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Abstract. An investigation of an epidemic of respiratory disease in a remote region of northwest Thailand revealed Influenza A/H_3N_2 as the etiologic agent. This epidemic would not have been recognized were it not for an unusual increase in respiratory disease following a meeting attended by residents of many villages. The influenza strains isolated most closely resembled A/Port Chalmers/1/73 when tested by hemagglutination inhibition but showed consistent antigenic differences when tested by quantitative neutralization. It is suggested that the differences noted between this strain and influenza viruses isolated elsewhere may have been due to the sequential transmission of influenza through partially immune people.

Since the pandemic of 1968, influenza has not been a major public health problem in Southeast Asia. There have, however, been minor outbreaks of disease reported from urban areas,[1-3] and from military installations.[4] There are no descriptions of influenza among residents of rural and isolated portions of Asia. As it has been suggested that the last two pandemic strains of influenza developed in rural Asia,[5,6] the epidemiology of this infection in this environment is worthy of investigation. In the spring of 1974 we had the opportunity to study an outbreak of influenza-like disease in an isolated region of northwest Thailand, among the people of the Karen hill tribe. The results of that study are presented here.

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, 350 meters above sea level (Fig. 1). 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 (Fig. 2). The climate of this region is influenced by the southern monsoon winds, with the wet season from May to October and the dry season from November to March. The people living in the area mostly belong to the Karen hill tribe.[7] They live in small isolated villages of 10 to 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 neighboring villages and rarely, in emergencies, to nearby towns. Educational opportunities are rare and there is little understanding of simple health measures; malnutrition, vitamin deficiency, and parasitic infections are common problems.

The Christian Medical Unit (CMU) of the American Baptist Mission is located in Mae Sariang. It is a ten-bed hospital with one full time physician (BES), and it provides medical service to an estimated 20,000 people who live within 6 days' walk of the town. Since 1973 the hospital has used a mobile medical unit to make visits every 6 weeks to hill tribe villages as far as 3 days' walk from the road.

INITIAL OBSERVATIONS

In the 3rd week of March 1974 an increase in respiratory disease was reported in Karen villages. The onset of the outbreak was temporally related

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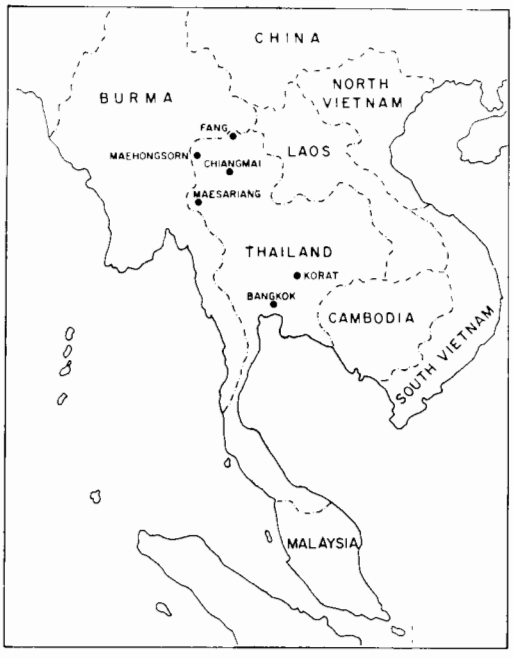

FIGURE 1. Map of Thailand showing the town of Mae Sariang in relation to Chiang Mai, Mae Hong Sorn, Fang, Korat and Bangkok.

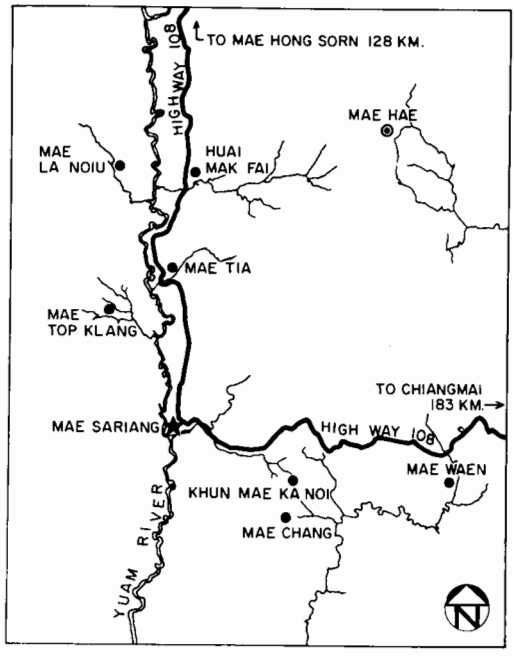

FIGURE 2. Location of the town Mae Sariang ★, in relationship to the Yuam River, the highway ——, the sentinel villages ● and the site of the meeting of the Karen Baptist Association at Mae Hae ⊙.

to a 2-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 (Fig. 2). This village is composed of 40 to 50 houses with an estimated population of 280 residents. At the time of the meeting 60 to 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. During the 2 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.

MATERIALS AND METHODS

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

Clinical studies. In order to monitor disease among the hill tribe people, villages were selected along migration routes. Four villages were chosen to the north and three to the south of the Mae Sariang-Chiang Mai Road (Fig. 2). People in these villages with respiratory symptoms were interviewed and examined. Blood was collected for serology. Throat washes or swabs were obtained for virus isolation using Hanks' balanced salt solution with 0.4% bovine albumin. Throat samples were sealed in plastic tubes, transported to the laboratory on Dry Ice, and stored at −70° C until isolation. Blood smears were prepared on older patients for estimation of the white blood cell count and differential counts. Blood cultures were taken when indicated and throat swabs were obtained from all patients for bacteriological culture. Due to the remoteness of the area no follow-up specimens could be obtained.

Laboratory studies. The techniques for isolation and identification of influenza viruses have appeared elsewhere.[8] Briefly, viruses were isolated in embryonated chicken eggs and primary monkey kidney (MK) tissue culture (*Macaca mulatta*).

TABLE 1

Hemagglutination inhibition cross titrations of influenza virus strains

Antigen*	Reciprocal hemagglutination inhibition antibody titers of antisera† to					
	MS/868/74	A/Pt Ch/1/73	A/Eng/42/72	A/H K/1/68	A/Jap/305/57	B/Lee/40
MS/868/74	320	320	160	80	<10	<10
A/Pt Ch/1/73	320	320	320	160	<10	<10
A/Eng/42/72	80	160	160	160	<10	<10
A/H K/1/68	80	80	80	320	10	<10
A/Jap/305/57	40	20	80	80	160	<10
B/Lee/40	<10	<10	<10	<10	<10	1,280

* Hemagglutination inhibition test used eight hemagglutinating units.
† Specific rooster antisera.

The presence of virus was recognized by hemagglutination or hemadsorption of guinea pig red blood cells. Reference 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 reference strains were prepared in roosters.[8] Isolates were identified by hemagglutination inhibition (HI) using eight hemagglutinating units of antigen and homologous and heterologous rooster antisera. Neutralization (N) tests used 100 $TCID_{50}$ of virus and quantitative neutralizations were done using dilutions of each virus against dilutions of rooster antisera.[9] Comparisons between influenza strains utilized the method reported by Schild,[10] and the degree of cross-immunological relationship was calculated by the method of Hilleman and Horsfall.[11] Sera were separated from blood within 4 hours of collection, and antibodies to influenza viruses were detected by HI.

RESULTS

Among villages surveyed, disease was evident only in those to the north of the road between Mae Sariang and Chiang Mai (Fig. 2). Most of the inhabitants of these villages were Christians. Residents of each affected village 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 an increase in respiratory disease among infants and children was observed; however, respiratory disease among older people was reported to have occurred approximately 2 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 reported in these villages for several months prior to the time of the survey.

The clinical presentation of the illness was determined by interviewing and examining 25 patients. 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 rhonchi to evidence of consolidation. In 13 patients studied, white blood cell counts of 10,000 or less were found in nine and differentials showed an absolute lymphocytosis (35–84%) in eight.

Despite difficulties in transportation and storage, nine hemagglutinating viruses were isolated from pharyngeal secretions of the 25 patients (36%). Isolations were made in 6 of 8 patients in one village. The viruses were easily passed in embryonated eggs or MK cells. Cytopathogenic effect was not seen in MK cells after as long as 14 days of incubation. The nine viruses were homogenous by HI and N using rooster antisera prepared against two of the isolates.

The identity of this virus (Mae Sariang/74) was established using rooster antisera against the isolates and reference influenza strains. Cross HI

TABLE 2

Neutralization cross titrations of influenza virus strains

Antigen*	Reciprocal neutralizing antibody titers with antisera† to			
	MS/868/74	A/Pt Ch/1/73	A/Eng/42/72	A/HK/1/68
MS/868/74	160	160	20	20
A/Pt Ch/1/73	20	160	20	20
A/Eng/42/72	20	80	160	20
A/H K/1/68	20	40	40	160
A/Bangkok/2/73	20	160	20	ND‡
A/Bangkok/1/74	20	320	20	ND

* Neutralization tests used 100 $TCID_{50}$ of the appropriate virus.

† Specific rooster antisera.

‡ ND, not done.

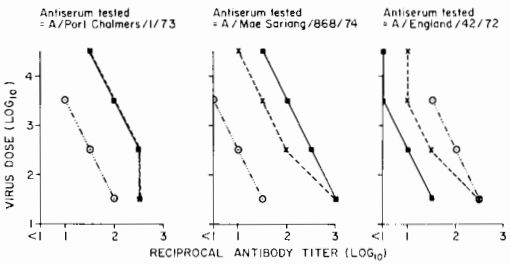

FIGURE 3. Quantitative relationships between recently isolated A/Influenza/H_3N_2/strains. Comparisons of neutralization reactions of A/Port Chalmers/1/73, A/Mae Sariang/868/74 and A/England/42/72 showing dissimilar patterns of neutralization. Viruses tested ×----× A/Port Chalmers/1/73, ■——■ A/Mae Sariang/868/74, ⊙—••—•⊙ A/England/42/72.

and N tests identified Mae Sariang/74 as influenza type A/H_3N_2 (Tables 1 and 2). Antigenic similarity between reference A/England/42/72, A/Port Chalmers/1/73 and Mae Sariang/74 was suggested by the HI tests (Table 1). The N test has been reported to be more sensitive than the HI test for detecting antigenic differences between influenza strains.[8] When antisera were tested by this method antigenic disparities were revealed between A/England/42/72, A/Port Chalmers/1/73 and Mae Sariang/74. A differentiation could easily be made between A/England/42/72 and the other two strains. Despite the neutralization of both strains equally by antisera to A/Port Chalmers/1/73, antisera to Mae Sariang/74 repeatedly titered eightfold higher against homologous Mae Sariang virus than it did against heterologous A/Port Chalmers/1/73 virus (Table 2). This suggested an antigenic difference between A/Port Chalmers/1/73 and Mae Sariang/74,[10] with a cross-immunologic relationship between the two strains of 35%.[11]

To further explore the differences between Mae Sariang/74 and A/Port Chalmers/1/73 quantitative neutralization studies were performed (Fig. 3). A difference could be shown between the two strains when dilutions of antisera to A/England/42/72 or Mae Sariang/74 were tested. Dilutions of antisera to A/Port Chalmers/1/73, however, could not differentiate between the two strains. These results suggest that the Mae Sariang/74 virus has antigenic characteristics between those of A/England/42/72 and A/Port Chalmers/1/73 and that the Mae Sariang strain may represent an intermediate step in the antigenic drift from A/England/42/72 to A/Port Chalmers/1/73.

The Mae Sariang/74 and reference influenza antisera were also tested for neutralization against two virus strains isolated in Bangkok. A/Bangkok/2/73 and A/Bangkok/1/74 were isolated in October 1973 and July 1974, respectively, and were supplied to us by Dr. Kanai Chatiyanonda, WHO National Influenza Center for Thailand. Both Bangkok strains were found to resemble the reference A/Port Chalmers/1/73 strain more closely than the Mae Sariang isolates (Table 2). This suggested that infections occurring in Bangkok were not related to the epidemic in northwestern Thailand.

Antibody to Mae Sariang/74 was found in the sera of 10 of the 16 people from whom no virus could be isolated. No antibody against this virus could be identified in the nine people who yielded virus; however, three of this group did have antibody to the previously epidemic influenza strain A/Hong Kong/1/68.

Blood cultures for bacteria were obtained from only three patients; *Streptococcus pneumoniae* was recovered from one of these. This individual had developed illness about 1 week prior to being seen and had an acute exacerbation of his symptoms 8 hours prior to examination. A high HI titer of antibody against Mae Sariang/74 virus was found in his serum. No other bacterial pathogens, including beta hemolytic streptococcus, were identified in either the blood or the throat cultures.

DISCUSSION

In Thailand, over the past several years, influenza has usually been reported during September, October or November in Bangkok[1-3] or at military bases up-country.[4] These outbreaks occurred at a time when resurgence of disease was occurring in other parts of Asia, Europe and North America and were probably introduced into Thailand from these areas. Two isolated 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 (Fig. 1) in April 1971 (R. Snitbhan, unpublished data) and this one in Mae Sariang. That this epidemic was an outbreak of influenza has been amply demonstrated. Influenza A/H_3N_2 viruses were isolated from the throat secretions of people who were acutely ill. These viruses showed antigenic differences from both A/England/42/72 and A/Port Chalmers/1/73; but their configuration was more closely related to the latter. The differences demonstrate the antigenic variability among influenza A/H_3N_2 viruses that was described by Schild et al.[12] and suggest that the Mae Sariang viruses are intermediate between the two reference strains.

We have no information as to the source of this epidemic. The virus may have been introduced into the hills from the central plains of Thailand. A mild outbreak of influenza occurred in Bangkok in August 1972 during which viruses similar to A/England/42/72 were isolated;[1] however, the virus isolated in October 1973 from Bangkok was more closely related to A/Port Chalmers/1/73 than to the A/England/42/72 or the Mae Sariang strain. Alternatively, the virus may have spread south through the hills from Burma, Laos or China. Consistent with the latter hypothesis was a report of respiratory illness in hill tribe villages near Fang (Fig. 1), 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 circumstances. Rare in itself was the gathering of individuals from many villages at the KBA meeting. The almost exclusive involvement of Christian villages, as opposed to non-Christian villages, implicate this meeting as a point source for the local epidemic. The presence of disease at the KBA meeting led to the infection of people from widely scattered villages and ultimately to a simultaneous increase in disease over a large area. Further, 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.

Following the acute outbreak in March and April, only occasional cases of influenza-like disease were reported to the CMU. Due to poor communications it is impossible to say whether the transmission of virus ceased or whether it resumed the slow progression which apparently occurred prior to the March 17th meeting. Sequential passage of influenza through partially immunized people may permit changes to occur in the viral antigenic characteristics.[5, 13] The antigenic differences noted between the Mae Sariang strains and the strains found elsewhere may have resulted from such a mechanism.

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