Development of diagnostic method of influenza virus infection by the detection of antibodies to the NS protein

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Equine influenza outbreaks occur worldwide. The international spread of equine influenza is caused mainly by transport of race horses. As most horses for racing and breeding purposes are vaccinated, conventional methods of diagnosis by the detection of antibodies to the virus can not differentiate between the horses infected and vaccinated. The aim of the present study is to develop a diagnostic method to disclose horses that have been infected with influenza virus by the detection of antibody response specific to infection. Using mice and horses experimentally infected with influenza A viruses, their antibody response to the nonstructural (NS) protein of the virus was examined.

The NS gene was cloned from A/equine/Miami/1/63 (H3N8) influenza virus into plasmid pPRO EX-1 and expressed in *E. coli*. Using the recombinant NS1 protein, it was examined whether anti-NS1 antibodies were detected in the sera of mice infected with A/Aichi/2/68 (H3N2). In the sera, production of anti-NS1 antibodies was demonstrated by ELISA. On the other hand, anti-NS1 antibodies were not detected in the sera of mice injected with the inactivated virus preparation.

It was then examined whether anti-NS1 antibodies were detected or not in the sera of horses infected with A/equine/Newmarket/1/77 (H7N7), A/equine/Tokyo/2/71 (H3N8), A/equine/Kentucky/1/81 (H3N8), or A/equine/La Plata/93 (H3N8). Anti-NS1 antibodies were detected in the sera of infected horses showing clinical signs. On the other hand, anti-NS1 antibodies were not detected in the sera of horses vaccinated. These results indicate that the present method of detection of anti-NS1 antibodies can be used to differentiate horses between infected and vaccinated.

The method for the detection of anti-NS1 antibodies, developed in the present study, is useful to disclose horses infected with influenza virus as a rapid method for diagnosis.