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Avian influenza A viruses of southern China and Hong Kong: ecological aspects and implications for man*

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Continuous surveillance of the influenza viruses isolated from domestic poultry from southern China and Hong Kong over more than 4 years resulted in the isolation of influenza viruses possessing 46 different combinations of haemagglutinin (H) and neuraminidase (N) subtypes. Of these, 43 were obtained from ducks from China. In all cases, infection appeared to be asymptomatic. The antigenic combination found most commonly in the viruses isolated was H4N6, which accounted for approximately one-quarter of the duck isolates, its occurrence being more frequent than expected from a statistical analysis of the observed frequencies of the haemagglutinin and neuraminidase genes among all the isolates. Some combinations of H and N occurred less frequently than expected or not at all. Influenza viruses tended to be isolated more frequently from ducks during the summer months in comparison with paramyxoviruses, which were more commonly encountered in the winter. Possible reasons for the great antigenic diversity of influenza A viruses in the poultry, especially the ducks, in the region are discussed together with the potential significance of these viruses to the emergence of human influenza pandemics.

An appreciation of the ecology of influenza A viruses (*1*) may be pertinent to understanding the emergence of pandemic influenza. It may also draw attention to particular avian and animal influenza A viruses that might be of potential importance as progenitors of pandemic influenza in man (*2*, *3*).

Results of three years' surveillance of domestic poultry from southern China and Hong Kong (*4* – *6*) showed that more influenza viruses were isolated from ducks, in this case domestic ducks, than from any other avian or animal species (*7*). This article reports the results of the fourth year of surveillance and examines aspects of influenza ecology, particularly the importance of the duck isolates obtained over the four years.

MATERIALS AND METHODS

Sampling of domestic poultry was conducted on a weekly or fortnightly basis from November 1978 to October 1979. The origin of the poultry, the protocol for sample collection, and details of the virus isolation and identification procedures have been reported previously (*4*, *8*, *9*). In the studies reported here, these procedures differed in two respects: (*a*) sisomicin (Schering) was occasionally used instead of gentamicin in the transport medium, and (*b*) swabs were held at – 70 °C instead of 4 °C before assay in embryonated eggs.

Haemagglutination-inhibition (HI) tests were carried out on duck, goose, chicken, and human sera treated with receptor-destroying enzyme (RDE) (*10*). The viruses used were chosen for their avidity with reference antisera and were local isolates, with the exception of the North American H8N4 and H12N5 viruses (*11*, *12*) obtained from Dr V. S. Hinshaw, St Jude Children's Research Hospital, Memphis, USA.

The frequency of occurrence of a given antigen subtype (haemagglutinin or neuraminidase) was calculated as the number of positive isolates divided by the number of swabs taken from any given group of samples. Tests for non-randomness of association of haemagglutinin and neuraminidase subtypes were reported in an earlier study (*13*).

RESULTS

Isolates

The number of isolations made in the fourth year has been combined in Table 1 with the numbers of iso-

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Table 1. Overall frequency of isolation of viruses from domestic poultry at a Hong Kong dressing plant during a four-year period of continuous surveillance, November 1975–October 1979[a]

			Influenza viruses		Paramyxoviruses[b]	
Type of poultry	Source of samples	No. of samples	No. of isolates	%	No. of isolates	%
Duck	Trachea	3776	183	4.8	83	2.2
	Cloaca	3297	275	8.3	152	4.6
	Total	7073	458	6.5	235	3.2
Goose	Trachea	654	6	0.9	4	0.6
	Cloaca	627	9	1.4	24	3.8
	Total	1281	15	1.2	28	2.2
Chicken	Trachea	662	3	0.5	22	3.3
	Cloaca	987	4	0.4	32	3.2
	Total	1649	7	0.4	54	3.3

[a] Data derived from November 1978 to October 1979 and references *4 – 6*.

[b] Avian paramyxoviruses: PMV-1 (Newcastle disease virus-like), PMV-4 (duck/Hong Kong/D3/75-like), PMV-6 (duck/Hong Kong/199/77-like), see references *8* and *9*.

lations made in the previous three years. The overall frequencies of isolation of influenza from ducks, geese, and chickens were in the proportions of 16:3:1, respectively, underlining the importance of ducks as a reservoir of these viruses. Influenza viruses were isolated more frequently than paramyxoviruses from ducks, but less frequently from geese and chickens. Overall, more isolates were obtained from cloacal than tracheal swabs. The frequency of isolation of influenza viruses from poultry originating from China was greater than that from Hong Kong poultry, this difference being most evident in ducks (Table 2).

Table 2. Origin of influenza A viruses isolated from domestic poultry at a Hong Kong dressing plant during four years' continuous surveillance, November 1975–October 1979

			Influenza virus isolations	
Type of poultry	Origin	No. of samples	No.	%
Duck	China	3236	361	11.2
	Hong Kong	3837	97	2.5
Goose	China	722	12	1.7
	Hong Kong	559	3	0.5
Chicken	China	170	1	0.6
	Hong Kong	1479	6	0.4

Antigenic combinations

Seven isolates with combinations of surface antigens (haemagglutinin and neuraminidase) not previously detected in Hong Kong were recognized in the fourth year of the study—H1N3, H4N1, H6N3, H10N3, H10N4, H10N8, and H10N9. A total of 43 combinations was recognized over the 4 years (Table 3) of which 21 were seen in the first, 10 in the second, and 5 in the third years (*4 – 6*). The slight increase in the number of newly detected antigenic combinations in the fourth year, following the gradual decline in numbers over the previous three years, is probably due to the larger number of samples taken in the fourth year, in particular the 3073 samples taken from ducks compared with the total of 4000 duck samples taken over the previous three years.

All the combinations found in the goose and chicken isolates, apart from H3N9, were also recorded in duck isolates. The most frequently encountered combinations in duck isolates were H4N6, which accounted for approximately one-quarter of the total, and H3N8.

Haemagglutinin and neuraminidase subtypes

The antigenic combinations detected over the four years comprised all previously described neuraminidases and haemagglutinins with the exception of H8

Table 3. Summary of influenza A viruses isolated from domestic poultry during four years' surveillance at a Hong Kong poultry dressing plant, November 1975–October 1979

Antigenic combinations[a]	No. of isolates	Antigenic combinations[a]	No. of isolates
Duck:[b]		Duck:[b] (continued):	
H1N1	1	H10N1	2
H1N2	2	H10N2	4
H1N3	1	H10N3	12
H2N2	5	H10N4	4
H2N3	1	H10N5	25
H2N9	1	H10N8	2
H3N2	22	H10N9	4
H3N3	3	H11N2	3
H3N4	9	H11N3	2
H3N6	26	H11N9	8
H3N8	50		458
H4N1	1	*Goose:*	
H4N2	20	H1N1	1
H4N3	1	H3N2	1
H4N4	4	H3N8	1
H4N5	3	H4N2	1
H4N6	111	H4N5	1
H4N7	1	H4N6	2
H4N8	9	H5N3	1
H5N2	1	H6N1	2
H5N3	16	H6N2	3
H6N1	29	H6N4	1
H6N2	23	H6N9	1
H6N3	5		15
H6N4	4	*Chicken:*	
H6N5	3	H1N1	1
H6N6	1	H3N2	3
H6N8	18	H3N6	1
H6N9	3	H3N9	1
H7N2	1	H6N4	1
H9N2	16		7
H9N6	1		

[a] Subtype designations are in accordance with the revised system of nomenclature for influenza viruses (*14*). The 43 antigenic combinations listed above would have numbered 46 in the previous system.

[b] H7N1 and H7N2 viruses have been isolated from both duck faeces and pond water and H9N3 from duck faeces on a local duck farm (*15*). Two isolations of H12N5 were made during random sampling in June 1980.

and the newly described H12 (*12*) (Table 3). However, during random sampling in May 1980, two isolations of H12N5 were made from the cloacae of ducks from China.

Sera from 150 ducks, 111 geese, and 155 chickens, randomly collected from poultry from southern China, were negative (titres less than 1:10) in HI tests with viruses of the H8 and H12 subtypes. However, this finding does not exclude the presence of H8 in the region and can at best only be taken as a guide, especially when the H12 data are taken into consideration; absence of detectable antibody does not necessarily indicate that the birds have not been infected.

Association of haemagglutinin and neuraminidase genes

The data in Table 3, which lists the isolates by phenotype (antigenic combination) and number, are presented in Table 4 by antigenic subtype (haemagglutinin and neuraminidase). The haemagglutinin subtypes that occurred most frequently were H4 (gene frequency 0.33), H3 (0.24), and H6 (0.19) and the

Table 4. Gene frequencies of antigenic subtypes of haemagglutinin and neuraminidase found in ducks at a Hong Kong dressing plant from November 1975 to October 1979

Antigenic subtype	No. of isolates	Gene frequency
Haemagglutinin		
H1	4	0.01
H2	7	0.02
H3	110	0.24
H4	150	0.33
H5	17	0.04
H6	86	0.19
H7	1	0.00
H9	17	0.04
H10	53	0.12
H11	13	0.03
Neuraminidase		
N1	32	0.07
N2	98	0.21
N3	43	0.09
N4	22	0.05
N5	30	0.07
N6	137	0.30
N7	1	0.00
N8	79	0.17
N9	16	0.04

Table 5. Statistical evaluation for non-random association of haemagglutinin genes and neuraminidase genes[a] in influenza viruses isolated from ducks during four years' surveillance at a Hong Kong dressing plant, November 1975–October 1979

Haemagglutinin subtypes[b]	Neuraminidase subtypes[b]						
	N1	N2	N3	N5	N6	N8	P_N
H3	0 (7.7)[c]	22 (23.5)	2 (10.3)	0 (7.3)[c]	26 (32.9)	50 (18.9)[d]	9 (9.3)
H4	1 (10.5)[c]	20 (32.1)	1 (14.1)[c]	3 (9.9)	111 (44.9)[d]	9 (25.8)[c]	5 (12.8)
H6	29 (6.0)[d]	23 (18.4)	5 (8.1)	3 (5.7)	1 (25.7)[c]	18 (14.8)	8 (7.3)
H10	2 (3.7)	4 (11.4)	12 (5.0)	25 (3.5)[d]	0 (15.9)[c]	2 (9.1)	8 (4.5)
P_H	1 (4.1)	28 (12.5)	20 (5.5)	0 (3.9)	1 (17.5)	0 (10.1)	9 (5.0)
	χ^2_g = 589.4		$P < 0.001$				

[a] Data are the number of isolates obtained and, in parentheses, the number of isolates expected on the basis of calculation from gene frequencies.

[b] These haemagglutinin and neuraminidase subtypes comprised 87.1% and 91.5% of the subtypes recorded. The remaining subtypes were pooled and designated as P_H and P_N.

[c] Major contribution to non-randomness by occurring less frequently than expected.

[d] Major contribution to non-randomness by occurring more frequently than expected.

most frequent neuraminidase subtypes were N6 (0.30), N2 (0.21), and N8 (0.17). The observed gene frequencies were used to obtain the expected phenotype frequency, on the assumption that there was random assortment of these genes within the population (Table 5). Combinations such as H3N2 and H3N6 occurred at about the expected frequencies. However, there was a certain amount of non-random association between particular genes, certain isolates occurring more frequently than expected (H3N8, H4N6, H6N1), others less frequently (H4N1, H4N3, H4N8, H6N6), and some not at all (H3N1, H3N5, H10N6). Some non-random associations also occurred in the remaining gene pool.

Isolation pattern

The pattern of isolations could be examined best in ducks, as these birds provided most (458/480) of the influenza virus isolates (Table 1). These viruses were regularly isolated over the four-year period. The pattern of isolation of paramyxoviruses was also examined as they comprised one-third of the duck isolates. In Fig. 1 the data are expressed in 4-month periods: March–June and July–October (humid, warm or hot weather) and November–February (dry, mild or cool weather). The isolation rate tended to be cyclic and seasonal although the pattern was spoilt by an isolation rate for influenza viruses that was higher than expected in the period November 1977–February 1978 and a lower than expected rate for paramyxovirus isolations in the period November 1978–February 1979. Generally, the isolation rate for influenza viruses was the converse of that for paramyxoviruses, the former being more frequently isolated in the summer months, the latter in the winter months.

Although influenza virus isolations generally predominated in the summer months, this was not true of all subtypes. Whereas, for example, H4 was most frequently detected in the summer, H3 predominated in the winter. Others such as H6 and N2 showed peak isolation rates in either season (data not shown). Thus, seasonal factors may contribute to the extent and availability of the gene pool (Table 4) and thus of the different antigenic combinations.

Human serology

A serological study was undertaken of people living in the rural and semi-rural areas of the New Territories, Hong Kong, to see whether avian viruses had infected human subjects. The viruses tested for were ones formerly designated Hav1 – Hav10 now represented by the H3 – H12 subtypes (*14*).

Of 355 sera tested, 106 reacted positively in the HI test with titres of 10 – 80, the majority with titres of 10 – 20. Seventy-one of the sera were positive for the H3 subtype, this high frequency presumably being due to infection by the Hong Kong strain of influenza A (H3N2) virus. There was no general correlation between frequency of the low-titre, presumptive antibody in man and the frequency of haemagglutinin subtypes in domestic ducks.

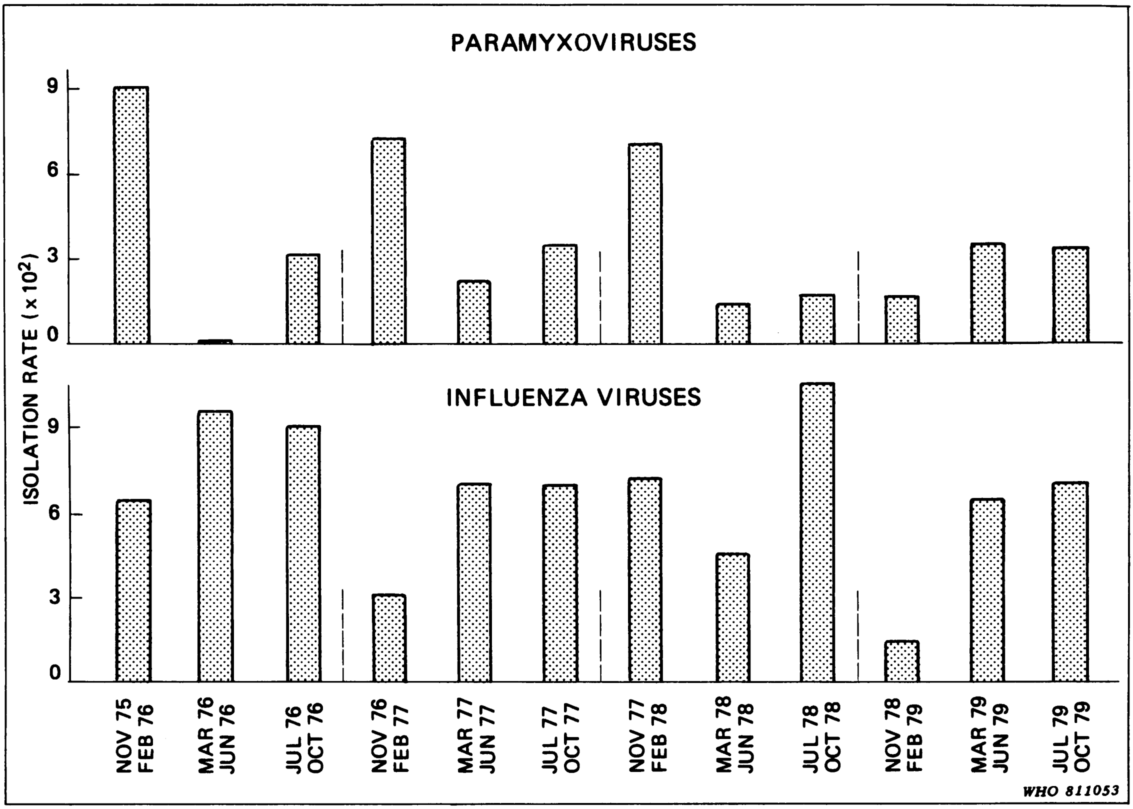

Fig. 1. Isolation rates of influenza viruses and paramyxoviruses (PMV-1, PMV-4, and PMV-6) assessed by 4-monthly intervals from November 1975–October 1979.

DISCUSSION

Extensive surveillance of domestic poultry originating in either southern China or Hong Kong over more than four years resulted in the isolation of influenza A viruses exhibiting 46 of the 108 antigenic combinations possible with the revised system of nomenclature (*14*). All but one of the combinations were recorded in duck isolates, thus emphasizing the importance of ducks in influenza ecology. Furthermore, the frequency of isolation of influenza viruses surveyed at a poultry dressing plant was in the ratio of 16:3:1, for ducks, geese, and chickens, respectively.

Several factors may account for the great diversity of influenza viruses in the duck population in this region: (*a*) the mass production of ducks for human consumption on innumerable small ponds, particularly throughout the delta of the Pearl River; (*b*) the possibility of maintaining transmission of the predominantly intestinal influenza viruses by the faecal–water–oral route to the new susceptible ducklings that are continually being introduced onto the ponds (*15*), and (*c*) the unique role that this region plays in the migratory patterns of birds from many different zones (*16*). This set of circumstances could provide a suitable environment for the introduction of new antigenic combinations of influenza viruses into the domestic duck population, and their subsequent maintenance.

The closeness of human habitations to the farms in southern China suggests that this would be an ideal situation for an avian influenza virus to cross the species barrier to man. This view is supported by evidence that some human pandemic viruses appear to have originated in southern China and that influenza viruses were isolated from the ducks from southern China much more frequently than from ducks from Hong Kong (ratio 4.5:1).

Human influenza is predominantly a winter disease. In contrast, although influenza viruses were isolated from asymptomatic ducks all the year round, the frequency of isolation was greater in the summer. If avian influenza were an exclusively respiratory infection, seasonal variation of factors such as temperature and humidity might affect virus transmission and thus the isolation pattern. However, as water-borne transmission seems to be more important, the link between seasonal factors and transmission is unclear, especially in view of the pattern of isolation of paramyxoviruses, which were more frequently isolated in the winter.

Assuming that ducks represent a major source of genetic material pertinent to human influenza, it might be reasonable to assert that the summer would be the most likely time for suitable viruses to infect man. Thus, it may be worth considering as candidate viruses those whose antigenic combinations occur most commonly and exhibit greatest stability. The frequently isolated viruses H4N6 and H3N2 were found to be considerably more stable in pond water than some of the less frequently encountered combinations and than the paramyxoviruses (*15*; Cheung & Shortridge, unpublished data, 1981). It is of interest that H3, the Hong Kong influenza virus haemagglutinin, formerly designated as Hav7 (*14*), was the second most commonly encountered haemagglutinin subtype.

Increasing evidence suggests that for a "non-human" virus to cause a pandemic it would be necessary for the virus to acquire the genes controlling transmission and virulence in man. These have not been clearly defined (*7*), but it seems that they may be acquired by recombination with the prevailing human virus (*3*). Thus, on numerical grounds, and taking into account seasonal differences in prevalence, a potential pandemic virus could be either one with the H4N6 antigenic combination, if there was merely reassortment of the avian genes, or a recombinant of H4 incorporating the prevailing human neuraminidase antigen, N1 or N2. Other haemagglutinins that have been detected frequently, and therefore of pandemic potential, are H6 and H10. However, it does not necessarily follow that the most frequent isolates will be the most likely to start a pandemic, since for recombination to occur, there must be a suitable ecological niche that satisfies all the conditions necessary for the propagation of the emerging recombinant.

The range of antigenic combinations viable enough to predominate on a farm is still a matter for conjecture. It might be inferred from a statistical analysis of the isolates obtained over the four-year period of surveillance that not all combinations are likely to occur, or that if they do they are selected against in nature. This means that only some antigenic combinations may be able to survive long enough in a particular host or environment to contribute to the postulated recombination with a human virus. It is of interest to note that, in a year-long study of a Hong Kong duck farm, H3N2 virus predominated over this period (and beyond) whereas other antigenic combinations, possibly arising as the result of recombination between this virus and ones apparently introduced by migratory and transient birds, failed to become established (*15*). All haemagglutinin subtypes except H8 were recorded among the isolates obtained over the four-year period; however, one cannot assume that this subtype, and others yet to be described, were absent from the region.

A serological study of rural and semi-rural people in Hong Kong did not provide convincing evidence of infection by viruses of avian origin, nevertheless more information might be gained by examining the sera of those specifically involved in raising ducks.

While the role of birds and animals in the emergence of human pandemic influenza is circumstantial, no attempt has been made to pre-empt the spread of viruses from such sources to man. As the most frequently encountered antigenic combination in the poultry of the region, the H4N6 virus might be a candidate for a study on the production of prospective vaccines by both the conventional egg-embryo, and the more recently described gene-cloning, techniques.

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RÉSUMÉ

VIRUS GRIPPAUX AVIAIRES A DE LA CHINE MÉRIDIONALE ET DE HONG KONG: ASPECTS ÉCOLOGIQUES ET CONSÉQUENCES POUR L'HOMME

Comme on a suggéré que de «nouveaux» virus pandémiques humains pourraient naître d'une recombinaison entre un virus d'origine animale (notamment aviaire) et une souche humaine et comme, d'autre part, l'Asie du Sud-Est a été le lieu d'origine des pandémies H2N2 et H3N2, une surveillance continue des virus grippaux de la volaille

domestique dans le sud de la Chine (y compris Hong Kong) a été entreprise. Le présent rapport expose les résultats d'une étude qui a duré plus de quatre ans.

Dans le nouveau système de nomenclature des virus grippaux A, il y a actuellement 108 combinaisons possibles de sous-types d'hémagglutinine et de neuraminidase. L'échantillonnage aléatoire de la volaille provenant de Chine et de Hong Kong, dans une entreprise d'habillage de Hong Kong, a permis d'identifier 46 combinaisons antigéniques différentes, dont 43 chez des canards provenant de Chine. On a découvert, au cours de la quatrième année et ensuite, des isolements qui n'avaient pas été signalés auparavant, à savoir H1N3, H4N1, H6N3, H10N3, H10N4, H10N8, H10N9 et H12N5, tandis que H9N6 a été isolé dans un élevage local de canards.

Les divers facteurs susceptibles d'expliquer la grande diversité de combinaisons antigéniques parmi les isolements sont: 1) la position géographique de la région en ce qui concerne les itinéraires de migration des oiseaux, 2) l'élevage intensif — particulièrement des canards — dans d'innombrables petits étangs, et 3) la propagation des virus par la voie fécale-hydrique-orale, virus qui sont entretenus dans les étangs par l'introduction continue de canetons. Du fait qu'on a observé que l'isolement des virus grippaux à partir des canards tendait à être plus fréquent en été, alors que celui des paramyxovirus était supérieur en hiver, et que l'habitat de l'homme et celui des canards sont très proches, on a émis l'hypothèse que cet environnement constituerait une situation idéale pour qu'un virus aviaire franchisse la barrière spécifique le séparant l'homme et donne des virus recombinants ayant un éventuel potentiel pandémique. Il est suggéré de produire des vaccins prospectifs contre les virus aviaires qui, d'après les données numériques et saisonnières, semblent les protagonistes les plus probables de recombinaisons de ce type.

REFERENCES

1. Webster, R. G. et al. *Journal of general virology*, **32**: 217 – 225 (1976).
2. Webster, R. G. & Laver, W. G. Antigenic variation of influenza viruses. In: Kilbourne, E. D., ed. *Influenza viruses and influenza*, New York, Academic Press, 1975, pp. 269 – 314.
3. Scholtissek, C. et al. *Virology*, **87**: 13 – 20 (1978).
4. Shortridge, K. F. et al. *Bulletin of the World Health Organization*, **55**: 15 – 20 (1977).
5. Shortridge, K. F. et al. *Bulletin of the World Health Organization*, **57**: 465 – 469 (1979).
6. Shortridge, K. F. *Research in veterinary science*, **28**: 296 – 301 (1980).
7. Laver, W. G. & Webster, R. G. *British medical bulletin*, **35**: 29 – 33 (1979).
8. Shortridge, K. F. & Alexander, D. J. *Research in veterinary science*, **25**: 128 – 130 (1978).
9. Shortridge, K. F. et al. *Journal of general virology*, **49**: 255 – 262 (1980).
10. Fazekas de St Groth, S. & Webster, R. G. *Journal of experimental medicine*, **124**: 331 – 345 (1966).
11. Lang, G. et al. *Bulletin of the World Health Organization*, **47**: 515 – 519 (1972).
12. Hinshaw, V. S. & Webster, R. G. *Journal of general virology*, **45**: 751 – 754 (1979).
13. Gardner, I. D. & Shortridge, K. F. *Reviews of infectious diseases*, **1**: 885 – 890 (1979).
14. *Bulletin of the World Health Organization*, **58**: 585 – 591 (1980).
15. Markwell, D. D. & Shortridge, K. F. *Applied and environmental microbiology*, **43** (in press, 1982).
16. McClure, H. E. *Migration and survival of the birds of Asia.* United States Medical Component, SEATO Medical Project, Bangkok, Thailand, 1974, 476 pp.