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# PERSISTENCE IN NATURE OF INFLUENZA VIRUS A/eq/PRAHA/56 (Heq1Neq1)

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*Summary.* — Equine influenza occurred in Czechoslovakia 14 years after the last epizootic in horses that had returned from abroad. Six strains A (Heq1Neq1) antigenically related to, but not identical with, strain A/eq/Praha/56 were isolated from 10 washings. Seroconversion was demonstrated with paired sera, but the antibody increase was more marked against the newly isolated strain.

*Key words*: *equine influenza*; *antigenic variation*; *seroconversion*

## *Introduction*

Influenza virus A/equi/Praha/56 (Heq1Neq1) was isolated mostly in connection with racings in the spring or autumn (Romvary *et al.*, 1965; Rose *et al.*, 1974). There have been much less reports on epizootics caused by this subtype as compared with the A(Heq2Neq2) subtype, in the last 15 years probably also due to intensive vaccination which is an efficient prophylactic measure.

In Czechoslovakia, in addition to the original isolation in 1956, the participation of the A (Heq1Neq1) subtype was demonstrated serologically in an epizootic that had involved in 1965—66 about 1500 horses in various parts of the country (Vařejka *et al.*, 1968). Epidemiologically, this epizootic was connected with the return of the horses from racings abroad, like a localized outbreak of influenza of the same subtype in 1978, which is the subject of the present communication.

## *Materials and Methods*

Washings and acute blood samples were taken on the 2nd day of illness and convalescent blood samples were taken 15 days later. Attempts at virus isolation were carried out by intra-amniotic inoculation of chick embryos. The isolated viruses were identified serologically with strain-specific rat, guinea pig (Tůmová *et al.*, 1979) and rabbit sera. The rabbits were immunized subcutaneously into the foot pads with four 0.3 ml-doses of virus concentrated by centrifugation at 75000×g for 3 hr, at an interval of 3 weeks. Gel immunodiffusion (ID), haemagglutination

**Table 1. Characterization of influenza strains A (Heq1Neq1) isolated in 1978**

| Immune | sera | HI test 184 | eq/56 | eq/2/78 | eq/4/78 | eq 1 | eq 3 | eq 5 | eq 6 | NI test 185 | eq/56 | eq2/78 | eq4/78 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rabbit | R-185 | — | — | — | — | — | — | — | — | **>640** | >640 | >640 | 640 |
| G.pig* | R-T/e | — | — | — | — | — | — | — | — | NT | NT | >640 | 640 |
| G.pig* | R-X42 | 640 | 1280 | 160 | 1280 | 1280 | NT | NT | 640 | — | — | — | — |
| Rat | R-184 | **640** | 320 | 80 | 40 | 80 | 80 | 80 | 40 | — | — | — | — |
| G.pig* | R-184 | **>320** | 320 | 160 | 80 | 160 | NT | NT | 80 | — | — | — | — |
| Rat | eq/Praha/56 | 160 | **320** | 160 | 80 | 80 | 80 | 80 | 80 | 160 | **180** | 80 | 90 |
| Rat | eq/Brno 2/78 | 160 | 160 | **640** | 320 | 320 | 320 | 320 | 320 | 15 | 15 | **640** | 640 |
| Rat | eq/Brno 4/78 | 40 | >40 | 160 | **80** | 160 | 80 | 80 | 80 | 40 | 50 | 250 | **280** |

*Postinfection sera.
— means no cross-reactions.
NT = not tested.

**Table 2. Antibody to RNP, H and N antigens of influenza virus A (Heq1Neq1) in paired horse sera**

| Horse | | ID | HI test eq/56 | HI test eq 4/78 | NI test eq/56 | NI test R 184 | Virus isolation | Designation of strain |
|---|---|---|---|---|---|---|---|---|
| Hakim | I | — | <10 | <10 | <5 | <5 | + | No.1 |
| | II | + | 160 | 640 | <5 | <5 | | |
| Larsen | I | — | 20 | 40 | <5 | <5 | + | A/eq/Brno |
| | II | + | 160 | 320 | 50 | 30 | | 4/78 |
| Olina | I | — | 10 | 20 | <5 | <5 | + | No. 3 |
| | II | + | 160 | 1280 | <5 | <5 | | |
| Sybila | I | — | 40 | 40 | 10 | 5 | ND | |
| | II | + | 160 | 640 | 500 | 250 | | |
| Symplex | I | — | 80 | 80 | 50 | 20 | | |
| | II | + | 160 | 1280 | 400 | 180 | — | |
| Sahib | I | — | 10 | 20 | <5 | <5 | + | No. 5 |
| | II | + | 320 | 320 | 160 | 35 | | |
| Svahil | I | — | 10 | 10 | <5 | <5 | + | No. 6 |
| | II | + | 320 | 1280 | 5 | 5 | | |
| Ferda | I | — | 10 | 10 | <5 | <5 | + | A/eq/Brno |
| | II | + | 80 | 1280 | 15 | 15 | | 2/78 |
| Diana | I | — | 10 | 20 | <5 | <5 | — | |
| | II | — | 160 | 160 | 30 | 15 | | |
| Karel | I | — | 40 | 160 | <5 | <5 | — | |
| | II | — | 160 | 640 | <5 | <5 | | |
| Šárka | I | — | 10 | 10 | <5 | <5 | — | |
| | II | — | 80 | 1280 | <5 | <5 | | |

I and II: acute and convalescent, serum collected 4 and 19 Oct., respectively.
ND — not done.
Strains Nos 1, 3, 5, 6 were not included into the strain collection.

inhibition (HI) and neuraminidase inhibition (NI) tests were carried out and the antigens prepared according to Palmer *et al.* (1975). The antigens used were: A-RNP from A/PR8 (H0N1); A/eq/Praha/56 (Heq1Neq1); and recombinants R-184 (H3Neq1); R-185 (Heq1N2); X42 (Heq1N2); and T/e (H3Neq1) — serum.

## *Results*

The first cases of an acute febrile illness were recorded on 2—4 October 1978 at the clinic of the Veterinary University, Brno. The infection involved 15 hospitalized horses 3—5 days after acceptance of 3 horses that had been kept, before admission to the clinic, together with race horses from an international meeting in Sofia. The acute febrile illness was accompanied with temperature of 41 °C and cough; bronchitis occurred after 2 days in 2 horses. Nasal washings were taken from 10 horses; in chick embryos they yielded 6 influenza virus strains A (Heq1Neq1). Antigenic characterization of the isolated strains by HI and NI tests (Table 1) revealed minor, though clear-cut differences of both envelope antigens from the strain A/eq/56. The degree of the difference can be evaluated from cross-reactions with virus recombinants which exclude the possibility of the titres being affected by steric inhibition.

ID tests revealed specific A-RNP antibody indicative of an overcome acute infection in 8 of 11 horses examined (Table 2). An antibody increase to the strain A/equi/Praha/56 and a very marked, up to 64-fold antibody increase to the newly isolated strain was demonstrated in all horses by the HI test. With the exception of 4 horses, all showed also a specific increase in anti-neuraminidase antibody.

Low antibody titres in acute sera suggested a previous contact with this or a similar antigen. In horses Larsen, Svahil, Ferda and Diana, primary immune reaction with a low increase in anti-neuraminidase antibody was involved. Negative ID and NI tests suggest that a type of infection with incomplete virus replication had occurred in horses Karel and Šárka.

## *Discussion*

The present findings allow the following conclusions. The virus was introduced to the clinic by horses that had been in contact with animals from an interneational meeting. All hitherto described epizootics had this anamnesis and were observed at relatively long time intervals, during which the infested population was gradually replaced by young non-immune animals. This was confirmed by the study of Vařejka *et al.*, (1968) in Czechoslovakia and Powell *et al.* (1974) reported a similar case from England. The present outbreak was not an isolated one. Cases of equine influenza A (Heq1Neq1) were observed (Trunkát, personal communication) and serologically confirmed in various localities in Bohemia and Moravia, mostly in connection with transport of race and breed horses. Once introduced, the infection can be expected to spread everywhere where vaccination is not carried out continuously.

The virus thus spreads and is maintained for a certain time in a susceptible animal population. But we cannot explain in this way the long-term persistence of the virus in nature. So far, there is no evidence of a virus carrier state or latent infection. Romvary *et al.* (1965) and Gerber (1970) considered the possibility of asymptomatic infections by which the virus would spread and persist in groups of horses with a different degree of immunity. In this way we could explain occasional sudden outbreaks of infection in horse stables without any connection with concentration of horses during racings (Tůmová *et al.*, 1972). In any case, the circulation of A (Heq1Neq1) virus in nature is slow, which conclusion is also supported by the comparative stability of the properties of the virus.

The antigenic relationship between the newly isolated strains and the classical A/eq/Praha/56 strain was demonstrated serologically. This is in accordance with the findings by Powell *et al.* (1974) and Rose *et al.* (1974) who found strains isolated in 1972—73 to be related to the 1956 strain with minor difference in the haemagglutinin. Serological tests with paired sera markedly demonstrated these differences by the increase in titres of antibody against the classical and the new strains. In the A (Heq2Neq2) subtype, antigenic differences have been repeatedly demonstrated since 1972 (Pereira *et al.*, 1972). An antigenic change of the A (Heq1Neq1) virus as

late as 23 years after the original isolation therefore represents an unusual phenomenon in influenza and confirms a low frequency of this virus in nature.

Based on the present results it is justified to assume that the effectiveness of vaccine containing the classical A/eq/Praha 1/56 strain will become limited with time.

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