Antibody Responses of Japanese Horses to Influenza Viruses in the Past Few Years

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ABSTRACT. A total of 305 horse sera collected in the Hidaka district of Hokkaido in the years 1988–90 were tested for the presence of hemagglutination-inhibition (HI) antibodies to A/equine/Newmarket/1/77 (H7N7), A/equine/Tokyo/2/71 (H3N8) and A/equine/Kentucky/1/81 (H3N8, Kentucky) strains of equine influenza (EI) virus. Antibodies to the 3 strains were detected in hardly of the 45 sera from 2-years-old horses which were collected before vaccination. Many of the 51 horses, after vaccination with inactivated EI virus, had HI antibodies to the 3 strains in 37 to 88 per cent. However, the number of positive reactors among the horses aged 3 to 23 years gradually decreased with the advance in age. In particular, no antibody response was found in the 60 horses over 9 years of age, except for 3 cases with HI antibody of low titer, with the Kentucky strain which has recently been prevalent among the horse populations in many countries. In a complement-fixing test, antibodies to the soluble antigen of type A influenza virus were detected in the sera collected from the horses which were exposed to an outbreak of EI virus infection, but not in the sera from the vaccinated horses examined.—**KEY WORDS:** antibody, horse, influenza virus.

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Equine influenza (EI) is apparently a common disease in horses and is widely distributed throughout the world. In Japan, the first occurrence of EI was recognized in December, 1971, when it was presumed that the disease was introduced into horses in our country by some imported horses [1, 6]. In previous reports, we conducted seroepidemiological studies on horse sera collected in the period from 1965 to 1971 [3] and from 1966 to 1976 [4]. The results showed a remarkable difference between the antibody response of horses before and after the first outbreak of EI virus infection in 1971. Although the occurrence of EI has not been reported in horses since the first outbreak of the disease in Japan, EI viruses are prevalent among horses raised in European, American, African and Asian countries [9–11, 13–15]. The purpose of this study is to provide information on the antibody responses to EI viruses in the sera collected from Japanese horses during recent years. Such a surveillance study is important in drawing up an efficient program to vaccinate horses against the invasion of EI virus from abroad.

MATERIALS AND METHODS

Sera: The horse sera used in this study were collected from the horses before and after the

vaccination with inactivated viruses at random in the Hidaka district of Hokkaido during the years 1988 to 1990, and in the Kanto area in 1988, respectively. Some horse and human sera were also used for comparative examination in serological tests. The horse sera were obtained when they were exposed to the first epidemic of EI infection in Tokyo Racing Course in 1971. These sera in the Kanto and Tokyo areas were supplied by courtesy of the Epizootic Research Station, Equine Research Institute, Japan Racing Association, Tochigi. The human sera were collected from patients in the acute and convalescent phases in the epidemics occurring with type B virus in 1985 and with subtype H3N2 of type A virus in 1990, respectively. These sera were supplied by courtesy of the Hokkaido Institute of Public Health, Sapporo. An antiserum to each of the virus strains used in the present study was prepared in chickens by a single intravenous injection of 5 ml (128–512 hemagglutination units/ml) of virus-infected allantoic fluid of embryonated hens' eggs. These sera were stored in a deep freezer at –30°C until tested.

Virus strains: Three reference strains of EI virus, A/equine/Newmarket/1/77 (H7N7, Newmarket), A/equine/Tokyo/2/71 (H3N8, Tokyo) and A/equine/Kentucky/1/81 (H3N8, Kentucky), and two human strains, A/Hokkaido/4/90 (H3N2, A-Hokkaido) and B/Hokkaido/2/82 (B-Hokkaido) were used in this

study. The equine strains were supplied by courtesy of the Epizootic Research Station, Equine Research Institute, Japan Racing Association, Tochigi. The human strains were isolated from patients in each of the epidemics occurring in Hokkaido in 1982 and 1990, and supplied by courtesy of the Hokkaido Institute of Public Health, Sapporo. These strains were used for serological tests after at least 5 to 10 passages in 10-day-old embryonated hens' eggs.

Hemagglutination-inhibition (HI) test: HI test in a microtiter system was carried out according to our previous papers [2, 4]. The virus antigens were Newmarket, Tokyo and Kentucky strains. An HI antibody titer of $>1:8$ was recorded as positive. The above-mentioned chicken antisera to viruses served as positive controls throughout the experiments. For HI testing, the serum samples were treated with both potassium periodate and receptor-destroying enzyme (Takeda Chemical Industries Ltd., Osaka) [2].

Complement-fixing (CF) test: A CF test was carried out in a microtiter system and CF-soluble (S) antigens were prepared with human A-Hokkaido and B-Hokkaido strains according to the method described in a previous paper [3]. The CF-S antigens of type A and B type-specifically reacted with each of the paired sera of human subjects suffering from the type A or B virus infection during the epidemics described above (data not shown).

RESULTS

Antibody response of horses to EI viruses in

Fig. 1. Age distribution of horses with HI antibodies to Newmarket (1), Tokyo (2) and Kentucky (3) strains in 1990. Total number tested was 166.

Table 1. HI antibodies to Newmarket, Tokyo and Kentucky strains in horse sera collected in 1988-90

Date of serum		H I antibodies to indicated strains								
collection	Number	Newmarket/1/77 (H7N7)			Tokyo/2/71 (H3N8)			Kentucky/1/81 (H3N8)		
(Age in years)	tested	No. pos. ^{a)}	(%)	gmt ^{b)}	No. pos.	(%)	gmt	No. pos.	(%)	gmt
[Hidaka district]										
Aug-Sep, 1988 (2-3)	21	20	(95.2)	56.9	15	(71.4)	47.9	17	(80.2)	58.2
Apr-Jun, 1990 (2)	45	6	(13.3)	25.1	0			0		
Aug-Sep, 1990 (2)	51	45	(88.2)	19.1	19	(37.3)	13.2	24	(47.1)	17.8
Jan-Dec, 1990 (3-23)	188	108	(57.4)	17.4	55	(29.3)	14.0	31	(16.5)	20.7
[Kanto area]										
1988 (6-8)	20	20	(100)	86.1	20	(100)	53.8	20	(100)	43.7

a) Number of positive sera (>8).

b) Geometric mean titer of positive sera.

Table 2. HI and CF-S antibodies to equine and human influenza viruses in horse sera collected in Tokyo Racing Course in 1971 and Hidaka district in 1988

Serum No.	HI antibody titers to indicated strains		CF-S antibody titers to indicated strains	
	A/equine/ Tokyo/71	A/equine/ Kentucky/81	A/Hokkaido/ 4/90 (A)	B/Hokkaido/ 2/82 (B)
[Tokyo in 1971]				
1	64	32	16	<4
2	8	8	8	<4
3	32	<8	<4	<4
4	32	<8	128	<4
5	32	<8	64	<4
[Hidaka in 1988]				
1	32	32	<4	<4
2	128	128	<4	<4
3	32	64	<4	<4
4	64	64	<4	<4
5	64	128	<4	<4

Table 3. Distribution of CF-S and HI antibodies to equine and human influenza viruses in horse sera collected in Tokyo Racing Course in 1971 and Hidaka district in 1988

Antigen	No. of sera with CF-S antibodies at indicated dilution							
	<4	4	8	16	32	64	>4	(%)
[Horse sera in Tokyo]								
CF-S (A/Hokkaido/90)	4	2	6	3	3	2	16/20	(80.0)
CF-S (B/Hokkaido/82)	20	0	0	0	0	0	0/20	(0)
[Horse sera in Hidaka]								
CF-S (A/Hokkaido/90)	21	0	0	0	0	0	0/21	(0)
CF-S (B/Hokkaido/82)	21	0	0	0	0	0	0/21	(0)
Antigen	No. of sera with HI-S antibodies at indicated dilution							
	<8	8	16	32	64	128	>8	(%)
[Horse sera in Hidaka]								
Newmarket/1/71 (H7N7)	1	1	1	5	7	6	20/21	(95.2)
Tokyo/2/71 (H3N8)	5	2	1	5	4	4	16/21	(76.2)
Kentucky/1/81 (H3N8)	4	2	2	2	2	7	17/21	(80.0)

1988–90: A total of 325 horse sera were collected on 4 occasions in the Hidaka district and once in the Kanto area in the years 1988–90 and tested for the presence of HI antibodies to the Newmarket, Tokyo and Kentucky strains of EI virus (Table 1). Most of the sera collected from horses 2 to 3 years of age in the Hidaka district in August and September, 1988 showed HI activity against the 3 strains with mean titers of 1:48 to 1:58. Antibodies to the strains were scarcely found in the sera of 45 2-year-old horses in April to June, 1990. In August and September, 1990, however, a considerable number of horses possessed HI antibodies to the 3 strains with mean

titers of 1:13 to 1:19 in 51 2-year-old horses. The same tendency was observed in sera collected from 188 horses 3 to 23 years old throughout the year 1990 with some differences in the positive rate for the antibodies. On the other hand, in the Kanto area all of 20 horses, aged 6 to 8 years, had high titers of HI antibodies to the 3 strains with mean values of 1:44 to 1:86.

Figure 1 shows the age distribution of horses with HI antibodies to the Newmarket, Tokyo and Kentucky strains of EI virus in 166 horse sera collected in the Hidaka district from January to December, 1990. These sera were from 51 2-year-old horses and

115 of 188 horses 3 to 23 years old. Data for the remaining 73 mares are not shown since their exact ages could not be confirmed. Although the positive antibody titers to the 3 strains in the 2-year-old horses ranged widely from 1:8 to 1:256, the number of horses with antibodies gradually decreased with age, especially in the case of the antibody to the Kentucky strain.

Presence of CF-S antibody in horses: Two groups of horse sera were tested for the presence of CF-S antibodies to type A or B. One consisted of the sera of horses which were exposed to the first outbreak of EI epidemic at Tokyo Racing Course in 1971, and the other consisted of the sera of horses vaccinated twice with inactivated viruses in the Hidaka district in 1988. Table 2 shows HI and CF antibody titers to equine and human influenza viruses in the horse sera of both groups. In the former group all 5 horses had positive HI antibodies to Tokyo strain, and only 2 of these were positive for the Kentucky strain. At CF test 4 of the 5 horse sera showed on antibody titer of 1:8 to 1:128 to S antigen of type A, and no antibodies to the antigen of type B were detected. In the latter group all 5 horses had antibody titers of 1:32 to 1:128 to both strains in an HI test, whereas no antibodies were detected to either S antigen in a CF test. Antibodies to CF-S antigen of type A, as shown in the upper part of Table 3, were detected only in the horse sera obtained during the EI epidemic in Tokyo Racing Course in 1971. No CF-S antibody was detected in the horse sera collected in the Hidaka district in 1988, although many horses (76–95 percent) had HI antibodies to the Newmarket, Tokyo and Kentucky strains (the lower part of Table 3). No antibodies to the CF-S antigen of type B were detected in the horse sera from the Tokyo area and the Hidaka district.

DISCUSSION

The importation of horses from European, American and other countries to Japan is increasing yearly, because of the growing fondness of the people for racing. Seven hundred and thirty two horses were imported in 1987, 1,587 in 1988, 2,122 in 1989, and 2,646 in 1990 [12]. In the years 1989–90 high or enzootic occurrence of EI infection with H3N8 virus was recognized in horses raised in foreign countries [9, 10]. Therefore, it is possible that horses in our country are exposed to an invasion of the disease from abroad, though all the imported

horses are kept in quarantine and checked. This fear was realized in the serological results in the present study since EI infection spreads rapidly among susceptible horses and affects horses of all ages.

Most of the present study was carried out on the horses reared in the Hidaka district which is the greatest breeding area in Japan. Although many horses had HI antibodies to the Newmarket, Tokyo and Kentucky strains after vaccination with inactivated EI virus, the number of positive reactors gradually decreased with age. In horses over 9 years of age in particular, no antibody response was found to the Kentucky strain. This result apparently indicates that the antibody-negative horses were not given EI vaccine containing the Kentucky strain used as one of the standard strains for the H3N8 virus since 1985 in our country. On the contrary, in the Kanto area all the horses had HI high titers of antibodies to the 3 strains, because they were vaccinated many times as required by law for their transfer. It is therefore quite possible that a suitable vaccination program could provide the horses with the high antibody level [7, 8] required for protection against homologous virus infection.

In the present study, no antibodies to CF-S antigen of type A were detected in the horses vaccinated with EI virus, although they had considerably high titers of HI antibodies to the virus strains. Antibodies to CF-S were detected only in the sera obtained from horses which were exposed to an EI epidemic. These findings are essentially similar to what is known about the influenza virus infection in man, viz, the antibody titers develop in 1 to 2 weeks and disappear 2 to 3 months after the infection. Therefore, the detection of CF-S antibodies is a useful method to use in the serological diagnosis of recent EI viral infection in horses, even though the horses had HI antibodies acquired by vaccination or previous infection with the virus.

All of the 5 affected horses during the first EI epidemic in Tokyo in 1971 and the 5 horses vaccinated in Hidaka in 1988 (Table 2) had HI antibodies of 1:8 to 1:128 to the Tokyo strain. All the vaccinated horses had HI antibodies of 1:32 to 1:128 to the Kentucky strain, whereas only 2 of the 5 epidemic cases had HI antibodies of 1:8 or 1:32 to the same strain. In the age distribution of horses with HI antibodies (Fig. 1), the 16 vaccinated horses aged over 9 years had antibodies to the Tokyo strain at titers of 1:8 to 1:16. Of these horses, however, only 3 had antibodies to the Kentucky strain at a

titer of 1:8. These results are of interest with regard to the antigenic correlation between the Tokyo and Kentucky strains. Comparative genetic analysis of the hemagglutinins of H3N8 influenza viruses showed that A/equine/Johannesburg/86 (H3N8) strain differed from the Tokyo strain in 136 nucleotides and from A/equine/Miami/1/63 (H3N8) strain in 101 nucleotides [5].

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REFERENCES

1. Akiyama, Y., Kumanomido, T., Hirasawa, K., Okuda, Y., and Tabuchi, E. 1972. Studies on outbreak of equine influenza in Japan in 1972 — conditions of outbreak and investigation of hemagglutination inhibiting antibody. *Exp. Rep. Equine Health Lab.* 9: 10–28.
2. Goto, H. and Shimizu, K. 1977. Antigenic relationship between the Tokyo and Miami strains of equine influenza subtype 2 virus. *Jpn. J. Vet. Sci.* 39: 571–574.
3. Goto, H., Shimizu, K., Abe, T., and Kanamitsu, M. 1975. Sero-epidemiological study on equine influenza in Japan. *J. Clin. Microbiol.* 2: 89–93.
4. Goto, H., Shinagawa, M., Shimizu, K., Taya, Y., Noda, H., and Tokunaga, T. 1978. A serological survey on equine influenza for the past ten years. *Jpn. J. Vet. Sci.* 40: 367–374.
5. Kawaoka, Y. and Webster, G. R. 1989. Origin of the hemagglutinin on A/Equine/Johannesburg/86 (H3N8): the first known equine influenza outbreak in South Africa. *Arch. Virol.* 106: 159–164.
6. Kono, Y., Ishikawa, K., Fukunaga, Y., and Fujino, K. 1972. The first outbreak of equine influenza in Japan. *Natl. Inst. Anim. Health Q. (Jpn.)* 12: 183–187.
7. Kumanomido, T. and Akiyama, Y. 1975. Immuno-effect of serum and nasal antibody against experimental inoculation with influenza A-equi-2 virus. *Exp. Rep. Equine Health Lab.* 12: 44–52.
8. Mumford, J. A., Wood, J. M., Folkers, C., and Schild, G. C. 1988. Protection against experimental infection with influenza virus A/equine/Miami/63 (H3N8) provided by inactivated whole virus vaccines containing homologous virus. *Epidem. Infect.* 100: 501–510.
9. OIE. 1989. Animal Disease Tables. *Animal Health Year-book* pp. 1–180.
10. OIE. 1990. Animal Disease Tables. *Animal Health Year-book* pp. 1–180.
11. Rogers, A. L. 1988. A-equi-2 influenza in horses in the Republic of South Africa. *J. S. Afr. Vet. Med. Assoc.* 59: 123–125.
12. Statistics on Animal Hygiene. 1990. Livestock Industry Bureau, Ministry of Agriculture, Forestry and Fisheries. pp. 52–53. Tokyo (in Japanese).
13. Uppal, P. K. and Yadav, M. P. 1987. Outbreak of equine influenza in India. *Vet. Rec.* 121: 569–570.
14. Uppal, P. K., Yadav, M. P., and Oberoi, M. S. 1989. Isolation of A/Equi-2 virus during 1987 equine influenza epidemic in India. *Equine Vet. J.* 21: 364–366.
15. Webster, R. G. 1991. New influenza virus in horses. *Nature (Lond.)* 351: 527.