Are Seals Frequently Infected with Avian Influenza Viruses?

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Influenza A virus isolates of the H4N5 subtype (which has previously been detected only in birds) were recovered from harbor seals dying of viral pneumonia on the New England coast from June 1982 through March 1983. When these isolates were compared with other mammalian and avian viruses in serological assays and RNA-RNA competitive hybridization, it was found that the seal viruses were most closely related antigenically and genetically to recent avian virus strains and were readily distinguishable from mammalian viruses, including H7N7 isolates recovered from seals in 1980. Unlike any previous isolates from mammals, these recent seal viruses replicate in the intestinal tracts of ducks, a characteristic of avian viruses. The association of avian viruses with influenza outbreaks in seals suggests that transmission of avian viruses to seals is occurring in nature. Potentially, this may be an example of the adaptation of avian viruses to mammals, which would represent an intermediate step in the evolution of new mammalian strains.

Avian species represent a major reservoir of influenza A viruses in nature (4). It has been postulated that these avian viruses play a role in the appearance of new strains in mammals, e.g., new human pandemic strains, by contributing genes via genetic reassortment between avian and human viruses (10). In support of this hypothesis are antigenic and genetic data that suggest that the hemagglutinin of the pandemic strain A/HK/1/68 (H3N2) originated from an avian virus like A/Dk/Ukr/1/63 (H3N8). In examining the role of avian viruses in disease outbreaks in mammals, we now describe the isolation and characterization of an H4N5 influenza A virus strain (previously detected only in birds) isolated from dead harbor seals in Massachusetts in 1982 to 1983.

During the winter of 1979 to 1980, an H7N7 influenza virus had been associated with a severe outbreak of pneumonia in the New England seal population (3). In view of this, we had been monitoring animals in this as well as other areas for increased mortality or evidence of influenza or both. From 1981 to 1982, there was no increase in seal deaths or strandings observed in Massachusetts; however, 16 lung samples from seals with evidence of pneumonia at autopsy were collected for virus isolation. One sample from an emaciated adult seal stranded on Plum Island, Mass., in June 1982 yielded an influenza A virus strain. Hemagglutination inhibition assays and neuraminidase inhibition tests (6) enabled the identification of the virus as H4N5 (Table 1). These surface antigens have previously been detected only on avian viruses (4). Comparisons with other avian strains (data not shown) by use of chicken and hyperimmune rabbit antisera indicated that the hemagglutinin of the seal virus was closely related to isolates from ducks, e.g., A/Dk/A1b/ 686/82 (H4N6), and turkeys, e.g., A/Ty/Mn/28/78 (H4N8), and that the neuraminidase was indistinguishable from the prototype for N5, i.e., A/Shearwater/Aust/1/75 (H6N5). These findings indicated that this new virus strain, A/Seal/ Mass/133/82, found in seals, was most closely related antigenically to recent avian isolates and was clearly different from the previous H7N7 seal isolates recovered from 1979 to 1980.

The H7N7 seal virus was shown to have derived all of its genome RNA segments from avian influenza strains (9). Although the surface proteins of the H4N5 seal isolate were clearly distinct from those of the earlier seal virus strain, it was not certain as to whether its internal genes were derived from the previous seal virus or from some other source. To clarify this question, competitive RNA-RNA hybridizations were performed with the RNA segments that code for the internal proteins of the H4N5 virus in comparison with a panel of avian and mammalian virus strains. With this assay, relative homologies are determined by the efficiency with which the various viral RNAs compete with the annealing of the labeled RNA segment and its homologous complementary RNA (1). The results of one of these analyses, in which the nucleoprotein gene of Seal/82 was used, are shown in Fig. 1. Turkey/Minn/833/79 competed with the labeled Seal/ 82 RNA more efficiently than did the earlier seal virus. Two other avian strains, Mallard/Minn/39/79 (H4N2) and Duck/Alberta/60/76 (H12N5), competed at a level similar to that of Turkey/Minn/833/79. Equine and human influenza virus strains competed poorly with the nucleoprotein gene probe. These results (data not shown), as well as the relative homologies of Gull/Md/5/77 and Ty/Ore/1/71 with the other strains, are consistent with a recent analysis of a large number of influenza virus nucleoprotein genes (1).

With each of the other RNA segments, two to four of the seven avian virus strains competed with the labeled H4N5 viral RNA more efficiently than did the RNA from the H7N7 seal virus. These results indicate that the RNA segments coding for the internal genes of the H4N5 seal virus were derived from avian virus strains and not from the preceding H7N7 seal virus.

Since Seal/82 was antigenically and genetically most closely related to avian viruses, we examined its ability to replicate in an avian host. In ducks inoculated orally, Seal/82 replicated to high titers (10^9.8 to 10^9.3) 50% egg infective doses [EID50] per g of feces) in the intestinal tracts of ducks, a tropism common to avian but not mammalian viruses (11). This result contrasted markedly with those obtained with the

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earlier H7N7 seal virus, which replicated poorly, if at all, in avian species and was not enterotropic in birds (9). The new H4N5 seal virus was, therefore, biologically more similar to avian viruses.

Since Seal/82 was essentially an avian virus and had, at that time, been recovered from only one animal, it was important to establish its ability to infect and replicate in seals. To test this, we inoculated six adult seals (1 to 9 years old) intranasally with approximately 5.0 × 10^7 EID_{50} virus in a ground-lung suspension from which Seal/82 had been isolated. To determine whether different species of seals were susceptible to infection with the virus, we used one harbor seal (Phoca vitulina), two ringed seals (Phoca hispida), and three harp seals (Phoca groenlandica) for this study. Nasal, corneal, and anal swabs were collected daily for the first 3 days postinfection (p.i.) and then on alternate days until 9 days p.i. Two seals were sacrificed at 3 days p.i. and one at day 5 p.i., and tissues, including brain, spleen, lung, liver, kidney, colon, thymus, and different lymph nodes (gastric, bronchial, mesenteric, hilar, and mandibular) were collected for virus isolation. Although there were no overt disease signs or histological evidence of pneumonia in the experimental animals, virus was recovered from nasal and corneal swabs from five of the six animals; no virus was recovered from one harp seal. At 9 days p.i., virus was still being recovered from nasal and corneal swabs taken from two of the three remaining seals. Virus was detected in anal swabs from two of the six animals; however, the recovery was sporadic. From the sacrificed animals, low levels (10^{1.5} EID_{50} per g of tissue) of virus were recovered from lung and lymph nodes (bronchial, mesenteric, and mandibular) from two of the animals, and no virus was recovered from either swabs or tissues from the other animal. Before inoculation, the seals had no antibodies to H4N5; by 2 weeks, however, the three remaining animals had detectable antibodies in both hemagglutination inhibition and neuraminidase inhibition assays; these antibodies were still present but declining by 30 days p.i. These findings indicate that Seal/82 infects and replicates in seals, the species from which it was recovered, and that at least three different species of seals are susceptible to infection with this virus.

To determine whether there was any prior evidence of H4N5 viruses in seals, sera from 200 seals collected from 1975 to 1982 were tested in hemagglutination inhibition tests for antibodies to the recent H4N5 virus; however, no antibodies (hemagglutination inhibition titer, <1:10) were detected. Individuals autopsying the seals and working with the viruses had no disease problems and had no detectable antibodies to the H4N5 virus in hemagglutination inhibition tests, suggesting that, in contrast to the H7N7 seal virus strain (8), there was no transmission of this virus to humans.

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<tr>
<td>Antigenic subtypes of influenza virus isolates</td>
<td>H7N7</td>
<td>H4N5</td>
</tr>
<tr>
<td>Estimated mortality</td>
<td>500</td>
<td>60</td>
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<tr>
<td>Histopathological findings</td>
<td>Necrotizing Bronchopneumonia</td>
<td>Necrotizing Bronchopneumonia</td>
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<td>Virus recovery from the following tissues of dead seals</td>
<td>Lung, brain, hilar lymph nodes</td>
<td>Lung, brain, hilar lymph nodes</td>
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<tr>
<td>Documented human infection</td>
<td>Yes</td>
<td>No</td>
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<tr>
<td>Concurrent weather conditions</td>
<td>Mild winter</td>
<td>Mild winter</td>
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<td>Virus recovery from experimentally inoculated animals</td>
<td></td>
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<tr>
<td>Seals (nasal passage and eyes)</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Ferrets (nasal passages)</td>
<td>+ (10^7.9 EID_{50}/ml)</td>
<td>+ (10^{3-5} EID_{50})</td>
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<td>Ducks (intestinal tract)</td>
<td>–</td>
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outbreak of influenza in seals and the first evidence that an influenza virus with all of the properties of an avian influenza virus can be involved in naturally occurring influenza in mammals. The earlier epizootic of viral pneumonia in seals in 1979 to 1980 (3) involved H7N7 viruses which were antigenically and genetically related to avian strains but replicated poorly, if at all, in avian species and were not enterotropin in birds. However, this virus grew quite well in a variety of mammals, including squirrel monkeys (5), and caused conjunctivitis in humans (8), indicating that the host range of the H7N7 seal virus was not limited to marine mammals. The detection of the H4N5 strain in seals raises questions as to whether this avian virus has been newly introduced and, as a step in the evolution of influenza viruses in nature, is currently adapting to a mammalian host or whether avian viruses are frequently transmitted to seals and associated with disease outbreaks in these mammals.

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LITERATURE CITED