Bulletin of the World Health Organization, 59 (1): 75-78 (1981)

Evidence for the natural transmission of influenza A virus from wild ducks to swine and its potential importance for man*

M. PENSAERT,[1] K. OTTIS,[2] J. VANDEPUTTE,[3] M. M. KAPLAN,[4] & P. A. BACHMANN[5]

In 1979, epidemics of influenza occurred in pigs in Belgium from which were isolated strains of influenza A (HswlN1) virus antigenically closely related to HswlN1 strains previously isolated from ducks in North America and the Federal Republic of Germany. This finding is considered as the first supportive evidence that an influenza A virus in an avian species might have been transmitted to mammals.

In January 1976, a swine strain (HswlN1) of the influenza virus A/New Jersey/76 caused the death of one soldier, and illness in several others, in an army camp at Fort Dix, New Jersey, USA, and apparently infected some 500 other soldiers in the camp (*1*). This gave rise to a fear that a pandemic might occur similar to the one in 1918 that killed some 20 million people and was believed to have been caused by a related influenza strain (*2*). Fortunately, no further spread of the Fort Dix swine strain occurred, but the incident aroused increased interest in influenza in animals, particularly in swine and birds. It has been suggested that these animal species in particular provide reservoirs for influenza strains which could themselves, or as a result of mutation or recombination with human strains, lead to influenza pandemics in human populations (*2*).

We report here an outbreak of naturally occurring influenza in pigs in Belgium from which were isolated strains of an HswlN1 virus antigenically closely related to the HswlN1 viruses obtained from wild ducks in North America and the Federal Republic of Germany in 1976–77. This finding suggests that an influenza strain harboured by the birds may have crossed the "species barrier" to produce infection in mammals.

* This study was supported by the Federal Ministry for Youth, Family and Health of the Federal Republic of Germany. Requests for reprints should be addressed to Professor P. A. Bachmann.

[1] Professor of Virology, Faculty of Veterinary Medicine, University of Ghent, Belgium.

[2] Research Associate, WHO Collaborating Centre for Collection and Evaluation of Data on Comparative Virology, Institute for Medical Microbiology, Infectious and Epidemic Diseases, Veterinary Faculty, University of Munich, Federal Republic of Germany.

[3] Research Associate, Laboratory of Virology, University of Ghent.

[4] General Secretary, Pugwash Conferences on Science and World Affairs, Geneva, Switzerland.

[5] Director, WHO Collaborating Centre. Professor of Microbiology and Infectious Diseases. Institute of Medical Microbiology, Infectious and Epidemic Diseases, Veterinary Faculty, University of Munich, Königinstrasse 49, 8000 Munich 22, Federal Republic of Germany.

MATERIALS AND METHODS

Influenza outbreaks

Starting in January 1979, several outbreaks of influenza were observed in swine in Belgium (*3*). The outbreaks were characterized by fever, dry cough, and anorexia. The majority of sows and weaned pigs became sick, whereas suckling pigs were not at all or only slightly affected. Mortality was low (less than 1%) and recovery was uneventful. As described below, influenza virus was isolated from two pigs that died of the disease, and serum specimens were tested from survivors and contact pigs.

Virus isolation

A 20% suspension was prepared in phosphate-buffered saline of portions of the lungs of a feeder pig (about 6 months old) that died of respiratory disease; 0.2-ml aliquots of this suspension were inoculated into the amniotic cavities of 10-day-old embryonated eggs. Amniotic fluid was harvested after 4 days' incubation and showed haemagglutinating activity (HA) with chicken red blood cells (RBCs). Three allantoic passages were made to increase the titre. The allantoic fluid from the third passage constituted the virus stock used for further identification, and was designated A/swine/Belgium/1/79.

A second isolate, designated A/swine/Belgium/2/79, from another farm, was similarly obtained from

a pooled sample of lungs, trachea, and nasal mucosa obtained from a sow that died after showing fever and respiratory distress.

Serological tests

Samples of haemagglutinating allantoic fluid of the two isolates were typed as influenza A by the immunodouble-diffusion (IDD) test according to standard procedures (*4*). Matrix (M) protein antigens of, and goat antiserums against, influenza A and B strains[a] were used in the test. Paired porcine sera were obtained from two farms at the time of illness and again 23 and 25 days, respectively, after acute respiratory disease was first observed. For haemagglutination-inhibition (HI) tests these sera were heat-treated at 56 °C for 30 min and then 1 volume of serum was mixed with 2 volumes of 50% kaolin at 20 °C for 20 min to remove non-specific inhibitors. This was followed by adsorption with RBCs to remove non-specific haemagglutinins (*4*). Each swine serum was tested against 4 HA units of each virus isolate.[b]

The HI and neuraminidase inhibition (NI) tests were done by standard methods (*4*, *5*) using microtitration plates. All tests were repeated at least twice.

RESULTS

In the IDD tests the Belgian isolates showed distinct precipitation lines with the goat antiserum against influenza A, but not with antiserum against influenza B, when tested in parallel with the appropriate controls. This clearly indicates that influenza A was involved.

Five paired serum specimens from each of the two infected farms showed rises in HI titres against the A/swine/Belgium/1/79 isolate from less than 1:8 to between 1:24 and 1:96 on one farm and to between 1:64 and 1:128 on the second farm. This confirmed that an influenza outbreak had occurred.

Characterization of the Belgian swine strains

The neuraminidase antigen of the swine influenza isolates was demonstrated to be of the N1 subtype by neuraminidase inhibition tests with antiserum prepared from a recombinant virus Heq1N1 produced by recombining A/equi/Prague/1/56 (for the Heq1 component) with A/New Jersey/8/76 (for N1). Inhibition was not observed with antisera to N2 neuraminidase.

The results of HI tests on the two virus isolates A/swine/Belgium/1/79 and A/swine/Belgium/2/79 with antisera to selected reference strains are shown in Table 1. There were no notable differences between the reactions of the two A/swine/Belgium/79 strains. Both strains reacted to significant titres with chicken antiserum to homologous A/swine/Belgium/1/79 virus and with sera to two duck strains, A/duck/Alberta/76 and A/duck/Bavaria/77. However, they failed to react, or gave low-titre reactions, with antisera to swine (Hsw1N1) and human influenza A viruses. Antigenic differences between A/duck/Alberta/76 and A/duck/Bavaria/77 were suggested by differences in the HI reactions of antisera to these strains with A/swine/Cambridge/39 virus.

DISCUSSION

Classical swine influenza is caused by Hsw1N1 strains which are designated according to the character of the H (haemagglutinin) and N (neuraminidase) antigens (*6*). These antigens in swine influenza viruses have remained relatively stable for many years although some variation ("drift") has been noted in the Hsw1 antigen (*7*). In 1976–77, Hsw1N1 strains were isolated from wild ducks in Canada, Hong Kong, the United States of America and in the Federal Republic of Germany (*8*) and these findings posed questions as to whether such strains, residing in an avian species, would be capable of causing natural infection in pig herds and thus provide evidence concerning involvement of birds in the transmission of influenza viruses to lower mammals and perhaps man.

Many avian species, including wild birds and domestic poultry, are naturally infected with a large variety of influenza A strains and provide immense reservoirs of infection (*2*). Until now, however, transmission of influenza viruses across a postulated "species barrier" from birds to mammals with resultant clinical disease has not been demonstrated. Such a species barrier has been assumed to exist because of the lack of evidence of natural infection in mammals with avian influenza A viruses despite the many hundreds of strains isolated from wild and domestic avian species during the past two decades. Our results provide suggestive evidence that transmission of an avian virus was associated with the outbreak of swine influenza in Belgium in 1979. The avian virus is closely related to virus strains previously isolated from wild ducks in the Federal Republic of Germany and Alberta, Canada.

In previous studies, we have shown that an Hsw1N1 strain isolated from wild ducks can infect pigs following intranasal inoculation in the laboratory and that the virus was transmitted from inoculated animals to

[a] Kindly supplied by the WHO Collaborating Centre for Reference and Research on Influenza, Atlanta, GA, USA.

[b] Kindly supplied by the WHO Collaborating Centres for Reference and Research on Influenza in Atlanta and London, and by Dr Virginia Hinshaw, St Jude Children's Hospital, Memphis, TN, USA.

Table 1. Results of haemagglutination-inhibition (HI) tests[a] with Belgian swine strains and selected human, swine, and avian strains of influenza A virus

Antisera to	Antigens							
	Swine/ Belg./ 1/79	Swine/ Belg./ 2/79	Duck/ Alb./ 35/76	Duck/ Bav./ 1/77	Human/ N.J./ 8/76	Swine/ Wis./ 1/67	Swine/ Camb./ 39	Swine/ Iowa/ 15/30
A/swine/Belg./1/79(Hsw1)[b]	320	160	80	80	40	80	10	80
A/duck/Alb./35/76(Hsw1)[c]	1280	640	1280	320	2560	2560	640	2560
A/duck/Bav./1/77(Hsw1)[b]	320	160	80	320	160	80	10	320
A/human/N.J./8/76(Hsw1)[b]	<10	<10	<10	<10	160	80	20	80
A/swine/Wis./1/67(Hsw1)[b]	<10	20	20	<10	160	320	<10	80
A/swine/Camb./39(Hsw1)[b]	<10	<10	<10	<10	<10	<10	1280	<10
A/swine/Iowa/15/30(Hsw1)[b]	<10	<10	<10	<10	80	160	<10	320
A/USSR/90/77(H1)[b]	<10	<10	<10	<10	<10	<10	10	<10
A/human/PR/8/34(H0)[d]	20	<20	nd[e]	nd	nd	nd	nd	nd
A/human/FM/1/47(H1)[d]	40	40	nd	nd	nd	nd	nd	nd
A/human/Sing./1/57(H2)[d]	<20	<20	nd	nd	nd	nd	nd	nd
A/human/HK/1/68(H3)[d]	<20	<20	nd	nd	nd	nd	nd	nd

[a] Reciprocal of serum dilution inhibiting 4 haemagglutinating (HA) units of antigen.
[b] From chicken.
[c] From rabbit.
[d] From goat.
[e] nd = not done.

their contacts although clinical signs of disease in infected animals were minimal (*8*). Nevertheless, it is uncertain whether the actual disease reported here that was observed during the natural outbreak in pigs in Belgium was caused by the avian type of virus, or whether the avian type virus was a passenger in the disease process.

Since swine have been shown to be sources of human influenza infection, the knowledge that avian influenza may be transmitted to swine may assume considerable importance in the future if it has not already done so.

ACKNOWLEDGEMENTS

We acknowledge with appreciation the excellent technical assistance of Angela Pfleger.

RÉSUMÉ

ARGUMENTS EN FAVEUR DE LA TRANSMISSION NATURELLE DU VIRUS GRIPPAL A DE CANARDS SAUVAGES À DES PORCS ET IMPORTANCE POTENTIELLE DE CE PHÉNOMÈNE POUR L'HOMME

Deux souches de virus grippal A ont été isolées à partir de porcs malades dans différentes fermes de Belgique en 1979. Ces souches se sont révélées identiques et les épreuves d'inhibition de l'hémagglutination et d'inhibition de la neuraminidase ont montré qu'il s'agissait de souches Hsw1N1. D'après l'épreuve d'inhibition de l'hémagglutination, les deux souches étaient étroitement apparentées aux souches Hsw1N1 isolées de canards sauvages au Canada et en République fédérale d'Allemagne en 1976 et 1977, respectivement. Nos résultats plaident fortement en faveur de

l'hypothèse selon laquelle une souche aviaire peut avoir été transmise au porc. Néanmoins, on ne sait pas encore avec certitude si la maladie observée au cours de l'épizootie naturelle chez les porcs en Belgique était due au virus aviaire ou bien si la présence de ce virus aviaire n'était que passagère dans le processus morbide.

REFERENCES

1. *Weekly epidemiological record*, **51**: 108-109 (1976).
2. KAPLAN, M. M. & WEBSTER, R. G. The epidemiology of influenza. *Scientific American*, **237** (6): 88-106 (1977).
3. VANDEPUTTE, V. ET AL. Serologische diagnose en onderzoek naar verspreiding var het varkens—influenzavirus in Belgie. *Vlaams Diergeneeskunde Tijdschrift*, **49**: 1-7 (1980).
4. *Advanced laboratory techniques for influenza diagnosis*. Atlanta, Georgia, US Department of Health, Education, and Welfare, Public Health Service, Center for Disease Control, Bureau of Laboratories, 1975, pp. 65-75 (Immunology Series No. 6, Procedural Guide).
5. AYMARD-HENRY, M. ET AL. Influenzavirus neuraminidase and neuraminidase-inhibition test procedures. *Bulletin of the World Health Organization*, **48**: 199-202 (1973).
6. Reconsideration of influenza A virus nomenclature: a WHO Memorandum. *Bulletin of the World Health Organization*, **57**: 227-233 (1979).
7. KENDAL, A. P. ET AL. Swine influenza viruses isolated in 1976 from man and pig contain two coexisting subpopulations with antigenically distinguishable hemagglutinins. *Virology*, **82**: 111-121 (1977).
8. OTTIS, K. & BACHMANN, P. A. Occurrence of HswlN1 subtype influenza A viruses in wild ducks in Europe. *Archives of virology*, **63**: 185-190 (1980).