Influenza (A2/Hong Kong/68) in the Baboon (*Papio* sp.) (34214)

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The role of animal reservoirs in the maintenance and transmission of agents capable of causing disease in man is well known. Questions still remain unanswered with regard to animal reservoirs, transmission, and the specific involvement of many agents. For example, of all the viruses causing infections in man, perhaps most perplexing has been the animal–man relationship of the influenza group of viruses. Three major factors undoubtedly enter into any attempt to understand and interpret this relationship: (a) the extreme antigenic variability of influenza viruses; (b) the recognition of the existence of type A influenza viruses in swine, equines, and a variety of avian species; and (c) the antigenic sharing which has been demonstrated between certain strains of human origin with those recovered from lower animals (1–3). Of current interest is the marked antigenic sharing shown by the newest human variant, the Hong Kong (A2/Hong Kong/68) strains, and equine 2 viruses (4).

Although a number of myxoviruses have been found to produce infections in nonhuman primates either naturally or under experimental conditions (5–13), little is known regarding the possibility of zoonoses and more specifically anthroponoses involving influenza viruses. Suggestions of such a possibility have been found in studies conducted by the authors and by others (5, 14). The recent appearance in the United States of the Hong Kong (A2/Hong Kong/68) strain led to a study of transmissibility of this virus among nonhuman primates. This report concerns infection and cage to cage transmission of this virus in the Kenya baboon (*Papio* sp.).

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Materials and Methods. Animals. Six baboons—five infants and one adult—were separated into two groups of three test and three control animals. Two of the control baboons had been previously on corticosteroid[2] therapy, 3 mg/kg of body weight daily, as uninoculated controls in a Rous sarcoma virus tumor study, the third animal was a "normal" adult. All six animals were handled as a group and kept in adjoining cages. Specimens were collected from all animals as indicated in Tables Ia and b. All the infants had been born at the Southwest Foundation for Research and Education; the one adult animal originally came from Africa (Kenya), but had been maintained in captivity for several years.

Virus. A freshly isolated strain, A2/Phila. 101/68, which was recovered in chick embryo allantoic fluid, served as inoculum when it was only one egg passage from its human donor. This material, which had a HA titer of 1:256 per 0.4 ml, was maintained at —70° until it was dropped intranasally (approximately 0.5 ml/animal) into the three test baboons under light ether anesthesia. This isolate was antigenically similar to the new Hong Kong strains of influenza virus.

Serology. All sera were simultaneously tested in each procedure. Until time of testing they were stored at —20°. Prior to testing, they were inactivated at 56° for 30 min. Aliquots of sera to be assayed by the hemagglutination-inhibition (HI) test, were further treated with trypsin and periodate as well as by adsorption with chicken erythrocytes to minimize reactions due to nonspecific inhibitors.

Antigen for the HI and complement-fixation (CF) tests was made from infected

[2] Celestone, Schering Corporation.

TABLE Ia. Virus Recovery and Serologic Testing of Baboons Inoculated with a Hong Kong Strain of Influenza Virus.[a]

Animal no.	Day no.	HI	CF: Commercial antigen	CF: Hong Kong	Virus isolation
B-127	0	0	0	0	—
	1	×	×	×	—
	3	×	×	×	320[b]
	5	×	×	×	640
	7	0	0	0	—
	9	×	×	×	—
	11	×	×	×	—
	14	160[c]	40	40	×
	21	160	10	40	×
	49	40	10	10	×
B-128	0	0	0	0	—
	1	×	×	×	40
	3	×	×	×	—
	5	×	×	×	160
	7	0	0	0	—
	9	×	×	×	80
	11	×	×	×	—
	14	40	40	40	×
	21	40	10	40	×
	49	×	10	10	×
B-177	0	0	0	0	—
	1	×	×	×	—
	3	×	×	×	—
	5	×	×	×	40
	7	0	0	0	40
	9	×	×	×	—
	11	×	×	×	—
	14	40	>160	>160	×
	21	160	>160	>160	×
	49	80	40	40	×

[a] 0 = <10; — = negative; and × = not done.
[b] HA titer of allantoic fluid harvest.
[c] Reciprocal of dilution.

allantoic fluid. The CF antigen containing type A soluble antigen was also obtained through commercial sources. Procedures used both for standardizing the antigens and reference antisera as well as for the tests themselves have been described in detail elsewhere (15). Microtiter procedures were used for the CF test. Because of difficulties in reading the HA pattern in microplates, the macro procedure in serology tubes was used for the HI test.

One of us (F. S. L.) found that hemadsorption neutralization tests (HAD-N) with most myxoviruses can be performed in a continuous line of African green monkey (*Cercopithecus*) kidney cells, (BSC-1) employing as test viruses either tissue culture of egg-adapted strains (unpublished data). With the A2 viruses especially, it was preferable to use a strain nonsensitive to serum inhibitors rather than to treat the sera under examina-

TABLE Ib. Virus Recovery and Serologic Testing of Uninoculated Control Baboons.[a]

Animal no.	Day no.	HI	CF: Commercial antigen	CF: Hong Kong	Virus isolation
B-094[d]	0	0	0	—	—
	1	×	×	×	—
	3	×	×	×	—
	5	×	×	×	—
	7	0	0	—	—
	9	×	×	×	—
	11	×	×	×	160[b]
	14	0	0	—	×
	21	±20[c]	10	10	×
	49	80	40	40	×
B-123[d]	0	0	0	0	—
	1	×	×	×	—
	3	×	×	×	—
	5	×	×	×	80
	7	0	0	0	—
	9	×	×	×	—
	11	×	×	×	—
	14	0	0	0	×
	21	80	10	40	×
	49	40	10	40	×
1106[e]	0	0	±10	0	—
	1	×	×	×	160
	3	×	×	×	—
	5	×	×	×	—
	7	0	0	0	—
	9	×	×	×	—
	11	×	×	×	—
	14	0	10	0	×
	21	80	>160	>160	×
	49	20	40	>160	×

[a] 0 = <10; — = negative; and × = not done.
[b] HA titer of allantoic fluid harvest.
[c] Reciprocal of dilution.
[d] Cortisone treated.
[e] Adult.

tion for removal of inhibitors. Hence the HAD-N tests were carried out in BSC-1 cells with a nonsensitive variant of A2/Phila. 101/68 which was selected out from the parent isolate according to methods described previously (16). Monolayers were propagated in stationary tubes in Eagle's minimum essential medium prepared in Hanks' balanced salt solution (BSS), containing 12% fetal calf serum. Before inoculation with the test materials they were washed free of growth medium and refed with medium 199. Neutral mixtures were made with equal volumes of serial twofold dilutions of serum and eight hemadsorbing units of virus. Subsequent to overnight storage at 40° 0.2-ml amounts of each dilution were inoculated into duplicate tubes and allowed to incubate at 36° for 3 days. Preparation of the tubes for visualization of hemadsorption was made in the usual manner by the addition of 0.5% guinea pig red cells. The initial dilution of serum which completely inhibited the appearance of hemadsorption was taken as the neutralizing antibody titer. Control titrations of virus were carried out with each test.

Virus isolation. At the designated times (Table Ia and b) each animals was sedated with Sernylan (Parke, Davis & Co.) and irrigated through the nares with approximately 5 ml of BSS. Using a syringe to which a short rubber catheter had been attached, this fluid was collected as it passed over the nasopharyngeal area. Approximately 4.0 ml of washing was collected per animal. A separate syringe and tube were used for each irrigation.

The washings were placed in tubes and were immediately quick frozen in an alcohol–CO_2 bath and then stored at —70° until tested. For testing, each washing was inoculated at a dilution of 1/10 in BSS; 0.1 ml was introduced amniotically and 0.2 ml allantoically. The embryonic fluids were harvested after approximately 40-hr incubation at 37° and tested for the presence of hemagglutinins. Strain identification was made with known reference serum in an HI test. No specimen was considered negative unless it had undergone two blind passages.

Rectal swabs were collected in BSS at the same time as the throat washings. Samples of both were inoculated into baboon (*Papio*) kidney cell cultures (BKC).

TABLE II. Hemadsorption-Neutralization (HAD-N) Test Results on Sera from Baboons Infected with A2/Hong Kong Strain of Influenza Virus.

Animal no.	Day no.	HAD-N titers		
		A2/Hong Kong/68	A2/Phila. /57	A/Equine 2
Inoculated				
B-127	0	0	0	0
	7	0	0	0
	14	64	0	0
	21	64	0	0
	49	128	0	±8
B-128	0	0	0	0
	7	0	0	0
	14	32	0	0
	21	32	0	0
	49	128	0	0
B-177	0	0	0	0
	7	0	0	0
	14	128	0	±8
	21	64	0	±8
	49	128	0	8
Controls				
B-094	0	0	0	0
	7	0	0	0
	14	0	0	0
	21	16	0	0
	49	16	0	0
B-123	0	0	0	0
	7	0	0	0
	14	0	0	0
	21	32	0	0
	49	16	0	0
1106	0	0	0	0
	7	0	0	0
	14	0	0	0
	21	32	0	0
	49	32	0	0

Results. Antibody responses and virus recovery. Listed in Table Ia and b are the serologic results obtained in the HI and CF tests along with virus recovery. Table II presents the HAD-N test results.

Inoculated animals. Antibody rises to the inoculated strain were observed in all three inoculated animals by all test procedures in approximately 2 weeks. The HI and HAD-N

findings ran remarkably in parallel. Since the allantoic fluid CF antigen also contained S antigen, it is not surprising that the two CF antigens gave comparable although not identical results.

Recovery of virus was accomplished twice in two of the inoculated animals and three times in the third baboon. This latter animal also differed from the other two in that virus was detected 9 days following inoculation. Virus isolation was made on first passage in all instances.

Control animals. The findings in the three control animals were similar to the test animals with these slight differences: (a) seropositives were not detected until the third week of the test, and (b) only one virus recovery was made from each animal. Also of interest was the fact that virus was not isolated from one young baboon (B094) until approximately 10 days after seeding the test animals. However, in the adult, virus was recovered only from the 24-hr sample. Viral isolates were identified as A2/Hong Kong/68 using human acute and convalescent sera.

In confirmation of earlier demonstrations of the antigenic relationship existing between the human Hong Kong and the equine 2 viruses, it was found by HAD-N that antibody to the latter also became detectable in two of the inoculated baboons. No antibody rises to a prototype A2 strain (A2/Phila./57) were demonstrable.

Throat washings inoculated into BKC were all negative. An enterovirus was isolated from one animal (B123) on days 7, 9, and 11. No other isolates were obtained. Tests for SV5 and SV41 HI antibodies showed a titer of 1:40 for SV5 and $<1:10$ for SV41 throughout the experiment.

Clinical findings. None of the animals exhibited any marked degree of clinical illness. All of the animals except B123 and B127 ran an intermittant low-grade fever from day 3 past inoculation to day 6. The temperature being in the normal range in the mornings and elevated 2–3°F in the late afternoon. In addition, animals 1106 and B127 exhibited partial anorexia during this same time period. There were no symptoms suggestive of upper respiratory disease.

Discussion. The data obtained in this study indicate that infection of a nonhuman primate with an influenza virus (A2/Phila. 101/68) as well as transmission to "cagemates" can be accomplished. The significance of these findings, however, is still open to interpretation. Although never demonstrated previously, the data may be interpreted as indicating that a nonhuman primate may act as either a vector or even possibly a host for human influenza viruses. The unexpected finding that virus excretion persisted for as long as 9 days (B128) needs confirmation because of its importance in the increased possibility for influenza transmission from animal to animal and possibly to man. Other myxoviruses have been associated either naturally or experimentally with simian hosts. We have previously demonstrated serologic evidence of infection of most primates with myxoviruses (5). Other investigators have recovered a number of the myxoviruses-measles (17), respiratory syncytial virus (18), and human and simian parainfluenza viruses (7, 9, 11, 19) from "naturally" infected nonhuman primates. Experimental infection of monkeys with mumps virus has been well established and indeed the first mumps vaccine was derived from infected monkey tissue (20).

Inoculation of BKC with throat and rectal swab specimens and serum testing helped to rule out the possibility that a virus other than the inoculated influenza virus was responsible for the observed results. It is also important to emphasize that it is difficult to use "clinical symptoms" as an indication of illness. While overt evidence of illness at times may be manifested, *e.g.*, diarrhea, more often animals will not demonstrate any evidence of illness (even to the trained clinician) until moribund.

The findings reported here are in keeping with those observed for the other myxoviruses as well as diverse viruses. It would appear that the more important question to be raised concerns lack of experimental data for influenza virus infection of simians. Is this the result of failure to use freshly isolated strains or are the results reported here unique to the new Hong Kong variant of

Type A? Further studies are necessary in order to fully answer this question.

Summary. A freshly isolated strain of human influenza virus (A2/Phila. 101/68), shown to be similar to the A2/Hong Kong/68 variants, produced infection in Kenya baboons (*Papio* sp.) accompanied by seroconversion and shedding of virus over a period of days. Uninoculated control animals in neighboring cages developed a similar pattern of seroconversion and virus shedding.

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