THE IDENTIFICATION AND DISTRIBUTION OF INFLUENZA A VIRUS IN INDONESIA

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ABSTRACT

Ten influenza A virus isolates were obtained from seven domesticated ducks, and one each from a migrating pelican, a native wild parrot and man. Agar gel precipitation (AGP)test for typing, and haemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests for subtyping were performed on the virus isolates. Besides, another AGP test using matrix (M) antigen of a locally isolated influenza A virus was also carried out to determine the influenza A antibody contents in the sera of birds and mammals qollected from several areas in Indonesia. The results showed that the nine virus isolates obtained from birds and one from human were all type A influenza. Two of the isolates from duck in Tangerang, West Java (Pro-38) and from the pelican (Plk-220) contained H4N6 subtype. The other duck isolates (Kdr-9,Kdr-10,Kdr-11, Kl-15, Kl-17 from Bali and Pro-40 from Bogor) and from the parrot (Nr) contained H4N2 subtype, while the one from man cantained H4 where its N was not identified yet. It was also shown that influenza A virus had a wide distribution in several areas of Indonesian and might infect poultry, such as improved and native chickens, ducks and muscovy ducks; and wild cockatoes; as well as horses, pigs and man. The infection rates in the intensive farms, such as improved chicken farms and piggeries were higher than those found in the extensive farms, such as small holding of native chickens and moscovy ducks.
HI tests using several virus isolates for the detection of antibody contents against H4 showed that influenza A (H4N6) virus was commonly distributed in the Western while influenza A (H4N2) in the Eastern part of the Indonesian archipelago.

INTRODUCTION

During the Dutch administration in Indonesia, a disease in poultry called *hoenderpest* or fowl pest occurred. The virus causing the disease was successfully isolated and further identification after a long period of isolation showed that the virus was an influenza A, containing Hav_1 Neq_1(Tumova and Pereira, 1968) or H_7N_7 (WHO,1980). The virus is also known as A/FPV/Dutch/27 or Dutch strain and is still kept in several reference laboratories as one of the standard avian influenza A virus for certain comperative study purposes (Alexander *et al.*, 1978; Onta *et al.*, 1978, Yamane *et al.*, 1979).

At the same time, another important disease in poultry named *pseudohoenderpest* or Newcastle disease was also observed in Indonesia (Kraneveld, 1926). This disease seemed more likely to attract scientists to be interested in, since it may cause a great deal of economical loss from time to time, even up to the present time (Ronohardjo,1984). The development of influenza A research in Indonesia at that time was not as good as one could expect. Therefore, data of the disease were also very rare, except those originating from a pathogenicity study of the virus in mice and chickens which were achieved (kraneveld and Nasoetion, 1939).

After a long period of relapse, Prodjohardjono (1977) described a disease with a sinusitis syndrome in local young ducks occurring in Yogyakarta. Later a disease locally called *cengesan* was also observed by Ronohardjo *et al,*(1978) in Tegal, Bali and Alabio ducks in dense duck populated areas of Indonesia. Further intensive study of the disease showed that the prevalence rate was sometimes high,up to more than 60%, and the virus was isolated from the respiratory organs of diseased ducks (Ronohardjo, 1980). The influenza virus was identified through biological and electron microscopical study of isolates (Ronohardjo, 1983).

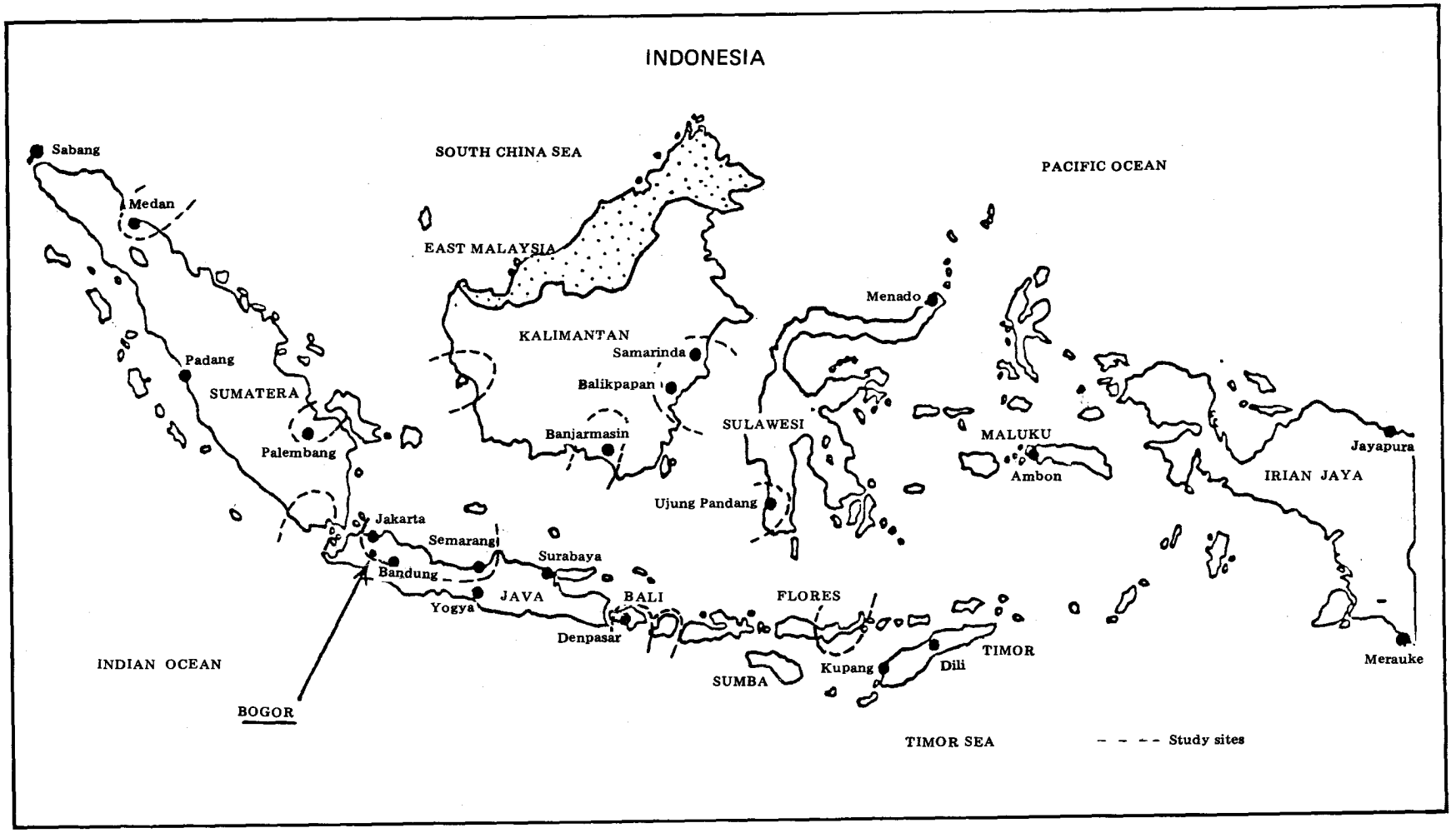
Due to the traditional system of duck farming in this country (Ronohardjo, 1982) contact between domesticated ducks and wild water birds during search for feed in the rice fields can occur quite often. Because the same site of rice fields may be used by both domestic ducks and wild water birds. It is considered that migrating wild water birds could be infected by human or animal influenza A virus (Slepuskin *et al.*, 1972; Zakstelkaja *et al.*, 1972). Besides, some influenza A viruses of human and avian origins can be isolated also from the domestic duck (Higgins and Schild, 1972; Shortridge *et al.*,1977). Furthermore, some influenza pandemics have started from South East Asia (Beveridge, 1977; Webster and Laver 1972).
Therefore, a study of influenza A virus in Indonesia (Figure 1) will give a beneficial information on the source of the genetical influenza A virus in nature, particularly from one of its site of origin.

MATERIALS AND METHODS

Specific Antigen and Antisera

Matrix (M) antigen of M x-31 (A/Hong Kong/1/68 x PR/81) and antiflu MP (A/Port Chalmers x PR/81) serum were obtained from Dr D Higgins, Pusat Peneli-

FIGURE 1. Map showing all the main islands of the Indonesians Archipelago and the study sites



tian Bio Medis (Research Centre for Bio Medical) in Jakarta. Besides, antisera of A/NSW/(H_1), A/PR/8/34(H_1), A/FM/1/47/(H_1) A/Swine/Iowa/15/30(H_1), A/Singapore/1/57(H_2), A/Hongkong/1/68(H_3), A/equine/Miami/1/63(H_3), A/duck/Ukr/1/63(H_3), A/duck/Czech/56(H_4), A/tern/S.Afric/61(H_5), A/ty/Ukr/Mess/65(H_6), A/FPV/Dutch/27(H_7), A/equine/Prag/1/56(H_7), A/ty/Out/6118/68(H_8), A/chick/Germ/N/49(H_{10}) and A/duck/Engl/56(H_{11}) were generously supplied by Dr R G Webster, WHO Collaborating Centre for Ecology of Influenza, Tennessee, USA.

Influenza A Virus Isolates

All virus isolates were obtained from field specimens and processed according to RIAD virological standard (Ronohardjo, 1983). They were harvested from the allantoic fluids and then kept in a 4 C refrigerator before use or freeze dried if the allantoic fluid containing suspected isolates was to be sent to the World Influenza Centre in London or for longer storage.

Field Animal Sera

Sera for this study were collected from slaughter houses, farms and hospitals. These sera were stored at -20°C before use.

Red Blood Cells Suspension

Chicken red blood cells (RBC) were obtained from local cocks through the wing venous. Chicken whole blood was mixed with the same volume of Alsever solution (Hsiung, 1973) and washed in pH 7.2 PBS at least for three times. The clean RBC were then resuspended with the same PBS to a 10% concentration and stored in 4° C refrigerator before use. For the haemagglutination inhibition (HI) test, 0.5% RBC in PBS were used.

Influenza A Matrix Antigen

To make M antigen, 100 ml allantoic fluid containing more than 128 haemagglutinin units/0,025 ml were used. To remove cellular debris from the allantoic fluid, the material was centrifuged at 500 g velocity in a 4° C refrigerated centrifuge. Further work was carried out according to the method of Palmer *et al.* (1975).

Influenza A Antiserum

Two healthy local cocks and free of specific antibody to influenza A virus were inoculated slowly with 5 ml allantoic fluid containing more than 128 haemagglutinin units of influenza A virus / 0.025 ml into the wing veins. Serum samples were taken fourteen days after inoculation and then followed by the performance of HI test for determination of antibody titres If the HI titre in the serum was found less than 160 a second inoculation with the same allantoic fluid was given as a booster dose. If the HI titre was found more than 160 the cocks were bled, sera were pooled and stored at - 20° C before use.

Agar Plate

Two grams of Noble agar were put into an Erlenmeyer flask containing 98 ml solvent (Palmer *et al.*, 1975 Kwapinski, 1972) and then boiled until all agar melted. Thereafter, it was shifted into a water bath at 56°C in order to keep the agar still melting. The melting agar was then distributed onto clean microscope slides and each slides received 4 ml liquid agar and left to harden. Before the clean microscope slides were used a thin layer of the same 25% Noble agar was put on each slide with a painting brush. The hardened agar layers on the microscope slides were kept in a humid box if not used in the same day. Agar layers that were not used within 30 days were renewed.

Agar Gel Precipitation

For the agar gel precipitation (AGP) test seven wells were made on agar, one in the centre and the other six wells surrounding it. The distance between the central and each side wells was 3 mm. For virus identification the central well was filled in with a standard M influenza A antiserum and the side wells each with a standard M influenza A antigen, unknown M antigen and normal allantoic fluid prepared in the same way as in the production of M antigen and PBS. For identifying anti influenza A in animal sera, the central well was filled in with a standard M influenza A antigen and the side wells with a standard M influenza A antiserum, normal serum and unknown animal sera to be detected. These reactants in the agar wells were then placed in a humid box and incubated at 37°C for 72 hours and checked every morning. The results of precipitation in the agar were stained, if needed, with amidoblack after Kwapinski's method (1972).

Haemagglutination Inhibition

All HI test were carried out in a microtitrating plastic tray with the double serum dilution and constant virus antigen dilution method according to Palmer *et al* (1975). Before the test sera were absorbed with 20% chicken RBC (Nakamura *et al.*, 1972) and treated with trypsin periodate (Hsiung, 1973). The HI titre was expressed in the highest dilution of the tested serum but still giving clear HI reaction.

RESULTS

Virus Type

The precipitating lines in agar between Flu MP antiserum and Pro-22, Pro-38 and M x-31 antigens fused identically without any cross or spure lines. It indicated that all tested antigens were those of the influenza A virus (Figure 2).

The same AGP test was also performed for the other suspected virus isolates obtained from a parrot (Nr) and a pelican (Plk-220) and also from a laboratory technician who carried out influenza work and showed a mild clinical sign (Pro-42). While the other isolates were all obtained from duck specimens. The same precipitating lines were observed in all of these isolates (Table l). It meant that the same influenza A virus was successfully isolated from man, wild birds and ducks originating from different places of Indonesia.

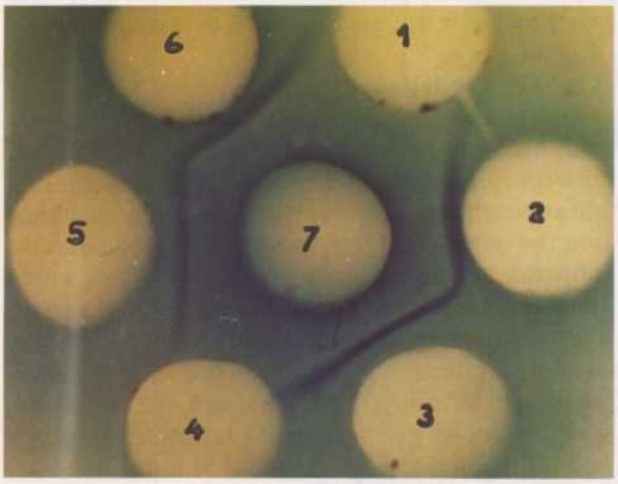


Figure 2. Agar gel precipitation test for determining the types of influenza A virus

1 : CA normal
2 : M Pro-38 antigen
3 : M x-31 antigen
4 : PBS
5 : M x-31 antigen
6 : Pro-22 antigen
7 : anti Flu MP serum

Virus Subtype

The subtyping of all virus isolates was achieved by means of HI and NI (neuraminidase inhibition) tests. But due to technical difficulties not all of these virus isolates were identified. Only ten of the isolates were H subtyped and 9 of them were N subtyped. The HI tests were performed in the virology department of RIAD and confirmed by the World Influenza Centre in London. However, the NI tests were carried out by this World Influenza Centre.

Table 1. Result of type determination of influenza virus by means of agar gel precipitation test

Species	Code	Origin of sample	Type A (AGP)
Duck	Pro-38	Ciputat, Tangerang	+
	Pro-40	Bogor	+
	Pro-22	Negara, Bali	+
	Kdr-9	Kediri, Bali	+
	Kdr-10	Kediri, Bali	+
	Kdr-11	Kediri, Bali	+
	Kl-15	Klungkung, Bali	+
	Kl-17	Klungkung, Bali	+
	A-7	Alabio, South Kalimantan	+
	A-10	Alabio, South Kalimantan	+
	Lp-1	Lampung	+
	Lp-2	Lampung	+
Pelican	Plk-220	Animal Quarantine, Jakarta	+
Parrot	Nr	Animal Quarantine, Jakarta	+
Man	Pro-42	Human, Bogor	+

Results of HI tests showed that all viruses under study contained H_4. While the NI results indicated that Pro-38 from Ciputat, West Java and Plk-220 virus contained N_6 and the rests of the isolates contained N_2 including that from the parrot (Table 2).

Table 2. Indonesian influenza A virus strains

Code	HI test at RIAD	Strain
Kdr-9	$H_4N?$	A/duck/Indonesia/Kdr/9/79 (H_4N_2)
Kdr-10	$H_4N?$	A/duck/Indonesia/Kdr/10/79 (H_4N_2)
Kdr-11	$H_4N?$	A/duck/Indonesia/Kdr/10/79 (H_4N_2)
Kl-15	$H_4N?$	A/duck/Indonesia/Kl/15/79 (H_4N_2)
Kl-17	$H_4N?$	A/duck/Indonesia/Kl/17/79 (H_4N_2)
Pro-40	$H_4N?$	A/duck/Indonesia/Bo/40/78 (H_4N_2)
Nr	$H_4N?$	A/parrot/Indonesia/Jkt/Nr/80 (H_4N_2)
Pro-38	$H_4N?$	A/duck/Indonesia/Tg/38/78 (H_4N_6)
Plk-220	$H_4N?$	A/pelikan/Indonesia/Jkt/220/78(H_4N_6
Pro-42	$H_4N?$	A/Bogor/Indonesia/40/81 ($H_4N?$)

Influenza A virus Infection in Animals

For the study of influenza A virus infection in animals, a total of 1,462 sera of improved chickens, cockatoos, local chickens, ducks, moscovy ducks, pigs and horses were collected from several places of Indonesia. AGP tests were performed using M antigen of the Pro-38 influenza A virus isolate (Figure 3). The results showed that high reactors were mostly found in pig and improved chicken sera, where the prevalence of reactors reached up to 38.01% and 37.56% respec-

tively. Local chickens and moscovy ducks were the most rarely infected species of poultry compared with the first two animal species. Their infection rates were only as high as 9.30% and 3.80% respectively (Table 3).

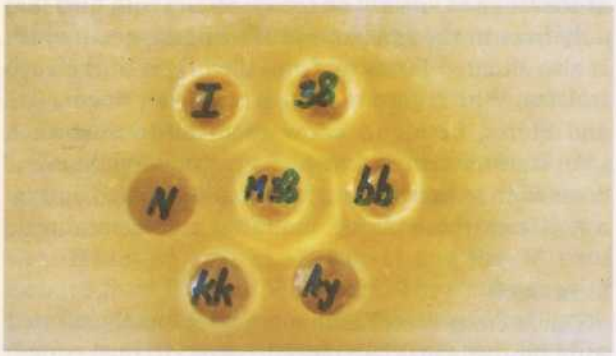


Figure 3. Agar gel precipitation test for determining types with influenza A anti virus on sera

Pro-38 : Pro-38 antiserum
bb : pig serum
Ay : chicken serum
kk : cockatoo serum
N : normal duck serum
I : infected duck serum
M-38 : M Pro-38 antigen

Table 3. Reactors of animal sera against M antigen of influenza A virus Pro - 38

Animal species	Serum	
	Reactor *)	%
Improved chicken	154/410	37,56
Cockatoo	20/101	19,80
Duck	117/656	17,84
Native chicken	8/ 86	9,30
Muscovy duck	1/26	3,80
Pig	65/171	38,01
Horse	2/12	16,67
Total	367/1.462	25,10

*) numbers of positive reactors /numbers of samples examined

1. Infection in Pig.

To know the spread of influenza A virus infection in pig with different farming systems a total of 171 sera were collected from commercial breeding farms, slaughter houses, and small holdings. Pigs for slaughter at the abattoir were originated from different farms, both from the commercial and small farms. Lampung was the place of choice where small pig farming was common,holding 2-5 pigs kept traditionally loose in the yard.

Results of the AGP test showed that the tendency of the commercial farm to be infected with influenza A virus was higher than that of the traditional small farm. Commercial farms in the areas of Solo and Tangerang indicated that 83.33% and 54.55% of their pigs, respectively were infected. While the prevalence of influenza infections in the Lampung area was 2.70% only. Data collected from the slaughter houses were found variable where Bogor had the highest (74.00%), Pontianak moderate (40.47%) and Bandung the lowest infection rates (Table 4).

Table 4. Reactors of pig sera against M antigen of influenza A virus Pro-38

Location	Serum		Remarks
	Reactor*)	%	
Solo	10 / 12	83,33	commercial farms
Bogor	18 / 24	74,00	slaughter house
Tangerang	12 / 22	54,55	commercial farms
Pontianak	22 / 54	40,47	slaughter house
Bandung	1 / 22	9,09	slaughter house
Lampung	1 / 37	2,70	small holdings

*) numbers of positive reactors/numbers of samples examined

2. Infection in Improved Chicken

For the study of influenza in improved chickens, sera were collected from the areas of Bogor, Semarang Denpasar , Medan, Surabaya and Palembang, covering 68 farms of which 18 (26.47%) were infected. Improved chicken farms in the area of Bogor had the highest infection (71.43%), and the lowest infection rate was found in the Palembang area where only 1 out of the 15 farms visited (6.67%) was infected (Table 5).

All sera collected from those infected farms were analised. The results showed that serum samples of improved chicken breeds deriving from 5 infected farms in the Bogor area, 127 out of 172 samples tested (73.68%) reacted positively against Pro-38 M antigen. While sera collected from different infected farms in the other places all had lower infection rates than those in Bogor. The lowest infection rate was found in the Palembang area (3.13%)

Table 5. Reactors of improved chicken sera against M antigen of influenza A virus Pro-38

Location	Reactor			
	Farm*)	%	Serum	%
Bogor	5 / 7	71.43	126 / 171	73,68
Semarang	1 / 10	10.00	13 / 53	24.53
Denpasar	1 / 8	12.50	2 / 13	15.38
Medan	3 / 14	21.43	9 / 68	13,24
Surabaya	1 / 14	7.14	3 / 65	4,62
Palembang	1 / 15	6.67	1 / 32	3.13

*) numbers of positive reactors/numbers of samples examined

3. Infection in Duck

Duck sera were collected from duck farms in the dense populated areas of Indonesia, such as West and South Kalimantan, West and Central Java, South Sulawesi, Lombok,Bali and Lampung. As it were in improved chickens, AGP test were also performed for the duck sera.

The results showed that among the lll farms visited 43 of them (38.74%) were infected. Generally, duck farms in West Kalimantan and Central Java had worse infection,viz 69.23% and 76.19% respectively than those in the other areas (Table 6).

Like in improved chickens duck sera from the infected farms were also analised. The results showed that the possibility of duck kept in the most affected areas had more infections with influenza A virus than those of the other low affected areas. The infection rates for ducks in West Kalimantan and Central Java farms were as high as 46.56% and 33.33% respectively. While duck farms in the other areas had much lower infection rates (Table 6).

Table 6.. Reactors in duck sera against M antigen of influenza A virus Pro-38

Location	Reactor			
	Farm*)	%	Serum*)	%
West Kalimantan	9 / 13	69,23	30 / 65	46,15
Central Java	16 / 21	76,19	34 /102	33,33
South Sulawesi	NA	NA	10 / 67	14,93
Lombok	8 / 28	28,57	14 / 96	14,59
South Kalimantan	2 / 13	15,38	8 / 64	12,50
Lampung	2 / 13	15,38	7 / 66	10,61
Bali	5 / 21	23,81	12 / 114	10,53
West Java	1 / 2	50.00	2 / 82	2,44

*) numbers of positive reactors/numbers of samples examined

NA = not available

Influenza A (H_4N_6) and (H_4N_2) Virus Infection

After surface antigens of the isolated influenza A virus were determined, some of these viruses were used for the HI tests on duck, cockatoo and human sera. Duck sera were collected from duck farms in West Kalimatan, an area situated far away from the origin of the isolated virus. The cockatoo is a wild bird that only lives in the Eastern islands of Indonesia which is also situated far away from the origin of the virus isolates. With regard to human sera from Bogor, Bali and Flores, beside to know about bird influenza A virus transmission to human, its distribution in several areas both in areas where the virus originated and far away from these places, could then be determined.

1. In duck

Ducks from West Kalimantan were mostly infected with the influenza A (H_4N_6) virus of duck origin (Pro-38) and that of the pelican bird (Plk-220) isolated from West Java.The respective reactors were 43.2% and 30.4% . It was surprisingly to note that the Plk-220 antigen used in the HI test for obtaining titres were mostly higher (2,560) than of Pro-38.

The same ducks from the West Kalimantan area also contained influenza A (H_4N_2) antibodies. The numbers of duck sera containing H_4N_6 of Kl-15 were found higher (30.4%) than those of Kdr-10 and Pro-40 (3.2%). It was also important to know that Kl-15 and Kdr-10 virus were isolated from Bali and the Pro-40 virus from Bogor, West Java (Table 7). It was also noted that 83% out of these positive tested sera possessing 2,560 HI titre against Plk-220, did not give any positive result against the other antigens used.

2. In cockatoo

From 30 cockatoo sera which were HI tested with both influenza A (H_4N_6) and (H_4N_2) virus antigens,

Table 7. Infections with influenza A virus isolates on ducks in West Kalimantan

Antigen	Reactor		HI titre						
			40	80	160	320	640	1280	2560
			%						
Pro-38 (H_4N_6)	54/125	43,2	23,2	6,4	—	3,2	10,4	—	—
Plk-220 (H_4N_6)	38/125	30,4	3,2	—	—	—	3,2	—	24,0
Kl-15 (H_4N_2)	38/125	30,4	19,2	9,6	3,2	—	—	—	—
Kdr-10 (H_4N_2)	4/125	3,2	—	3,2	—	—	—	—	—
Pro-40 (H_4N_2)	4/125	3,2	—	3,2	—	—	—	—	—
Kl-17 (H_4N_2)	0/125	0,0	—	—	—	—	—	—	—

*) numbers of positive reactors/numbers of sample examined

it was shown that many positive results were achieved if Kl-15 and Kl-17 virus containing H_4N_2 were used. The numbers of positive results in the test using both antigens were the same (53.3%).

Among the two influenza A (H_4N_6) virus antigens used in the HI test only Pro-38 gave positive results (13.3%). It was also seen that its antibody titre was lower than with the influenza A (H_4N_2) virus antigens (Table 8).

3. In human

To study the transmission of influenza A (H_4 N_6) and (H_4N_2) virus of birds to human being, 99 human sera from Flores 100 from Bali and 36 from Bogor were HI tested with Kl-17, Kdr-11 and Pro-38 virus isolated from Bali and West Java. The results showed that people in Flores were most likely infected by influenza A (H_4N_2) virus originated from Bali,Kl-17 in particular. The total positive reactors was as high as 47,5%, generally with low titres. The highest HI titre was 320 represented only by 1% positive reactors. Eventhough some of the sera from Flores also reacted against Pro-38 but the HI titres were much lower than against Kl – 17 because all of the 15.2% positive reactors against Pro-38 antigen had antibody titres of 20 only.

It was also shown that people in Bali mostly had Kl-17 virus infection like those from Flores. The positive reactors were as high as 28%, while that of Kdr-11 and Pro-38 were only 16.0% and 12.0% respectively. The HI titres obtained by using Kl-17 and Kdr-11 were relatively higher than Pro-38.

Meanwhile, HI tests which were performed on sera of people from Bogor indicated that high positive reactors were obtained with the Pro-38 antigen (25%) rather than with Kl-17 and Kdr-11 antigens (Table 9).

Table 8. Infections with influenza virus isolates on cockatoo

Antigen	Reactor*)		HI titre						
			40	80	160	320	640	1280	2560
			%						
Pro-38 (H_4N_6)	4/30	13.3	13.3	—	—	—	—	—	—
Plk-220 (H_4N_6)	0/30	0.0	—	—	—	—	—	—	—
Kl-15 (H_4N_2)	16/30	53.3	13.3	26.6	13.3	—	—	—	—
Kl-17 (H_4N_2)	16/30	53.3	20.0	33.3	—	—	—	—	—
Kdr-10 (H_4N_2)	0/30	0.0	—	—	—	—	—	—	—
Pro-40 (H_4N_2)	0/30	0.0	—	—	—	—	—	—	—

*) numbers of positive reactors/numbers of samples examined

Table 9. Infections with influenza A virus isolated from duck on man

Location	Antigen	Reactor*)	HI titre					
			20	40	80	160	320	640
			%					
Flores	Kl-17 (H_4N_2)	47/99	47.5	25.3	12.1	6.1	3.0	1.0	—
	Kdr-11 (H_4N_2)	18/99	18.2	9.1	8.1	1.0	—	—	—
	Pro-38 (H_4N_6)	15/99	15.2	15.2	—	—	—	—	—
Bali	Kl-17 (H_4N_2)	28/100	28.0	5.0	15.0	3.0	4.0	—	1.0
	Kdr-11 (H_4N_2)	16/100	16.0	7.0	7.0	—	2.0	—	—
	Pro-38 (H_4N_6)	12/100	12.0	12.0	—	—	—	—	—
Bogor	Kl-17 (H_4N_2)	5/36	13.9	—	2.7	5.6	5.6	—	—
	Kdr-11 (H_4N_2)	4/36	11.1	—	8.3	2.8	—	—	—
	Pro-38 (H_4N_6)	9/36	25.0	—	2.8	11.1	11.1	—	—

*) numbers of positive reactors/numbers of samples examined

The study on the transmission of influenza A virus of duck to human being in those three areas, showed that both influenza A (H_4N_6) and (H_4N_2) virus were evidently trasmitted to man. But people living in the Eastern parts of Indonesia were most likely to be infected with influenza A virus isolated from Bali, while those living in the Bogor area were infected with the virus from Ciputat. Tangerang-West Java, a place situated nearby Bogor.

DISCUSSION

Typing and subtyping of the isolates to date resulted in the identification of two influenza A virus strains viz influenza A (H_4N_6) and influenza A (H_4N_2) The influenza A(H_4N_6) virus which was isolated from a duck in Tangerang (Pro-38) and the other one from a pelican (Plk-220) were both pure avian influenza A virus similar to the A/duck/C2/56 (H_4N_6) strain (Pereira *et al.*, 1966). This finding suggests that the avian influenza A virus is widely distributed in several regions of the world and migrating waterbirds such as pelicans may play an important role in natural transmission and distribution of the virus (Slepuskin *et al.*, 1972). While the influenza A (H_4N_2) strain which was obtained from Bali ducks (Kdr-9, Kdr-10, Kdr-11, Kl-15 and Kl-17); from a duck in Bogor (Pro-40) and from a parrot (Nr) was a hybrid virus resulting from a genetical recombination process (Webster and Laver, 1972). In this case, the H_4 surface antigen originated from the avian and the N_2 from the human influenza virus, either A/Singapore/57 (H_2N_2) or A/Hong Kong/68 (H_3N_2), both pandemic virus strains of South East Asia origin (Beveridge, 1977). It means that some wild birds, either native or exotic, and domesticated ducks in Indonesia beside being able to contract infection with influenza A virus, they may also actively conserve the N_2 surface antigen of pandemic influenza A virus from the environment. And most likely the genetical recombination process itself may proceed in these birds (Webster and Laver, 1972).

Influenza A virus infection seems to occur not only in these birds but also in some other animal species, including pigs and horses. From the AGP tests using M Pro-38 local strain antigen, it was shown that sera from improved chickens, cockatoos, native chickens, moscovy ducks,pigs and horses, as well as from ducks were found containing antibodies against this influenza A virus (Table 3). The infection rates in pigs (38.01%) and improved chickens (37.56%) were found higher than in the native chickens (9.30%) and moscovy ducks (3.80%). This may be due to the fact that the first two animal species are held intensively in the farms, while the latter two species are kept extensively loose in yards (Table 3). These data were more or less the same as those obtained from pigs in the Lampung area (Table 4). It seems that animal density in the farms and regular contact between affected animals and their neighbours are positively influencing virus transmission and reinfection (Davenport, 1972; Nakamurra *et al.*, 1972). Besides, the problem of contamination with diseased excretions on feed, drinking water and farm tools used in the animal houses may occur more often in the intensive rather than in the extensive farming system (Alexander *et al.*, 1978; Slemons and Easterday, 1979).

This problem of contamination was also proved appearing in poultry houses according to the result of tests run on sera of improved chickens which were collected from poultry farms in several areas of Indonesia. Poultry farms in the area of Java which were mostly located in the big cities or near by, and developed earlier and were older than those found in other parts of the country were worsely infected with the virus. The highest incidence of influenza was especially found in farms in the Bogor area rather than those, for example, in Palembang and Surabaya (Table 5).

It was also observed that the wet season influenced the infectivity of influenza. Long heavy rains during the collection of duck sera in the regions of West Kalimantan induced the achievement of a high reactor rate of virus infection in the duck.

Eventhough, the two influenza A (H_4N_6) and (H_4N_2) virus strains contained the same surface antigen of H_4, but the result of HI tests using these two strains run on sera ducks from West Kalimantan, cockatoos from the Eastern islands and human from Bogor, Bali and Flores showed variabilities of titres and numbers of reactors. It seemed that the N surface antigens of these viruses and the existence of virus variants in the nature affected the HI results (Pereira *et al.*, 1966; Schhild *et al.*, 1973).

HI results on duck sera from West Kalimantan obtained by the use of influenza A (H_4N_6) virus strain isolated from a pelican indicated more frequencies of positive reactors and high titres compared with the use of other isolates. It seemed most unlikely that migrating wild birds such as pelicans did not take an important role in virus transmission in the nature (Rosenberger *et al.*, 1974; Zakstelkaja *et al.*, 1972). The data also supported the result of isolating influenza A virus from parrots and the antibody contents in sera of cockatoos.

From the HI results of duck, cockatoo and human sera collected from several areas of Indonesia, it was shown that influenza A (H_4N_2) virus was distributed mostly in the western part of the country, while in-

fluenza A (H_4 N_2) virus in the eastern islands (Goto *et al.*, 1978; Pereira *et al.*, 1972). Data also pointed out tnat West Kalimantan duck sera and human sera from Bogor mostly contained influenza A (H_4N_6) virus antibodies of the Tangerang duck (Pro-38) and pelican (Plk-220) strains and higher HI titres were achieved. While cockatoo and human sera from Bali and Flores contained antibodies of the influenza A (H_4N_2) virus strains of Bali ducks (Kl-17 and Kl-15).

AKNOWLEDGEMENT

The authors thank Dr R G Webster from the WHO Collaborating Centre for Ecology of Influenza, Tennessee-USA and Dr D Higgins, Pusat Penelitian Bio Medis, Jakarta for their generous supply of influenza A antigens and antisera used in this study. They also thank Dr J J Skehel WHO Influenza Centre, London for subtyping of the virus isolates.

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