

## Some observations on the circulation of influenzaviruses in domestic and wild birds

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*Four viruses isolated from poultry in the USSR and Poland were identified as influenza A strains. One strain was closely related to fowl plague virus, the second showed an antigenic relationship to A/chicken/Scotland/59 (Hav5N1), and two others were antigenically related to A/duck/Ukraine/1/63 (Hav7Neq2) and A/duck/England/56 (Hav3Nav1). Antibodies to different strains of influenzavirus were detected in sera collected from poultry and from wild birds in the northern area of the USSR. More of the sera collected from migratory sea birds in the autumn gave HI reactions than did those collected in the spring. HI reactions were of higher titre when recent locally isolated viruses were used than with reference strains of influenzavirus. Sera collected in the autumn of 1969 from wild sea birds (mostly herring gulls, ducks, arctic loons, and long-tailed ducks) frequently showed HI activity not only with avian influenzavirus but also with A/equine/Miami/1/63 (Heq2 Neq2) and A/Hong Kong/1/68 (H3N2). The significance of these findings is discussed.*

The nature and mechanism of changes in the surface antigens of human influenzaviruses are still not fully understood. Recently, antigenic relationships have been discovered between the antigenic variant A/Hong Kong/1/68 (H3N2) and some strains isolated from birds and horses (Coleman et al., 1968; Kasel et al., 1969; Tumová & Easterday, 1969; Zakstel'skaja et al., 1969). The investigation of the antigenic structure and biological properties of influenzavirus strains isolated from birds and mammals is of interest and might have some bearing on antigenic variation in the human influenzavirus.

This paper reports the results of studies on the antigenic structure of 4 new influenzavirus strains isolated from poultry and the results of serological studies on domestic and wild birds.

### MATERIALS AND METHODS

#### Viruses

The strains A/chicken/USSR/314/67 and A/chicken/USSR/68 were isolates from chickens (Surin et al., 1970). Two duck strains A/duck/Poland/1891/67 and

A/duck/Poland/3362/67 were received from the Institute of Immunology, Polish Academy of Science. All strains were isolated and propagated in fertile eggs.

#### Antigens

Inactivated influenza antigens prepared from animal influenzaviruses by treatment with 2-oxetanone ( $\beta$ -propiolactone) were used for the serological survey.

#### Sera

Immune antisera against newly isolated and reference strains were prepared in rats by the method described by Gorbunova & Sokolov (1960). Sera from 392 chickens (hens and cocks) were collected in 1968 and 1969 in the central region of the USSR. Sera from 314 migrating wild birds were collected in the northern part of the European region of the USSR in the spring or autumn of 1969. To destroy inhibitors all sera were treated with carbon dioxide and heated for 30 min at 56°C or were treated with receptor-destroying enzyme (RDE) (Gorbunova & Sokolov, 1960).

Haemagglutination inhibition (HI) tests were performed according to the technique described by the WHO Expert Committee on Influenza (1953) or, for the serological survey, by a microtitration tech-

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nique. Virus neutralization tests were carried out in embryonated eggs (Gorbunova & Sokolov, 1960).

#### RESULTS

The results of preliminary studies showed that all four virus isolates contained influenza type A ribonucleoprotein antigens. The typical morphological structure of influenzaviruses was recognized by electron microscopy. HI tests showed that the 4 isolates studied differed from human type A influenzaviruses and were not identical with one another (Table 1).

Strain A/chicken/USSR/314/67 was antigenically related to A/chicken/Scotland/59 (Nav5N1) but showed no relationship to an antigenically related strain, A/tern/South Africa/61 (Hav5Nav3). The isolate A/chicken/USSR/68 was found to be related to fowl plague virus (A/FPV/Dutch/27(Hav1Neq1). Hyperimmune rat serum to this virus reacted with tern/South Africa/61 and chicken/Scotland/59 viruses in addition to FPV. A/duck/Poland/3362/67 virus reacted with antisera to A/duck/Czechoslovakia/56 (Hav4Nav1), A/duck/England/56 (Hav3Nav1), A/duck/England/62 (Hav4Nav1), and A/duck/Ukraine/1/63 (Hav7Neq2), but the antiserum to A/duck/Poland/3362/67 reacted exclusively with A/duck/Ukraine/2/60 (Hav7Neq2). Strain A/duck/Poland 1891/67 showed a relationship to strain A/duck/Ukraine/1/63.

In order to seek evidence of influenza infection in domestic poultry, 222 sera collected in 1968 and 170 sera collected in 1969 from chickens were examined in the HI tests. It was found that not all samples possessed HI activity and that the frequency of positive results differed according to the antigen used.

In sera collected in 1968, HI activity to A/chicken/USSR/314/67 and to A/tern/South Africa/61 was detected with similar frequency (10.8% and 13.4%, respectively) while HI antibody to A/chicken/USSR/68 was found in only 5.7% of the chickens.

In the sera collected in 1969, HI activity to A/chicken/USSR/314/67 was found in 11.2% of birds; to A/chicken/Scotland/59 in 18.8%; to A/duck/Ukraine/2/60 in 17.6%; to A/duck/Ukraine/1/63 in 17.6%; to A/duck/Ukraine/1/60 in 14.7%; to A/duck/England/62 in 10.6%; to A/duck/England/56 in 3.5%; and to A/duck/Czechoslovakia/56 in 5.3%.

HI activity against some influenzaviruses was also detected in sera collected from wild birds (Table 2).

Sera collected in the spring contained only low levels of HI activity against duck and chicken

influenzaviruses. Negative results were obtained with all the sera tested with Heq1N1, Heq2N2, and A/duck/Poland/3362/67 antigens.

The sera collected in the autumn, before the departure of the birds to their wintering grounds, gave positive results in a higher percentage of cases. In 47 of 255 sera (mostly sera from herring-gulls, ducks, Arctic loons, long-tailed ducks) HI activity was detected for equine-2 antigen (18%) and 27 sera reacted with the FPV antigen (10.6%). The HI titres ranged from 1:20 to 1:40. There was a large increase in the proportion of sera positive with duck viruses (Poland/1891/67, Poland/3362/67, England/56, Ukraine 2/60, and Ukraine 1/63).

In sera collected in the autumn from herring-gulls HI activity to the Heq2Neq2 and duck/Ukraine/1/63 viruses was frequently detected. Such HI activity was not present in sera collected in the spring.

Sera collected from wild birds in the autumn of 1969 were also investigated in HI tests with antigens prepared from the following human influenzaviruses, A/PR/8/34 (H0N1), A/Pan/52 (H1N1), A/Singapore/1/57 (H2N2), and A/Hong Kong/1/68. Positive results were detected predominantly with sera from *Clangula hyemalis*, *Gavia arctica* and *Larus argentatus* and it is notable that the current epidemic strain A/Hong Kong/68 virus reacted with a proportion of the sera from all three species (21 of 197 sera). Strain A/Singapore/57 reacted with 10 of 150 sera and A/PR/8/34 with only 3 of 150 sera. No HI reactions were detected with the virus containing H1 haemagglutinin.

#### DISCUSSION

Our results show clearly that the newly isolated strains were not identical with the reference strains. Additional one-way and two-way cross-reactions were detected between them and some other members of the chicken and duck subgroups of viruses. These findings suggest that there are broad strain variations in the antigenic structure of the surface antigens of avian influenzaviruses.

The results of the serological survey showed the presence of some haemagglutination-inhibiting activity in sera of domestic and wild birds. The question arises as to whether these are antibodies or non-specific inhibitors. The data support our assumption that they are antibodies: first, some but not all birds of the same species show such activity; and secondly the proportion of positive sera in the same species sharply increased after the period of nesting.

Thus in our opinion the haemagglutination-inhi-

Table 1. Antigenic analysis of influenzaviruses of avian origin by means of haemagglutination inhibition and neutralization tests <sup>a</sup>

| Antigens                           | Antisera |                     |                     |                             |                |                   |                         |                         |                           |                           |                             |                             |
|------------------------------------|----------|---------------------|---------------------|-----------------------------|----------------|-------------------|-------------------------|-------------------------|---------------------------|---------------------------|-----------------------------|-----------------------------|
|                                    | FPV      | chicken/<br>USSR/68 | chicken/<br>USSR/67 | chicken/<br>Scotland/<br>59 | tern/<br>SA/61 | duck/<br>Czech/56 | duck/<br>England/<br>56 | duck/<br>England/<br>62 | duck/<br>Ukraine/<br>1/63 | duck/<br>Ukraine/<br>2/60 | duck/<br>Poland/<br>3362/67 | duck/<br>Poland/<br>1891/67 |
| Haemagglutination inhibition tests |          |                     |                     |                             |                |                   |                         |                         |                           |                           |                             |                             |
| FPV                                | 1 280    | 80                  |                     |                             |                |                   |                         |                         |                           |                           |                             |                             |
| A/tern/South Africa/61             |          | 80                  | 10                  | 640                         | 160            |                   |                         |                         |                           |                           |                             |                             |
| A/chicken/Scotland/59              |          | 80                  | 320                 | 1 280                       | 80             |                   |                         |                         |                           |                           |                             |                             |
| A/chicken/USSR/314/67              |          | 20                  | 640                 | 80                          |                |                   |                         |                         |                           |                           |                             |                             |
| A/chicken/USSR/C/68                | 320      | 320                 | 320                 |                             |                |                   |                         |                         |                           |                           |                             |                             |
| A/duck/Czechoslovakia/56           |          |                     |                     |                             | 160            |                   |                         |                         |                           |                           |                             |                             |
| A/duck/England/56                  |          |                     |                     |                             | 20             | 320               |                         |                         |                           |                           |                             |                             |
| A/duck/England/62                  |          |                     |                     |                             |                |                   | 160                     |                         |                           |                           |                             |                             |
| A/duck/Ukraine/2/60                |          |                     |                     |                             |                |                   |                         | 40                      | 160                       | 160                       |                             |                             |
| A/duck/Ukraine/1/63                |          |                     |                     |                             |                |                   |                         | 640                     | 40                        |                           |                             |                             |
| A/duck/Poland/1891/67              |          |                     |                     |                             |                |                   |                         | 160                     |                           |                           |                             | 1 280                       |
| A/duck/Poland/3362/67              |          |                     |                     |                             |                |                   |                         | 320                     |                           |                           | 640                         |                             |
| Virus neutralization tests         |          |                     |                     |                             |                |                   |                         |                         |                           |                           |                             |                             |
| A/chicken/USSR/314/67              |          | 40                  | 160                 | 40                          |                |                   |                         |                         |                           |                           |                             |                             |

<sup>a</sup> The values given reciprocals of the serum dilutions: blank spaces denote <10. Sera were treated with RDE.

Table 2. Results of a serological survey of migratory birds in the Archangel region in the spring and autumn

| Species of bird |                               | Antigens    |          |                 |               |                 |                   |
|-----------------|-------------------------------|-------------|----------|-----------------|---------------|-----------------|-------------------|
|                 |                               | Heq1Neq1    | Heq2Neq2 | duck/England/56 | duck/Czech/56 | duck/England/62 | duck/Ukraine/2/60 |
|                 |                               | Autumn sera |          |                 |               |                 |                   |
| gull            | <i>Larus argentatus</i>       | 5/78        | 15/78    | 7/64            | 1/64          | 1/54            | 3/78              |
|                 | <i>Stercorarius pomarinus</i> |             | 2/15     | 3/15            |               |                 |                   |
| duck            | <i>Clangula hyemalis</i>      | 4/58        | 16/58    | 6/46            | 6/47          |                 | 7/58              |
|                 | <i>Aythya marila</i>          |             | 3/9      | 2/9             |               |                 |                   |
|                 | <i>Podiceps ruficollis</i>    | 1/6         |          |                 |               |                 |                   |
|                 | <i>Mergus serrator</i>        |             |          |                 | 1/1           |                 |                   |
| loon            | <i>Gavia arctica</i>          | 3/56        | 11/56    | 8/56            | 5/51          |                 | 2/55              |
| snipe           | <i>Philomachus pugnax</i>     |             |          |                 |               |                 |                   |
| other species   |                               |             |          |                 |               |                 |                   |
|                 |                               | Spring sera |          |                 |               |                 |                   |
| gull            | <i>Stercorarius pomarinus</i> |             |          | 1/17            | 2/17          |                 |                   |
|                 | <i>Larus fuscus</i>           |             |          | 2/15            | 2/15          | 1/15            | 1/15              |
|                 | <i>Larus argentatus</i>       |             |          | 1/9             | 2/9           | 1/9             |                   |
|                 | <i>Anas acuta</i>             |             |          |                 |               |                 |                   |
| duck            | <i>Mergus serrator</i>        |             |          |                 |               |                 |                   |
|                 | <i>Melanitta nigra</i>        |             |          |                 |               |                 |                   |
| other species   |                               |             |          |                 |               |                 |                   |

<sup>a</sup> All sera were treated with carbon dioxide. All the spring sera were negative with antigens Heq1Neq1, Heq2Neq2, and duck/Poland/3362

biting activity of the blood can be considered as evidence for the circulation of influenzaviruses in bird populations. In this respect it is of interest that the haemagglutination-inhibiting activity to duck influenzaviruses, especially to A/duck/Ukraine/1/63 and A/duck/Ukraine/2/60, were demonstrated along with those to A/chicken/USSR/314/67 and A/chicken/Scotland/59.

Also of importance are the data illustrating the appearance of haemagglutination-inhibiting activity to mammalian and human influenzaviruses in blood taken from migrating birds after the period of

nesting. Thus it should be noted that the events described occurred during the autumn of 1969, i.e., prior to the period of epidemic activity of Asian influenza virus among the human population and simultaneously with epizootics among horses in the USSR caused by Heq2Neq2 virus (Ivanova et al., 1970).

The data discussed above confirm the evidence reported earlier (Pysina & Gorbunova, 1969); Zakstel'skaja et al., 1969) suggesting that birds can play some part in the distribution of influenzaviruses, but further study is required to confirm these conclusions.

## RÉSUMÉ

### QUELQUES OBSERVATIONS SUR LA CIRCULATION DES VIRUS GRIPPAUX CHEZ DES OISEAUX DOMESTIQUES OU SAUVAGES

Quatre virus isolés chez des oiseaux de basse-cour en URSS et en Pologne ont été identifiés comme étant des virus grippaux A. L'un d'eux (A/chicken/USSR/68) était

antigéniquement apparenté au virus « classique » de la peste aviaire; un autre (A/chicken/USSR/314/67) au virus A/chicken/Scotland/59; les deux derniers, aux

f 1969. The figures indicate the number of positive sera ( $\geq 1:20$ ) and the total number tested <sup>a</sup>

| Antigens              |                             |                             |            |                         |                         |      |       |       |
|-----------------------|-----------------------------|-----------------------------|------------|-------------------------|-------------------------|------|-------|-------|
| duck/<br>Ukraine/1/63 | duck/<br>Poland/<br>1891/67 | duck/<br>Poland/<br>3362/67 | tern/SA/61 | chicken/<br>Scotland/59 | chicken/<br>USSR/314/67 | FPV  | swine | total |
| Autumn sera           |                             |                             |            |                         |                         |      |       |       |
| 13/78                 | 11/78                       | 5/78                        | 1/78       | 2/78                    | 1/62                    | 6/78 | 5/63  | 78    |
| 3/15                  | 1/15                        | 1/15                        |            |                         |                         |      |       | 15    |
| 12/58                 | 4/58                        | 6/58                        | 2/58       | 4/58                    | 3/58                    | 9/58 | 14/47 | 58    |
| 4/9                   |                             | 1/9                         | 1/9        |                         |                         | 1/9  | 1/7   | 9     |
| 1/6                   |                             |                             |            |                         |                         | 2/6  |       | 6     |
|                       |                             |                             |            |                         |                         |      | 1/1   |       |
| 11/55                 | 4/56                        | 3/56                        | 1/56       | 4/56                    | 2/43                    | 8/56 | 5/43  | 56    |
| 1/7                   | 2/7                         | 1/7                         | 1/7        |                         |                         |      | 1/7   | 7     |
|                       |                             |                             |            |                         |                         |      |       | 24    |
| Spring sera           |                             |                             |            |                         |                         |      |       |       |
|                       | 1/17                        |                             |            | 1/17                    |                         | 1/17 | 2/17  | 17    |
|                       |                             |                             | 1/15       |                         |                         | 3/15 |       | 15    |
|                       | 1/9                         |                             |            |                         |                         |      |       | 9     |
| 1/6                   |                             |                             |            |                         |                         | 1/6  | 1/6   | 6     |
|                       |                             |                             |            |                         |                         | 1/4  | 1/4   | 4     |
|                       |                             |                             |            |                         |                         | 1/2  |       | 2     |
|                       |                             |                             |            |                         |                         |      |       | 6     |

Blank spaces denote titre <20.

souches A/duck/Ukraine/1/63 et A/duck/England/56.

Des sérums prélevés chez 392 oiseaux de basse-cour et chez 313 oiseaux migrateurs dans le nord de l'URSS ont été examinés en épreuves d'inhibition de l'hémagglutination (IH). Des anticorps IH actifs contre de nombreuses souches de la grippe aviaire ont été trouvés avec une fréquence variant selon l'espèce animale, la saison choisie pour le prélèvement des sérums et la souche utilisée comme antigène.

Chez les oiseaux migrateurs, les sérums prélevés en automne étaient plus souvent positifs que les sérums prélevés au printemps. Des titres plus élevés ont été obtenus

lorsqu'on a utilisé comme antigène les souches isolées récemment que lorsqu'on a employé des souches grippales de référence. Les sérums prélevés en automne 1969 chez des oiseaux migrateurs (principalement mouettes argentées, grèbes, canards sauvages, canards à longue queue) renfermaient des anticorps IH actifs contre les virus de la grippe aviaire mais aussi contre les virus A/equine/Miami/1/63 et A/Hong Kong/1/68.

Les auteurs discutent l'importance de ces informations au regard de la circulation et des variations antigéniques des virus grippaux.

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