Identification of the genetic attenuation-marker of canine parvovirus vaccine and methodological and epidemiological studies in canine serious infectious diseases

Abstract of Doctoral Thesis

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Here, we describe basic studies for development and improvement of vaccines to prevent serious and fatal infectious diseases such as canine parvovirus (CPV) infection, canine distemper, as well as the highly incident canine infectious respiratory disease (CIRD) in owners of multiple dogs. Many challenges remain in the development of more effective and safe vaccines. Regarding live attenuated vaccines for CPV infections, the genetic markers that define the attenuated phenotype have not yet been identified. Evaluation of canine distemper virus (CDV) dynamics using quantitative reverse transcription and polymerase chain reaction (qRT-PCR) did not reveal any relationship to infection outcomes in experimentally infected dogs. In Japan, studies on the surveillance of pathogens that cause CIRD have been limited. The first study identified the minimal determinant for the attenuation of the CPV vaccine strain 9985-46. It showed that attenuation of the 9985-46 strain was defined by at least two mutations in residues 300 and 389 of the VP2 capsid protein. These results are important for the quality control of the 9985-46 live attenuated vaccine strain, and provide insights regarding the rational design of second-generation live attenuated vaccine candidates. The second study evaluated the usefulness of qRT-PCR in investigating the dynamics of CDV in experimentally infected dogs and the relationship between the change and outcome of the infection. The qRT-PCR results showed that CDV replicated irrespective of the degree of clinical manifestation in dogs, as qRT-PCR was more sensitive than virus titration in cell cultures of rectal and nasal sheddings. We also demonstrated that the qRT-PCR results correlated with viral titers in cell culture at the peak of viral RNA. In addition, the peak of viral RNA in symptomatic dogs was consistent with the onset of symptoms. These observations suggested that the peak of viral RNA reflected active CDV replication. This assay will be useful for comparing the multiplication and dissemination among different CDV strains and for determining the protective efficacy of vaccines. The third study conducted an etiological investigation on CIRD in Japan and evaluated the

efficacy of its vaccine in the field. The results suggested that *Bordetella bronchiseptica*, canine parainfluenza virus (CPIV), and canine respiratory coronavirus (CRCoV) were the major pathogens that caused CIRD as single or multiple pathogens. In addition, CPIV, canine adenovirus type 2, and CDV were detected less frequently in dogs previously administered with the multivalent live vaccine that included these viruses when compared to unvaccinated dogs, suggesting that the vaccine effectively prevented canine infections. The findings obtained in these studies provide useful and insightful information regarding the development of improved vaccines against important infectious canine diseases.