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AETIOLOGIC STUDY ON AN INFLUENZA-LIKE EPIDEMIC IN HORSES IN CHINA

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Summary. - About thirty thousands horses were affected and hundreds of them died in an apidemic caused by equine 2 influenza virus (H3N8) in China. The estimated morbidity and mortality accounted for 81% and 2%, respectively. The viral protein and RNA electrophoresis patterns revealed that the new isolates were antigenically different from the prototype strain influenza A/eq/Miami/1/63(H3N8). Therefore, the representative strain of the equine 2 subtype of influenza A virus recommended for producing reference reagents, vaccines, and for serological diagnosis must have been altered by antigenic drift.

Key words: equine influenza virus; horses; strain typing

A line of evidence indicates that the origin of pandemic influenza viruses is closely related to animal or avian influenza viruses (Webster *et al.*, 1975) and that the origin of H3N2 subtype of human influenza A virus could be derived from the recombination between A/equine 2 virus and the H2N2 subtype of human influenza A virus (Webster *et al.*, 1975). Serological studies also show that A/equine 2 virus may have circulated in man in the past, possibly during the epidemic in 1900 (Kilburne *et al.*, 1975).

Severe outbreaks of influenza epidemics in horses associated with great losses due to influenza A/equine 2 virus have been reported in many countries (Hinshow *et al.*, 1983). So influenza surveillance in horses has attracted more

Table 1. Identification of the haemagglutinin of two isoiates

Antigens	Antisera	
	A/Eq/Prague/1/56(H7N7)	A/Eg/Miami/1/63(H3N8)
Eq/Jilin/1/89	<20	60
Eq/Jilin/2/89	<20	100
A/Eq/Prague/1/56(H7N7)	640	<20
A/Eq/Miami/1/63(H3N8)	<20	1280

Table 2. Identification of neutraminidase of the two isolates

Antigens	Antisera		
	N7	N8	A/Beijingl1/68(H3N2)
Eq/Jilin/1/89	<10	>2560	<10
Eq/Jilin/2/89	<10	>2560	<10
A/Eq/Prague/1/56(H7N7)	320	<10	<10
A/Eq/Miami/1/63(H3N8)	<10	640	<10
A/Beijing/1/68(H3N2)	<10	<10	640

and more attention.Here we analyse an epidom c of influenza-like disease among horses in Northeastern China from March 1989.

The epidemic had broken out studdenly at the boundary between Heilongjiang and Jilin provinces at the beginning of March 1989 and spread rapidly from horse to horse. It peaked from March to April, afterward the incidences decreased, the epidemic stopped completely at the beginning of June 1989. The horse populations in 13 and 15 different places in Heilongjiang and Jilin provinces were affected respectively within two months and all age groups were involved. The morbidity of 81% and the mortality of 2% in lew horse populations were recorded. No other domestic animals (donkeys or mules) nor human beings were affected.

The incubation period was 1 to 3 days, the symptoms inactivity, fever ($\geqq 40\ ^0C$), cough, purulent pharyngitis, purulent conjunctivitis, sinusitis, and hasal discharge. Recovery began 5 to 6 days after the onset of illness, but a complete convalescence lasted about two weeks. Death was always associated with bacterial infections, such as pneumonia or enteritis.

Altogether 24 nose swab samples were collected and inoculated into ten day old embryonated eggs. The methods of incubation, HA, and HI tests for viral identification were performed as described (Robinson *et al.*, 1969). The NA and NI test were used as described (Henry *et al.*, 1973). The haemagglutinating agents were isolated from the specimens. The reference sera to A/eq/Prague/1/56(H7N7), A/eq/Miami/1/63(H3N8), N7 and N8 were used for viral identification (kindly provided by Dr. Jude Webster, ST Children's Research Hospital, Memphis, U. S. A.). The results were shown in Tables 1 and 2. The two isolates were identified to be of H3N8 subtype, they were designated as A/eq/Jilin/1/89(H3N8) and A/eq/Jilin/2/89(H3N8), respectively.

The 42 convalescent-phase serum specimens were taken from the horse neck vein after two months of the onset of illness; 12 control serum samples were collected from healthy horses. The A/eq/Jilin/1/89(H3N8), A/eq/Miami/1/63(H3N8), and A/eq/Prague/1/56(H7N7) antigens were used for testing. The results with HI test were shown in Table 3. No antibody to these three antigens was detected in any of normal sera. Of 42 convalescent sera, 35

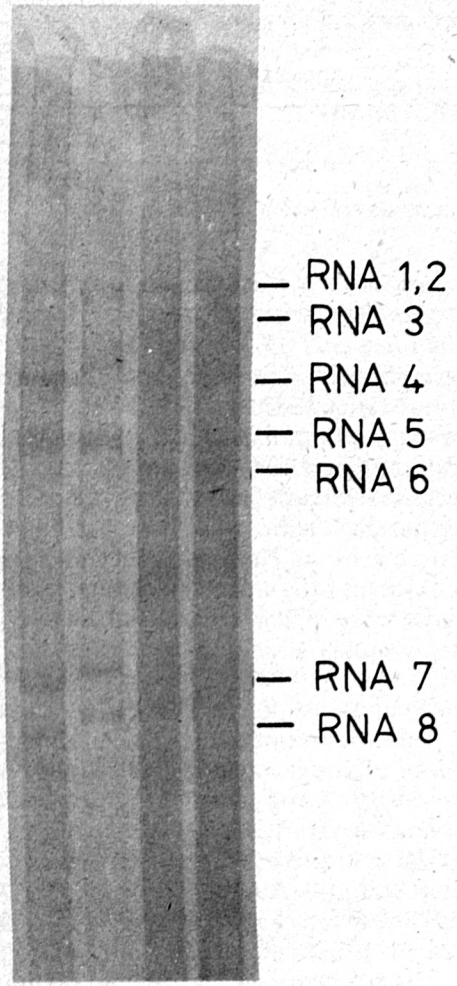

Fig. 1
Comparison of RNAs migration pattern between isolates and prototype strains
1 - A/eq/Miami/1/63(H3N8)
2 - A/Sichuan/2/87/(H3N2)
3 - A/eq/Jilin/2/89(H3N8)
4 - A/eq/Jilin/1/89(H3N8)

had antibodies against A/eq/Jilin/1/89(H3N8) (HI titres from 15 to 1600) but only 5 to A/eq/Miami/1/63(H3N8) (HI titres 15 to 320 - positive rates 83.3% and 11.9 %, respectively). No one convalescent serum had antibody to A/eq/Prague/1/56(H7N7) (HI titres $\geqq$ 10). These results indicate that the epidemic was caused by influenza A/eq/Jilin/1/89(H3N8)-like virus and that the prototype strain A/eq/Miami/1/63(H3N8) was not suitable for seroepidemiologic survey.

Table 3. The results of HI tests

Sera	Tested antigens		
	A/Eq/Prague/1/56 (H7N7)	A/Eq/Miami/1/63 (H3N8)	A/Eq/Jilin/1/89 (H3N8)
Convalescence	0/42	5/42	35/42
Normal	0/42	0/12	0/12

The figures in denominator mean the total No. testing serum
The figures in numerator mean the No. sera with HI titres ≧10

For excluding the effect of nonspecific inhibitors in the horse sera, radial haemolysis test (SRH) was used with A/eq/Jilin/1/89(H3N8) antigen (Guo *et al.*, 1981). The results showed that all 35 sera (with HI ≧ 10) revealed zones of haemolysis with diameters from 5.0 to 12.0 mm. However, other sera did not cause any haemolysis zone. Thus, the results of HI test were more reliable than SRH, but the latter also confirmed the role of an A/eq/Jilin/1/89(H3N8)-like strain.

The haemagglutinin and neuraminidase analyses of isolated viruses are results shown in Tables 4 and 5, respectively. Table 4 indicates that the two isolates were antigenically different from prototype strain A/eq/Miami/1/63(H3N8), but they were antigenically similar to each other. Table 4 indicates that the euraminidases of the isolates were different from those of A/eq/Miami/1/63(H3N8).

Virus purification, RNA extraction, RNA gel electrophoresis and gel staining have been described previously (Guo *et al.*, 1984). The results shown their migration pattern was different from that of the prototype strain.

Table 4. The results of cross HI test among different H3N8 viruses

Antigens	Antisera		
	A/Eq/Jilin/1/89	A/Eg/Jilin/2/89	A/Eg/Miami/1/63
A/Eq/Jilin/1/89	3200	960	60
A/Eq/Jilin/2/89	5120	3200	120
A/Eq/Miami/1/63	100	60	1280

Table 5. The results of eross NI test among different H3N8 viruses

Antigens	N8	a	b	c	1	2	3	4	5
A/Eq/Jilin/1/89	2560	1280	1280	160	160	160	160	80	80
A/Eq/Jilin/2/89	2560	1280	1280	160					
A/Eq/Miami/1/63	640	80	160	640	10	25	10	10	<10

Antisera 1-5 mean horse convalescent sera

Equine influenza epidemic in horses has first occurred in China in 1974 (Deng *et al.*, 1980). There are some differences betweeb tge two epidemics: the first was caused by equine 1 virus which was antigenically similar to prototype strain A/eq/Prague/1/56(H7N7), while the second by equine 2 virus which was antigenically different from the prototype strain A/eq/Miami/1/63(H3N8). The horses in many provinces and cities were affected in the first epidemic, while in the second one only two provinces (Heilongjing and Jilin) were in the second one only two involved. The mortality in latter epidemic was higher than that in the first one. Donkeys and mules were affected in the first epidemic but they were not involved in the second one. However, the epidemiologic features and clinic appearances were very similar to each other.

The equine 2 virus activities are increasing recently in the world, especially in Asia, it seems that it could cause and epidemic in human population as well. However, where did equine 2 virus come from in horses in China? There seem to be two possibilities. One is that it was transmitted from the U.S.S.R. to China by horse, the other is that it came from another source, such as avian influenza virus. However, it was well known that equine 2 virus HA antigens were antigenically distinguishable from avian (H3) and human (H3) viruses (Kilbourne, 1987), so the first possibility could be more probable.

The representative strain of equine 2 virus used for producing reference reagents, vaccines, and for serologic diagnosis, could have altered by an antigenic drift.

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