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Epidemiological studies of A/Hong Kong/68 virus infection in dogs*

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Experimental inoculation of dogs with the A/Hong Kong/68 influenzavirus resulted in subclinical infection. The virus was readily passed to contacts in the same cage when the latter were exposed in the same inoculation room 24 hours after experimental infection. Removing the site of contact to a noncontaminated room or delaying contact until 48 hours after experimental inoculation greatly reduced the possibility of infection in contact animals. A survey of 271 canine serum samples obtained after a human epidemic from different geographical areas of the USA and the United Kingdom showed that 5.9 % of the samples were positive; no positive reactions were found among 111 pre-epidemic samples. These studies demonstrated the laboratory and natural susceptibility of dogs to the Hong Kong variant and suggest the possible role of dogs in the epidemiology of human influenza.

The animal populations usually investigated for epidemiological associations with man have been swine, horses, and birds, species known to be hosts of influenza A virus. There has been little interest regarding the role of dogs as reservoirs of human influenzavirus. Their potential role in the epidemiology of human influenza was suggested by studies demonstrating in the laboratory the susceptibility of dogs to certain human influenzaviruses, i.e., the A1, Asian, and B strains (Ado & Titova, 1959; Todd & Cohen, 1968) and by unconfirmed reports of the detection of antibodies to human influenza strains in canine sera (Todd & Cohen, 1968; Lundgren et al., 1969).

With the advent of the Hong Kong/68 virus, a new pandemic subtype, it was of interest to continue the study of the role the dog might play in the epidemiology of human disease caused by this virus.

This study was undertaken to determine whether dogs were susceptible to laboratory infection with a Hong Kong strain of influenzavirus and whether such an infection could be transmitted spontaneously to contacts in the same cage. Serological studies were also undertaken to determine whether infection of dogs with Hong Kong influenzavirus had occurred in nature.

MATERIAL AND METHODS

Viruses

The A/Hong Kong/8/68 (H3N2), A/equine/Pennsylvania/64 (Heq2Neq2), and A/Philadelphia/2946/57 (H2N2) reference viruses were laboratory stock strains maintained in embryonated chicken eggs. The virus used for exposure of the dogs in experiments A and B consisted of fresh undiluted allantoic fluids from the third egg passage of a Hong Kong influenza virus, A/Philadelphia/101/68 (H3N2), that was isolated in our laboratory from a case of disease in man. The same virus at the same passage level had been used previously for experimental infection of baboons (Kalter et al., 1969) and of equines (Todd et al., 1970). The virus employed in experiment C was fresh undiluted allantoic fluid representing a pool of virus that had been reisolated from 2 dogs of experiment A, i.e., it had undergone one passage in canines.

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The materials used for inoculations had haemagglutination (HA) titres of 1 : 128 to 1 : 256 per 0.4 ml and egg infectivity titres (EID_{50}) of $10^{8.0}$ – $10^{8.3}$ per ml.

Viruses for haemadsorption–neutralization (HAD–N) tests were also propagated in the allantoic cavity of embryonated chicken eggs. Before use in the test, samples of allantoic fluid harvests, which had been stored in 2-ml ampoules at −85°C, were thawed and their haemadsorption titres in BS-C-1 cells were established as follows: 0.2 ml of serial twofold dilutions were inoculated into duplicate tubes containing the monolayers from which the growth medium had been removed and replaced with serum free medium. After incubation for 72 hours at 36°C without rotation, the monolayers were thoroughly washed and tested for the presence of haemadsorption (HAD) with guineapig red blood cells. The highest dilution of virus that produced haemadsorption was considered to be the endpoint (Lief & Henle, 1959).

Cell cultures

A continuous cell line of African green monkey (*Cercopithecus aethiops*) kidney cells (BS-C-1) was used for the HAD–N test (Lief & Somburanasin, unpublished data).

Primary cultures of canine kidney cells (PCK) were used for the attempts to isolate any extraneous virus with which the experimental animals could have been infected prior to the start of the experiment.

All cell cultures were propagated without rotation in the presence of Eagle's minimum essential medium (MEM) in Earle's buffered salt solution containing 10% of inactivated calf serum and 100 IU of penicillin, 78 IU of streptomycin and 4.7 IU of amphotericin B per ml. Before inoculation with any test material, the growth medium was removed and the monolayers were washed and replaced with serum-free medium.

Experimental animals and their housing

Altogether 39 dogs without previous known history of exposure to human influenza were purchased from commercial breeders. These dogs were of mixed breeds and of both sexes, ranging in age from 5 to 9 months.

The dogs were maintained in controlled environment isolation units for at least 3 weeks before the start of the experiment. Upon arrival they were immunized against canine distemper and canine infectious hepatitis. They were sorted at random into the experimental groups (exposed, contact, control) 24 hours before inoculation. Each group was placed in a separate room that had been recently cleaned and disinfected. The dogs belonging to the exposed and contact groups were kept in the isolation area. The control group was kept in a room outside the isolation area in order that they might act as sentinels for accidental disease outbreak in the experimental rooms and to minimize inadvertent contamination from the animals exposed to virus.

During the course of each experiment, contact with man was restricted to the investigator and one assistant and the person responsible for cleaning. The animal quarters were cleaned, and specimens were collected, in the following order: (1) the control group; (2) the group that were later to serve as contacts; and (3) the group already exposed to virus either by inoculation or contact.

Inoculation of virus

Dogs to be inoculated with virus were anaesthetized with thiamylal sodium after a fasting period of 24 hours. A 2-ml quantity of undiluted infective allantoic fluid was sprayed directly into the pharynx of each dog with a hand atomizer and 0.5 ml of the same material was instilled into each nostril. Dogs of the control group were given normal allantoic fluid in the same quantities and in the same way.

Clinical examinations

For 7 days before and for 10 days after the inoculation of the virus, or after contact with an exposed dog, each animal was examined daily for signs of respiratory disease such as apathy, anorexia, nasal and ocular discharge, tonsillitis, pharyngitis, and cough. Rectal temperatures were also taken daily and always before the collection of specimens.

Virus recovery

The techniques used for attempting to recover virus from the dogs were the same as previously described (Todd & Cohen, 1968). The preexposure specimens were inoculated amniotically as well as allantoically into embryonated eggs and in addition were introduced in 0.2-ml quantities into each of 4 tubes containing monolayers of PCK cells. The PCK tissue culture tubes were examined daily for the presence of cytopathic effect (CPE). On the seventh day, 2 of the 4 tubes were tested for the presence of HAD. The other 2 tubes inoculated with

the individual specimens were subpassaged into another series of 4 tubes of PCK cell cultures. A specimen was considered negative if no CPE or HAD was detectable after 3 blind passages.

Collection of serological specimens

Preexposure blood samples were drawn from the jugular vein of all experimental animals upon arrival and immediately before the experiment was started. Postexposure samples were taken after 7, 14, 21, 28, 45, and 60 days. All sera were inactivated at 56°C for 30 minutes and were then stored at −20°C until tested.

Serological tests

All serum samples drawn from the same dog, but at different times, were assayed simultaneously in a given test procedure.

Haemadsorption–neutralization test (HAD–N)

The techniques used have been developed by Lief & Somburanasin.[1] Sera were diluted in twofold steps in MEM containing 100 IU of penicillin and 78 IU of streptomycin per ml: 0.4 ml of each dilution was then mixed with an equal amount of the particular seed virus diluted so that 0.2 ml of the mixture would contain 8–16 haemadsorbing units of the virus. The serum–virus mixtures were then incubated overnight at 4°C and 0.2-ml amounts of each dilution were inoculated into duplicate tissue culture tubes containing monolayers of BS-C-1 cells. The tubes were incubated at 36°C for 72 hours without rotation and were then tested for the presence of HAD. The highest dilution of serum that completely inhibited the appearance of haemadsorption was considered to be the neutralizing antibody endpoint. Viral infectivity titrations were carried out with each test, as a control for the test.

Complement fixation (CF) test. The methods used for preparing antigens and performing the CF test have been described (Lief & Henle, 1959). In addition, before use in the CF test, all dog sera were heated at 60°C for 20 minutes and adsorbed with packed sheep red blood cells in order to eliminate nonspecific reactions. Most of the tests were performed in microplates employing 0.025 ml of serial twofold dilutions of sera mixed with an equal amount of the appropriate (S) or (V) antigen.

Canine sera for the serological survey

The 382 canine serum samples that were assayed for evidence of infections with influenzavirus in nature were obtained from several centres in the USA and from Cambridge, England.

[1] Unpublished data.

RESULTS

Experimental infections

Experiment A—infectivity and transmission to primary contacts. In the first experiment 12 dogs of mixed breeds and both sexes, approximately 8–9 months old, were employed as follows: (1) 5 dogs were inoculated with $3 \times 10^{8.2}$ EID_{50} of the Hong Kong virus A/Philadelphia/101/68; (2) 5 dogs were paired with the inoculated dogs 24 hours later; and (3) 2 dogs were given normal allantoic fluid at the same time as group (1) and acted as sentinel controls.

None of the dogs in this experiment developed signs of respiratory disease. However, all the animals inoculated with virus showed elevated temperatures on the following day ranging from 39.9°C to 40.6°C.

Virus was recovered from nasopharyngeal secretions of all 5 inoculated dogs (Table 1) beginning 24 hours after exposure and on the following 4–6 days. Of the 5 contact dogs 4 also yielded virus, 3 at 24 hours and the other at 48 hours after contact and for 3–7 days thereafter. Most isolations were accomplished at the first egg passage. The recovered viruses were antigenically indistinguishable from the virus strain inoculated. No virus isolations were made from any of the preexposure specimens or from specimens obtained from control dogs.

All dogs of the inoculated and contact groups responded with significant rises in HAD–N and CFV (anti-V) antibodies (Table 2). These antibodies were directed against both the A/Hong Kong/68 and the equine strains but the levels produced against the latter were lower and in the case of the HAD–N antibodies became detectable later than those to the A/Hong Kong/68 virus. Indeed in 3 of the inoculated dogs HAD–N antibodies to Hong Kong virus could be detected in moderate to high titres as early as 7 days after experimental infection. By 2–3 weeks after exposure, however, both HAD–N antibody (at titres ranging from 1 : 8 to 1 : 256) and CFV antibodies (at titres ranging from 1 : 16 and 1 : 128) were present in all dogs of the inoculated and contact groups. They were still detectable without much change at 60 days. Dog no. 14, from which no virus could be recovered, nevertheless developed high titres of HAD–N antibodies by 30 days after exposure. In contrast, contact dog no. 9, which shed virus for the shortest time, showed only a slight

response to the Hong Kong virus. In these 2 dogs no reaction to the equine virus was observed.

All the inoculated and 3 of the contact dogs developed detectable but low levels of antibodies to S antigen (anti-S). Contact dogs no. 9 and no. 14 remained negative with respect to this antibody. None of the animals developed antibodies against an early prototype Asian strain (A/Philadelphia/2946 / 57) as revealed by CF tests and none of the control animals developed detectable antibodies by any test to any of the influenzavirus strains tested.

Experiment B—transmission to secondary contacts. A second experiment was designed to discover

Table 1. Recovery of virus from dogs exposed to an A/Hong Kong/68 influenzavirus by inoculation or contact [a]

Experiment	Dog no.	Method of exposure	Days after experimental inoculation or contact										
			0	1	2	3	4	5	6	7	8	9	10
	2	inoculated	—	E1	E1	E1	E1	E1	—	—	—	—	—
	14	contact	—	—	—	—	—	—	—	—	—	—	—
	4	inoculated	—	E1	E1	E1	E1	E1	—	—	—	—	—
	7	contact	—	E1	E1	E1	E1	E1	E1	E1	—	—	—
A	6	inoculated	—	E1	E1	E1	E1	E1	—	—	—	—	—
	9	contact	—	E1	—	E2	—	—	—	—	—	—	—
	12	inoculated	—	E1	E1	E1	E1	—	—	—	—	—	—
	8	contact	—	E1	E1	E1	—	E1	—	—	—	—	—
	13	inoculated	—	E1	E1	E2	E1	E1	E1	—	—	—	—
	5	contact	—	—	—	E1	E1	E1	E1	—	—	—	—
	02	inoculated	—	E1	E1	E1	—	—	—	—	—	—	—
	05	primary contact	—	—	—	—	—	—	—	E1	—	—	—
	Q3	secondary contact	—	—	—	—	—	—	—	—	—	—	—
	Q1	inoculated	—	E1	E2	E1	E1	E1	—	—	—	—	—
	Q3	primary contact	—	—	—	E2	—	—	—	—	—	—	—
	01	secondary contact	—	—	—	—	—	—	—	—	—	—	—
B	Q4	inoculated	—	—	E1	E1	—	E1	—	—	—	—	—
	R3	primary contact	—	—	—	—	—	—	—	E2	—	—	—
	Q2	secondary contact	—	—	—	—	—	—	—	—	—	—	—
	R1	inoculated	—	E1	E1	E1	E1	E1	—	—	—	—	—
	Q5	primary contact	—	—	E1	—	—	—	—	—	—	—	—
	P3	secondary contact	—	—	—	—	—	—	—	—	—	—	—
	1	inoculated	—	E1	—	—	E1	E1	—	—	—	—	—
	7	primary contact	—	—	—	—	—	—	—	—	—	—	—
	5	secondary contact	—	—	—	—	—	—	—	—	—	—	—
	3	inoculated	—	E1	E2	—	E1	—	—	—	—	—	—
	6	primary contact	—	—	—	—	—	—	—	—	—	—	—
C	2	secondary contact	—	—	—	—	—	—	—	—	—	—	—
	14	inoculated	—	E1	E1	E2	—	E2	—	—	—	—	—
	8	primary contact	—	—	—	—	—	—	—	—	—	—	—
	13	primary contact	—	—	—	—	—	—	—	—	—	—	—
	10	secondary contact	—	—	—	—	—	—	—	—	—	—	—
	12	secondary contact	—	—	—	—	—	—	—	—	—	—	—

[a] E1 = recovered at the first egg passage; E2 = recovered at the second egg passage.

whether an influenza infection established in dogs could be transmitted in series to secondary as well as to primary contacts. Accordingly, 5 pedigree beagles and 9 mongrels, of both sexes, aged 5–6 months, were randomly divided into the following groups: (1) 4 dogs were inoculated intranasopharyngeally with 3 ml of the virus used in the first experiment; (2) 24 hours later the inoculated dogs were transferred to a new room where they were put in cage contact with 4 new dogs, which now comprised the primary contact group, and these 4 pairs of dogs were maintained in close contact for 3 days; (3) on the fourth day the 4 dogs of the primary contact group were transferred to a second room and were placed in cage contact with 4 new dogs, which became the secondary contact group (the primary and secondary contact groups stayed together for 10 days); and (4) 2 control dogs were inoculated with normal allantoic fluid in the same way as group (1).

As before, all of the dogs remained free of signs of respiratory disease, although dogs in the inoculated group had a rise in body temperature on the day immediately following exposure.

Virus was readily recovered from the nasopharyngeal secretions of all of the inoculated dogs for 3–5 days, beginning 24–48 hours after infection (Table 1). However, virus was recovered at the first egg passage from only 2 primary contact dogs (no. Q5 and no. O5) and at the second egg passage from 2 other primary contact dogs (no. Q3 and no. R3). All virus recoveries from primary contact dogs were limited to a single postexposure day for each of the contact dogs. No virus was isolated from any of the dogs of the secondary contact group or from the controls. All isolates were antigenically identical with the virus administered to the inoculated dogs.

As in experiment A all inoculated dogs responded with a rise in HAD–N and CFV antibodies to both the Hong Kong and the equine strains. Again the HAD–N antibodies to Hong Kong virus were detected as early as 7 days after exposure, reached a peak by the 28th day; significant titres were still observed after 60 days. Antibodies to the equine virus appeared later and at a lower level, reaching a peak 28–45 days after exposure. In the dogs of the primary contact group that shed virus, only dog no. O5 responded with the production of HAD–N and CFV antibodies (Table 2). Anti-S responses also developed in these dogs, but at levels that decreased somewhat by the 60th day. None of the animals developed measurable antibodies to the A/2946/57 strain. The secondary contacts and the controls remained negative to all tests for influenza antibodies.

Experiment C—infection with reisolated virus. This experiment was performed (1) to verify the pattern of the infection evoked in dogs by an A/Hong Kong/68 virus reisolated from experimental dogs; and (2) to determine the effect on transmission when contact between dogs was established later than 24 hours after infection.

Thus 13 dogs of mixed breeds and both sexes, ranging in age from 5 to 7 months, were randomly divided into the following groups: (1) 3 dogs were inoculated intranasopharyngeally with $3\times10^{8.0}$ EID_{50} of virus that consisted of a pool of isolates recovered from dog no. 7 on postexposure days 5 and 6 and from dog no. 5 on postexposure day 5; (2) 48 hours later 4 dogs comprising the primary contact group were placed in cage contact with the inoculated dogs and two dogs (no. 8 and no. 13) were placed in contact with inoculated dog no. 14; (3) 3 days later the primary contact dogs were transferred to a second room where they were put into cage contact with another set of 4 dogs for a period of 10 days, the latter then becoming the secondary contact group; (4) 2 control dogs were inoculated with normal allantoic fluid as in the early experiments.

Except for moderate rises in body temperature, which were observed in all 3 inoculated dogs on the first day following inoculation, none of the animals in this experiment developed clinical signs of disease. Virus was recovered from all inoculated dogs at the first egg passage 24 hours after the introduction of virus. Dog no. 1 yielded virus again on the fourth and fifth days, dog no. 3 on the second and fourth days, and dog no. 14 on the second, third, and fifth days. The latter isolations were accomplished at the first or second egg passage. No virus was recovered from the primary or secondary groups or from the control dogs.

Only the inoculated animals developed HAD–N antibodies to the infecting virus and one of these showed cross-reacting antibody to the equine virus (Table 2). The homologous antibody appeared as early as 7 days after exposure and reached maximum titres by the third week, while the cross-reacting antibody observed in the one dog was detected in the third week. Inoculated dogs showed rises in anti-V to the Hong Kong and equine strains as well as in anti-S, which appeared after 2 weeks.

Table 2. Antibody titres of dogs responding positively after exposure to A/Hong Kong/68 influenzavirus by inoculation or contact [a]

Experiment	Dog no.	Method of exposure	HAD–N test				CF test					
			Hong Kong antigen				Hong Kong V			Type A soluble		
			Days after exposure:				Days after exposure:			Days after exposure:		
			7	14	28	60	14	28	60	14	28	60
A	2	inoculated	256	256	256	256	64	64	32	8	8	4
	14	contact	0	0	128	128	32	16	8	0	0	0
	4	inoculated	0	32	256	512	8	128	32	0	16	4
	7	contact	0	128	128	1 024	32	32	32	0	4	4
	6	inoculated	9	1 024	256	256	128	64	32	8	8	4
	9	contact	0	0	8	8	8	4	4	0	0	0
	12	inoculated	128	128	128	128	16	64	16	0	16	4
	8	contact	0	64	128	256	32	32	16	8	8	4
	13	inoculated	0	64	256	256	32	32	32	16	16	8
	5	contact	0	64	128	256	64	64	32	0	8	8
B	02	inoculated	8	128	256	256	64	32	32	16	16	8
	05	contact	0	8	32	32	16	16	8	16	8	4
	Q1	inoculated	16	64	128	32	32	32	16	8	8	4
	Q4	inoculated	16	32	128	32	16	16	4	8	4	0
	R1	inoculated	32	64	128	64	32	32	16	16	8	4
C	1	inoculated	32	32	128	ND	32	16	ND	4	4	ND
	3	inoculated	8	32	128	ND	16	16	ND	4	0	ND
	14	inoculated	32	128	128	ND	64	16	ND	4	4	ND

[a] The values given are the reciprocals of the serum dilutions; 0 = <1 : 4; ND = not done. All animals were negative at the start of the experiment.

Tables giving the data for animals with a negative response and those for the responses to the equine antigen have been deposited in the WHO library, and copies may be obtained on request to the Chief Librarian, World Health Organization, 1211 Geneva, Switzerland.

However, the anti-S titres were barely measurable (1 : 4). None of the dogs developed antibodies to A/2946/57 virus. Antibodies were not detected in sera from contact dogs, in sera from control dogs, or in sera collected from dogs prior to the start of the experiment.

Serological survey of canine sera

A total of 382 serum samples from dogs living under natural conditions were tested by HAD–N and CF tests for the presence of specific antibodies to the A/Hong Kong/8/68 virus.

No antibodies were found in the 111 serum samples collected in 1968 prior to November. Antibodies were, however, detected in 3 of 71 serum samples collected between November 1968 and April 1969. Among the 200 serum samples collected during February and March 1970, 11 positive sera were found in the 164 samples from the USA and 2 in the 36 serum samples received from the United Kingdom (Table 3).

Of the 16 sera showing HAD–N antibodies to the A/Hong Kong/68 variant, 6 also possessed cross-reacting antibody to the equine virus (Table 4).

The titres of antibody to the Hong Kong variant varied from 1 : 8 to 1 : 256, and those of antibody to the equine strain from 1 : 4 to 1 : 64. All sera that had HAD–N antibodies to Hong Kong virus also had antibody to the same virus detectable by CF. However, anti-S titres could be measured in only 3 of these samples (dogs no. 387, no. 179, no. 182). No antibodies to the older A/2946/57 human influenza virus were detected by either test. There was no significant difference in frequency of

Table 3. Distribution of neutralizing antibodies to A/Hong Kong/68 influenzavirus in canine sera according to the presence or absence of human disease in the community

Serum samples		Human cases of Hong Kong influenza in the community	No. positive/ no. tested	Percentage positive
Date	Place			
Jan.–April 1968	Columbus, Ohio	none	0/36	0
Jan.–May 1968	Boston, Mass.	none	0/40	
Jan.–June 1968	New York, N.Y.	none	0/7	
May–Oct. 1968	Philadelphia, Pa.	very few	0/28	
Nov. 1968–Apr. 1969	Philadelphia, Pa.	severe epidemic	3/71	4.2
Feb.–March 1970	Philadelphia, Pa.	mild outbreak	9/122	6.7%
	Montgomery Co., Pa.	mild outbreak	0/22	
	Collingswood, N.J.	mild outbreak	2/20	
Feb.–April 1970	Cambridge, England	epidemic	0/9	5.5%
	London, England	epidemic	1/7	
	Glasgow, Scotland	epidemic	1/20	

occurrence of A/Hong Kong/68 antibody in dogs of different age groups.

DISCUSSION

The experimental inoculation of dogs with a Hong Kong variant of human influenzavirus by intranasal instillation and aerosol resulted in viral infection as demonstrated by the recovery of virus over a period of days, and by the appearance of specific antibodies. Examination of nasopharyngeal specimens taken just prior to the experimental infection did not reveal the presence of any virus that would render these conclusions unreliable and furthermore the reisolated virus was identified as being the same as that inoculated. The inoculation of dogs with Hong Kong virus ended in the establishment of an inapparent infection.

The data suggest that the amount of virus excreted from inoculated dogs was inadequate to establish infection in contacts and that residual virus in the contaminated room was at least partially responsible for infections of the contacts in experiment A. With the limited period of virus shedding it was not unexpected that the secondary contacts in experiment B failed to become infected.

In experiment C, virus reisolated from an infected dog was successfully employed experimentally to infect others. However, with this inoculum virus recovery was more difficult, i.e., reisolation could not be achieved on each postexposure day and in several instances was accomplished only at the second egg passage. Furthermore, no transmission of the infection was observed in the contact groups. This would suggest that, under the conditions of this experiment, mechanical transmission of the agent could not take place after a delay of 48 hours from artificial exposure. This is further supported by the fact that virus was shed by the carrier animals for 3–5 days following exposure and yet they were not apparently contagious during this period.

The factors that decide whether infections are subclinical or clinical are not fully understood. Nevertheless it has been observed with a number of respiratory viruses that stress enhances the risk of acquiring a clinical illness (Shope, 1943; McNamara et al., 1962; Pierce, 1963). Whether physically or pharmacologically induced stress could produce clinical disease in dogs infected with Hong Kong influenzavirus remains to be determined.

In general, virus recovery was accompanied by the development of HAD–N and CFV antibodies, which were still detectable at good levels 60 days after exposure. The anti-S responses were measurable but low. The lack of serological response in 3 of the

Table 4. Antibody titres to A2/Hong Kong/68 influenzavirus found in dogs infected in nature [a]

Place	Dog		Sampling date	Antibody titres against different antigens					
				HAd-N test		CF test			
	No.	Age years		Hong Kong	Equine-2	Hong Kong V	Equine-2 V	A2/2946/ 57 V	Type A Soluble
Philadelphia, Pa.	387	2¼	28 Jan. 1969	128	0	16	0	0	8
	372	6	10 Feb. 1969	128	16	ND	ND	ND	ND
	406	11	1 April 1969	256	64	32	16	0	0
Philadelphia, Pa.	7	5	9 Feb. 1970	8	0	4	0	0	0
	439	2	24 Feb. 1970	256	16	16	0	0	0
	67	7	24 Feb. 1970	8	0	8	0	0	0
	114	2	5 Mar. 1970	8	0	8	0	0	0
	116	7	5 Mar. 1970	16	0	4	0	0	0
	146	1½	6 Mar. 1970	8	0	4	0	0	0
	179	4 months	12 Mar. 1970	32	4	16	4	0	0
	182	8 months	12 Mar. 1970	32	0	16	0	0	0
	189	2¾	12 Mar. 1970	64	4	16	8	0	0
Collingswood, N.J.	154	4	10 Mar. 1970	16	0	8	0	0	0
	165	6	10 Mar. 1970	64	4	4	0	0	0
Glasgow, Scotland	14	9	7 April 1970	16	0	4	4	0	0
London, England	35	4	7 April 1970	16	0	AC	AC	AC	AC

[a] The values given are the reciprocals of the serum dilutions; 0 = <1 : 4; AC = anti-complementary; ND = not done because of insufficient material.

primary contact dogs in experiment B from which virus was recovered could have reflected insufficient replication of virus to provide adequate antigenic stimulation.

The results of the serological survey indicate that dogs also became infected in nature in the presence of human cases of influenca in the community. Approximately 6% (16 of 271) of the animals were found to have specific antibodies for Hong Kong virus when their sera were tested by at least two procedures. The titres of neutralizing and CF antibodies found in the dogs infected in nature were comparable in magnitude to the titres observed in experimental dogs 60 days after exposure. As a result of the present study it is suggested that dogs are worthy of consideration as an animal species that might participate in the epidemiology of human influenza, possibly as a reservoir of infection.

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RÉSUMÉ

ÉTUDES ÉPIDÉMIOLOGIQUES SUR L'INFECTION PAR LE VIRUS A/HONG KONG/68 CHEZ LE CHIEN

Des chiens âgés de 5 à 9 mois ont été exposés à l'infection par le virus A/Hong Kong/68 soit par instillations intranasales et inhalations d'aérosols soit par contact avec des animaux déjà infectés.

Tous les animaux inoculés directement ont contracté l'infection grippale prouvée par les isolements de virus pendant plusieurs jours et l'apparition d'anticorps spécifiques décelée par les épreuves de fixation du complément et d'hémadsorption-neutralisation. Tous les chiens mis en contact avec des animaux infectés 24 heures après l'inoculation du virus ont été contaminés lorsque l'exposition a eu lieu dans le local où avait été pratiquée l'inoculation primaire. Un seul chien sur 4 mis en contact avec un congénère infecté 24 heures après l'inoculation, mais dans un local non contaminé, a contracté l'infection. Aucune transmission n'a été observée lorsque le contact a été réalisé 48 heures après l'infection expérimentale.

D'autre part, 382 sérums canins recueillis en 1968/1970 en différents endroits des Etats-Unis d'Amérique et du Royaume-Uni ont été examinés: 111 d'entre eux, prélevés avant l'épidémie de grippe de Hong Kong de 1968, ne renfermaient pas d'anticorps spécifiques pour le virus A/Hong Kong/68, alors que 16 (5,9%) des 271 sérums prélevés après l'épidémie étaient positifs à l'égard de ce virus.

La démonstration d'une réceptivité naturelle et expérimentale au virus A/Hong Kong/68 chez le chien suggère un rôle possible de cet animal dans l'épidémiologie de la grippe de Hong Kong chez l'homme justifiant de plus amples recherches.

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