Sero-Epizootiological Study of Racehorses with Pyrexia in the Training Centers of the Japan Racing Association

Takeo SUGIURA, Tomio MATSUMURA, Yoshio FUKUNAGA, and Kiyoshi HIRASAWA

Epizootic Research Station, Equine Research Institute, Japan Racing Association. 1400-4 Shiba, Kokubunji-machi, Tochigi 329-04, Japan

(Received 21 October 1986/Accepted 3 September 1987)

ABSTRACT. A sero-epizootiological study was conducted on horses which showed clinical pyrexia at two training centers, each of which maintained 1,200 to 2,100 racehorses for training, of the Japan Racing Association. It continued from 1980 to 1985 to clarify the cause of pyrexia, so that measures might be considered for the prevention of infectious disease. A total of 3,849 horses were found to be affected with pyrexia for the 6 years. Of them, 2,852 horses were tested to equine herpesvirus type 1 (EHV-1), equine rhinovirus type 1 (ERhV-1), rotavirus and equine adenovirus (EAdV) by collecting paired serum samples. As a result, 669 (23.5%) horses showed seroconversion to these viruses; that is, 491 horses (73.4%) to EHV-1, 91 (13.6%) to ERhV-1, 64 (9.6%) to rotavirus and 23 (3.4%) to EAdV. EHV-1, ERhV-1 and rotavirus exhibited a seasonal characteristic in spread. EHV-1 and rotavirus spread in winter and early spring. ERhV-1 spread not only in the cold season, but in changing times of season, such as early spring and early autumn. The percentage of 3 years old in the horses showing seroconversion to EHV-1 was two times higher than those in the horses showing seroconversion to other viruses. All of the horses showing seroconversion to ERhV-1 and EAdV were under 4 or 3 years old, respectively. The mean age of the horses showing seroconversion to EHV-1, rotavirus, ERhV-1 and EAdV were 2.96, 2.73, 2.44 and 2.25, respectively.—**KEY WORDS:** herpesvirus, horse, pyrexia, rhinovirus, sero-epizootiology.

Jpn. J. Vet. Sci. 49(6): 1087–1096, 1987

The Japan Racing Association (J. R. A.) has two training centers (T. C.). Racehorses are transported to race courses from T. C. every time for each race. In contrast to functional convenience, such concentrated accomodation system as the T. C. inevitably harbors great risk of inducing infectious diseases. Actually, on account of a large-scale epizootic of equine influenza over a period from December, 1971 to January, 1972 [19], horse races were canceled for two months around this period. Also, the spread of Getah virus infection interfered the horse races, because training was suspended in a number of horses in 1978 [7]. From these experiences, all of the racehorses are injected with several vaccines, such as equine influenza, Japanese encephalitis and Getah virus vaccines. And, horses exhibiting clinic-

al pyrexia or some symptoms of infectious diseases are serologically examined for viruses, such as equine herpesvirus type 1 (EHV-1), equine rhinovirus type 1 (ERhV-1), rotavirus and equine adenovirus (EAdV). These agents are known as major causative ones of epizootics, but vaccines against them are never applied among racehorses in Japan.

This paper reports the results of the serological survey to detect the cause of pyrexia of racehorses for considering the counterpart to prevent the infectious diseases in the T. C.

MATERIALS AND METHODS

Horse populations: The Ritto T. C. is in Shiga Prefecture in the western part of

Table 1. Number of racehorses in the Ritto Training Center in 1980

Age	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
2	18	36	86	610	485	521	524	643	895	1041	1106	1149	6644
3	1101	1109	933	1300	731	619	338	474	538	434	507	484	8396
4 and more	884	847	656	998	556	301	256	352	445	424	428	413	6560
Entered ^{a)}	258	223	276	370	347	282	228	207	367	397	301	268	3424

a) Almost the same number of horses left.

Japan, and Miho T. C. is in Ibaraki Prefecture in the eastern part of Japan. At each T. C., 1,200 to 2,100 racehorses are trained and accommodated in 114 to 123 sheds in 42 to 48 ha. The racehorses are transported to race courses except local ones from the T. C. every time for each race. During the season of local races, racehorses are transported and stay long in local race courses mainly in July and August. On the other hand, about 200 to 400 racehorses are replaced with new horses every month. The age distribution of racehorses and the number of replaced horses at the Ritto T. C. in 1980 are shown in Table 1.

Serum samples: Paired serum samples were taken from horses exhibiting clinical pyrexia with body temperature exceeding 38.5°C at the clinic of each T. C., that is acute stage and the convalescent stage. The convalescent stage began about 2 weeks after the acute stage. The samples were stored -20°C until use for testing.

Complement fixation (CF) test: Sera were tested for antibodies against the HH-1 strain of EHV-1 (provided by Dr. T. Shimizu, of the National Institute of Animal Health; NIAH), the T-1 strain of EAdV-1 (provided by Dr. W. H. McCollum, of the University of Kentucky), and the Lincoln strain of bovine rotavirus (provided by Dr. M. Kodama, of the NIAH) by CF test.

Antigens used were prepared as follows. The infected culture fluid and cell debris of RK-13, MDCK or MA-104 cells were concentrated by polyethylene glycol No. 6,000

at 10% (w/w) and sodium chloride at 3% (w/w) and treated with ultrasonication. The resulting product was centrifuged at 6,000 × g for 30 min. The supernatant obtained was used as antigen.

The comparative CF test was conducted by the CH₅₀ method as developed by the Centers for Disease Control of the U. S. A. and modified by Inoue [15]. In the modified method, 0.05 ml of five units of guinea pig complement, 0.025 ml of four units of antigen, 0.025 ml of a serum sample, from which serial two-fold dilutions were prepared, and 0.05 ml of 1.7% sensitized sheep red blood cells were used. The antibody titer was expressed with the reciprocal of the highest dilution that had inhibited hemolysis more than 50%.

Virus neutralization (VN) test: Antibody against the NM-11 strain of ERhV-1 (provided by Dr. W. H. McCollum, of the University of Kentucky) was detected by the VN test. A microtiter assay essentially the same as that described by Bibrack and Hartl [1] was used. In it, 0.05 ml of virus (100 TCID₅₀) was mixed with an equal amount of a serial two-fold diluted serum in flat-bottomed microplate (Nunc, Roskilde, Denmark). Vero cells (1×10⁶ of cells in 0.075 ml) were added to the resulting mixture after incubation at 37°C for 1 hr. The antibody titer was expressed as the reciprocal of the highest serum dilution that had neutralized the virus completely.

Status of the infection: When a horse showed a serum antibody titer in the con-

Table 2. Distribution of horses with pyrexia in each month in the Ritto and Miho Training Centers (T. C.) from 1980 to 1985

Year	T. C.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
1980	Ritto	29	36	15	13	11	16	49	24	44	10	17	19	283
1980	Miho	196	39	17	20	19	15	11	15	20	23	15	39	429
1981	Ritto	27	23	14	4	54	48	15	22	46	33	62	55	443
1981	Miho	100	59	27	26	74	38	15	14	19	66	66	43	547
1982	Ritto	60	75	29	43	42	27	38	40	46	25	39	36	500
1982	Miho	20	19	20	25	10	24	24	22	56	37	24	37	318
1983	Ritto	55	49	33	25	29	40	66	24	28	23	10	6	388
1983	Miho	80	44	26	14	6	10	21	33	27	27	17	14	319
1984	Ritto	34	15	11	16	17	31	11	1	13	16	17	5	187
1984	Miho	17	17	12	10	11	18	7	0	7	3	6	2	110
1985	Ritto	10	4	21	15	16	23	57	18	13	19	12	5	213
1985	Miho	5	4	4	11	14	5	16	11	18	7	10	7	112
Total	Ritto	215	202	123	156	169	185	236	129	190	126	157	126	2014
Total	Miho	418	182	106	106	134	110	94	95	147	163	138	142	1835
Total	Both T. C. 's	633	384	229	262	303	295	330	224	337	289	295	268	3849

Fig. 1. Pyrexia ratio of horses stabled in the Ritto Training Center. The ratio was calculated with total number of horses with pyrexia/total number of horses in training center in each month. —●— 1980, ---●--- 1981, —●— 1982, —■— 1983, ---■--- 1984, —■— 1985.

Fig. 2. Pyrexia ratio of horses stabled in the Miho Training Center. The ratio was calculated with total number of horses with pyrexia/total number of horses in training center in each month. —●— 1980, ---●--- 1981, —■— 1982, —■— 1983, ---■--- 1984, —■— 1985.

valescent stage which was eight times as high as that shown in the acute stage, the horse was regarded as infected with the virus tested.

RESULTS

Number of horses with pyrexia in each month: The numbers of horses with pyrexia

Table 3. Distribution of horses with seroconversion to equine herpesvirus type 1 in each month in the Ritto and Miho Training Centers (T. C.) from 1980 to 1985

Year	T. C.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
1980	Ritto	7	9	0	0	0	0	0	0	0	0	0	0	16
1980	Miho	96	5	0	0	0	0	0	0	0	0	0	0	101
1981	Ritto	1	0	0	4	5	0	0	0	0	0	0	0	10
1981	Miho	53	23	7	0	4	0	0	0	0	0	0	0	87
1982	Ritto	21	47	1	0	0	0	0	0	0	0	0	8	77
1982	Miho	0	1	4	4	0	0	0	0	0	0	0	17	26
1983	Ritto	20	21	8	0	0	0	0	0	0	0	0	2	51
1983	Miho	47	8	1	0	0	0	0	0	0	0	0	0	56
1984	Ritto	23	7	0	0	0	0	0	0	0	0	0	1	31
1984	Miho	0	5	3	1	0	0	0	0	0	0	0	1	10
1985	Ritto	0	0	3	6	0	0	0	0	0	0	0	5	14
1985	Miho	1	1	2	7	0	0	0	0	0	0	0	1	12
Total	Ritto	72	84	12	10	5	0	0	0	0	0	0	16	199
Total	Miho	197	43	17	12	4	0	0	0	0	0	0	19	292
Total	Both T. C.'s	269	127	29	22	9	0	0	0	0	0	0	35	491

at both T. C.'s in every month are presented in Table 2. The maximum total number per year was observed in 1981 at the Miho T. C. and in 1982 at the Ritto T. C. It declined year by year thereafter. In 1985, however, it was a little larger than in 1984. A total number of horses which manifested pyrexia among present survey was 3,849.

In Figs. 1 and 2, the percentages of horses with pyrexia per total number of horses accommodated in each T. C. in a month were presented. The mean value of each month was 0.0501 ± 0.0395 ($M \pm S.D.$). The upper 90% confidence limit was 0.115. Among these peaks 9 peaks in July, 1980, 1983 and 1985, and February, 1982 in the Ritto T. C., and in January, 1980, and in January, May, October to November, 1981, and in January, 1983 in the Miho T. C. exceeded the upper 90% confidence limit.

Serology for EHV-1: Outbreaks of EHV-1 infection were demonstrated at both T. C.'s in winter to spring every year (Table 3). At the Miho T. C. in January and February,

1980, 101 horses showed seroconversion; that is the widest spread of this infection in the survey. At the Miho T. C. 83 horses exhibited seroconversion over the period from January to March, 1981. Thirty-two to 73 horses showing seroconversion were observed at the Ritto T. C. in January to March, 1982, in December, 1982 to March, 1983, and in December, 1983 to February, 1984, and at the Miho T. C. in December, 1982 to March, 1983. Four to 16 horses showed seroconversion in other seasons, some time from February to May.

Serology for ERhV-1: Epizootics of ERhV-1 were demonstrated (Table 4). At the Miho T. C. over the period from December, 1980, to February, 1981, 12 horses exhibited seroconversion; that is, the widest spread in the present survey. In the other spread, 1 to 3 horses showed seroconversion in one month.

Serology for rotavirus: Epizootic of rotavirus was demonstrated at the Miho T. C. in May to July and November to December,

Table 4. Distribution of horses with seroconversion to equine rhinovirus type 1 in each month in the Ritto and Miho Training Centers (T. C.) from 1980 to 1985

Year	T. C.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
1980	Ritto	1	1	0	0	1	0	0	0	1	0	0	1	5
1980	Miho	3	2	0	1	2	1	0	0	1	0	0	2	12
1981	Ritto	2	1	0	2	2	0	0	0	3	0	0	0	10
1981	Miho	7	3	0	2	0	0	0	0	2	2	3	2	21
1982	Ritto	0	0	0	1	1	2	0	0	0	0	0	0	4
1982	Miho	1	1	1	2	0	0	2	0	1	2	0	0	10
1983	Ritto	0	2	2	1	0	0	0	0	0	0	2	0	7
1983	Miho	1	0	0	0	0	0	0	0	0	1	0	2	4
1984	Ritto	1	0	2	1	3	0	0	0	0	2	0	0	9
1984	Miho	1	0	2	0	1	1	0	0	0	1	0	0	6
1985	Ritto	0	0	0	0	0	0	0	0	0	0	0	0	0
1985	Miho	0	1	0	0	1	0	0	0	1	0	0	0	3
Total	Ritto	4	4	4	5	7	2	0	0	4	2	2	1	35
Total	Miho	13	7	3	5	4	2	2	0	5	6	3	6	56
Total	Both T. C.'s	17	11	7	10	11	4	2	0	9	8	5	7	91

Table 5. Distribution of horses with seroconversion to rotavirus in each month in the Ritto and Miho Training Centers (T. C.) from 1980 to 1985

Year	T. C.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
1980	Ritto	0	0	0	0	0	0	0	0	0	0	0	0	0
1980	Miho	0	0	0	0	0	0	0	0	0	0	0	0	0
1981	Ritto	0	0	0	0	0	0	0	0	0	0	0	0	0
1981	Miho	0	0	0	0	6	2	1	0	0	0	3	5	17
1982	Ritto	0	3	1	1	0	0	0	0	0	0	0	1	6
1982	Miho	1	0	0	1	0	0	0	0	0	0	0	0	2
1983	Ritto	1	1	0	0	0	0	0	0	0	0	0	0	2
1983	Miho	12	7	0	0	0	0	1	0	0	1	2	1	24
1984	Ritto	0	0	0	1	1	0	0	0	0	0	0	0	2
1984	Miho	0	0	0	0	0	1	0	0	0	0	0	0	1
1985	Ritto	0	0	0	1	1	0	2	0	0	0	0	0	4
1985	Miho	0	0	0	0	0	0	0	0	1	1	1	3	6
Total	Ritto	1	4	1	3	2	0	2	0	0	0	0	1	14
Total	Miho	13	7	0	1	6	3	2	0	1	2	6	9	50
Total	Both T. C.'s	14	11	1	4	8	3	4	0	1	2	6	10	64

Table 6. Distribution of horses with seroconversion to equine adenovirus in each month in the Ritto and Miho Training Centers (T. C.) from 1980 to 1985

Year	T. C.	Jan.	Feb.	Mar.	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Total
1980	Ritto	0	0	0	0	2	0	0	0	0	0	0	0	2
1980	Miho	1	0	0	0	0	0	0	0	0	0	0	0	1
1981	Ritto	1	0	0	0	0	1	0	0	0	0	0	0	2
1981	Miho	0	0	0	0	0	0	0	0	0	0	0	0	0
1982	Ritto	0	1	0	0	0	0	0	0	0	0	2	3	6
1982	Miho	0	0	0	1	1	1	2	1	0	1	0	0	7
1983	Ritto	0	0	0	0	0	0	0	0	1	0	0	0	1
1983	Miho	1	0	0	0	0	0	0	0	0	0	0	0	1
1984	Ritto	0	0	0	0	0	0	0	0	0	0	0	0	0
1984	Miho	0	0	0	0	0	0	0	0	0	0	0	0	0
1985	Ritto	0	0	0	0	0	0	0	0	0	0	1	0	1
1985	Miho	0	0	0	0	0	0	1	0	1	0	0	0	2
Total	Ritto	1	1	0	0	2	1	0	0	1	0	3	3	12
Total	Miho	2	0	0	1	1	1	3	1	1	1	0	0	11
Total	Both T. C.'s	3	1	0	1	3	2	3	1	2	1	3	3	23

Table 7. Age distribution of racehorses showing seroconversion to viruses

Age in Years	EHV-1 ^{a)}	Rota ^{b)}	ERhV-1 ^{c)}	EAdV ^{d)}
2	94	34	63	19
3	338	20	20	7
4	48	6	8	0
5	11	4	0	0
Total	491	64	91	26
Mean	2.96	2.73	2.44	2.27

a) Equine herpesvirus type 1.

b) Rotavirus.

c) Equine rhinovirus type 1.

d) Equine adenovirus.

1981, and January to February, 1983 and at the Ritto T. C. in February to April, 1982, and in December, 1982 to February, 1983 (Table 5). The number of horses showing seroconversion on one occasion was larger in rotavirus infection than in ERhV-1 infection. The total number of horses infected with rotavirus was smaller than that with

EHV-1 or ERhV-1 in the present survey.

Of the horses showing seroconversion, 55.0% was found in winter, from December to February.

Serology for EAdV: A total 23 horses showed seroconversion to EAdV (Table 6). The maximum number of horses showing seroconversion in one month was only three at the Ritto T. C. in December, 1982.

Age distribution of infected horses: The age distribution of horses infected with the viruses is shown in Table 7. More than 50% of horses infected with ERhV-1, EAdV, and rotavirus were 2 years old, although 68.9% of EHV-1-infected horses were 3 years old. Of the EAdV-infected horses, 73.1% (19 in 26) were 2 years old and none were more than 4 years old. The mean age of infected horses were 2.96 years for EHV-1, 2.73 for rotavirus, 2.44 for ERhV-1 and 2.27 for EAdV.

Percentages of virus as cause of infection: Of 3,849 pyretic horses, paired serum samples were taken from 2,852 horses and

examined for four viruses. And, 491 (17.2%), 91 (3.2%), 64 (2.2%) and 23 (0.8%) of examined horses showed seroconversion to EHV-1, ERhV-1, rota and EAdV, respectively. In the remaining 76.5% of examined horses, the cause of pyrexia was not determined.

DISCUSSION

From the present sero-epizootiological study conducted for 6 consecutive years since 1980, it was strongly suggested that in 23.8% of all the horses with pyrexia, this symptom might have been caused by some viral agents. Although the spread of viruses did not threaten races actually, the infection influenced training and participation in races of individual racehorses. Since pyrexia is a symptom of respiratory or systemic infectious disease, it is important to determine the cause of pyrexia to prevent such infectious disease. In 76.5% of horses the cause of pyrexia was not determined. As the cause of pyrexia in these horses, heat stroke, fatigue, bacteria and unknown viral infections were considered. On the other hand, nine peaks that exceeded the 90% confidence limit of percentages of horses with pyrexia per total stabled horses were observed. Among these, four peaks observed in January and February from 1980 to 1983 were considered to be due to infection with EHV-1 and other respiratory agents. Three peaks observed in July, 1980, 1983 and 1985 were considered to be due to shipping fever or heat stroke. Remaining one peak was observed in May, 1981 in the Miho T. C., and a smaller peak was observed in the Ritto T. C. at the same time. In this season, from spring to early summer, such phenomenon was sometime observed among horse population. The other symptoms were diarrhea, edema of hind legs and rashes on several parts of the body, although diseased horses did not show

increase of antibody titers at the convalescent stage to any viruses tested, including Getah and Japanese encephalitis viruses. It is possible that there may be an unknown infectious disease in this season.

Of the horses showing seroconversion, 74.9% manifested pyrexia caused by EHV-1. This fact suggests that pyrexia in some racehorses may have been prevented by vaccination against EHV-1. In Japan EHV-1 vaccine is only one biological product which is officially permitted to use for the prevention of abortion by this virus. It might be necessary to apply already or newly developed vaccine to the prevention of the respiratory disease at the T. C.

The spread of EHV-1 has been reported to be worldwide [2, 4-6, 10, 12, 16, 26, 32]. In Japan, subtype 1 of EHV-1 was introduced from the U.S.A. in 1967 by imported broodmares [18]. Before that time only subtype 2 had been found in Japan [27]. In the present survey, it was clarified that the spread of this infection showed a seasonal occurrence in winter to early spring, from December to March with a peak incidence in January, although on rearing or breeding farms the virus was found to spread in any season of the year [23, 29]. The percentage of 3 years old among horses showing seroconversion to EHV-1 was two times higher than those to other viruses. The mean age of horses showing seroconversion to EHV-1 was the highest among the examined viruses.

The spread of ERhV-1 on rearing farms of the J. R. A. [21, 29], as well as on a breeding and rearing farms [23], was reported by several groups of investigators. As a result, it was made clear that this virus had been spread almost every year among rearing horses, although the number of infected horses was relatively small at the T. C. The infected horses were rather young, or under four years old. From these facts, it was considered that infection with ERhV-1

might be rare among racehorses. Most racehorses had already antibodies to this virus, since they had been exposed to infection on rearing or breeding farms.

Serological reactors against the Lincoln strain of bovine rotavirus were found in 65.7% of foals and 27.0% of racehorses in Japan in 1979 [14]. After that, equine rotavirus was isolated from foals associated with diarrhea in tissue culture by Imagawa *et al.* [13]. Actual epizootics had never been noted among racehorses before the present survey was conducted. Horses showing seroconversion were relatively old. As there is a tendency for this virus to infect human beings repeatedly [22], it is considered that reinfecting horses may have been included in those horses showing seroconversion.

In Japan a sero-epizootiological study on EAdV was undertaken by Kamada in the Tokyo Racecourse in 1977 [17]. As the results, 44.3% of racehorses had CF antibody, although the number of sero-positive horses was very small in the present survey. From these results, it is considered that the spread of this virus has been much reduced in the past 13 years. The mean age was lower in horses showing seroconversion to EAdV than in horses showing seroconversion to any other virus studied in the present survey.

EHV-1, ERhV-1 and rotavirus exhibited a seasonal characteristic in spread. EHV-1 spread in winter and early spring, from December to May. Rotavirus spread in winter, November to February. In contrast, ERhV-1 spread not only in the cold season, but in April to May, and in September. In Japan, the daily variations of atmospheric temperature are large in these months. Clinical signs of infection with this virus were rarely observed on a rearing farm, even when antibody titer increased in most horses on the farm [21, 23, 29]. These phenomena suggest that the pathogenicity of this virus may be very weak. If the

condition of an infected horse is affected with stress from changes of weather, the horse will show clinical signs.

There are two papers published to report about sero-epizootiological studies on viruses in race tracks in countries other than Japan. In Canada, the spread of equine influenza virus and EHV-1 was reported to have occurred from 1973 to 1975 [11] and from 1977 to 1978 [24]. Serological surveys were conducted on the horse population, except horses kept in race tracks, in these countries. They revealed the spread of equine influenza virus types 1 and 2 [2, 24, 32], EHV-2 [12, 24], EAdV-1 to 3 [2, 23, 32], ERhV-1 to 3 [2, 11, 24], reovirus type 1 to 3 [3, 28, 32] and parainfluenza virus type 3 [12, 31]. In Japan, an epizootic of equine influenza occurred from 1971 to 1972 [19]. Since then, vaccines of A/equine/Prague/56, A/equine/Miami/63 and A/equine/Tokyo/71 were used in most racehorses in autumn every year up to 1985. These strains were changed to A/equine/Newmarket/77, A/equine/Tokyo/71 and A/equine/Kentucky/81, respectively, in 1985. No epizootics of equine influenza have occurred in Japan yet. Serological reactors to reovirus types 1 to 3 [Imagawa *et al.* oral presentation at the 100th Meetings for Japanese Society of Veterinary Science, Tokyo], EHV-2 [20, 30], Akabanevirus [9], picornaviruses [8], and *Chlamydia psittaci* [Ogawa *et al.* oral presentation at the 97th Meetings for Japanese Society of Veterinary Science, Tokyo] have been reported in Japanese horse population. We are now testing these agents, including toxoplasma reported in the U.S.A. [25] and *Chlamydia trachomatis*, to survey for serum samples examined in present study. Results showing that these pathogens rarely affect racehorses will be realized in the near future.

Introduction of enzyme-linked immunosorbent assay (ELISA) has been made progress in the titration of antibody to

infectious agents of horses [31]. ELISA is highly sensitive and can be used for the evaluation of a large number of serum samples. It is a powerful means for serological study.

REFERENCES

1. Bibrack, B., and Hartl, G. 1971. Mikrotest zum Nachweis neutralisierender antiherpes gegen das Rhinopneumonitis virus der Pferde. *Zbl. Vet. Med. B.* 18: 517-526.
2. Burrows, R., Goodridge, D., Denyer, M., Hutchings, G., and Frank, C. J. 1982. Equine influenza infections in Great Britain, 1979. *Vet. Rec.* 110: 494-497.
3. Corner, M., Kalica, A., Kita, J., Quick, S., Schiff, E., Joubert, J., and Gillespie, J. 1984. Isolation and characteristics of an equine reovirus type 3 and antibody prevalence survey to reoviruses in horses located in New York State. *Vet. Microbiol.* 9: 15-25.
4. Dinter, Z., and Klingeborn, B. 1976. Serological study of an outbreak of paresis due to equid herpesvirus 1 (EHV-1). *Vet. Rec.* 99: 10-12.
5. Doll, E. R., and Bryans, J. T. 1963. Epizootiology of equine rhinopneumonitis. *J. Am. Vet. Med. Assoc.* 142: 31-37.
6. Fretz, P. B., Babiuk, L. A., and McLaughlin, B. 1979. Equine respiratory disease on the Western Canadian racetracks. *Can. Vet. J.* 20: 58-61.
7. Fukunaga, Y., Ando, Y., Kamada, M., Imagawa, H., Wada, R., Kumanomido, T., Akiyama, Y., Watanabe, O., Niwa, K., Takenaga, S., Shibata, M., and Yamamoto, T. 1981. An outbreak of Getah virus infection in horses. Clinical and epizootiological aspects at the Miho Training Center in 1978. *Bull. Equine Res. Inst.* 18: 94-102.
8. Fukunaga, Y., Kumanomido, T., Imagawa, H., Ando, Y., Kamada, M., Wada, R., and Akiyama, Y. 1981. Isolation of picornavirus from horses associated with Getah virus infection. *Jap. J. Vet. Sci.* 43: 569-572.
9. Furuya, Y., Shoji, H., Inaba, Y., and Matumoto, M. 1980. Antibodies to Akabane virus in horses, sheep, and goats in Japan. *Vet. Microbiol.* 5: 239-242.
10. Greenwood, R. E. S. 1979. Equine rhinopneumonitis outbreak at Newmarket. *Vet. Rec.* 104: 534-535.
11. Hamre, D. 1966. Virologic studies of acute respiratory disease in young adults. IV. Virus isolations during four years of surveillance. *Am. J. Epidemiol.* 83: 238-249.
12. Hofer, B., Steck, F., Gerber, H., Lohrer, J., Nicklet, J., and Paccaud, M. F. 1972. An investigation of the etiology of viral respiratory disease in a Remount Depot. *Proc. 3rd. Int. Conf. Equine Infectious Diseases*, 1972: 527-545.
13. Imagawa, H., Ando, Y., Sugiura, T., Wada, R., Hirasawa, K., and Akiyama, Y. 1981. Isolation of foal rotavirus in MA-104 cells. *Bull. Equine Res. Inst.* 18: 119-128.
14. Imagawa, H., Ando, Y., and Akiyama, Y. 1979. A survey on complement fixation antibody against bovine rotavirus in light horses in Japan. *Exp. Rep. Equine Hlth Lab.* 16: 23-29.
15. Inoue, S. 1978. Complement fixation test. pp. 73-83. In: Examination of virus and rickettsia., 2nd ed. (Yanagisawa, K. ed.), Society for Japanese Public Hygiene, Tokyo (In Japanese).
16. Jana, A. M., Pandya, G., and Rao, K. M. 1980. Evidence of complement fixing antibodies against equine rhinopneumonitis virus in army horses and mules. *Curr. Sci.* 49: 575-576.
17. Kamada, M. 1978. Comparison of the four serological tests for detecting antibodies against equine adenovirus. *Exp. Rep. Equine Hlth Lab.* 15: 91-96.
18. Kawakami, Y., Nakano, K., Kume, T., Hiramune, T., and Murase, N. 1970. Abortion by equine rhinopneumonitis virus in Hidaka, Hokkaido district in Japan. *Bull. Nat. Inst. Anim. Hlth* 61: 9-16. (In Japanese).
19. Kono, Y., Ishikawa, K., Fukunaga, Y., and Fujino, M. 1972. The first outbreak of equine influenza in Japan. *Nat. Inst. Anim. Hlth Quart.* 12: 183-187.
20. Kono, Y., and Kobayashi, K. 1964. Cytopathogenic equine orphan (CEO) virus in horse kidney cell culture. II. Immunological studies of CEO virus. *Nat. Inst. Anim. Hlth Quart.* 4: 21-27.
21. Kumanomido, T., and Akiyama, Y. 1979. Serological survey of equine rhinovirus serotype 1 among light horses in Japan. *Exp. Rep. Equine Hlth Lab.* 16: 15-22.
22. Mata, L., Simhon, A., Urrutia, J. J., Kronmal, R. A., Fernandez, R., and Garcia, B. 1983. Epidemiology of rotaviruses in a cohort of 45 Guatemalan Mayan Indian children observed from birth to the age of three years. *J. Infect. Dis.* 148: 452-461.
23. Matsumura, T., Komano, M., Sugiura, T., and Fukunaga, Y. 1986. Sero-epizootiological study on viral diseases among horses in a breeding farm of Japan during a period from 1981 to 1985. *Exp. Rep. Equine Hlth Lab.* 23: 28-34.
24. Powell, D. G. 1975. Equine infectious respiratory disease. *Vet. Rec.* 96: 30-34.
25. Riemann, H. P., Smith, A. T., Stormont, C., Ruppanner, R., Behymer, D. E., Suzuki, Y.,

- Franti, C. E., and Verma, B. B. 1975. Equine toxoplasmosis: A survey for antibodies to toxoplasma gondii in horses. *Am. J. Vet. Res.* 36: 1797-1800.
26. Sherman, J., Mitchell, W. R., Martin, S. W., Thorsen, J., and Ingram, D. G. 1979. Epidemiology of equine upper respiratory tract disease on Standardbred racetracks. *Can J. Comp. Med.* 43: 1-9.
27. Shimizu, T., Ishizaki, R., and Matsumoto, M. 1963. Survey of antibody to equine rhinopneumonitis virus in horse population in Japan. *Bull. Nat Inst. Anim. Hlth* 44: 11-17 (In Japanese).
28. Sturm, R. T., Lang, G. H., and Mitchell, W. R. 1980. Prevalence of reovirus 1, 2 and 3 antibodies in Ontario racehorses. *Can. Vet. J.* 21: 206-209.
29. Sugiura, T., Ando Y., Masuzawa, H., Kuriyama, H., Ogawa, A., and Hirasawa, K. 1983. Sero-epizootiological studies on rearing horses on the Hidaka Rearing Farm from 1980 to 1983. *Bull. Equine Res. Inst.* 20: 48-54. (In Japanese).
30. Sugiura, T., Fukuzawa, Y., Kamada, M., Ando, Y., and Hirasawa, K. 1983. Isolation of equine herpesvirus type 2 from foals with pneumonitis. *Bull. Equine Res. Inst.* 20: 148-153.
31. Sugiura, T., Matsumura, T. and Fukunaga, Y. 1985. Diagnosis of equine infectious anemia by enzyme-linked immunosorbent assay with viral antigen purified by affinity chromatography. *Bull. Equine Res. Inst.* 23: 42-48.
32. Zmudzinski, J., Baczynski, Z., and Skukmowska-Kryazkowska, D. 1980. Serological examinations of horses for the presence of antibodies against pneumotropic viruses. *Bull. Vet. Inst. Pulawy* 24: 1-4.

要 約

日本中央競馬会トレーニング・センターにおける発熱性疾患馬の血清疫学調査：杉浦健夫・松村富夫・福永昌夫・平澤 澄（日本中央競馬会競走馬総合研究所栃木支所）——1980～1985年に日本中央競馬会トレーニング・センターに所属する競走馬の発熱性疾患について血清疫学的に調査した。発熱馬総数は3,849頭で、血清が採取された2,852頭中669頭（23.7%）に、馬ヘルペスウイルス1型（EHV-1）、馬ライノウイルス1型（ERhV-1）、ロタウイルスおよび馬アデノウイルス（EAdV）に対する抗体の上昇が認められ、EHV-1が491頭（73.4%）、ERhV-1が91頭（13.6%）、ロタウイルスが64頭（9.6%）、EAdVが23頭（3.4%）であった。EHV-1とロタウイルスに対する抗体は、冬～初春に上昇し、ERhV-1抗体は冬のみならず、初春および初秋にも上昇がみられた。EHV-1抗体上昇馬のうち、満3歳馬の占める割合は他のウイルスの約2倍であり、ERhV-1とEAdVの抗体上昇馬はすべて満4または3歳以下であった。EHV-1、ロタ、ERhV-1およびEAdVの抗体上昇馬の平均年齢はそれぞれ2.96、2.73、2.44および2.27歳であった。