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Characterization of Two Influenza A Viruses from a Pilot Whale

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Influenza A viruses of the H13N2 and H13N9 subtypes were isolated from the lung and hilar node of a pilot whale. Serological, molecular, and biological analyses indicate that the whale isolates are closely related to the H13 influenza viruses from gulls.

During the fall of 1984, there were two major strandings of pilot whales (*Globicephala melaena*) on the New England coast; one, in October at Eastham, Mass., involved 97 whales, and one in November at Wellfleet, Mass., involved 23 animals. Since a number of different influenza viruses, antigenically and genetically closely related to viruses from avian sources, have been isolated from the common seal (*Phoca vitulina*) (3, 4, 6) and from lung samples of striped whales (family Balaenopteridae) (5), it occurred to us that stranded whales may also be infected with influenza viruses. We now report the isolation of two influenza A viruses from the lung and hilar node of a sick pilot whale. These results suggest that further studies should be done to determine whether influenza viruses play a role in marine strandings.

A pilot whale that was in poor condition and had been swimming close to shore for 2 weeks near Portland, Maine, in late October 1984 was "herded" ashore by skin divers, removed from the water, and taken inland before it was killed and subjected to a postmortem. The animal had sloughing skin and extreme emaciation and had difficulty in swimming, surfacing, and diving. Postmortem examination revealed a hilar node approximately five times normal size with no evidence histologically of a germinal center. The lungs were hemorrhagic, and the liver was small and friable. Hemagglutinating viruses were isolated from a 10% suspension of disrupted lung and hilar node; only two of the six embryonated eggs inoculated with lung suspension yielded virus, and results were similar with the hilar node suspension. The viruses were reisolated from the tissue a second and third time by independent investigators, and on these occasions, only 1 or 2 of 30 embryonated eggs contained hemagglutinating agents. The initial isolation of hemagglutinating viruses was therefore at the very lower limit of detection, reflecting the possibility that infection had occurred sometime earlier and that the level of virus was declining. Both lung and hilar node tissues yielded H13N2 influenza virus, and the hilar node yielded a second influenza virus, H13N9 (Table 1). Other samples from 19 dead pilot whales from the Wellfleet stranding were badly autolysed and did not yield virus. Comparison of the two influenza virus isolates from the whale with H13 influenza virus from gulls (*Larus* species) (2), using monospecific sera to the hemagglutinin (Table 1) and monoclonal antibodies to the neuraminidase (data not shown), provides evidence that the hemagglutinin from the whale viruses is closely related to H13 gull viruses and that the neuraminidases are similar to the prototype N2 and N9 isolates from avian species.

Competitive RNA-RNA hybridization analyses (Fig. 1) (1) indicate that the nucleoproteins of the H13 influenza viruses from gulls and the pilot whale are closely related and readily distinguishable from other influenza A viruses. The H13 viruses are also biologically distinguishable from other avian

TABLE 1. Isolation and antigenic characterization of influenza A viruses[a] isolated from whale tissues[b]

Source of virus	Antigenic subtype	Designation	HI titer with antisera to:			NI titer with antisera to NA from:	
			HA from Gull/Md/704/77 (H13)[c]	Gull/Md/1824/78 (H13N9)[c]	Whale/Maine/1/84 (H13N9)[d]	RI/5+/57 (N2)[e]	Dk/Mem/546/74 (N9)[e]
Hilar node	H13N9	A/Whale/Maine/1/84	160	1,280	1,280	<20	2,000[f]
	H13N2	A/Whale/Maine/2/84	320	2,560	640	300[f]	<20
Lung	H13N2	A/Whale/Maine/2B/84	160	1,280	640	200	<20
Reference strain	H13N6	A/Gull/Md/704/77	320	1,280	640	<20	<20
Unrelated avian strain	H3N8	A/Pintail/Alb/276/84	<20	<20	<20	<20	<20

[a] Influenza viruses were isolated from the tissues in embryonated chicken eggs and characterized antigenically as described elsewhere (6). Other tissues from the same whale yielded no virus.
[b] HI, Hemagglutination inhibition; NI, neuraminidase inhibition; HA, hemagglutinin; NA, neuraminidase.
[c] Prepared in rabbits.
[d] Prepared in chickens.
[e] Prepared in goats.
[f] Titers were indistinguishable from those of the reference N2 and N9 viruses.

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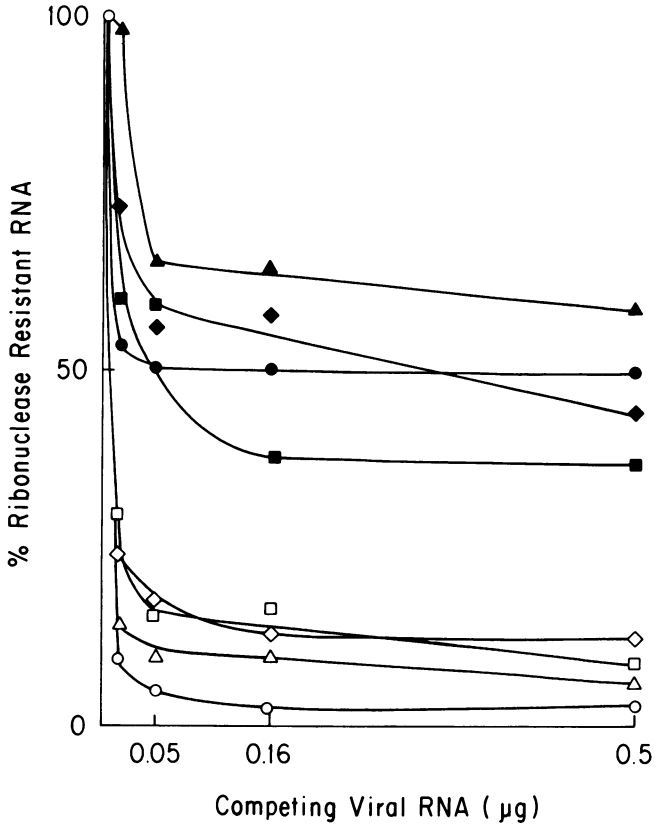

FIG. 1. Comparison by competitive hybridization of RNAs from avian and whale virus isolates with RNA segment 5 (nucleoprotein gene) from the prototype H13 gull virus isolate. RNA segment 5 from A/Gull/Md/704/77 was labeled with ^{125}I and annealed with homologous cRNA in the presence of increasing amounts of genome RNA from the homologous virus strain and other strains. The degree of relatedness between the labeled probe and the corresponding RNA segment of the other virus strains is indicated by the efficiency with which each RNA competes with the annealing of the labeled RNA and its homologous cRNA. Methods for the isolation and iodination of RNA segments and the isolation of cRNA and details of the hybridization conditions have been described previously (1). The RNA mixtures (15 μl) were annealed for 40 h at 15°C below the homologous melting temperature. Symbols: ▲, Gull/Md/5/77 (H11N9); ●, Seal/Mass/133/82 (H4N5); ◆, Dk/Mem/546/74 (H11N9); ■, Ty/Minn/833/79 (H4N2); ◇, Gull/Md/1824/78 (H13N9); □, Whale/Me/2/85 (H13N2); △, Whale/Me/1/85 (H13N9); ○, Gull/Md/704/77 (H13N6).

influenza viruses in that they are not enterotropic in ducks (2). Thus, ducks inoculated orally with the two whale isolates showed no disease signs or virus replication in the intestinal tract; rectal inoculation of ducks with either of the whale isolates resulted in virus replication in the lower intestinal tract (10^4 to 10^5 50% egg infective doses per g.) This property is similar to those of earlier H13 gull viruses (2) and distinguishes these viruses from the majority of other avian influenza viruses, which can transit the low pH of the crop of ducks and replicate in the intestinal tract after oral inoculation. Evidence for replication of these viruses in other mammalian species was obtained by intranasal inoculation of ferrets; the viruses replicated with peak titers of 10^4 50% egg infective doses per ml in the nasal wash 3 days postinfection.

Since H13N2 influenza viruses have never previously been isolated from any species, the possibility of laboratory contamination seems unlikely. The removal of the pilot whale from the water and beach area before postmortem minimized the likelihood of contamination from seawater or gull droppings. Removal of the tissue samples immediately after killing of the whale also ruled out the possibility of contamination with droppings from scavenging gulls. The isolation of both viruses from the hilar node indicates that the viruses must have replicated in the whale to reach this site and did not originate directly from contaminated water.

The antigenic, genetic, and biological comparisons of both of the whale influenza virus isolates with other influenza A viruses suggests that the whale viruses probably originated from gulls. Since avian influenza viruses are shed in high concentrations in the feces, contamination of seawater would provide a likely method of transmission. Despite the vast dilution of fecal material in the ocean, the feeding activities of gulls and whales often place them in close contact, increasing the probability of fecal-oral transmission of virus through seawater. Alternatively, H13 influenza viruses may originate from whales and be spread to gulls in the vast aerosols produced by whales. These studies provide the first evidence for the isolation of influenza viruses from sick whales and for the isolation from the same whale of two different influenza viruses, one of which has never previously been reported. This work provides additional evidence for transmission of avian influenza viruses to mammals in nature. The reason(s) for marine mammal strandings is still obscure; the isolation of influenza viruses from a pilot whale suggests that future studies are needed to determine if influenza viruses play a role in strandings.

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