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Characterization of a novel haemagglutinin subtype (H13) of influenza A viruses from gulls*

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*Influenza A virus strains isolated in the United States of America from ring-billed and Franklin gulls (*Larus delawarensis, L. pipixcan*) were found to possess a haemagglutinin (HA) antigen distinct from those of the twelve previously designated haemagglutinin subtypes of influenza A virus. Serological assays with antisera to reference strains representing the HA subtypes 1–12 and to a gull isolate, A/gull/Maryland/704/77, showed that the haemagglutinin of the gull virus was not related antigenically to the previously designated subtypes. In addition, comparison of the nucleotide sequences, and deduced amino acid sequences, of the 3' region of the RNA genes coding for haemagglutinin indicated that the gull viruses represent a genetically distinct group. We propose that this new HA antigen, which has so far been detected only in gulls, be designated H13 and that A/gull/Maryland/704/77 (H13N6) be designated the reference strain for this subtype.*

Extensive investigations in several countries, coordinated by the World Health Organization (*1*), have revealed that a wide range of birds, including domestic and feral species, harbour influenza A viruses. Although many viruses have been isolated from migratory waterfowl and from some Laridae (terns), there has been no report of isolates from gulls. In the studies reported here, samples for virus isolation were collected from gulls in three different areas of the United States of America. During 1977–80, tracheal, cloacal, and fecal specimens were collected from populations of ring-billed gulls (*Larus delawarensis*) and Franklin gulls (*Larus pipixcan*) in Maryland, Massachusetts, and Minnesota. A total of 64 haemagglutinating agents were isolated from droppings presumed to be from gulls. Three isolates were made from cloacal swabs. These 67 haemagglutinating agents were identified as influenza A viruses in double immunodiffusion (DID) tests with antisera to influenza A ribonucleoprotein (*2*).

ANTIGENIC ANALYSIS

Further antigenic classification of these isolates by haemagglutination inhibition (HI), neuraminidase inhibition (NI), and DID tests with antisera specific to isolated surface antigens of reference influenza viruses (*3*, *4*) indicated the presence of several different antigenic subtypes, including: H2N9, H5N9, H6N2, H6N8, H6N4, H9N5, H11N2, H11N8, and H11N9. However, there were 41 isolates that possessed haemagglutinin antigens that could not be identified in these tests. Among these viruses, three different neuraminidase antigen subtypes were identified, i.e., N6, N8, and N9.

Since the haemagglutinins of these 41 isolates were not inhibited in HI tests with antisera to the 12 reference subtypes, hyperimmune rabbit antisera were prepared (*4*) to (*a*) a representative isolate, A/gull/Maryland/704/77, which had been cloned twice by limiting dilution in embryonated chicken eggs, and (*b*) a laboratory-derived reassortant virus possessing haemagglutinin derived from Gull/Md/77 virus, i.e., gull/Md/704/77(H)-Bel/1/42 (N1)R. In HI tests with these antisera, all the gull viruses with unidentified HA antigens reacted to high titre ($\geqslant 1:2560$), suggesting that they shared antigenically related haemagglutinins. In contrast, reference influenza virus strains representing the twelve established HA subtypes (*3*) were not inhibited by these sera.

Reciprocal DID tests (*4*) were done, in which (*a*) purified gull/Md/77 virus was tested against specific anti-HA antisera to the twelve established HA subtypes, and (*b*) antiserum to the reassortant gull-Bel (H?N1) virus, adsorbed with Bel/1/42 virus to remove antibodies to cross-reactive influenza A antigens other than HA, was tested against purified reference virus for each of the HA subtypes. No lines of precipitation were observed in the gels except

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between gull/Md/77 virus and homologous antisera, indicating that this antiserum was specific for the HA of gull/Md/77 virus and that the HA of this virus is unrelated to the twelve previously designated subtypes. The above antigenic studies were conducted by the laboratories in both Tennessee and London; the results of both groups confirmed the antigenic uniqueness of the HA of the gull viruses.

NUCLEOTIDE SEQUENCING

Nucleotide sequence analyses of the genes of influenza viruses provide valuable information on genetic relationships between strains and subtypes of virus (*5*). The HA gene of gull/Md/77 virus was analysed and compared with the corresponding genes of viruses representing the twelve established HA subtypes. Viral RNA segment 4, coding for HA, was isolated and sequence analysis of cDNA, primed by a synthetic dodeconucleotide that was complementary to the terminal twelve nucleotides common to the 3′ end of all segments of influenza A virus RNA, was carried out using the dideoxy method (*6*).

Comparisons of regions of the genes coding for haemagglutinin, extending some 20% of the gene length from the 3′ end, and the corresponding deduced amino acid sequences of the twelve HA subtypes showed 20–74% amino acid variation between subtypes (*6*) and, in general, less than 10% variation within subtypes. To characterize the gull viruses, the 3′ end of the genes coding for HA of gull/Mass/26/80 and gull/Md/704/77 were sequenced. For unknown reasons, the sequence of the HA gene of gull/Md/704/77 was not easily read from the sequence gels, and some nucleotides could not be assigned unambiguously. Therefore, the more reliable predicted amino acid sequence of gull/Mass/26/80 virus was compared with corresponding sequences of viruses belonging to the twelve established HA subtypes. The sequence was found to be distinct from that of any other subtype. To obtain quantitative relationships, the predicted amino acid sequences of the two gull HAs were compared with 30 other HA sequences from viruses representing the twelve subtypes. Because the signal peptide sequences are so variable, the analysis was from the N-terminal aspartic acid (*6*) or the corresponding position in the amino acid sequence. The comparisons confirmed that the haemagglutinins of the two gull viruses are closely related, and are distinct from those of subtypes H1 to H12 ($\geqslant 38\%$ amino acid sequence difference), though closest to viruses of the H11 subtype.

Full technical details of these investigations have been reported elsewhere (*7*). We propose that a new haemagglutinin subtype, H13, be designated and that A/gull/Maryland/704/77(H13N6) be designated as the reference strain.

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RÉSUMÉ

CARACTÉRISATION D'UN NOUVEAU SOUS-TYPE D'AGGLUTININE (H13) DE VIRUS DE LA GRIPPE A CHEZ DES MOUETTES

On a constaté dans des isolements de virus de la grippe A provenant de mouettes (*Larus delawarensis* et *Larus pipixcan*) des Etats-Unis d'Amérique la présence d'un antigène hémagglutinant (HA) distinct des douze sous-types d'hémagglutinines précédemment reconnus pour le virus grippal A. Des épreuves sérologiques, avec des antisérums correspondant aux souches de référence qui représentent les sous-types HA 1 à 12 et à un isolement de virus chez une mouette (A/Gull/Maryland/704/77), ont montré que l'hémagglutinine du virus de mouette n'avait pas de relation antigénique avec les hémagglutinines des sous-types précédemment identifiés. De plus, la comparaison des séquences de nucléotides (et des séquences d'acides aminés qui en ont été déduites) de la région 3′ des gènes ARN codant pour l'hémagglutinine de ces virus de mouette montre qu'il s'agit d'un groupe génétiquement distinct. Les auteurs proposent d'appeler H13 ce nouvel antigène HA, qui jusqu'ici n'a été trouvé que chez les mouettes, et de désigner la souche A/Gull/Maryland/704/77 (H13N6) comme souche de référence pour ce sous-type.

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