A Serologic Survey on Equine Influenza for the Past Ten Years

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Abstract. Over a period of 1966 to 1976, a serologic survey was performed on the serum samples of native and imported horses raised in Hokkaido and Tokyo. No hemagglutination-inhibition (HI) antibodies against the subtype 1 (Prague strain) and the subtype 2 (Miami and Tokyo strains) of equine influenza (EI) virus were detected in any of the sera from 587 native draft horses throughout the observation period. When examined by the HI test over a period of 1966 to 1970, 28 native racing horses had no such antibodies. Twelve and 16 (55 and 73%) of 22 native racing horses tested in 1972 and 1973, respectively, and 67 (72%) of the 93 native racing and riding horses tested in 1976, had HI titers of 1:8 to 1:512 against the strains. Of 80 imported racing horses tested over a period of 1966 to 1970, 48 (60%) had positive titers for the Prague strain and 23 (29%) for the Miami strain. Almost the same percentage was recognized against each of the strains in 28 imported horses tested in 1972 and 1973. In 1976, 12 to 14 (67 to 78%) of 18 imported horses were reactive in positive titers to the strains. These results indicated that there had been a marked difference in the antibody response of racing and riding horses between the time before and the time after the first outbreak of EI infection in 1971.

Equine influenza (EI) is an acute infectious respiratory disease of horses which commonly occurs in epizootic form. The first outbreak of EI infection in Japan was recognized in racing and riding horses during December, 1971 and January, 1972 [1, 6]. Since then, a majority of horses have been vaccinated with inactivated EI viruses. This vaccination is required by law, especially for racing and riding horses which

are transported widely. As mentioned in the authors' preceding report [5], a sero-epizootiological survey was performed on equine sera collected for several years just before the first outbreak of the disease. It indicated that some of the horses imported from England, France and U.S.A. had antibody against the subtype 1 or 2 strain of EI virus. On the contrary, no antibodies against both strains were detected in any

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of the serum samples of native horses examined. The present study was, therefore, undertaken to investigate whether the first epizootic had a considerable effect on the serological pattern of the equine population in Japan. Such study is very important for planning an efficient vaccination program for horses against EI infection.

Materials and Methods

Viruses: The strains of EI virus used were A/equine/Prague/56, Heq1 Neq1 (Prague), A/equine/Miami/63, Heq2 Neq2 (Miami) [5], and A/equine/Tokyo/71, Heq2 Neq2 (Tokyo). The Tokyo strain was isolated from a diseased horse in the outbreak of EI infection in the vicinity of Tokyo in 1971 [7], and supplied by the courtesy of Dr. Koichi Takehara, of the Nippon Institute for Biological Science, Tachikawa, Tokyo. Those strains were employed at the 15th to 25th passage level in 10-day-old embryonating hen's eggs.

Sera: The equine sera used in this study were derived from animals of several herds mostly in Hokkaido and Tokyo. They were collected during three periods, from 1966 to 1970, from 1972 to 1973, and in 1976. All the animals were apparently healthy, ranging from 1 to 29 years of age, and included both males and females. The serum samples were stored at -20°C until use. The result of the survey on sera collected over a period from 1966 to 1970 was cited partially from the authors' previous paper [5].

Hemagglutination-inhibition (HI) test: The test was performed by the microtiter method with a twofold dilution of the treated serum, 8 hemagglutinating units of virus antigen in 0.025 ml each, and 0.5% chicken erythrocytes in 0.05 ml volume.

All reagents were prepared with 0.01 M phosphate-buffered saline, pH 7.2. Serum samples used for the test were treated by the same method as that described elsewhere [4]. Virus antigen was fresh allantoic fluid from embryonating hen's eggs infected with the virus strains used. The titer of the test serum was expressed as reciprocal of the highest initial dilution of serum completely inhibiting hemagglutination.

Results

1. Antibody response of native horses to EI viruses

The native horses examined in the present study were divided into two groups, draft horses and racing or riding ones. Serum samples collected from 452 draft horses were used for the first observation over a period from 1966 to 1970, those from 72 for the second observation in 1972 to 1973, and those from 63 for the third observation in 1976. No antibody titers were detected against the Prague, Miami and Tokyo strains of EI virus in any serum sample of draft horses used for the three observation periods. These findings strongly suggest that the first epizootic of EI infection may not have spread among the draft horses in Japan.

As shown in Table 1, 28 racing and riding horses contained no serum HI antibodies against the Prague and Miami strains during the first observation period

Table 1. HI antibodies against EI virus in native racing and riding horse sera

Year(s) of serum collection	Antigen	No. of sera with antibody at indicated dilution									
		<8	8	16	32	64	128	256	512	≥ 8	%
1966-70	Prague	28	0	0	0	0	0	0	0	0/28	0
	Miami	28	0	0	0	0	0	0	0	0/28	0
1972-73	Prague	10	2	7	2	1	0	0	0	12/22	54.5
	Miami	6	2	2	4	4	2	2	0	16/22	72.7
	Tokyo	6	1	3	3	6	1	2	0	16/22	72.7
1976	Prague	26	4	8	11	27	11	5	1	67/93	72.0
	Miami	26	6	8	6	25	18	2	2	67/93	72.0
	Tokyo	26	0	6	6	7	22	20	6	67/93	72.0

Fig. 1. Age distribution of Prague, Miami and Tokyo antibodies in native horse sera collected in 1976

mentioned above, or just before the first epizootic. Of 22 serum samples collected from racing horses over the second observation period, twelve had an HI titer of 1:8 or higher against the Prague strain, and sixteen against the Miami and Tokyo strains. The titers ranged from 1:8 to 1:256. Over the third observation period, positive titers against all the 3 strains were detected in 67 of the 93 racing and riding horses. They extended from 1:8 to 1:512 and were higher than those determined over the second observation period.

Fig. 1 gives the age distribution of HI antibodies against the Prague, Miami and Tokyo strains of EI virus in 87 racing and riding horses, except 6 foals, tested in 1976. Almost all the 37 horses 4 to 8 years of age

had an HI titer of 1:8 or higher against the 3 strains. Then the titers were in a wide range of 1:8 to 1:512 against each of the strains. In 50 horses over 9 years of age, however, the HI titers of the 3 kinds of antibodies were divided into two groups. One group consisted of high titers ranging from 1:64 to 1:512 in 24 to 31 horses (48 to 62%), and the other of titers of less than 1:8 in 17 or 18 horses (34 or 36%). This tendency was strong in the distribution of Tokyo antibody.

2. Antibody response of imported horses to EI viruses

The horses imported from Australia, England, France and U.S.A. were all racing horses, except a few which were riding

Table 2. HI antibodies against EI viruses in imported horse sera

Year(s) of serum collection	Antigen	No. of sera with antibody at indicated dilution									%
		<8	8	16	32	64	128	256	512	≥8	
1966-70	Prague	32	7	12	6	6	8	5	4	48/80	60.0
	Miami	57	5	4	3	7	2	2	0	23/80	28.8
1972-73	Prague	13	5	2	3	3	2	0	0	15/28	53.6
	Miami	19	1	4	2	0	2	0	0	9/28	32.1
	Tokyo	18	1	3	3	1	0	0	2	10/28	35.7
1976	Prague	4	1	1	2	6	3	1	0	14/18	77.8
	Miami	6	1	2	3	3	2	1	0	12/18	66.7
	Tokyo	5	2	2	2	0	3	2	2	13/18	72.2

Fig. 2. Distribution of Prague and Miami antibodies in imported horse sera collected over a period from 1966 to 1970

ones. Serum samples were collected for tests over the same period as those from the native horses (Table 2). During the first observation period, 48 of 80 imported horses had an HI titer of 1:8 or higher against the Prague strain and 23 against the Miami strain. The titers ranged from 1:8 to 1:512. Of 28 sera collected during the second observation period, 15 were shown to have the positive titers against the Prague strain, 9 against the Miami

strain, and 10 against the Tokyo strain. These titers were in a narrow range, from 1:8 to 1:128, except those of 2 sera, as compared with those during the first observation period. Of 18 sera collected during the third observation period, 14 presented positive titers against the Prague strain, 12 against the Miami strain, and 13 against the Tokyo strain. These titers extended from 1:8 to 1:256 or 1:512.

The titers of HI antibodies against the

Fig. 3. Distribution of Prague and Miami antibodies in imported horse sera collected over a period from 1972 to 1976

Prague and Miami strains were plotted against the years of importation of horses. Figs. 2 and 3 give the results of HI tests with sera collected over a period from 1966 to 1970 and over a period from 1972 to 1976, respectively. In the upper part of Fig. 2 are shown the HI titers of Prague antibody of horses imported over a period from 1952 to 1969. Some of the horses brought to Japan over a period from 1952 to 1965 had moderate titers ranging from 1:8 to 1:64, while a majority of the horses imported over a period from 1966 to 1969 had relatively high titers in a wide range of 1:16 to 1:512. The lower part of Fig. 2 presents the distribution of HI titers of Miami antibody. All the horses imported over a period from 1952 to 1965 had HI titers of less than 1:8, whereas many of those imported over a period from 1966 to 1969 had HI titers ranging from 1:8 to 1:256. These results indicate a distinct difference in distributions between antibody against the Prague strain and that against the Miami strain. Horses imported in 1966 and later presented a higher level

of EI antibodies than those imported in 1965 and earlier. On the contrary, as shown in Fig. 3, there was no marked difference in distribution or level of titer between the two kinds of antibody when HI tests were performed with 46 sera collected over a period from 1972 to 1976. Most of the horses imported over a period from 1958 to 1974 had HI titers ranging from 1:8 to 1:256 against both strains. There were, however, no distinct correlation between the distribution of antibody titers and the year of importation of horses.

3. Antibody response of mares and her young to EI viruses

An experiment was carried out to investigate the antibody response of foals to the strains of EI virus, since a passive transfer of antibodies from an immune mare to her suckling foal was considered. In it serum samples were collected from 20 dams and their young in August and December, 1976. Table 3 shows antibody titers against the Prague, Miami and Tokyo strains demonstrated in HI tests. Of the 20 mares,

Table 3. HI antibody titers against EI viruses with paired sera from mares and their foals

Mare No.	HI titer in mare			HI titer in foal		
	Prague	Miami	Tokyo	Prague	Miami	Tokyo
M 11	128-128 ^{a)}	64-64	128-128	32-0 ^{b)}	8-0	16-0
M 19	64-64	64-64	128-128	16-0	16-0	32-0
M 18	64-64	64-64	128-128	8-0	16-0	32-0
M 27	64-64	64-32	64-64	16-0	8-0	16-0
M 23	32-NT ^{c)}	16-NT	32-NT	8-0	0-0	8-0
M 24	0-0	16-8	64-32	0-0	„	8-0
M 7	32-16	8-8	16-16	„	„	0-0
M 4	8-8	8-8	16-16	„	„	„
M 28	16-16	16-8	16-16	„	„	„
M 25	0-8	8-8	16-16	„	„	„
M 2	16-32	0-0	0-0	„	„	„
M 30	8-16	„	16-16	„	„	„
M 29	8-8	„	0-0	„	„	„
M 8	0-0	„	0-8	„	„	„
M 3, M 10,						
M 12, M 15,	„	„	0-0	„	„	„
M 16, M 22						

Remarks.

a) : HI antibody titers in August (preceding hyphen) and December (following hyphen), 1976.

b) : HI antibody titers in 3 months (preceding hyphen) and 7 months (following hyphen) after birth.

0=less than 1:8.

c) : NT=Not tested.

14 had HI titers of 1:8 or higher against all or at least one of the strains. These titers ranged from 1:8 to 1:128. They were almost the same in August and December. In the sera of six of the 20 foals tested in August, or the third month after birth, HI antibody titers of 1:8 to 1:32 were detected against the strains of EI virus. The 6 foals were nursed through May to November by their immune dams with HI titers of 1:32 to 1:128. Their titers, however, decreased to less than 1:8 in December, or the 7th month after birth. No HI antibody titer was seen in any suckling foal from immune dams with an HI titer of 1:16 or lower, or from non-immune dams.

Discussion

The results of this serologic survey indicate that there is a considerable difference

in EI antibody pattern in native horses between the time before and the time after the first outbreak of EI infection in 1971. None of 480 horses, consist of 452 draft and 28 racing horses, tested over a period from 1966 to 1970 had HI antibody against some of the strains of EI virus employed. During a period from 1972 to 1973, however, HI titers of 1:8 and higher were recognized in 12 to 16 (55 to 73%) of 22 racing horses. In 1976, when the test was again conducted in 93 racing and riding horses, the same positive rates were obtained from these horses, except some which had higher titers. Moreover, HI antibodies were distributed in horses with higher titers than in those examined over a period from 1972 to 1973. Distribution of HI antibodies by age was surveyed in native horses in 1976, but no increase in

antibody titer was found in relation to the age of horses. On the other hand, 37 horses 4 to 8 years old showed a wide range of HI titers from 1:8 to 1:512, while more than half of 50 horses over 9 years old, which might have been exposed to the first outbreak of the disease in Japan, showed high titers ranging from 1:64 to 1:512. These results suggest strongly that the antibody response of native racing and riding horses in Japan may have been influenced greatly not only by the current vaccination of EI virus, but also by the natural infection in 1971.

Of 80 imported horses tested over a period from 1966 to 1970, 48 (60%) had HI titers of 1:8 to 1:512 against the Prague strain, and 23 (29%) HI titers of 1:8 to 1:256 against the Miami strain. No increase was observed in the positive percentage of antibodies in sera tested in 1972 and 1973, since the sera were collected immediately after the first outbreak of EI infection. The HI titers of these sera were in a narrower range, or from 1:8 to 1:128, in 1972 and 1973 than over a period from 1966 to 1970. The authors' previous paper [5] revealed that the mean HI titer of EI antibody in horses imported into Japan decreased with the lapse of time after importation, even though such lapse of time had no effect on the positive rate of antibody. Thus, it is clear that the imported horses escaped from infection with EI virus in the first epizootic of the disease. In 1976, however, imported horses had very high positive rates of 67 to 78%, indicating a wide range of titer from 1:8 to 1:512. This may be due to the frequent vaccination of EI virus after the first outbreak. The same may be said in the comparison of distribution of antibody titers, especially against the Miami strain, among the years of importation (Figs. 2 and 3).

Under natural conditions, it is known that the incidence of EI infection among horses is governed by the status of immunity due to infection or vaccination [3, 8]. That is, each horse with frank illness had a significant HI antibody conversion from negative ($<1:5$ to $<1:10$) to positive (1:40 to 1:1280) against the infecting strain of virus, while horses with an HI titer of 1:5 or higher had no clinical signs of the disease, but some of them exhibited secondary responses [3, 10]. During the widespread outbreak of EI infection in England in 1973 [8], regularly vaccinated horses exposed to viruses failed to have clinical or serologic evidence of infection. In addition, no clinical signs of infection were reported from 25 out of 26 training stables throughout England, where a surveillance progress and vaccination had been carried out regularly. As described above, 67 to 78% of 93 native and 18 imported horses, consisting of racing and riding ones, became reactive to EI viruses in 1976, exhibiting HI titers of 1:8 to 1:512. A large number of horses have still been vaccinated regularly in the recommended manner. Thus, it is apparent that these antibody responses have been protecting horses from a severe epizootic of EI infection in Japan since 1972.

No HI antibody titers against the EI viruses employed were detected at all in the 587 sera collected from draft horses during the observation period. In the sera of all the 20 foals tested, these titers decreased to less than 1:8 in December of the year of birth, although six of these foals had titers of 1:8 to 1:32 in August of the same year. These findings indicate that a large number of horses susceptible to EI virus always exist in the equine population in Japan. A complete lack of immunity in draft horses, however, may not have been

the cause of occurrence of EI virus among horses in a large area, because draft horses were not assembled together in one place, different from racing horses which are concentrated in a race track. For the immunization of foals, it has been recommended in U.S.A. [2, 9] that a vaccination program begin on the farm with two injections of vaccine 6 to 12 weeks apart and that each year's crop of foals be vaccinated before weaning and given a booster injection before being sent from the farm for sale or training. This method may be very useful as means of immunizing foals also in Japan.

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