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Serologic Evidence of Influenza A Infection in Marine Mammals of Arctic Canada

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ABSTRACT: A serologic survey of influenza A antibodies was undertaken on 1,611 blood samples from five species of marine mammals collected from Arctic Canada from 1984–98. Sampling was done in 24 locations throughout the Canadian Arctic encompassing Sachs Harbor (72°N, 125°W), Northwest Territories in the west to Loks Land (63°N, 64°W), Nunavut in the east, to Eureka (80°N, 86°W), Nunavut in the north to Sanikiluaq (56°N, 79°W), Nunavut in the south. A competitive ELISA using a monoclonal antibody (Mab) against influenza A nucleoprotein (NP) was used. Five of 418 (1.2%) belugas (*Delphinapterus leucas*) and 23 of 903 (2.5%) ringed seals (*Phoca hispida*) were serologically positive. None of the 210 walruses (*Odobenus rosmarus rosmarus*), 76 narwhals (*Monodon monoceros*) and four bowhead whales (*Balaena mysticetus*) had detectable antibodies to influenza A. Positive belugas were identified from communities on southeast Baffin Island while positive ringed seals came from communities in the eastern, western and high Arctic. Virus isolation attempts on lung tissue from a seropositive beluga were unsuccessful. We believe that influenza A infection in marine mammals is sporadic, the infection is probably self-limiting, and it may not be able to be maintained in these animals. Although the predominant hemagglutinin (H) type was not determined and therefore the pathogenicity of the strains to humans is unknown, the hunting and consumption of marine mammals by the Inuit may put them at risk for influenza A infection.

Key words: Competitive ELISA, influenza A, marine mammals, serologic survey, zoonotic infection.

Influenza A viruses, family Orthomyxoviridae, genus *Orthomyxovirus*, infect a variety of animals including swine, humans, horses, birds and marine mammals (Lamb and Krug, 1995). Influenza A viruses are subdivided by virtue of antigenic differences in their hemagglutinin (H) and neuraminidase (N) proteins (Murphy and Webster, 1995). Aquatic wild birds are the reservoir of all influenza A viruses, however interspecies transmission plays an important role in the pathogenesis and maintenance of the virus in both animal and human populations (Webster et al., 1992).

Marine mammal strains of influenza A were first isolated from the lung and brain of sick and dying harbor seals (*Phoca vitulina*) on the northeastern coast of the USA in 1970–80 (Geraci et al., 1982). This H7N7 strain was associated with mortality of about 20% of the seal population and also showed potential for causing conjunctivitis in humans but was not transmitted from human to human. Subsequently, another strain (H4N5) was isolated from lung and brain of sick and dying harbor seals from the coast of New England (USA). This strain caused mortality estimated to be between 2 to 4% and was found to be genetically and serologically related to avian strains (Hinshaw et al., 1984). Continuing surveillance on the New England coast resulted in the isolation of two additional influenza A virus strains (Callan et al., 1995). Both were associated with an increase in harbor seal mortality and the pathology was consistent with that described for previous seal influenza outbreaks. The first strain, isolated in January 1991 was of the H4N6 subtype and the second was isolated in January 1992 was of the H3N3 subtype. Further genetic analysis indicated that they too were both of avian origin and that transmission from birds to seals was the most likely mode of infection.

Indirect evidence of influenza A infection have also been reported from a number of other seal species. Influenza A antibodies have been detected in both harp

seals (*Phoca groenlandica*) and hooded seals (*Cystophora cristata*) of the Barents Sea (Steuen et al., 1994). Forty-six of 1,095 samples of "seals and sea lions" from the North and Bering Seas had antibodies to influenza A as determined by ELISA. Further testing revealed exposure to H1, H3, H4, H7 and H12 subtypes (de Boer et al., 1990). One of 32 ringed seal (*Phoca hispida*) samples from Alaska gave a positive result for influenza antibodies of the H3 and H7 subtypes (Danner and McGregor, 1998).

Influenza A isolations have also been made from cetacean species. A dual infection (H13N2 and H13N9) has been reported from a pilot whale (*Globicephala macrorhynchus*). Serological, molecular, and biological analyses indicate that these isolates were closely related to influenza A viruses isolated from gulls (Hinshaw et al., 1986). Isolations (H1) have also been made from lung and liver of Balaenopteridae whales in the South Pacific (Lvov et al., 1978). These findings also suggest that influenza A strains are regularly being introduced into both pinniped and cetacean populations by aquatic birds but that they do not become enzootic (Webster et al., 1992).

The purpose of this study was to determine the prevalence of antibodies to influenza A virus in five species of marine mammals from Arctic Canada between the years 1984–94. Samples were obtained from 24 locations that encompassed an area bounded by Sachs Harbour (72°N, 125°W), Northwest Territories (NWT) in the west to Loks Land (63°N, 64°W), Nunavut in the east to Eureka (80°N, 86°W), Nunavut in the north to Sanikiluaq (56°N, 79°W), Nunavut in the south.

Material for testing consisted of 1,611 archived whole blood samples collected from hunter killed animals. Serologic evidence of influenza A was detected by using a competitive ELISA (C-ELISA) specific to influenza A nucleoprotein (NP) as described by Zhou et al. (1998). Briefly, microtiter plates coated with recombinant NP were incubated with a 1:5 dilution of each serum sample immediately followed by addition of the monoclonal antibody to NP. After incubation, monoclonal antibody binding was detected using conjugated goat anti-mouse IgG conjugated to horseradish peroxide (HRP). Enzyme substrate and chromogen were added and the colored end product was measured photometrically. Test serum containing NP specific antibodies would compete with the monoclonal antibody for antigenic sites thereby inhibiting the binding of those antibodies and resulting in diminished color development. Samples were considered positive for antibodies to influenza A NP when the percentage of inhibition was greater than 30%. Since samples consisted of hemolyzed blood, determination of the hemagglutinin type by the hemagglutination inhibition (HI) test was unsuccessful. Virus isolation using lung tissue from a seropositive beluga by inoculation of nine-day-old embryonated chicken eggs and routine virological techniques (Payment and Trudel, 1993) was also unsuccessful.

Five of 124 (4.0%) belugas tested from the southeast coast of Baffin Island, were positive for the presence of antibodies to influenza A (Table 1). Positive belugas were identified from 34 animals tested from the sampling in 1,990/91 but none were identified from the 29 belugas tested in 1987/89, or the 62 tested in the subsequent 3 yrs (1,992/94). This would suggest that though belugas are susceptible to infection by some influenza A strains, it is probably an infrequent event. This is the first serologic record of influenza A in beluga. A total of 293 belugas tested negative from samples collected between 1984–1997 from nine communities, including Grise Fjord (76°N, 82°W, $n = 27$) in the high Arctic, Pangnirtung (66°N, 65°W, $n = 66$) from central Baffin Island, Arviat (61°N, 94°W, $n = 66$), Coral Harbor (64°N, 83°W, $n = 11$), and Sanikiluaq (56°N, 79°W, $n = 28$) from Hudson Bay, and East Whitefish (69°N, 133°W, $n = 24$), Hendrickson Island (69°N, 133°W, $n = 40$),

TABLE 1. Influenza antibody prevalence in beluga whales (*Delphinapterus leucas*) landed at Cape Dorset, Lake Harbour, and Iqaluit in Southeast, Baffin Island.

Location and approximate co-ordinates	Year	Number	Positive[a]
Southeast Baffin Island, Nunavut	1987	1	0
64°N, 76°W to 63°N, 64°W	1989	28	0
	1990	5	1 (2.0)
	1991	29	4 (13.8)
	1992	1	0
	1993	39	0
	1994	22	0
Total		124	5 (4.0)

[a] Number positive (% positive).

Husky Lakes (69°N, 132°W, $n = 27$), and Shingle Point (60°N, 137°W, $n = 4$) in the western Canadian Arctic.

The situation regarding seroprevalence of influenza A is also similar for ringed seals, although antibody prevalence was higher and more widespread. Seropositive seals were identified from four locations representing areas in the eastern, western and high Arctic (Table 2). Twenty-three of the 903 (2.5%) ringed seals had detectable levels of antibodies to influenza A. They were identified from two of the four sampling locations in the high Arctic and both Resolute Bay and Arctic Bay had seropositives in more than one year (Table 2). In the eastern Arctic only one location (Pangnirtung) was sampled and seropositives were identified from only one of the five years that samples were taken. In the west-

TABLE 2. Sampling locations and serum antibody prevalence of ringed seals (*Phoca hispida*) tested for influenza A antibodies.

Location and approximate co-ordinates	Year	Number	Positive[a]
High Arctic, Nunavut			
Resolute Bay, 75°N, 95°W	1992	37	1 (2.7)
	1993	38	7 (18.4)
Arctic Bay, 73°N, 85°W	1993	37	2 (5.4)
	1997	57	2 (3.5)
Grise Fjord, 76°N, 83°W	1993	13	0
Eureka, 80°N, 86°W	1994	17	0
Eastern Arctic, Nunavut			
Pangnirtung, 66°N, 65°W	1990	47	0
	1992	77	3 (3.9)
	1993	57	0
	1995	72	0
	1996	9	0
Western Arctic, Northwest Territories			
Holman, 71°N, 118°W	1993	31	8 (25.8)
	1994	29	0
Paulatuk, 68°N, 123°W	1993	10	0
	1994	34	0
Sachs Harbour, 72°N, 125°W	1993	3	0
Hudson Bay, Nunavut			
Arviat, 61°N, 94°W	1990	4	0
	1991	148	0
	1992	129	0
Total		903	23 (2.5)

[a] Number positive (% positive).

ern Arctic seropositive seals were identified from Holman in 1993 but not in 1994 while all the samples from the two nearby locations of Paulatuk, and Sachs Harbour were seronegative. All 281 seals sampled between 1990–92 from Arviat in western Hudson Bay were also seronegative. It is unknown whether the same influenza subtype or multiple infections are responsible for the seropositive results obtained at the different sampling locations or even at the same location at different times. Seropositive seals were identified in all seasons of the year and no association was apparent between seroprevalence and sex or age. Seroprevalence in Canadian ringed seals (0–25.8%) was in the range that has been reported by other investigators studying other species. Harp seals had a prevalence that ranged between 10.0–27.1% while prevalence in hooded seals ranged between 0–11.4%. Both species were sampled from populations in the Barents Sea in 1991–92 (Steuen et al., 1994). Seroprevalence of influenza A in "seals and sea lions" from the North and Bering Seas of 0.4% and 12.7% has also been reported (de Boer et al., 1990).

No seropositive animals were identified among the 76 narwhals, four bowhead whales or the 210 walruses that were also tested. The narwhals were sampled between 1986 and 1994 and came from five locations in the eastern and high Canadian Arctic. They included Iqaluit (63°N, 68°W, $n = 24$), Pond Inlet (72°N, 77°W, $n = 25$), Arctic Bay (73°N, 85°W, $n = 3$), Repulse Bay (66°N, 86°W, $n = 6$), and Igloolik (68°N, 81°W, $n = 18$). The bowheads were sampled between 1994 and 1998 and were from Igloolik (68°N, 81°W, $n = 1$), Repulse Bay (66°N, 86°W, $n = 1$), Pangnirtung (66°N, 65°W, $n = 1$), and Shingle Point (60°N, 137°W, $n = 1$). The walruses were sampled between 1984 and 1996 and included samples from five locations encompassing most of their range in Canada. The sampling sites were Igloolik (68°N, 81°W, $n = 138$), Hall Beach (67°N, 80°W, $n = 58$), Grise Fjord (76°N, 83°W, $n = 5$), Loks Land (63°N, 65°W, $n = 5$), and Resolute Bay (75°N, 95°W, $n = 4$).

The sporadic seroprevalence pattern that was observed in both ringed seals and belugas supports the theory that the virus is being introduced periodically from virus infected birds but is not able to be maintained within these species enzootically (Webster et al., 1992). It is also possible that other animal species may be involved in perpetuating influenza A viruses in Arctic marine mammals. No evidence is available indicating that influenza A infection causes disease and mortality in either beluga or ringed seals and all the animals in this study were apparently healthy when sampled. However, the finding that a high proportion of both ringed seals and belugas do not have antibodies to influenza, yet are susceptible to infection, suggests that they may also be at risk for a limited epizootic as was reported in harbor seals off the Northeastern coast of United States in 1970–80 (Geraci et al., 1982).

A conservative estimate of the population of ringed seals in Canadian waters was made in 1990 (Kingsley, 1990). At least two million animals were accounted for but the actual number could be easily twice that given the difficulty in conducting censuses. A large die-off of ringed seals though tragic in the short term would probably not endanger the seal population as a whole. The situation for belugas is quite different. Some Canadian stocks of belugas are considered endangered, including the southeast Baffin Island (Richard, 1991), Ungava Bay (Smith and Hammill, 1996) and St. Lawrence estuary stocks (Kingsley, 1998). Even a small self-limiting epidemic affecting any of these stocks could impact on their chances of long term survival. Though no large-scale epidemics have been reported in cetacean species, influenza A has been implicated as the cause of stranding in at least one case (Hinshaw et al., 1986). Influenza A may also be a contributing factor to mortality in Arctic cetaceans.

Influenza A virus is found in a number

of Arctic waterfowl species (Hinshaw et al., 1980). Marine mammals have ample opportunity to become infected with these viruses by coming in contact with the birds directly or by ingesting feces in the water. There are reports of both seals (Lucas and McLaren, 1988) and whales (Odlum, 1948) preying on seabirds. Perpetuation of infection among members of the same species, or between species of marine mammals is probably accomplished by direct transmission.

Canadian Inuit have traditionally relied on marine mammals as a source of food and continue to do so to the present time. Although diet varies from community to community, inhabitants of most coastal communities consume marine mammals. The hunting of marine mammals is regulated in the Northwest Territories and Nunavut for all species except for ringed seals. In 1995–96, 523 belugas, 251 Narwhals and 174 walruses were harvested (Department of Fisheries and Oceans, 1997). Though the annual harvest of ringed seals has never been accurately determined it is estimated that in the order of about 10,000 seals are taken every year (Smith, 1987), (Smith and Taylor, 1977). These animals undergo no official inspection for food safety and may be infected with a number of zoonotic diseases including brucellosis (Nielsen et al., 1996) and trichinosis (MacLean et al., 1992) in addition to influenza A. Northerners who butcher and consume raw meat as well as researchers who work with marine mammals would, through the course of their activities come in contact with these diseases. Though there are no reported human cases of influenza A originating from a marine mammal source in the Canadian Arctic, the potential for such transmission exists, though it is probably very low given the very small percentage of marine mammals that carry the virus.

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