Isolation of an Influenza A Virus from Seals

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Summary

Influenza A virus of serotype Hav1 Neq1 (H7N7 by the 1980 revised influenza typing system proposed by WHO experts) was repeatedly isolated from lung and brain tissues taken from harbor seals (Phoca vitulina) found suffering from pneumonia on Cape Cod Peninsula (U.S.A.) in the winter of 1979–1980. The seal isolates, although of a serotype identical to some fowl plague virus strains, were harmless to chickens and turkeys in transmission experiments. An earlier human infection by a Hav1 Neq1 influenza virus and the serologic relatedness of this avian serotype with the equine 1 serotype are cited in support of the view that influenza viruses with these antigenic characteristics seem to have a facility to pass from birds to mammals.

Introduction

Influenza A viruses in nature infect a wide range of avian species, but among mammals only humans, pigs and horses. While interspecies virus exchange is frequent in birds (7), it has only been reported infrequently for the mammalian influenzas. Furthermore, despite antigenic and genetic relatedness between avian and mammalian virus strains (20, 17), there has been little evidence of direct interspecies transmission of influenza A viruses between birds and mammals.

In December 1979, biologists from the New England Aquarium in Boston, MA, observed a sudden increase of stranded and dead harbor seals (Phoca vitulina) on Cape Cod Peninsula (8). The peak mortality occurred in January 1980, and is still continuing in the seals. The number of deaths was estimated at 500 seals which represented a mortality of at least 20 percent in the affected population.
We report here the isolation and characterization of an influenza A virus of antigenic type Hav1Neq1 from the lungs and brains of harbor seals that died of the pneumonia epizootic.

**Materials and Methods**

**Clinical Material**

Harbor seals (*Phoca vitulina*) found dying or dead on the coast of Cape Cod (U.S.A.) during December 1979 and January 1980 were taken to the New England Aquarium for examination. The predominant finding on necropsy was acute hemorrhagic pneumonia, and a viral etiology was suspected. Selected lung and brain tissues, as well as serum samples from affected live seals were prepared for virus isolation.

**Virus Isolation Procedure**

Allantoic passages in nine-day-old chicken embryos followed standard virological practices, using as starting inocula 10 percent tissue extracts in antibiotic broth. Yolk sac passages were included in the first isolation attempts, in order to determine the presence of chlamydia. Inoculated eggs were incubated at 39°C. Embryo mortality was determined by daily candling, and allantoic fluids from dead embryos were examined for hemagglutinins to chicken erythrocytes.

**Serology**

Reference antisera to influenza H and N types were prepared in chickens. Antisera to the seal isolates were obtained from chickens and turkeys used in pathogenicity studies. Hemagglutinin (HA) titrations and hemagglutination-inhibition (HI) tests were carried out on plastic trays by standard techniques (13). Neuraminidase titrations and inhibition tests followed the procedure of **Aymard-Henry et al.** (13.)

**Results**

The virological diagnosis of the seal pneumonia involved two laboratories. Nineteen seals were examined of which 13 yielded virus. From ten seals both lung and brain material was studied and virus was found either in both sites (seven seals) or not at all (three seals). From the remaining nine seals, only lung was examined with six positive and three negative isolation results. In the first chicken embryo passages of seal tissue inocula, the presence of virus was evident following yolk sac inoculation by heavy embryo mortality during the first four days but irregular hemagglutinating activity of their allantoic fluids, while in parallel allantoic passages mortality was low but hemagglutination was a regular feature. Brain and lung inocula from one seal differed from this pattern, in that neither mortality nor hemagglutination was noted at the first passage level, but virus was detected in the second and subsequent passages from both tissues. Embryo mortality increased with increasing passage levels as did hemagglutination titers of infected embryonic fluids (from 1:160 to 1:1280). Infected embryos were usually congested but frank hemorrhages were rare. Examination of infected yolk sacs for chlamydia by the Gimenez staining procedure (9) did not reveal such agents.

**Virus Identification**

Morphological study of the isolated agent under the electron microscope revealed typical orthomyxovirus particles in respect to size, surface peplomer