banks islands during 1993 to 1996 and to determine if these populations are potential reservoirs for the disease. We collected pharyngeal swabs to determine whether live, healthy snow geese had been exposed to *P. multocida*. We also collected serum samples to determine whether birds had become infected with *P. multocida* serotype 1 and produced antibodies against the bacterium.

## MATERIALS AND METHODS

### Study areas and populations

Several breeding populations of lesser snow geese winter in the Pacific Flyway of North America (Johnson, 1996). Two of the largest colonies of birds nest on Banks Island (Northwest Territories, Canada; 72°20'N, 125°10'W) and on Wrangel Island (Russia; 71°20'N, 179°40'W), the only major snow goose colony in the Palearctic (Bousfield and Syroechkovsky, 1985; Syroechkovsky and Litvin, 1986). These two populations generally follow separate migration pathways between their breeding and wintering areas (Bellrose, 1976). Most geese from Wrangel Island migrate south along the Pacific coast and divide into a northern subpopulation that winters in British Columbia (Canada) and Washington (USA) and a southern subpopulation that winters in the Central Valley of California (USA). Banks Island geese migrate through prairie Canada and a large portion of this population moves through Montana (USA) to northern California and the Central Valley. Spring migration begins from the Central Valley during February and March. In general, birds of both populations follow a

spring migration that reverses their autumn route, although most Wrangel Island geese in the southern subpopulation migrate through Montana and prairie Canada.

The Banks Island population has increased considerably in the past two decades (Kerbes et al., 1999), similar to other lesser snow goose populations in the central Canadian arctic. Breeding surveys indicate that the population has increased from approximately 165,000 breeding birds in 1976, to 200,000 by 1987, and to 480,000 by 1995. In contrast, the Wrangel Island population, which may be the remnant of a large population in Russia (Subcommittee on White Geese, 1992), apparently declined from >200,000 in the early 1960's (Bousfield and Syroechkovsky, 1985), to about 150,000 in the 1980's, and to about 75,000 breeding adults in the 1990's (V. Baranyuk, unpubl. data). Consistent winter counts for the northern subpopulation suggest that most of this decline has occurred in the southern subpopulation which winters in California. The cause of the apparent decline in this vulnerable population is unknown, but harvest mortality and avian cholera epizootics (which occur almost annually in California), may have been contributing factors.

Avian cholera epizootics have been frequently reported from winter and spring migration areas used by Banks Island geese and by geese from the southern subpopulation of Wrangel Island (Wobeser, 1992; National Wildlife Health Center [NWHC], unpubl. data). In recent years, avian cholera epizootics have occurred at the Banks Island snow goose colony, with mortality estimates ranging from 5,000 birds in 1991 (Nieman and Trost, 1991) to >20,000 birds in 1995 and 1996 (Samuel et al., 1999). In contrast, avian cholera epizootics have never been documented on Wrangel Island, although the colony has been intensively studied by Russian biologists for over 25 yr. In addition, avian cholera has not been reported at winter or migration areas used by geese from the northern subpopulation of Wrangel Island.

### Pharyngeal swab samples

Molting adult and gosling lesser snow geese were captured during July–August in the vicinity of the breeding colonies at Wrangel and Banks islands using either ground-based (Cooch, 1953) or helicopter (Timm and Bromley, 1976) drive techniques, respectively. Birds were captured in groups, banded with U.S. Fish and Wildlife Service (Laurel, Maryland, USA) legbands, and released in groups. During banding, body weight, a pharyngeal swab, blood sample, and morphological measurements were taken on a portion of the birds. To

reduce injuries to goslings, they were processed first and held in a separate enclosure until release of the entire group.

We swabbed the pharyngeal area using Culture Swab Transport Systems (Amies medium with charcoal, rayon tip, Difco Laboratories, Detroit, Michigan, USA). Swabs were maintained at cool (ambient arctic) temperatures until they were transported to NWHC. At NWHC, swabs were held at room temperature (22–25 C) until they were cultured (3–5 wk after collection) for the presence of *P. multocida* using the methods described in Samuel et al. (1997). In brief, *P. multocida* selective broth (PMSB) was inoculated from each swab to enhance the growth of *P. multocida* present in the sample (Moore et al., 1994) and reduce the expression of competitive organisms. A portion of the PMSB culture was streaked for isolation onto sheep blood agar plates (BAP) (product #01-202, Remel, Lenexa, Kansas, USA). After 18 to 24 hr of incubation at 37 C, the plates were held at room temperature and examined periodically over 4 days. Suspect *P. multocida* colonies were identified based on colony morphology, restreaked on BAP, and incubated to produce growth for further examination. Suspect isolates were Gram stained. Gram-positive isolates and Gram-negative isolates that were lactose positive on MacConkey agar or grew on Columbia colistin-nalidixic acid (CNA) agar containing polymyxin were discarded. Isolates with lactose negative growth or that failed to grow on MacConkey or Columbia CNA were inoculated into tubes of sulfide, indole, and motility (SIM) medium. Motile isolates were discarded and the resulting Gram-negative isolates fitting the *P. multocida* criteria were identified using the analytical profile index (API) 20E or API (NFT or NE) identification systems (bioMerieux, St. Louis, Missouri, USA). *Pasteurella multocida* isolates were serotyped by the agarose gel precipitin (AGP) test (Heddleston et al., 1972).

Some of the recovered isolates were tested for pathogenicity. A 6 hr Bacto brain heart infusion (BHI) broth (Difco) culture of each isolate was prepared ( $1.4 \times 10^5$  to  $2.5 \times 10^9$  CFU/ml). Four 4- to 6-wk-old Pekin (*Anas platyrhynchos*) ducks were injected subcutaneously in the dorsal caudal region of the neck with 0.2 ml of each culture (Price, 1985). Ducks that died following challenge were necropsied to identify gross lesions characteristic of avian cholera (Rosen, 1971; Friend, 1987) and livers were cultured for *P. multocida*.

#### Serum samples

We obtained blood samples from adult and gosling lesser snow geese by jugular venipunc-

ture using 5 cc Monovet® syringes (Sarstedt, Princeton, New Jersey, USA) and 23 ga, 2.5 cm needles. Serum was collected by centrifugation at 1,500 g for 5 to 10 min and kept chilled until arrival at NWHC, when samples were frozen at -14 C until tested for antibody concentrations.

Serum antibody against *P. multocida* serotype 1 was measured using an enzyme-linked immunosorbent assay (ELISA) procedure described by El Tayeb (1993) and Voller et al. (1979), with modifications in the antigen preparation and conjugate type. Instead of a live antigen, bacteria were killed with Beta-propiolactone (Sigma Chemical Company, St. Louis, Missouri) at a final concentration of 0.5%, prior to disrupting them through a French press. Cellular debris was removed by centrifugation (1 hr at 20,000 g) and the antigen extract protein content was determined using the Bradford/Biorad micro protein assay (Bradford, 1976). We found that affinity purified goat anti-turkey IgG (H + L) peroxidase-labeled antibody (Kirkegaard and Perry Laboratories [KPL], Gaithersburg, Maryland, USA) provided a greater positive to negative ratio between our positive and negative reference sera with less non-specific binding than either affinity purified goat anti-chicken or anti-duck IgG (H + L) peroxidase-labeled antibody (NWHC, unpubl. data).

The ELISA was conducted following the procedure used by El Tayeb (1993). Briefly, from frozen aliquots, an antigen dilution of 8.25 µg/ml in carbonate/bicarbonate buffer pH 9.6 ± 0.2 was used to coat the inner 60 wells of each 96-well Falcon 3915 Pro-Bind Assay microtitration plate (Becton Dickinson and Company, Union Park, New Jersey). The plates were covered and incubated overnight at 4 C, then washed four times with PBS/Tween buffer pH 7.2 ± 0.2. The test sera and the reference sera were diluted 1:100 in PBS/Tween buffer (pH 7.2 ± 0.2), mixed well, and 50 µl was added to individual wells in triplicate. A no-serum control containing only the PBS/Tween buffer also was added to three wells to check for non-specific binding. The plates were covered and incubated at 37 C for 30 min and then washed as before. Affinity purified goat anti-turkey IgG(H + L) peroxidase-labeled antibody (KPL), initial concentration of 0.1 mg/ml, was diluted 1:500 in PBS/Tween buffer, and 50 µl were added to each well. The plates were covered and incubated at 37 C for 30 min, followed again by four washings. Equal volumes of ABTS® (2,2'-azino-di[3-ethyl-benzthiazoline sulfonate (6)]) peroxidase substrate and peroxidase solution B (H<sub>2</sub>O<sub>2</sub>) (KPL) were mixed, warmed to room temperature, and 100 µl was

added to each well, including the blanking wells (first column of the microtiter plates). The plates were covered and placed in a light tight container and incubated for 1 hr at room temperature for color development. The reaction was stopped with 100  $\mu$ l/well ABTS<sup>TM</sup> peroxidase stop solution (KPL). The stop solution (5% sodium dodecyl sulfate in water) was diluted one part ABTS<sup>TM</sup> peroxidase stop solution with four parts reagent quality water to produce a 1% solution. Absorbance (optical density [OD]) measurements, zeroed on the blanking wells in the first column of the microtiter plates (containing only the substrate and stopping solution), were obtained on a Biokinetics reader (model EL 312E, Bio-Tek Instruments, Inc., Highland Park, Winooski, Vermont, USA) at a 405 nm wavelength.

The mean absorbance measurements of the triplicate test sera were expressed as the percent positivity relative to the positive reference sample; the ELISA value percent (EV%) = [(Test OD - Negative reference OD)/(Positive reference OD - Negative reference OD)]  $\times$  100 (de Savigny and Voller, 1980). Reference sera for conducting the ELISA was obtained from captive lesser snow geese vaccinated with *P. multocida* bacterin (Price, 1985). Antibody response peaked 2 wk post-vaccination, declined substantially by 8 wk post vaccination, and then appeared to stabilize or decline slightly until about 20 wk post-vaccination (El Tayeb, 1993; NWHC, unpubl. data). Sera obtained pre-vaccination, 8 wk post-vaccination, and 3 wk after challenge (anamnestic response) with a pathogenic *P. multocida* culture provided the negative (ELISA OD range = 0.07–1.3), low positive, and high positive reference sera (ELISA OD range = 1.2–1.6), respectively. Each reference serum consisted of a pool ( $n = 4$ ) of snow goose sera. We used the mean EV% (approximately 10%) for birds at 8 wk post-vaccination as the low positive reference value in our ELISA procedure and as the cut-off for indicating positive *P. multocida* antibody level in wild birds (see Wright et al., 1993).

Sensitivity evaluation for the ELISA was difficult to conduct because we had no source of birds with known *P. multocida* infection and antibody response, other than snow geese vaccinated with the *P. multocida* bacterin. For a negative population, we used sera obtained from lesser snow goose goslings during 1993 and 1995 at Wrangel Island, where no avian cholera epizootics have been recorded. Most of these goslings had ELISA EV% values  $<0\%$ . An evaluation of the ELISA data using vaccinated lesser snow geese (positive population) and Wrangel Island goslings (negative population) indicated that the 10% threshold we used

to determine seropositive birds may underestimate the recent infection rate because some vaccinated birds had antibody levels that were in the seronegative range ( $<10\%$ ) at 8 wk post-vaccination.

The specificity of the serotype 1 antigen ELISA was tested against chicken antisera (National Veterinary Sciences Laboratory, Ames, Iowa, USA) for all 16 *P. multocida* serotypes. Chicken antisera against serotypes 1, 6, 7, 8, 9, 11, 15, and 16 produced OD values higher than our low positive reference serum while the antisera for serotypes 2, 3, 4, 5, 10, 12, 13 and 14 produced OD values below the low positive reference serum and near the negative reference serum. Despite the potential for cross reaction with other serotypes, we believe the ELISA has appropriate specificity for detecting antibody levels to *P. multocida* serotype 1 in the snow geese we tested from the Pacific Flyway because rarely have isolations of those serotypes that would produce positive OD values been reported from wetland birds outside the Atlantic Flyway.

#### Statistical analysis

We used logistic regression methods (Hosmer and Lemeshow, 1989) to analyze seroprevalence of snow geese to *P. multocida* based on breeding colony, year of sampling, and sex class. Additional analyses of seroprevalence were also conducted using  $\chi^2$  analysis (Zar, 1984). We analyzed the serum antibody levels from seropositive snow geese using Analysis of Variance (ANOVA) based on ranks of the antibody EV% values (SAS, 1989). These non-parametric ANOVA's were used because the antibody levels were positively skewed and did not follow a normal distribution.

#### RESULTS

Except for occasional samples with little or no growth, numerous bacteria were cultured from the swab samples collected from lesser snow geese. The only isolate of *P. multocida* from  $>750$  adult snow geese at Wrangel Island was serotype 12 with moderate cross reactions to serotypes 5, 14, and 15 from one adult female in 1995 (Table 1). No isolates were recovered from 59 goslings sampled in 1993 or from 32 gosling sampled in 1995. Among  $>2,300$  adult snow geese sampled at Banks Island, *P. multocida* was isolated each year (1994–1996). No isolates were recovered from 10 goslings sampled in 1994 or from 30 gos-

TABLE 1. *Pasteurella multocida* isolates obtained from pharyngeal swabs of adult lesser snow geese captured on Wrangel and Banks islands during the nesting seasons of 1993–1996.

Breeding colony	Year	Number sampled	<i>P. multocida</i> serotype 1 isolates	<i>P. multocida</i> serotype 3 isolates	<i>P. multocida</i> serotype 12 isolates
Wrangel Island Russia	1993	301	0	0	0
	1994	222	0	0	0
	1995	250	0	0	1 <sup>a</sup>
Banks Island Canada	1994	288	1	1	0
	1995	972	0	3	3 <sup>b</sup>
	1996	1,052	0	1	0

<sup>a</sup> Isolate also identified as having moderate cross reaction with serotypes 5, 14, and 15 in AGP test.

<sup>b</sup> Two isolates also identified as having cross reaction with serotype 3 in AGP test.

lings sampled in 1996. *Pasteurella multocida* serotype 1 was isolated from an adult male snow goose in 1994 (Samuel et al., 1997), serotype 3 was isolated from five adults (two females, three males) during 1994–1996, and serotype 12 was isolated from three adults (one male, two females) in 1995. Two of these serotype 12 isolates also had cross reactions with serotype 3. Two birds with serotype 12 isolates also were seropositive by ELISA, indicating previous infection with *P. multocida* serotype 1. All the remaining birds with *P. multocida* isolates were serologically negative for serotype 1.

The 1994 Bank's Island serotype 1 and serotype 3 isolates were tested previously for pathogenicity (Samuel et al., 1997). We tested pathogenicity of the serotype 12 isolate obtained from Wrangel Island, and the remaining serotype 3 and serotype 12 isolates obtained from Banks Island in 1995 and 1996. The serotype 1 isolate was the only pathogenic isolate; none of the serotype 3 or serotype 12 isolates killed or caused morbidity in any of the ducks challenged.

Median antibody levels (EV%) for seropositive snow geese at Banks Island (Table 2) were similar among years, by sex class, or for year by sex interaction (all  $P > 0.3$ ). Median antibody levels were also similar among years, subpopulations, sex classes, and all interactions ( $P > 0.13$ ) at Wrangel Island (Table 2), but the number of seropositive geese was smaller at Wrangel

than at Banks Island and probably reduced the statistical power of these analyses. Although median antibody levels were generally low during all years (12–19 EV%), 10 birds had antibody levels exceeding 40 EV%. Extrapolating from our captive, vaccinated snow geese, these antibody levels indicate very recent *P. multocida* infection or an anamnestic response. One of the 10 snow geese with a high antibody level (a female) was from the southern subpopulation on Wrangel Island. The remaining nine geese were from Banks Island (three males, six females) and three of these birds had levels  $\geq 70$  EV%. Eight of the geese with high antibody levels were sampled during 1995 and 1996, following the avian cholera epizootics on Banks Island.

Antibody levels (EV%) were negative for 60 goslings sampled on Wrangel Island in 1993 and 31 goslings sampled in 1995, no goslings were sampled in 1994. Antibody levels (EV%) were also negative for 28 goslings sampled at Banks Island in 1995, no goslings were sampled in 1994 or 1996.

Logistic regression analyses of the antibody prevalence (Table 2) indicated that *P. multocida* infection was similar among years ( $P = 0.19$ ) and between subpopulations ( $P = 0.41$ ) on Wrangel Island. However, females had a higher ( $P < 0.02$ ) prevalence (4.8%) than males (2.1%). At Banks Island, the primary difference in prevalence was among years ( $P < 0.01$ ); preva-

TABLE 2. *Pasteurella multocida* serum antibody prevalence and median antibody levels of adult lesser snow geese captured on Wrangel and Banks islands during the nesting seasons of 1993–1996.

Breeding colony	Year	Subpopulation	Sex	Number sampled	<i>P. multocida</i> seropositive <sup>a</sup>	Median antibody level (EV%) <sup>b</sup>
Wrangel Island Russia	1993	North	Male	85	1 (1.2%)	14.4
			Female	79	4 (5.1%)	12.1
		South	Male	147	2 (1.4%)	18.3
			Female	141	3 (2.1%)	17.6
	1994	North	Male	57	0 (0.0%)	—
			Female	66	2 (3.0%)	14.6
			Female	169	13 (7.7%)	12.8
	1995	North	Male	35	2 (5.7%)	11.9
			Female	35	1 (2.9%)	11.7
			Female	75	3 (4.0%)	13.6
		South	Male	93	5 (5.4%)	17.2
			Female	93	5 (5.4%)	17.2
Female			93	5 (5.4%)	17.2	
Banks Island Canada	1994		Male	141	2 (1.4%)	13.1
			Female	144	6 (4.2%)	15.9
	1995		Male	462	36 (7.8%)	13.6
			Female	517	47 (9.1%)	14.4
	1996		Male	503	42 (8.4%)	14.2
			Female	516	38 (7.4%)	15.1

<sup>a</sup> Number and % positive.<sup>b</sup> Median antibody level for seropositive snow geese as determined by ELISA.

Prevalence was higher during epizootic (8.2%) than non-epizootic (2.8%) years. At Banks Island, males (8.1%) and females (8.2%) had similar prevalence during epizootic years (1995 and 1996), but during 1994 the prevalence was greater in females than males, similar to the pattern found at Wrangel Island. Over all non-epizootic years (Wrangel Island and Banks Island in 1994), prevalence was more than twice as high in females (4.7%) than in males (2.0%).

#### DISCUSSION

We cultured *P. multocida* serotype 3 and serotype 12 isolates from snow geese at both Wrangel and Banks islands, but none of these isolates were pathogenic to Pekin ducks. We have also isolated *P. multocida* other than serotype 1 from wetlands used by waterfowl and none these isolates which have been tested were pathogenic (NWHC, unpubl. data). Thus, we emphasize the importance of serotyping and pathogenicity testing for *P. multocida* iso-

lates recovered from healthy birds or from the environment.

*Pasteurella multocida* is believed to colonize the upper respiratory tract and spread to the lungs before invading the bloodstream (Christensen and Bisgaard, 1997:466), and bacteria have been isolated from the respiratory tracts of waterfowl (Titcher, 1979). However, we generally were unsuccessful in our attempts to isolate the bacteria using pharyngeal swabs and we found no association between avian cholera epizootics and the isolation of *P. multocida* serotype 1 from pharyngeal swabs. Serotype 1 was isolated from one bird on Banks Island, but this bird was seronegative in our ELISA test. This bird was exposed to *P. multocida* but may not have developed an infection or was too recently infected to develop antibody response. The bird was known to be alive >2 yr after banding. We suspect that pharyngeal swabs may underestimate the number of birds that are exposed, infected, or carrying *P. multocida* because the bacterium

may be difficult to isolate from live birds, not all exposed birds become infected (Wobeser, 1992), and the bacterium may be harbored in other tissues (e.g., lungs) that are difficult to sample in live birds. The lengthy time required before laboratory processing might have reduced our ability to isolate *P. multocida* from the samples; however, we have been able to culture *P. multocida* from mixed cultures held in Amies media for >2 yr (NWHC, unpubl. data).

Even when mortality events were not detected on the breeding colonies, approximately 3% of snow geese had antibody titers indicative of infection with *P. multocida*. This prevalence most likely represents recent exposure (<2–4 mo) of birds to *P. multocida* due to enzootic transmission of disease within snow goose populations, and we suspect that low-level mortality is associated with these infections. We offer several lines of supporting evidence that our ELISA measures recent infection. First, birds in general have poor immune response to bacterial infection and short-term IgG antibody response was observed in captive waterfowl (including snow geese) vaccinated with a *P. multocida* bacterin (El Tayeb, 1993; NWHC, unpubl. data). Second, if epizootics produced prolonged high antibody levels in snow geese we would have expected to find higher median antibody levels in infected geese following mortality events at Banks Island. Third, we found no statistical difference in prevalence or antibody levels between northern and southern subpopulations of snow geese nesting at Wrangel Island, despite the evidence that only the southern subpopulation experiences winter and spring avian cholera epizootics.

Antibody prevalence was higher in female snow geese (4.7%) than in males (2.0%), when summer avian cholera epizootics did not occur. This gender-based difference in prevalence was not found at Banks Island in 1995 and 1996, following avian cholera epizootics. We are uncertain about the reasons for this higher preva-

lence in females, but we suspect that several factors could be involved. First, there may be different infection rates on breeding areas based on behavioral differences between nesting females and territorial males; however, we would generally expect that females would have less opportunities for exposure because >95% of their time is spent incubating eggs (Afton and Paulus, 1992). Second, males may have a lower antibody response to *P. multocida* than females. Third, males may be more susceptible to mortality from *P. multocida* infection. These latter two factors may be linked. We found that vaccinated male snow geese produced lower *P. multocida* antibody levels than females (NWHC, unpubl. data) and higher antibody levels were associated with survival during laboratory challenge studies (El Tayeb, 1993; NWHC, unpubl. data). In addition, Windingstad et al. (1998) reported that adult male Canada geese (*Branta canadensis*) had higher mortality than adult females during avian cholera epizootics and McLandress (1983) observed a higher frequency of male Ross' and lesser snow geese among birds dying from avian cholera, compared to hunter killed birds. Further investigation is needed to determine the cause and significance of lower seroprevalence in males than females.

Antibody prevalence increased significantly to >8% in snow geese sampled approximately 1 mo following avian cholera epizootics on Banks Island in 1995 and 1996, compared with 2.8% in 1994 when an epizootic did not occur. In addition, Samuel et al. (1999) estimated that 9% and 5% of the breeding colony at Banks Island died from avian cholera during the 1995 and 1996 epizootics, respectively. Considering the estimated colony size of approximately 400,000 geese (Kerbes et al., 1999), it appears that 50,000 to 70,000 birds were infected with *P. multocida* during each epizootic, and about half of the infected geese survived. This mortality was much smaller than previously believed for such a peracute disease (Collins, 1977;

Hunter and Wobeser, 1980; Friend, 1987; Wobeser, 1981). We suspect that the survivors may be carriers of the bacteria and play an important role in transmitting the organism to susceptible birds.

Snow geese may be particularly important in the epizootiology of avian cholera because some populations of these birds have dramatically increased during the past decade (Ankney, 1996), they are frequently involved in larger avian cholera epizootics (NWHC, unpubl. data), the birds associate in dense winter aggregations that may enhance disease transmission, and these birds nest in colonies which facilitate continuation of the disease cycle during summer (Wobeser, 1992; Samuel et al., 1999). Further research is needed to determine the immune status of birds infected with *P. multocida*, how susceptibility varies between males and females, whether snow geese are actually carriers of the bacterium, how prevalence of carriers changes spatially and temporarily, and how the disease is transmitted.

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