

Epidemic of Equine Influenza among Vaccinated Racehorses in Japan in 2007Takashi YAMANAKA¹*, Hidekazu NIWA¹, Koji TSUJIMURA¹, Takashi KONDO¹ and Tomio MATSUMURA¹¹Epizootic Research Center, Equine Research Institute, Japan Racing Association, 1400-4 Shiba, Shimotsuke, Tochigi 329-0412, Japan

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ABSTRACT. On August 2007, we encountered equine influenza epidemic by Florida sub-lineage strain (H3N8) in Japan Racing Association's facilities where 4142 racehorses in total were stabled. The number of new febrile cases sharply increased, but the occurrence was rapidly calmed down within 2 weeks. The morbidity rate in these facilities was 12.8% and the subclinical infection rate of healthy racehorses examined by rapid antigen detection tests was 19.4% at the early stage of epidemic. The serological studies along with the low morbidity rate and the existence of numbers of asymptotically infected racehorses, suggested that the cross-reactivity of the antibodies provided by vaccination against the epidemic strain contributed to reducing the morbidity rate and duration of epidemic.

KEY WORDS: equine influenza, racehorses, vaccine.

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Equine influenza virus (EIV) is one of the most important respiratory pathogens of horses, due to its rapid spread among susceptible animals. Infected horses with EIV develop typical respiratory disorders including acute onset of pyrexia, nasal discharge, coughing and depression [10, 12]. This viral infection has led to severe economic loss to the horse industries, for example, in Hong Kong in 1992 [9] and in South Africa in 1986 [3], horseracing was obliged to be cancelled for several months. Also in Japan, from 1971 to 1972, the outbreak of EI was occurred and the horseracing was cancelled for 2 months [5]. Owing to extensive vaccination for horses after 1972, no EI occurrences had been reported in Japan. In recent years, since the EI occurrences by EIV which is classified into Florida sub-lineage diverged from American lineage were reported in some foreign countries [7, 8], the Office International des Epizooties (OIE) recommends A/equine/Ohio/1/03-like strain classified into the same sub-lineage as vaccine strain (<http://www.oie.int/eng/Eq%20inf%20conc%20%20recs%202005.pdf>). Strains currently contained in the Japanese inactivated whole vaccine are A/equine/La Plata/93 (H3N8 American lineage, La Plata/93), A/equine/Avesta/93 (H3N8 Eurasian lineage, Avesta/93), and A/equine/Newmarket/77 (H7N7). The Japanese vaccines against EIV have not contained the recommended strain yet. On August 2007, the authors met an epidemic of EI in the facilities of the Japan Racing Association (JRA), Miho Training Center (Miho TC, Ibaraki prefecture), Ritto Training Center (Ritto TC, Shiga prefecture), Sapporo Racecourse (Sapporo RC, Hokkaido prefecture), Hakodate Racecourse (Hakodate RC, Hokkaido prefecture) and Kokura Racecourse (Kokura RC, Fukuoka prefecture). Each facility stabled 1599, 1367, 494, 532 and 150 racehorses registered by JRA at the onset of the epidemic, respectively. All horses were stabled under high standards of management and veterinary care by JRA, and received

booster vaccinations at intervals of six months following a primary vaccination in mandatory. All horses were lastly vaccinated for EI in May 2007. This note provides the overview of the epidemic.

On 15th August, 19 horses which were stabled in 4 different facilities (Miho TC, Ritto TC, Sapporo RC and Hakodate RC) showed acute pyrexia ($\geq 38.5^{\circ}\text{C}$). The nasal swabs from these horses immersed in 2.5 ml of lactate broth medium containing 0.005% (w/v) gentamicin sulfate were tested by the rapid antigen detection (RAD) kit for influenza type A and B viruses (ESPLINE Flu A+B, Fujirebio, Japan) [11]. As a result, 12 samples showed positive results for influenza type A virus (Fig. 1). Immediately, the movement

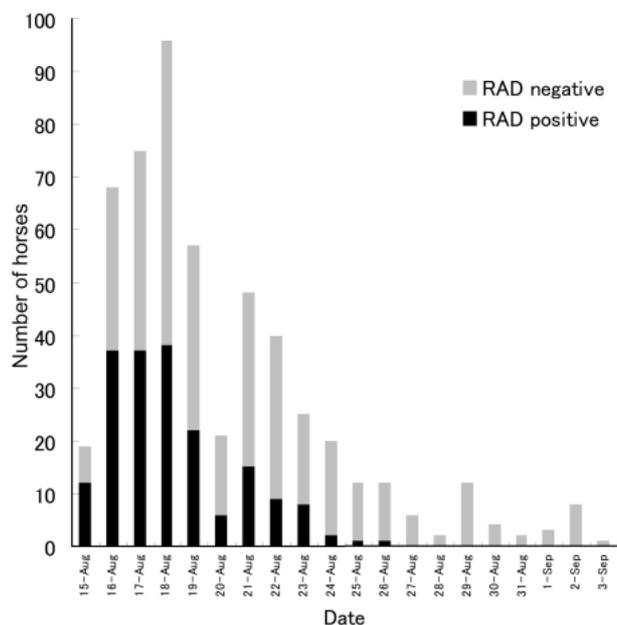


Fig. 1. Changes of daily number of new febrile cases with number of positive horses by the RAD kit, in the JRA facilities between 15th August and 3rd September, 2007.

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of horses between the JRA and non-JRA facilities was banned and the RAD tests were carried out for all new febrile cases. Although the number of new febrile cases and the positive results by the RAD kit sharply increased and reached the peak on 18th August, the number of new febrile cases was rapidly decreased and the positive result by the RAD kit was not observed after 27th August. The movement of horses was partially resumed with strict restriction on 4th September. Between 15th August and 3rd September, each morbidity rate at Miho TC, Ritto TC, Sapporo RC, Hakodate RC and Kokura RC was 10.9%, 17.8%, 13.2%, 8.3% and 16.7%, respectively. The average of morbidity rate in these facilities was 12.8% (531/4142). In addition, nasal swab samples collected from 967 horses that did not show any clinical signs were tested by the RAD kit on 16th and 17th August to grasp the overview of EIV infection in the JRA facilities. As a result, samples from 188 horses (19.4%) showed the positive results, suggesting that many racehorses infected with EIV subclinically during the epidemic.

To confirm the diagnosis by RAD kit, 85 nasal samples taken from febrile horses with on 15th and 16th August were tested by reverse transcriptase-PCR (RT-PCR) with primer pair described previously by Newton and others [8]. By RT-PCR, the presence of hemagglutinin (HA) 1 gene of EIV was recorded in 59 samples. Moreover, attempts to isolate virus from the 11 nasal swabs showing positive results by the RAD kit and RT-PCR were conducted by inoculation into the allantoic cavities of ten-day-old embryonated hen's eggs. The harvested allantoic fluids were tested for hemagglutination activity, using 0.5 (v/v)% chicken erythrocytes. As a result, the allantoic fluids derived from 4 horses in the 3 different facilities showed hemagglutination activities. And, we confirmed that these isolates are influenza type A virus by RAD test and RT-PCR. These isolates were named A/equine/Ibaraki/1/07 (Ibaraki/1/07), A/equine/Ibaraki/2/07, A/equine/Shiga/1/07, and A/equine/Kitakyushu/1/07. The RT-PCR products of the isolates were purified using QIAquick PCR Purification kit (QIAGEN, Tokyo, Japan). The nucleotide sequences of the purified RT-PCR products (1021 nucleotides) were determined using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, U.S.A.) with DNA sequencer (PRISM 310, Applied Biosystems). Although one synonymous nucleotide substitution on the region of signal peptide in one strain (A/equine/Shiga/1/07) was found, the HA1 nucleotide sequences of these 4 isolates were sharing >99.9% identity. The HA1 sequence of Ibaraki/1/07 will be available in DDBJ/EMBL/GenBank under accession number AB360549. A BLAST search of the HA1 sequence of Ibaraki/1/07 showed the highest similarity (99.3%) to the sequences of A/equine/Ohio/1/03 and A/equine/Wisconsin/1/03 classified into Florida sub-lineage (H3N8) [1, 7]. On the other hand, the HA1 sequence homologies of Ibaraki/1/07 to La Plata/93 (accession number: D30686) and Avesta/93 (accession number: Y14057) were 96.9% and 95.4%, respectively.

Paired sera collected from 20 febrile racehorses at acute (the early stage of epidemic between 15th and 17th August) and convalescent phases (approximately 3 weeks later) were tested by hemagglutination inhibition (HI) assay as previously described [4]. We have used the infected allantoic fluid of La Plata/93 (seed virus for the vaccine manufactures in Japan, kindly provided from Kaketsuken, Kumamoto, Japan) and Ibaraki/1/07 as HA antigens. Both HA antigens were treated with Tween 80 and diethyl ether as previously described by Livesay and others [6]. The results of HI assays are shown in Table 1. The similarities in HI antibody titers and the response against both antigens were observed in the paired sera of each horse. Except a horse (Horse 3) which showed 8-fold increase of HI titer only against Ibaraki/1/07 strain, all horses showed the same tendency of antibody response to both antigens, in which significant increase of HI titers (4-fold or more) between paired sera was observed in 17 horses, and not in 2 horses (Horses 11 and 18). The antibody increases against the epidemic strain in 18 of 20 febrile horses strongly suggested that the epidemic was caused by the infection with Florida sub-lineage strain of EIV. Furthermore, the geometric mean titers of the 20 horses in acute phase for La Plata/93 and Ibaraki/1/07 were 1:80.0 and 1:62.8, respectively, suggested that the HI antibody provided by the vaccine strain (La Plata/93) cross-reacted well against the epidemic strain.

When EI outbreak occurred by antigenically distinct strain (A/equine/Hong Kong/92) from the old vaccine strains (A/equine/Miami/63 and A/equine/Kentucky/81) in Hong Kong in 1992 [2], the morbidity rate was 37% (352/

Table 1. HI titers against the vaccine strain (La Plata/93) and the isolated strain (Ibaraki/1/07) of the paired sera collected from 20 febrile horses in acute and convalescent phases

Horse	La Plata/93		Ibaraki/1/07	
	Acute	Convalescent	Acute	Convalescent
1	1:80	1:640	1:80	1:1280
2	<1:10	1:640	<1:10	1:1280
3	1:80	1:160	1:80	1:640
4	1:160	1:1280	1:80	1:1280
5	1:80	1:640	1:80	1:1280
6	1:40	1:640	1:20	1:1280
7	1:40	1:640	1:20	1:1280
8	1:160	1:640	1:80	1:640
9	1:160	1:640	1:80	1:320
10	1:80	1:1280	1:80	1:1280
11	1:320	1:640	1:320	1:640
12	1:40	1:1280	1:80	1:1280
13	1:40	1:640	1:20	1:640
14	1:80	1:640	1:80	1:1280
15	1:80	1:640	1:40	1:1280
16	1:160	1:640	1:80	1:1280
17	1:80	1:640	1:40	1:1280
18	1:320	1:320	1:320	1:320
19	1:80	1:320	1:80	1:640
20	1:80	1:320	1:160	1:640
GMT ^{a)}	1:80.0	1:597.1	1:62.8	1:905.1

a): Geometric mean titer.

955) [9]. By contrast, the morbidity rate (12.8%) recorded in this epidemic was apparently lower than that in Hong Kong. The low morbidity rate along with the existence of many asymptotically infected horses with EIV during the epidemic suggests that the HI antibodies provided by vaccination would cross-protect well from manifestation of the symptoms in horses infected with the epidemic strain.

From the results of this study, the mandatory vaccination conducted every 6 months to all racehorses registered by JRA seemed to have contributed to reducing the morbidity rate and duration of the epidemic. The authors believe that this is the first report on occurrence of EI caused by Florida sub-lineage strain in Asia. Further surveillance on the occurrence of EI among horses in non-JRA facilities is underway.

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