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Epidemiology, genetic diversity, and association of canine circovirus infection in dogs with respiratory disease

DANKAONA W. [1,2], *MONGKHOLDEJ E.* [3], *SATTHATHUM C.* [3], *PIEWBANG C.* [1,2], *TECHAGAMSUWAN S.* [1,2]

[1] *Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand;* [2] *Animal Virome and Diagnostic Development Research Unit, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand;* [3] *Division of Dentistry and Maxillofacial Surgery, Kasetsart University Veterinary Teaching Hospital, Bangkok, Thailand*

Introduction
CanineCV is a non-enveloped, closed-circular, single-stranded DNA virus, belonging to the genus Circovirus of the family Circoviridae. The virus genome contains two major genes which are replicase (Rep) and capsid (Cap) genes. The virus-associated systemic vasculitis and hemorrhagic gastroenteritis and diarrhea have been described in several studies (1-3) but the role of the virus in canine respiratory disease remained unclear. This study aims to investigate the role of this virus in canine respiratory disease by epidemiological approach through genome-based assay and histopathological examination.

Methods
Nasal and oropharyngeal swabs collected from a total of 190 dogs including healthy (n=76) and respiratory disease (n=114) were tested for CanineCV. The polymerase chain reaction (PCR) method using primer pairs targeting the Rep and Cap genes was used for CanineCV genome detection. Co-detection with other respiratory viruses was identified as previous study (4). The association between presenting of CanineCV and various factors including respiratory signs, signalment (age, sex, breed, sterilization status), sampling route, and other co-detected respiratory viruses were determined by chi-squared test. The strength of the association was assessed by odds ratio. The SPSS version 22.0 was used for statistical analyses. The formalin-fixed tissues from positive-CanineCV dog carcasses that had died with respiratory disease were processed for histopathological workup and stained with hematoxylin and eosin for microscopic inspection. The In situ hybridization (ISH) technique was employed to localize virus genome and interpreted with histopathological lesions. This study was approved by the Institutional Animal Care and Use Committee (IACUC) (No. 2031014) and the Institutional Biosafety Committee (IBC) (No. 1931036) of Chulalongkorn University.

Results
The occurrence of PCR-positive CanineCV was 8.95% (17/190), consisting of 2.63% (2/76) from the healthy group and 13.16% (15/114) from the respiratory disease group. The PCR-positive CanineCV was associated with respiratory illness (p= 0.013) in which the respiratory disease dogs were more likely to be positive with CanineCV compared to the healthy dogs (odds ratio=5.606). Additionally, presenting of CanineCV was aged-related (p=0.005). The junior dogs were prone to be positive for CanineCV compared to the older age of dogs (odds ratio=3.83). No association was found between other factors and positive CanineCV. The common microscopic findings from positive cases were hemorrhagic pneumonia and hemorrhagic histiocytic lymphadenitis of tracheobronchial nodes (Fig. 1A). ISH examination from the lung sections showed the viral DNA signals within the nucleus of alveoli and endothelial cells of capillary blood vessels (Fig. 1B). The DNA signals were also labeled in the cytoplasm of infiltrated mononuclear cells resembling pulmonary alveolar macrophages or lymphocytes. In the tracheobronchial lymph node, CanineCV DNA labeling was intensely localized within the cytoplasm of lymphoid cells in the lymphoid follicles (Fig. 1C).

Conclusion
Our findings indicated that CanineCV is associated with canine respiratory disease with an evidence of canine respiratory cell tropism locating in pneumocytes and endothelial cells as well as in the infiltrated mononuclear cells in the lung of infected dogs.