THE INVESTIGATION OF INFLUENZA IN THE ARMY IN 1957

BY

Royal Army Medical Corps

From the Virus Department of the Royal Army Medical College

The Virus Laboratory of the Royal Army Medical College investigated outbreaks of influenza throughout 1957. Those occurring during the early part of the year were due to the Dutch (1956) strain of virus A, and involved units in Eastern and Western Commands and Germany. The greatest number of specimens came from London District. The attack rate appears to have been generally below 10 per cent and many units escaped the infection. In April there was an outbreak in Hong Kong due to the Asian (1957) strain of virus A. This virus appeared in China in February, spread rapidly through the Far East, and by the end of the year had visited every continent. During July cases were landed in England from outbreaks on board troopships coming from the Far and Middle East. It might have been expected that the disease would be spread from these ships, but influenza did not occur in Army units until the following month. A unit in Western Command, far from ports and with no known connection with other cases, suffered a sharp outbreak. The disease spread rapidly through the Home Commands and Germany, reaching its climax in September. Few units escaped infection and the average attack rate was in the region of 20 per cent. The only outbreak due to influenza virus B during 1957 occurred in an apprentices’ unit in Northern Command in May. No cases due to influenza virus C were seen.

COLLECTION AND TRANSMISSION OF SPECIMENS

It was suggested that material for virus isolation should be obtained during the first three days of the disease from six to twelve of the first cases in each outbreak, and that paired sera should be taken from these cases and from as many others as was desired.

Throat swabs or garglings were used for virus isolation. Throat swabs were taken on wooden mounts and broken off into quarter-ounce bottles containing 2 ml. of buffered salt solution containing 0.25 per cent lactalbumen hydrolysate. These bottles were supplied by the Virus Laboratory, and stocks were held by Command and Hospital Laboratories. Garglings were made with 10 ml. of isotonic saline and were collected in one-ounce bottles containing 5 ml. of inactivated serum broth. Both types of specimen were frozen immediately after collection in solid carbon dioxide and dispatched to the Virus Laboratory in thermos flasks by courier, passenger train or air freight. If solid carbon dioxide was not available, ice was used for packing specimens which could be sent to the Virus Laboratory in a few hours. Owing to the fact that broth is
often toxic to eggs, pathologists were encouraged to send throat swabs rather than garglings in 1957. It was thought that this would increase the chances of virus isolation, and the results of 220 consecutive attempts at the isolation of influenza virus in 1956-7 shown in Table 1 appear to bear out this theory. However, these specimens were sent under a variety of conditions, and the majority of the throat swabs were from cases due to the Asian (1957) virus, whilst most of the garglings were from cases due to A prime strains, so that too close a comparison would not be valid. That the problem of the collection and transmission of these specimens requires further investigation is shown by the failure of the laboratory to isolate virus from some outbreaks, and by the receipt of large numbers of toxic throat swabs from two of the centres dispatching specimens.

**Table 1. Results of attempts at influenza virus isolation in 1956-7**
**(Figures in parentheses are percentages)**

<table>
<thead>
<tr>
<th>Type of Specimen</th>
<th>Total</th>
<th>Positive</th>
<th>Negative</th>
<th>Toxic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garglings</td>
<td>34</td>
<td>8 (23)</td>
<td>20 (59)</td>
<td>6 (18)</td>
</tr>
<tr>
<td>Throat Swabs</td>
<td>186</td>
<td>74 (40)</td>
<td>87 (47)</td>
<td>25 (13)</td>
</tr>
</tbody>
</table>

First specimens of serum were taken as early as possible in the disease, and second specimens generally in the third week. Blood from cases retained in their own units was taken to the nearest laboratory, where the serum was separated. It was found more satisfactory for the paired sera to be sent together to the Virus Laboratory than for them to be sent separately. Little difficulty was experienced from contaminated serum, although the specimens were dispatched by post (airmail from overseas), but specimens of whole blood received by the Virus Laboratory were almost invariably badly haemolysed and frequently anticomplementary.

**LABORATORY INVESTIGATIONS**

Virus isolation was attempted from garglings or from the fluid surrounding throat swabs. Specimens were held at \(-70^\circ C\) until they were required, when they were thawed, treated with antibiotics and inoculated into the amniotic cavities of ten-day-old chick embryos. The eggs were incubated for three days and the amniotic fluid was harvested and tested for the presence of virus by adding it to suspensions of fowl and human group O red blood cells. If haemagglutination occurred, an extract of the embryo was made by freezing and thawing, and the virus was identified by using this extract as antigen in complement fixation tests with sera known to contain antibody to influenza viruses A and B.

Amniotic fluids in which virus was not detected were inoculated into the amniotic sacs of a further batch of eggs. Two passages were generally considered sufficient, but virus was not recovered after three amniotic passages of material from any of the ten serologically proved cases, examined from the outbreak of influenza B.
Amniotic fluids containing virus were inoculated allantoically into eggs to provide sufficient virus for typing by haemagglutination-inhibition. The allantoic fluids were dispatched to the World Influenza Centre, Mill Hill, where typing of virus A was undertaken. Only one isolation was made from the majority of outbreaks. Seventy-eight isolations of virus A were made from throat swabs and garglings during 1957, 21 being of the Dutch (1956) type and 57 of the Asian (1957) type.

Pieces of lung removed post mortem from fatal cases were ground up and examined bacteriologically. Suitable antibiotics were added to inhibit bacterial growth and the fluid was inoculated allantoically. Five of the nine specimens examined yielded virus A, all of the Asian type. The monthly incidence of virus isolations is shown in Table 2.

Serological investigations were limited to complement-fixation tests. Except when the epidemic was at its height in September and October, the second specimen of each pair was "screened" at a dilution of 1/8 against the following antigens: Influenza A, B and C; Sendai; Adenovirus; Psittacosis; Rickettsia burneti (Q fever).

Where a positive result was obtained in this screen test, the two sera from the case were titrated with the antigen concerned, using another antigen of the same type as a control. Thus, a positive screen test with influenza A soluble antigen was investigated by titrating the pair of sera in doubling dilutions from 1/4 to 1/128 with influenza A soluble antigen and from 1/4 to 1/16 with influenza B soluble antigen. Controls of all reagents used were set up at the same time. A fourfold or greater rise in titre was considered diagnostic.

While the epidemic was at its height, paired sera were titrated against influenza A and B antigens, and only those giving negative results were tested against the other antigens. This may have caused some mixed infections to be diagnosed as due to influenza A, but this was a minor disadvantage when weighed against the saving of time and antigens which enabled many more tests to be carried out without delay.

During the summer four troopships arrived at English ports from the Far and Middle East, after experiencing outbreaks during the voyage. It was too late to take specimens for virus isolation, as all the cases had recovered. However, a retrospective diagnosis was made by examining serum of convalescent cases. Several typical cases were selected in consultation with the medical officer of the ship, and specimens of blood obtained before they disembarked. The sera were titrated against influenza A and B. Most specimens gave titres of 1/32 or higher to influenza A, and this was considered adequate evidence of infection. Later, two ships arrived with outbreaks still in progress. Convalescent sera were used to make the diagnosis the day after the ships docked, and the cases in the acute phase of the disease were investigated in the ordinary way.

1,196 cases had diagnostic titres of antibody to influenza virus A, and 31 to influenza virus B. Table 2 gives the monthly incidence of these findings. The cases occurring in the first three months of the year were from outbreaks in Europe caused by the Dutch (1956) strain. Four of the seven serological positives
in April were also from these outbreaks. The remaining three cases and the two isolations were from Hong Kong, where the Asian strain of virus first attacked British troops. The remainder of the influenza outbreaks during the year were due to this strain.

Table 2. Monthly incidence of positive results with influenza A in 1957

<table>
<thead>
<tr>
<th>Month</th>
<th>Virus Isolations</th>
<th>Complement Fixation Tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>January</td>
<td>4 (Dutch '56)</td>
<td>8</td>
</tr>
<tr>
<td>February</td>
<td>13 (Dutch '56)</td>
<td>58</td>
</tr>
<tr>
<td>March</td>
<td>4 (Dutch '56)</td>
<td>54</td>
</tr>
<tr>
<td>April</td>
<td>2 (Asian '57)</td>
<td>7</td>
</tr>
<tr>
<td>May</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>June</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>July</td>
<td>4 (Asian '57)</td>
<td>23</td>
</tr>
<tr>
<td>August</td>
<td>14 (Asian '57)</td>
<td>168</td>
</tr>
<tr>
<td>September</td>
<td>35 (Asian '57)</td>
<td>593</td>
</tr>
<tr>
<td>October</td>
<td>2 (Asian '57)</td>
<td>207</td>
</tr>
<tr>
<td>November</td>
<td>0</td>
<td>16</td>
</tr>
<tr>
<td>December</td>
<td>0</td>
<td>26</td>
</tr>
<tr>
<td>Totals</td>
<td>78</td>
<td>1,196</td>
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</tbody>
</table>

(Cases are grouped under the month in which the disease commenced)

SUMMARY

The experiences of the Virus Laboratory, Royal Army Medical College, in the investigation of influenza during 1957 are described.

The typing of strains of influenza virus A was carried out at the World Influenza Centre, and the co-operation of Drs. C. H. Andrewes and Alick Isaacs was greatly appreciated. The technical assistance of Mr. G. W. Thompson and Ptes. A. R. Fergie and C. W. Potter made this investigation possible. Too many medical officers to mention individually went to considerable lengths to provide specimens and information.