EXPERIENCE WITH INFLUENZA VACCINATION IN ICELAND, 1957

JÚLÍUS SIGURJÓNSSON, Dr. med.
Professor of Hygiene, Faculty of Medicine,
University of Iceland, Reykjavík

BJÖRN SIGURDSSON, Dr. med.
Director, Institute for Experimental Pathology,
University of Iceland, Reykjavík

HALLDÓR GRÍMSSON, Fil. mag.
Research Associate, Institute for Experimental Pathology,
University of Iceland, Reykjavík

SYNOPSIS

An epidemic of Asian influenza which occurred in Iceland in October and November 1957 is briefly described. About 8000 persons were vaccinated with two injections of a vaccine containing 100 CCA units of Asian virus per dose. The inactivated virus was adsorbed on aluminium phosphate. Data are given on the antibody production after vaccination.

The results of a survey indicate that vaccination with this type of vaccine, when performed early enough, reduced the incidence of influenza by some 67%. Moreover, the cases which did occur in vaccinated persons seemed to be of less than average severity.

An epidemic of Asian influenza occurred in Iceland in the fall of 1957. The first verified case occurred in a passenger arriving in Reykjavík on a boat coming from the USSR on 26 August. A strain of Asian virus was isolated and later sent to the World Influenza Centre in London, where the diagnosis was confirmed.

There was no epidemic spread of influenza in Reykjavík in the following weeks. Cases of so-called "influenza" were reported throughout September, as many as 113 in the week ending 28 September, but the correctness of diagnosis is open to doubt, and in the few cases in which isolation of virus was attempted the outcome was negative. The first positive throat washings were collected from patients on 23 October, and the Asian strain was again recovered.

Shortly before this, it had become apparent from clinical and epidemiological observations that a real epidemic of influenza was in the making. From then on the number of weekly cases reported in Reykjavík increased rapidly until a maximum of 1042 cases was reached in the week from
27 October to 2 November. In the last week of November only 78 cases were reported and from then on the epidemic petered out.

As shown in the figure, the curve of weekly incidence, based on the reporting of cases, was remarkably symmetrical. The weekly returns represent obviously only a minority of cases actually occurring during the epidemic. Absenteeism in schools had reached 35%-50% on 28 October, when they were temporarily closed, and this gives an indication of the high morbidity during the peak of the epidemic.

**CASES OF INFLUENZA REPORTED IN REYKJAVIK, AUTUMN 1957**

On the whole the epidemic was not of a serious character, although the morbidity was high. However, in Reykjavik 29 deaths were attributed to influenza; of these only three were of persons under 60 years of age, all in poor health.

As soon as it had been established that the influenza epidemic which started in eastern Asia in the spring of 1957 was caused by a virus quite distinct in antigenic composition from the previously known strains and also that the epidemic spread rapidly and extensively, the epidemic was viewed with some apprehension by the health authorities in Iceland as in other countries.

Gradually it became apparent that the epidemic was reaching pandemic proportions; and, although it was on the whole reported as being mild and to have caused only relatively few deaths, the health authorities con-
sidered the possibility of producing a certain amount of vaccine to protect not only those expected to have particularly low resistance but also certain groups of personnel in order to prevent the breakdown of important services in case the epidemic should spread rapidly on reaching Iceland.

Commercially available vaccine prepared from earlier strains could not be expected to be of much value and vaccine prepared from the new variant could not be expected to become commercially available early enough to be useful for the expected epidemic, particularly as any vaccine-producing country would be likely to reserve for its own use the vaccine produced for the first few months.

At the request of the Director of Public Health in Iceland the Institute for Experimental Pathology in Reykjavik undertook the preparation of influenza vaccine from the Asian strain, and experimental production on a small scale was started in the beginning of July. The first batch was released and vaccination begun early in September; the total production amounted to 16 000 doses, sufficient for the two-course vaccination of 8000 persons, or approximately 5% of the population.

Preparation and Dosage of Vaccine

The vaccine was prepared from a strain of Asian virus obtained from the Statens Seruminstitut in Copenhagen. It had been passed through a number of egg passages, and was passed once more after it had been received in the Reykjavik laboratory. The pooled allantoic fluid thus obtained agglutinated chick red cells to a titre of 1:1000 by the pattern method. It was distributed in small tubes and stored at —50°C.

When required for inoculation one tube was removed from the deep-freeze and a dilution of 1:1000 was prepared in Hank’s salt solution, pH 7.2. Penicillin and streptomycin were added so that 1000 units and 100 μg respectively were contained in 1 ml of the inoculum. Ten-day-old hen’s eggs were inoculated into the allantois with 0.1 ml of this dilution. After further incubation for 45 hours the allantoic fluid was collected, incubated at 37°C for 1-2 hours to free virus from red blood cells and tissue debris and then centrifuged at 1500 revolutions per minute (r.p.m.) to sediment cells.

The fluid thus obtained usually titrated 1:2000-1:4000 in the pattern test. When centrifuged without further treatment at sufficiently high speed to sediment the virus it was found that too much of the impurities were also carried down. In order to eliminate these impurities the cell-free fluid was therefore first centrifuged at 10 000 r.p.m. (8300 x g) for 7 minutes, whereupon the supernatant was formalinized by adding formalin 1:4000 and storing at 3°C for 4 days. The fluid was then centrifuged again, now at 24 600 r.p.m. (50 700 x g) for half an hour to sediment the virus. The
virus thus obtained was relatively free of extraneous protein. It was then resuspended in 0.05 M tris(hydroxymethyl)-aminomethane buffer and made up to such volume that one ml contained 100 chicken cell agglutinating (CCA) units (tube test)\(^1\) after Merthiolate (1:10000) and aluminium phosphate (4 mg/ml) had been added.

The protein content of the fully prepared vaccine was about 0.05 mg/ml, or 5 mg\%. The prescribed dose of this vaccine was two subcutaneous injections of 1 ml each at a two-week interval.

**Rise in Antibody after Vaccination**

The antigenic potency of the vaccine was tested by subjecting serum samples from a group of people to the haemagglutination-inhibition test. Samples were taken just before vaccination and then about two weeks after each of the two injections. Comparative tests were made with same kind of vaccine without aluminium phosphate. Two such control groups were used; one of them received 100 CCA units subcutaneously, the other group received 20 CCA units intradermally. Both groups were injected twice with an interval of two weeks.

Sera to be tested for inhibition of haemagglutination were inactivated at 56°C for 30 minutes and then treated with potassium periodate according to the method of Lundbäck.\(^2\)

The lowest dilution of serum was 1:10. No samples taken before vaccination inhibited haemagglutination in that dilution.

The results obtained after the first and second injections are shown in Table 1.

A relatively high proportion (about 40\%) of those receiving alum-free vaccine failed to develop HI antibody to a titre of 10 or higher after one injection. This is true whether they received 20 CCA units intradermally or 100 CCA units subcutaneously. On the other hand, only two of a group of 16 receiving the alum-adsorbed vaccine failed to develop titratable antibody after one injection. However, there was no appreciable difference between the titre reached by the positives in all three groups.

The table also shows that the results achieved with the alum-containing vaccine after the second injection were appreciably better than those obtained with the alum-free vaccine.

The median value \(^3\) of the titres in each series, as shown in Table 1, was found to be a more suitable index for comparing the results obtained than either the arithmetic or the geometric mean.

---

3. When several of the observed values of the central range are identical, the central value (or values if the total number is not odd) is taken as median, although this is not strictly in conformity with the definition inasmuch as the numbers of greater and smaller values observed may not be equal.
TABLE 1. RISE IN HAEMAGGLUTINATION-INHIBITION TITRE IN HUMANS AFTER ONE AND TWO INJECTIONS OF VACCINE

<table>
<thead>
<tr>
<th>Number tested</th>
<th>Titres reached</th>
<th>Median value of titre</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>&lt;10</td>
<td>10-15</td>
</tr>
<tr>
<td>Two weeks after first injection</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 CCA units, intradermally</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>100 CCA units, subcutaneously</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>100 CCA with aluminium phosphate, subcutaneously</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td>Two weeks after second dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20 CCA units, intradermally</td>
<td>17</td>
<td>7</td>
</tr>
<tr>
<td>100 CCA units subcutaneously</td>
<td>20</td>
<td>2</td>
</tr>
<tr>
<td>100 CCA units with aluminium phosphate, subcutaneously</td>
<td>14</td>
<td>1</td>
</tr>
</tbody>
</table>

The geometric mean is obviously meaningless if cases falling below the lowest titre tested for are counted as zero values; but if these cases are excluded and the mean is calculated only for the cases reacting at or surpassing the threshold value it is obviously not representative of the series as a whole and may be grossly misleading. In computing the arithmetic mean of all cases no great error need result from the sub-threshold values being counted as zero, but unlike the median the mean is apt to be unduly influenced by single cases of an unusually high titre.

Table 1 shows that the antibody response—even after two injections of the alum-containing vaccine—was on the whole rather low. This, however, is in conformity with what appears to have been the common experience elsewhere—namely, that the capacity of the new virus variant to elicit antibody formation was lower than that of the previously known strains of influenza virus.

Sera were obtained from eight unvaccinated persons after an attack of Asian influenza in order to compare the antibody rise after an attack with that observed after vaccination. However, in only two of these cases had the clinical diagnosis been confirmed by virological examination. Two of the eight cases had serum titres below 1:10. The remaining six cases had titres from 1:15 to 1:240, with a median of 80. These titres observed after
a natural attack of Asian influenza are only slightly higher than those observed after vaccination with alum-containing influenza vaccine.

**Protective Effect of Vaccine**

Vaccination was begun on 10 September. Priority was given to people in poor health, tuberculosis patients and others expected to have low resistance to influenza and also to personnel of certain important services.

No generalized reactions were observed but local redness and a slight swelling at the site of injection persisting for a few days were of common occurrence.

Questionnaires on the occurrence of influenza were distributed to a number of households in Reykjavík in which some members had been vaccinated. In general only one or two members of these households had been vaccinated; the others were intended to serve as controls.

At the end of the epidemic 318 of these questionnaires, each covering one household, were returned after having been filled in. Of these, 87 had to be discarded because the information given in them was incomplete. In 94 of the remaining homes there had been no cases of influenza and in 45 additional homes influenza cases had occurred before vaccination was complete. If we exclude all these there remain 92 households where the vaccinated members had received the second dose at least one week before the first case of influenza occurred in that household. As regards social and economic status, these homes, in our opinion, conformed to the broad average for Reykjavík, where the common standard of living is fairly high. The most frequent family size was 4-5 members (in two-thirds of the homes it ranged from 3 to 6). Most of these families lived in good modern houses in which overcrowding could hardly be said to occur.

The total number of persons in the 92 households subjected to further study was 433; 118 had been vaccinated. All of them can be assumed to have been exposed to infection shortly after vaccination was completed.

Although several strains of Asian influenza virus were isolated during this epidemic, thus establishing the nature of the outbreak, the diagnosis of individual cases in the present study was based on clinical symptoms only. The criteria employed were those generally considered diagnostic during an influenza outbreak: abrupt onset with fever lasting for a few days with headache, a relatively severe constitutional reaction, slight respiratory symptoms, etc. During the height of an epidemic it seems reasonably safe to assume that the bulk of such cases are in fact influenza.

In Table 2 the age and sex distribution and the number of influenza cases occurring in each subgroup are shown separately for those vaccinated and for the unvaccinated controls. The table shows that the attack rate is uniformly much lower among those vaccinated than among the controls.
### TABLE 2. INCIDENCE OF INFLUENZA ACCORDING TO AGE AND SEX IN VACCINATED AND UNVACCINATED PERSONS

<table>
<thead>
<tr>
<th>Age-group (years)</th>
<th>Not vaccinated</th>
<th></th>
<th>Vaccinated</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>males</td>
<td>females</td>
<td>males</td>
<td>females</td>
</tr>
<tr>
<td></td>
<td>number</td>
<td>attacked</td>
<td>number</td>
<td>%</td>
</tr>
<tr>
<td>0-4</td>
<td>21</td>
<td>18</td>
<td>85.7</td>
<td>25</td>
</tr>
<tr>
<td>5-14</td>
<td>51</td>
<td>47</td>
<td>92.2</td>
<td>46</td>
</tr>
<tr>
<td>15-19</td>
<td>12</td>
<td>10</td>
<td>83.3</td>
<td>12</td>
</tr>
<tr>
<td>20-39</td>
<td>27</td>
<td>19</td>
<td>70.4</td>
<td>35</td>
</tr>
<tr>
<td>40-59</td>
<td>25</td>
<td>7</td>
<td>28.0</td>
<td>32</td>
</tr>
<tr>
<td>60 and over</td>
<td>12</td>
<td>6</td>
<td>50.0</td>
<td>17</td>
</tr>
<tr>
<td>All ages</td>
<td>148</td>
<td>107</td>
<td>72.3</td>
<td>167</td>
</tr>
<tr>
<td>20 and over</td>
<td>64</td>
<td>32</td>
<td>50.0</td>
<td>84</td>
</tr>
<tr>
<td>20-59</td>
<td>52</td>
<td>26</td>
<td>50.0</td>
<td>67</td>
</tr>
</tbody>
</table>

INFLUENZA VACCINATION IN ICELAND, 1957
In the vaccinated group at all ages (118 persons) a total of 22 cases of influenza were diagnosed, an attack rate of 18.6%; whereas among the 315 controls there were 219 cases, an attack rate of 69.5%.

These figures are, however, not quite comparable because the age distribution in the vaccinated and the unvaccinated groups differs. As seen from the table more than half of the controls, but only few (slightly less than 10%) of those vaccinated were below 20 years of age, and as the incidence of influenza was clearly higher in the younger age-groups an unqualified comparison including all the age-groups is not justified.

The incidence in the adult age-groups is more even, at least up to the age of 60 years. There is hardly a significant difference in incidence between the sexes, nor is it likely that the conspicuously low incidence shown among the controls by males in the age-group 40-59 years is significant.

Comparison between all vaccinated and unvaccinated persons (both sexes together) aged 20-59 years shows that the attack rate for the 101 vaccinated persons was 18.8% ± 3.89% (19 cases), whereas for the control group of 119 persons it was 58.0% ± 4.52% (69 cases), or about three times higher. The ratio of the observed difference to its standard error is 39.2:5.97, or 6.57:1. Thus it would appear that the effectiveness of the vaccination in giving full protection amounted to about 67% in this group.

In addition there was some evidence that the influenza cases in the vaccinated group were in general milder than those occurring in the control group, but the data obtained on this point were not found to be complete enough to be subjected to analysis.

It was mentioned earlier that the records from 45 homes had been discounted in the above study because influenza had occurred there before the vaccination had been completed. When exposed to infection, the vaccinated members in these households had only got such protection as would result from one injection; of 44 persons aged 20-59 years thus vaccinated, 13 (or 29.5%) came down with influenza, whereas the 43 unvaccinated persons of the same age-group and the same households had an attack rate of 55.8%.

ACKNOWLEDGEMENT

We wish to express our thanks to Dr Oli Hjaltested, Physician in Charge of the Chest Clinic of the Health Centre, Reykjavík, for invaluable help with the clinical side of this project.

RÉSUMÉ

Une épidémie de grippe A/Asia/57 a sévi en Islande en automne 1957. Le premier cas a été signalé le 26 août, importé par un bateau venant de l'URSS, mais il ne donna pas lieu à une épidémie dans les semaines qui suivirent. Faute d'avoir isolé le virus, on ne peut affirmer que les cas déclarés comme « grippe » en septembre l'aitent été sans conteste.
Le virus A/Asia/57 fut isolé d’un lavage de gorge à la fin d’octobre. Dès lors, le nombre des cas déclarés à Reykjavik augmenta rapidement, atteignant un maximum de 1042 dans la semaine du 27 octobre au 2 novembre. La dernière semaine de novembre, on ne signalait plus que 78 cas et depuis lors l’épidémie s’éteignit.

A la demande des autorités sanitaires d’Islande, l’Institut de Pathologie de Reykjavik entreprit de préparer un vaccin, à partir du virus A/Asia/57 provenant du Statens Serum-institut de Copenhague et passé plusieurs fois sur œuf. Le vaccin contenait du phosphate d’aluminium. Des essais comparatifs ont été faits avec du vaccin sans alun. La teneur du vaccin en virus était de 100 CCA/ml. La vaccination a débuté en septembre.


Le vaccin à l’alun a donné des résultats meilleurs que le vaccin sans alun. Toutefois, même après deux injections de vaccin à l’alun, les titres des sérums étaient faibles. Ils étaient à peine inférieurs aux titres des sérums de convalescents de grippe, non vaccinés. Ces données confirmaient la faible antigénicité du virus A/Asia/57, observée dans l’ensemble des pays touchés par la pandémie.

On a vacciné en priorité le personnel des services généraux et les personnes pour lesquelles la grippe pouvait être le plus dangereuse, tels que les sujets peu résistants, tuberculeux par exemple.

D’après les enquêtes de contrôle faites dans les foyers des personnes vaccinées, on estime que 67 % des personnes vaccinées ont été totalement protégées. En outre, les cas qui se sont déclarés dans ce groupe ont été moins graves que chez les non vaccinés.