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Pasteurella multocida Serotype 1 Isolated from a Lesser Snow Goose: Evidence of a Carrier State

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ABSTRACT: Pharyngeal swabs were collected from 298 lesser snow geese (*Chen caerulescens caerulescens*) at Banks Island (Northwest Territories, Canada) in the summer of 1994. *Pasteurella multocida* serotype 1 was isolated from an adult male bird and *P. multocida* serotype 3 was isolated from an adult female goose. Pathogenicity of the serotype 1 isolate was confirmed by inoculation in Pekin ducks (*Anas platyrhynchos*). The serotype 3 isolate was non-pathogenic in Pekin ducks. This is the first documented isolation of pathogenic *P. multocida* serotype 1 from apparently healthy wild snow geese.

Key words: Avian cholera, *Pasteurella multocida,* carriers, snow geese, *Chen caerulescens caerulescens.*

Disease losses have been estimated at >80% of the nonhunting mortality in fledged North American waterfowl (Stout and Cornwell, 1976). Avian cholera, caused by the bacterium *Pasteurella multocida,* is among the most common diseases affecting wild waterfowl. Mortality from this disease was first reported in wild North American waterfowl in 1943–1944 in Texas (Quortrup et al., 1946) and northern California (Rosen and Bischoff, 1949). Since these early outbreaks, avian cholera epizootics have been reported throughout North America, with mortality occurring in all four flyways, Canada, and probably Mexico (National Wildlife Health Center [NWHC], unpubl.). This disease naturally infects over 100 species of wild birds (Botzler, 1991) and typically occurs in explosive outbreaks which kill hundreds to thousands of birds. In addition, scattered mortalities that never develop into extensive epizootics occur (Botzler, 1991; Wobeser, 1992), but the extent of these low level losses is unclear.

Despite the purported importance of avian cholera as a mortality factor among waterfowl populations (Botzler, 1991) and the alleged importance of managing this highly virulent disease (Bolen et al., 1989), little is known about the reservoir for this infectious disease or how the disease is transmitted (Botzler, 1991; Wobeser, 1992). Two important reservoirs have been suggested as a source of avian cholera in waterfowl populations: carrier birds and enzootic sites. Neither one of these hypotheses has been thoroughly investigated; however, the majority of studies do not support the general hypothesis that site specific characteristics such as water or soil are important reservoirs for *P. multocida* (Botzler, 1991). The evidence for carrier birds among wild waterfowl is similarly inconsistent. *Pasteurella multocida* occurs in many mammals (Botzler, 1991) and the presence of virulent and non-virulent carriers in domestic poultry has been known for many years (Pritchett et al., 1930a, b; Wobeser, 1992). Titche (1979) isolated one *P. multocida* culture from a snow goose after collecting nasopharyngeal and tracheal swabs from 238 geese (species composition unknown). Titche (1979) also obtained *P. multocida* from macerated lung tissue of several dabbling duck species collected from four areas in California. In contrast, Donahue and Olson (1969) could not isolate *P. multocida* from the nasal pharynx of 400 wild waterfowl (including 89 snow geese) sampled in Missouri and Illinois. Although *P. multocida* isolates have been recovered from apparently healthy waterfowl (see Botzler, 1991; Wobeser, 1992), these isolates have not usually been serotyped. Likewise, the virulence of these isolates has not been tested in waterfowl (Botzler, 1991). Thus, most re-

searchers have generally concluded that the existence of carrier birds has not been sufficiently documented (Botzler, 1991; Wobeser, 1992).

Molting lesser snow geese (*Chen caerulescens caerulescens*) were captured during July 1994 in the vicinity of the Egg River nesting colony on Banks Island (Northwest Territories, Canada; 72°20′N, 125°00′W) using helicopter-drive trapping techniques (Timm and Bromley, 1976). Birds were captured in groups, banded, and released in groups. We collected pharyngeal swabs from 298 apparently healthy adult lesser snow geese to culture for *P. multocida*. Culture Swab Transport Systems (Amies medium with charcoal, rayon tip, Difco Laboratories, Detroit, Michigan, USA) were used for sampling. Swab samples were maintained at cool (ambient) temperatures during transport to NWHC. Once at NWHC, swab samples were stored at room temperature (approximately 22 C) until they were processed (<21 days after collection).

To process culture swab samples, a sterile cotton swab was immersed into the Amies tube and completely coated with its contents. The swab was transferred to a tube containing 5 ml of *Pasteurella multocida* selective broth (PMSB) (Moore et al., 1994) and incubated at 37 C with 5% CO_2 for 12–16 hr. A standard blood agar plate (BAP) (Acumedia Manufacturers, Inc., Baltimore, Maryland, USA) was swabbed and then streaked for isolation from the inoculated PMSB. Plates were incubated at 37 C for 20 to 24 hr with 5% CO_2. Following incubation, plates were held at room temperature and examined at 24, 48, 72 and 96 hr. Suspect *P. multocida* colonies were restreaked on BAP and incubated at 37 C for 20 to 24 hr with 5% CO_2. A Gram stain was performed on all isolates and Gram positive isolates were discarded. The remaining Gram-negative isolates were streaked onto Bacto MacConkey agar (Difco) and modified Bacto Columbia CNA agar (Difco) containing 17 units/ml of polymyxin B sulfate (Sigma Chemical Company, St. Louis, Missouri, USA) (McFaddin, 1985) to distinguish poorly staining streptococci from morphologically similar *P. multocida* coccobacillary bodies. Plates were incubated at 37 C for 20 to 24 hr with 5% CO_2. Isolates that were lactose positive or grew on Columbia CNA agar containing polymyxin were discarded. Isolates with lactose negative growth or that failed to grow on MacConkey or Columbia CNA were inoculated into a tube of sulfide, indole, and motility medium (Bacto SIM) (Difco). Motile isolates were discarded because *Pasteurella multocida* isolates are usually non-motile (Heddleston, 1975). The resulting Gram-negative isolates fitting the *P. multocida* criteria (Columbia CNA agar negative, lactose negative/MacConkey growth positive, or MacConkey growth negative, or non-motile) were identified using the API 20E or API NFT identification system (bioMerieux Vitek, Inc., Missouri, USA). *Pasteurella multocida* isolates were serotyped by the gel diffusion precipitin test (Heddleston et al., 1972).

Except for occasional samples with little or no growth, numerous bacteria were cultured from the swab samples collected from geese. We isolated *P. multocida* from two swab cultures. One isolate from an adult male snow goose was identified as serotype 1; the other isolate from an adult female was identified as serotype 3 with multiple crosses. The isolates were tested for virulence using a 6-hr Bacto brain heart infusion (BHI) broth (Difco) culture of each isolate (1.4×10^5 CFU serotype 1 and 4.8×10^5 CFU serotype 3). Four 28-day-old Pekin (*Anas platyrhynchos*) ducklings were injected subcutaneously in the dorsal caudal region of the neck with 0.2 ml of each culture (Price, 1985). All of the four ducklings challenged with the serotype 1 isolate died within 48 hrs, indicating it was pathogenic. At necropsy, all of these ducklings had gross lesions characteristic of avian cholera (Rosen, 1971; Friend, 1987), including petechial hemorrhages on the heart or necrotic foci on

the liver. *Pasteurella multocida* was cultured from the livers of each bird. A culture from one bird was identified as serotype 1, serotyping was not conducted for cultures from the remaining three birds. Ducklings challenged with the serotype 3 culture survived and remained healthy.

The importance of snow geese and other waterfowl species in the epizootiology of avian cholera remains uncertain. While a number of species may be involved in transmission of this disease, considerable evidence indicates that snow geese may play a role in carrying and transmitting the disease in the Pacific and Central flyways. Rosen (1972) believed that waterfowl carry *P. multocida* from the wintering grounds to breeding areas where additional mortality occurs. Wobeser et al. (1979, 1982) reported that avian cholera occurs among northward migrating geese in Saskatchewan each spring. In these outbreaks, the disease was mostly confined to lesser snow and Ross' geese (*Chen rossii*). Brand (1984) found that avian cholera mortality in the Central and Mississippi flyways tracked the migration patterns of snow geese following a breeding ground outbreak along the coast of Hudson Bay, Canada. At other wintering areas in California it has been noted that avian cholera mortality typically follows the arrival of snow geese (J. G. Mensik, pers. comm.). Snow geese from Banks Island are a predominant population at these wintering areas in California. Breeding ground outbreaks of avian cholera in snow geese also have been demonstrated at Banks Island in 1991 (Nieman and Trost, 1991), 1995, and 1996 (NWHC, unpubl.) and at other breeding areas in Canada (Wobeser et al., 1983). Finally, data on avian cholera mortality events indicate that snow geese are frequently (>50%) involved in larger outbreaks and constitute a majority of the recorded mortality in these outbreaks (M. D. Samuel, unpubl.).

Future investigations may require improved techniques that are more sensitive to detecting birds that are carriers of *P. multocida*. We suspect pharyngeal swabs collected in this study underestimate the number of infected birds because birds may harbor *P. multocida* in tissues that are difficult to sample in live birds. In addition, storing and transporting swabs is probably not as reliable as immediate culture from tissues, and isolation of *P. multocida* from mixed cultures is often difficult. All these factors probably increase the number of false negative results. Additional research is needed to document the prevalence of carriers in waterfowl populations, how prevalence changes annually and after avian cholera outbreaks, and how the disease is transmitted among birds.

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