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Antigenic heterogeneity among influenza A(H3N2) field isolates during an outbreak in 1982/83, estimated by methods of numerical taxonomy

BY W. E. P. BEYER AND N. MASUREL

Department of Virology and WHO Influenza Centre, Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands

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SUMMARY

Fourteen influenza A(H3N2) field isolates, mainly obtained during the first weeks of the 1982/83 influenza epidemic in The Netherlands, and nine influenza A(H3N2) reference strains were examined by means of haemagglutination inhibition (HI) tests with 23 polyclonal ferret sera. The resulting HI patterns were subjected to various methods of numerical taxonomy using, among others, taxonomic distance and correlation between strains for resemblance coefficients. Marked differences between distance and correlation coefficients were found in strains which differed in avidity only. The field isolates could be divided into four groups in respect of their taxonomic resemblance to the reference strains. The same grouping was found for five of the field isolates by testing these against 200 human sera.

INTRODUCTION

Apart from the well-known heterogeneity of influenza A and B viruses in time and place (Palese & Young, 1982), differences in antigenicity and biological properties among field isolates, even during one epidemic (intra-epidemic variation), have recently aroused interest. When, in 1977, Pereira & Chakraverty (1977), while examining large numbers of influenza A and B isolates in Great Britain from the period 1968–76, discovered that 'on occasion two or even three antigenic variants have circulated concurrently' they called this finding 'unexpected'. Indeed, it seemed to contradict the customary view of an epidemically spreading infectious disease as being the result of time- and place-clustered host-to-host transmission of a single infectious agent.

Meanwhile, the existence of antigenically, genetically or biologically different strains during one influenza epidemic has been confirmed by many investigators (Young, Desselberger & Palese, 1979; Ortin *et al.* 1980; Glezen, Couch & Six, 1982; Oxford *et al.* 1983; Velazco *et al.* 1983; World Health Organization, 1984*b*). Three main lines of reasoning have been followed.

(1) One 'mother strain' starts the epidemic and undergoes rapid genetic mutations in the course of the epidemic, resulting in the selection of several 'daughter strains' (Palese *et al.* 1981).

(2) Several different strains exist before the epidemic starts; they become active

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at the same time or join successively and co-circulate independently (Bao-Lan *et al.* 1983).

(3) The infectious agent causing the epidemic is a mixture of several antigenically distinguishable 'substrains' (Schild *et al.* 1983; J. C. de Jong and colleagues, in preparation).

To obtain further insight, Bao-Lan *et al.* (1983) recommended the characterization of field isolates at the very start of a closed or semi-closed outbreak. Such a study has recently been reported by Oxford *et al.* (1983). A recent epidemic in The Netherlands enabled us to follow up this point.

In The Netherlands the impact of influenza has not been strong since the severe epidemic of 1977/78. In 1982/83 there was a mild epidemic caused by influenza A(H3N2) (Masurel & Beyer, 1983), which started explosively in the middle of November 1982, reaching its peak at the end of December 1982 with 44 cases of influenza-like illness per 10000 inhabitants per week, as monitored by the sentinel stations of the Dutch Continuous Morbidity Registration (H. Bijkerk, personal communication), and decreasing rapidly in the course of January 1983. We examined field strains mainly isolated in a limited area and during the first weeks of the epidemic, together with a number of influenza A(H3N2) reference strains by means of elaborate haemagglutination inhibition (HI) tests with human sera and post-infection ferret sera. To quantify the resemblance between the strains as determined on the basis of the test results, various methods of numerical taxonomy have been employed. The value of the approach is discussed in this paper.

MATERIALS AND METHODS

Isolation and propagation of viruses

Virus isolations from human samples (sputum, nasal secretions, throat swabs) were usually performed in cultures of Rhesus monkey kidney cells and, in one case (A/Nederland/242/82) in tertiary Cynomolgus monkey kidney cells. For further analysis, 14 arbitrarily chosen A(H3N2) viruses were inoculated in the allantoic cavity of 10- to 12-day-old embryonated hen's eggs (for nomenclature see Table 1*a–b*). The strains A/Nederland/239/82 to A/Nederland/258/82 (abbreviated throughout this paper as A/Ned/239, etc.) were isolated during the first weeks of the epidemic (November/December 1982), while only A/Nederland/261/83 (A/Ned/261) was isolated later (February 1983). With the exception of A/Ned/242 (kindly provided by Dr J. C. de Jong, Bilthoven) and A/Ned/240, which were isolated in the provinces of Utrecht and Zeeland respectively, all strains were obtained from patients who presented at the University Hospital in Rotterdam.

Laboratory reference influenza A(H3N2) strains A/Hong Kong/2/68 (A/HK/68), A/Port Chalmers/1/73 (A/PC/73), A/Victoria/3/75 (A/Vic/75), A/Texas/1/77 (A/Tx/77), A/Bangkok/1/79 (A/BK/1/79), A/Bangkok/2/79 (A/BK/2/79), A/England/496/80 (A/Eng/80), A/Shanghai/31/80 (A/Sha/80), and A/Philippines/2/82 (A/Phil/82) were kindly provided by Dr J. J. Skehel, WHO World Influenza Centre, London, England, and propagated in chicken eggs. The strains are drift variants of the A(H3N2) subtype which have demonstrated epidemic potential throughout the world (Pereira, 1979; World Health Organization, 1983).

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Serology

Antisera were obtained by intranasal infection of ferrets with virus-containing egg fluids followed by bleeding of the animals after 18 days.

From a collection of sera obtained from healthy volunteers of all ages in 1980, 200 were randomly selected and examined in the HI test for antibody against A/BK/1/79, A/Phil/82, and the field isolates A/Ned/239, A/Ned/248, A/Ned/251, A/Ned/252 and A/Ned/257.

All sera were pre-treated with *Vibrio cholerae* filtrate at 37 °C for 16 h and inactivated at 56 °C for 1 h (Masurel, 1962). The HI test was performed in microvolumes as previously described (Dowdle, Kendal & Noble, 1979). Titres were expressed as the reciprocal of the serum dilution showing 50 % haemagglutination by four haemagglutination units of virus. If no titre was observed (< 9), it was arbitrarily recorded as 5 for calculations. Each HI test was performed twice. The entries in Table 1 *a–b* are the geometric mean titres (GMTs) of the two experiments.

Numerical taxonomy

In the chessboard HI test the virus strains can be considered as taxonomic units and their reactions with the antisera as multivariate observations (Lee, 1968) or character states (Sneath & Sokal, 1973). Thus test results could be subjected to methods of numerical taxonomy.

The logarithmic titre values were programmed in a Hewlett-Packard 41CV computer, which calculated a 'sufficient fit' and two different resemblance coefficients for each combination of two virus strains in respect of all antisera.

Definitions

(1) *Sufficient fit.* If two strains did not show fourfold or greater difference with all 23 antisera, they were considered not distinguishable according to Dowdle, Kendal & Noble (1979) ('sufficient fit'). If the difference was fourfold or greater with at least one antiserum, they were considered distinguishable ('no sufficient fit').

(2) *Pearson product–moment correlation coefficient* r_{jk}.

$$r_{jk} = \frac{\sum_{i=1}^{n} (X_{ij} - \bar{X}_j)(X_{ik} - \bar{X}_k)}{\sqrt{\left[\sum_{i=1}^{n} (X_{ij} - \bar{X}_j)^2 \sum_{i=1}^{n} (X_{ik} - \bar{X}_k)^2\right]}},$$

where i = any antiserum, n = number of antisera, j and k = two virus strains compared with each other, X_{ij}, X_{ik} = $\log_{10}$ of the titre value of strains j and k, respectively, against antiserum i, $\bar{X}_j$, $\bar{X}_k$ = mean of all logarithmic titre values of virus strains j and k, respectively. For further calculations (averaging) the Fisher z transformation of r_{jk} had to be used, because the variance of the correlation coefficient is not constant, but depends on its magnitude, a disadvantage which is ruled out by the transformation (Sokal & Sneath, 1963):

$$z_{jk} = \tfrac{1}{2} \ln \frac{1 + r_{jk}}{1 - r_{jk}}.$$

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(3) *Taxonomic distance coefficient* d^2_{jk}.

$$d^2_{jk} = \frac{\sum_{i=1}^{n} (X_{ij}-X_{ik})^2}{n-1} \quad \text{(symbols as mentioned above).}$$

The d^2_{jk} was also calculated for standardized titre values, i.e. every logarithmic entry X_{ij} of the chessboard HI table was transformed by the following equation:

$$X'_{ij} = \frac{X_{ij}-\bar{X}_i}{S_i},$$

where $\bar{X}_i$ and S_i = mean and standard deviation of all reactions with antibody i.

Thus, three different matrices of resemblance coefficients (one z_{jk} and two d^2_{jk} for original and transformed data, respectively) were obtained (not shown), each of which were subjected to the weighted pair-group method using arithmetic averages, a numerical technique for defining groups (clusters) of related organisms based on high similarity. The whole procedure has been described in detail by Sneath & Sokal (1973). In short, the two viruses producing the highest coefficient of the matrix were selected. Subsequently, their coefficients with each of the remaining viruses were replaced by the arithmetic average of those two coefficients (a pair). Thus a new matrix was formed with a reduced number of coefficients of the resting viruses and a new structure (cluster) representing the average resemblance of the two closest viruses in respect of all other viruses, and being quantitatively defined by the coefficient of these two viruses. Then the procedure was repeated with the highest coefficient of the new matrix. A cluster of viruses, once established, was treated as a single virus, regardless of its content (equal weighting of clusters and single organisms). This was repeated until all viruses and clusters were linked together. The results were visualized by a so-called dendrogram which showed the virus strains (abscissa) forming clusters along a coefficient scale (ordinate). The clusters in terms of the Fisher z transformation were recalculated to give correlation coefficients.

RESULTS

Chessboard HI test

The original titre values of 9 reference strains and 14 field isolates against 23 ferret antisera (Tables 1*a* and 1*b*, respectively) are difficult to interpret because of their large number.

A general idea of strain resemblance can be obtained by the sufficient fits. Fig. 1 presents the symmetric matrix indicating a sufficient fit by '+' and no sufficient fit by a blank for each combination of two viruses. The sequence of the strains was changed to make cluster-forming visible. All 9 reference strains were distinguishable from each other (no sufficient fit). Each of the field isolates was associated with either 1 or 2 of the reference strains. For instance, A/Ned/252 and A/Ned/258 formed a cluster both with A/Tx/77 and A/Eng/80, A/Ned/255 with A/BK/2/79, and A/Ned/242, A/Ned/247, A/Ned/251, A/Ned/257 and A/Ned/261 with A/Sha/80. The remaining field strains were grouped with

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A/Eng/80, in the course of which 3 of these (A/Ned/240, A/Ned/248, and A/Ned/254) seemed to show some connexion with A/Sha/80 and A/Ned/242 as well.

Thus, the matrix structure suggests four groups of field isolates (A/Tx/77-, A/Eng/80-, A/Sha/80-, and A/BK/2/79-associated strains) with existing 'intermediate' positions of some strains. Here the qualification 'intermediate' is not used in the sense of phylogeny, but purely descriptive. No field strain was connected with A/BK/1/79 or A/Phil/82.

Further insight in the taxonomic structure is given by the coefficient matrices and the resulting dendrograms. Fig. 2 shows the dendrogram based on the transformed d^2_{jk} matrix. As could be expected, reference strains show no close relationship with each other, forming common clusters only at large distance coefficients. The smallest cluster limit where two clusters containing reference strains are joined is 0·24 (A/Tx/77 × A/Eng/80). Thus, a phenon line drawn at 0·24 may be supposed to separate closely related strains and clusters joining at a taxonomic distance $< 0{\cdot}24$ from less related strains and clusters joining at a distance $\geqslant 0{\cdot}24$. On this condition, the 14 field isolates are found to be separated into four groups, each connected with one reference strain. These groups are identical with those obtained by the sufficient-fit matrix, except that the expression of the 'intermediate' character of some strains by the dendrogram is less clear.

The dendrogram based on original d^2_{jk} coefficients (not shown) is nearly identical. Group composition of the dendrogram based on correlation coefficients (Fig. 3) is also in marked agreement with results observed in Fig. 2, regardless of small differences in the internal structure of some of the clusters. Although in both dendrograms the sequence of the reference strains is essentially obtained by the cluster method, it also reflects in part the antigenic drift of the reference strains from 1968 to 1982. An obvious difference between the two dendrograms is the position of A/BK/1/79. Whereas this reference strain is not closely related to any of the other viruses in the sufficient-fit matrix and in the d^2_{jk} dendrogram, it shares in the A/Sha/80-group of the r_{jk} dendrogram. This disparity is the taxonomic expression of the phenomenon of avidity (Schild & Dowdle, 1975). As can be seen in Table 1*a*, titres of the A/BK/1/79 virus are all higher than those of A/Sha/80, namely 3- to 8-fold in most cases. Thus A/Sha/80 is following the same pattern as A/BK/1/79 on a lower titre level (paralellism). The relatively constant factor between both patterns, resulting in a high taxonomic distance, is ignored by the correlation coefficient, as can be derived from the formulas in Materials and Methods.

Thus the combined interpretation of three taxonomic systems derived from original test results leads to a characterization of the field isolates in respect of grouping with reference strains, intermediate position and avidity, as summed up in Table 2.

HI test on 200 human sera

Table 3 shows the number of positive and negative sera for each virus tested, and the overall geometric mean titre (GMT) per virus. The differences between the logarithmic titres of all combinations of two viruses have been tested for significance by the paired t test. All strains differ significantly ($P < 0{\cdot}01$) from

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Table 1*a*. *Chessboard HI tests of nine influenza A(H3N2) reference strains against 23 ferret antisera. Entries are GMTs of two experiments. Titres < 9 are recorded as 5*

Ferret antisera	Viruses								
	A/HK/68	A/PC/73	A/Vic/75	A/Tx/77	A/BK/1/79	A/BK/2/79	A/Eng/80	A/Sha/80	A/Phil/82
A/HK/68	*4876*	287	91	5	5	5	12	5	5
A/PC/73	271	*1422*	1267	543	128	24	261	63	5
A/Vic/75	22	813	*2659*	5166	2844	448	2172	813	48
A/Tx/77	271	913	2509	*5166*	4344	2172	5267	1536	305
A/BK/1/79	5	30	457	1086	*11202*	564	4180	3448	136
A/BK/2/79	5	29	256	1086	2844	*7564*	2583	887	271
A/Eng/80	5	48	1581	4344	17376	8201	*17376*	3870	543
A/Sha/80	5	34	271	305	16720	543	2633	*4445*	136
A/Phil/82	34	12	305	1086	4344	543	2172	1086	*8201*
A/Ned/239	5	34	2438	4344	8045	2172	7303	1536	543
A/Ned/240	5	48	543	1935	3254	543	2301	430	271
A/Ned/242	5	484	4876	16402	13271	2172	4344	3072	609
A/Ned/247	5	24	1724	2172	7306	1536	4602	2172	543
A/Ned/248	5	5	913	2172	30966	1219	6143	3653	2172
A/Ned/249	5	34	1086	4344	16402	1536	6143	2583	271
A/Ned/250	5	12	2172	8688	17438	1086	4344	2301	271
A/Ned/251	5	34	1086	4344	4344	1086	3072	768	305
A/Ned/252	5	136	2301	8688	16402	2438	12286	2050	543
A/Ned/254	5	136	1292	7741	5799	1086	4602	1086	543
A/Ned/255	5	68	1086	2438	4101	543	3072	786	271
A/Ned/257	5	136	407	1086	17375	192	4101	3072	271
A/Ned/258	5	5	1086	2172	3254	543	3072	543	271
A/Ned/261	5	30	1086	2172	30966	609	8688	4344	484

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Table 1*b*. *Chessboard HI tests of* 14 *Dutch influenza A*(*H3N2*) *field isolates from the season* 1982/83 *against* 23 *ferret antisera. Entries are GMTs of two experiments. Titres* < 9 *are recorded as* 5

	Viruses													
Ferret antisera	A/Ned /239	A/Ned /240	A/Ned /242	A/Ned /247	A/Ned /248	A/Ned /249	A/Ned /250	A/Ned /251	A/Ned /252	A/Ned /254	A/Ned /255	A/Ned /257	A/Ned /258	A/Ned /261
A/HK/68	12	9	5	5	17	5	7	5	5	5	5	5	13	5
A/PC/73	121	68	30	30	136	96	68	14	136	68	30	17	192	34
A/Vic/75	1935	1151	362	768	1086	1086	1086	271	2899	1086	271	271	2172	271
A/Tx/77	3871	2583	609	711	4344	2956	2172	543	6143	2172	1086	543	4344	543
A/BK/1/79	3653	2900	1283	3072	2172	1724	1219	1450	2051	1086	384	1935	1086	1935
A/BK/2/79	1150	1086	384	457	2172	2090	1219	384	2050	1086	4344	271	1086	384
A/Eng/80	15483	6144	3871	2172	8688	8360	4344	1627	7741	7741	6144	2172	4344	2172
A/Sha/80	2900	2301	1086	3072	2172	1659	1086	1627	1217	1086	271	2172	1086	2172
A/Phil/82	2172	2172	1536	1086	2172	6143	2172	543	2438	2172	543	1086	1086	1086
A/Ned/239	*5107*	5267	1219	1267	4344	3870	4602	610	7448	4101	2899	1086	5166	768
A/Ned/240	2438	*2509*	646	430	1368	1329	1536	223	2737	1086	513	287	2172	271
A/Ned/242	5267	4514	*1792*	2534	3653	3653	4344	1721	5473	3653	1219	2172	8688	2172
A/Ned/247	5267	4344	1219	*996*	3072	3072	3072	768	4180	2172	768	813	5799	1086
A/Ned/248	5166	4344	2899	3072	*5267*	6143	3072	1536	3653	2172	1536	2172	2583	1086
A/Ned/249	5166	4344	3072	2172	4344	*4942*	3072	1368	4344	3072	2172	2172	2301	2172
A/Ned/250	3254	4344	2583	1827	4344	4737	*3072*	1368	4344	2900	1086	1627	3072	1536
A/Ned/251	2583	3072	913	513	1536	4602	3072	*543*	4101	2900	913	768	2172	609
A/Ned/252	9474	4876	1724	2257	4344	8688	6143	1086	*8688*	5799	1827	1219	4344	1086
A/Ned/254	3072	3072	1086	768	2172	6508	3072	543	4344	*3072*	768	768	2172	609
A/Ned/255	2172	2172	862	543	1536	2984	3653	384	3072	1086	*896*	543	1536	543
A/Ned/257	3072	2172	3072	2583	3072	3072	3072	1724	2583	1536	287	*2090*	1086	2172
A/Ned/258	3072	1536	513	513	2050	2737	2900	484	3653	1450	543	543	*3448*	543
A/Ned/261	8688	4875	4875	4344	8688	6144	8688	3072	7741	3871	1086	4344	2172	*2900*

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Table 2. *Taxonomic relation between reference strains and field isolates*

Reference strains	Field isolates	Number of field isolates (percentage)
A/Tx/77	A/Ned/258*	1 (7%)
A/BK/2/79	A/Ned/255	1 (7%)
A/Sha/80†	A/Ned/242, A/Ned/247, A/Ned/251, A/Ned/257, A/Ned/261	5 (36%)
A/Eng/80	A/Ned/239, A/Ned/240,‡ A/Ned/248,‡ A/Ned/249, A/Ned/250, A/Ned/252,* A/Ned/254‡	7 (50%)

* 'Intermediate' between A/Tx/77 and A/Eng/80.
† Strains of this group are non-avid with respect to A/BK/1/79.
‡ 'Intermediate' between A/Sha/80 and A/Eng/80.

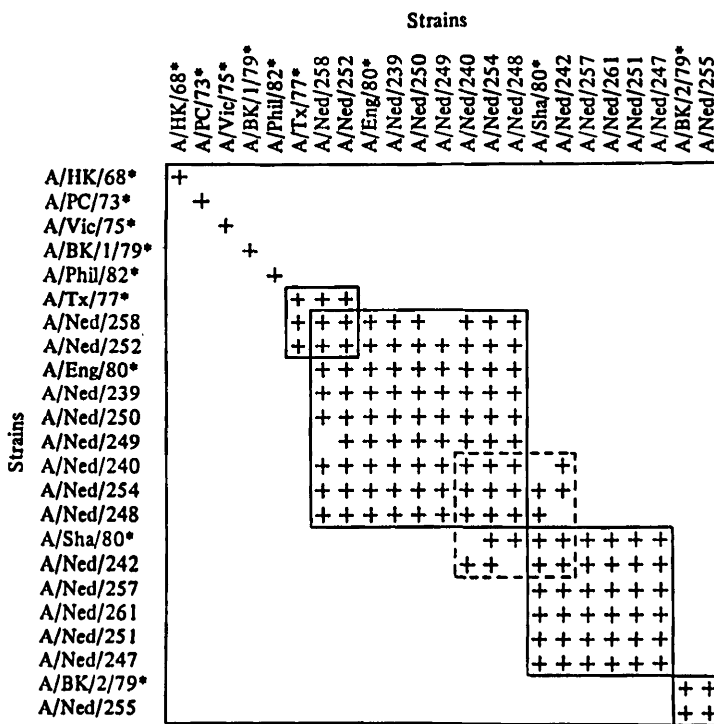

Fig. 1. Matrix of sufficient fits. '+' means a sufficient fit as defined in Materials and Methods. Blank means no sufficient fit. Sequence of strains has been changed to make clusters (encased) visible. * Reference strains.

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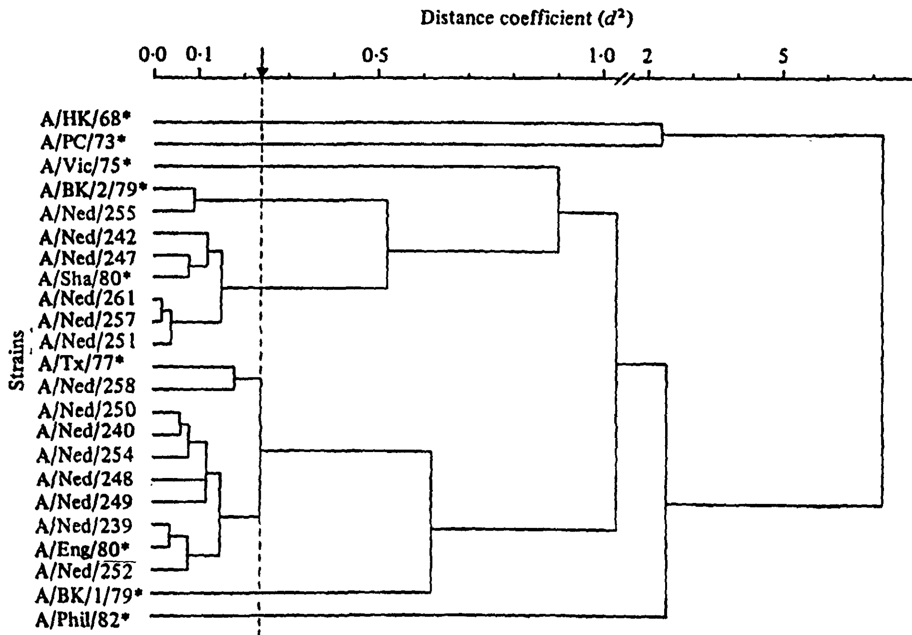

Fig. 2. Dendrogram based on taxonomic distance coefficients (d^2) of transformed titre values. For threshold line at $d^2 = 0{\cdot}24$, see text. * Reference strains.

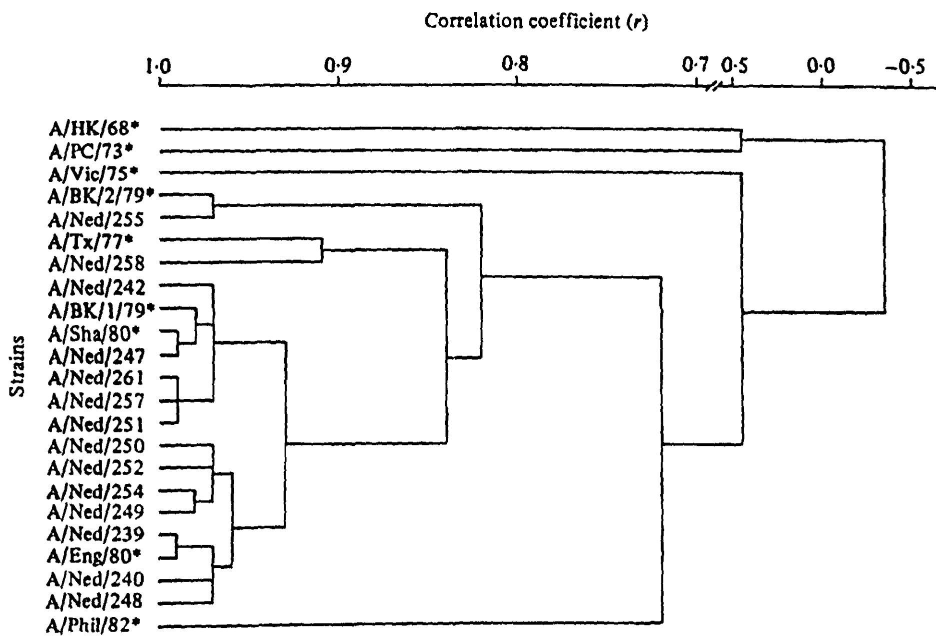

Fig. 3. Dendrogram based on correlation coefficients (r). * Reference strains. Note position of A/BK/1/79.

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Table 3. *Results of testing of two reference strains and five field isolates against 200 human sera collected in* 1980

	Viruses						
	A/BK /1/79	A/Phil /82	A/Ned /239	A/Ned /248	A/Ned /251	A/Ned /252	A/Ned /257
Number of sera with titre < 9	64	88	65	67	80	75	77
Number of sera with titre ⩾ 9	136	112	135	133	120	125	123
Overall geometric mean titres* ($n = 200$)	58	21	34	35	22	34	24

* Titres < 9 are recorded as 5.

A/BK/1/79 and, except for A/Ned/251, from A/Phil/82. A/Ned/239, A/Ned/248 and A/Ned/252 show no mutual significant differences, but do differ from A/Ned/251 and A/Ned/257, and vice versa, which is in accordance with results presented in Table 2, where A/Ned/239, A/Ned/248 and A/Ned/252 are indeed found to belong to a reference strain-related group different from that of A/Ned/251 and A/Ned/257.

DISCUSSION

The findings presented in this paper indicate the existence of antigenetically distinguishable influenza A(H3N2) strains, even during a short period and in a limited area. Although only 14 field isolates are involved in this study, it is of interest that even among this small number four different groups can be established (Table 2), all related to a reference strain. None of the groups is close to the recently worldwide increasing A/Phil/82 strain (World Health Organization, 1983). Two field strains resemble reference strains from 1977 and 1979, respectively, while 12 out of the 14 isolates are related to the more recent reference strains from 1980, suggesting that the quantitative proportion of older strains is decreasing in favour of the more recent ones.

It is well known that during the first six years of the subtype H3N2 period, the subsequent epidemic strains replaced each other (1968–72: A/Hong Kong/1/68; 1972–73: A/England/42/72; from 1973 onwards: A/Port Chalmers/1/73), while this has not been true since 1974, when two or more strains started to 'co-circulate' together (Pereira & Chakraverty, 1977). By reviewing the literature (World Health Organization, 1975–82; 1984*a*), the suggestion arises that since 1977 the number of H3N2 variants that are co-circulating each year seems to increase constantly. At this moment it is not clear whether this phenomenon is a sign of the dying down of the H3N2 subtype or, alternatively, of a more advantageous strategy to persist and survive.

Of the three possible explanations for intra-epidemic variation mentioned in the Introduction, the first one suggesting the successive mutation of a 'mother strain' and subsequent selection of 'daughter strains' in the course of an epidemic, does not seem to be applicable to our study. The strains used by us were mainly isolated during the first weeks of the epidemic, and all resemble the older reference strains.

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The second concept, claiming the co-circulation of several independent strains, cannot be excluded by our test results. However, a number of questions remain, for example: do all strains emerge at the same time; can they remain in circulation in a dense population without any form of interference or recombination; is each strain indeed linked with independent host-to-host transmission?

The third approach, suggesting a mixture of different 'substrains' in original patients' specimens, has recently gained interest (Kendal, Noble & Dowdle, 1977; Kilbourne, 1978; Schild *et al.* 1983; J. C. de Jong and colleagues, in preparation). According to the latter authors, the four groups among the isolates would represent components or substrains of a mixture which is thought to be transmitted from host to host as a whole and to remain essentially stable in its qualitative composition in the course of an epidemic. The quantitative portions of the mixture, however, may vary in each individual host, depending on the presence of antibodies against any of the components formed in response to earlier infections. On the basis of this hypothesis, research on original patients' material to determine its initial quantitative and qualitative composition and the study of an animal model are in progress.

In this context it should be mentioned that influenza virus heterogeneity has already been described as a change from an original (O) to a derivate (D) form by Burnet & Bull (1943). Quantitation of antigenic variants in cloned influenza pools was first published by Yewdell, Webster & Gerhard (1979).

In this paper the basic method to establish the four groups of isolates is an HI cross-tritration with ferret antisera against each of the virus strains. This offers the advantage of recognizing the smallest differences between recent isolates, whereas monoclonal antibodies, which are usually formed against some older reference strains, may theoretically fail to do so. On the other hand, polyclonal animal antisera present a huge amount of cross reactions caused by overlapping antibodies (T. F. Weijers and colleagues, in preparation). Another reason for cross-reactions between the field isolates might be the presence of shared substrains in low concentrations. All this might cause the 'intermediate' position of some isolates in the sufficient-fit matrix (Fig. 1), which is based only on 'significant' differences between strains, i.e. titre values differing more than fourfold. The taxonomic coefficient matrices, however, use all titre values of Table 1*a*–*b*, even if there is no 'significant' difference between these. This is possible if the number of entries is large enough (for discussion on this point see Sneath & Sokal, 1973). Additional confirmation is acquired by the agreement between the sufficient-fit matrix and the dendrogram based on the distance coefficients.

Taxonomic methods to clarify antigenic relationships among influenza virus strains from large experimental data similar to those presented here were introduced by Lee (1968), Lee & Tauraso (1968), and Dowdle *et al.* (1969) for HI chessboard titrations, and later also for neutralizing, complement fixation, and neuraminidase inhibition cross-titrations (Meier-Ewert, Gibbs & Dimmock, 1970; Chakraverty, 1971). In most of these studies only the Pearson product–moment correlation coefficient was used as a measure of similarity. This is easy to calculate but may suffer from theoretical (for review see Hall, 1967) and, in influenza serology, practical disadvantages, as it cannot discriminate between closely related strains, i.e. those with similar antibody patterns, and strains which are related by avidity only (A/BK/1/79 and A/Sha/80 in our study). Avidity is a well-known

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and frequent phenomenon in influenza serology (for review see Schild & Dowdle, 1975), but has not yet been sufficiently explained.

Distance coefficients in influenza serology, already used by Underwood (1982) for classification of a large number of monoclonal antibodies against an A(H3N2) strain and by T. F. Weijers and colleagues (in preparation), cannot recognize avid and non-avid strains either, considering these as poorly related strains. Thus, in our opinion, the combined use of correlation and distance coefficients takes into account the particularity of influenza serology. For establishing groups of related viruses within the coefficient matrices, the weighted pair-group cluster method was chosen instead of the alternative unweighted clustering (for review see Sokal & Sneath, 1963). During each clustering cycle the latter recomputes resemblance coefficients based not on the previous matrix but on the initial matrix. Thus in our study the clusters with large numbers of strongly related viruses would artificially have attracted even less related viruses and, therefore, decreased the distance between close and non-close strains, being unfavourable for discrimination. Equal weighting of clusters and single strains at every clustering cycle had avoided this problem.

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REFERENCES

BAO-LAN, L., WEBSTER, R. G., BROWN, L. E. & NEROME, K. (1983). Heterogeneity of influenza B viruses. *Bulletin of the World Health Organization* **61**, 681.

BURNET, F. M. & BULL, D. R. (1943). Changes in influenza virus associated with adaptation to passage in chick embryos. *The Australian Journal of Experimental Biology and Medical Science* **21**, 55.

CHAKRAVERTY, P. (1971). Antigenic relationship between influenza B viruses. *Bulletin of the World Health Organization* **45**, 755.

DOWDLE, W. R., COLEMAN, M. T., HALL, E. C. & KNEZ, V. (1969). Properties of the Hong Kong influenza virus. 2. Antigenic relationship of the Hong Kong virus haemagglutinin to that of other human influenza A viruses. *Bulletin of the World Health Organization* **41**, 419.

DOWDLE, W. R., KENDAL, A. P. & NOBLE, G. R. (1979). Influenza viruses. In *Diagnostic Procedures for Viral, Rickettsial and Chlamydial Infections*, 5th ed. (ed. E. H. Lennette and N. J. Schmidt), pp. 585–609. Washington: American Public Health Association.

GLEZEN, W. P., COUCH, R. B. & SIX, H. R. (1982). The influenza herald wave. *American Journal of Epidemiology* **116**, 589.

HALL, A. V. (1967). Methods for demonstrating resemblance in taxonomy and ecology. *Nature* **214**, 830.

KENDAL, A. P., NOBLE, G. R. & DOWDLE, W. R. (1977). Swine influenza viruses isolated in 1976 from man and pig contain two coexisting subpopulations with antigenically distinguishable hemagglutins. *Virology* **82**, 111.

KILBOURNE, E. D. (1978). Genetic dimorphism in influenza viruses: characterization of stably associated hemagglutin mutants differing in antigenicity and biological properties. *Proceedings of the National Academy of Sciences USA* **75**, 6258.

LEE, A. M. (1968). Numerical taxonomy and influenza B virus. *Nature* **217**, 620.

LEE, A. M. & TAURASO, N. M. (1968). A method for the formulation of influenza virus vaccine using numerical taxonomy. *Bulletin of the World Health Organization* **39**, 261.

https://doi.org/10.1017/S0022172400061179 Published online by Cambridge University Press

MASUREL, N. (1962). Studies on the Content of Haemagglutination Inhibiting Antibody for Swine Influenza Virus A in Sera from People Living in The Netherlands in 1957–1958. Thesis, University of Leiden.

MASUREL, N. & BEYER, W. E. P. (1983). Influenza in the winter 1982–1983, caused by the A-H3N2-virus [in Dutch]. *Nederlands Tijdschrift voor Geneeskunde* **127**, 487.

MEIER-EWERT, H., GIBBS, A. J. & DIMMOCK, N. J. (1970). Studies on antigenic variations of the haemagglutinin and neuraminidase of swine influenza virus isolates. *Journal of General Virology* **6**, 409.

ORTIN, J., NAJERA, A., LOPEZ, C., DAVILA, M. & DOMINGO, E. (1980). Genetic variability of Hong Kong (H3N2) influenza viruses: spontaneous mutations and their location in the viral genome. *Gene* **11**, 319.

OXFORD, J. S., ABBO, H., CORCORAN, T., WEBSTER, R. G., SMITH, A. J., GRILLI, E. A. & SCHILD, G. C. (1983). Antigenic and biochemical analysis of field isolates of influenza B virus: evidence for intra- and inter-epidemic variation. *Journal of General Virology* **64**, 2367.

PALESE, P., BRAND, C., YOUNG, J. F., BAEZ, M., SIX, H. R. & KASEL, J. A. (1981). Molecular epidemiology of influenza viruses. In *Perspectives in Virology*, vol. XI, pp. 115–127. New York: Alan Liss.

PALESE, P. & YOUNG, J. F. (1982). Variation of influenza A, B, and C viruses. *Science* **215**, 1468.

PEREIRA, M. S. (1979). Global surveillance of influenza. *British Medical Bulletin* **35**, 9.

PEREIRA, M. S. & CHAKRAVERTY, P. (1977). The laboratory surveillance of influenza epidemics in the United Kingdom 1968–1976. *Journal of Hygiene* **79**, 77.

SCHILD, G. C. & DOWDLE, W. R. (1975). Influenza virus. Characterization and diagnostic serology. In *The Influenza Viruses and Influenza* (ed. E. D. Kilbourne), pp. 315–372. New York: Academic Press.

SCHILD, G. C., OXFORD, J. S., DE JONG, J. C. & WEBSTER, R. G. (1983). Evidence for host-cell selection of influenza virus antigenic variants. *Nature* **303**, 706.

SNEATH, P. H. A. & SOKAL, R. R. (1973). The aims and principles of numerical taxonomy. In *Numerical Taxonomy. The Principles and Practice of Numerical Classification*, by P. H. A. Sneath and R. R. Sokal, pp. 68–75, 121–128. San Francisco: Freeman.

SOKAL, R. R. & SNEATH, P. H. A. (1963). *Principles of Numerical Taxonomy*, pp. 290–319. San Francisco: Freeman.

UNDERWOOD, P. A. (1982). Mapping of antigenic changes in the haemagglutinin of Hong Kong influenza (H3N2) strains using a large panel of monoclonal antibodies. *Journal of General Virology* **62**, 153.

VELAZCO, J. G., COUCH, R. B., SIX, H. R. & GLEZEN, W. P. (1983). Surveillance of influenza in Houston, Texas, USA: gradual transition from A/Victoria/75(H3N2) to A/Texas/77(H3N2) predominance and antigenic characterization of 'intermediate' strains. *Bulletin of the World Health Organization* **61**, 345.

WORLD HEALTH ORGANIZATION (1975). Influenza in the world, October 1973–September 1974. *Weekly Epidemiological Record* **50**, 29; (1976) Influenza in the world, October 1974–September 1975. *Weekly Epidemiological Record* **51**, 29; (1977) Influenza in the world, October 1975–September 1976. *Weekly Epidemiological Record* **52**, 37; (1978) Influenza in the world, October 1976–September 1977. *Weekly Epidemiological Record* **53**, 16; (1979) Influenza in the world, October 1977–September 1978. *Weekly Epidemiological Record* **54**, 25; (1980) Influenza in the world, October 1978–September 1979. *Weekly Epidemiological Record* **55**, 17; (1981) Influenza in the world, October 1979–September 1980. *Weekly Epidemiological Record* **56**, 33; (1982) Influenza in the world, October 1980–September 1981. *Weekly Epidemiological Record* **57**, 41; (1982) Influenza in the world, October 1981–September 1982. *Weekly Epidemiological Record* **57**, 389; (1983) Recommended composition of influenza vaccines for use in the 1983–1984 season. Influenza in the world, October 1982–February 1983. *Weekly Epidemiological Record* **58**, 53; (1984*a*) Influenza in the world, October 1982–September 1983. *Weekly Epidemiological Record* **59**, 5; (1984*b*) Recommended composition of influenza vaccines for use in the 1984–1985 season. Influenza in the world, October 1983–February 1984. *Weekly Epidemiological Record* **59**, 53.

YEWDELL, J. W., WEBSTER, R. G. & GERHARD, W. U. (1979). Antigenic variation in three distinct determinants of an influenza type A haemagglutinin molecule. *Nature* **279**, 246.

YOUNG, J. F., DESSELBERGER, U. & PALESE, P. (1979). Evolution of human influenza A viruses in nature: sequential mutations in the genomes of new H1N1 isolates. *Cell* **18**, 73.

https://doi.org/10.1017/S0022172400061179 Published online by Cambridge University Press