

Epidemic Influenza In A Hill Tribe in Northwest Thailand

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OBJECTIVE: To investigate an epidemic of respiratory disease in the Karen hill tribes of northwest Thailand.

BACKGROUND: The town of Mae Sariang is located on the banks of the Yuam River in a mountainous region of northwestern Thailand (97° 52' longitude, 18° 10' north latitude, at 350 meters above sea level). From Mae Sariang a partially paved road runs north along the river 140 Km to the provincial capital of Mae Hong Sorn. Another road runs 193 Km through the mountains east from Mae Sariang to the city of Chiang Mai (Figure 1 and 2).

The people of this region are largely Karen. They live in small isolated hillside villages of 10–500 houses and are subsistence farmers. Due to the isolation of the villages, travel is largely by foot; it is usually limited to occasional visits to local villages and rarely, in an emergency, to nearby towns. Educational opportunities are rare and there is little understanding of simple health measures. Malnutrition, vitamin deficiency and parasitic infestations are common problems. The climate of this area is influenced by the southern monsoon winds, with the wet season from May to October, and the dry season from November to March. The Christian Medical Unit (CMU) of the American Baptist Mission is located in Mae Sariang. It is a ten-bed hospital with one fulltime physician (BES) and it provides medical service to an estimated 20,000 people who live within a six day walk. Since 1973 the hospital has used a mobile medical unit to make visits every six weeks to hill tribe villages up to three day walk from the road.

In the third week of March 1974, an increase in respiratory disease was reported in Karen villages. The onset of the outbreak was temporally related to a two day meeting of the Karen Baptist Association (KBA) which was attended by an estimated 300 residents of Karen villages. The meeting was held in the village of Mae Hae, located approximately 38 Km northeast of Mae Sariang, 10 hours on foot from the nearest road (Figure 2). This village is composed of 40-50 houses with an estimated population of approximately 280 residents. At the time of the meeting 60–75 residents (23–27 %) of the village were acutely ill with respiratory symptoms. Many people in surrounding villages also had acute respiratory disease and one village reported seven deaths. Over the two weeks following this meeting 237 patients were seen by the CMU in villages north of Mae Sariang. Many of these were people who had been present at the KBA meeting including one of the CMU staff.

On 5 April the SEATO Medical Research Laboratory was requested by the staff of the CMU to help determine the etiology of this epidemic.

DESCRIPTION: From 7–9 April 1974 a field team was deployed from the SEATO Medical Research Laboratory to substantiate reports of increased respiratory disease among the Karen people.

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Clinical studies: People were examined in villages selected along migration routes so as to monitor past and current disease along these routes. Four villages were selected to the north and three villages to the south of the Mae Sariang — Chiang Mai road (Figure 2). The clinical presentation of the illness was determined by interviewing and examining sick patients with respiratory symptoms. Clinical samples were taken from all people examined and blood was obtained for serology.

Laboratory studies: Throat washes or swabs were obtained on all patients for virus isolation. The techniques for isolation and identification of influenza viruses have appeared elsewhere (1). Briefly, viruses were isolated in embryonated chicken eggs and primary monkey kidney (MK) tissue culture (*Macaca mulatta*). The presence of virus was recognized by hemagglutination or hemadsorption of guinea pig red blood cells. Prototype strains of previously isolated influenza viruses were obtained from Dr. Franklin H. Top, Jr., Walter Reed Army Institute of Research, Washington, D.C. Specific antisera to both the isolates and the prototype strains were prepared in roosters. Isolates were identified by hemagglutination inhibition (HI) using eight hemagglutinating units of antigen and homologous and heterologous rooster antisera. Neutralization tests used 100 TCID₅₀ of virus and quantitative neutralization tests were done using 10 fold dilutions of each virus against dilutions of rooster antisera (2).

Blood was obtained for serology on all patients; sera were tested for antibody to influenza by an HI test. Blood smears were obtained on older patients for estimation of the white blood count (WBC) and differential counts. Blood cultures were taken when indicated and throat swabs were obtained from all patients for bacteriological culture.

PROGRESS:

Distribution of cases: Among villages surveyed, disease was evident only in those to the north of the road between Mae Sariang and Chiang Mai. The inhabitants of these villages were largely Christian. Residents of each had attended the KBA meeting and many had developed respiratory disease during the meeting or shortly after returning to their homes. At the time of the survey there was a marked increase in respiratory disease among infants and children; however, respiratory disease among older people was reported to have occurred approximately two weeks earlier. Village headmen estimated that between 10 and 40% of the people in four villages had recently been sick. People in the three villages south of the road were not Christians. No one from these villages had attended the KBA meeting and there was no respiratory disease seen or reported for several months prior to the time of the survey in these villages.

Characteristics of illness: The clinical presentation of the illness was determined by interviewing and examining 25 patients. People of all ages were sick (Table 1). Twelve of the 25 were less than 10 years old and the oldest was 46 years old. Patients seen were said to have been ill from one to 17 days. All had a history of fever and the older ones complained of headache, malaise and prostration. All developed a characteristic hacking cough which in some cases was productive of sputum. The majority had hyperemic throats, and one child had a mild exudative tonsillitis. Eight of the 25 patients had chest findings ranging from scattered ronchi to evidence of consolidation. In 13 patients studied, white blood counts of 10,000 or less were found in nine and differentials showed an absolute lymphocytosis (35–84%) in eight.

Bacterial cultures: Bacterial cultures at blood were obtained from three patients; pneumococcus was recovered from one of these. This individual had first developed illness about one week prior to being seen and had an acute exacerbation of his symptoms eight hours prior to examination. No other bacterial pathogens including beta hemolytic streptococcus were identified in either the blood or the throat cultures.

Virus isolation and identification: Despite difficulties in transportation and storage, nine virus strains were isolated from the pharyngeal secretions of the 25 patients (36%) (Table 1). In one village, isolations were made in six of the eight patients examined. Isolates were easily passed in MK cells or embryonated eggs. No evidence of cytopathogenic effect was noted in the MK cells after as long as 14 days of incubation.

Table 1. Age Distribution of Respiratory Disease and Influenza Isolates of 25 Patients Examined In Northwest Thailand

Age (Years)	Patients Examined	Influenza Isolates
0-9	12	5
10-19	1	0
20-29	3	1
30-39	5	1
≥ 40	4	2
TOTAL	25	9 (36%)

Antisera prepared in roosters against two of the isolates had an HI titer of 1:320 when tested against the homologous antigens and filtered within a two-fold dilution when tested against the other strains (Table 2). These results indicate that there were no significant antigenic differences among the isolates from this epidemic.

To determine the extent of the differences in antigenic configuration between the current strain (Mae-Sarlang/74) and earlier isolated strains, rooster antisera prepared against the isolates and prototype influenza strains were tested against homologous and heterologous viruses. The HI test demonstrated a close relationship between the current strain and prototype A/Port Chalmers/1/73 (Table 3).

It has been suggested that the neutralization test is more sensitive to antigenic variation than is the HI test (2). When antisera were tested by neutralization, a disparity was revealed in the antibody activity of antisera prepared against these strains. Antisera to A/Port Chalmers/1/73 equally neutralized at high titers both the homologous and the current strains. However, when antisera to the current strains were used, neutralization of 100 TCID₅₀ of A/Port Chalmers/1/73 repeatedly required 8-fold more antisera than did the current strain (Table 4). The degree of disparity was not sufficient to differentiate a new influenza strain when analyzed by the method of Archetti and Horsfall (3). These findings, however, do suggest minor antigenic differences between the prototype strain and the present isolate. Quantitative neutralization tests using three viruses against antisera prepared against them substantiated these minor differences (Figures 3 & 4).

HI antibody response: Serum samples were obtained from 25 patients. Unfortunately, due to the remoteness of the area, convalescent samples were not available. In all the individuals from whom virus was isolated the titers were < 1:10. Antibody was present in 14 of the remaining 16 people.

DISCUSSION: That this epidemic was an outbreak of influenza has been amply demonstrated. Influenza virus, closely resembling A/Port Chalmers/1/73 was isolated from 36% (9/25) of the throat secretions collected from acutely ill patients.

The magnitude and extent of the epidemic and the incidence of disease could not be accurately assessed. The population of the hill tribes can only be roughly estimated and the number and distribution of the villages affected is unknown. An incidence of influenza might be inferred from the attack rates reported

for the people of Christian sentinel villages, where 10–15% of the population was said to have been involved.

In Thailand, over the past several years, influenza has usually appeared during September, October or November in Bangkok or at the Royal Thai Air Force Bases. It occurred at a time when resurgence of disease was occurring in other parts of Asia, Europe and North America and was probably introduced into Thailand from these areas. Two epidemics have been studied in the spring when the incidence of influenza was low elsewhere. Both of these were noted first in rural areas; one in Korat in April 1971 and this one in Mae Sariang. We have no information as to the source of this epidemic. The virus may have been introduced into the hills from the central valley of Thailand; a mild outbreak of influenza occurred in Bangkok in October and November of 1973, from which a virus similar to the A/Port Chalmers /1/73 strain was isolated. Alternately, the virus may have spread south through the hills from Burma, Laos or China. Consistent with this hypothesis is the occurrence of respiratory illness in hill tribe villages near Fang, 200 Km to the north of Mae Sariang in February 1974 (personal communication: Prince Pisadej Rachanee, Director, His Majesty's Hill Tribe Project, Chiang Mai, Thailand).

Influenza may have resulted in a recognizable epidemic through a series of unusual and fortuitous circumstances. Rare in itself was the gathering of individuals from many villages at the KBA meeting.

Table 2. Hemagglutination Inhibition Test on Nine Virus Strain from Isolated Patients in Mae Sariang Using Rooster Antisera Prepared to Two of Them

Antiserum Antigen	Reciprocal Hemagglutination Titers	
	SM/898/74	MS/913/74
MS/862/74	320	320
MS/868/74	320	320
MS/871/74	320	320
MS/872/74	320	320
MS/874/74	320	320
MS/877/74	320	320
MS/878/74	320	320
MS/883/74	320	320
MS/886/74	320	320
MS/913/74	320	320
MS/916/74	640	640

Table 3. Comparison by Cross Hemagglutination Inhibition of Current Influenza Strains with Prototype Strains of Previously Isolated Influenza Viruses

Antiserum ^a		Reciprocal Hemagglutination Inhibition Titers					
Antigen ^b	A/MS/868/74	A/P.Chal/1/73	A/Eng/42/72	A/H.K./1/68	A/Jap/305/57	B/Lee/40	
A/MS/868/74	<u>320</u>	320	160	80	<10	<10	
A/P.Chal/1/73	320	<u>320</u>	320	160	<10	<10	
A/Eng/42/72	80	160	<u>160</u>	160	<10	<10	
A/H.K./1/68	80	80	80	<u>320</u>	10	<10	
A/Jap/305/57	40	20	80	80	<u>160</u>	<10	
B/Lee/40	<10	<10	<10	<10	<10	<u>1280</u>	

^a Specific rooster antisera

^b Hemagglutination inhibition test used 8 hemagglutinating units.

Table 4. Comparison by Cross Neutralization of Current Influenza Strains with A Influenza/H₃N₂/viruses

Antigen ^b \ Antisera ^a	Reciprocal of Neutralizing Antibody Titer			
	A/MS/868/74	A/P. Chal/1/73	A/Eng/42/72	A/H.K./1/68
A/MS/868/72	<u>160</u>	160	20	20
A/P.Chal/1/73	20	<u>160</u>	20	20
A/Eng/42/72	20	80	<u>160</u>	20
A/H.K./1/68	20	40	40	<u>160</u>

^a Specific rooster antisera

^b Neutralization tests used 100 TCID₅₀ of the appropriate virus.

The almost exclusive involvement of Christian villages, as opposed to non-Christian villages, implicate this meeting as a point source for the local epidemic. This led to the infection of people from widely scattered villages and ultimately to a simultaneous increase in disease over a large area. The epidemic probably would not have been recognized were it not for the activities of the CMU mobile unit with its program of medical service to isolated villages.

The data collection for this study is complete. We are awaiting the final identification of strains of influenza virus isolated in Bangkok during the summers of 1973 and 1974. Upon receipt of this information this work will be analysed and prepared for publication.

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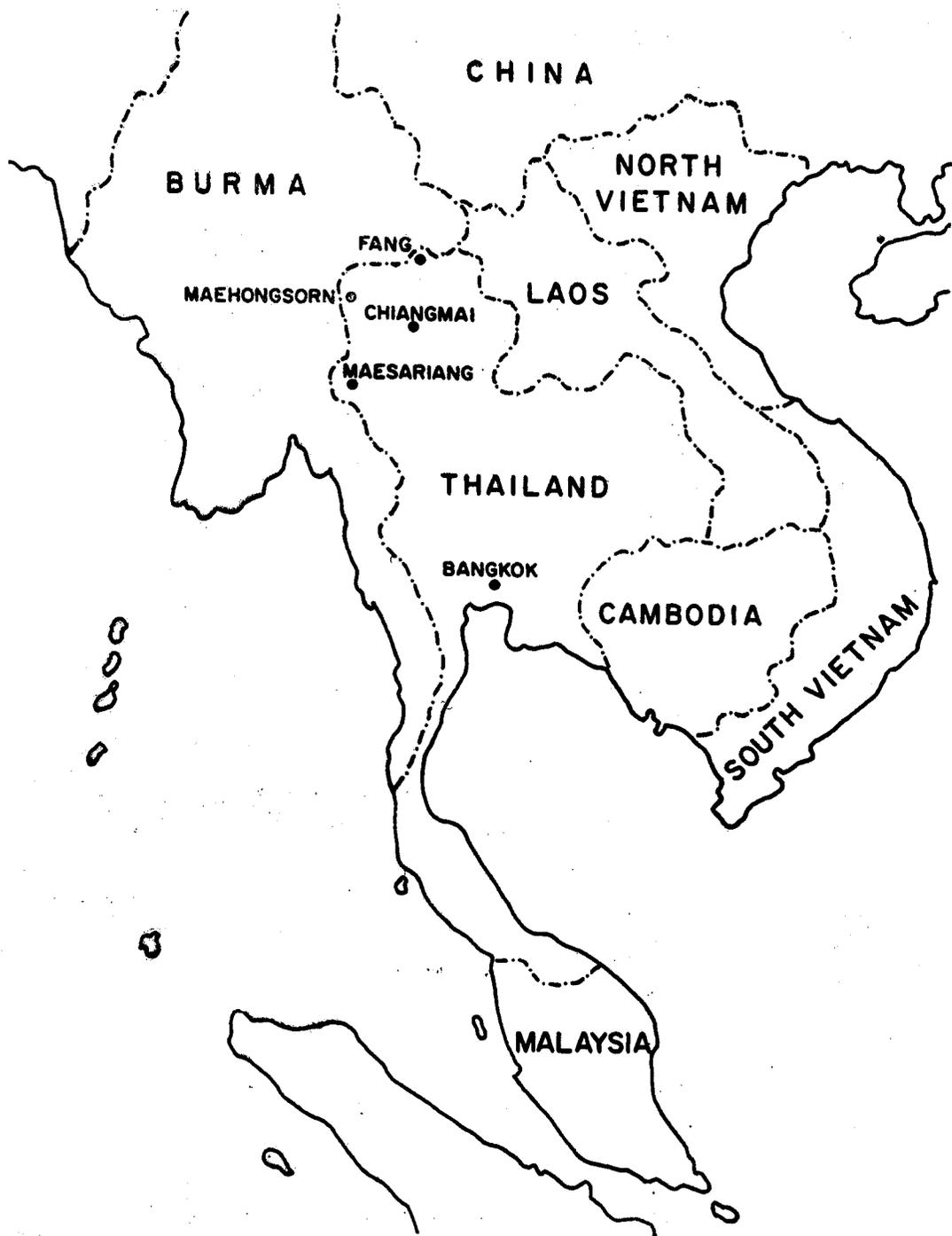


FIGURE 1 MAP OF THAILAND SHOWING THE TOWN OF MAE SARIANG IN RELATION TO CHIENG MAI, MAE HONG SORN, FANG AND BANGKOK.

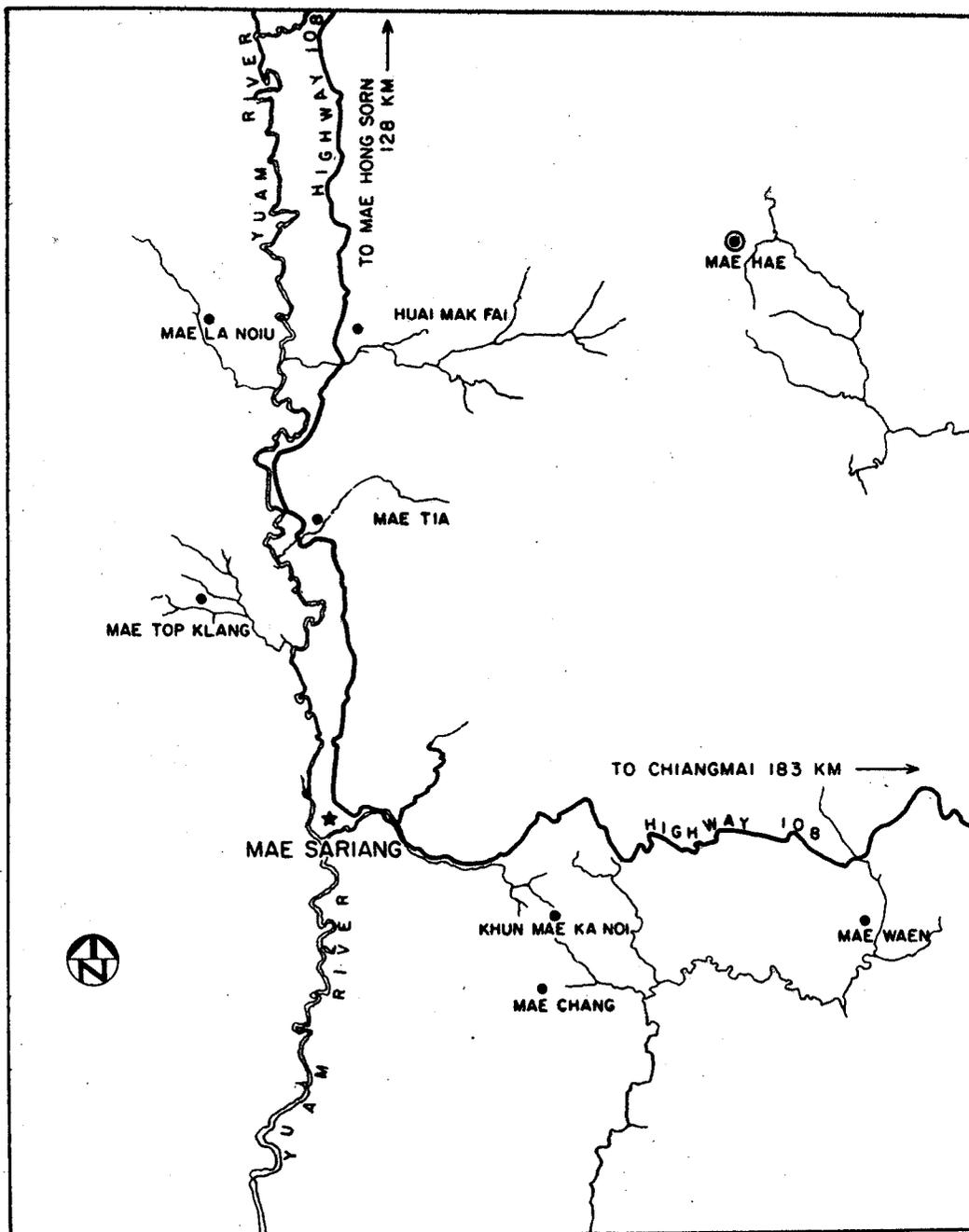


FIGURE 2. LOCATION OF THE TOWN MAE SARIANG (★), IN RELATIONSHIP TO THE YUAM RIVER (=), THE HIGHWAY (—), THE SENTINEL VILLAGES (●) AND THE SITE OF THE MEETING OF THE KAREN BAPTIST ASSOCIATION AT BAN MAE HAE (●).

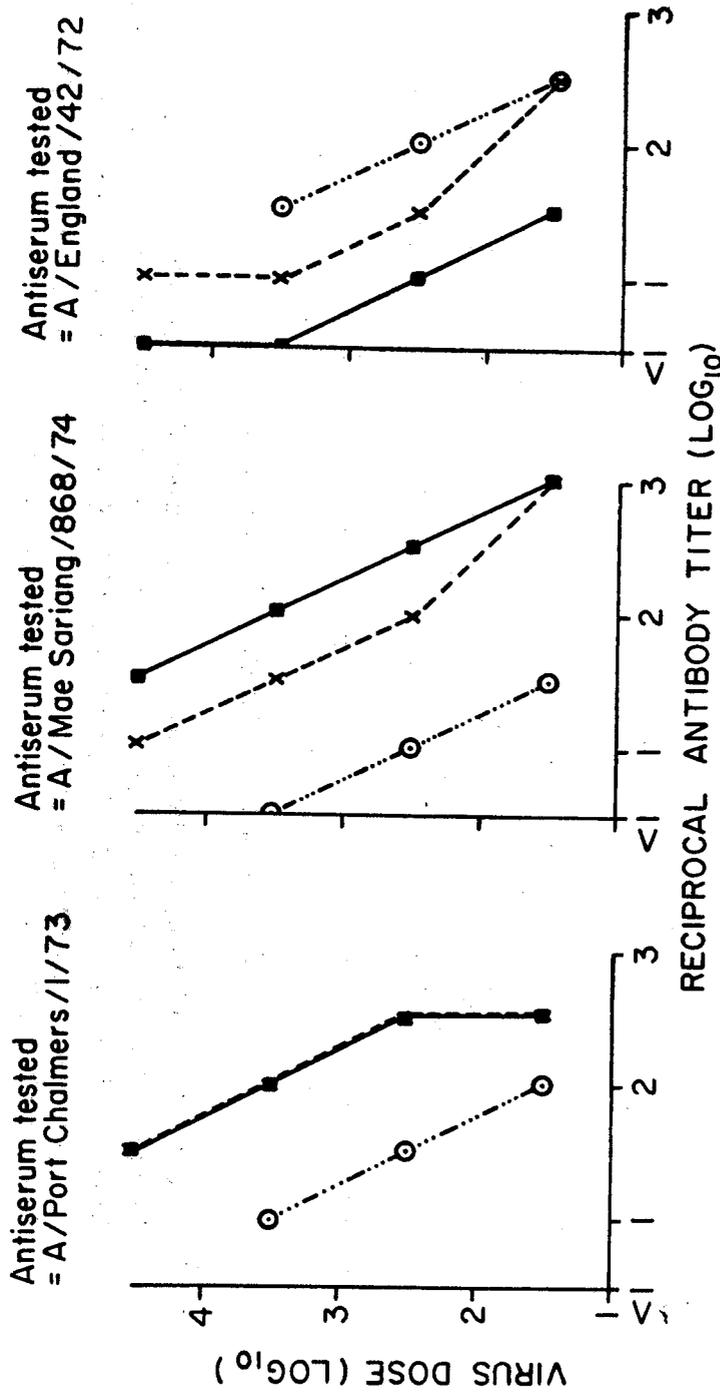


Figure 3. Quantitative relationships between recently isolated A/Influenza/H₃N₂/strains. Comparisons of neutralization reactions of A/Port Chalmers/1/73, A/Mae Sariang/868/74 and A/England/42/72 showing dissimilar patterns of neutralization. Viruses tested x---x A/Port Chalmers/1/73, ■---■ A/Mae Sariang/868/74, ○---○ A/England/42/72

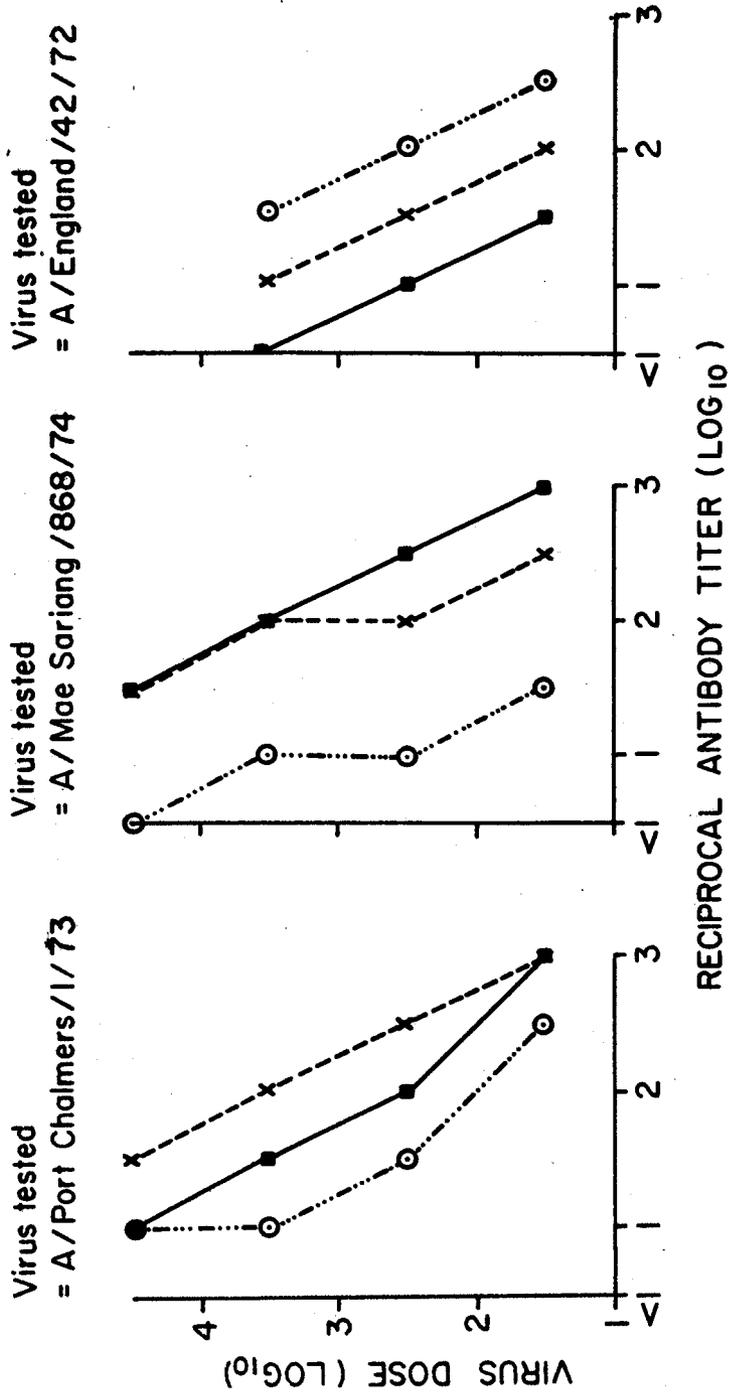


Figure 4. Quantitative relationships between recently isolated A/Influenza/H₃N₂/ strains. Comparisons of neutralization reactions of A/Port Chalmers/1/73, A/Mae Sariang/868/74 and A/England/42/72 showing dissimilar patterns of neutralization. Antisera tested x---x A/Port Chalmers/1/73, ■—■ A/Mae Sariang/868/74, ○—○ A/England/42/72