

Study of recently identified porcine
parvoviruses in pig herds of Japan and
Thailand

(日本とタイの養豚に感染している新規豚
パルボウイルスの研究)

Summary of Doctoral Thesis

Prayuth Saekhow

(Supervised by Prof. Hidetoshi Ikeda)

Graduate School of Veterinary and Life Science
Nippon Veterinary and Life Science University

Summary of Doctor Thesis

This thesis describes my study on porcine parvoviruses during the PhD course.

There are many parvoviruses infecting a variety of animals including insects and vertebrates. Parvoviruses are non-enveloped, isometric viruses with a linear single stranded DNA of approximately 4–6 kb in size. According to the proposed taxonomy of the family *Parvoviridae* (Cotmore et al., 2014), the family *Parvoviridae* is divided into two subfamilies; *Parvovirinae* infecting vertebrates and *Densovirinae* infecting invertebrates. The subfamily *Parvovirinae* has been subdivided into eight genera. There are several parvoviruses important in the field of veterinary medicine, such as porcine parvovirus (PPV), bovine parvovirus, canine parvovirus, canine minute parvovirus, Aleutian mink disease virus and feline panleukopenia virus, which belong to different genus in the subfamily. Most of parvoviruses are thought to have common characters of the resistance to antiseptic substances and extreme environmental conditions like heat and pH and the requirement of cellular DNA synthesis for its viral replication. These are related to the difficulty to eradicate diseases associated with the viruses and their pathogenesis.

I have studied porcine parvoviruses infecting pigs. The parvovirus initially discovered in pigs is porcine parvoviruses (PPV), which was reported in the 1960s and is now enzootic in most pig-producing countries. PPV mainly causes reproductive failure in naïve dams, and manifestation characterized

by stillbirth, mummification, embryonic death and infertility. In contrast, PPV infection of adult pig causes only a subclinical or mild disease.

Besides PPV, several other parvoviruses or their genomes have been recently identified in pigs. The detection of these newly identified porcine parvoviruses was reported in several countries, but their association with diseases is still not known. However, as more multifactorial or idiopathic disorders in pig farms are suspected, these new parvoviruses should be further characterized. I have studied the classical and four newly identified porcine parvoviruses.

In the chapter I, I describe my study of the characterization of porcine parvovirus 2 (PPV2) detected in Japanese pig farms. PPV2 was first detected in specimens from domestic pigs in Myanmar in 2001. The genome was subsequently reported from China, Hungary, the USA and Germany. The prevalence of Japan was 58% in healthy pigs and 100% in sick pigs, which was equivalent to or higher than other countries. When I started to study PPV2, six near full genome sequences of PPV2 have been reported, and there were variations in sizes of ORF1 and ORF2. I therefore molecularly cloned a near complete genome of PPV2, termed JPT68, from the tonsil of a healthy domestic pig. Based on the comparison with other PPV2 sequences, I amplified and sequenced a DNA fragment of a variable region of PPV2 detected from 41 Japanese pigs, and then compared with those of other countries using phylogenetic analyses. The analysis showed that diverged PPV2 strains exist in Japan and clearly distinct strains of PPV2 were detected in 7 of the 10 pig farms. Circulating multiple strains within a farm

may be a risk for generating emerging virus as reported in other parvoviruses.

In the chapter II, I describe my study of the five classical and newly identified porcine parvoviruses detected in Thailand. Thailand had not been investigated for the classical PPV or new porcine parvoviruses except for the seroprevalence of PPV (Tummaruk, et al., 2012). I examined the 80 tonsil samples of pigs collected at a slaughterhouse in the Chiangmai area of Thailand. I investigated the prevalence of the five porcine parvoviruses and characterized the genetic diversity of PPV2 and PPV3. The prevalence was 53% (42/80) for PPV (PPV-Kr or -NADL2 as the new abbreviation), 83% (66/80) for PPV2 (CnP-PARV4), 73% (58/80) for PPV3 (P-PARV4), 44% (41/80) for PPV4 (PPV4) and 23% (18/80) for PBo-likeV (PBoV7). Over 60% of 80 pigs carried more than 3 of the 5 viruses. The phylogenetic analyses for PPV2 and PPV3 indicated the existence of two and one clade(s) of viruses, respectively, suggesting an invasion from a limited source for both viruses.

In the chapter III, I describe the characterization of the infection status of porcine parvoviruses in Japanese pigs and their genetic diversity of PPV3. I previously investigated PPV2 of Japanese pigs, as described in chapter I. With the same specimens, my colleagues and I extended such a screening for 14 other viral genomes including 4 porcine parvoviruses. Only 5 virus genomes were detected; four were the member of the family *Parvoviridae* and another one was PCV2. The prevalence in 120 apparently healthy pigs aged about 6 months was 67% (80/120) for PPV (PPV-Kr or -NADL2 as the new abbreviation), 39% (47/120) for PPV3 (P-PARV4), 33% (32/120) for PPV4

(PPV4), 55% (66/120) for PBo-likeV (PBoV7) and 80% (96/120) for PCV2. PCV2 is a causative agent of porcine circovirus associated disease (PCVAD) and PPV has been considered as one of the cofactors for PCVAD. In the screening, the detection of PCV2 was significantly coincidental with either detection of PPV, PPV2 or PPV3. A coincidental detection of PPV and PPV4 was also observed. Although the exact reason is not known, the concurrent infection with PCV2 and porcine parvoviruses in the subclinically infected pigs may relate to the clinical manifestations of PCVAD. Additionally, I performed a phylogenetic analysis of PPV3 which suggested that Japanese PPV3s showed a slight variation, and possibly, there were farms harboring homogeneous or heterogeneous PPV3s.

Finally, this study shows the infection status of newly identified porcine parvoviruses in pig herds of Japan and Thailand. Since these viruses are not known regarding the association with any disease, our investigation should provide useful information for further studies.