replace the detailed appraisal of each individual case but can provide an overall picture of the intensity of disabilities and the proportions of patients in each range of disability in a " mass " campaign or in a control programme.

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Hong Kong Influenza in the Sudan

by A. R. SALIM,[1]

Influenza-like diseases have been known to occur in the Sudan for a long time, but the true nature of these diseases has not been investigated. In 1957, there was an epidemic of an influenza-like disease which physicians attributed to the Asian influenza type of virus that was causing epidemics in other countries at that time. However, no virus isolations or serological studies were performed in the Sudan at that time.

In the winters of 1968–69 and 1969–70, there were epidemics of an influenza-like disease throughout the country. Laboratory investigations were carried out to establish the nature of the causative organism(s) of these epidemics.

The epidemic of 1968–69

During the summer months of 1968, there were no reports of influenza-like diseases in the country. Such diseases were first observed in December. In February 1969, there was a sudden increase in the number of patients with influenza-like disease and eventually a large proportion of the population in all age-groups was affected.

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In Khartoum, a town with a population of about 100 000 persons, all the out-patient departments in hospitals and health centres are attended by qualified medical officers and the incidence of influenza-like diseases in the period from January 1968 to December 1969 is shown in Table 1. These figures represent the number of patients who were sufficiently ill to seek medical advice. The epidemic began to die out gradually in July 1969

The epidemic of 1969–70

Few cases of influenza-like diseases were reported during the period from June to September 1969. In December 1969, the number of cases began to rise and by January 1970, reports came from all parts of the country of an epidemic of an influenza-like disease. In Khartoum, the epidemic reached its maximum intensity in the second week of January 1970 and then began to subside gradually, until it died out at the end of May 1970 (see Fig. 1). The epidemic was more widespread and the cases were more severe than in the previous winter; the monthly incidence of influenza-like diseases reported in Khartoum town are shown in Table 1.

There were very few complications from these two epidemics; this is indicated by the absence of a

Table 1. Monthly incidence of influenza-like diseases in Khartoum, 1968-70

Year	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
1968	13	10	73	103	98	140	128	0	0	0	0	12
1969	0	4 145	1 016	1 267	395	197	98	193	112	234	237	237
1970	6 333	3 139	616	227	174	157	120					

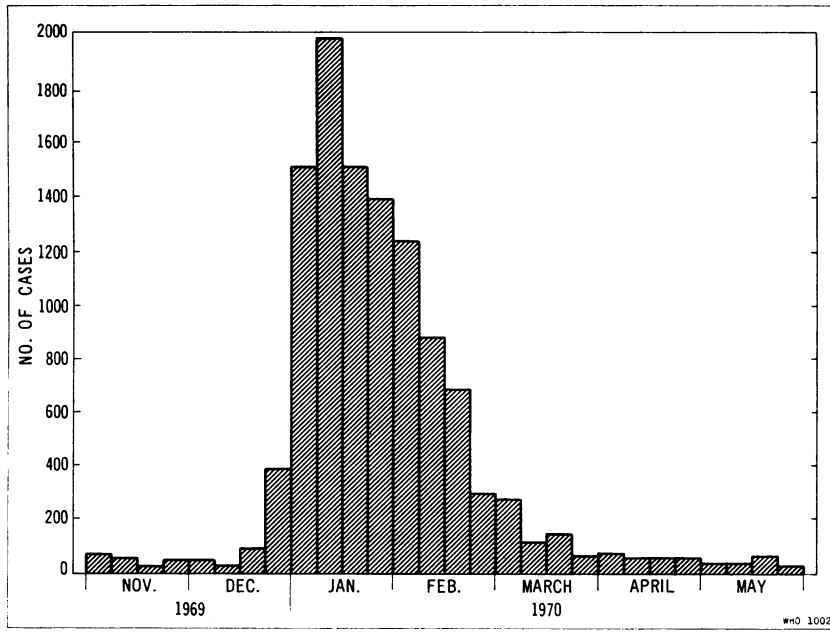

Fig. 1. Weekly incidence of influenza-like diseases in Khartoum from November 1969 to May 1970

significant rise in admissions of pneumonia cases in the Khartoum hospitals and the absence of a significant rise in mortality from respiratory diseases.

Materials and methods

Laboratory investigations. During January 1970, at the peak of the epidemic, students at the University of Khartoum were selected for laboratory investigations. Students were chosen because they were more readily available for follow-up studies. All students who reported to the University health centre were examined by qualified medical officers and each student was asked to report back after 3, 7, and 15 days. Those students who suffered from an influenza-like disease were asked if they would volunteer to take part in the investigation and only those willing to co-operate were included in these tests.

Isolation of virus. Each patient was asked to gargle 3 times, using sterile nutrient broth (about 10 ml). The washings were collected in sterile bottles and these were transported to the laboratory on ice in vacuum flasks.

Specimens were cultured on blood-agar plates, which were incubated either aerobically or anaerobically, and in nutrient broth. Penicillin and streptomycin were then added to the specimens to give final concentrations of 1000 IU/ml and 1000 μg/ml, respectively. The specimens were left for 1 hour at 4°C and were either inoculated into embryonated eggs immediately or stored at −20°C until tested later.

Embryonated white Leghorn eggs, 13 days old, were inoculated by the amniotic-cavity route according to the method described by Busby, House & Macdonald (1964, p. 170). At the end of the incubation period the amniotic fluids were collected aseptically and tested for the presence of virus by the haemagglutination test, employing human group 0 and fowl red blood cells (RBC). Blind passages were done only once for negative specimens.

Haemagglutination (HA) test. Haemagglutination tests were performed in plastic plates (WHO pattern) following the method of Busby, House & Macdonald (1964, p. 170). Doubling dilutions of amniotic fluids were made in normal saline, starting at 1 : 10 dilution.

Allantoic cavity inoculation. When a haemagglutinating agent was obtained from the amniotic fluids, 10^{-2} dilutions of the fluids were made in nutrient broth containing 1000 IU of penicillin and

1000 μg of streptomycin per ml. The mixtures were then inoculated with a syringe into the allantoic cavities of 10–11-day-old embryonated eggs, using the method described by Busby, House & Macdonald (1964, p. 86, method 2). Volumes of 0.25 ml were used to inoculate each egg, 6 eggs being used for each specimen. The eggs were incubated at 35°C for 48 h and then chilled at 4°C overnight; the allantoic fluids were then harvested and tested for the presence of haemagglutinating agents by the HA test described above, except that incubation during the HA test was done at room temperature only and human group O RBC alone were used.

Haemagglutination inhibition (HAI) test. Two samples of sera were obtained from patients suffering from influenza-like diseases; one sample was taken at the acute stage and the second sample 15 days later. Treatment of sera with trypsin and heat was found to be the best way of removing non-specific inhibitors from sera (Sampaio & Isaacs, 1953). The trypsin solution was prepared by dissolving 40 mg of trypsin (Difco 1 : 250) in phosphate-buffered saline, at pH 8.2; fresh trypsin solution was prepared for each day's tests. Mixtures of equal volumes (0.2 ml) of trypsin solution and sera were shaken and placed in a water-bath at 56°C for 30 min; 1.2 ml of saline were then added to give final serum dilutions of 1:8.

The infected allantoic fluids were titrated against human group O RBC as described above for the HA test and the highest dilution of the allantoic fluid that gave 50% haemagglutination was taken as 1 unit; 4 units of this antigen were used in the HAI test.

Firstly, twofold dilutions of the treated sera, starting at 1 : 8 dilution, were added to wells in haemagglutination plates in 0.2-ml volumes. Then, 4 HA units of the antigen, in a volume of 0.25 ml, were added to all the wells containing the sera, and the two reagents were mixed throughly and were allowed to stand at room temperature for 30 min. A volume of 0.25 ml of 1.0% human group O RBC was added to all the wells and was mixed thoroughly; the plates were kept at room temperature for 1 hour and the test was read after the controls had settled. The titre of the serum was taken as the reciprocal of the highest dilution that showed 50% inhibition of haemagglutination; 4-fold or greater rises in titre were taken as significant.

The controls for this test were:

(1) 1 : 8 serum dilution alone with RBC,

(2) RBC alone in saline,

(3) titration of the antigen used; and

(4) a positive convalescent serum from a patient from whom an agent had been isolated.

Results

Virus isolations. From a total of 33 specimens from throat gargles tested, 9 (27%) haemagglutinating agents were recovered in embryonated eggs. These agents always gave higher HA titres against human group O RBC than against fowl RBC when incubation was carried out at room temperature or at 4°C. Thus the isolates could be tentatively identified as influenza type A viruses.

Virus typing. Dr H. G. Pereira, Director, World Influenza Centre, London, England, kindly agreed to type our virus isolates. He reported that all the isolates were strains of influenza A2 virus and that they were indistinguishable from the A2/Hong Kong/1/68 variant (Coleman et al., 1968).

Serological examinations. Two specimens of sera were obtained from only 25 of the 33 patients examined with influenza-like disease. Sera were stored at —20°C until tested. When the typing of the viruses was reported to us, these paired sera were tested by the HAI test in order to reach a diagnosis in those cases from which no viruses had been isolated. The strain identified as A2/Sudan/5/70 was selected for the tests, because it gave the highest haemagglutination titre.

Of the 25 paired sera examined, 18 showed 8-fold or greater rises in titre (72%) and 7 were negative. Thus, the number of cases diagnosed serologically was three times the number diagnosed by virus isolation. Significant rises in titre were obtained in 8 out of 9 cases from which viruses were isolated. This shows that this strain of Hong Kong virus was the cause of the influenza-like disease.

All the acute-phase sera, taken within 1–3 days of onset of symptoms, contained antibody to this virus, with titres ranging from 1 : 8 to 1 : 128.

Bacteriological examinations. Of the 33 throat gargles only 3 samples showed the presence of β haemolytic streptococci and only 2 showed coagulase-positive staphylococci.

Discussion

The status of influenza in the Sudan has not been well understood in the past, owing mainly to the lack of laboratory facilities and perhaps also to the mild nature of this disease in a country where major causes of fever are still rife, e.g., malaria, kala azar,

and typhoid. Certainly the epidemics of influenza-like diseases of 1957 and the last two epidemics of 1968–69 and 1969–70 were severe enough not to pass unnoticed.

The first isolations of influenza viruses in the Sudan reported in this paper will no doubt add greatly to our knowledge of some of the influenza-like diseases that have occurred in this country in the past.

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