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INFORMATION

Hokkaido University conferred the degree of Doctor of Philosophy (Ph.D) in Veterinary Medicine on June 29, 2001 to 4 recipients.

The titles of their theses and other information are as follows :

Studies on the diagnosis and prevention of equine influenza

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Equine influenza viruses are circulating among horses in the world. International transport of racing horses leads the increase of the risk of introduction of equine influenza viruses in Japan. In the present study, rapid and sensitive methods for diagnosis were established to detect virus-infected horses at the quarantine service. In addition, to provide information for the selection of vaccine strains, antigenic analysis of equine influenza virus hemagglutinin (HA) was carried out.

Antibodies to the nonstructural protein (NS 1) of A/equine/Miami/1/63 (H3N8) influenza virus were detected exclusively in the sera of horses infected with equine H3 influenza viruses, but not in those of the animals immunized with the inactivated viruses by ELISA using a recombinant NS 1 as antigen. The results indicate that the present method is useful for serological diagnosis to disclose horses infected with equine H3 influenza viruses from those vaccinated.

A rapid and highly sensitive method for diagnosis of influenza by detecting viral antigens using immuno-PCR has been developed. The sensitivity of the immuno-PCR for the detection of the NS 1 which is antigenically com-

mon among influenza A viruses was $10^{1.0-2.0}$ times higher than that of RT-PCR for the detection of the viral genome. For the detection of the HA subtype-specific antigen, this assay using anti-HA monoclonal antibodies attained a sensitivity of up to $10^{7.0-8.0}$ times higher than those by virus isolation or by RT-PCR.

To provide information on the antigenic variation of the HA among equine H3 influenza viruses, 26 strains isolated from horses in different areas in the world during the 1963-1996 period were analyzed using a panel of monoclonal antibodies recognizing at least 7 distinct epitopes on the H3 HA molecule of the prototype strain A/equine/Miami/1/63 (H3N8). The reactivity patterns of the virus strains with the panel indicate that antigenic drift of the HA has occurred with the year of isolation, but less extensively than that of human H3N2 influenza virus isolates, and that different antigenic variants cocirculate. To assess immunogenicity of the viruses, antisera from mice vaccinated with each of the 7 representative inactivated viruses were examined by neutralization and hemagglutination-inhibition tests. The results emphasize the importance of monitoring the antigenic drift

in equine influenza virus strains and to introduce current isolates into vaccine.

Original papers of this thesis appeared in "Jpn. J. Vet. Res." Vol. 48 : 177-186 and 186-195 (2001) and "Vet. Microbiol." Vol. 20 : 111-119 (2001).

Iron acquisition systems in *Pseudomonas aeruginosa* :
Their contribution to bacterial growth and virulence

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Pseudomonas aeruginosa is known to possess high-affinity iron acquisition systems mediated by iron chelators produced by the bacterium, generally called siderophores, or operating via heme uptake. This organism produces pyoverdinin and pyochelin as siderophores. To investigate the significance of each siderophore for *P.aeruginosa* infection, the author constructed a set of mutants from wild-type strain PAO 1 by allelic exchange, which were deficient in producing one or both of the siderophores. Results of animal experiments using the mutants suggested that both pyoverdinin and pyochelin were required for efficient bacterial growth and full expression of virulence in *P.aeruginosa* infections, although pyoverdinin might be comparatively more important. However, the siderophores were not always required for the infections. Considering results of in vitro experiments, it was thought that iron acquisition via heme uptake might also play an important role in *P.aerugi-*

nosa infections.

High-affinity iron acquisition systems of gram-negative bacteria involve specific receptors in the outer membrane. The transport of iron-siderophore complexes and heme bound to the receptors into the periplasm is generally thought to be dependent on the function of the cytoplasmic protein, TonB. To clarify the contribution of the TonB protein to high-affinity iron acquisition in *P.aeruginosa*, the author constructed *tonB*-inactivated mutants from strain PAO 1 and its siderophore-deficient derivative. Results of in vitro growth assays of them indicated that the TonB protein was essential for iron acquisition mediated by pyoverdinin and pyochelin and via heme uptake in *P.aeruginosa*. In addition, results of animal experiments suggested that the TonB-dependent iron acquisition might be essential for *P.aeruginosa* to infect the animal host.

Original papers of this thesis appeared in "Infect. Immun." Vol. 68 : 1834-1839 and 4498-4504 (2000).