

*Short Communication*

**ISOLATION AND SEROLOGICAL CHARACTERIZATION OF  
INFLUENZA A VIRUS FROM A PIG IN THAILAND**

CHATIYANONDA KANAI, KUPRADINUNT SUWICHA, SANGKAWIBHA NADHIRAT,  
KUNIAKI NEROME\*, MIKIO NAKAYAMA\* and AKIRA OYA\*

*Department of Medical Science, Virus Research Institute, Yod-se, Bangkok 1,  
Thailand and \*Department of Virology and Rickettsiology, National  
Institute of Health, Kamiosaki, Shinagawa-ku, Tokyo 141*

(Received January 30, 1981. Accepted April 27, 1981)

*Reprinted from Japanese Journal of Medical Science and Biology*

*Vol. 34, No. 3, pp. 175-178 June, 1981*

*Printed and Published in Japan*

*Short Communication*

**ISOLATION AND SEROLOGICAL CHARACTERIZATION OF  
INFLUENZA A VIRUS FROM A PIG IN THAILAND**

CHATIYANONDA KANAI, KUPRADINUNT SUWICHA, SANGKAWIBHA NADHIRAT,  
KUNIAKI NEROME\*, MIKIO NAKAYAMA\* and AKIRA OYA\*

*Department of Medical Science, Virus Research Institute, Yod-se, Bangkok 1,  
Thailand and \*Department of Virology and Rickettsiology, National  
Institute of Health, Kamiosaki, Shinagawa-ku, Tokyo 141*

(Received January 30, 1981. Accepted April 27, 1981)

**SUMMARY:** A hemagglutinating agent was isolated from a pig in Thailand in 1978 during the early febrile stage of an influenza-like illness and identified as influenza A virus. The isolate contained hemagglutinin and neuraminidase antigens that were antigenically indistinguishable from those of A/Tokyo/6/73 (H3N2), a Port Chalmers-like strain isolated in Japan.

Serological tests also indicated that prevalence of H3N2 virus in the swine population in Thailand.

Since the first recorded isolation of H3N2 influenza virus from swine (Kundin, 1970), a number of H3N2 strains have been isolated from pigs in the world. Most of them were antigenically very similar to A/Hong Kong/1/68 (Kundin, 1970; Shortridge et al., 1979; Shortridge and Webster, 1979). Some swine isolates were more or less related to A/England/42/72 (Shortridge et al., 1979; Shortridge and Webster, 1979), A/Port Chalmers/1/73 (Shortridge and Webster, 1979), A/Victoria/3/75 (Shortridge et al., 1976; Shortridge et al., 1979), or A/Texas/1/77 (Shortridge et al., 1979), but were clearly distinguished from respective strains, except one virus strain which was indistinguishably close to A/Victoria/3/75 (Hinshaw et al., 1978).

In the present paper, we present the evidence to prove that the H3N2 virus very similar to A/Port Chalmers/1/73 was prevalent among swine population in Thailand in 1978.

For virus isolation, 60 nasal swabs were obtained from 26 swine herds in the vicinity of Bangkok (Nakhom Pathom, Suphan Buri, Ratchaburi) from November to December, 1978. Virus isolation was performed by intra-amniotic inoculation of 9-day-old fertile hen's eggs.

Sera were collected from the swine herds at the time the nasal swabs were obtained. All sera were treated with 0.8% trypsin and 0.011 M potassium

periodate by the method described by Jensen (1961).

The hemagglutination-inhibition (HI) tests were performed on microtiter plates using 0.5% chicken erythrocytes.

Neuraminidase titration and neuraminidase-inhibition (NI) tests were done by the method recommended by the WHO Expert Committee (1973), except that antigen-antibody reaction was performed in the presence of 0.5% Triton X-100 to prevent steric hindrance by antibody to hemagglutinin (Russ *et al.*, 1974).

Double immunodiffusion tests were done in 1% agarose, as described previously (Nerome *et al.*, 1978).

The following strains of human and animal influenza viruses were used in the tests: A/Aichi/2/68 (H3N2), A/Tokyo/1/72 (H3N2, A/England/42/72-like strain), A/Tokyo/6/73 (H3N2, A/Port Chalmers/1/73-like strain), A/Victoria/3/75 (H3N2), A/Kumamoto/22/76 (H3N2, A/Victoria/3/75-like strain), A/Tokyo/1/77 (H3N2, A/Texas/1/77-like strain), A/Bangkok/1/79 (H3N2), and A/equine/Miami/1/63 (Heq2 Neq2).

A hemagglutinating agent was isolated from a nasal swab taken from a pig in Nakhom Pathom located 60 km south of Bangkok in November, 1978. The pig from which the agent was isolated showed clinical signs of typical influenza, such as fever, cough and extreme weakness, and died on the following day.

The agent possessed a high level of neuraminidase activity when examined with fetuin as substrate, indicating that it belongs to orthomyxovirus or paramyxovirus.

In double immunodiffusion tests, the virus isolate reacted with antiserum to influenza ribonucleoprotein antigen and was identified as type A influenza virus (data not shown).

Cross HI tests with post-infection ferret sera to various strains of influenza A

TABLE I

*Cross hemagglutination-inhibition tests with the isolate from a swine and reference strains of influenza A virus*

Test virus	Hemagglutination-inhibition by antisera to						
	Aichi/* 2/68	Tokyo/* 1/72	Tokyo/* 6/73	Victoria* /3/75	Kumamoto* /22/76	Tokyo* 1/77	Bangkok* /1/79
A/Aichi/2/68	1,024	2,048	2,048	2,048	128	—#	—
A/Tokyo/1/72	64	1,024	1,024	1,024	64	—	—
A/Tokyo/6/73	32	256	2,024	2,024	128	—	—
A/Victoria/3/75	—	—	1,024	4,096	4,096	32	—
A/Kumamoto/22/76	—	—	1,024	4,096	2,024	64	128
A/Tokyo/1/77	—	—	—	512	512	512	1,024
A/Bangkok/1/79	—	—	—	512	1,024	1,024	8,192
Isolate	32	256	2,048	2,048	128	—	—

\* Post infection ferret sera.

# less than 32.

TABLE II

*Identification of the neuraminidase antigen on the isolate from swine*

Test virus	Neuraminidase inhibition by antisera to purified neuraminidase from:		
	Aichi/2/68 (N2)	Victoria/3/75 (N2)	eq/Miami/1/63 (Neq2)
A/Aichi/2/68	16,384	64	<40
A/Victoria/3/75	512	1,024	<40
A/eq/Miami/1/63	<40	<40	4,096
Isolate	512	512	<40

Results are expressed as the terminal serum dilution causing 50% neuraminidase activity.

virus indicated that hemagglutinin antigen on the isolate belonged to H3 subtype antigen and was closely related to that of A/Tokyo/6/73 (Table I).

The neuraminidase antigen of the virus was characterized in NI tests with antisera to purified neuraminidases of A/Aichi/2/68, A/Victoria/3/75, and A/equine/Miami/1/63. The neuraminidase activity of the isolate was inhibited by antiserum to neuraminidase of A/Victoria/3/75 at a titer similar to the homologous titer (Table II).

The results of the present study showed that the virus possessed the same surface antigens as A/Tokyo/6/73, A/Port Chalmers/1/73-like strain. The virus we described was designated A/swine/Bangkok/9/78 (H3N2). In addition to the above-mentioned virus, another influenza virus antigenically similar to A/Tokyo/6/73 was isolated from a pig showing severe clinical signs of influenza in Bangkok in 1979.

On the other hand, the serological tests with 248 swine sera showed that a large number of pigs (49.6%) in Thailand became infected with human H3N2 influenza (data not shown). Thus, both serological and virus isolation studies suggest that the influenza viruses similar to A/Port Chalmers/1/73 were circulating among pigs in Thailand.

#### ACKNOWLEDGEMENT

We would like to thank Faculty of Veterinary Medicine, Chulalongkorn University for providing facilities. This work was supported in part by the grant from Department of Medical Science, Ministry of Public Health of Thailand.

#### REFERENCES

- HIGGINS, D. A. AND SCHILD, G. C. (1972): Characterization of the hemagglutinin and neuraminidase antigens of some recent avian type A influenza virus isolated from Hong Kong. *Bull. WHO*, *47*, 531-534.
- HINSHAW, V. S., JEAN, W. J., WEBSTER, R. G. AND EASTERDAY, B. C. (1978): The Prevalence of influenza viruses in swine and the antigenic and genetic relatedness of influenza viruses from man and swine. *Virology*, *84*, 51-62.

- JENSEN, K. E. (1961): Diagnosis of influenza by serologic methods. *Amer. Rev. Resp. Dis.*, **83**, 120-124.
- KUNDIN, W. D. (1970): Hong Kong A2 influenza virus infection among swine during a human epidemic in Taiwan. *Nature*, **228**, 957-958.
- NEROME, K., NAKAYAMA, M., ISHIDA, M., FUKUMI, H., BUTTERFIELD, W. K., WEBSTER, R. G. AND CAMPBELL, C. H. (1978): Isolation and serological characterization of influenza viruses from birds that were dead on arrival at Tokyo Airport. *Arch. Virol.*, **57**, 261-270.
- RUSS, G., VARECKOVA, E. AND STYK, B. (1974): Steric effects in the action of influenza virus neuraminidase with antibodies. *Acta Virol.*, **18**, 299-306.
- SHORTRIDGE, K. F., WEBSTER, R. G., BUTTERFIELD, W. K. AND CAMPBELL, C. H. (1976): Persistence of Hong Kong influenza virus variants in pigs. *Science*, **196**, 1454-1455.
- SHORTRIDGE, K. F. AND WEBSTER, R. G. (1979): Geographical distribution of swine (Hsw1N1) and Hong Kong (H3N2) influenza virus variants in pigs in South East Asia. *Intervirology*, **11**, 9-15.
- SHORTRIDGE, K. F., CHERRY, A. AND KENDAL, A. P. (1979): Further studies of the antigenic properties of H3N2 strains of influenza A isolated from swine in South East Asia. *J. Gen. Virol.*, **44**, 252-254.
- WHO Report (1973): Influenza virus neuraminidase and neuraminidase-inhibition test procedures. *Bull. WHO*, **48**, 199-202.