Epidémiol. santé anim., 1997, 31-32

SURVEILLANCE OF INFLUENZA VIRUS IN PIGS AND HORSES IN THE CZECH REPUBLIC*

Pospíšil Z. [1], Lány P. [1], Zendulková D. [1], Tumová B.[2], Jahn P. [1], Cíhal P. [1]

La recherche sur le virus de la grippe porcine a une longue histoire en République tchèque. A la fin des années 50, de nombreuses souches de grippe porcine A (H_1N_1) ont été isolées ; des études de pouvoir pathogène et des essais d'adaptation de souches de grippe humaine A/Hong Kong/68 (H_3N_2) ont été réalisés.
En 1956, la première souche de grippe équine A equi 1/Prague/56 (H_7N_7) fut isolée et devint une souche prototype. Le sous-type 2 A/equi 2/Brno/89 (H_3N_8) ne fut obtenu qu'en 1989. Nos études sur la grippe ont été résumées en 1995. Pendant longtemps, aucune souche de virus grippal n'a pu être isolée du porc, ni des anticorps détectés. Il n'y avait pas d'évidence d'infection grippale dans les populations porcines. Nos essais n'ont pas réussi à montrer la présence d'anticorps contre les nouveaux variants de virus grippal A (H_1N_1) issus d'oiseaux, alors que dans le passé ils étaient fréquents chez le porc en Allemagne, en Belgique, France, Italie et aux Pays-Bas. Cependant, il a été montré que les sérums de porcs contenaient des anticorps contre la souche de virus A/Prague 625/95 (H_3N_2) isolée en décembre 1995 en République tchèque lors d'une épidémie de grippe. Chez le cheval, plusieurs souches de virus A equi 2 ont été isolées lors d'une flambée à l'automne 1995. Le test d'inhibition de l'hémagglutination (HIT), utilisant des immunosérums spécifiques de souches sous-type A/equi 2 Miami 1/63, Newmarket 10/76 et Brno 2/89 a révélé que nos isolats différaient beaucoup dans leurs propriétés antigéniques de la souche prototype Miami, isolée en 1963, mais étaient semblables aux souches de Newmarket 10/76 et à nos souches Brno 2/89. Le séquençage de la partie HA_1 du gène de l'hémagglutinine a été comparé avec ceux de 43 souches de grippe du cheval isolées dans le monde pendant la période allant de l'isolement de la souche prototype Miami à nos jours ; il a été démontré que l'unique substitution sur notre souche était sur la position du 156ème acide aminé sur 329 positions étudiées.

RESEARCH ON SWINE INFLUENZA

The first epidemic outbreaks of swine influenza, recorded in the mid-fifties, were apparently associated with the process of accummulating animals in large swine farms. It launched the long-term research of swine influenza at the Veterinary Research Institute in Brno. A haemagglutinating virus, classified later as a swine influenza A H1N1 virus, was isolated from several acute and chronic outbreaks of disease.

Some of the isolates were highly virulent and, in experimentally infected piglets, they produced clinical signs and lesions typical of natural infection. A series of experiments aimed at elucidation of the pathogenesis of swine influenza was conducted in the subsequent years. Research concentrated on the dynamics of infection, virus multiplication sites, development of lesions, antibody response, ways of transmission, etc.

As could be expected, the apparent similarity of human and animal influenza raised the question of the role of animals in the ecology of influenza viruses. Discussions were started in connection with the outbreak of swine influenza in the United States, which was concurrent with a large epidemic of human influenza in 1918. A WHO programme on the zoonotic character of influenza was launched in 1957. As Kaplan and Beverige explained later, the programme was uderrated and taken as an odd attempt rather than accepted as a serious effort to find an important link in the epidemiology of human influenza.

The question of interspecies transmission was raised again at the turn of the sixties, this time in connection with isolation of a strain from pigs in Taiwan which was antigenically identical with the human influenza virus strain A/Hong Kong/68 (Kundin, 1970). Soon, antibodies to this strain were demonstrated in porcine blood sera in several countries.

At that time, studies of pathogenesis and adaptation of human influenza virus strains to swine were started in the above mentioned institute. Hysterectomy-derived, colostrum-deprived, 5-day-old piglets from an SPF herd, reared in incubators under aseptic conditions, received 5x 10^6 $CEID_{50}$ of A/Hong Kong/68 virus intranasally under general anaesthesia. The piglets responded to the infection by a transient (two-day) elevation in body temperature to 40,5°C, inappetence, increased respiration rate and mild serous nasal discharge. No coughing was observed. The clinical signs culminated on post-infection day 3.

Using the fluorescent antibody technique and re-isolation in chick embryos, the presence of virus was demonstrated in sacrificed piglets from day 1 to day 8 after infection. Bronchopneumonic lesions, showing a tendency to extend between post-infection days 2 and 8, resembled those found in conventional piglets of the same age infected with the swine influenza virus strain A/sw/Mirotín (Hsw1N1), (Pospíšil et al., 1973).

Attempts to adapt the human A/Hong Kong/68 strain to swine revealed a gradual decrease of virulence in the 2nd and 5th passages, as manifested by both the subsidence of macroscopic lung lesions, which almost disappeared

* Supported by the Grant Agency of the Czech Republic (Grant No. 508/95/0183).
1 University of Veterinary and Pharmaceutical Sciences, Faculty of Veterinary Medicine, Palackého 1-3, 612 42 Brno, Czech Republic
2 National Institute of Public Health, Šrobárova 48, 100 42 Prague, Czech Republic

at the 5th passage, and the decreasing number of cells showing specific fluorescence from the 5th passage onwards. The lung lesions increased in size again, extending from the apical to the caudal lobes and the number of fluorescent cells also increased, exceeding even the amount seen in the 1st passage (Menšík et al., 1976).
In the early seventies, blood sera of sows in three large swine herds affected with respiratory disease were investigated for the presence of antibodies to the A/Hong Kong/68 strain. Positive reactions with titres ranging from 1:40 to 1:1,280 were found in 62 to 83% of the sows. Considering the complete absence of antibodies in blood sera collected in 1966, this result was interpreted as indirect evidence of the transmission of human influenza virus to swine.
Transmission of human influenza virus to animals was also confirmed by our subsequent studies. A very close antigenic relationship was found between porcine isolates and A/Hong Kong/68 and, later, A/Port Chalmers/73 and A/Victoria/75 strains. While antibodies to variants of A/Hong Kong/68 (H3N2) were found only in older sows, antibodies to A/Port Chalmers/73 and A/Victoria/75 strains , which appeared in the human population only later, were detected also in younger animals. The results were interpreted as evidence suggesting a stepwise penetration of influenza virus into swine herds in relation to the appearance of influenza infection in the human population. They also provided a direct support for the hypothesis that pigs might serve as a "mixing vessel" for the generation of pandemic strains of human influenza virus (Tùmová et al., 1980).
The systematic research of swine influenza was later discontinued, but rather accidentally, an influenza virus strain, identical antigenically with the classic type A/sw (Hsw1N1), was isolated from a piglet suffering from bronchopneumonia in 1991.
In the studies resumed in 1995-1996, for a long time, we were neither able to detect any specific antibodies in pig populations nor to isolate influenza virus which would prove influenza infection. It seems likely that the frequent respiratory diseases affecting pig populations nowadays are caused by other aetiologic agents but that the whole complex of respiratory infections is still simply referred to as „influenza".
For detection of specific antibodies by haemagglutination inhibition test (HIT), various strains of swine influenza A/sw (H1N1) or A/sw (H3N2), particularly A/sw/Brno 2/75 (H1N1) and A/sw/Gent/86 (H3N2), were used in our last studies. However, examinations of all the sera tested gave only negative results. Because the survey covered large-scale pig farms in many regions of southern and notherm Moravia and southern Bohemia, our findings indicated that, at the time of investigation, swine influenza virus was absent in the major part of the Czech Republic.
Our results were confirmed by those of examination of the sera collected in our study in the National Reference Laboratory for Respiratory Diseases of the National Institute of Public Health in Prague. In addition to strains of swine influenza, the sera were also tested against new variants of influenza A(H1N1)viruses which were of avian origin but were isolated from pigs (A/sw/Germany/87 and a/sw/Italy 45/79). These isolates were frequently found in pig herds in Germany, the Netherlands, Belgium, France and Italy (Castruci et al., 1993, Ludwig et al., 1994). All attempts to detect specific antibodies against these strains in the pig sera failed. However, pig sera from 3 out of 9 farms showed antibodies against the human influenza A/Praha 625/95 (H3N2) strain isolated during the influenza epidemic in humans in December 1995. While in December 1995 all pig sera were negative, in January 1996 they showed HI antibodies at titres ranging from 1:80 to 1:160. In April 1996, average values of titres decreased to 1:20 and in June 1996 all the sera were negative again. It could be concluded that the animals under study were exposed to human influenza through their breeders whose sera were also shown to have antibodies against this strain.
From the dynamics of antibody titres it could be assumed that, during the human influenza epidemic, pigs were infected and antibody response was elicited but the virus was not established in the pig population and gradually disappeared. This was corroborated by observations on the health status of pigs during the epidemic. The owners and breeders did not notice any health problems related to influenza disease during or after the epidemics.

RESEARCH ON HORSE INFLUENZA

Although it is evident from historical records that a respiratory disease with a clinical pattern corresponding to that of equine influenza has been occurring on the European continent at least since the seventeenth century, its causal agent was isolated and identified as late as in 1956. We used to state with a certain pride that the first successful isolation was achieved by Dr. Sovinová from the Institute of Hygiene and Epidemiology in Prague and that the isolate, designated A/equi 1/Prague/56 (H7N7), (Sovinová et al., 1958), became the subtype 1 reference and prototype strain. Recently, however, the subtype 1 has failed to be isolated any more from outbreaks of respiratory disease and it is open to question whether the strain has disappeared completely from or is persisting in the partly immune or immunized equine population.
A second subtype of equine influenza virus, designated A/equi 2/Miami/63 (H3N8), was detected by Waddell et al., (1963) in the United States. In the area of the former Czechoslovakia, this subtype was isolated for the first time by our team from an outbreak of a respiratory disease in a group of horses that had participated in racing events along with horses from seven European countries in 1989 (Pospíšil et al., 1991). This newly isolated strain A/equi 2 /Brno/89 differed, to a certain extent, from the strain A/equi 2/Miami/63 and the subtype developed drift variants similarly to the human influenza virus.
Further isolates of influenza A/equi 2 virus were recovered from a flare-up of infection in autumn 1995 (Lány et al., 1995). The HIT with specific immune sera against selected influenza viruses, subtypes A/equi 2 Miami 1/63, Newmarket 10/76 and Brno 2/89, showed that our isolates were antigenically different from the prototype Miami strain isolated in 1963, but were similar to Newmarket 10/76 isolates and our previous Brno 2/89 strains. By using monoclonal antibodies was with kind assistance of the Animal Health Trust in Newmarket, Great Britain

found, that our isolates could be grouped with the strains possessing the subtype Miami HC1 heamagglutinin but failed to react with the subtype Miami HC4 and showed only a week reaction with the sybtype Fontainebleau HC2 strain. A sequencing of the HA1 portion of the HA gene was performed in one of our isolates (A/equi 2/M-2/95). A comparison of our strain with 43 equine influenza strains isolated in the period between the first isolation of the prototype Miami strain and today showed that both our strains and strains recently isolated abroad differed from the previous isolates. The difference, in comparison with all the other strains, was that an amino acid substitution unique to our strain was in the 156th out of 329 positions examined. Another interesting finding was that our isolate showed the greatest similarity to European isolates obtained between the end of eighties and the beginning of nineties. This can be explained by only a limited opportunity for interaction between our horses and those from Western Europe in the past.

BIBLIOGRAPHY

Castrucci M.R., Donatelli I., Sidoli L., Barigazzi G., Kawaoka Y., Webster R.G., 1993. Genetic reassortment between avian and human influenza A viruses in Italian pigs. Virology-New-York 193, 503-506.

Kundin W.D., 1970. Hong Kong A-2 influenza virus infection among swine during a human epidemic in Taiwan. Nature 228, 857-867.

Lány P., Pospíšil Z., Zendulková D., Èíhal P., Jahn P., 1997. An equine influenza outbreak in the Czech Republic in the fall of 1995: an epidemiological study. Vet. Med.-Czech 42, 39-42.

Ludwig S., Haustein A.., Kaleta E. F., Scholtissek C., 1994. Recent influenza A (H1N1) infections of pigs and turkeys in northern Europe. Virology 202, 281-286.

Menšík J., Valíèek L., Pospíšil Z., 1971. Pathogenesis of swine influenza infection produced experimentally in suckling piglets in the presence of colostrum-derived specific antibody in their blood stream. Zentbl. Vet. Med. B 18, 665-678.

Menšík J., Pospíšil Z., Machatková M., Tùmová B., Franz J., Štumpa A., 1976. Adaptation of human influenza A/Hong Kong/68 (H3N2) virus to colostrum deprived, specific-pathogen-free piglets. Zentbl. Vet. Med. B 23, 638-651.

Pospíšil Z., Menšík J., Tùmová B., Štumpa A., Èerný M., 1973. Experimental infection of colostrum - deprived, specific-pathogen-free piglets with A/Hong Kong/68 (H3N2) influenza virus. Zentbl. Vet. Med. B 20, 139-152.

Pospíšil Z., Tùmová B., Ulmann L., Zendulková D., Faltejsek J., 1991. Epizootics of equine influenza in Czechoslovakia caused by the type A/equi 2 (H3N8), and the effects of vaccination. Acta vet. Brno 60, 153-159.

Sovinová C., Tùmová B., Poustka F., Nìmec J., 1958. Isolation of virus causing respiratory disease of horses. Acta virol. 2, 52-61.

Tùmová B., Vì níková D., Menšík J., Štumpa A., 1980. Surveillance of influenza in pig herds in Czechoslovakia in 1974-1979. Zentbl. Vet. Med.B 27, 517-523.

Waddell G.H., Theigland M.B., Siegel M.M., 1963. A new influenza virus associated with equine respiratory disease. J. Am. vet. med. Ass. 143, 587-590.