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ANTIBODIES TO NEW VARIANTS OF SUBTYPE A(H3 N2) INFLUENZA VIRUS IN PIGS

B. TŮMOVA, A. ŠTUMPA, *V. MÁDR, *J. MENŠÍK

Czechoslovak Influenza Centre, Institute of Hygiene and Epidemiology, 110 42 Prague; *Research Institute of Veterinary Medicine, Brno, Czechoslovakia

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Summary. — Following an explosive epidemic of A(H3N2) influenza among the human population of Czechoslovakia in 1983, haemagglutination-inhibiting antibodies (titre range 10—640) against strains A/Texas/77, A/Bangkok/79 and A/Philipines 2/83 were detected in 93% of sera collected from 135 pigs on three farms. Only 6.6% of sera were negative. Anti-neuraminidase antibodies were detected at rates of 81% and 23% in two and one of the herds, respectively. Antibodies against A/RNP were demonstrated by the immunodiffusion test in only one of the herds in 10out of 45 sera tested. This herd was also found to possess antibodies against both envelope antigens of a human A(H1N1) subtype strain. Haemagglutination-inhibition tests with strains A/Hong Kong/68 (H3 N2), A/sw/Shope 15/31, A/sw/Bavaria 2/77 and A/New Jersey 8/76 (H1 N1) were negative in the sera from all three herds.

Key words: antibodies; influenza virus A (H3 N2); influenza virus A (H1 N1); swine influenza; interspecific transmission

In 1969—1979, we demonstrated subtype A(H3 N2) influenza virus and corresponding antibodies in herds of swine, for the most part in association with the presence of this subtype among human population (Tůmová *et al.*, 1980).

In 1983 an explosive epidemic of this subtype swept over Czechoslovakia, culminating in the first week of March. The total of 388 isolates represented a very heterogenous group of antigenic relationship to the variants A/Texas/77, A/Bangkok/79 and A/Philipines 2/83. Six weeks after the culmination of this epidemic, a total of 135 blood specimens were collected from pigs (age 6 months to 2 years) on three farms to be tested for antibodies to the above influenza virus variants. Herds I and II were conventional herds numbering about 200 animals each, farm III was specialized in the production of healthy breeding gilts.

The immunoassays comprised the haemagglutination-inhibition test (HIT), neuraminidase-inhibition test (NIT) and the gel immunodiffusion test (IDT) with A/RNP antigen performed by the method of Palmer *et al.* (1975). For HIT the sera were treated with RDE and nonspecific agglutinins were absorbed by chicken erythrocytes. In this test all of the sera were negative for strains

Table 1. Haemagglutination-inhibiting antibody titres against subtype A(H3N2) strains in pig sera

Strain	Titre					
	negat.	1:10	1:20	1:40	1:80	1:160+
A/Texas	9 (6.6%)	4 (2.9%)	42 (31.1%)	47 (34.8%)	21 (15.5%)	12 (8.8%)
A/ Philip.	7 (5.1%)	3 (2.2%)	57 (42.2%)	46 (34.0%)	17 (12.5%)	5 (3.7%)
A/Bangkok	3 (0.4%)	0	12 (18.4%)	32 (49.2%)	17 (26.1%)	1 (0.1%)

Table 2. Antibodies against N2 neuraminidase in pig sera

Herd	No. of sera	NI antibody titres					Total positive
		negat.	1:10	15—25	30—45	1:50+	
I.	43	8	6	20	6	3	35 (81.3%)
II.	22	4	4	9	3	2	18 (81.8%)
III.	26	20	5	1	0	0	6 (23.0%)
	91						59 (64.0%)

A/sw/Shope 15/31, A/sw/Bavaria 2/77, A/New Yersey 8/76 (H1 N1/Hsw N1) and strain A/Hong Kong/68 (H3 N2). However, antibody titres in a range of 10—640 were found against strains A/Texas/77 and A/Philipines 2/83 in all three herds (135 sera) and against strain A/Bangkok/79 (herds I and II were tested; serum total 65). The titre distribution presented in Table 1 was the same in all three herds.

Anti-neuraminidase (N2) antibodies were tested in only 91 sera from all three herds; the recombinant R(H7 N2/Heq1 N2Bang) was used. Titres of 10—80 were obtained in 59 (64%) of the sera (Table 2). In contrast to HI antibodies, considerably lower titres and a rate of only 23% were obtained for herd III; in herds I and II the positivity rate was equal 81%.

Antibodies against A/RNP antigen were detected by IDT in herd I sera only, at a rate of 10/43 sera (23%), and were evidence of recent type A influenza infection in the herd. Three sera from this herd also exhibited antibodies against both envelope antigens of the human A/Ostrava 11/80 (H1 N1) strain. The other two herds were negative against this antigen.

From the above results the following information may be drawn:

— The new subtype A(H3 N2) variants preserve the ability of this subtype to infect not only man but also pigs, as evidenced by finding of antibodies to nucleoprotein and both envelope antigens of the subtype strains in question.

— Strain A/Hong Kong/68 evidently does not persist in pig herds, contrary to the expectations following the 1974—1977 isolations (Shortridge *et al.*, 1977; Tůmová *et al.*, 1980; Ottis *et al.*, 1982) at a time when this strain had already been replaced among the human population by a new drift variant. Similarly was interpreted the isolation of the A/England 42/72 variant from pigs in 1977 as persistence of virus A(H3 N2) in pig herds. However, it is improbable that the antigenic drift should be of the same character in man and swine. Especially in conventional herds the conditions for influenza virus circulation in immune populations, leading to drift, are widely different and limited in time.

— An interesting finding is that of antibodies to the envelope antigens of human A/Ostrava 11/80 (H1 N1) strain, confirming that pigs may be infected even by this subtype. It is in line with the isolation of this subtype from pigs by Hannoun and Gourre (1980) at a time of its sporadic prevalence in man. These results deserve further systematic study.

— No virus of classical swine influenza has been demonstrated in the pig herds under our observation in recent time. An antigenically related virus was, however, incidentally isolated in another herd in 1980 (Mádr, Věžníková and Tůmová, unpublished data), so that the present negative findings may be transient. This virus has in recent years also been demonstrated in several European countries (Ottis *et al.*, 1981; Pensaert *et al.*, 1981; Sinnecker *et al.*, 1983); there is presumably a possibility of its importation into swine herds by migrating birds, in which a virus of identical antigenic properties was demonstrated antecedent to an outbreak in pigs.

The difference between the other authors' and our results may also have been influenced by the different husbandry in pig rearing. In particular, the swine

influenza virus variant isolated from migrating water birds in America, Asia and Europe could have more readily been introduced into open herds than to herds kept in pig houses as is usual in Czechoslovakia.

Both the previous and contemporary findings imply that virus A(H3 N2) variants are transferred into pig herds from the human population in connection with the presence of this subtype in man. Quite recent reports by Japanese authors demonstrate that pig herds may become reservoirs of strains arisen by recombination of classical swine influenza virus and an A(H3 N2) strain identical with strains from the human population (Nerome *et al.*, 1981; 1983). For this reason long-term and systematic follow-up of pig herds is important for learning about the ecology of swine influenza and the development of new variants potentially pathogenic for lower mammals and for man.

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