TYPE B INFLUENZA IN SINGAPORE

By M. Yin-Coggrave
(Dept. of Bacteriology, University of Singapore.)
and
Z. N. Kadri
(Dept. of Student Health, University of Singapore)

A presumptive diagnosis of influenza can be made on clinical grounds in acute and short-termed febrile illness characterised by respiratory signs, headaches, fever, malaise, muscle and bone aches. However, the definitive diagnosis and actual incidence can only be made by virus isolation or serological tests. Although strains of Asian (A2) influenza virus have been isolated on several occasions since their appearance in Singapore in 1957 (Lim et al. 1957), no isolation of type B influenza was reported until recently when an outbreak occurred in a university student population.

MATERIALS AND METHODS

Collection of Specimens

Each patient was given 10 ml. of Lemco broth containing antibiotics (500 units penicillin and 500 μgm. streptomycin per ml.) for throat garglings. The garglings were collected in sterile screw-capped bottles and stored frozen at —20°C.

Egg Passages

A 0.25 ml. of throat garglings was inoculated into the amniotic cavity of each 10 days old embryonated (hen) eggs. A batch of four eggs was used for each specimen. The eggs were incubated at 37°C. and candled daily. The amniotic fluid from each egg was collected on the day the embryo died or 3-4 days after inoculation.

Haemagglutination Tests

A spot test for haemagglutination (HA) was carried out for each sample of amniotic fluid collected by adding 2 drops of 1 per cent red blood cells (RBCs) to 2 drops of amniotic fluid in a perspex cup at room temperature and reading the result after 30 minutes. Amniotic fluids which agglutinated fowl and/or guinea pig RBCs were passaged three times. Those which attained high HA titers were then identified by haemagglutination-inhibition (HI) technique described in the World Health Organisation Technical Report Series (1959). Amniotic fluids which failed to agglutinate the RBCs were discarded.

Antigens and Immune Sera

The prototype antigens (A2/Asia/'57 and B/Johannesburg/'58) and immune sera used in the identification of the isolates were supplied by the World Health Organisation.

RESULTS

Clinical Findings

The ninety cases reported here were seen as out-patients at the University Clinics. Diagnosis in these cases rests on the clinical picture they presented and on the epidemiological evidence of their occurrence.

Table I depicts the clinical manifestations. The patients ranged from eighteen to twenty-three years of age. Fever in the majority was associated with sensations of cold or chilliness. Their raised temperatures on clinical examinations ranged from 37.5°C. to 40°C. In some cases, the relatively low temperature recordings could be attributed to their having taken anti-pyretic drugs prior to seeking treatment. On the average, the febrile illness lasted five days, but it ran a biphasic course; that is to say, the disease was interposed by an interval of 24 to 48 hours during which the patients were afebrile and felt as if they were on their way to recovery.

Seventy-two patients complained of varying degree of headache, and fifty-four had myalgia which was either generalised or confined to the back or lower limbs. Symptoms

TABLE I

ANALYSIS OF CLINICAL MANIFESTATIONS OBSERVED IN NINETY PRESUMED CASES OF INFLUENZA DUE TO TYPE B VIRUS

Fever	90	Nausea	36
Nasal Catarrh	48	Lassitude and Lethargy	42
Cough	30	Drowsiness	12
Headache	72	Anorexia	24
Myalgia	54	Congested or Inflamed Throat	90
Giddiness	24	Palpable Submandibular Lymphnodes	66

TABLE II

HAEMAGGLUTINATION-INHIBITION TESTS

FERRET ANTISERA

Isolates	**B/Lee**	**B/Bon**	**B/Eng/ 9/54**	**B/Eng/ 1/64**	**B/Amakusa/ /1/64**	**B/Johannes- burg/33/58**	**B/Singa- pore/3/64**	**B/Taiwan/ 4/62**
B/Lee	+1280	20	*—	—	—	—	—	—
B/Bon	240	480	—	—	—	—	—	—
B/England/9/54	—	—	30	30	30	—	—	—
B/England/1/64	—	—	15	320	960	60	40	15
B/Amakusa/1/64	—	—	15	240	1280	40	40	—
B/Johannesburg/ 33/58	—	—	—	80	320	480	160	40
B/Singapore/3/64	—	—	—	120	160	120	1280	20
B/Taiwan/4/62	—	—	—	—	—	—	—	240

+Receiprocal of highest dilution of serum showing inhibition.

* — = < 10

of malaise, lassitude and lethargy affected forty-two patients. Stuffiness of nose or rhinorrhoea was observed in forty-eight cases and dry irritating cough was present in thirty cases. Thirty-six patients experienced feelings of nausea, twenty-four dizziness, and twelve felt slightly drowsy during the early stage of illness.

On physical examination, all the ninety individuals showed signs of congestion of varying degrees of inflammation of throat. The submandibular lympn nodes were enlarged and tender in sixty-six cases. In several cases, the heart rate was accelerated. No significant positive findings were elicited in the lungs. Hospitalisation was not required in any case nor were any complications encountered.

Virus Isolation

Nineteen specimens of throat garglings were inoculated into batches of eggs. Amniotic fluids collected from 8 batches gave positive spot haemagglutination with guinea-pig RBCs. After three amniotic passages, one isolate gave a HA titer of 1 in 160, two a titer of 1 in 320 and one a titer of 1 in 640. These viruses produced good HA titers on allantoic passages. Amniotic fluids from 4 batches of eggs had HA titers of less than 1 in 10 and did not increase in titer on allantoic passage. The HA titers for fowl RBCs were generally two to fourfold lower than for the guinea-pig RBCs in the first two passages. No difference in this affinity for the latter was detected on subsequent amniotic or allantoic passages.

Identification of Isolates

The HI tests for 4 of the 8 haemagglutinating agents showed them to be influenza B virus. Although the influenza B immune sera inhibited the agglutination of these 4 isolates, the inhibition was markedly less effective than for the prototype B/Johannesburg/33/'58 strain.

Antigenic Comparison with other Influenza B Strains

Two of our Singapore influenza B isolates sent to WHO, World Influenza Centre in Mill Hill, London, were compared with type B strains from other countries. The Singapore strains proved to be antigenically similar to but not identical with the prototype B/Johannesburg/33/'58 strains (Table II).

DISCUSSION

The Singapore influenza B isolates were found to be antigenically similar to but not identical with the prototype B Johannesburg strains. The difference however is not considered sufficient to justify the designation of the Singapore strains as a new subtype. It is noteworthy that the Singapore strains are also antigenically different from those isolated previously in Malaya whose haemagglutinability were inhibited by sera to B/Bon and B/England/9/'54 (Gordon Smith and Thompson, 1956).

Although the Asian influenza epidemic which struck Singapore in 1957 was said to be due to the spread of the A2 influenza virus from epidemics in Taiwan and Hong Kong, it is apparent that the Influenza B outbreak here is not related to the prior occurrence of Taiwan influenza B outbreak reported by Green et al. in 1962 (a year previously). As shown in Table II, immune sera against the Singapore strains inhibited the haemagglutinability of the Taiwan strains, the haemagglutinability of the Singapore strains were not affected by immune sera prepared against the Taiwan strains. The latter has since been designated as influenza B2. Variations in the antigenic characters seen in the influenza A virus are clearly seen also to occur among the type B.

In influenza epidemics that have been studied in the past, it has been observed that young people showed much higher incidence of morbidity than those above the age of thirty years. It is easy to see why the university student population is potentially susceptible to this infection. Prolonged continuous contacts between individuals during their course of studies exposed them to a hazard of rapid spread of infection. Thus influenza which is noted for its ability to spread rapidly from person to person once introduced into such a community immediately results in rapid and wide spread morbidity and sickness absenteeism.

SUMMARY

This is the first report of the isolation of influenza B virus in Singapore during an outbreak in December 1963—February 1964. The Singapore influenza B isolates were found

to differ slightly in antigenicity from the prototype B/Johannesburg/'58 strain.

An analysis of the clinical picture of cases studied is presented.

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