

CDC INFLUENZA REPORT
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SPECIAL NOTE

Information contained in this report is a summary of data reported to CDC by State Health Departments, Epidemic Intelligence Service Officers, National Office of Vital Statistics, collaborating influenza diagnostic laboratories, and other pertinent sources. Much of it is preliminary in nature and is primarily intended for those involved in influenza control activities. It is understood that the contents of these reports will not be released to the press, except by the Office of the Surgeon General, Public Health Service, U. S. Department of Health, Education, and Welfare. State Health Officers, of course, will judge the advisability of releasing any information from their own states.

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I. Summary of Information

With confirmation of the Grinnell, Iowa, outbreak as Far East strain influenza, a chain of infection is described which commences with an outbreak at Davis, California, extends to Grinnell, Iowa, and to now confirmed cases in Kentucky and suspect cases in New Mexico, Illinois, Indiana, Connecticut, and Colorado. Although no significant spread of the virus outside of special population groups has yet been reported, a general seeding of the Far East strain influenza virus throughout the United States is certainly occurring.

Far East strain influenza has also been confirmed in the foreign exchange students visiting in Salt Lake City, Utah. These students began their bus tour in San Francisco, an area where a number of focal outbreaks have been reported.

At the International Boy Scout Jamboree, Valley Forge, Pennsylvania, an influenza-like illness has to date affected about 350 of the 53,000 scouts. These cases are occurring primarily among California delegates, who began noting cases en route. In several instances a single individual or known contacts have given rise to subsequent focal outbreaks. In this instance, again the "provoking factor" of crowding in trains and buses appears to be important in the spread of infection. Cases among Illinois and Indiana scouts seem closely related to the proximity of a Los Angeles group during a train trip from New York to Valley Forge. No generalized outbreak is presently apparent. The lack of crowding in close quarters in this pup tent encampment may impair further spread of the illness.

Far East strain influenza has been confirmed in the numerous areas indicated by the appended map. Laboratory confirmation of cases is pending in several widely separated areas.

An influenza associated death in California is described in this report. Laboratory studies are not yet complete but the impression at post-mortem examination was that of death due to a toxic myocarditis with acute circulatory collapse and cardiac failure. Additional details will be presented as they are made available.

II. Epidemics and Case Reports

3-A. KENTUCKY, Louisville

(Reported by Dr. Russell E. Teague, Kentucky State Department of Health.)

On July 5 a civilian traveller en route to Washington, D. C., by air deplaned in Louisville with symptoms of an influenza-like illness. Throat washings from this patient yielded a Far East strain of influenza virus. No marked upswing in febrile respiratory disease has been noted in the Louisville area recently.

3-B. NEW MEXICO, Las Vegas

(Reported by Dr. John Mason, New Mexico Department of Public Health.)

Three students recently returned from the Grinnell, Iowa, confer-

ence (CDC Influenza Report 1-J) developed an influenza-like illness shortly after returning home. Several other similar illnesses have appeared among contacts in the Las Vegas area.

3-C. CONNECTICUT, Milford

(Reported by Dr. Stanley H. Osborn, Connecticut State Department of Health.)

Two Connecticut residents who attended the Grinnell, Iowa, conference (CDC Influenza Report 1-J) subsequently developed an influenza-like illness. Both left Grinnell by auto on July 2. On the following day one experienced the sudden onset of chills, fever, headache, malaise, and cough. She arrived home on July 5. The other student experienced a similar illness on July 10. A Rhode Island delegate developed a similar but milder illness on July 4. Specimens from the Connecticut delegates are presently under laboratory study.

3-D. PENNSYLVANIA, Valley Forge

(Reported by Dr. W. D. Schrack, Jr., Pennsylvania State Department of Health; Dr. Eugene Green, PHS, Medical Officer in Charge, International Boy Scout Jamboree; Dr. James Mosley, CDC Epidemic Intelligence Service.)

To date about 350 cases of febrile respiratory illness have occurred among 53,000 Boy Scouts presently attending the International Jamboree at Valley Forge, Pennsylvania. The encampment continues through July 18, with the exodus of all campers being complete by July 21. Cases so far are confined to 5 or 6 regional groups. No extensive spread of the illness is presently apparent.

The California delegation of 970 scouts has experienced over 200 cases of the influenza-like illness. Groups involved originated in San Francisco, Sacramento, and Los Angeles. In each instance illnesses occurred en route to Valley Forge. Among the San Francisco group were five boys who had recently come from a camp in Sonoma County where, during two weeks in June, 250 cases of febrile respiratory illness had occurred among 500 campers. Several Hawaiian scouts, recently arrived by boats on which outbreaks were reported, accompanied this group to Valley Forge.

A single case of febrile respiratory illness on the day of departure preceded the outbreak in the Los Angeles group. During the seven-day railroad trip to Valley Forge, 56 scouts became ill. Six were immediately hospitalized upon arrival at the Jamboree. Due to the failure of air conditioning equipment in the railroad coaches, 400 miles of the trip were travelled under extremely hot, oppressive conditions. Cases have continued to occur in a subdivision of this group.

Thirty-two cases of an influenza-like illness occurred during the 10-day railroad trip of the Sacramento group to Valley Forge. No index case could be identified with certainty in this group. Additional illnesses appeared after arrival at the Jamboree.

Several Los Angeles scouts shared a railroad coach from New York to Valley Forge with the Indiana and Illinois delegations. Forty-eight to 72 hours later, influenza-like illnesses appeared among the group having occupied seats nearest to the Los Angeles scouts.

The Louisiana Jamboree scouts, numbering 160, travelled to the encampment in four buses. Several had recently left a scout camp which experienced an extensive outbreak of febrile respiratory illness. During the trip an influenza-like illness appeared in the bus carrying these scouts. Eventually, all boys in this bus suffered similar illnesses.

Additional illnesses have occurred among Texas scouts en route to Valley Forge.

To date, only two state groups have experienced febrile respiratory illnesses at the Jamboree with no cases prior to arrival. At present these are few in number and may not be significant in terms of a potential major outbreak.

Throat washings and acute blood specimens are under laboratory study at Walter Reed Hospital and the National Institutes of Health. Medical care facilities have been well provided. Six hundred hospital beds are available at Valley Forge Army hospital, in addition to several field hospitals if needed. Scouts are divided by region and locale into 38 sections, each with about 1400 individuals. Affected scout groups have been carefully screened upon arrival and, where influenza-like illnesses have prevailed, relative isolation has been imposed.

At the Jamboree two scouts are housed in a pup tent. The crowding together of large groups in close quarters, such as has appeared instrumental in the spread of influenza in other recent outbreaks, seems to have been avoided. Careful surveillance is being maintained at the encampment.

3-E. CALIFORNIA, Fresno, Los Angeles, Sonoma Counties

(Reported by Dr. P. K. Condit, California Department of Public Health.)

In the above noted outbreaks, three summer children's camps, with about 800 children, have experienced a total of over 100 cases of febrile respiratory illness. Health officers have been alerted to the possibility of influenza in such camp groups. Careful surveillance is being maintained.

3-F. CALIFORNIA, Los Angeles

(Reported by Drs. A. C. Hollister and P. K. Condit, California Department of Public Health.)

An outbreak of febrile respiratory illness appeared in the City Jail, beginning about July 7. Thirty-seven cases have been reported to

to date. Specimens are presently under laboratory study. Little difficulty is anticipated in collecting convalescent blood specimens.

III. Progress Reports

3-G. UTAH, Salt Lake City (see also CDC Influenza Report No. 1-K)

(Reported by Dr. J. P. Kessler and Mr. Russell S. Fraser, Utah State Department of Health.)

Throat washings from 2 acute cases of febrile respiratory illness among the group of foreign exchange students who became ill in Salt Lake City were found positive for Far East strain influenza virus. The Hemagglutination-Inhibition test was positive with Japan 305 chicken serum. Among approximately 64 home contacts exposed to the students early in July, 11 secondary cases of influenza-like illness have appeared. Throat washings from these patients are presently under laboratory study.

3-H. IOWA, Grinnell (see also CDC Influenza Report No. 1-J)

(Reported by Dr. Ralph H. Heeren, Iowa State Health Department, and Dr. A. P. McKee, State University of Iowa, College of Medicine.)

Far East strain influenza virus has been isolated by Dr. McKee from throat washings obtained from cases at the Grinnell conference. Identification was made with Japan 305 ferret serum. Five convalescent bloods from patients living in California also were found to have diagnostic titer rises to the Far East strain virus.

3-I. KENTUCKY (see also CDC Influenza Report No. 2-B)

(Reported by Drs. Russell E. Teague and S. Stephen Chapman, Kentucky State Department of Health.)

In addition to the previously noted isolation of Far East strain influenza virus, 5 others have been obtained from students who attended the Grinnell conference. Of 30 Kentucky students who were present at the gathering, 24 have subsequently experienced episodes of influenza. Very few secondary cases among contacts have been noted.

3-J. CALIFORNIA, Davis (see also CDC Influenza Report No. 1-G)

(Reported by Drs. A. C. Hollister, P. K. Condit, D. Welti, California Department of Public Health, and Mrs. Dorothy Calafiore, CDC Epidemic Intelligence Service.)

Between June 17 and June 25, 224 of 391 high school girls attending a conference at Davis, California, developed a febrile respiratory illness shown to be due to influenza virus, Far East strain (CDC Influenza Report 1-G).

A subsequent report from California indicates that the conference, American-Legion-sponsored Girls State, was attended by girls from all

parts of California who traveled to Davis via 10 chartered buses, which left designated points in the state on June 17 and arrived at Davis the same evening. On arrival, each girl was assigned to one of 12 "city" groups in which she worked through the meeting. Temperatures were taken and recorded for each girl at the time of arrival. Meals were consumed in a common dining room; sleeping facilities were in dormitories, two girls to each room. Activities called for one or more "general" sessions each day, remaining activities being in small "city" groups.

Dates of onset of illness were as follows:

	<u>No. of Cases</u>		<u>No. of Cases</u>
June 18	2	June 23	53
June 19	1	June 24	113
June 20	4	June 25	9
June 21	4	Unknown	7
June 22	35	Total	<u>228</u>

Among 391 girls, there were 224 known illnesses (attack rate 57%); among 24 adults on the staff, 4 illnesses. Of the two cases with onsets June 18, little is known of one case since the girl returned home almost immediately. The second case complained of headache and sore throat. Her temperature was 99.4°. The June 19 case was the first with symptoms typical of succeeding cases. Peaking of cases on June 24 may be explained in part by the institution of twice daily temperature recordings on that date. The sharp decline in cases on June 25 may be due to the fact that the girls learned that those ill would not be allowed to go home.

Attack rates and dates of onset were determined both by bus groupings and by "city groups." There were no striking differences between the different groups. Attack rates by "city groups" ranged from 47% to 69%.

The illness onset was characteristically sudden. Usually a prodrome of "not feeling well" preceded the acute onset by 2 to 4 hours. Following the prodrome moderately severe supra-orbital headache, sore throat, and malaise customarily occurred. Cough was a frequent symptom but not a troublesome complaint. Catarrh and coryza, when present, were mild. Nausea and vomiting were infrequent, diarrhea almost nonexistent. It is doubtful that true chills occurred. Fever above 104° was noted in but few cases. Physical findings were limited to fever, questionable lymphoid adenopathy and pharyngeal injection in some, and nontender submaxillary and cervical adenopathy in about half. Lungs were clear to auscultation except for a few patients with bronchial cough.

Complications were limited to two patients. One had a mild otitis media; the second, with rales in the chest, had minimal pneumonitis by X-ray. Ten other chest X-rays, taken because rales were heard, were normal.

There appeared to be a minimal spread of illness into the community. There were at least 5 possible contact cases and three possible secondary contact cases. One case occurred in a 22-year-old nurse, who took care of the girls.

A 57-year-old woman, who served as director of the activities at Girls' State, returned from the conference on June 29 and became ill that day with headache, malaise, some nausea and diarrhea, and temperature of 101°. She sought medical attention the following day. She continued to have symptoms and, on July 3, complained of extreme fatigue and weakness. Her systolic pressure was found to be 80; she was hospitalized immediately. Her condition steadily became worse. She developed auricular fibrillation and cardiac failure and died July 4. A blood count taken July 3 showed 67,000 white cells per mm³ with 80% lymphocytes. A white count obtained three hours before death showed 127,000 cells per mm³, 80% lymphocytes.

Post-mortem examination was performed on July 5. Both the attending physician and the pathologist felt the clinical and pathological findings to be consistent with an acute toxic myocarditis of the type associated with viral infections. The heart was markedly enlarged and flabby. There was no evidence of pneumonia other than the usual terminal findings. Spleen, lymph nodes, and coronary arteries were normal. The attending physician stated that the blood count prior to death showed no evidence of anemia and there was nothing in the patient's history to suggest leukemia. Bone marrow studies are in progress.

IV. Summary Tables - Cases and Outbreaks

TABLE I

Confirmed Outbreaks and Cases of Influenza Due to Far East Strains, United States
June 1--July 15, 1957

Dates of Outbreaks	Location	Type of Population	Population at Risk	No. with Influenza-like illnesses	Deaths	Laboratory Diagnosis Virus Isolation	Serology	CDC Influenza Report Number
May 20-- June 18	CALIFORNIA San Francisco	Naval and passenger ships in harbor recently arrived from Far East	c. 9500	800 $\frac{1}{2}$	1 pneumonia		Yes	1-A
Early June	RHODE ISLAND Newport	Crews of several Naval vessels	?	Attack rates by ships 18 - 45%	0	Yes		1-B 2-C
Mid June	CALIFORNIA San Diego	Naval Training Station Recruits Station Personnel	c. 2890 c. 5000	2251 Attack rate 7%	0	Yes (6-21-57)		1-C
Early June	CALIFORNIA San Diego	Crew members of a Naval vessel	130	78	0	Yes		1-C
Mid June	OHIO Cleveland	Military man recently returned from Far East	Single Case		0	Yes		1-D
June	HAWAII	Military personnel Military dependents Civilians	?	527 $\frac{1}{2}$ 103 $\frac{1}{2}$ 300 $\frac{1}{2}$	0 0 0		Yes	1-E
June 17-25	CALIFORNIA Davis	High school girls and adult leaders	391 24	224 4	0 1	Yes		1-G 2-J
Late June	CALIFORNIA Monterey	Fort Ord Army Base	?	400 $\frac{1}{2}$	0		Yes	1-H 2-F
June 26-- July 2	IOWA Grinnell	College students and adult leaders	1688	200 $\frac{1}{2}$	0	Yes	Yes	1-J
July 1--5	UTAH Salt Lake City	High school students Exposed residents	37 64	30 11	0 0	Yes		1-K 2-E
June 24	OHIO Cleveland	Hospital orderly	Single Case		0	Yes (6-24-57)		2-A

TABLE I (Continued)

Dates of Outbreaks	Location	Type of Population	Population at Risk	No. with Influenza- like Ill- ness	Deaths	Laboratory Diagnosis by		CDC Influenza Report Number
						Virus Isolation	Serology	
Early July	KENTUCKY Lexington	Students returning from Grinnell, Iowa	30	24	0	Yes		2-B
July 5	KENTUCKY Louisville	Traveler from Philippines	Single Case		0	Yes		3-A

TABLE II

Unconfirmed Influenza-like Illness, Outbreaks - United States
June 1--July 15, 1957

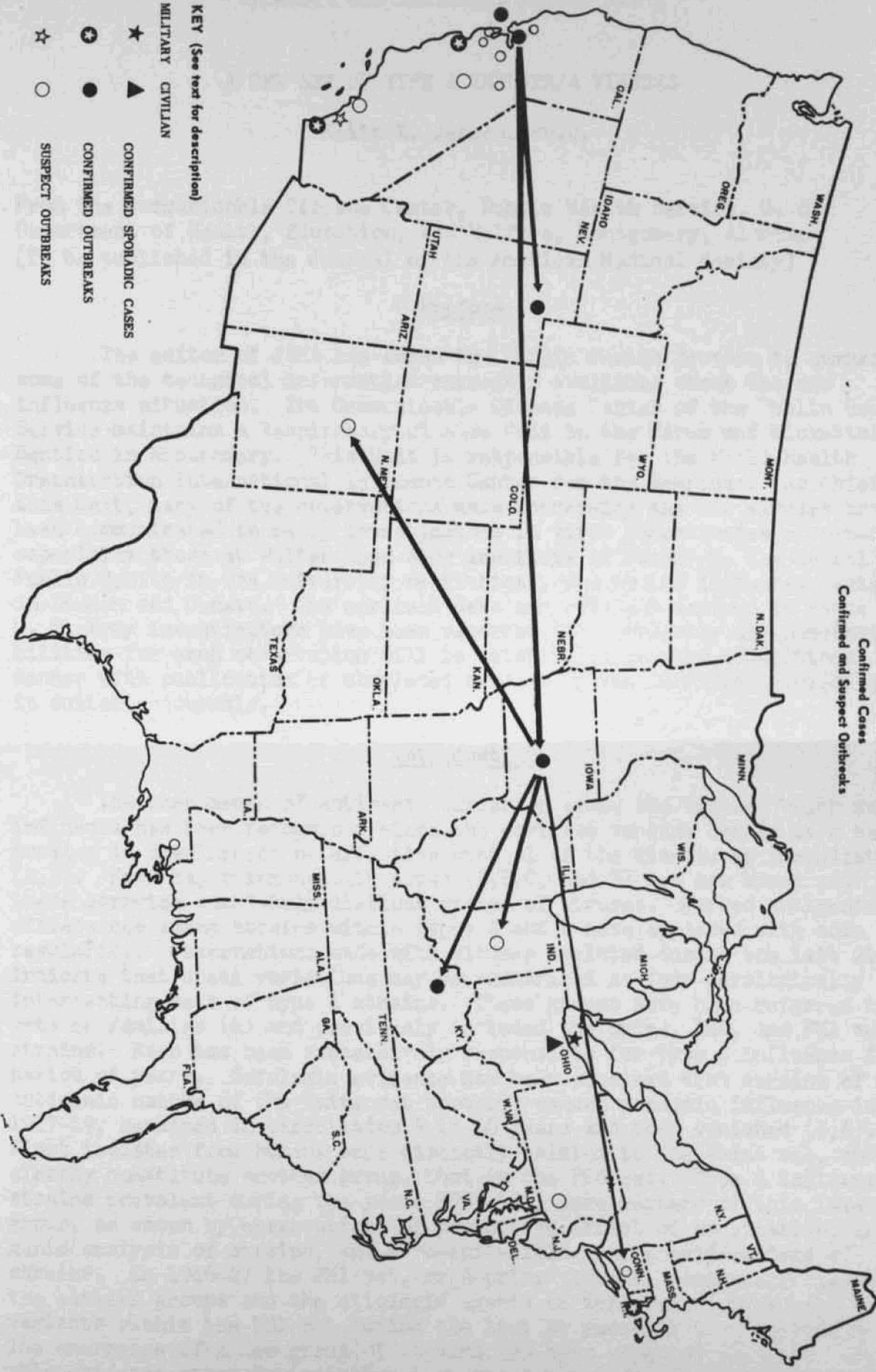
Dates of Outbreaks	Location	Type of Population	Population at Risk	No. with Influenza-like Illness	Deaths	Specimens Obtained		CDC Influenza Report Number
						Throat Washings	Blood	
June 20-25	CALIFORNIA San Mateo Co.	Boys camp 15-17 year olds	53	36	0	Yes	Yes	1-F
Late June	CALIFORNIA Solano Co.	Mare Island Naval Base	?	200 $\frac{1}{2}$	1 from bacterial pneumonia	Yes	Yes	1-I
Early July	INDIANA Indianapolis Evansville	College students who attended Grinnell, Iowa, Conference	?	?	0	Yes		2-C
Early July	CALIFORNIA Oceanside	Camp Pendleton	40,000	Attack rate 3 $\frac{1}{2}$ %	0	Yes	Yes	2-D
Mid July	NEW MEXICO Las Vegas CONNECTICUT	College students who attended Grinnell, Iowa Conference	?	2	0	Yes	Yes	3-B 3-C
July 11-15	PENNSYLVANIA Valley Forge	International Boy Scout Jamboree	53,000	c.350	0	Yes	Yes	3-D
Mid July	CALIFORNIA Fresno, Sonoma, Los Angeles Counties	Three summer children's camps	800	c.100	0		Yes	3-E
July 7-9	CALIFORNIA Los Angeles	City jail	?	37	0	Yes	Yes	3-F

TABLE III

Outbreaks of Febrile Respiratory Disease - Etiology Other Than Influenza or No Specimens Obtainable
June 1--July 15, 1957

Dates of Outbreaks	Location	Type of Population	Population at Risk	No. with Influenza- like Ill- ness	Deaths	Specimens Obtained		CDC Influenza Report Number
						Throat Washings	Blood-	
Early July	MISSOURI Columbia	Townspeople	?	200 $\frac{1}{2}$	0	Yes	Yes	1-L
Late June	CALIFORNIA San Mateo, Santa Cruz, Sonoma, and Tuolumne Counties	Seven summer chil- dren's camps	505 $\frac{1}{2}$	123 $\frac{1}{2}$	0	0	0	1-M
								Negative for in- fluenza

INFLUENZA - FAR EASTERN STRAIN - 1957
 Confirmed Cases
 Confirmed and Suspect Outbreaks



KEY (See text for description)

- ★ **MILITARY CIVILIAN**
- ▲ **CONFIRMED SPORADIC CASES**
- **CONFIRMED OUTBREAKS**
- **SUSPECT OUTBREAKS**

Arrows indicate probable spread from foci of infection

A NEW SET OF TYPE A INFLUENZA VIRUSES

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Preface

The editor of JAMA has asked the Public Health Service to summarize some of the technical information currently available about the new influenza situation. The Communicable Disease Center of the Public Health Service maintains a Respiratory Disease Unit in the Virus and Rickettsia Section in Montgomery. This Unit is responsible for the World Health Organization International Influenza Center for the Americas. As Chief of this Unit, many of the observations made concerning the new viruses have been communicated to me by investigators in virus laboratories elsewhere, especially those at Walter Reed Army Institute of Research, the School of Public Health in the University of Michigan, and by the Influenza Centers in London and Geneva. The earliest data and evidence derived by teams of U. S. Army investigators have been reported (1). Priority and responsibilities for each observation will be established in this conventional manner with publication of completed studies by the individual investigators in suitable journals.

Introduction

The phenomenon of antigenic variation among the viruses which cause influenza has been recognized since the earliest vaccine trials as a basic problem in development of effective control of the disease by immunization (2,3). Four major immunologic types (A,B,C, and D) are now known and these comprise completely distinct groups of viruses. Marked antigenic differences among strains within types A and B have appeared with some regularity. Observations made with viruses isolated during the last 24 years indicate that these variations may be summarized as four serologically intersecting sets of type A strains. These groups have been referred to as sets or families (4) and previously included the Swine, PR8, and FM1 sets of strains. Each has been successively responsible for type A influenza for a period of years. Serologic evidence has been obtained that strains of the antigenic nature of the Swine set probably caused pandemic influenza in 1917-19, remained in circulation 5 to 10 years and then vanished (5,6). The first isolates from humans were distantly related to the Swine set, and clearly constitute another group, that is the PR8 set. Type A influenza strains prevalent during the years 1933-1943 were members of this later group, as shown by observations of protective effect of vaccination, antigenic analysis of strains, and sero-epidemiological considerations of strains. In 1946-47 the FM1 set, or A-prime viruses, completely replaced the earlier groups and the etiologic agents of influenza A have been variants within the FM1 set during the last 10 years on a world-wide basis. The emergence of a new group of strains has been expected (2,3,4). Available evidence strongly indicates that the fourth set of strains has evolved

this year and can be expected to replace the FM1 set or A-prime isolates. These new viruses were first isolated in May 1957 from epidemics of influenza in Singapore and are now termed Far East (FE) influenza A (1,7,8). This designation is greatly preferred over an A-double prime or similar name.

Antigenic Relationships

The modifications of antigenic structure among strains in earlier sets have been followed with interest (4). Early in 1956, virus isolates from Holland, Canada, the United States, England, India, Hawaii, and elsewhere, were found to be sharply different from strains circulated in previous years, although they were clearly in the FM1 set. This group was called the "Dutch 56" by English investigators (7,9). Strains isolated in Japanese epidemics in December 1956 were antigenically similar to the Dutch 56 strains. Influenza cases which occurred from January through March 1957 in many areas of the U. S., Europe, and Asia, were caused by influenza A viruses closely related to the Dutch 56 strains. The FE isolates are distinctly different in antigenic comparisons with all earlier type A viruses. Typical results of hemagglutination-inhibition tests with sera from convalescent ferrets and immunized chickens and several strains of types A, B, C, and D influenza are presented in Table 1. Although different titers will be obtained with the use of various lines of each virus and sera from other animals or from bleedings at different times after immunization, these results appear to indicate to fair degree the antigenic relationships which exist among these viruses. Comparable data have been obtained with sera against several other type A isolates.

The isolates have been given designations by Influenza Centers which indicate the antigenic type, geographical location of the laboratory which made the isolation or where the case occurred and the year of isolation. A number before the year differentiates between isolates and may identify the laboratory responsible for obtaining the virus. Thus, strains with the block of numbers 300-400 have been forwarded by the Walter Reed Army Institute of Research. Exceptions to this rule are A/Swine/1976/31 which is the virus isolated from pig number 1976 by Dr. Shope and the C/JJ/50 where the letters "JJ" are initials from a patient's name.

With the possible exception of a slight reaction between the A/Denver/57 virus and antiserum against the FE isolate A/Japan/305/57, no antigenic similarity is evident between the FE virus and other influenza strains. In contrast to these results, titers obtained with the A/Hawaii/56 and A/Denver/57 isolates indicate a very close antigenic relationship. These viruses also cross-react to a high degree with "Dutch 56" viruses and all influenza virus isolations made during January - April 1957 from many areas of the world. The dotted line is to draw attention to the reactions noted between these isolates and the FM1/47 and Mal/54. These results indicate all four are in a set but the 1956-57 isolates may be sufficiently different to alter the formula for a polyvalent influenza vaccine. Antigenic intersection between later sets and those of which Swine/31 and PR8/34 are prototype strains can be seen by the low antibody titers. No reactions were found between the strains from different types (A,B,C, and D).

The FE viruses are also set apart by results obtained with virus neutralization tests using virus infection in embryonate eggs as the index of serum-virus interaction.

At least 20 isolates have been typed as members of the new set, including agents from epidemics in Asia and Europe and sporadic cases in the U. S. and elsewhere (8). Although slight differences are noted in cross-tests among these viruses, all are closely related antigenically and comprise a new group or the fourth set of type A influenza viruses.

The relationship to the older type A strains is evident from results of complement fixation tests. Both allantoic fluids and membrane extracts from embryonate eggs infected with the new agent have been demonstrated to contain high concentrations of influenza A antigens. Data presented in Table 2 confirm other observations that the A/Japan/305/57 (FE) strain is a type A influenza virus and that it may be more closely related to A/Denver/1/57 than to A/PR8/34. These results were obtained using a standard overnight fixation method and demonstrate that viral antigens in allantoic fluids gave a more specific reaction than did the membrane extracts which contained a high concentration of "soluble" antigen. The membrane extracts had been absorbed with erythrocytes to remove viral particles.

Growth in eggs, tissue culture, mice, and ferrets

Strains of the FE set have a host range and in vitro behavior similar to other type A influenza viruses. Isolations from throat washings are readily accomplished by standard methods using the chick embryo amniotic sac (10). Allantoic fluids harvested from infected embryonate eggs contain concentrations of 10^8 to 10^9 egg infectious doses per ml. No notable differences in titers were obtained when eggs were incubated at 35°C rather than 37°C . Concentrations of virus as measured by hemagglutination of guinea pigs, human, or chicken erythrocytes have been comparatively low (100 to 200) even when the eggs were injected with highly diluted seed or treated with cortisone. An exception to this observation has been with the recently acquired isolate, A/Formosa/313/57. Eleven-day embryonate eggs inoculated with this strain by the allantoic route and incubated for 48 hours at 37°C have repeatedly yielded pools of allantoic fluids with hemagglutinin titers of 1:800.

Fluids from eggs inoculated with strains of the FE set contain virus particles in the filamentous and spherical forms (1,7). Similar pleomorphism has been demonstrated with many type A strains by electron photomicrography or with the light microscope using darkfield illumination or staining with impregnation procedures (9). These observations may account for the usually low hemagglutinin titers obtained and antigenic behavior noted with several of the FE isolates. Investigations concerning the significance of these forms in fluids from infected eggs have demonstrated that the long fibers will agglutinate cells and have the antigenic specificity of spherical virus particles but are believed infective only at one end (11). Future work with these viruses is indicated to clarify the nature of the various forms.

It was of interest to find that the FE isolates, A/Japan/305/57, would agglutinate sheep erythrocytes. Following observations in complement fixation tests with the virus in allantoic fluids as the antigen, comparative hemagglutination titrations were carried out with 1% suspensions of sheep erythrocytes and 0.5% chicken red blood cells suspensions at 4° C and room temperatures. At either temperature this virus in allantoic fluids diluted in the usual phosphate buffered saline caused the formation of the typical patterns with both sheep and chicken cells in titers of 1:100. In contrast the several other type A strains isolated in previous years did not agglutinate the sheep cells. In this respect the FE isolate behaves more like type B strain Lee/40 which also agglutinated sheep cells at both temperatures. The significance of these observations is not yet clear. Although it has been suggested that the strength of the virus-cell bond varies with strains of virus and the species of animals from which the erythrocytes are obtained (12), further work must be carried out to permit additional speculation.

Very limited information has been obtained concerning the growth of FE viruses in tissue culture, mice and ferrets. Virus in allantoic fluids from infected eggs will multiply in monolayer monkey kidney cultures as seen by an increase in hemagglutinin infectivity and slight cytopathogenic changes. The concentration of virus produced is similar to that found with many egg-adapted type A strains with hemagglutinin titers in the 10 - 100 range (13).

The new viruses also grow in mice and ferrets following intranasal inoculations. Characteristic lung consolidation was found in mice 3 to 10 days after being given allantoic fluids containing 10⁰ egg infectious doses of any of several FE isolates. The viruses have been maintained by serial lung to mice transfers, however, after 5 passages lesions were no longer noted, indicating that much of the pathologic changes in the first mice were probably due to toxicity as seen with other influenza viruses (14). Studies concerning the virulence of these viruses are being continued.

Serologic Behavior in Laboratory Tests

It was early recognized that many of the FE influenza isolates were very sensitive to nonspecific inhibitors in heated sera which are not antibody and therefore interfere with interpretation of results from hemagglutination-inhibition (H.I.) tests. Furthermore, the treatment of sera with trypsin, periodate or Vibrio cholerae extracts (10) did not remove these troublesome inhibitors. Two of the isolates, A/Japan/305/57 and A/Formosa/313/57, were not sensitive, however, and a majority of the tests have been carried out with these strains. Lines of these viruses established by transfers in ferrets and mice and subsequently in eggs appear more capable of combining with antibody than those which have been carried only in eggs but have a greatly increased sensitivity to nonspecific inhibitors (1,4,9,13).

These considerations make it difficult to draw conclusions from data derived by H.I. or virus neutralization tests. Future studies utilizing complement fixation, serum antibody absorption or antibody-virus precipitation methods may demonstrate a closer antigenic relationship between the FE viruses and earlier type A strains.

It has been reported (15) that serum obtained from people in Holland in the age range 70-94 contained antibody against the FE strain. Rises in these antibody titers were found in this group following injection of type A vaccines prepared with strains isolated since 1933. This observation is of particular interest because it suggests that viruses prevalent during the pandemic of 1889 contained antigens shared with these most recent A strains which would be similar to interrelationships demonstrated with the Swine, PR8 and FM1 sets of strains (4,6,16,17). Although sera taken from people of similar age groups in Alabama (13), Washington, D. C. (18), Michigan (19) and Missouri (20) did not contain inhibiting antibody measurable with FE viruses, additional tests with comparable post-vaccination sera and other lines of FE viruses are indicated. Studies of this nature are being continued.

Antigenic Potency

The evidence is firm that the production of antibody stimulated by injections of preparations containing killed influenza viruses is correlated with immunization against the disease (2,3). Vaccine specifications have been on the basis of viral concentrations determined by chicken cell agglutination (CCA) activity. Early results of studies with the prototype strain A/Japan/305/57 by six vaccine manufacturers in the U. S. indicated that products containing more than 200 CCA units per ml would be difficult to obtain (21). Results of studies by the Commission on Influenza, Armed Forces Epidemiological Board (22) with previously isolated A viruses have made it clear that an antigenic mass represented by 750 to 1,000 CCA units was most effective. The lower specification of 200 units for military and civilian uses (21,22) is governed by the practical consideration of the yields obtained in experimental lots by vaccine manufacturers with the A/Japan/305/57 virus.

There is reason to believe at this time that considerably more antigen is contained in preparations of this strain than the CCA titer indicates. Chickens immunized with allantoic fluids which contain about 50 CCA units have responded by producing antibody measured by H.I. tests in titers as high as 1:800 which is in the range obtained with greater concentrations of immunizing fluids containing previously isolated A viruses (1,13). When allantoic fluids were titrated by complement fixation methods a much higher concentration of antigen was measured than with similar allantoic fluids from eggs infected with other type A viruses. Representative results of these tests are presented in Table 3. It will be noted that although the hemagglutinin titers were lower with A/Japan/305/57, the highest CF antigen concentrations were found with this strain. Failure of the PR8 strain antigen to fix complement with antibody in these sera indicates the degree of specificity which may be encountered using sera from young age groups. This is in keeping with previously recorded observations concerning age-specific antibody (6,17). In view of these results and the other considerations additional studies are under way to assess the value of CF titers as a means of indicating antigen concentration and potency of influenza vaccines (23).

Several studies have been initiated to learn the minimal CCA dose of the A/Japan/305/57 strain necessary to elicit antibody in groups of humans. At least three methods of virus concentration and vaccine manufacture are in use and representative preparations have been made available by several

pharmaceutical companies for these tests in adult civilian and military populations. Early results (13,24) have indicated that antibody production can be stimulated with this virus.

Serological Diagnosis

The earliest tests (1,25) demonstrated that comparatively low antibody titers were found in serum from patients convalescent from infection with the FE viruses. This may be due to the behavior of these strains in H.I. tests or, more likely, due to the nature of antibody response when a new antigen is experienced (22). It is evident that serologic diagnosis can be made using both H.I. and CF test methods (1,13,25) however, several cases not detectable using the Japan/305 virus in H.I. tests were found by CF tests using that virus in allantoic fluid or membrane extracts containing soluble antigen (13). Previous experience has indicated that serological diagnosis is more often made when both H.I. and CF tests are carried out rather than either one (22).

Availability of Materials and Information

Diagnostic sera, antigens and seed viruses have been sent to approximately 100 laboratories in the Americas which collaborate with the WHO Influenza Program. Other interested laboratories are urged to join the network and offer their assistance. Any competent laboratory will be supplied materials and information concerning the laboratory aspects of influenza by the Center upon request.

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Summary

Current influenza epidemics in the Far East and sporadic outbreaks in the U. S. and elsewhere, are caused by viruses which are type A. The antigenic relationships and other technical aspects of laboratory diagnosis and strain study are described in this report. The new variants are sufficiently different to warrant inclusion in a new formula vaccine. Some of the problems concerned with developing and testing this vaccine are discussed.

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Table 1

Results of Cross Hemagglutination-Inhibition Tests
Antisera

Isolates	Swine/31	PR8/34	FML/47	Mal/54	Haw/56	Den/57	(FE)Japan/305*	GL/54	JJ/50	Send/52
A/Swine/31	1600	0**	50	0	0	0	0	0	0	0
A/PR8/34	0	1600	50	0	100	400	0	0	0	0
A/FML/47	50	0	3200	200	50	50	0	0	0	0
A/Malaya/54	0	0	1600	1600	100	400	0	0	0	0
A/Hawaii/56	0	0	50	0	3200	6400	0	0	0	0
A/Denver/57	0	0	0	0	1600	12,800	50***	0	0	0
A/Japan/305/57	0	0	0	0	0	0	800	0	0	0
B/GL/54	0	0	0	0	0	0	0	400	0	0
C/JJ/50	0	0	0	0	0	0	0	0	800	0
D/Sendai/52	0	0	0	0	0	0	0	0	0	1600

*Chicken serum against prototype strain of Far East A; other sera are from ferrets after infections with the designated isolates.

**0 = No inhibition of 4 HA units by 1:50 serum dilution (final).

***Inhibition noted with 2 pools of chicken sera but not with 2 other pools.

Table 2

Antibody Titers from Complement Fixation Tests
With Sera from Recently Infected Humans* or Ferrets

Antigens	Human Sera		Ferret Sera	
	Acute	Convalescent	Acute	Convalescent
<hr/>				
A/Japan/305/57				
Allantoic fluid	< 8	64	< 8	64
Membrane extract	< 8	128	< 8	64
A/Denver/1/57				
Allantoic fluid	< 8	64	< 8	128
Membrane extract	< 8	64	< 8	64
A/PR8/34				
Allantoic fluid	< 8	< 8	< 8	< 8
Membrane extract	< 8	64	< 8	32
B/GL/1739/54				
Allantoic fluid	< 8	< 8	< 8	< 8
Membrane extract	< 8	< 8	< 8	< 8

*Infections with strains similar to A/Denver/1/57.

Table 3

Comparative Hemagglutinin and Complement Fixation
Antigen Titers of Allantoic Fluids

Virus Strain	Hemagglutinin Titer*	CF Antigen Titers**
A/Japan/305/57	100	128
A/FM1/47	800	16
A/Malaya/54	800	32
A/Ned/56	200	16
A/PR8/34	1200	48

* Reciprocal of highest dilution giving positive pattern test with 0.5% suspension of chicken erythrocytes.

** Conventional tests using overnight incubation at 4° C and pooled serum from persons convalescent from A infections in March 1957.