

## SEROLOGIC DISCOVERY OF AN INAPPARENT OUTBREAK OF INFLUENZA IN MALAYSIA\*

S K LAM MSc, PhD\*\* A RUDNICK M S, PhD\*\*\* AND V RAJAGOPAL MBBS\*\*\*\*

### Summary

As part of a rural dengue fever study, paired serum specimens were routinely collected from trainees residing at a youth training centre in Selangor, who presented with pyrexias of undetermined origin. In June 1976, there was an unusual increase in such cases. After completing serologic and virologic studies, we could not significantly associate dengue or other arboviruses with the fevers. Although influenza was not originally suspected, further serologic tests revealed that influenza A/Victoria was the cause of the outbreak. This finding emphasises the need for laboratory support in the diagnosis of undifferentiated fevers.

As part of an ongoing study of arbovirus infections in Malaysia, the Arbovirus Research Unit, University of California International Center for Medical Research, arranged with the medical unit of the Dusun Tua Youth Training Centre, Selangor, to obtain paired serum samples from trainees who developed pyrexias of undetermined origin (PUO). This centre is situated in the Sungei Langat Valley, 16 miles south of Kuala Lumpur. It occupies 64 acres of rural land and conducts residential courses for youths between 15 and 25 years of age.

In June 1976, there was a noticeable increase in PUO cases at the centre. The outbreak continued for approximately 1 month. Patients often had fevers as high as 41°C. Cough, headache and sore throat were sometimes reported. Patients recovered rapidly, usually within 2 or 3 days. Influenza was not suspected and paired serum samples were obtained for arbovirus serology. When laboratory tests for arbovirus infection proved to be negative in all but two patients, the samples were tested for antibodies against influenza antigens. At the time of the outbreak, there were approximately 800 trainees from all over Malaysia residing at the centre.

In Malaysia, there are two peak periods for

influenza activity annually. The first period usually begins in May and lasts for about 10 weeks. The second period starts in mid-November and extends into the early weeks of January. At the time of the Dusun Tua outbreak, influenza A strains antigenically related to A/Victoria/3/75 were being isolated sporadically from patients in Kuala Lumpur. This antigenic strain of influenza A was first isolated in Malaysia in December 1975<sup>1</sup>, and was responsible for a moderately severe outbreak. The clinical symptoms associated with this virus were rather mild: the illness began with a cough, followed by low-grade fever and marked dyspnoea. Patients took 10 to 14 days to recover. The clinical picture was quite distinct from that reported for patients at the centre.

### MATERIALS AND METHODS

Acute and convalescent serum samples were obtained from 64 patients with PUO and single acute samples from 4 patients with PUO of the total population of 800 trainees at the centre. The acute samples were taken a day or two after onset of illness and the convalescent samples 2 to 3 weeks later. We attempted to isolate arboviruses from all acute specimens.

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\*\*Associate Professor, Department of Medical Microbiology, University of Malaya, Kuala Lumpur, Malaysia, (Address for reprint requests).

\*\*\*Adjunct Professor and Program Director, University of California International Center for Medical Research, University of California San Francisco, California 94143, USA.

\*\*\*\*Medical Officer, Dusun Tua Youth Training Centre, Selangor, and Sports Medical Unit, Ministry of Culture, Youth and Sports, Kuala Lumpur, Malaysia.

Arbovirus serology was performed on all specimens from the 68 patients, and influenza serology on specimens of 61 of the 64 patients from whom pairs were obtained.

Specific influenza reference antisera were supplied by the World Health Organization (WHO). Arbovirus control antisera were prepared in mice by a series of five intraperitoneal inoculations of live or formalinised mouse-brain vaccine for each antigen used.

#### *Virus isolation*

Each acute serum specimen was diluted 1:4 in 0.75% bovine plasma albumin in borate saline before inoculating 0.01 ml intracerebrally and 0.03 ml intraperitoneally into 2 litters of 1- to 2-day-old Swiss albino mice. The mice were observed for signs of illness for 21 days. Brains were harvested from all sick mice, suspended and inoculated into new groups of mice. When no illness developed by day 8 postinoculation, brains were harvested from two mice and stored at  $-70^{\circ}\text{C}$  pending results of dengue challenge tests. Mice surviving for 4 weeks were challenged with 100 LD<sub>50</sub> of dengue type 2 virus to determine if immunity had developed as a result of the original inoculum. If challenge resistance occurred, the blind-harvested brains were to be serially passed in mice to adapt the virus isolate to the stage where it could be identified in further tests.

#### *Serology*

Haemagglutination inhibition (HI) tests

*Arboviruses:* The HI test was performed according to the method of Clarke and Casals<sup>2</sup> adapted to the microtitre method of Sever.<sup>3</sup> Male goose erythrocytes were employed. Sera were extracted with acetone and adsorbed with goose cells prior to testing. The following nine antigens, extracted with sucrose-acetone, were tested against serial 2-fold serum dilutions: dengue 1 (Hawaiian), dengue 2 (Trinidad 1751), dengue 3 (H-87), dengue 4 (H-241), Japanese encephalitis (Nakayama), tembusu (AMM-1775), and Zika (B24982) in serogroup B; chikungunya (original) and Sindbis (P-886) in serogroup A. Serum titres of 1:20 and greater were considered positive for the presence of antibody. All of these viruses are known to be present in Malaysia, except chikungunya, which is active in neighboring countries.

*Influenza:* The microtitre HI system for influenza recommended by WHO was used. Serial twofold dilution of each serum were tested against four haemagglutinating units of the viral antigens. To a mixture of 0.025 ml virus and 0.025 ml serum, 0.05 ml of a 0.7% suspension of human group 0 red blood cells was added. The usual controls and a back titration of the viral antigens were included in each run. The plates were incubated at room temperature for one hour before readings were taken. HI titres of 1:10 and greater were considered positive.

The influenza antigens employed were A/Victoria/3/75, A/Port Chalmers/1/73, A/New Jersey/8/76 and B/Hong Kong/5/73. A/Port Chalmers was the prevalent strain in Malaysia<sup>4</sup> prior to the appearance of A/Victoria in 1975. A/New Jersey, commonly referred to as swine influenza virus, was responsible for a localised outbreak of influenza in a military camp in New Jersey, in the United States, in 1976, but because of its antigenic similarity to the strain responsible for the 1918 pandemic, all WHO centres were requested to include this antigen in serologic testing. All the antigens were standardised to four haemagglutinating units for the HI test.

#### Plaque-reduction neutralisation test (PRNT)

Serum samples from 16 patients were tested for PRNT antibody to Umbre virus, strain IG 1424. Umbre is a member of the Turlock serogroup of arboviruses and does not produce a haemagglutinin. It was previously isolated from chickens and mosquitoes at the youth centre. The test was performed by the method of Russell et al<sup>5</sup> with the following modifications. The VERO cell line in the 254th passage was used in six-unit multidishes with 35 mm diameter wells. Minimal Essential medium with 10% foetal calf serum was used as the growth medium, with the addition of Eagles' overlay medium containing glutamine. The incubation period was 1 hour at room temperature and absorption was carried out for 1 hour at  $37^{\circ}\text{C}$ . A second agar overlay was added on day 7. Plaques were counted on day 9 postinfection.

#### RESULTS

No viruses were isolated in suckling mice from the acute-phase serum of any of the 68 patients. Results of arbovirus HI and PRNT serology were negative for 62 patients and in-

conclusive for the four patients from whom only acute-phase serum was obtained. Paired sera of two patients showed a fourfold or greater increase in arbovirus serogroup B antibodies, indicating that infection with one of the viruses of the group had occurred.

In the HI test against influenza antigens, 55 (90.2%) of the 61 patients tested showed a fourfold or greater increase in titres against A/Victoria antigen, including the two who were shown to be positive for serogroup B arbovirus antibodies. None of the sera reacted against A/New Jersey antigen nor was there a significant increase in titres against B/Hong Kong. Forty-two (76.3%) of the 55 positive patients also showed an anamnestic response to A/Port Chalmers.

#### DISCUSSION AND CONCLUSIONS

Although isolation of influenza virus was not attempted from trainees at the centre (because that phase of the study had been retrospective), several strains of A/Victoria virus were isolated from students at a university in Kuala Lumpur during the same period. This lends support to the conclusion, based on the positive serology, that the outbreak at the centre was caused by this virus.

None of the sera from the young trainees, ranging in age from 17 to 25 years, reacted with A/New Jersey antigen. In March 1975, 30 sera from young healthy blood donors tested for antibodies to a similar virus (A/Mayo Clinic/103/74) were all negative.<sup>6</sup> However, 76% of this series of 30 were positive for A/Port Chalmers antibodies.

A/Port Chalmers virus was first isolated in Kuala Lumpur in 1974<sup>6</sup> and was the predominant strain responsible for influenza outbreaks until it was replaced by A/Victoria in December 1975. The anamnestic response to A/Port Chalmers of many of the patients at the centre might account for the differences in the clinical

picture seen.

In Malaysia, arboviruses are responsible for many undifferentiated fevers. Our data show that influenza A/Victoria virus was the cause of one such febrile outbreak, and recent studies by Brown et al<sup>7</sup> revealed that in rural areas, scrub typhus is a common cause of fevers that could not be diagnosed clinically. Such findings emphasise the importance of using laboratory diagnostic procedures for differential diagnosis of fevers when clinical diagnosis fails to discern their cause.

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