

Infection with A2 Hong Kong Influenza Virus in Domestic Cats*

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The antigenic relationship of A2 Hong Kong influenza virus with equine influenza virus, and its ability to infect horses and baboons, have led to studies on the susceptibility of domestic animals to the virus.

In this study it was found that cats could be infected with A2 Hong Kong influenza virus by intranasal inoculation or by contact with an infected cat or with a human influenza patient. There was no clinical illness, but infected animals shed the virus from the throat for 1 week and developed haemagglutination-inhibiting antibodies. A survey of normal cat sera showed that 6 out of 28 sera inhibited haemagglutination by A2 Hong Kong influenza virus.

The results suggest that domestic cats may act as vectors in the transmission of influenza virus. Experimental infection in cats may be used as a laboratory model for influenza.

There has been much speculation about the possible role of animals in the epidemiology of human influenza. It has been suggested that pandemic strains of human influenza virus may perhaps originate in animals (Andrewes, 1959; Fenner, 1968). Many animals and birds have been shown to suffer from natural influenza, and porcine, equine and avian influenza viruses have been isolated and characterized (Andrewes, 1964). Antigenic relationships have been established among the haemagglutinins and neuraminidases of some human, avian and animal influenza viruses (Pereira, Tumova & Webster, 1957; Webster & Pereira, 1968; Kasel, Fulk & Couch, 1969). The experimental production of recombinants between human and animal influenza viruses, besides indicating their common ancestry, suggests that similar hybrids may occur in nature also (Tumova & Pereira, 1965; Easterday et al., 1969). While no interspecies transfer of influenza infection has been convincingly demonstrated in nature, it has been shown that man may be experimentally infected with equine influenza virus, and horses and baboons with human influenza A2 virus (Kasel & Couch, 1969; Kalter et al., 1969). The demonstration of infection by human influenza viruses in an animal species in close contact with

man would perhaps be more relevant in understanding the role of animals in human influenza. We report the susceptibility of domestic cats to A2 Hong Kong influenza virus.

MATERIALS AND METHODS

Virus strains

Influenza virus strains isolated in this laboratory were used. Throat washings from influenza patients were divided into aliquots and stored at -30°C . Virus isolation was by direct allantoic inoculation into chick embryos (Paniker & Nair, 1969). The isolates were shown to be antigenically identical with A2/Hong Kong/68 influenza virus by comparison with standard strains obtained from the Government of India Influenza Centre, Pasteur Institute, Coonoor, South India.

Animals and animal inoculation procedures

The animals used were adult domestic cats (*Felis catus* L.) and kittens under 8 weeks of age housed in large wire cages. In contact experiments, the cages were kept 1 ft (30.5 cm) apart.

For experimental infection, approximately 0.2 ml of virus-infected allantoic fluid from the first egg passage (haemagglutinin (HA) titre ranging from 20 to 80) was inoculated intranasally into cats under light ether anaesthesia, within a few hours after collection. Blood samples were collected by

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cardiac puncture before and after infection, and the rectal temperature was recorded. For virus isolation by direct allantoic inoculation, throat washings were collected periodically after infection from the cats under anaesthesia.

Haemagglutination inhibition (HI) test

All sera were treated with 0.011 M potassium periodate and heated at 56°C for 30 min prior to testing. Previous tests had shown this to be the most effective method for removing nonspecific inhibitors to A2 Hong Kong virus in cat sera. For the survey of HI antibodies in normal cat sera, combined treatment with trypsin and periodate (Ananthanarayan & Paniker, 1960) was also used for inactivation of nonspecific inhibitors. HI tests were done in plastic trays using 4 units of virus and 0.5% fowl erythrocytes. The tests were run in parallel using A2/Hong Kong/68 influenza virus and the locally isolated strains used for infection. In no case was a difference in titre greater than 2-fold observed between the two. Since 3 different strains had been used for infection in different experiments, only HI titres against A2/Hong Kong/68 virus are shown in the results. All titres are expressed as reciprocals of the dilution.

RESULTS

Experimental infection of kittens

Two kittens (no. 1 & 2), both 3 weeks old, were inoculated intranasally with virus-infected allantoic fluid and housed in one cage. Serial throat washings were collected, and blood samples were obtained before and 15 days after infection. Virus could be recovered from both animals, the period of virus shedding lasting for up to 6 days; no virus could be isolated 13 days after infection. The kittens had no antibodies to the virus before infection, but post-infection sera had HI antibody titres of 160 and 320.

Transmission of infection to cage mate

A kitten (no. 3) from the same litter was left as a contact in the same cage. Serial throat washings and paired serum samples were collected. Virus was recovered from the contact kitten for up to 6 days (on day 1 and day 6, but not on day 4) and the HI antibody titre rose from <10 to 120.

Reinfection in kittens

Kittens no. 1, 2 and 3 were reinfected with the same virus 2 months after the initial infection. No

virus was isolated from serial throat washings, but the HI antibody titre increased from 80, 80 and 20, to 640, 160 and 1280, respectively, 12 days after infection.

Experimental infection in adult cats

Two adult cats (no. 4 & 5) were intranasally infected with the virus. Both animals shed the virus and they developed HI antibody titres of 640 and 320, respectively.

Transmission of infection to animals in adjacent cages

For these experiments, infected and contact animals were kept in separate cages 1 ft (30.5 cm) animals were kept in separate cages 1 ft (30.5 cm) apart. One kitten (no. 6), 3 weeks old, was intranasally infected and another (no. 7), 6 weeks old, was left in the adjacent cage. The contact kitten shed the virus (on day 5 and day 8 after exposure) and developed an HI antibody titre of 320.

Two adult cats (no. 8 & 9) were left as contacts in separate cages adjacent to animals no. 4 and 5, from the time the latter were infected. Virus could be recovered from one of the contact animals (on days 3 and 5 after exposure), which also developed an HI antibody titre of 640. The other animal did not shed the virus, though it developed an HI antibody titre of 20.

Transmission of infection by contact with influenza patient

Two kittens (no. 10 & 11), 6 weeks old, under light ether anaesthesia were directly exposed for about 2 min to a patient suffering from influenza, identified by virus isolation and serology, on the first day of his illness. The patient was asked to cough and sneeze so that the animals were exposed to the spray of droplets. One of the kittens (no. 10) did not develop antibodies and no virus could be recovered from its throat, but the other (no. 11) shed the virus up to 8 days (on days 3 and 8, but not on days 1 and 5) after contact and developed an HI antibody titre of 80.

HI antibodies in normal cat sera

Sera from 28 cats, 15 adults and 13 kittens, were tested for HI antibodies after treatment with periodate alone and with periodate and trypsin. The results were not significantly different after the two procedures. Inhibition of haemagglutinin was seen in 7 sera (see accompanying table).

RESULTS OF HI TESTS WITH A2/HONG KONG/68 INFLUENZA VIRUS AND NORMAL CAT SERA

Animals	No. of sera with HI titres							Total
	<10	10	20	40	80	160	320	
Adult cats	11	0	0	0	1	1	2	15
Kittens	10	1	0	0	0	2	0	13
Total	21	1	0	0	1	3	2	28

DISCUSSION

The results reported show that cats are susceptible to A2/Hong Kong influenza virus infection. Not only can they be infected by intranasal challenge, but they also transmit the infection to contacts kept either in the same cage or in neighbouring cages. The infected animals showed no evident clinical illness. There was no rise of temperature, nor was there any observable discharge from the nose, coughing or sneezing. But the virus was shed from the throat for at least 1 week after infection and HI antibodies were produced. Infection appeared to produce local immunity, as after reinfection no virus could be recovered from the throat, though there was an increase of antibody titre.

Of particular interest was the finding that one of two cats exposed to a human influenza patient developed the infection, indicating direct interspecies transfer of the virus. This may be of epidemiological significance and suggests that cats, because of their close contact with man, may act as vectors in the chain of virus transmission.

If transmission of infection from man to cats were a common event in nature, cat serum surveys should reveal antibodies to human influenza viruses. But most animal serum surveys have not included cats. Meenan, Boyd & Mullaney (1962) found that 4 out of 20 cat sera inhibited haemagglutination by A2/Asia/57 virus. Though the sera were pretreated with periodate, they ascribed this to non-specific inhibitors as there was no inhibition of haemagglutination by an inhibitor-insensitive strain. Out of 28 cat sera tested in this study, 6 inhibited haemagglutination in high titres (≥ 80); 4 out of 15 adults and 2 out of 13 kittens. However it has not been established whether these represent antibodies or non-specific inhibitors.

The susceptibility of cats to influenza virus may have other applications. The need for a convenient laboratory model for influenza has long been felt. Mice have been used for studying the experimental epidemiology of influenza (Schulman, 1998), and cats may provide an alternative model.

RÉSUMÉ

INFECTION PAR LE VIRUS GRIPPAL A2 HONG KONG CHEZ LE CHAT DOMESTIQUE

On a inoculé par voie intranasale à des chats domestiques un virus grippal isolé chez l'homme et offrant les mêmes caractéristiques antigéniques que le virus A2/Hong Kong/68.

Les animaux n'ont présenté aucun signe clinique de maladie, mais ils ont éliminé le virus dans les sécrétions pharyngées pendant une semaine environ et ont réagi par la production d'anticorps inhibiteurs de l'hémagglutination. Des chats inoculés ont transmis l'infection à des congénères placés dans la même cage ou dans une cage voisine. Sur deux chatons mis en contact pendant 2 minutes avec un malade atteint de grippe, un a con-

tracté l'infection, avec excrétion de virus pendant 8 jours et élaboration d'anticorps IH.

Par ailleurs, sur 28 sérums prélevés chez des chats bien portants, 6 inhibaient l'hémagglutination par le virus A2/Hong Kong/68. On n'a pu cependant établir si le phénomène était dû à la présence d'anticorps ou à l'action d'inhibiteurs non spécifiques.

Ces observations démontrent la réceptivité du chat domestique au virus grippal humain. Outre leur intérêt épidémiologique, elles donnent à penser que le chat pourrait servir de modèle pour l'étude de l'infection grippale au laboratoire.

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