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Experimental infection of animals with influenza-virus types A and B *

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The knowledge that domestic cats were susceptible to infection with freshly isolated A/Hong Kong/68 influenzavirus led to studies on the susceptibility of some other animal species to this virus, as well as to studies on the ability of egg-passaged Hong Kong virus and an Asian virus to infect cats. The ability of a recent isolate of influenzavirus B to infect these animals was also studied. Macaca radiata *monkeys could be infected with fresh isolates of A/Hong Kong virus by intranasal instillation or by contact with infected animals. They could also be infected with influenzavirus B by intranasal challenge, but contact transmission was not demonstrated. Mongrel dogs were found to be susceptible to A/Hong Kong/68 virus by intranasal instillation, but not to type B virus. Domestic cats could be infected with A/Hong Kong/68 virus passaged 6 times in eggs. They were also susceptible to infection with an established laboratory strain of Asian virus. Cats could be infected with influenzavirus B either by intranasal challenge or by contact with infected animals. In no case was clinical illness found following infection, but the infected animals shed virus from the throat and developed haemagglutination inhibiting antibodies.*

The possibility that influenza infection in man and in animals may be interrelated has been strengthened by the demonstration that man may be experimentally infected with equine influenzavirus (Couch et al., 1969) and that animals may be infected with human influenzavirus A. Horses (Kasel & Couch, 1969), baboons (Kalter et al., 1969), and domestic cats (Paniker & Nair, 1970) have been found to be susceptible to experimental infection with A/Hong Kong/68 virus. Direct interspecies transmission of Hong Kong influenzavirus infection from man to the domestic cat has also been reported (Paniker & Nair, 1970). Kundin (1970) has reported natural infection with A/Hong Kong /68 virus among swine. Isolates from such swine have been found to infect human volunteers (Beare et al., 1971).

All the natural influenza infections in animals investigated so far have been caused by type A viruses. However, serum surveys have demonstrated antibodies to influenza B virus in horses (Ditchfield et al., 1965) and in swine (Takatsy et al., 1967). Experimental infection with influenzavirus B has been induced in chincoteague ponies (Kasel et al., 1968) and in swine (Takatsy et al., 1969).

This paper reports studies on the susceptibility of monkeys, dogs, and domestic cats to experimental infection with influenzavirus types A and B.

MATERIALS AND METHODS

Virus strains

Hong Kong influenzavirus. Three different isolates of virus antigenically identical to A/Hong Kong/68 (H3N2) virus were obtained in this laboratory by direct allantoic inoculation of human throat washings (Paniker & Nair, 1969). Unless otherwise stated, isolates were used for animal inoculation within 3 hours of harvesting.

Influenzavirus A/Ann Arbor/4/63(H2N2). This strain, which was isolated at the Strain Study Centre, School of Public Health, Ann Arbor, Mich., USA, was used after several allantoic passages.

Influenzavirus B. A recently isolated strain of influenzavirus B (B/Coonoor/S.103/70) obtained from the Government of India Influenza Centre, Pasteur Institute, Coonoor, South India, was used.

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Animals and inoculation procedures

The following animals were used:

(1) Locally trapped *Macaca radiata* monkeys that had been in captivity for about 5 years. They were housed in individual cages.

(2) Stray mongrel dogs, 2–3 weeks old, which were kept together in one room.

(3) Stray domestic cats (*Felis catus* L.), which were housed in individual cages.

For experimental infection, approximately 0.2 ml of allantoic fluid infected with virus (haemagglutination titre, 40–80) was instilled intranasally into anaesthetized animals. The monkeys and puppies were anaesthetized with pentobarbital sodium, and the cats with ether. Blood samples were collected by cardiac puncture before infection and again 2 weeks later. Throat washings were collected periodically under anaesthesia, for virus isolation by direct allantoic inoculation. Specimens yielding no virus by allantoic inoculation were retested amniotically, but no additional isolates could be obtained. The animals were inspected daily for any evident signs of illness, and their rectal temperatures were recorded.

Haemagglutination inhibition (HI) test

Sera were pretreated with kaolin to remove non-specific haemagglutination inhibitors (Spence, 1960). The HI tests were performed as described earlier (Paniker & Nair, 1970).

RESULTS

Experimental infection in monkeys

Two monkeys were infected intranasally with Hong Kong influenzavirus and three animals were left as contacts in adjacent cages. The intranasally infected animals shed virus from the throat from the second day of infection until the sixth day. Infection was transmitted to all three contacts and they shed virus from the throat from the fourth day of the experiment until the eighth day. All animals developed HI antibodies. The monkeys infected intranasally had preinfection HI titres of <10 and 10, and these rose to 160 and 240 after infection. In the contact monkeys, HI antibody titres rose from <10, 10, and 20 to 160, 60, and 60, respectively.

Three monkeys were infected intranasally with influenzavirus B and one animal was kept as a contact in an adjacent cage. All the intranasally inoculated monkeys developed HI antibodies (the preinfection titres <10, <10, and 10, rose to titres of 80, 80, and 40, respectively, after infection), and two of them shed virus from the throat for up to 4 days. The contact monkey did not shed virus, nor did it show a significant rise of HI antibodies (the HI titres were 20 and 60 before and after infection respectively).

Experimental infection in dogs

Four dogs were infected intranasally with A/Hong Kong/68 virus. All developed infection and shed virus from the throat for 4 days. The dogs did not have HI antibodies to the virus before infection, but after infection the HI titres were 320, 320, 160, and 80.

Three dogs were infected intranasally with influenzavirus B and two were kept as contacts. None of the animals showed any evidence of infection either by virus shedding or HI antibody response.

Experimental infection in cats

Egg-passaged A/Hong Kong/68 virus. Two cats were infected intranasally with a strain of A/Hong Kong/68 virus after 6 serial allantoic passages. Both animals developed infection and shed virus from the throat for 6–8 days. One animal died before convalescent serum could be collected, but the other showed an HI antibody response, the preinfection titre being <10 and the 10-day postinfection titre 40.

Asian influenzavirus. Three cats were infected intranasally with a strain of Asian influenzavirus (A/Ann Arbor/4/63) that had undergone several allantoic passages. One of these animals did not shed any virus for the period of 11 days that it was under observation. It escaped before convalescent serum could be collected. The other two shed virus from the throat, one of them for up to 10 days. One of these animals died before the second sample of serum could be collected; the other developed HI antibodies, the titre increasing from 80 to 320.

Influenzavirus B. Three cats were infected intranasally and two were left as cagemate contacts. All the intranasally infected animals shed virus from the throat, one of them for up to 8 days. Two of these developed HI antibodies (preinfection titre <10 and postinfection titre 240 in both); the third animal died before the convalescent serum could be collected. From one of the two contacts, virus could be recovered on the sixth day of infection; this animal also showed a rise in HI antibody titre from <10 to 80. The second contact animal did

not shed any virus, nor did it show serological evidence of infection.

DISCUSSION

Domestic cats were found to be susceptible to infection with fresh isolates of A/Hong Kong/68 virus in an earlier study (Paniker & Nair, 1970). The present study shows that the virus would still produce infection in cats even after six serial allantoic passages. Cats were also found to be susceptible to infection with an Asian strain of virus that had undergone many allantoic passages over a period of 8 years. Hong Kong virus was found to infect *Macaca radiata* monkeys both by intranasal instillation and by contact with infected animals. Dogs could also be infected with the virus intranasally, but contact transmission was not tested.

Influenzavirus B infected monkeys following intranasal challenge, but there was no clear evidence of transmission by contact. It also infected domestic cats both by intranasal instillation and by contact. Dogs were not susceptible to influenzavirus B infection.

Kalter et al. (1969) suggested that it may be essential to use freshly isolated virus strains for experimental infection of animals, but the present results indicate that domestic cats may be infected with strains that have undergone several serial allantoic passages. The use of domestic cats as experimental models for A/Hong Kong influenza infection has been suggested earlier (Paniker & Nair, 1970) and now their use for experiments with the earlier A and B strains can also be recommended.

RÉSUMÉ

INFECTION EXPÉRIMENTALE D'ANIMAUX PAR DES VIRUS GRIPPAUX DES TYPES A ET B

Des singes *Macaca radiata* se sont montrés réceptifs à l'infection par une souche fraîchement isolée du virus grippal A/Hong Kong/68 inoculée par voie intranasale. L'infection a pu être transmise à d'autres singes par contact avec les animaux infectés. On a obtenu l'infection par instillation intranasale d'un virus grippal B, mais un essai de transmission par contact n'a pas abouti.

On a réussi à infecter des chiens par inoculation intranasale d'un virus A/Hong Kong/68 récemment isolé. Par contre les tentatives d'infection expérimentale par un virus du type B ont échoué.

Des chats domestiques se sont montrés réceptifs à l'infection par une souche de virus A/Hong Kong/68 ayant subi une série de 6 passages sur membrane chorio-allantoïde, ainsi que par une souche de laboratoire A/Ann Arbor/4/63. Des chats ont été infectés par un virus de type B après inoculation intranasale ou après contact avec des animaux infectés.

Dans aucun cas, l'infection par les virus grippaux n'a entraîné de manifestations cliniques; les animaux infectés ont éliminé le virus dans les sécrétions pharyngées et ont élaboré des anticorps inhibant l'hémagglutination.

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