Research Recherche

Bulletin of the World Health Organization, 62 (6): 871 – 878 (1984)

Antigenic comparisons of swine-influenza-like H1N1 isolates from pigs, birds and humans: an international collaborative study

V. S. HINSHAW,[1] D. J. ALEXANDER,[2] M. AYMARD,[3] P. A. BACHMANN,[4] B. C. EASTERDAY,[5] C. HANNOUN,[6] H. KIDA,[7] M. LIPKIND,[8] J. S. MACKENZIE,[9] K. NEROME,[10] G. C. SCHILD,[11] C. SCHOLTISSEK,[12] D. A. SENNE,[13] K. F. SHORTRIDGE,[14] J. J. SKEHEL,[15] & R. G. WEBSTER[16]

The objective of this international collaborative study was to compare recent swine isolates of influenza viruses and determine whether significant antigenic differences among isolates from different areas of the world could be detected. H1N1 viruses isolated from pigs, birds and humans in 12 different countries were compared in haemagglutination-inhibition assays with post-infection ferret sera and monoclonal antibodies to H1N1 strains. Using A/NJ/8/76 as the reference strain, we found that recent swine isolates from Hong Kong, Italy, Japan, and the USA possess a haemagglutinin virtually indistinguishable from that of viruses typically associated with pigs, i.e., A/NJ/8/76. In contrast, recent swine isolates from several European countries (Belgium, Denmark, France, Federal Republic of Germany, and Spain) were distinguishable from A/NJ/8/76, as demonstrated by tests in the various laboratories. These studies suggest that the H1N1 viruses in pigs are antigenically heterogeneous and that the circulation of particular variants is associated with the geographical location of the animals. These results raise the question of whether these viruses originated from the same source, i.e., pigs, and have undergone antigenic drift or, alternatively, were introduced from other hosts, such as birds.

[1] Associate Member, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38101, USA. Requests for reprints should be sent to this author.

[2] Virologist, Central Veterinary Laboratory, Weybridge, Surrey, England.

[3] Professor of Microbiology and Director, Centre National de la Grippe — France-Sud, Lyons, France.

[4] Professor, Institute of Medical Microbiology, Ludwig-Maximilians University, Munich, Federal Republic of Germany.

[5] Professor, Department of Veterinary Sciences, University of Wisconsin, Madison, WI, USA.

[6] Professor, Unit of Viral Ecology, Centre National de la Grippe — France-Nord, Pasteur Institute, Paris, France.

[7] Associate Professor, Department of Veterinary Hygiene and Microbiology, Hokkaido University, Sapporo, Japan.

[8] Associate Professor, Department of Biochemistry, Kimron Veterinary Institute, Beit Dagan, Israel.

[9] Associate Professor, Department of Microbiology, University of Western Australia, Nedlands, Western Australia, Australia.

[10] Chief, Respiratory Virology Division, The National Institute of Health, Tokyo, Japan.

[11] Head, Biological Standards and Control, Hampstead Laboratories, London, England.

[12] Professor, Virology Institute, Justus-Liebig University, Giessen, Federal Republic of Germany.

[13] Microbiologist, National Veterinary Science Laboratories, Ames, IA, USA.

[14] Professor, Department of Microbiology, University of Hong Kong, Hong Kong.

[15] Head, WHO Collaborating Centre for Influenza Reference and Research, National Institute for Medical Research, London, England.

[16] Professor, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, Memphis, TN, USA.

Influenza disease outbreaks in pigs involving H1N1 viruses represent a significant problem in these animals throughout the world (*28*). Pigs in the USA have experienced a high incidence of influenza for many years, more recently involving H1N1 strains antigenically similar to A/NJ/8/76 (*6*); however, this has not been the case in other areas of the world. In Europe, there was little indication of swine influenza until 1976 (*17*); since then, disease outbreaks in European pigs have increased in severity and frequency, (*1-3, 5, 19, 21, 23, 25, 27*). Although some outbreaks have been associated with the importation of pigs from the USA (*17*), this is not always the case. In view of the increased circulation of H1N1 viruses in pigs in different countries (*28*), an international collaborative study was undertaken to examine the distribution of these viruses and to characterize the isolates from these animals.

Antigenic characterization of the H1N1 viruses was of particular interest because previous studies (*28, 29*) had suggested that isolates from pigs in Belgium were more closely related to avian H1N1 viruses whereas viruses from pigs in France were more like human H1N1 strains. Since antigenically related viruses exist in humans and birds (*9*), it was possible that these H1N1 viruses in pigs originated from other sources. Recent studies also suggested that the European swine isolates were antigenically (*28*) and genetically (*22*) different from the viruses from US pigs. In view of these findings and a recommendation by the participants at a WHO meeting on the ecology of influenza viruses (*29*), collaboration between influenza laboratories was established to compare the viruses involved in influenza disease outbreaks in pigs throughout the world. To accomplish this, reference antigens and antisera were distributed to investigators who then tested isolates from pigs, birds and humans available in their repositories in haemagglutination-inhibition tests. The results of these tests indicate that the viruses circulating in pigs include H1N1 viruses closely related to those typically associated with pigs and, in addition, those typically associated with birds. The only avian species harbouring swine-influenza-like viruses were turkeys in the USA. These studies indicate that pigs harbour antigenically distinct H1N1 viruses.

MATERIALS AND METHODS

Viruses. Influenza A virus isolates were grown in 10–11-day old embryonated chicken eggs and characterized serologically as H1N1 with hyperimmune goat antiserum, post-infection ferret serum, and rabbit antisera (*6, 7*) in the individual laboratories. A total of 118 H1N1 swine isolates obtained during 1977–83 were examined, including viruses from Belgium, Denmark, England, France, Federal Republic of Germany, Hong Kong, Italy, Japan, Spain and the USA. A total of 60 avian isolates with an H1 haemagglutinin isolated during 1976–83 were examined; these included viruses from ducks, turkeys, chickens, coots and geese from Australia, Canada, England, Federal Republic of Germany, Hong Kong, Israel, Japan and the USA. In view of the number of viruses included, they will not be listed individually; however, representative strains of particular interest will be described in the Results section. Prototype strains —A/NJ/8/76 and A/Dk/Alb/35/76—were grown in embryonated chicken eggs and provided to each laboratory as control antigens.

Serological assays and antisera. Haemagglutinin (HA) titrations and haemagglutination-inhibition (HI) tests were performed in micro- or macrotitration plates with receptor-destroying enzyme-treated sera (*20*). Post-infection ferret sera were prepared, as already described (*20*). It should be noted that ferrets inoculated with A/Dk/Alb/35/76 received an intraperitoneal injection of 5×10^7 EID_{50} of the same virus at 12 days post-inoculation and were bled 10 days later. The intraperitoneal booster was necessary to produce a significant antibody response (HI titre > 1:80).

Monoclonal antibodies. Hybrid cell lines producing antibodies to the haemagglutinin of A/NJ/8/76 (X-53A), A/USSR/90/77 or A/Brazil/-11/78 were selected following fusion of myeloma cells P3/X-63/Ag8 or SP2/0 AG14 (*15*) with immune spleen cells from Balb/c mice, according to the method of Kohler & Milstein (*14*), as previously described for influenza virus (*26*). The mice had been immunized with one intraperitoneal injection of purified intact influenza virus (10 μg haemagglutinin protein) and the fusion was done 1–2 months later after a similar booster injection of antigen 4 days earlier (*26*). The hybridoma cells were screened for antibody production in HI assays (*20*) and enzyme-linked immunosorbent assay (ELISA) (*12*). Cultures producing antibody to the HA were cloned in soft agar and injected intraperitoneally into pristane-treated mice (*26*). Ascitic fluid was collected 7–10 days later and diluted 1:100 in phosphate-buffered saline for use in the assays.

Protocol. Each laboratory was provided with prototype strains, A/NJ/8/76 and A/Dk/Alb/-35/76, as well as receptor-destroying enzyme-treated ferret antisera to the reference strains and ascitic fluids diluted in phospate-buffered saline, and requested to use standard macro- or micro-haemagglutination-inhibition tests in comparing their isolates with the control prototype strains.

Table 1. Haemagglutination-inhibition reactions of representative swine H1N1 viruses with monoclonal antibodies and ferret antiserum to A/NJ/8/76

Virus	Country of origin[a]	Relative titres with ferret antiserum to A/NJ/8/76[b]	Reaction with the following monoclonal antibodies to A/NJ/8/76 (X-53A)[c]				
			6/1	36/3	40/3	72/3	117/2
Group 1							
Sw/TN/1/75	USA (36)	1	+	+	+	+	+
Sw/Wis/32/83		1	+	+	+	+	+
Sw/Hong Kong/37/77	Hong Kong (11)	1	+	+	+	+	+
Sw/Hong Kong/97/79		0.5	+	+	+	+	+
Sw/Italy/45/79	Italy (3)	0.25	+	+	+	−	−
Sw/Italy/26/83		1	+	+	+	±	+
Sw/Hokkaido/2/81	Japan (20)	0.5	+	+	+	−	−
Sw/Niigata/2/77		0.5	+	+	+	+	+
Group 2							
Sw/Belgium/426/79	Belgium (5)	0.06	±	±	−	−	−
Sw/Belgium/11/82		0.12	±	−	−	−	−
Sw/Denmark/3801/81	Denmark (1)	0.12	±	−	−	−	−
Sw/Arnsberg/6554/79	Federal Republic of Germany (4)	0.25	−	−	−	−	
Sw/Germany/1/82		0.12 − 0.25	−	−	−	−	−
Sw/Mar/2897/79	France (34)	0.06	±	−	−	−	−
Sw/Finistere/52/82		0.12	−	−	−	−	−
Sw/Spain/1/82	Spain (1)	0.06	±	−	−	−	−
Reference virus							
A/NJ/8/76 (X-53A)		1	+	+	+	+	+
A/NJ/8/76		0.5	+	−	+	+	+

[a] Number in parenthesis is the number of isolates tested from that country.

[b] The number indicates the ratio, HI titre to isolate: HI titre to A/NJ/8/76.

[c] The monoclonal antibodies were prepared as described in Materials and Methods; they were diluted 1 : 100 for the tests. Homologous titres for A/NJ/8/76 (X-53) are: 12800 (6/1); 12800 (36/3); 1600 (40/3); 12800 (72/3) 12800 (117/2). − = < 1 : 100; + = 1 : 100; + = > 1 : 100.

RESULTS

Comparison of recent swine isolates with monoclonal antibodies and ferret antiserum to A/NJ/8/76

The primary objective of this study was to compare recent swine isolates and determine whether significant antigenic differences among isolates from different areas of the world could be detected. These viruses had initially been classified as H1N1 with hyperimmune rabbit or goat antisera; however, hyperimmune sera are not sufficiently sensitive for discriminating between closely related viruses. Since post-infection ferret sera and monoclonal antibodies are clearly more specific than hyperimmune antisera, it was anticipated that they would be useful for detecting minor antigenic changes in influenza viruses in nature.

To compare the different viruses, isolates were examined in HI tests with 5 monoclonal antibodies and post-infection ferret antiserum to A/NJ/8/76. Although A/NJ/8/76 is a human isolate, it had been shown in earlier studies (*6*, *11*, *13*) to be virtually antigenically indistinguishable from concurrently circulating swine viruses in the USA. Since this study involved 15 different laboratories, variation in HI titres among laboratories was anticipated even though the same reference antigens and antisera were used. Although there were variations in titres among the laboratories, the values related to the prototype strains were very consistent. In addition, many isolates were examined by more than one laboratory and the basis for classifying the viruses as different was a lack of reactivity with 3 or more of the monoclonal antibodies and a greater than 4-fold difference in inhibition of the isolate with ferret antiserum as compared to the results with A/NJ/8/76 in that same laboratory.

From these serological comparisons, the viruses could be divided into two groups; representative strains are presented in Table 1. The viruses in the first group are indistinguishable from A/NJ/8/76 with ferret antiserum and react with 3–5 of the monoclonal antibodies. These viruses do not react identically, e.g., Sw/Hokkaido/2/81; however, based on both reactivity with monoclones and ferret antisera, they appear more closely related to each other than the viruses in the other group. These results indicate that swine viruses closely related to A/NJ/8/76 are still circulating in the USA but are also present in Italy,

Table 2. Reactions in haemagglutination-inhibition tests between H1N1 influenza A viruses and monoclonal antibodies to human and swine H1N1 strains

Test virus	Reactivity in HI tests with monoclonal antibodies to:					
	Human H1N1 viruses (pool)	NJ/8/76 (X-53A)[a]				
		6/1	36/3	40/3	72/3	117/2
Brazil/11/78	+ (25600)	−	−	−	−	−
USSR/90/77	+ (12800)	−	−	−	−	−
NJ/8/76	− (< 100)	+	±	+	+	+
Dk/Alb/35/76	− (< 100)	−	−	−	−	−
Dk/Bavaria/2/77	− (< 100)	−	−	−	−	−
Ty/Ks/4880/80	− (< 100)	+	+	+	+	+
Sw/Ia/15/30	− (< 100)	+	+	−	−	+
Sw/Cam/39	− (< 100)	−	−	−	−	−
Sw/Wis/1/67	− (< 100)	+	+	+	+	−
Sw/Tn/1/75	− (< 100)	+	+	+	+	+
Sw/Bel/1/79	− (< 100)	−	−	−	−	−
Sw/Mar/2897/79	− (< 100)	±	−	−	−	−
Sw/Stephan/80	− (< 100)	+	+	−	−	−
Sw/Wis/1/83	− (< 100)	+	+	+	+	+
Sw/Ger/1/82	− (< 100)	−	−	−	−	−

[a] The monoclonal antibodies were prepared as described in Materials and Methods; they were diluted 1 : 100 for the tests. Homologous titres for A/NJ/8/76 (X-53) are: 12800 (6/1); 12800 (36/3); 1600 (40/3); 12800 (72/3); 12800 (117/2). − = < 1 : 100; ± = 1 : 100; + = > 1 : 100.

Japan and Hong Kong. These viruses will be referred to as "US viruses". The viruses in the second group react to lower titres (4–8-fold difference) with the ferret antiserum and react poorly, if at all, with the monoclonal antibodies, indicating that they are antigenically distinguishable from the US viruses. These viruses in the second group were isolated from pigs within a relatively localized area in Europe; the earliest of these isolates was in Belgium in 1979, and a related virus is obviously still present in that country, as well as in the Federal Republic of Germany, France and Denmark. These viruses will be referred to as "European viruses".

One aspect which is not evident from Table 1 is that, although 116 swine viruses were examined (including multiple isolates from the different countries), there was only one country, i.e., France, which had viruses representative of both groups, e.g., Sw/Stephan/80 which reacts more like A/NJ/8/76, whereas the majority of isolates from that area (e.g., Sw/Mar/2897/79) resemble the European viruses.

The above results suggest that currently circulating H1N1 viruses in pigs include at least two antigenically distinct groups and that the presence of one or the other is associated with the geographical location of the animals.

Comparison of antigenically distinct swine viruses with H1N1 isolates from different species

Since the above results indicated that antigenically distinct H1N1 viruses were circulating in pigs in different areas, the question to be addressed was whether similar viruses existed currently in other hosts or previously in pigs. Since antigenically related H1N1 viruses are present in humans and birds (*9*), a series of these viruses were selected for comparison with the antibodies used above. In addition, a pool of monoclonal antibodies to the haemagglutinin of recent human strains (A/Brazil/11/78 and A/USSR/90/77) and ferret antiserum to an avian virus (A/Dk/A1b/35/76) were included in these studies. Results with representative strains are presented in Table 2.

(*a*) *Human viruses.* Recent human H1N1 epidemic strains, e.g., Brazil/11/78 and USSR/90/77, failed to react with the monoclonal antibodies to A/NJ/8/76, and thus resembled the European swine viruses (Table 2). Since it was possible that the swine viruses could have been of human origin, the swine and human viruses were examined with the monoclonal antibody pool to human strains. The human viruses, including 45 recent human isolates from France, reacted to high titres (HI titre 6400–25600) with this pool whereas the swine viruses in group 2 failed to react (HI titre < 100). These results, as well as reciprocal HI tests with ferret antisera (not shown), indicated that the European swine viruses were not closely related to currently circulating H1N1 viruses in humans.

On occasion, H1N1 influenza viruses resembling those typically associated with pigs have been isolated from humans, as in the case of A/NJ/8/76 (*6*), and antigenic variation in these isolates (both from humans and from pigs) has been convincingly demonstrated by Kendal (*11*) and Kilbourne (*13*). The variants described by Kendal termed subgroups 1 and 2, were compared with the 5 monoclonal antibodies to A/NJ/8/76. In HI tests with these monoclonal antibodies, subgroup 1 reacted with the 5 preparations whereas subgroup 2 reacted with 4 to high titres but to a very low level with antibody 36/3. These results indicate that the antigenic variants described by Kendal are not comparable to the antigenic differences observed between the European and US swine viruses examined in this study.

(*b*) *Avian viruses.* In 1976, H1N1 viruses related to swine viruses were first described in avian species (*7*). Such viruses have been detected in birds in North America, Europe and Asia (*4*, *7*, *18*). In examination of the avian viruses with the monoclonal antibodies to A/NJ/8/76, it was evident that the majority of the avian strains, similar to the European swine viruses, failed to react with these antibodies (Table 2).

Since ferret antiserum to Dk/A1b/35/76 inhibited both European and US swine viruses (results not shown), it was not very helpful in distinguishing these viruses. This serum is more cross-reactive because the ferrets were, of necessity, boosted to obtain adequate HI titres. Even so, some avian viruses reacted to low titres, or not at all, with the ferret antiserum, indicating antigenic variation among the avian viruses. This has been observed in other avian strains (*16*), including H1N1 isolates from the same bird population and even with viruses cloned from the same stock (Hinshaw, unpublished observation). Antisera to recent turkey isolates from France, which fail to react with the monoclonal antibodies in this test, react to high titres with recent European swine isolates and to a much lower titre with US swine isolates (results not shown). The more striking observation was that the avian viruses, similar to the European swine viruses in Europe, failed to react with the monoclonal antibodies (Table 2); selected isolates were also examined with 12 other monoclonal antibodies to A/NJ/8/76 and with the monoclonal antibody pool to the human strains and they also failed to react with these. These findings might suggest that some current swine viruses have a haemagglutinin more closely related to the avian strains, rather than those viruses typically associated with pigs.

It should be noted that avian strains are frequently termed "non-avid" in that they react to low levels in

HI tests, even with homologous antisera (*16*). In view of this, selected viruses, e.g., Dk/A1b/35/76, were tested in ELISA with the monoclonal antibodies to determine whether the antibodies were binding but failing to inhibit haemagglutination. The results of these tests (not shown) indicated that the monoclonal antibodies failed to bind to the avian virus although binding to A/NJ/8/76 was quite efficient. This suggests that the determinants are not hidden or masked but are absent from the avian viruses, indicating that the HI tests are providing an accurate analysis of the antigenic determinants on these viruses.

Although the majority of avian viruses failed to react with the monoclonal antibodies to A/NJ/8/76, there were some exceptions. The most notable included several turkey isolates from the USA which reacted to high titres with the antibodies. Previous studies (*10*) had indicated that these turkey viruses were antigenically and genetically closely related to swine viruses and A/NJ/8/76, thus it was not surprising that they would react like the US swine viruses. Turkey viruses from England, Israel and France reacted more like the European swine viruses, indicating that all H1N1 strains in turkeys are not the same. The only other avian virus which reacted with the monoclonal antibodies was A/Dk/Miyagi/66/77 (H1N6) from Japan (results not shown); the other duck isolates from this area failed to react.

(*c*) *Porcine viruses.* To determine whether viruses like the US and European groups existed previously in pigs, earlier swine isolates (Sw/Ia/15/30, Sw/Cam/-39, Sw/Wis/1/67, Sw/Tn/1/75) were examined in HI tests with 18 monoclonal antibodies to A/NJ/8/76, which included the 5 used in this study. There was only one swine virus which failed to react with any of the monoclonal antibodies, similar to the avian strains, and this was A/Sw/Cam/39. This virus has previously been described as antigenically distinct from other swine viruses (*11*) and the results here agree with that conclusion. Ferret antisera to Sw/Cam/39 (results not shown) reacted to low titres with both US and European swine viruses and failed to react with Dk/A1b/35/76; however, Sw/Cam/39 was inhibited to low titres with ferret antisera to A/NJ/8/76 and Dk/A1b/35/76. These results suggest that Sw/Cam/39 is more closely related to the current European rather than US swine viruses.

DISCUSSION

Collaborative studies on H1N1 isolates from swine in different countries throughout the world indicate that at least 2 distinct antigenic variants of these viruses are currently circulating in pigs. Based on serological assays, the viruses termed "US viruses" (which included isolates from pigs in Hong Kong, Italy, Japan, and the USA) are most closely related to A/NJ/8/76, whereas the other viruses termed "European viruses" (which were detected in pigs in Belgium, England, France, the Federal Republic of Germany, and Spain) are antigenically distinguishable. In comparisons of this latter group with other current H1N1 viruses in humans and birds, they appear to be more similar to the avian viruses. Possible explanations for these findings are that the haemagglutinin on the European swine viruses has undergone antigenic variation or is possibly of avian origin.

It has been suggested that pigs imported from the USA have served as a source of swine viruses for pigs in other countries, e.g., Italy and Japan during the late 1970s. The type of viruses detected in pigs in these countries would agree with this idea, i.e., they are antigenically very similar to those present in the US pigs. This does not appear to be the case with the more commonly detected viruses in European pigs. These viruses are distinguishable from the viruses in US pigs and are localized to pig populations in European countries in close proximity to each other. This might suggest that the European viruses originated from a different source to that of the US viruses; a potential source, in this case, could be birds in that area which harbour H1N1 strains.

The likelihood of interspecies transmission of viruses between birds and pigs in nature cannot be predicted; however, laboratory studies (*8*) have demonstrated that avian viruses, including H1N1 strains, are capable of infecting and replicating in pigs, and even being transmitted to other pigs. It is also known that swine viruses infect birds; for example, the swine-influenza-like viruses in turkeys in the USA are apparently of swine origin and they have been associated with disease outbreaks in turkeys (*10*). Recent disease outbreaks in turkeys in France involve viruses very similar to the European swine viruses, and the birds are located in close proximity to swine herds (*1*). These findings lend support to the possibility that H1N1 viruses are being exchanged between birds and pigs and, therefore, antigenically related viruses may be detected in both groups.

Although interspecies transmission of H1N1 viruses represents a potential explanation for the appearance of antigenically distinct viruses in pigs, it is certainly possible that swine viruses themselves have undergone sufficient antigenic variation to explain the differences. The finding that Sw/Cam/39 reacts similarly to the current European swine viruses could be interpreted to mean that these viruses have existed in pigs in the past and have been maintained. Alternatively, could Sw/Cam/39 represent an avian

virus? These questions cannot be answered within the context of this study but underline the need for further studies to examine and compare related viruses from different hosts.

The present study was focused on the antigenic relatedness of the haemagglutinins of these viruses, but there are obvious limitations in this approach. Monoclonal antibodies recognize only a very small portion of the HA molecule, so a comparison of the genes coding for the haemagglutinins is needed to obtain a more definitive evaluation of their relatedness. Recent studies on the HA genes of H1N1 viruses by Scholtissek (*22*) would support the serological evidence that the haemagglutinins of the European swine and avian viruses are genetically more closely related to each other than to the recent US swine viruses or recent human H1N1 strains. It is also necessary to compare other genes in these viruses to determine whether the HA gene alone, or other genes, may be similar to those of avian strains. Preliminary studies on the nucleoprotein of Sw/Cam/39 with a panel of monoclonal antibodies to the nucleoprotein of an avian virus (Hinshaw, unpublished observation) indicate that the nucleoprotein gene of this virus is more closely related to swine rather than avian strains. However, genetic comparisons of these genes need to be done. Similar studies on recent isolates would be informative as to the genetic make-up of these antigenically distinct swine viruses. The possibility that genetic reassortment between avian and swine viruses might occur, thus resulting in the introduction of one or more genes, must also be considered. A reassortant between human and swine viruses (H1N2) has been isolated from pigs in Japan (*24*); the possibility that avian and swine viruses could also reassort is not unreasonable.

The H1N1 viruses circulating in pigs and birds may well be of significance to humans, particularly since contact between domestic species and humans does occur. It is well established that swine viruses infect humans in the natural setting and, even if the swine viruses possessed a haemagglutinin from avian strains, there is no known reason that these would not be exchanged as well. In fact, the laboratory studies discussed earlier would lend credence to such a possibility. Since H1N1 viruses are still circulating in humans, it is important to consider that all H1N1 isolates may not be "typical" human strains but may represent viruses from animal sources.

These studies were conducted with the primary goal of determining whether antigenic variation existed in swine isolates in different areas. The results indicate that antigenic variants are circulating in pigs in different areas of the world. Whether these variants shared different origins, in the one case from pigs and in the other from birds, is a possibility that requires further investigation.

ACKNOWLEDGEMENTS

This work was supported in part by grant AI 02649 from the National Institutes of Allergy and Infectious Diseases, by Childhood Cancer Center support grant CA-21765 from the National Cancer Institute, and by ALSAC (St. Jude Children's Research Hospital).

RÉSUMÉ

ETUDE COLLECTIVE INTERNATIONALE EN VUE DE LA COMPARAISON ANTIGÉNIQUE DE VIRUS H1N1 ANALOGUES À CEUX DE LA GRIPPE PORCINE ET ISOLÉS CHEZ DES PORCINS, DES OISEAUX ET DES SUJETS HUMAINS

L'objet de cette étude collective internationale était de comparer des isolements récents de virus grippaux obtenus chez le porc et de déterminer l'existence éventuelle de différences antigéniques significatives selon la provenance géographique. Pour comparer les virus H1N1 isolés chez des porcs, des oiseaux et des sujets humains dans douze pays différents, on a procédé à des réactions d'inhibition de l'hémagglutination en utilisant comme matériel des sérums de furets infectés et des anticorps monoclonaux dirigés contre les souches H1N1. En utilisant A/NJ/8/76 comme souche de référence, on a montré que l'hémagglutinine des isolements en provenance de Hong Kong, d'Italie, du Japon et des Etats-Unis était pratiquement indiscernable de celle du virus typiquement associé au porc, c'est-à-dire A/NJ/8/76. En revanche, les isolements récents obtenus dans divers pays d'Europe (Belgique, Danemark, Espagne, France et République fédérale d'Allemagne) présentaient des différences par rapport à la souche A/NJ/8/76, à en juger d'après les épreuves pratiquées dans les différents laboratoires. Ces résultats suggèrent que les virus H1N1 présents chez les porcins sont antigéniquement hétérogènes et que la circulation de certains variants est liée à la localisation géographique des animaux. Il s'agit donc maintenant de savoir si ces virus proviennent de la même source, c'est-à-dire des porcins, et ont subi un glissement antigénique ou s'ils ont été introduits par d'autres hôtes, tels que des oiseaux.

REFERENCES

1. AYMARD, M., ET AL. *Rapport d'activité du Centre National de Référence de la Grippe—France-Sud.* 1981.
2. BACHMANN, P. ET AL. *Pro. Veterinara*, **1**:4 (1981).
3. BIRONT, P. ET AL. *Vlaams Diergeneeskundig Tijdschrift*, **49**: 8-11 (1980).
4. BUTTERFIELD, W. K. ET AL. *J. inf. dis.*, **5**: 138, 686-689 (1978).
5. GOURREAU, J. M. ET AL. *Ann. virol. (Inst. Pasteur)*, **132** E: 287-294 (1981).
6. HINSHAW, V. S. ET AL. *Virology*, **84**: 51-62 (1978).
7. HINSHAW, V. S. ET AL. *J. gen. virol.*, **41**: 115-127 (1978).
8. HINSHAW, V. S. ET AL. *Infect. immun.*, **34**: 354-361 (1981).
9. HINSHAW, V. S. ET AL. *Arch. virol.*, **67**: 191-201 (1981).
10. HINSHAW, V. S. ET AL. *Science*, **220**: 206-208 (1983).
11. KENDAL, A. P. ET AL. *Virology*, **82**: 111-121 (1977).
12. KIDA, H. ET AL. *Virology*, **122**: 38-47 (1982).
13. KILBOURNE, E. T. *Proc. Natl Acad. Sci. (USA)*, **75**: 6255-6262 (1978).
14. KOHLER, G. & MILSTEIN, G. *Eur. j. immunol.*, **6**: 511-519 (1976).
15. KOPROWSKI, H. ET AL. *Proc. Natl Acad. Sci. (USA)*, **74**: 2895-2988 (1977).
16. LU, B. ET AL. *Infect. immun.*, **38**: 530-535 (1982).
17. NARDELLI, L. ET AL. *Zbl. Vet. Med. B*, **25**: 853-857 (1978).
18. OTTIS, K. & BACHMANN, P. A. *Arch. virol.*, **63**: 185-190 (1980).
19. OTTIS, K. ET AL. *Tierarztl. Umschau*, **36**: 608-612 (1981).
20. PALMER, D. F. ET AL. *Advanced Laboratory Techniques for influenza diagnosis.* Atlanta, GA, US Department of Health, Education & Welfare, 1975 (Immunology Series No. 6).
21. PENSAERT, M. ET AL. *Bull. Wld Hlth Org.*, **59**: 75-79 (1981).
22. SCHOLTISSEK, C. ET AL. *Virology*, **129**: 521-523 (1983).
23. SORENSON, K. J. ET AL. *Dansk Veterinaertidsskrift*, **64** (21): 826-829 (1981).
24. SUGIMURA, T. ET AL. *Arch. virol.*, **66**: 271-274 (1980).
25. VANDEPUTTE, J. ET AL. Swine influenza in Belgium: virus isolation and experimental infection. *Vlaams Diergeneesk. Tijdschrift*, **50**: 291-300 (1981).
26. WEBSTER, R. G. ET AL. *Virology*, **96**: 258-264 (1979).
27. WITTE, K.N. ET AL. *Tierärtzl. Umschau*, **36**: 591-606 (1981).
28. AYMARD, M. ET AL. Epizootic swine influenza on animal farms in Brittany. *Revue d'épidémiologie et de santé publique*, **31**: 311-327 (1983).
29. The ecology of influenza viruses: a WHO Memorandum. *Bull. Wld Hlth Org.*, **59**: 869-873 (1981).