Antibody Responses Induced by Japanese Whole Inactivated Vaccines against Equine Influenza Virus (H3N8) Belonging to Florida Sublineage Clade2

Takashi YAMANAKA1*, Hiroshi BANNAI1, Manabu NEMOTO1, Koji TSUJIMURA1, Takashi KONDO1 and Tomio MATSUMURA1

1)Epizootic Research Center, Equine Research Institute, Japan Racing Association, 1400–4 Shiba, Shimotsuke, Tochigi 329–0412, Japan

NOTE. Virology

Equine influenza virus is one of the family Orthomyxoviridae, and can lead to an acute respiratory disease in horses [9]. Although viruses with two subtypes, H7N7 and H3N8, have been isolated from horses since 1979 [21]. It is currently widely accepted that the H7N7 virus may be extinct [19]. On the other hand, the H3N8 virus is still circulating among horses worldwide [5, 19].

Vaccination using whole/subunit inactivated vaccines is widely used as a prophylaxis measure against equine influenza [16]. Whole/subunit inactivated vaccines provide protection by inducing an antibody to the viral surface glycoproteins, in particular, the hemagglutinin [16]. The efficacy of protection induced by an equine influenza vaccine strain against another strain depends on the antigenic differences between them, as found with other influenza A viruses [23]. Therefore, the composition of available equine influenza vaccines is reviewed annually by the World Organisation for Animal Health (OIE) according to the antigenic characteristics of circulating viruses. In 2009, OIE recommended A/equine/South Africa/4/03-like strains as a vaccine strain [14]. A/equine/South Africa/4/03 (South Africa03) was classified as a Florida sublineage Clade (Fc)1 [3, 14], which has diverged from the American lineage since around 1996 [10, 11]. In 2010, OIE recommended the inclusion of an Fc2 strain of equine influenza virus (H3N8), which is represented by A/equine/Richmond/1/07 (Richmond07) [15], in equine influenza vaccines because Fc2 viruses have been widely circulating in the United Kingdom and Eurasia [1, 4, 6, 18, 20], and the antigenic characteristics of Fc1 and clade2 are distinguishable. Fc2 appears to have diverged from Fc1 since the first introduction of the latter into the United Kingdom in 2003 [12].

All racehorses in Japan are bi-annually vaccinated following a primary vaccination with commercially available whole inactivated equine influenza vaccines. The current Japanese vaccines contain A/equine/Avesta/93 (Avesta93, Eurasian lineage), A/equine/La Plata/93 (La Plata93, American lineage) and A/equine/Ibaraki/1/07 (Ibaraki07). Avesta93 and La Plata93 were introduced into the vaccines in 2004 and 1996, respectively [8, 13]. Ibaraki07 was isolated from a horse during the equine influenza epidemic in Japan in 2007 and classified as Fc1 [3, 22]. Ibaraki07 was introduced into the Japanese vaccines in October 2009. However, they do not contain an OIE-recommended Fc2 virus at present. Therefore, this raises concerns that the current Japanese vaccines are not effective against Fc2 viruses. Here, we evaluated the antigenic differences between Japanese vaccines and Richmond07 by hemagglutination inhibition (HI) assays in order to assess the efficacy of current Japanese vaccines against Fc2 viruses.

HI assays were performed as previously described [7], using infected allantoic fluids as hemagglutinin antigens and 96-well microplates. Briefly, the antisera were treated with trypsin-heat-potassium metaperiodate to remove non-specific inhibitors as previously described by Beardmore et al. [2]. Then the required final dilution of treated antiserum (1:10) was prepared with phosphate buffered saline (pH: 7.4, PBS) and adsorbed with packed chicken erythrocytes. Two-fold dilutions of the antiserum with PBS were prepared; 25 µl of the diluted serum was used in each well of microplate. To each well, 25 µl of virus containing 4 hemagglutination units was added, and the microplate was incubated at room temperature for 30 min. Then 50 µl of 0.5% chicken erythrocytes was added to each well. The results were read after incubation at room temperature for
was produced by exposing 2–3 ferrets to each virus (10^7.6–10^8.3 50% egg infectious dose) in a chamber (7 m^3) for 20 min using an ultrasonic nebulizer (SONICLIZER305; ATOM, Tokyo, Japan), except for the antiserum against Richmond07 provided by Dr. Debra M. Elton (Animal Health Trust, U.K.) [4].

The HI antibody titers with the ferret antisera are shown in Table 1. The reactivity of ferret antiserum raised to Avesta93 was limited to the homologous virus. The ferret antiserum raised to La Plata93 reacted with all the tested viruses at the same titer, with the exception of Avesta93. The reactivity of ferret antiserum raised to South Africa03 was limited to the homologous virus. The HI antibody titers of ferret antiserum raised to South Africa03 were 4 to 16 times lower than the homologous titer. The HI antibody titer of ferret antiserum raised to Ibaraki07 against South Africa03 was only 2 times lower than the homologous titer. However, the HI antibody titers of antiserum raised to Ibaraki07 against the heterologous viruses were 4 to 16 times lower than the homologous titer. The HI antibody titer of ferret antiserum raised to Richmond07 against the heterologous viruses were 4 to 8 times lower than those of the other ferret antiserum. The HI antibody titer of the ferret antiserum raised to La Plata93 was 4 to 8 times lower than those of the other ferret antiserum, the geometric mean HI antibody titer against La Plata93 of the horses was higher than those against the other vaccine strains (Avesta93 and Ibaraki07).

From these above, it is suggested that not only the Japanese vaccine strains possess the adequate immunogenicities in horses, but also they can induce an antibody reacting well with Richmond07.

Bryant et al. [4] mentioned that the antibody induced by an American lineage strain may protect against Fc2 viruses because the two American lineage strains (A/equine/Newmarket/1/93 and A/equine/Kentucky/98) are antigenically similar to the Fc2 viruses including Richmond07. Also in this study, the ferret antiserum raised to La Plata93 reacted with Richmond07 at the same titer against the homologous virus. Taken together, the geometric mean HI antibody titer of the horses against Richmond07 is probably due to a cross-reactivity of antibody induced by La Plata93 belonging to American lineage.

Our data showed that the antibody induced by the Japanese equine influenza vaccines containing La Plata93 reacts well with Richmond07. Therefore, we can expect the antibody induced by the current Japanese equine influenza vaccine to provide some protection against Richmond07-like viruses.

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