Fatal Systemic Canine Herpesvirus Infection Associated with Acute Death in Neonatal Puppies

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Introduction
Canine herpesvirus (CHV) belongs to Alphaherpesviridae family and defines as a member of canine infectious respiratory disease complex (CIRDC), causing respiratory distress such as nasal discharge, coughing and sometimes pneumonia in infected adult dogs. Several studies revealed the co-infection of other CIRDC-associated viruses and CHV leading to severity. Routes of CHV transmission in neonatal puppy can occur either transplacentally from infected bitch or directly contact with infected oronasal secretion. Clinical manifestations are age-dependent; affected adult dogs often show asymptomatic signs or mild rhinitis and pharyngitis, whilst neonatal or young puppies might show hemorrhagic and necrotic diseases. The virus can evade host immune response and persists in various nerve plexuses, leading to latent infection and shedding when immune-compromised.

So far, there is no incidence of CHV infection in Thailand. This report described pathological and molecular investigations of CHV infection in a dog breeding farm. This might raise awareness of CHV infection occurring in Thailand.

Materials and Methods
Case history: Seven 14-day-old Labrador retrievers puppies consecutively died within 6 hours after clinical signs including anorexia, lethargy and sudden respiratory distress, were observed. While their bitch showed neither respiratory problems nor depression. In detail, the bitch was semi-naturally mated with a commercial breeder male dog. The dead puppies were submitted to Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University for routine postmortem examination.

Pathology and sampling: The necropsy was conducted on 3 submitted puppies. Tissues including heart, brain, tracheobronchial lymph nodes, trachea, lung, tonsil, liver, kidney, spleen, and small intestine were collected in 10% neutral buffered formalin for histopathological study. Nasal and oropharyngeal swabs, and pleural effusion were collected for bacterial and viral identifications. Moreover, the vaginal swab and blood samples were collected from their bitch and one neighboring dog in 3 months after parturition.

Molecular assay: The collective swabs, fluid and blood that mentioned above were viral genomic extracted. Multiplex polymerase chain reactions (mPCR) were done for CIRDC–associated virus detection composing of canine influenza virus (CIV), canine parainfluenza virus (CPIV), canine distemper virus (CDV), canine respiratory coronavirus (CRCoV), canine adenovirus type 2 (CAV-2) and canine herpes virus (CHV). The PCR conditions are available upon request. Subsequently, the amplified PCR product (136 bp) was submitted for sequencing.

Results and Discussion
Gross findings: All carcasses were fresh with good body condition. Diffusely patchy red-tinged foci and rubbery wedge-shaped area were revealed on kidney surface and its cut surface, respectively (Fig.1A-B). Livers were enlarged, congested, rubbery-like firm with diffused white foci (Fig.1C). More than 80% of lung parenchyma were dark-red, firm, wet, enlarged with diffusely mottled white focci, and blood oozing on cut surface (Fig.1D). Tracheas were fully occupied with foamy turbid fluid throughout its lumen. All puppies contained intra-thoracic serosanguineous fluid at an amount of 30-45 ml. Reddish enlarged tracheobronchial lymph nodes and tonsils were evident. Splenomegaly with diffusely reddish pin-pointed foci was observed (Fig.1E).

Figure 1 Disseminated petechial hemorrhage with massively diffuse necrotic foci in multiorgan.
Histological findings: Severe multifocal necrotizing nonsuppurative pneumonia with pulmonary congestion was presented (Fig. 2). Alveoli and pulmonary parenchyma were occupied with macrophages, some neutrophils and plasma cells. Moderate acute fibrinous bronchiolitis and alveolitis were shown with peri-bronchiolar edema and mild bronchial epithelium sloughing that resulted in airway occlusion. Moderate hemorrhagic histiocytic lymphadenitis and tonsillitis were detected. Severe hepatic congestion with multifocal necrosis was markedly seen. Severe acute tubular necrosis with nonsuppurative interstitial nephritis and massive congestion at both cortex and serosa were described. Multifocal necrotic foci with histiocytic splenitis were noted.

Figure 2 Multifocal necrotizing nonsuppurative pneumonia with severe pulmonary edema (H&E, 400x)

Detection of CHV: Bacterial culture was negative in the representative samples of necropsied puppies. The mPCR detecting the CIRDC viruses was positive only for the CHV (Fig. 3). The sequencing result of 136-bp amplicon were 100% homology to canid he pervirus 1 glycoprotein B gene which was deposited in GenBank database. However, the samples from vaginal swabs and blood samples from the bitch and neighboring dog were negative for CHV.

Figure 3 CHV glycoprotein B (136bp) was detected from both nasal and oral swabs of the puppies. Negative results were noted in the samples of bitch and neighboring dog. (M: marker 100 bp; NP: nasal swab puppy; OP: oral swab puppy; B: bitch; ND: neighboring dog; -ve: negative control; +ve: positive control)

Our findings showed that all necropsied puppies exhibited massively hemorrhagic and necrotizing diseases, corresponding to the CHV infection. The partial CHV glycoprotein B was detected in all nasal and oropharyngeal swabs and effusions without evidence of other respiratory viruses and bacteria, confirming that CHV is a primary cause of the disease. Unfortunately, the CHV could be neither detected in vaginal swab or blood samples from their bitch and neighboring dogs. This result might be explained by the latent infection and immune evasion of CHV due to delaying sampling after acute onset in puppies. However, how the puppies were infected via transplacenta, during parturition or direct contact with oronasal secretions could not be clarified in this study.

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