AVIAN CHOLERA IN WATERFOWL: THE ROLE OF LESSER SNOW AND ROSS'S GEESE AS DISEASE CARRIERS IN THE PLAYA LAKES REGION

Authors: Samuel, Michael D., Shadduck, Daniel J., Goldberg, Diana R., and Johnson, William P.

Source: Journal of Wildlife Diseases, 41(1) : 48-57

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-41.1.48

Journal of Wildlife Diseases, 41(1), 2005, pp. 48–57

AVIAN CHOLERA IN WATERFOWL: THE ROLE OF LESSER SNOW AND ROSS'S GEESE AS DISEASE CARRIERS IN THE PLAYA LAKES REGION

Michael D. Samuel,[1,2,4] Daniel J. Shadduck,[1] Diana R. Goldberg,[1] and William P. Johnson[3]

[1] US Geological Survey, National Wildlife Health Center, 6006 Schroeder Road, Madison, Wisconsin 53711, USA
[2] Wisconsin Cooperative Wildlife Research Unit, 204 Russell Laboratories, 1630 Linden Drive, Madison, Wisconsin 53706, USA
[3] Texas Parks and Wildlife Department, PO Box 659, Canyon, Texas 79015, USA
[4] Corresponding author (email: mdsamuel@wisc.edu)

ABSTRACT: We collected samples from apparently healthy geese in the Playa Lakes Region (USA) during the winters of 2000–01 and 2001–02 to determine whether carriers of *Pasteurella multocida,* the bacterium that causes avian cholera, were present in wild populations. With the use of methods developed in laboratory challenge trials (Samuel et al., 2003a) and a serotype-specific polymerase chain reaction method for identification of *P. multocida* serotype 1, we found that a small proportion of 322 wild birds (<5%) were carriers of pathogenic *P. multocida.* On the basis of serology, an additional group of these birds (<10%) were survivors of recent avian cholera infection. Our results confirm the hypothesis that wild waterfowl are carriers of avian cholera and add support for the hypothesis that wild birds are a reservoir for this disease. In concert with other research, this work indicates that enzootic infection with avian cholera occurs in lesser snow goose (*Chen caerulescens caerulescens*) populations throughout their annual cycle. Although fewer Ross's geese (*Chen rossii*) were sampled, we also found these birds were carriers of *P. multocida.* Even in the absence of disease outbreaks, serologic evidence indicates that chronic disease transmission and recent infection are apparently occurring year-round in these highly gregarious birds and that a small portion of these populations are potential carriers with active infection.

Key words: Avian cholera, carriers, *Chen caerulescens caerulescens, Chen rossii,* lesser snow geese, *Pasteurella multocida,* Ross's geese, wild waterfowl.

INTRODUCTION

Avian cholera, caused by the bacterium *Pasteurella multocida,* was initially reported in North America in domestic poultry during the late 1800s (Botzler, 1991) and in wild waterfowl in Texas and California (USA) in 1944 (Quortrup et al., 1946; Rosen and Bischoff, 1949). Since that time, the disease has been reported in wild birds from all flyways and has become the most important infectious disease affecting North American waterfowl (Friend, 1999). Avian cholera outbreaks occur almost annually as acute outbreaks at waterfowl concentration areas in the Central Valley of California, the Rainwater Basin of Nebraska, areas of Texas and Minnesota, and western Canada (Wobeser et al., 1982; Botzler, 1991) and in snow goose (*Chen caerulescens caerulescens*) breeding colonies (Samuel et al., 1999a). In addition, chronic transmission and infection might occur year-round in some snow goose populations (Samuel et al., 1999b). During avian cholera outbreaks, it is not uncommon for thousands of waterfowl to die (Brand, 1984; Botzler, 1991; Samuel et al., 1999a), with mortality from the most severe epizootics exceeding 20,000 birds (Brand, 1984; Friend, 1999; Samuel et al., 1999a).

Two competing hypotheses have been proposed to explain the recurrent pattern of avian cholera outbreaks: 1) *P. multocida* persists in specific wetlands year-round in water, soil, or other reservoirs and 2) waterfowl carriers of *P. multocida* initiate disease outbreaks as migratory birds congregate in staging and wintering areas. The absence of *P. multocida* in these wetland ecosystems provides evidence that wetlands are not a likely reservoir for cholera outbreaks (Samuel et al., 2004). In contrast, researchers have argued that carrier birds (healthy birds with virulent *P. multocida*) might serve as reservoirs (long-term source) for the bacteria and initiate

epizootics (Botzler, 1991; Samuel et al., 2003a); however, convincing evidence that waterfowl are an important reservoir has also been weak. Serologic samples obtained from snow geese at breeding colonies on Wrangel Island (Russia) and at Banks Island (Canada) when avian cholera outbreaks did not occur indicated that birds had been recently (within 3–4 mo) infected with *P. multocida* and survived infection (Samuel et al., 1999b). These summer outbreaks in colonial nesting species might help maintain the disease cycle through carrier birds (Botzler, 1991; Wobeser, 1992; Samuel et al., 1999b). Although serologic techniques might be useful in assessing recent infection from *P. multocida* (Samuel et al., 1999b), they only suggest that wild birds that survive infection could retain bacteria and become potential carriers of *P. multocida,* which could be transmitted later to susceptible birds. *Pasteurella multocida* obtained from oral swab samples showed that carrier snow geese occurred (Samuel et al., 1997), but prevalence of detected carriers was very low (Samuel et al., 1999b).

Previous investigators have suspected that lesser snow and Ross's geese (*Chen rossii*) (light geese) could be the primary source of *P. multocida* because outbreaks have been associated with their movement and distribution patterns (Wobeser et al., 1979, 1982; Brand, 1984; Samuel et al., 2001), they suffer outbreaks and chronic mortality every year (Mensik and Samuel 1995), outbreaks that might perpetuate the disease cycle occur in snow goose breeding areas (Samuel et al., 1999a), and the magnitude of mortality in other species has been associated with snow goose mortality. This evidence suggests that snow and Ross's geese, and possibly other species, could serve as a reservoir for *P. multocida.* However, to confirm that carrier birds occur and are important in the transmission of avian cholera, additional research was needed that focused on isolating live *P. multocida* from the tissues of birds and determining how and when the organism is transmitted to susceptible birds (Samuel and Mensik, 2000). The objectives of this study were to determine whether lesser snow and Ross's geese are carriers of *P. multocida,* the frequency of carriers in healthy bird populations, and what tissues should be sampled to successfully recover the bacterium.

FIGURE 1. Playa Lakes region of Colorado, Kansas, New Mexico, Oklahoma, and Texas (USA).

MATERIALS AND METHODS

Study area

Our research targets primarily lesser snow geese of the western Central Flyway which breed in the western Canadian Arctic (Kerbes et al., 1999) and are often involved in avian cholera outbreaks in wintering and migration areas. These geese winter in the Playa Lakes Region of the United States (Fig. 1), an area encompassing 364,000 km^2 in northwestern Texas and adjacent portions of eastern New Mexico, southeastern Colorado, southwestern Kansas, and western Oklahoma (Playa Lakes Joint Venture, 1994). This area has a semiarid climate and is relatively flat and open, and playa lakes are the dominant wetland habitat feature. The region is affected heavily by agriculture, including both cultivation and grazing. Detailed descriptions of the area are found in reports by the US Fish and Wildlife Service (1988), Bolen et al. (1989a, b), and Haukos and Smith (1992). The Playa Lakes Region is important to migrating and wintering waterfowl in the Central Flyway (Buller, 1964; Bolen et al., 1989a), and light geese are increasing in this area (Ray and Miller, 1997). Since the 1970s, estimates of light geese in this region have increased from less than 100 to more than 150,000 in 1999 (Texas Parks and Wildlife Department, unpubl. data). These increases have been especially apparent during the last 10 yr, when harvest estimates in the Playa Lakes Re-

gion have averaged over 12,000 light geese per year.

Field collection and testing

Light geese from the Playa Lakes Region were collected during the winters of 2000–01 and 2001–02. Geese were collected primarily by shooting live, apparently healthy birds. A blood sample for antibody analysis was collected from the body cavity or heart of each carcass within 8 hr and centrifuged (10–15 min at 1,500 × G). Sera were removed and frozen at −20 C before shipment on dry ice to the National Wildlife Health Center (NWHC; Madison, Wisconsin, USA), where they were stored frozen until processing. On the basis of a laboratory study of mallards (*Anas platyrhynchos*; Samuel et al., 2003a), two swab samples were taken for *P. multocida* culture from the oral, nasal, and cloacal cavities; leg joint; and eye surface from each goose collected. Swabs were swirled in cryovials containing 1.25 ml of either brain heart infusion (BHI) broth (Difco Laboratories, Detroit, Michigan, USA) or 10% dimethylsulfoxide (DMSO), and swab tips were broken off into the vial. The cryovials were stored in liquid nitrogen (−210 C) within 2 hr of sampling and sent in a liquid nitrogen dry shipper to the NWHC for subsequent laboratory analysis.

Frozen samples were thawed at room temperature (16–20 C), and the contents of each vial were transferred into a culture tube containing 5 ml of BHI broth. Each tube was incubated for 2–2.5 hr at 37 C with shaking (100 revolutions per minute) for pre-enrichment. Following incubation, 2 ml of the broth was transferred to a tube containing 5 ml of *P. multocida* selective broth (PMSB; Moore et al., 1994) and incubated for 12–16 hr at 37 C with 5–10% CO_2. Following this second incubation, a portion of the liquid culture was streaked onto a blood agar plate (BAP; Becton Dickinson, Sparks, Maryland, USA). The BAP were incubated for 20–24 hr at 37 C with 5–10% CO_2 and examined for colonies resembling *P. multocida.* Suspect *P. multocida* colonies were examined by Gram stain, serotyped by the agarose gel precipitin test (Heddleston et al., 1972), and identified with the analytical profile index (API) 20E identification system (bioMerieux, St. Louis, Missouri, USA) as indicated by Samuel et al. (2003b).

Each *P. multocida* isolate was tested for virulence in four adult mallard ducks (Whistling Wings, Hanover, Illinois, USA) by subcutaneously injecting 0.2 ml of the challenge inocula (6.2×10^5 to 1.7×10^6 cells) in the dorsal caudal region of the neck (Samuel et al., 2003b), a method previously used to standardize our challenge trials with similar results to naturally infected ducks (Samuel et al., 2003a). Birds challenged with serotype 1 isolates were housed in individual stainless steel cages (76 × 61 × 41 cm; Lab Products, Inc., Aberdeen, Maryland) to reduce the risk of disease transmission among birds. Mallards challenged with non–serotype 1 isolates were maintained together, but in a separate 22-m^2 isolation room on 1.3 × 2.5 cm gauge diamond Tenderfoot® (Tandem Products, Inc., Minneapolis, Minnesota, USA) because mortality was not expected from these isolates. Ducks were observed for illness, morbidity, and mortality for 7 days, and challenged survivors were euthanized by cervical dislocation. The livers of all birds that died or were euthanized were cultured for *P. multocida,* and isolates were identified by API 20E and serotyped as described in the previous paragraph.

We performed a polymerase chain reaction (PCR) analysis on 1 ml of PMSB cultures that were frozen at the same time initial culturing was started. We used the PCR procedure described by Rocke et al. (2002) with the following modifications. Briefly, PMSB enrichment culturing time was increased to 12–16 hr, and normal flora from the oral cavity of the mallard ducks, consisting of five or six bacterial species, was used as a negative control. We spiked the negative control with a mixture of 25 *P. multocida* cultures to provide our positive test control and used a second positive control to ensure that DNA extraction procedures worked correctly. Electrophoresis of the PCR products was performed with the Horizon 11-14 gel electrophoresis apparatus (Life Technologies, Inc., Rockville, Maryland, USA).

Sera from wild geese were tested with the same enzyme-linked immunosorbent assay (ELISA) procedures described for mallard ducks (Samuel et al., 2003a), with three changes to improve test consistency. The antigen and conjugate preparations were done gravimetrically, rather than by pipetting. Room temperature was standardized to 18.5 C ± 1.5 C, which required a reduction of the substrate incubation time to 35 min. These procedural changes were tested with the use of our snow goose reference sera (Samuel et al., 1999b) to ensure consistency in detecting positive antibody levels with our previous ELISA procedures.

RESULTS

Fifty-five Ross's and 266 snow geese were collected in the Playa Lakes Region and tested for *P. multocida* during January,

February, and March 2001 and 2002 (Table 1). We recovered 11 *P. multocida* serotype 1 isolates from five geese. Six serotype 1 isolates were recovered from a single adult male Ross's goose from Texas in 2001; the bacterium was isolated from nasal, oral, cloacal, and both leg joint samples. Three serotype 1 isolates were recovered from two adult male snow geese from Colorado in 2002: from an eye swab from one goose and the cloacal and leg joint swabs from the second bird. Serotype 1 isolates were recovered from two adult female snow geese from Texas in 2001, from a nasal swab, and from an oral swab. Other *P. multocida* serotypes were also isolated from an adult snow goose collected in Kansas in 2001 and Texas in 2002 (Table 1); however, these serotypes are not commonly pathogenic in waterfowl (Friend, 1999). Estimated prevalence of carriers in light geese on the basis of culture samples was approximately 2% in Texas and 4% in Colorado, compared with 0% in the remaining states. Population prevalence was 2% in Ross's geese compared with 2% in snow geese. Prevalence of carriers was 2% in adult geese compared with 0% in immature geese and 2% in males compared with 1% in females. The PCR and culturing techniques produced similar results; all culture-positive samples were PCR positive. The PCR analysis detected one additional positive bird from oral swabs taken from an immature female snow goose from Texas in 2002.

We recovered at least one *P. multocida* serotype 1 isolate from each of the tissues we sampled in wild geese (Table 2). We also recovered the bacterium from swabs preserved in both BHI and DMSO. Serotype 1 isolates were recovered from both preservation methods (BHI and DMSO) for two tissues from the positive Ross's goose. All other serotype 1 isolates were recovered from either the DMSO or BHI swabs, but not both. The number of positive samples was insufficient to determine which preservation method or tissue swab was preferred for detection of carriers.

TABLE 1. Ross's and snow geese collected for *Pasteurella multocida* isolation from the Playa Lakes Region (USA) in January–March 2001 and 2002.

State	Collection dates	Ross's geese				Snow geese				Birds with *Pasteurella multocida* serotype 1	Birds tested
		Adult		Immature		Adult		Immature			
		M	F	M	F	M	F	M	F		
Colorado	March 2001 and March 2002	3	1	0	1	25[a,b]	16[c]	1	3	2	50
Kansas	February–March 2001	1	0	0	0	11	12[d]	1	0	0	25
New Mexico	February 2001 and February–March 2002	2	3	1	0	22	22	5	1	0	56
Oklahoma	March 2001 and February 2002	5	2	0	0	2	4	0	0	0	13
Texas	February–March 2001 and January–March 2002	5[a]	13	9	9	39	56[e,f]	26	20	3	177[g]
Total		16	19	10	10	99	110	33	24	5	321

[a] *Pasteurella multocida* serotype 1 was isolated from several swabs from one bird.
[b] *Pasteurella multocida* serotype 1 was isolated from one swab from one bird.
[c] *Pasteurella pneumotropica/haemalytica* isolated from one swab from one bird.
[d] *Pasteurella multocida* (three cultures, three distinct serotypes: 8/13, 3/4, and 3) was isolated from one swab from one bird.
[e] *Pasteurella multocida* serotype 1 was isolated from one swab from each of two birds.
[f] *Pasteurella multocida* serotype 3/12 was isolated from two swabs from one bird.
[g] An additional negative sample was collected from an adult snow goose in Texas; however, gender was listed as undetermined for that bird, so it was not included in this table.

TABLE 2. *Pasteurella multocida* serotype 1 isolates recovered from Ross's and lesser snow geese from the Playa Lakes Region (USA) in January–March 2001 and 2002. Swab samples were preserved in brain heart infusion broth (BHI) and dimethylsulfoxide (DMSO) and processed with *Pasteurella multocida* selective broth after liquid nitrogen storage. Pathogenicity trials were conducted by challenging four mallard ducks with each *Pasteurella multocida* isolate recovered.

Tissue sample	No. of birds sampled		*P. multocida* serotype 1 isolates			
			Recovered		Pathogenicity[a]	
	BHI	DMSO	BHI	DMSO	BHI	DMSO
Leg joint	320	318	1[b]	2[b]	1/1	0/1
Cloaca	322	319	0	2	None	1/2
Eye[c]	319	321	1	0	0/1	None
Oral	317	320	1[b]	2[b]	1/1	2/2
Nasal[d]	317	320	1	1	0/1	1/1
Birds tested[e]	322	322	3	3	1/3	3/3

[a] Number of serotype 1 isolates killing one or more of four mallards/number of isolates tested.
[b] *Pasteurella multocida* serotype 1 isolates were recovered from BHI and DMSO swabs for a Ross's goose.
[c] Three non–serotype 1 isolates were also recovered from a BHI swab in one bird. These isolates were nonpathogenic in mallard ducks.
[d] Non–serotype 1 *P. multocida* isolates were recovered from BHI and DMSO swabs from one bird. One of these isolates was nonpathogenic in mallard ducks; the other was not tested.
[e] Number of birds sampled, with ≥1 recovered *P. multocida* serotype 1 isolates and with ≥1 pathogenic isolate.

Ten of the 11 *P. multocida* serotype 1 isolates recovered from wild birds were tested for pathogenicity in mallards; we excluded one of two leg joint isolates recovered from a Ross's goose. Mortality occurred in six of the 10 challenge groups (Table 2). We tested five of the six isolates recovered from the Ross's goose from Texas. The oral swab isolate in DMSO killed three ducks, and the BHI isolate killed one; the leg joint isolate in BHI killed two ducks, and the isolate in DMSO was not tested; the isolate from the nares (DMSO) killed one duck. No mortality occurred in the challenge group inoculated with the cloacal isolate. The isolate from a snow goose oral swab (DMSO) collected in Texas caused mortality in three ducks, but the isolate from the nares (BHI) of the other Texas snow goose was nonpathogenic. Two isolates from one snow goose collected in Colorado were tested; the cloacal (DMSO) isolate killed one duck, and no deaths occurred from the leg joint (DMSO) isolate. No mortality occurred in the challenge group inoculated with the eye swab (BHI) isolate from Colorado. *Pasteurella multocida* serotype 1 was recovered from all the ducks that died during the challenge trial, and three ducks surviving challenge with serotype 1 isolates (an indication of infection), including one from a group in which no mortality occurred. No mortality or illness was observed in any of the ducks inoculated with the non–serotype 1 isolates (one serotype 3, one serotype 3/4, one serotype 3/12, and one serotype 8/13), and no *P. multocida* was recovered from these groups on necropsy.

We found significant correlation ($r=0.84$, $n=96$, $P<0.0001$) between our previous antibody titer levels measured for our reference sera from Bank's Island snow geese (Samuel et al., 1999b) and the antibody titers from the ELISA test used in this study. A threshold ELISA value of 17.5 to indicate seroconversion in this study provided good agreement (kappa = 0.75) between the two ELISA procedures. This threshold produced a slightly lower seropositive prevalence (41% positive) from the revised ELISA procedure than we found for our original ELISA (49%) in the 96 snow goose reference samples.

Recent exposure to *P. multocida* on the basis of positive ELISA antibody titer was indicated in three of the 52 Ross's geese and eight of the 266 snow geese sampled

(Table 3). Positive Ross's geese included one adult female from New Mexico and one adult female and one immature male from Texas. Positive snow geese included one adult male from Kansas; one adult male from New Mexico; and two adult males, two adult females, one immature male, and one immature female from Texas. As expected, the estimated seroprevalence of geese to *P. multocida* was low: 0% in Colorado, 3% in Kansas, 4% in New Mexico, 0% in Oklahoma, and 5% in Texas. Seroprevalence was 6% in Ross's geese compared with 3% in snow geese; 3% in adults compared with 4% in juveniles, and 4% in males compared with 3% in females. The number of samples collected by species, age, sex, or state was insufficient to statistically assess differences in prevalence. *Pasteurella multocida* was not isolated from any of the geese with positive ELISA antibody titers.

DISCUSSION

Samuel et al. (2003a) found that swab samples (oral, nasal, cloacal, eye, and leg joint) preserved in liquid nitrogen with either BHI or DMSO and cultured in PMSB provided the most successful method for recovering *P. multocida.* On the basis of these methods, wild geese in the Playa Lakes Region were collected and sampled for *P. multocida* during the winters of 2000–01 and 2001–02. Swab cultures from these birds confirmed that wild, apparently healthy lesser snow and Ross's geese can be carriers of *P. multocida* and therefore can transmit the bacterium that causes avian cholera to other birds and spread the disease to other locations. A persistent issue in the epizootiology of avian cholera has been the identification of a reservoir for the disease agent. Our study provides the strongest available evidence that birds serve as a reservoir for avian cholera. These findings suggest that further research might be warranted to determine how long birds can carry *P. multocida* and how the bacterium is transmitted to susceptible birds.

TABLE 3. Serum samples collected from Ross's and lesser snow geese[a] for *Pasteurella multocida* antibody from the Playa Lakes Region (USA) in January–March 2001 and 2002.

State	Collection dates	Ross's geese				Snow geese				No. seropositive	No. tested
		Adult		Immature		Adult		Immature			
		M	F	M	F	M	F	M	F		
Colorado	March 2001 and March 2002	3	1	0	1	25	16	1	3	0	50
Kansas	February–March 2001 and February–March 2002	0	1	0	0	17[b]	12	1	0	1	31
New Mexico	February 2001 and February–March 2002	2	3[b]	1	0	22[b]	22	5	1	2	56
Oklahoma	March 2001 and February 2002	4	2	0	0	1	2	0	0	0	9
Texas	February–March 2001 and January–March 2002	5	10[b]	10[a]	9	37[c]	56[c]	26[b]	19[b]	8	172[d]
Total		14	17	11	10	102	108	33	23	11	318

[a] F = female; M = male.
[b] A positive antibody response to *P. multocida* was found for one bird in this group.
[c] A positive antibody response to *P. multocida* was found for two birds in this group.
[d] An additional negative sample was collected from an adult snow goose in Texas; however, gender was listed as undetermined for that bird, so it was not included in this table.

We found no association between *P. multocida* culture-positive and serologic-positive geese. In a previous laboratory challenge study, we only found a weak association between the detection of carriers and seroconversion (Samuel et al., 2003a). In this and previous studies, we found that detectable antibodies peaked approximately 2 wk postinfection and declined to below background levels within 3–4 mo; thus, seroconversion represents recent infection with *P. multocida.* In challenged ducks, we found that 90% seroconverted within 2 wk postinfection (Samuel et al., 2003a). We suspect there are several possible reasons that none of our carrier birds had seroconverted: 1) these birds were commensal carriers of *P. multocida* but had not become infected, 2) carriers had been infected for at least 3–4 mo and antibody titers had declined below the detectable threshold, and 3) carriers were recently infected with *P. multocida* and had not seroconverted. We suspect the latter explanation is less probable because most birds would have detectable antibody within 1–2 wk postinfection. Further research is needed to understand the relationship between infection, seroconversion, and carrier status.

As in previous studies we conducted with *P. multocida* isolates (Samuel et al., 2003b), none of the non–serotype 1 isolates were virulent in ducks. Six of the 10 serotype 1 isolates we recovered from wild birds were pathogenic to mallards (at least one bird of four died). The remaining four isolates caused some illness, but no mortality. Four of the five isolates tested from the Ross's goose, all except the cloacal swab, were pathogenic. Two of five isolates tested from the snow geese were pathogenic. The cloacal isolate from a snow goose collected in Colorado was pathogenic, but not the leg joint isolate from this bird. Isolates from oral swabs were all pathogenic, but pathogenicity varied for cloacal, nasal, and leg joint swabs (one of two pathogenic for each). The single isolate from the eye swab was nonpathogenic. At present, we are uncertain why some of the serotype 1 isolates were not pathogenic in ducks. However, previous studies have concluded that pathogenicity of *P. multocida* strains can be highly variable (Christensen and Bisgaard, 2000) and that pathogenicity increases over a short time with repeated transmission (Matsumoto and Strain, 1993). We did not attempt to further characterize these isolates or to test isolates recovered from our challenged ducks.

Although *P. multocida* isolates have been recovered previously from wild waterfowl (see Botzler, 1991; Wobeser, 1992), only Samuel et al. (1997) determined the serotypes of the isolates and demonstrated that the isolates were virulent. Samuel et al. (1997) reported recovering only a single *P. multocida* serotype 1 isolate from oral swabs of more than 3,400 adult snow geese (Samuel et al., 1999b). On the basis of results presented in this study, we suspect the sampling and preservation methods used by Samuel et al. (1997) were insufficient to ensure adequate isolation of *P. multocida* in carrier birds. The methods we developed appear suitable for detecting avian cholera carriers among wild waterfowl; however, we also suspect the sampling and preservation methods developed in this study will fail to detect all birds that might be carriers. For most of the tissues we sampled for *P. multocida* isolates, we were only successful in recovering the bacterium in one of the two swabs collected (seven of nine tissues). Fortunately, in most of the positive geese (four of five), we were successful at recovering *P. multocida* in tissues (oral, nasal, cloacal) from which collection would be easy in live or dead birds. To increase the probability of detecting *P. multocida* in carrier waterfowl, we recommend testing at least two samples from a variety of tissues, with preservation at low temperatures in either BHI or DMSO.

Polymerase chain reaction provided only minimal enhancement of our ability to detect *P. multocida* organisms in our

field samples. As a result, we only recommend using PCR as a potential supplement to culture when the primary goal is to determine prevalence or number of carriers. The PCR alone would not be suitable if viable isolates were desired for virulence testing as recommended by Samuel et al. (2003b), if isolates were desired for further characterization, or if pre-enrichment with PMSB was not possible.

Avian cholera is of particular concern to wildlife managers because most species of waterfowl, raptors, and other birds of wetland ecosystems are susceptible (Botzler, 1991; Friend, 1999). Although the factors that trigger an outbreak are poorly understood, it is commonly believed that weather, stress, and high densities of susceptible birds are important contributors (Botzler, 1991; Windingstad et al., 1998). Increased densities of waterbirds, especially gregarious light goose species, probably increase the risk of disease transmission and outbreak events (Wobeser, 1992). Once an outbreak starts, wetland contamination from diseased birds is the primary source of infection to susceptible birds of all species, although other routes of transmission such as bird-to-bird contact are likely (Wobeser, 1992). Our research demonstrates that some species of waterfowl, especially light geese, are carriers of *P. multocida* and might be more disposed to avian cholera outbreaks that concurrently or subsequently affect other susceptible species. In addition, the increased abundance of light geese and the large-scale mixing of these populations could enhance the exchange and spread of avian cholera and other disease agents (Wobeser, 1992). Loss of habitat, increased abundance of light geese and other waterfowl, and increased densities of waterbirds are all factors that likely contribute to increasing the risk of avian cholera outbreaks, increasing the risk of infecting other waterbirds in the same wetlands, and increasing the continental distribution of this infectious disease.

Current management strategies to control avian cholera losses are reactive, consisting primarily of collecting and disposing of carcasses when outbreaks occur (Wobeser, 1992). Development of proactive or alternative disease management approaches to avian cholera has likely been hindered by uncertainty about the reservoir for the disease. The apparent importance of snow geese as carriers of avian cholera, coupled with the dramatic increase in abundance of midcontinent snow goose populations (Ankney, 1996; Abraham and Jefferies, 1997) and their propensity to occur in large aggregations throughout the year, amplifies the potential role of this species in avian cholera outbreaks. Although the proportion of *P. multocida* carriers in the midcontinent light goose population is relatively low (3–5%), these populations likely exceed 2.5 million birds (Kelley et al., 2001), containing an estimated 75,000–125,000 *P. multocida* carriers. Results from our research have implications for other areas in which snow geese occur in large numbers (e.g., California, coastal regions of Texas and Louisiana) and for areas in which avian cholera epizootics are problematic in waterfowl (e.g., Nebraska's Rainwater Basin). Strategies for prevention and control of avian cholera should consider that carrier birds are a likely source of disease outbreaks and disease spread. Management actions that decrease potential disease transmission by separating light geese (and other carrier species) from other species, reducing stress factors that might precipitate epizootic events, and reducing high concentrations of waterfowl on a limited number of wetlands might minimize the effect of avian cholera on waterfowl populations. Because avian cholera affects many waterbird species, further research is needed to determine whether other species, such as white-fronted geese (*Anser albifrons*) and northern pintails (*Anas acuta*), frequently associated with avian cholera outbreaks can also serve as a reservoir for this disease.

ACKNOWLEDGMENTS

We appreciate the dedicated assistance of many individuals who contributed to this proj-

ect. M. O'Meilia, J. Neal, J. Gammonley, T. Sanders, H. Hands, B. Price, A. Price, M. Sexson, K. Sexson, C. Swank, S. Baugh, T. Unruh, M. Torres, D. Fisher, T. Mitchusson, S. Denny, D. Cook, J. Zotter, J. Gentz, M. Gentz, and the Garden City Zoo assisted with collecting samples from wild geese. Funding for this study was provided by the US Fish and Wildlife Service, the Playa Lakes Joint Venture, Texas Parks and Wildlife, Ducks Unlimited, and the US Geological Survey. We thank J. Blanchong, D. Blehert, R. Botzler, and an anonymous referee for manuscript review.

LITERATURE CITED

ABRAHAM, K. F., AND R. L. JEFFERIES. 1997. High goose populations: Causes, impacts, and implications. *In* Arctic ecosystems in peril: Report of the arctic goose habitat working group, B. D. J. Batt (ed.). Arctic goose joint venture special publication. US Fish and Wildlife Service, Washington, D.C., and Canadian Wildlife Service, Ottawa, Ontario, Canada, pp. 7–72.

ANKNEY, C. D. 1996. An embarrassment of riches: Too many geese. Journal of Wildlife Management 60: 217–223.

BOLEN, E. G., L. M. SMITH, AND H. L. SCHRAMM, JR. 1989a. Playa lakes: Prairie wetlands of the Southern High Plains. BioScience 39: 615–623.

———, G. A. BALDASSARRE, AND F. S. GUTHERY. 1989b. Playa lakes. *In* Habitat management for migrating and wintering waterfowl in North America, L. M. Smith, R. L. Pederson, and R. M. Kaminski (eds.). Texas Tech University Press, Lubbock, Texas, pp. 341–365.

BOTZLER, R. G. 1991. Epizootiology of avian cholera in waterfowl. Journal of Wildlife Diseases 22: 257–259.

BRAND, C. J. 1984. Avian cholera in the Central and Mississippi Flyways during 1979–80. Journal of Wildlife Management 48: 399–406.

BULLER, R. J. 1964. Central flyway. *In* Waterfowl tomorrow, J. P. Linduska (ed.). US Fish and Wildlife Service, Washington, D.C., pp. 209–232.

CHRISTENSEN, J. P., AND M. BISGAARD. 2000. Fowl cholera. Revue Scientifique et Technique de Le Office International Des Epizooties 19: 626–637.

FRIEND, M. 1999. Avian cholera. *In* Field manual of wildlife diseases, general field procedures and diseases of migratory birds, M. Friend and J. C. Franson (eds.). US Geological Survey, Information and Technology Report 1999–001, pp. 74–92.

HAUKOS, D. A., AND L. M. SMITH. 1992. Ecology of playa lakes. *In* Waterfowl management handbook. US Department of the Interior, Fish and Wildlife Service, Fish and Wildlife Leaflet 13.3.7.

HEDDELSTON, K. L., J. E. GALLAGER, AND P. A REBERS. 1972. Fowl cholera: Gel diffusion precipitin test for serotyping *Pasteurella multocida* from avian species. Avian Diseases 16: 925–936.

KELLEY, J. R., D. C. DUNCAN, AND D. R. YPARRAGUIRRE. 2001. Distribution and abundance. *In* The status of Ross's geese, T. J. Moser (ed.). Arctic goose joint venture special publication. US Fish and Wildlife Service, Washington, D.C., and Canadian Wildlife Service, Ottawa, Canada, pp. 11–17.

KERBES, R. H., K. M. MEERES, AND J. E. HINES, eds. 1999. Distribution, survival and numbers of lesser snow geese of the western Canadian Arctic and Wrangel Island, Russia. Canadian Wildlife Service, Occasional Paper 98, Ottawa, Ontario. 120 pp.

MATSUMOTO, M., AND J. G. STRAIN. 1993. Pathogenicity of *Pasteurella multocida*: Its variable nature demonstrated by in vivo passages. Avian Diseases 37: 781–785.

MENSIK, G., AND M. D. SAMUEL. 1995. Studying snow geese and avian cholera. California Waterfowl (October/November): 16.

MOORE, M. K., L. CICNJAK-CHUBBS, AND R. J. GATES. 1994. A new selective enrichment procedure for isolating *Pasteurella multocida* from avian and environmental samples. Avian Diseases 38: 317–324.

PLAYA LAKES JOINT VENTURE. 1994. Guidelines for the implementation of the North American waterfowl management plan in the Playa Lakes Joint Venture. Special report, Playa Lakes Joint Venture Coordinator, Albuquerque, New Mexico, 25 pp.

QUORTRUP, E. R., F. B. QUEEN, AND L. J. MEROVKA. 1946. An outbreak of pasteurellosis in wild ducks. Journal of the American Veterinary Medical Association 108: 94–100.

RAY, J. D., AND H. W. MILLER. 1997. A concentration of small Canada geese in an urban setting at Lubbock, Texas. Southwestern Naturalist 42: 68–73.

ROCKE, T. E., S. R. SMITH, A. MIYAMOTO, AND D. J. SHADDUCK. 2002. A serotype-specific polymerase chain reaction for identification of *Pasteurella multocida* serotype 1. Avian Diseases 46: 370–377.

ROSEN, M. N., AND A. I. BISCHOFF. 1949. The 1948–1949 outbreak of fowl cholera in birds in the San Francisco Bay area and surrounding counties. California Fish and Game 35: 185–192.

SAMUEL, M. D., AND G. MENSIK. 2000. New insights on a deadly disease. California Waterfowl (April/May): 18–19.

———, D. R. GOLDBERG, D. J. SHADDUCK, J. I. PRICE, AND E. G. COOCH. 1997. *Pasteurella multocida* serotype 1 isolated from a lesser snow goose: Evidence of a carrier state. Journal of Wildlife Diseases 33: 332–335.

———, J. Y. TAKEKAWA, G. SAMELIUS, AND D. R.

GOLDBERG. 1999a. Avian cholera mortality in lesser snow geese nesting on Banks Island, Northwest Territories. Wildlife Society Bulletin 27: 780–787.

———, D. J. SHADDUCK, D. R. GOLDBERG, V. BARANYUK, L. SILEO, AND J. I. PRICE. 1999b. Antibodies against *Pasteurella multocida* in snow geese in the western arctic. Journal of Wildlife Diseases 35: 440–449.

———, K. A. CONVERSE, AND K. J. MILLER. 2001. Disease mortality events involving Ross's geese. *In* The status of Ross's geese, T. J. Moser (ed.). Arctic Goose Joint Venture special publication. US Fish and Wildlife Service, Washington, D.C., and Canadian Wildlife Service, Ottawa, Canada, pp. 33–42.

———, D. J. SHADDUCK, D. R. GOLDBERG, AND W. P. JOHNSON. 2003a. Comparison of methods to detect *Pasteurella multocida* in carrier waterfowl. Journal of Wildlife Diseases 39: 125–135.

———, D. J. SHADDUCK, D. R. GOLDBERG, M. A. WILSON, D. O. JOLY, AND M. A. LEHR. 2003b. Characterization of *Pasteurella multocida* isolates from wetland ecosystems during 1996 to 1999. Journal of Wildlife Diseases 39: 798–807.

———, D. J. SHADDUCK, AND D. R. GOLDBERG. 2004. Are wetlands the reservoir for avian cholera? Journal of Wildlife Diseases 40: 377–382.

UNITED STATES FISH AND WILDLIFE SERVICE. 1988. Playa Lakes Region waterfowl habitat concept plan, category 24 of the North American waterfowl management plan. US Department of the Interior, Albuquerque, New Mexico. 37 pp.

WINDINGSTAD, R. M., M. D. SAMUEL, D. D. THORNBURG, AND L. C. GLASER. 1998. Avian cholera mortality in Mississippi flyway Canada geese. *In* Biology and management of Canada geese. D. H. Rusch, M. D. Samuel, D. D. Humburg, and B. D. Sullivan (eds), Proceedings of the International Canada Goose Symposium. pp. 283–290.

WOBESER, G. 1992. Avian cholera and waterfowl biology. Journal of Wildlife Diseases 28: 674–682.

———, D. B. HUNTER, B. WRIGHT, D. J. NEIMAN, AND R. ISBISTER. 1979. Avian cholera in waterfowl in Saskatchewan, spring 1977. Journal of Wildlife Diseases 15: 19–24.

———, F. A. LEIGHTON, A. D. OSBORNE, D. J. NIEMAN, AND J. R. SMITH. 1982. Avian cholera in waterfowl in western Canada, 1978–1981. Canadian Field-Naturalist 96: 317–322.

Received for publication 2 April 2004.