

Possible Waterborne Transmission and Maintenance of Influenza Viruses in Domestic Ducks

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Two duck farms in Hong Kong were examined monthly for 1 year for the occurrence and persistence of influenza viruses within the duck communities. The predominant virus in one community was H3N2, a virus antigenically related to the pandemic Hong Kong strain. This virus was isolated monthly throughout the year from feces or pond water or both, indicating a cycle of waterborne transmission. Viruses of the same antigenic combination were isolated 1 and 2 years after the last sampling occasion, implying persistence in the community. Infection was asymptomatic. Maintenance of virus appeared to be dependent upon the continual introduction of ducklings susceptible to infection onto virus-contaminated water; the feces of ducks 70 to 80 days old were generally free of detectable virus despite the exposure of the ducks to virus in pond water. In the second community, in which ducklings were not introduced after the initial sampling, the prevailing viruses, H7N1 and H7N2, also present asymptotically, ceased to be detected once the ducks were 70 to 80 days old. The normal practice of raising ducks of different ages on the same farm, wherein the water supplies are shared, as typified by the first community, appears to be instrumental in maintaining a large reservoir of influenza viruses in the duck population of southern China.

The occurrence of influenza viruses in a variety of avian species is now well established, and in many cases, infection is asymptomatic. More influenza viruses have been isolated from wild and domestic ducks than from any other species (7, 13, 17, 18). Five years of surveillance of domestic poultry in Hong Kong has revealed that the duck population of the region provides a reservoir for a great diversity of influenza viruses, the majority of which were isolated from the cloaca. In all cases, the poultry appeared to be unaffected by the virus.

Influenza viruses have been shown to replicate in the intestinal tracts of experimentally infected domestic and feral ducks (24). These viruses, shed in high concentrations in the feces, were able to survive in river water for several days. Furthermore, influenza viruses have been isolated from unconcentrated lake water and from feces samples on the shores of Canadian lakes where wild ducks had congregated before winter migration (6). It would appear, then, that influenza viruses are a waterborne, intestinal infection of ducks.

The similarity of the antigenic and biochemical characteristics of avian and human influenza viruses has led to the hypothesis that human pandemic influenza has its origins in a nonhuman source (23). Studies fostered mainly by the World Health Organization (8) have led to the

belief that the ecology of the influenza viruses of lower animals and birds could prove useful in the anticipation of new human pandemic viruses. Southern China, including Hong Kong, has been implicated as the point of origin of the last two pandemics (H2N2 and H3N2), and it is more than coincidental that this area supports intensive duck farming in which the birds are raised in crowded conditions in close proximity to humans. Thus, this study provided an opportunity to examine the ecology of avian influenza viruses on typical farms in the region, with the possibility that the observations might have a bearing on the origins of human pandemic viruses.

MATERIALS AND METHODS

Duck farms. Two farms in the New Territories, Hong Kong, were studied. The ducks on farm 1 were purchased monthly as 1-day-old ducklings from a local hatchery which imported embryonated eggs from Taiwan, whereas farm 2 had its own, not very prolific, breeding stock. Pond water and feces samples were collected midmonthly on both farms. The ducks on these farms appeared healthy and were not vaccinated against avian paramyxovirus type 1 (PMV-1; Newcastle disease virus). One-day-old ducklings hatched in the laboratory from randomly purchased embryonated eggs were free of detectable influenza and avian paramyxoviruses.

The conditions on farm 1 were as follows. Samples

were collected monthly from July 1978 to June 1979, except for August 1978, when adverse climatic conditions prevented collection. Follow-up samplings were carried out in June 1980 and May 1981. Samples were collected from three categories of ducks, which were differentiated on the following approximate age basis: ducklings, <30 days old; growers, 30 to 70 days old; and finishers, 70 to 80-day-old sexually immature ducks of adult size held for approximately 10 days in preparation for market. There were approximately 1,000 ducks of each category throughout the study. The ducks of each category were housed in a separate compound containing a single pond. Three main compounds were used. Occasionally, alternative compounds were used during cleansing operations or to accommodate additional ducks. Well water was pumped into the pond of the growers, and the overflow, which was gravity-fed in series to the ponds of the finishers and ducklings, was then discharged into a nearby stream.

The conditions on farm 2 were as follows. The first samples were taken in June 1978 and then concurrently with farm 1 on seven other occasions, the last one being May 1979. Breeder ducks (sexually mature adults >10 months old) were sampled on all occasions. Because of unsuccessful breeding, growers and finishers were each sampled on two occasions only. There were approximately 800 ducks on the farm in June, but their numbers were reduced to about 100 in August because of adverse climatic conditions.

Collection of samples. Individual fecal specimens were collected during the latter part of the morning from the edge of the pond and the ground in the vicinity of congregated ducks as described (16). A 500-ml volume of water was collected from either the pond effluent or the surface for each duck category by the grab sample technique (1), transported on ice, and held at 4°C until it was processed the next morning.

Concentration of water samples. The protocol was essentially that described by Heyward et al. (4) with polyethylene glycol (PEG-6000; Sigma Chemical Co., St. Louis, Mo.) for the concentration of virus from infected allantoic fluid. A 400-ml volume of unclarified pond water reduced to 10 ml of supernatant and 2 ml of coarse pellet. Transport medium was used in lieu of buffer to suspend pellets after each centrifugation step. The final pellets and supernatant were assayed immediately.

Virus isolation. Volumes (0.2- to 0.3-ml) were inoculated into the allantoic cavities of 10- or 11-day-old chicken embryos. One embryo was used per feces sample. For each pond water sample, the total volume of the suspended pellet was used and inoculated in equal volumes into an average of six embryos, whereas four embryos were used for the supernatant. In

preliminary studies, virus was detected in the pellet rather than in the supernatant, implying solids association of virus (12). Thus, both phases of the concentrate were assayed throughout the study, the benefits of which are clearly illustrated for farm 1 pond water (Table 1).

Antigenic analysis of isolates. Hemagglutinating agents isolated in embryonated eggs were subtyped as influenza viruses in hemagglutination and neuraminidase inhibition tests with antisera specific for the isolated hemagglutinin (H) and neuraminidase (N) subunits of reference strains of influenza A viruses (22). Subtype designations are in accordance with the revised system of nomenclature for influenza viruses (25). Avian paramyxoviruses were similarly identified by using antisera to Newcastle disease virus (NDV; PMV-1) and prototype viruses, duck/Hong Kong/D3/75 (PMV-4) and duck/Hong Kong/199/77 (PMV-6) (14, 16).

Physicochemical tests. The physicochemical tests were carried out at the time of sample collection. The pH values of the water samples were determined in July only, with a Beckman electromagnate pH meter (Beckman Instruments, Inc., Palo Alto, Calif.). Feces pH was determined in March only, with pH indicator strips (Micro Essential Laboratory, Brooklyn, N.Y.). Temperature was measured during the 12 months for each water sample.

Bacteriology. Standard plate counts determined at 40°C to simulate duck body temperature were carried out on each water sample (1).

RESULTS

The results for farm 1 were as follows.

Isolates. Eighty-eight viruses comprising 84 (96%) influenza A viruses and 4 avian paramyxoviruses were isolated from 701 fecal samples derived from the three categories of ducks (Table 2). The overall isolation rate of 13% was due to the preponderance of influenza viruses (82/84) obtained from ducklings and growers whose influenza isolation rates were 14 and 17%, respectively. Of the pond water samples, 23 of 35 (55%) contained viruses, with influenza viruses isolated on all such occasions and paramyxoviruses isolated jointly on three. There were no pronounced differences in isolation frequencies for the pond water of ducklings (58%), growers (75%), and finishers (64%).

Antigenic analysis of virus isolates. The 151 influenza viruses isolated from feces and pond water occurred as six different antigenic combi-

TABLE 1. Distribution of viruses in pellet and supernatant after concentration of pond water with polyethylene glycol on farm 1

Water concentrate	No. of portions examined	Isolations		
		Influenza A	Paramyxoviruses	Overall
Pellet	189	64 ^a (34%)	5 (3%)	69 (37%)
Supernatant	139	3 (2%)	1 (1%)	4 (3%)

^a Data derived from the sum of isolations over 12 months for ducklings, growers, and finishers for each phase of the water concentrate.

TABLE 2. Virus isolations from duck feces and pond water during 12 months of surveillance on farm 1

Type of sample	Category of duck	No. of samples examined	No. of isolations		
			Influenza A	Paramyxoviruses	Overall
Feces	Duckling	261	37 (14%)	3 (1%)	40 (15%)
	Grower	258	45 (17%)	1 (<1%)	46 (18%)
	Finisher	182	2 (1%)	0	2 (1%)
		701	84 (12%)	4 (<1%)	88 (13%)
Pond water ^a	Duckling	12	7 (58%)	1 (8%) ^b	7 (58%)
	Grower	12	9 (75%)	1 (8%) ^b	9 (75%)
	Finisher	11	7 (64%)	1 (9%) ^b	7 (64%)
		35	23 (66%)	3 (9%)	23 (66%)

^a Water samples were concentrated, and the suspended pellets and supernatants were assayed for virus. A sample was considered positive if it yielded one or more isolates in the concentrates.

^b Paramyxovirus jointly isolated with influenza viruses from same water sample.

nations. Two hemagglutinin subtypes, H3 and H9, were recognized, the former comprising 92% of the fecal isolates and 90% of those from pond water (Table 3). The neuraminidase subtypes detected were N2, N3, and N6. The H3N2 antigenic combination was the predominant isolate from feces (90%) and pond water (88%). One fecal specimen yielded H3 in combination with N2, N3, and N6. The H9 subtype occurred in combination with N2 and N3 subtypes.

Ten avian paramyxoviruses, comprising seven PMV-1 and three PMV-6 strains, were found in feces and pond water (Table 3). Two of the PMV-6 viruses were jointly isolated with influenza H3N2 from the pond water of finisher ducks.

Occurrence of isolates. The predominant virus, influenza H3N2 (Table 3), was isolated on every sampling occasion (Table 4). Other viruses were isolated infrequently. Multiple influenza virus isolations were made in September 1978 (H3N2, H3N3, H3N6, H9N2) and from January to April 1979 (H3N2, H3N3, H9N2, H9N3). Avian paramyxoviruses were isolated on two sampling occasions only. Subsequent sampling of duckling

and grower feces in June 1980 and May 1981 confirmed the continued presence of H3N2 virus. The rate of isolation, expressed as the percentage of virus recovery from feces and water, was analyzed on a monthly basis for each duck category (Fig. 1). Ducklings and growers showed similar isolation patterns in that there was evidence of a trend of alternate high and low cycles of virus incidence irrespective of the season. However, this pattern was not observed with finishers whose feces were devoid of detectable virus apart from two possibly spurious isolations (Table 2). The frequencies for autumn and winter (November through February; dry, mild to cool) and spring and summer (March through October; humid, warm to hot) were 25 and 21% for water and 17 and 10% for feces, respectively.

Physicochemical measurements. The pond water of the three categories of duck showed little variation in pH: ducklings, 6.6; growers, 6.2; and finishers 6.2. Fecal pH ranged from 5.5 to 7.4, with means of 6.6, 6.0, and 6.3 for ducklings, growers, and finishers, respectively.

Pond water temperatures ranged from 12.0°C

TABLE 3. Identification of surface antigens on viruses isolated from feces and pond water on farm 1

Antigenicity of isolates	Feces				Pond water				Overall
	Ducklings	Growers	Finishers	Total	Ducklings	Growers	Finishers	Total	
H3N2	36	41	2	79 (90%)	15	25	24	64 (88%)	143 (89%)
H3N6		1 ^a		1					1
H3N3		1 ^a		1	1	1		2	3
H9N2		2		2			1	1	3
H9N3	1			1					1
PMV-1 ^b	3			3	3	1		4	7
PMV-6 ^c		1		1			2	2	3
Overall	40 (46%)	46 (52%)	2 (2%)	88 (100%)	19 (26%)	27 (37%)	27 (37%)	73 (100%)	161 (100%)

^a Jointly isolated with H3N2.

^b PMV-1 (avian paramyxovirus 1; Newcastle disease virus).

^c PMV-6 (avian paramyxovirus 6; duck/Hong Kong/199/79-like).

TABLE 4. Viruses isolated during 12 months of surveillance and on subsequent samplings on farm 1

Sampling date	Virus(es) isolated
July 1978	H3N2
August 1978 ^a	
September 1978	H3N2, H3N3, H3N6, H9N2
October 1978	H3N2
November 1978	H3N2
December 1978	H3N2
January 1979	H3N2, H9N3
February 1979	H3N2, H3N3, H9N2
March 1979	H3N2, H3N3
April 1979	H3N2, H3N3, PMV-1
May 1979	H3N2
June 1979	H3N2, PMV-1, PMV-6
June 1980	H3N2
May 1981	H3N2

^a Adverse climatic conditions prevented sampling in August.

in January to 31.5°C in July, with means of 22.1, 22.8, and 22.5°C for ducklings, growers, and finishers, respectively.

Bacteriology. Throughout the study, there was little variation in pond water bacterial counts, which ranged from 2×10^5 to 1.4×10^7 /ml, with means of 3×10^6 , 2.7×10^6 , and 3.1×10^6 /ml for ducklings, growers, and finishers, respectively.

The results for farm 2 were as follows.

Isolates. The only virus isolations were made from the feces and pond water of grower ducks in the June 1978 sampling. These isolates comprised H7N1 and H7N2 antigenic combinations, with the latter predominating (Table 5). In the subsequent July sampling, the grower ducks were of finisher age. No isolations were made from the feces of finisher or breeder ducks on any sampling occasion, the last one being May 1979, nor was virus detected in the pond water of breeders or finishers.

TABLE 5. Virus isolations from duck feces and pond water during 12 months of surveillance on farm 2

Type of sample ^a	Category of duck	No. of samples examined	Viruses isolated (no. of isolations)
Feces	Grower	40	H7N1 (2) ^b H7N2 (13)
	Finisher	26	— ^c
	Breeder	155	—
Pond water	Grower	2	H7N1 (1), ^b H7N2 (2)
	Finisher	7	—
	Breeder	7	—

^a Samples taken on seven occasions.

^b H7N1 virus jointly isolated with H7N2.

^c Virus not isolated.

DISCUSSION

These studies are the first to describe the ecology of influenza viruses under conditions of duck husbandry as practiced in southern China. The customary procedure is to introduce regularly young ducklings onto the ponds to provide a continuous supply of finisher ducks (70 to 80 days old) for the market. In the case of farm 1, introduction was on a monthly basis. Farm 2 was unusual in that ducklings (<30 days old) were not introduced after the first sampling occasion, and farm 2 represented a control with which to compare farm 1.

It has been established that avian influenza viruses replicate in both the respiratory and intestinal tracts of ducks (24) and it has been suggested that a significant mode of infection in these birds is by the waterborne route (6). In terms of farm layout, the results from farm 1 reveal that grower ducks (30 to 70 days old) were the main reservoir of influenza viruses which, by recurrent fecal contamination of pond water, were transmitted via interconnected ponds to other duck groups. Although viruses were isolated from the pond water of all three categories of duck, significant numbers were not detected in the feces of finisher ducks, implying that age was a factor in the ability of ducks to support virus replication. The findings on farm 2 were in accord with this view. It is not known whether early exposure to virus results in an intestinal immune response which prevents the establishment of subsequent infection.

The only other intensive study of similar birds has been on wild ducks assembled on Canadian lakes before migration, in which a virus isolation rate as high as 60% was recorded in ducks up to 1 year old (7). This is considerably higher than the rate observed in domestic ducks in Hong Kong both in the present study and in surveillance at a local dressing plant (13, 17, 18). However, the domestic ducks of southern China essentially represent a static population, in which virus is maintained all year round due to farming practices. In contrast, migratory ducks represent a transient population of susceptibles, in which introduced virus is amplified by close contact and rapid dissemination on shared water, especially after the breeding period. Once migration begins, virus is not so readily detectable in the birds at locations far removed from their assembly areas (7).

On both farms, infection was asymptomatic, and this is of interest in the case of farm 2, in which the hemagglutinin of the prevailing viruses (H7N1 and H7N2) was the same as that of the classical Fowl plague virus (H7N7). In so far as human influenza is concerned, the results from farm 1 have demonstrated the continuity of infection over 3 years with a virus antigenically

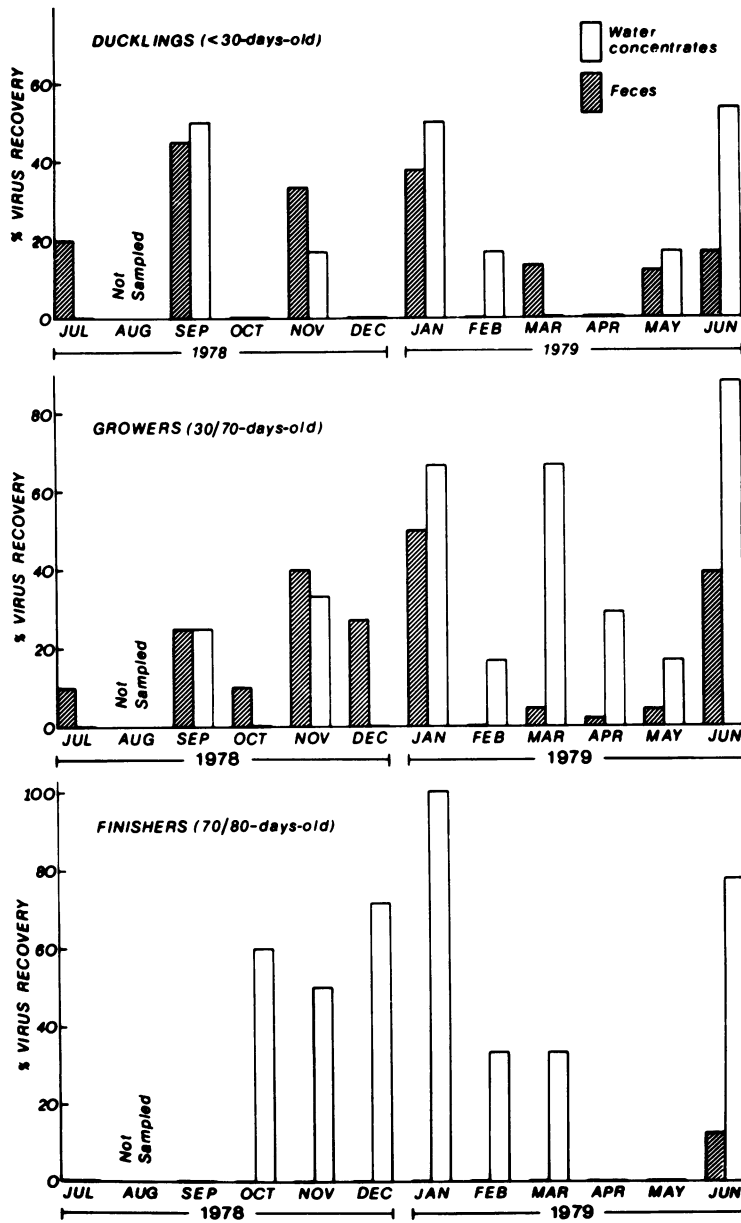


FIG. 1. Monthly frequency of virus isolation from feces and pond water concentrates of duckling, grower, and finisher ducks on farm 1. The percent virus recovery was determined as the (sum of the frequency of isolation of each antigenic combination per number of inoculations) \times 100.

related to a human influenza virus (H3N2), and this may be of relevance since the initial isolation of pandemic Hong Kong influenza was made in the vicinity in 1968. It should, however, be noted that these antigenic designations are in accordance with the revised system of nomenclature for influenza viruses and that the H3N2 virus would previously have been designated

Hav7N2 (25). It was the recognition of the antigenic and biochemical similarities of the Hav7 and H3 hemagglutinins (21) that led to the hypothesis that pandemic human influenza might be derived from a nonhuman source (10, 23).

Infrequently detected antigenic combinations may have arisen from recombination between the predominant H3N2 virus and an introduced

virus, exemplified by the occurrence in January 1979 of H3N2 and H9N3 viruses and the subsequent isolation of H3N3. The H9N3 combination has not been isolated previously from poultry in the region (13, 17, 18), lending support to introduction from a nonpoultry source. Multiple isolations from a single sample are not unprecedented (17); in this study, three isolations (H3N2, H3N3, and H3N6) were obtained from a single fecal sample, supporting the view that recombination can occur in duck intestines (5).

The isolation of different antigenic combinations in September 1978 and January to April 1979 coincided, respectively, with the presence of southbound migratory birds and birds from the immediate north overwintering in the area, thus suggesting a role for transient birds in the introduction and dissemination of viruses. The spread of PMV-1 has been attributed to wild birds (9), but whether the PMV-1 and -6 strains were introduced from such a source is unclear since PMV-1 isolates of high virulence have frequently been detected in apparently healthy ducks from southern China (15), indicating that the virus may be endemic in the region. The thermolability of one such isolate in particular (unpublished data) suggests that the virus may have been a vaccine strain (3) possibly introduced from a neighboring farm.

The apparent inability of viruses other than H3N2 to become established could be in part because of their inherent physical instability, which may be inferred from statistical analyses of the antigenic combinations of isolates obtained in long-term surveillance studies at a local poultry dressing plant (2; K. F. Shortridge, unpublished data). On the other hand, age may also be a contributing factor in that older domestic ducks such as those of finisher age or adult migratory ducks (7) appear to harbor fewer viruses. Thus, the occurrence and range of antigenic combinations recorded in Hong Kong surveillance studies (13, 17, 18) may only represent a portion of those present in the duck population of the region.

In water, factors affecting virus survival are complex. Whereas dilution effects and the presence of chemical and biological antiviral agents are known to affect adversely virus survival (19, 20), solids association of virus can be beneficial (11, 12). This study has demonstrated the survival in pond water of certain fecally shed influenza viruses. That these viruses could be solids associated and remain infectious can be inferred from the detection of 95% of the isolates in the pellets of concentrated pond water samples (Table 1). There was no convincing evidence that pond water temperature, bacterial content, or the pH of duck feces had any significant effect on the occurrence of virus.

Possible modes of virus transmission on duck farms may include the following: (i) respiratory; (ii) intestinal (fecal-oral due to coprophagous habits, fecal-water-oral from ingestion of water, and cloacal uptake of water); (iii) reproductive, i.e., during copulation or egg development in the magnum. The fecal-water-oral route is probably significant in view of the greater number of virus isolations from the cloaca than from the trachea of domestic ducks (13, 17, 18) and the fact that, in southern China, ducks are raised in large numbers on small ponds with generally limited surroundings. However, in view of the experimental demonstration that the establishment of intestinal infection of ducks with avian influenza viruses may be achieved by introduction of virus via the cloaca (24), it is possible that virus-infected water may gain entry to ducks by this route. The fact that both intestinal and reproductive tracts open into a common area, the cloaca, means that swabs taken from this site cannot identify the source of virus, and hence, the possibility of infection of the reproductive tract cannot be excluded.

These results imply that a cycle of waterborne transmission and maintenance of influenza viruses exists within the duck communities of southern China, and it is conceivable that virus transmission could occur in this manner to other susceptible animals, including humans. Control of influenza viruses in nature may be a way of preventing the emergence of human pandemic influenza, and this might be achieved by alterations in duck husbandry or alternatively, by virus inactivation or removal processes.

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