ISOLATION AND IDENTIFICATION OF AN INFLUENZA A VIRUS IN DUCKS IN TAIWAN

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In 1972, a severe outbreak of respiratory disease was observed in a farm with 800 ducks at Tansui, Taipei county, Taiwan. Severe respiratory signs were noted in ducks of 2-4-week-old, and 600 of them were dead of the disease.

A hemagglutinating RNA virus was isolated by means of embryonated duck eggs (DE) and duck kidney cell cultures (DK) inoculation. The isolate had a titer of $10^{7.0\text{-}7.5}$ EID_{50}/ml in DE and $10^{7.6}$ PFU/ml in DK, and could cause CPE in both DK and chicken kidney cell cultures (CK). The isolate could also cause agglutination of RBC of cattle, pig, goat, sheep, rabbit, guinea pig, rat, mouse, chicken, duck and goose. Growth curve of the isolate was studied in DK cultures. In liquid phase, virus could be detected at 4 hours postinoculation (PI) and reached peak level ($10^{6.1}$ PFU/ml) at 48 hours PI, while in cellular phase, firstly detected at 6 hours PI and peaked 11 hours PI with a titer of $10^{4.6}$ PFU/ml

The physico-chemical studies indicated that the isolate was heat labile and sensitive to ether, sodium deoxycholate, solution of pH 3.0 and pH 9.0, but resistant to trypsin treatment. The size of the virus was 110 nm as measured by electron microscope.

Hemagglutinin and neuraminidase determinations confirmed that the isolate consisted of Hav6 and N1 antigens, which were similar to the strains A/duck/Germany/1868/68 isolated in Germany and A/duck/Pennsylvania/486/69 isolated in the United States.

In 1955, Schafer firstly demonstrated that fowl plaque virus, highly pathogenic strain, was type A influenza virus. Since then, many strains of type A influenza

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virus were isolated from domestic fowls, terns and wild birds over many countries in Europe, America and Africa. Molecular biological studies revealed that avian influenza virus might potentially become the public health hazzard. Now it was considered as a new zoonosis.[13]

Type A influenza virus was frequently isolated from domestic or wild ducks which suffered severe respiratory disease. It was not until 1972, the similar disease was noted in this country. The present paper described the isolation and identification of the causative virus from the first case of the disease in this country.

MATERIALS AND METHODS

Virus isolation

Five 3-week-old moribund ducks were collected for virus isolation from a broiler duck farm at Tansui near by Tansui river. Each organ from the ducks was individually homogenized with motar pestle, made 10% emulsion in yeast-lactalbumin-Earle's solution supplemented with 100 IU/ml penicilline and 100 μg/ml streptomycin, then centrifuged at 3000 r.p.m. for 20 minutes. The supernate was used as inoculum.

Eight-day-old embryonated chicken eggs were inoculated with 0.2ml of the inoculum via allantoic cavity, candled every day for 7 days. Allantoic fluid of eggs died after 24 hours postinoculation was harvested for hemagglutination test.

Confluent duck kidney cell cultures were inoculated with 0.2ml of the inoculum, and observed for CPE for 7 days. The culture fluid was harvested and tested for HA titer when CPE was noted.

Physicochemical properties

Identification of the isolated virus was studied with physical, chemical, electron microscopy, hemagglutination inhibition (HI) and neuraminidase inhibition (NI) tests by the method introduced by Center for Disease Control (Atlanta, Georgia).[13]

Hemagglutination inhibition test

The tested sera were collected in 1971, 1972, 1973 and 1980 in Taiwan. HI test was conducted in plastic agglutinate plate, titer of 1:40 and higher were regarded as positive.

Growth curve

One step growth curve of the isolate was determinated on duck kidney cell culture by the method described previously.[5]

Plaque assay

The DK cultures were inoculated with virus preparations, incubated at 37°C

for 1 hour, added with 4 ml of first overlay and added again with second overlay 48 hours later as the method described before.[6]

RESULTS

Clinical signs

An infectious disease with high mortality was recognized in Tansui, Taiwan during the spring of 1972. The clinical signs were found in duckling with excessive lacrimation, eye closed and ataxia. The affected birds fell on their sides, emaciation, ruffled feathers, severe coughing, rales (Fig. 1), edema of head, face and sinus (Fig. 2), Watery diarrhea, some affected ducklings had nervous symptom. Duck hepatitis immune serum was used for treatment but was found in vain. Mortality was 75% (600/800).

Pathological findings

Excessive secretion of mucus in trachea, nasal cavity and sinus was observed. The affected lung was infiltrated with serofluid, air sac were thicken and clouded. Edema was found in the subcutaneous tissue.

Isolation of etiological agent

Influenza virus was isolated from the lung and trachea of one moribund duckling by means of embryonated chicken egg and primary duck kidney cell cultures inoculation.

The isolated virus was then named Taiwan strain.

Pathogenecity of isolate

Chorioallantoic fluid collected from the embryo died within 48-96 hours after the inoculation of the isolated virus was used to inoculate into 7 to 11-day-old embryonated duck eggs. The duck embryos that inoculate with the isolated virus at the dose of $10^{7.0\text{-}7.5}$ EID_{50}/0.1 ml showed extensive hemorrhage and died 48 to 96 hours postinoculation.

Cytopathic effect were noted at 24 hours after isolated virus was inoculated onto primary chicken kidney cell. Cells began rounding and peeled off within 30 hours postinoculation (Fig. 4).

Plaque formation on chicken and duck kidney cells was observed 4 days after isolated virus inoculation. The size of plaque was 0.5 to 3.5 mm in diameter, with infective titer of $10^{7.62}$ PFU/ml.

The isolated virus grew well in cell lines such as Vero, ESK, HmLu, MDBK, MDCK, MA104, BHK, SK-H, cc81, FLF, FLK-H and primary cell cultures of swine kidney cell and guinea pig kidney cell (Table 1).

Table 1. Growth and Hemagglutinin Producing of Avian Influenza A/duck/Taiwan/72 Virus in Different Primary Cells and Cell Lines

Type of Cells		Growth	HA
Primary Cell	SK	+	+
	CK	+	+
	GK	+	+
	DK	+	+
	GPK	+	+
Cell Line	VERO	+	+
	ESK	+	+
	MDBK	+	+
	MDCK	+	+
	MA104	+	+
	BHK	+	+
	SK-H	+	+
	CC81	+	+
	FLF	+	+
	FLK-H	+	+
	BEK	+	+
	HmLu	+	+
	MK2	−	−

Virus growth curves

The Taiwan strain appeared in the duck kidney cell culture fluid with titer of 10^3 PFU/ml at 4 hours postinoculation (PI), increased to the titer of $10^{5.5}$ PFU/ml at 8 hours PI, then reached the peak titer ($10^{6.1}$ PFU/ml) at 48 hours PI.

Intracellular viral titer was detected 1 hour PI (10^1 PFU/ml), then disappeared in the period of 2 to 5 hours PI, virus began to appear at 6 hours PI with the titer of $10^{1.7}$ PFU/ml and reached the peak titer ($10^{4.6}$ PFU/ml) at 11 hours PI. This peak titer was lasted for 24 hours PI, then began to decline, at 48 hours PI the intracellular viral titer was declined a log units (Fig. 5).

The resistance of the Taiwan strain virus to chemical agents, pH and heat

The virus was sensitive to 20% ethyl ether and 0.1% sodium deoxycholate, and was resistant to 0.1% trypsin. At pH 3, the virus titer was declined about log 4.25. At pH 9, the virus titer was declined log 2.0. The virus were losed partial of its infectious titer when treated with 2 M/$MgCl_2$ and distilled water at 56°C for 30 minutes. (Table 3)

Fig. 1. The affected duckling with the nervous disorder (arrowed) and respiratory sign.

Fig. 2. The affected duckling with the swollen face and sinus.

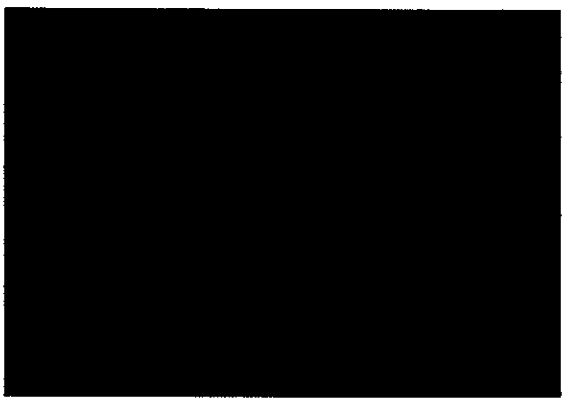

Fig. 3. Normal primary duck kidney cell at 4th days cultivation. (x50)

Fig. 4. Rounding of the Taiwan strain virus infected duck kidney cell. (24 hours after inoculation, x 50)

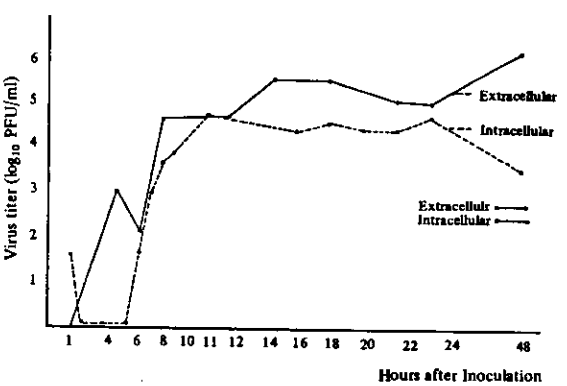

Fig. 5. Multiplication of A/duck/Taiwan/72 (Hav6 N1) in duck kidney cell culture.

Fig. 6. Electromicorscopic picture of A/duck/Taiwan /72 (Hav6 N1) (x101,465)

Table 2. Hemagglutination of New Isolate of Avian Influenza A/Duck/Taiwan/72 Virus and NDV Ishii Strain

Virus		A/Duck/Taiwan/72 (Hav6 NI)			NDV Ishii strain		
		Rom Temp. 1-2 hr.	4C, 2hr.	4C, 12hr.	Room Temp. 1-2 hr.	4C, 2hrs.	4C, 12hrs.
HA titer against Erthrocytes of.	Chicken	1280	1280	1280	320	320	320
	Goat	5	5	5	40	40	40
	Rabbit	1280	1280	1280	1280	1280	1280
	Dog	1280	1280	1280	320	160	160
	Duck	1280	1280	1280	320	320	160
	Horse	0	0	0	80	160	160
	Swine	1280	640	640	0	160	80
	Mouse	1280	1280	1280	80	160	160
	Sheep	1280	640	640	160	160	160
	Cow	640	320	320	80	40	40
	Goose	640	320	320	80	40	40
	G-pig	640	640	640	160	160	160
	Rat	640	1280	1280	320	160	160

Table 3. Physical and Chemical Properties of A/Duck/Taiwan/72 (Hav6 N1)

Virus Control		Treatment with						
		20% Ethyl ethar	0.2% Trypsin	0.1% SDC	pH 3.0	pH 9.0	2MMgCℓ 56°C, 30	Dist. water 56°C, 30
Taiwan[a]	7.5[d]	0	2.5	0	3.25	5.5	4.25	3.5
418[b]	5.0	4.25	4.75	5.0	4.25	4.5	4.5	3.0
Sato[c]	5.5	0	6.75	0.5	2.75	5.5	4.25	0

Note: a: A/duck/Taiwan/72 (Hav6 N1)
b: Duck Hepatitis Virus
c: NDV (Newcastle disease virus)
d: EID_{50} Log index

Type of nucleic acid in the Taiwan strain virus

The infectivity of isolated virus was not affected by the treatment of 5-isododeoxyuridin and it was proved to be a RNA virus.

Hemagglutination activity of the Taiwan strain virus

The Taiwan strain virus was noted to agglutinated red blood cells of chicken, goat, rabbit, dog, duck, pig, mice, sheep, cow, goose, guinea pig and rat either at 4°C or room temperature. The virus was observed not to agglutinate red blood cells of horse.

The antigenic nature of the Taiwan strain virus and its nomenclature

HI test was conducted with antiserum against Taiwan strain virus and 18 known subtype specific antigens of type A influenza virus. Antiserum against Taiwan strain virus had the HI titer $\geqslant$ 2560 against Taiwan strain virus and A/turkey/Mass./65 (Hav6 N2), the HI titer against other known subtype antigens were all under 320. (Table 4).

Another HI test was carried out. Taiwan strain virus was used as antigen, the HI titer against Taiwan strain virus of eighteen antiserum against known subtype antigen. It was found antiserum against A/turkey/Mass./65 (Hav6 N2) had the HI titer 32 against Taiwan strain virus. Antiserum against A/turkey/Canada/63 (Hav6 Neq2) had the HI titer 16 against Taiwan strain virus. (Table 5).

NI test of each subtype influenza antisera were conducted against Taiwan strain virus. It was found antisera against A/PR/8/34 (H0 N1), A/FM/1/47 (Hsw1 N1) and A/chicken/Scotland/59 (Hav5 N1) had the NI titer higher than 128 (Table 6). NI test of antiserum against Taiwan strain virus was conducted against each subtype antigen. It was noted that A/PR/8/34 (H0 N1), A/chicken/Scotland/59 (Hav5 N1) had the titer 200 and 80 respectively (Table 7). According to the results obtained above, the antigenic structure of the Taiwan strain was determined to be Hav6 and N1. The virus then nomenclatured as A/duck/Taiwan/72 (Hav6 N1).

Electron microscopic observation of A/duck/Taiwan/72 (Hav6 N1)

The infectious allantoic fluid of A/duck/Taiwan/72 (Hav6 N1) was negative stained with phosphotungstic acid and examined with electron microscope. It was found the virus was 110nm in diameter and had the envelope and spikes (Fig. 6).

Survey on antibody against A/duck/Taiwan/72 (Hav6 N1), A/duck/Czech./56 (Hav4 N1), A/duck/Eng./56 (Hav3 N1) and A/duck/Eng./62 (Hav4 N1)

The survey was carried out with the duck sera collected over a period from 1971 to 1973 and in 1980. The results obtained were described below: In 1971, one of 3 duck farms in Pintung, one of 4 duck farms in Miauli, one of 5 duck farms in Hsinchu, one of 3 duck farms in I-Lan, one of 8 duck farms in Ponfu, collectively,

Table 4. HI Test of each Subtype Antigen to Antisera of A/duck/Taiwan/72

Antigens	HI Titer
A/PR/8/34 (H0N1)	160
A/FM/1/47 (H1N1)	320
A/Singapore/1/57 (H2N2)	20 >
A/Aichi/2/68 (H3N2)	20 >
A/swine/Wisconsin/15/30 (Hsw 1N1)	320
A/equine/Praque/1/56 (Heq1Neq1)	20 >
A/equine/Miami/1/63 (Heq2Neq2)	20 >
A/turkey/England/56 (Hav1Nav3)	20 >
A/chicken/Germany "N"/49 (Hav2Neq1)	20 >
A/duck/England/56 (Hav3Nav1)	20 >
A/duck/Czech./56 (Hav4Nav1)	20 >
A/tern/South Africa/61 (Hav5Nav2)	20 >
A/chicken/Scot./59 (Hav5N1)	20 >
A/turkey/Mass./65 (Hav6N2)	2,560 ⩽
A/turkey/Canada/63 (Hav6Neq2)	320
A/duck/Ukraine/1/63 (Hav7Neq2)	20 >
A/turkey/Ontario/6118 (Hav8Nav4)	20 >
A/turkey/Wisconsin/66 (Hav9N2)	20 >
A/duck/Taiwan/72	2,560 ⩽

Table 5. HI Test of A/duck/Taiwan/72 Using Each Subtype of Antisera

Antigens	HI Titer
A/PR/8/34 (H0N1)	8 >
A/FM/1/47 (H1N1)	8 >
A/Singapore/1/57 (H2N2)	8 >
A/Aichi/2/68 (H3N2)	8 >
A/swine/Wisconsin/15/30 (Hsw1N1)	8 >
A/equine/Praque/1/56 (Heq1Neq1)	8 >
A/equine/Miami/1/63 (Heq2Neq2)	8 >
A/turkey/England/63 (Hav1Nav3)	8 >
A/chicken/Germany "N"/49 (Hav2Neq1)	8 >
A/duck/England/56 (Hav3Nav1)	8 >
A/duck/Czech./56 (Hav 4Nav1)	8 >
A/tern/South Africa/61 (Hav5Nav2)	8 >
A/chicken/Scot./59 (Hav5N1)	8 >
A/turkey/Mass./65 (Hav6N2)	32
A/turkey/Canada/63 (Hav6Neq2)	16
A/duck/Ukraine/1/63 (Hav7Neq2)	8 >
A/turkey/Ontario/6118 (Hav8Nav4)	8 >
A/turkey/Wisconsion/66 (Hav9N2)	8 >

Table 6. HI and NI Titer of Each Subtype Influenza Sera to A/duck/Taiwan/72

Anti-sera	HI and NITiter to A/duck/Taiwan/72		
	Subtype	HI Titer	NI Titer
A/PR/8/34	H0 N1	8 >	128 <
A/FM/1/47	H1 N1	8 >	128 <
A/Singapore/1/57	H2 N2	8 >	32 >
A/Aichi/2/68	H3 N2	8 >	32 >
A/swine/Wisconsin/15/30	Hsw1 N1	8 >	128 <
A/equine/Praque/1/56	Heq1 Neq1	8 >	32 >
A/FPV/Dutch/27	Hav1 Neq1	8 >	32 >
A/turkey/England/63	Hav1 Nav3	8 >	32 >
A/chicken/Germany "N"/49	Hav2 Neq1	8 >	32 >
A/duck/England/56	Hav2 Nav1	8 >	32 >
A/duck/Czeck. 56	Hav4 Nav1	8 >	32 >
A/tern/South Africa/61	Hav5 Nav2	8 >	32 >
A/chicken/Scotland/59	Hav5 N1	320	128 <
A/turkey/Ontario/7732/66	Hav5 Nav6	8 >	32 >
A/turkey/Mass./65	Hav6 N2	8 >	32 >
A/turkey/Canada/63	Hav6 Meq2	40	128 <
A/Shear Water/East. Aust. 1/72	Hav6 Nav5	8 >	32 >
A/duck/Ukraine/1/63	Hav7 Neq2	8 >	32 >
A/turkey/Ontario/6118/68	Hav8 Nav4	8 >	32 >
A/turkey/Wisconsin/66	Hav9 N2	8 >	32 >
A/duck/Taiwan/62	? ?	2,560 ⩽	128 <

Table 7. HI and NI Titer of each Type of Influenza Viruses to Rabbit Serum against A/duck/Taiwan/72

Anti-sera	Antiserum to Influenza A/duck/Taiwan/62		
	Subtype	HI Titer	NI Titer
A/PR/8/34	H0 N1	160	200
A/FM/1/47	H1 N1	320	
A/Singapore/1/57	H2 N2	20	80
A/Aichi/2/68	H2 N3	20	80
A/swine/Wisconsin/15/30	Hsw1 N1	160	
A/equine/Praque/1/56	Heq1 Neq1	20	80
A/equine/Miami/1/63	Heq2 Neq2	20	80
A/FPV/Dutch/27	Hav1 Nav3	20	80
A/chicken/Germany "N"/49	Hav2 Neq1	20	
A/duck/England/56	Nav3 Nav1	20	80
A/duck/Czeck./56	Nav4 Nav1	20	
A/tern/South Africa/61	Hav5 Nav2	20	80
A/turkey/Mass./65	Hav6 N2	640	1,280
A/duck/Ukraine/1/63	Hav7 Neq2	20	
A/turkey/Ontario/6118/68	Hav8 Nav4	20	80
A/turkey/Wisconsin/66	Hav9 N2	20	
A/turkey/Canada/63	Hav6 Neq2	80	320
A/chicken/Scotland/59	Hav5 N1	20	80
A/duck/Taiwan/72	? ?	2,560	2,560

6 out of 83 duck farms were found to have antibody against A/duck/Taiwan/72 (Hav6 N1). In 1972, one of 5 duck farms over three counties were antibody positive and the virus was isolated. In 1973, the sera collected from 6 duck farms over 2 counties were all antibody negative. In 1980, the survey on 11 duck farms over 2 counties showed the result of one positive duck farm in each county, collectively, 24 out of 340 sera had the antibody against Taiwan strain virus (Table 8, 9).

HI antibody against A/duck/Czech./56 (Hav4 N1) was not detected in all 83 duck farms in 1971, and 2 duck farms in 1972.

HI antibody against A/duck/Eng./56 (Hav3 N1) was noted in 2 out of 83 duck farms over 12 counties in 1971. In 1972, all 6 duck farms over 2 counties were antibody negative.

HI antibody against A/duck/Eng./62 (Hav4 N1) were found in all 4 duck farms over 2 counties in 1971. In 1972, one of 6 duck farms over 2 counties were antibody positive.

DISCUSSION

Natural infections of domestic duck with type A influenza viruses have been reported in Czechoslovakia,[4] USSR,[12] England,[10] Italy,[9] Canada,[7] Germany,[11] Yugoslavia,[8] and USA.[3]

In Taiwan, duckling with nasal discharge and coughing similar to influenza was found very often. From these ducklings Pasturella anatipestifer was isolated. These bacteria may be secondary to influenza virus infection. The duck farm in this outbreak was faced the Tansui river, where many egret and migratory waterfowls were gathered. Isolation of type A influenza virus from migratory waterfowl had been reported in many countries.[2,13] It was suggested that influenza viruse may be brought into the area by migratory waterfowls and spread to domestic duck.

In Czechoslovakia,[4] the outbreaks of influenza occurring in 10-21 days old ducklings. In England,[10] outbreaks of respiratory disease in ducklings due to type A influenza infections were also occurring in 2 to 3 weeks old ducklings, the mortality rate was 20%. The outbreak reported by Hwang[3] in USA was occurring in 10 weeks old Muscovy duck, the mortality rate was 10%. In the outbreak in Taiwan reported here, the mortality was 65%. This high mortality rate may due to the effects of some factors such as the poor management of the duck farm, duck species and the virus involved, secondary bacterial infection, the age of duckling during exposure and the course of the disease. The course of disease reported by Hwang[10] was 2 weeks. In the outbreak in Taiwan, it was noted that the course of the disease also lasted for more than 2 weeks.

In general, embryonated chicken eggs were inoculated via the amniotic route for the isolation of influenza virus. In this report, it was found that allantoic route of inoculation and primary duck kidney cell culture were also suitable for virus

Table 8. Neuraminidase Inhibition Test of each Subtype Antigen to Anti-A/duck/Taiwan/72 Sera

Antigen	Ni Titer
A/PR/83/34 (H0N1)	200
A/Singapore/1/57 (H2N2)	80 >
A/equine/Prague/1/56 (Heq1Neq1)	80 >
A/equine/Miami/1/63 (Heq2Neq2)	80 >
A/duck/England/56 (Hav3Nav1)	80 >
A/tern/South Africa/61 (Hav5Nav2)	80 >
A/turkey/England/63 (Hav1Nav3)	80 >
A/turkey/Ontario/6118/66 (Hav5Nav4)	80 >
A/shear water/East Aust/1/72 (Hav6Nav5)	80 >
A/turkey/Ontario/7732/66 (Hav5Nav5)	80 >
A/chicken/Scotland/59 (Hav5N1)	80
A/duck/Taiwan/72 (Hav6N1)	320

Table 9. Survey on HI Antibody against A/duck/Taiwan/72 (Hav6 N1), A/duck/Czech./56 (Hav4 Nav1), A/duck/Eng./59 (Hav3 Nav1) and A/duck/Eng./62 (Hav4 Nav1)

County	A/duck/Taiwan/72				A/duck/Czech./56		A/duck/Eng./56		A/duck/Eng./62	
	1971	1972	1973	1980	1971	1973	1971	1973	1971	1973
Hualien	0/6*				0/6		0/6		1/1	
Taitung	0/6				0/6		0/6		2/3	
Kaohsiung	0/5	0/3	0/5	1/1	0/5	0/5	0/5	0/5		0/5
Tainan	1/29				0/29		1/29			
Taichung	0/7				0/7		1/7			
Miauli	1/4				0/4		0/4			
Hsinchu	1/5				0/5		0/5			
Tauyen	0/2				0/2		0/2			
Taipei	0/5	1/1			0/5		0/5			
Ilan	1/3				0/3		0/3			
Ponfu	1/8				0/8		0/8			
Pintung	1/3	0/1	0/1		0/3	0/1	0/3	0/1		1/1

*No. of Positive farms/No. of duck farms survey

Table 10. Serological Survey on Antibody against A/duck/Taiwan/72 (Hav6 N1) in Ducks in 1980

District	Type of Fowl	Positive Rate (%)
Yen Shui	broiler duck	0/21
Tung Shan	broiler duck	0/20
Hsia Ying	broiler duck	0/32
Hsueh Chia	breeder duck	14/79
Liu Ying	broiler duck	0/19
Liu Chia	broiler duck	0/17
Yen Shui	broiler duck	0/17
Yen Shui	breeder duck	0/66
Hsin Hua	breeder duck	0/41
Hu Nei	breeder duck	10/26
Total		24/338 (7.1)

isolation.

In Schetter's report,[11] Duck/Germany/1968/65 was labile to ether, chloroform, heat (56°C) and pH 3.0, the virus was resistant to trypsin. Taiwan strain virus was found to share the same properties.

A/duck/Taiwan/72 (Hav6 N1) was 110 nm in diameter, with envelops and spikes, and was in accordance with the morphology of avian influenza virus described in textbook.[2]

A/duck/Taiwan/72 (Hav6 N1) grow readily in kidney cell cultures of chicken, duck, goose and guinea pig, and also grow well in some cell lines.

The type A influenza virus was divided into many subtypes on the basis of difference in the envelope antigen. The antigenic character of Taiwan strain virus had been analized by HI and NI test, and was determined to be Hav6 N1. Some virus strains had been isolated from duck were A/duck/Czech./56 (Hav4 N1), A/duck/Eng./56 (Hav3 N1), A/duck/Eng./62 (Hav4 N1), A/duck/pennsylvania/486/69 (Hav6 N1), A/duck/Germany/1868/68 (Hav6 N1). In England, the outbreaks occurring in 1956 and 1962 were caused by 2 viruses of different antigentic character. The antigenic character of Taiwan strain virus was identified to the causatic virus of the outbreak in Germany in 1968 and that in USA in 1969.

The result of serological survey on antibody against A/duck/Taiwan/72 (Hav6 N1), showed that the duck had been infected one year before the outbreak occurred. The positive sera still existed in Taiwan in 1980. No positive sera against A/duck/Czech./56 (Hav4 N1) was found in 1971 and 1973. Positive sera against A/duck/Eng./56 (Hav3 N1) and A/duck/Eng./62 (Hav4 N1) were found in 1971, 1971 and 1973 respectively. According to the results of this survey, it was suspecious that the disease had become enzootic in Taiwan. Further information was needed to understand the real epidemiology and the role played by the wild waterfowls.

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台灣肉鴨發生流行性感冒及其抗原性之鑑定

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1972 年 5 月，在台北縣淡水鎮一肉鴨場所飼養之鴨，在 2～4 週齡之間因發生嚴重呼吸症狀，致 800 隻鴨死亡 600 隻（ 75％）。

由病鴨使用鴨腎細胞及雞胚胎分離 7 株，具有紅血球凝集性之 RNA 病毒，分離毒對雞胚胎之感染價有 $10^{7.0-7.5}$ EID_{50}／ml；對鴨腎細胞，雞腎細胞會引起圓化之細胞病變，並會形成 0.5～3.5 mm 大小的斑點（ Plaque ），對鴨腎細胞的病毒感染價有 $10^{7.6}$ PFU／ml ，會凝集牛、猪、山羊、綿羊、兔、天竺鼠、家鼠、小白鼠、雞、鴨、鵝等紅血球，曾探討分離毒在鴨腎細胞的增殖情形，在液相，由病毒感染後 4 小時始能檢出，至 48 小時爲最高值有 $10^{6.1}$ PFU／ml ，細胞內的病毒增殖在感染後 6 小時開始，至第 11 小時爲最高感染價有 $10^{4.6}$ PFU／ml 。

分離毒對乙醚，Sodium deoxycholate 具有感受性，對Trypsin 有抵抗性，對 pH 3.0、9.0 以及對熱的處理均不甚穩定，病毒在電子顯微鏡下其大小有 110 nm 。

分離毒以Hemagglutinin及Neuraminidase 的抗原鑑定爲A／duck／Taiwan／72（Hav 6N1 ）對 1971 、 1972 、 1980 年間的鴨血清有陽性例的存在。又A／duck／Eng.／56（Hav 3 Nav 1 ），在 1971 年，又A／duck／Eng.／62（Hav 4 Nav 1 ）在 1971、1973 年鴨群中有 HI 抗體陽性群。

以上係本省首次自家禽分離流行性感冒病毒的抗原性鑑定，分離株的抗原性與發生在德國的A／duck／Germany／1868／68（Hav 6 N 1 ）及美國的A／duck／Pennsylvania／486／69（Hav 6 N 1 ）相同。

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