Acta Veterinaria-Beograd 2017, 67 (1), 11-24
UDK: 636.7.09:[616.98:578.825
DOI: 10.1515/acve-2017-0002

Research article

VIRAL MOLECULAR AND PATHOLOGICAL INVESTIGATIONS OF *CANID HERPESVIRUS* 1 INFECTION ASSOCIATED RESPIRATORY DISEASE AND ACUTE DEATH IN DOGS

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(Received 07 July, Accepted 04 November 2016)

Canid herpesvirus 1 (CaHV-1) is a member of the canine infectious respiratory disease complex (CIRDC). The outcome of CaHV-1 infection can be occasionally fatal. So far, no information on CaHV-1 circulation in Thailand has been reported resulting in a lack of preventive strategies. In this study, nasal (NS) and oropharyngeal (OS) swabs were collected from 100 live dogs with respiratory distress. Among them, 23 pleural effusions were aspirated. A panel of CIRDC-associated viruses was screened by (RT)-PCR, including CaHV-1, CIV, CPIV, CDV, CRCoV and CAdV-2, for all collected samples. The CaHV-1 was detected in 32 dogs. Additionally, CaHV-1 was consistently detected in six pleural effusions. Most CaHV-1 infected dogs were over 5 years of age (43.8%) and expressed a mild nasal discharge. Pathological results of four three-month-old puppies, naturally moribund from respiratory disease, revealed a severe multifocal necrotic-hemorrhagic disease in several organs without pathognomonic inclusion bodies. They were only found to be CaHV-1 positive by PCR. Phylogenetic analysis demonstrated concordant results of CaHV-1 circulation in Thailand. Although mostly found as a co-infection with other CIRDC viruses (68.8%) it also occurred alone. Therefore, rapid ante-mortem diagnosis might facilitate the investigation of unclassical CaHV-1 infection, which is fatal in neonates and causes illness in annually core-vaccinated adults.

Key words: *Canid herpesvirus* 1, CIRDC, pathology, respiratory distress, Thailand

INTRODUCTION

Canid herpesvirus 1 (CaHV-1) is an enveloped double stranded DNA virus that belongs to the family *Herpesviridae*, subfamily *Alphaherpesvirinae*, genus *Varicellovirus* [1]. The CaHV-1 has been defined as a member of the canine infectious respiratory disease

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complex (CIRDC), causing respiratory distress, such as nasal discharge, coughing and sometimes pneumonia in infected dogs. Several studies have revealed that the co-infection of CaHV-1 with other CIRDC viruses, such as canine influenza (CIV), canine parainfluenza virus (CPIV), canine distemper virus (CDV), canine respiratory coronavirus (CRCoV) and canine adenovirus type 2 (CAdV-2), often leads to an increased severity of the symptoms. However, the role of CaHV-1in CIRDC remains unclear [2,3].

Infection of dogs by CaHV-1 may be transplacental or after direct contact with infected material, such as uterine secretions during birth or oro-nasal secretions. Infection during the mid- to late-gestation period might cause abortion or mummification of the fetus. The incubation period of CaHV-1 varies from 6–10 days. The virus can evade the host immune response and persist in various nerve plexuses, resulting in latent infections and virus shedding when the host is immunocompromised [4,5]. Clinical manifestations are dependent on the age and immune status of the host, where infected adult dogs are typically asymptomatic or show mild rhinitis and pharyngitis, while infected neonates or young puppies might die within a few days after birth or infection [6,7].

After necropsy, the distinct lesion of CaHV-1 is seen as a generalized necrotizing and hemorrhagic disease, most obvious in the vital organs, such as the lung, liver, kidneys and spleen [8,9], although a similar finding is also evident in other organs, including the small intestine, adrenal glands, brain, heart, pancreas, stomach and eyes [10]. Eosinophilic intranuclear inclusion bodies might be present in the epithelial cells of the trachea, bronchi, lung, liver and kidneys, suggesting that inclusion bodies might be consistent with a fulminant CaHV-1 infection based on a high infection level of viral genomic DNA [11].

Taxonomically, CaHV-1 appears to be a monotypic virus that is genetically similar to feline herpesvirus-1 (FHV-1), phocid herpesvirus-1 (PhHV-1) and equine herpesvirus-1 (EHV-1) and -4 (EHV-4) [12,13]. However, the host range of CaHV is restricted to dogs only [14]. This virus multiplies only in permissive canine-derived cell lines, such as Madin-Darby canine kidney (MDCK) cells. Two stages in CaHV-1 attachment and invasion *in vitro* have been suggested. Firstly, the virus interacts with heparan sulfate on the cell surface, and it is then secondly internalized via an unidentified viral component and cellular receptor. In contrast, the virus appears to attach to non-permissive cells through the heparin-sensitive mechanism but does not enter the cell due to the lack of the correct cellular receptor [15].

Although there have been some reports to suggest that the respiratory problems in CaHV-1-infected dogs can be self-limited or even fatal worldwide [16], the fundamental data regarding CaHV-1 circulation in Thailand remains unknown. This study aimed to determine the prevalence of the CaHV-1 infections with genetic characterization in live respiratory distressed dogs as well as CaHV-1 investigations in acute dead puppies.

MATERIAL AND METHODS

Study population and sample collection

Live animals: one hundred dogs showing respiratory distress that had been brought to veterinary hospitals/clinics located in Bangkok, Thailand during 2013–2014 were included in this study. Nasal (NS) and oropharyngeal swabs (OS) were collected by sterile rayon tipped applicators (Puritan®, Maine, USA) and kept in sterile phosphate saline buffer. Among them, 23 pleural effusions (PE) diagnosed by radiographs were aspirated and collected in sterile 1.5-ml eppendorf tubes. All specimens were kept at-80°C until used in the molecular assay. Clinical signs were scored by certified veterinarians at the time of sampling as: +, nasal discharge; ++ mild cough; +++, cough and nasal discharge; ++++, cough, nasal discharge and inappetence; +++++, evidence of bronchopneumonia. Signalment, such as age and history of vaccination, were recorded for further analysis. All following procedures were approved by the Chulalongkorn University Animal Care and Use Committee (No. 1431005).

Additionally, two bitches that delivered dead puppies were sampled by NS, OS and vagina swabs (VS) at the first week and 3 months after parturition. All swabs were then screened for CaHV-1 by genomic DNA detection using polymerase chain reaction (PCR).

Pathological study

Dead animals: four naturally moribund puppies, aged up to 3 months, which had suffered from severe sudden respiratory distress followed by epistaxis, were submitted for routine necropsy at the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University. Clinical data and gross findings were recorded. Swabbed samples, including NS, OS and PE, were collected for molecular assay. Various tissues, including the brain, lung, trachea, bronchial lymph node, tonsil, liver, spleen, kidneys and intestines, were collected in 10% neutral buffered formalin for histopathology. Tissues were subjected to routine histological process and embedded in paraffin wax. Sections were cut at a 4-μm thickness, stained with hematoxylin and eosin (H&E), and then observed under a light microscope.

Virus detection

All sampled swabs (NS, OS and VS) as well as PE were screened for CaHV-1 by PCR. Viral nucleic acids were extracted using a Viral Nucleic Acid Extraction Kit II (GeneAid, Taipei, Taiwan) according to the manufacturer's recommendations. Genomic DNA was quantified and qualified using a Nanodrop™ Lite Spectrophotometer (Thermo Fisher Scientific Inc., Massachusetts, USA). Each PCR reaction was performed in a final volume of 25 μl and consisted of GoTaq® Hot Start Green Master Mix (Promega, Wisconsin, USA), DNA template, nuclease-free water and specific primers

as previously described [17]. The PCR thermal cycling was performed at 95ºC for 5 min, followed by 40 cycles of 1 min each at 95ºC, 58ºC and 72ºC, and then a final 72ºC for 10 min. After resolution by electrophoresis through a 2% (w/v) agarose gel, the PCR products (136 bp) were stained with 10% ethidium bromide and visualized under an ultraviolet illuminator. Potential co-infection with other CIRDC-related viruses (CIV, CPIV, CDV, CRCoV and CAdV-2) was ascertained using multiplex PCR (for DNA viruses) and multiplex reverse transcriptase PCR (RT-PCR; for RNA viruses) approaches (Table 1) [18].

Table 1. Primers for PCR amplification of CIRDC viruses

Virus	Primer name	Primer sequence (5'-3')	Target gene*	Product size (bp)
Canine influenza virus (CIV)	CIV_M_F151	CATGGARTGGCTAAAGACAAGACC	M	126
	CIV_M_R276	AGGGCATTTTGGACAAAKCGTCTA		
Canine distemper virus (CDV)	CDV_N_F768	AACAGRRATTGCTGAGGACYTAT	NP	290
	CDV_N_R1057	TCCARRRATAACCATGTAYGGTGC		
Canine adenovirus type 2 (CAdV-2)	CAdV_E3_F25073	TATTCCAGACTCTTACCAAGAGG	E3	551
	CAdV_E3_R25613	ATAGACAAGGTAGTARTGYTCAG		
Canine parainfluenza virus (CPIV)	CPIV_N_F428	GCCGTGGAGAGATCAATGCCTAT	NP	187
	CPIV_N_R614	GCGCAGTCATGCACTTGCAAGT		
Canine respiratory coronavirus (CRCoV)	CoV_16053_F	GGTTGGGAYTAYCCTAARTGTGA	S	542 (First round PCR)
	CoV_16594_R	TAYTATCARAAYAATGTCTTTATGTC		
	CoV_Pan_16510_R	TGATGATGGNGTTGTBTGYTATAA		458 (Second round PCR)
Canine herpesvirus (CaHV)	CaHV_GBF439	ACAGAGTTGATTGATAGAAGAGGTATG	GB	136
	CaHV_GBR574	CTGGTGTATTAAACTTTGAAGGCTTTA		

* M = Matrix, NP = Nucleoprotein, E3 = Early transcribed region, S = Spike protein, GB =Glycoprotein

Briefly, the extracted nucleic acids were divided into two samples. One pair of the nucleic acids was subjected to cDNA synthesis using the Omniscript® Reverse Transcription Kit (*Qiagen* GmbH, Hilden, *Germany*) according to manufacturer's recommendations. The cDNA was then used as a template for CIRDC-RNA virus detection by multiplex RT-PCR. The reaction was comprised of a mixture of 2x GoTaq® Hot Start Green Master Mix (Promega, Wisconsin, USA), 0.4 µM final concentration of each primer (Table 1) and 2 µl of cDNA, and made up to 25 µl with nuclease-free water. The reactions were performed using 3Prime G Gradient Thermal Cycle (Techne, UK). PCR was performed at 95ºC for 5 min, followed by 40 cycles of 1 min each at 95ºC, 58ºC and 72ºC, and then a final 72ºC for 10 min. The other pair of nucleic acids was subjected to PCR assay for CAdV-2 detection with the same cycling conditions as described above in the multiplex RT-PCR. The PCR products were resolved by 2% (w/v) agarose gel electrophoresis with 10% ethidium bromide in-gel staining and visualized by UV transillumination and compared to expected size of the PCR product

(Table 1). Likewise the potential infection with bacteria was screened from the tracheal discharge samples collected from the necropsied puppies.

Phylogenetic analysis

Six selected CaHV-1 PCR products from necropsied dogs were purified using a NucleoSpin Extract II™ kit (Macherey-Nagel, Düren, Germany) and then commercially sequenced at Solgent Co., Ltd. (Korea) for genetic sequencing. The nucleotide sequences were aligned using the BioEdit Sequence Alignment Editor Version 7.0.9.0 software and then compared with previously deposited nucleotide sequences in the GenBank database using the BLASTn algorithm. Phylogenetic analysis was performed using the Maximum Likelihood (ML) algorithm using the BioEdit Sequence Alignment Editor Version 7.0.9.0 software. Standard errors were calculated by the Bootstrap method using 1,000 replicates [19].

RESULTS

Clinical relevance of CaHV-1 infection

In total, CaHV-1 was detected in 32 out of 100 live dogs with respiratory distress, which were comprised of 17 dogs (53.1%) that were positive for both OS and NS, while nine and six dogs were only positive from the NS (28.1%) and OS (18.8%), respectively. In addition, CaHV-1 was detected in 6/23 (28.3%) of the PE samples. Infection frequencies of live dogs with CaHV-1 showed an age-dependent affect, being highest in dogs more than 5 years old (14/32, 43.8%), and decreasing with decreasing age at 8/32 (25%), 5/32 (15.6%) and 5/32 (15.6%) in 1–5 years old dogs, 3 months to 1 year old and <3 months old, respectively. The clinical expressions of CaHV-1-positive dogs varied from mild to severe symptoms. They mostly expressed a nasal discharge (9/32, 28.1%), followed by nasal discharge with a cough (7/32, 21.9%), nasal discharge with a cough and inappetence (6/32, 18.8%), bronchopneumonia (6/32, 18.8%) and, to a lesser extent, a cough only (4/32, 12.5%). In relation to their vaccination history, for which commercial vaccines are only available for CPIV/CDV/CAdV-2 prevention, a higher frequency of CaHV-1 infection was found in vaccinated dogs (20/32, 62.5%) than in unvaccinated dogs (12/32, 37.5%).

Single CaHV-1 infection and multiple infections with other CIRDC-associated viruses

Single CaHV-1 infections were observed in 31.3% (10/32) of dogs, whereas co-infection was at least one other CIRDC virus were found in 68.8% (22/32) of dogs (Table 2, Figure 1). Focusing only on the CaHV-1 positive dogs, the most frequent observations were found in adult dogs of >5 years old (5/10, 50%), minimally one-shot vaccinated (7/10, 70%), and clinically presenting mild respiratory signs, such as nasal discharge (3/10, 30%). However, this pattern of findings was similar in the co-infected CIRDC dogs, since they were most frequent in dogs >5 years old (8/22,

32.4%), vaccinated dogs (13/22, 59.1%) and those presenting with only a nasal discharge (6/22, 27.3%).

Table 2. Clinical data of *Canid herpesvirus 1* (CaHV-1) infected dogs detected by PCR

Age	Vaccination history*	Number of CaHV-1 infected live dogs Graded by clinical relevance** (n=32)				
		+	++	+++	++++	+++++
<3 months	Vaccinated	0	0	0	0	1‡
	Non-vaccinated	0	0	0	1	3‡
3 months to 1 year	Vaccinated	2‡	1	0	0	0
	Non-vaccinated	1	0	1	0	0
>1 year to 5 years	Vaccinated	2	1‡	2	0	0
	Non-vaccinated	0	0	0	2‡	1
≥5 years	Vaccinated	4§	2‡	2‡	2	1
	Non-vaccinated	0	0	2‡	1	0

*Dogs <1 year old were vaccinated against canine distemper (CDV), canine parainfluenza (CPIV), canine adenovirus type 2 (CAdV-2). Dogs >1 year old were vaccinated against CDV, CPIV, CAdV-2 and rabies virus; ** Clinical relevance of respiratory distress dogs was categorized by veterinarians at the time of sampling as: +, nasal discharge; ++ mild cough; +++, cough and nasal discharge; ++++, cough, nasal discharge and inappetence; +++++, evidence of bronchopneumonia; ‡ One and § two dogs were infected with CaHV-1 only, and the rest in each category were co-infected with other CIRDC-associated viruses.

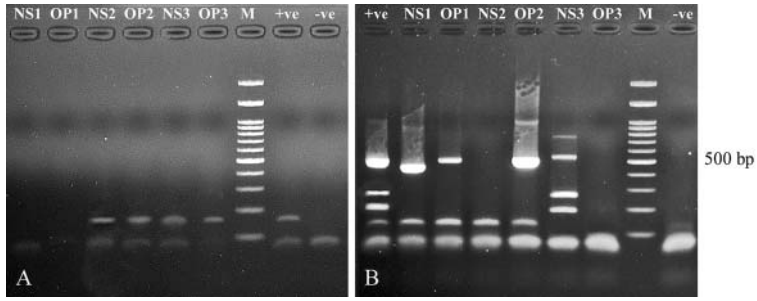

Figure 1. Canine infectious respiratory disease complex (CIRDC) associated viruses detected by (RT)-PCR. (A) Multiplex PCR showed the amplicon specific band of CaHV-1 (136 bp), but not CAdV-2 (551bp), in both nasal (NS) and oropharyngeal (OS) swabs of dog No. 2 and 3. (B) Multiplex RT-PCR revealed multiple infections of CIV (126 bp) and CRCoV (458bp) in dog No. 1 and 2, while CPIV (187 bp), CDV (290 bp) and CRCoV were found in the NS of dog No. 3. M: 100 bp DNA marker, +ve: positive control, -ve: negative control. Gels shown are representative of those seen from 2 independent PCR reactions.

Pathological findings of CaHV-1infected puppies

After necropsy, the macroscopic findings for the four puppies showed that more than 80% of the lungs were dark red, firm, edematous, non-collapsible and with blood oozing on the cut surface. Diffusely mottled white foci infiltrated the parenchyma (Figure 2A). The tracheas were fully occupied with frothy, turbid fluid throughout the lumen and particularly in the distal part. All puppies also had 30–45 ml intrathoracic serosanguineous fluid. Reddish enlarged tracheobronchial lymph nodes and tonsils

were presented. Their livers were enlarged, congested, firm, dark, rubbery with diffusely small white foci (Figure 2B). A diffusely patchy hemorrhage on the kidney surface and multiple wedge-shaped hemorrhages at the renal medullar layer were evident on the cut surface (Figure 2C).The spleen was enlarged with diffusely pinpoint reddish foci in all four puppies (Figure 2D). Moderate congestion of the brain and intestines was noted.

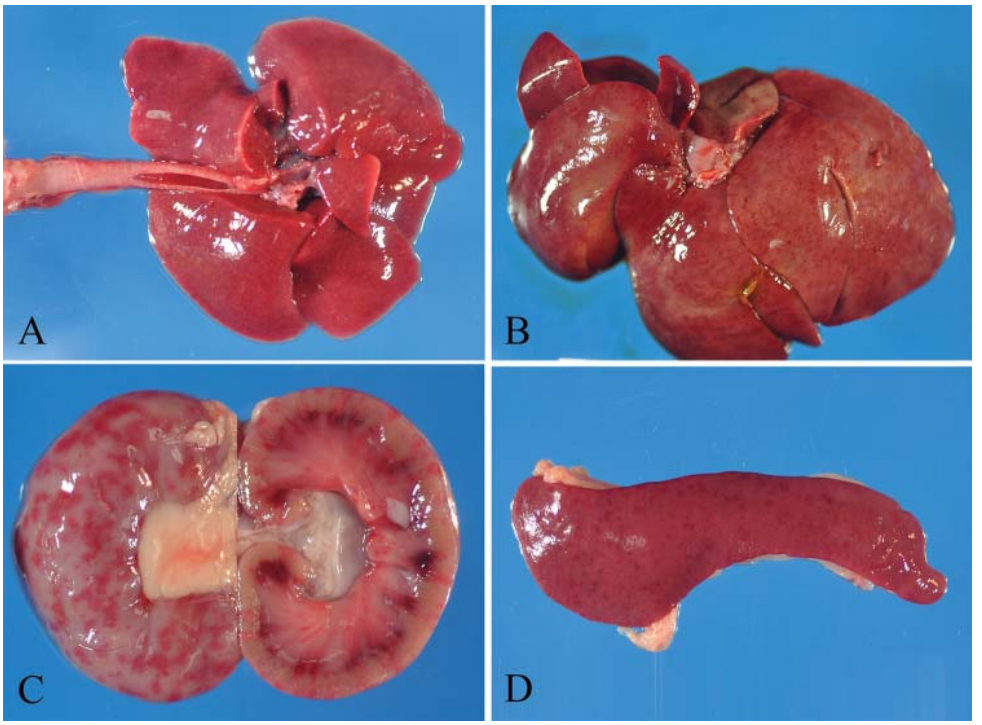

Figure 2. Post-mortem examination revealed (A) a dark red enlarged lung with diffuse pinpoint white foci, (B) liver congestion with diffuse white foci, (C) diffuse patchy hemorrhage on the renal surface and multiple wedge-shaped hemorrhages at the renal medulla on its cut surface and (D) marked splenomegaly with diffuse pin point red foci.

Histologically, prominent lesions composed of severe acute multifocal necrotizing pneumonia with pulmonary congestion were evident in the lung tissue (Figure 3A). Inflammatory cells, such as neutrophils, macrophages and some plasma cells, were infiltrated in the alveoli and pulmonary parenchyma. Moderate squamous metaplasia of the bronchiolar epithelium was often observed and moderate fibrinosis was generally trapped in both the alveoli and lumen of the small bronchioles. Bronchioles were edematous with mild bronchial epithelial lacerations that resulted in sloughing of such cells and tissue debris into the airways. No evidence of obvious foreign organisms or inclusion bodies was noted. Moderate hemorrhagic histiocytic lymphadenitis of the tracheobronchial lymph nodes and tonsillitis were observed. For the liver, severe multifocal necrotizing hepatitis with hepatic congestion was markedly seen. In addition, two of four dogs also revealed moderately centrilobular fatty degeneration. In the kidney,

severe acute tubular necrosis with massive congestion at both cortex and medulla were described (Figure 3B). Multiple necrotic foci with histiocytic splenitis were noted. Mild nonsuppurative encephalitis was characterized by a few layers of mononuclear cell perivascular cuffing. Moderate nonsuppurative enteritis accompanied by a mild degree of villous atrophy was also seen.

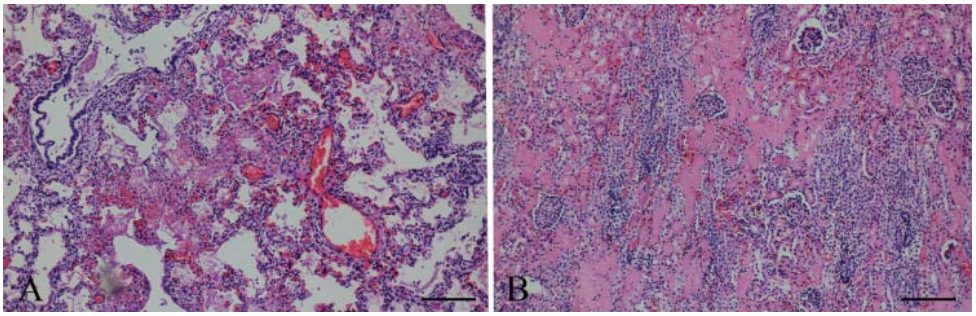

Figure 3. Histopathology revealed (A) severe acute multifocal pyogranulomatous necrotizing hemorrhagic pneumonia and (B) severe acute tubular necrosis with massive congestion. Bar= 200 mm.

Moreover, the NS, OS and PE samples from all necropsied puppies were found to be CaHV-1 positive by PCR detection. Co-infection with bacteria or other CIRDC viruses (CIV, CPIV, CDV, CRCoV and CAdV-2) was not detected in the respiratory samples by bacterial identification and PCR assays.

Interestingly, we were informed later by the owners that two bitches of these four dead puppies had been sick during pregnancy. One bitch showed mild nasal discharge during the first 2 weeks of pregnancy and the other had diarrhea in the first 3 weeks of pregnancy. After CaHV-1 positive PCR results were reported for these puppies, the NS, OS and VS of the respective bitches were collected the first-week and 3-months after parturition and screened for viruses. The presence of CaHV-1 DNA was detected in all the first-week samples, but was absent in all the 3-month specimens (data not shown).

Phylogenetic analysis

The sequences of the six selected amplified PCR products (CU1–6/TH/CaHV-1) displayed 100% homology to CaHV-1 strain KS-1 glycoprotein B (gB) gene (Accession number HQ846625), and were unrelated to other herpesviruses, suggesting that they are species specific. Phylogenetic analysis revealed that CaHV-1 circulating in Thailand (CU1–6/TH/CaHV-1) belonged to the *Alphaherpesviridae* and was genetically close to the PhHV-1 (Accession number Z68147) and Bovine herpesvirus 1 (Accession number JN787952) (Figure 4).

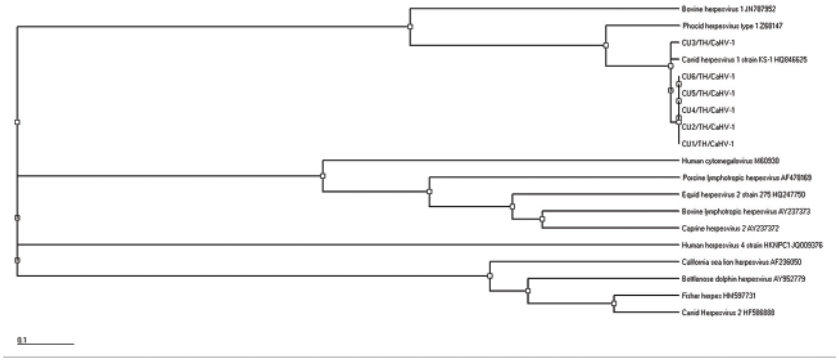

Figure 4. Maximum likelihood phylogenetic tree of CaHV-1 based on the 136 bp nucleotide sequence of the partial glycoprotein B gene. The CU1–6/TH/CaHV-1 isolates represent the six selected PCR products of CaHV-1 isolates in dogs in Bangkok, Thailand. They were compared with other herpesviruses from the indicated various hosts followed by their accession number.

DISCUSSION

Infection of dogs with CaHV-1 can manifest as a fatal systemic disease in neonatal dogs, particularly those of less than 6 weeks of age. Recently, CaHV-1 infection in clinically healthy adult dogs has been reported to cause severe acute respiratory problems [9], and hepatic necrosis without any respiratory signs [8]. However, the prevalence was quite rare. Despite that CaHV-1 has been recognized as one of the pathogens that causes CIRDC for decades, the prevalence of CaHV-1 infections circulating in Thailand has not been previously determined. This might be the result of a lack of CaHV-1 diagnostic tools in clinical use. In addition, this study also illustrated the fatal CaHV-1 infection associated with severe hemorrhagic and necrotizing respiratory disease in puppies. These findings showed evidence of unclassical CaHV-1 infection which was confirmed by PCR analysis.

Viruses belonging to the *Herpesviridae* family are well-recognized pathogens that are able to potentially persist in their particular hosts. CaHV-1 is extremely good at evading the host immune responses and is adapted for persistence in the peripheral ganglion of the nervous system at immune-privileged sites [4]. Later, the dormant virus reactivates for its replication and virion production without being recognized by host immune cells. The virus can spread its descendants from the infected organs in the lytic cycle when the host loses a proper immune function, such as under stress, pregnancy, anti-cancer and systemic corticosteroid treatment [19,20]. In addition, the latent infection of CaHV-1 can be reactivated during other infectious conditions, particularly with CIRDC [3].This is in agreement with our findings showing that most of the CaHV-1 infected dogs were co-infected with other CIRDC-viruses. Multiple CIRDC virus infections often increase the severity of the clinical outcome [3]. In addition, most routinely vaccinated dogs that were CaHV-1 positive and co-infected with CIV or CRCoV exhibited a low degree of severe respiratory problems, while those

that were unvaccinated and simultaneously infected with CDV and CAdV-2 expressed progressive clinical signs (data not shown). These findings might have resulted from the lack of neutralizing immunological responses against CDV and CAdV-2 derived from the vaccination which probably protects the CaHV-1 positive and vaccinated dogs from a progressive severity of the disease.

Most of the CaHV-1-infected dogs in this study were adults. Apparently, they showed a lower clinical severity when compared with the infected puppies and younger dogs. CaHV-1 infection in adult to senior dogs was previously reported to cause mild respiratory symptoms that were localized and self-limited [21]. However, the evidence that CaHV-1 infection can induce a fatal systemic disease in adult dogs is increasing [9,16,20]. These findings can be explained by immunocompromised conditions, including infection, disease, under stress and aging, which then resulted in viral reactivation and shedding.

According to the necropsy results of CaHV-1 infected puppies and positive PCR results of their bitches, the route of transmission could have occurred either by direct contact with vaginal secretion or transplacentally. Previously, CaHV-1 has been detected in the vaginal canal during whelping suggesting an infection risk to puppies [22]. Acute respiratory failure with disseminated hemorrhagic and necrotic foci in various organs, characterized by lesions in the puppies with CaHV-1 infection [22] is consistent with the results of this study that showed massive widespread necrohemorrhagic lesions in the lung, liver, spleen and lymph nodes. In addition, damage to the respiratory epithelium and exfoliation of tissue debris that corresponded to tubular necrosis of the kidneys were also described. Some of the disseminated necrosis in several organs might have resulted from severe hypoxia due to viral pneumonia [9]. Compared with herpesvirus infections in other animals, most clinical signs after infection were involving the respiratory system according to its host cellular tropism and host restrictive property [1]. However, other clinical signs affecting other organs were reported such as ocular diseases in feline herpesvirus-1 (FHV-1) infection [1], cardiac injuries in calves with bovine herpesvirus-1(BHV-1) infection [23], cauliflower-like mass on the skin and genital tract in dolphins with bottlenose dolphin herpesvirus (TTHV) infection [24], and abortion with encephalomyelitis in equine herpesvirus-1 (EHV-1) infection [25].

Typically, the diagnosis of a CaHV-1 infection is performed by microscopic analysis based on the presence of intranuclear inclusion bodies in a variety of epithelial cells, such as bronchial and renal epithelia, hepatocytes, myocytes and ganglia cells, and accompanied with massive focal necrosis and hemorrhaging of the internal organs. However, less specific alterations in puppies and other systemic disease involvement in older animals leads to diagnostic ambiguities and requires a specific method to confirm the CaHV-1 infection, including immunohistochemistry, immunofluorescence, virus isolation, electron microscopy, and molecular assays [9,22,26,27]. Typically, CaHV-1 infections are difficult to confirm using immunohistochemistry, which has a lower sensitivity than PCR [28], because of the instability and fragility of the virus in storage [29]. However, most CaHV-1-infected PCR-positive dogs presented with disseminated

necrotic and hemorrhagic lesions in the absence of herpesviral inclusion bodies [22], which was in agreement with this study. Moreover, a cross-sectional study of 57 dead puppies in Denmark revealed that 22.8% of the dead puppies were positive for CaHV-1 infections when assayed by real-time PCR (qPCR), but analysis of the histological lesions was not consistent and the *in situ* hybridization results were mostly negative, except for one sample that contained a few positive cells [11].

Several serological investigations have revealed the prevalence of CaHV-1 infections in household and colony breeding dogs in various countries at different time points, such as USA (12.8% during 2003–2004) [6], Italy (27.9%, assuming before 1998) [30], Netherlands (39.3% during 1997–1998) [31], Belgium (45.8% in 2000) [32] and England (88% in 1994) [33]. In Asia, a CaHV-1 serological survey was first reported in Korea and ranged from 18–70% depending on the region [34]. In Japan, PCR-based CaHV-1 detection showed 12.8% positive CaHV-1 infections in inpatient dogs that presented with nosocomial infections during hospitalization and caused acute tracheobronchitis [16].Therefore, this investigation increased the data on CaHV-1 prevalence in Asia using molecular and pathological characterizations in dogs in Thailand. Since 2003, a commercial inactivated subunit vaccine against CaHV-1 has been available in Europe and is recommended to use in pregnant bithces [35], but it is still unlicensed in several countries, including Thailand. Therefore, veterinarians should be aware of CaHV-1 infection levels and symptoms. A rapid and reliable genetic-based technique, such as PCR, might facilitate the ante-mortem diagnosis and prevent the CaHV-1 transmission in apparently clinically healthy animals.

Acknowledgements

This work was funded by The 100th Anniversary Chulalongkorn University Fund for Doctoral Scholarship to CP, The Research Chair Grant from The National Science and Technology Development Agency (P-15-50004) to YP, The Thailand Research Fund (TRG5780250), Grant for Joint Funding of External Research Project, Ratchadaphiseksomphot Endowment Fund, Veterinary Science Research Fund (RES_57_397_31_037), Chulalongkorn University and The National Research Council of Thailand (GRB_APS_38_59_31_02) to ST. Some of this information was presented in the World Veterinary Congress (WVC) 2015 in Istanbul, Turkey and supported by the Overseas Presentations of Graduate Level Academic Thesis Scholarship.

Authors' contributions

CP collected samples, carried out the experiment and drafted the manuscript. AR performed necropsy, provided critical comments on pathological findings and drafted the manuscript. YP participated in the design of the study and drafted the manuscript. ST participated in the design of the study, in the sequence alignment, and helped to draft the manuscript. All authors read and approved the final manuscript.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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VIRUSOLOŠKA MOLEKULARNA I PATOMORFOLOŠKA ISPITIVANJA RESPIRATORNE INFEKCIJE I AKUTNOG UGINUĆA KOD PASA INFICIRANIH HERPES VIRUSOM PASA TIP 1

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Herpes virus pasa tip 1 (CaHV-1) je jedan od uzročnika infektivnog respiratornog kompleksa pasa (CIRDC). Pojava infekcije sa CaHV-1 može da ima kao posledicu uginuće inficirane životinje. Do sada ne postoje informacije o cirkulaciji CaHV-1 na Tajlandu, što je posledica nepostojanja strategije prevencije ovog obolenja. U ovoj studiji uzimani su nosni (NS) i orofaringelni (OF) brisevi od 100 živih pasa sa simptomima obolenja respiratornog trakta. Od 23 psa, uzet je aspirat pleuralne šupljine. Kod svih uzoraka, obavljen je skrining CIRDC-povezanih virusa metodom (RT)-PCR koja je obuhvatala CaHV-1, CIV, CPIV, CDV, CRCoV i CAdV-2. CaHV-1 je dokazan u uzorcima poreklom od 32 psa. Pored toga, CaHV-1 je mogao da se dokaže u uzorcima pleuralne efuzije poreklom od 6 pasa. Većina pasa inficiranih sa CaHV-1, bili su stariji od 5 godina (43,8%) pri čemu je kod svih pasa zapažen blagi nosni iscedak. Patomorfološkim pregledom četiri šteneta stara tri meseca, uočene su multifokalne nekrotično-hemoragične promene u nekoliko organa. Međutim, ni u jednom slučaju nisu ustanovljena za herpes virus patognomonična inkluziona telašca. Inkluzije su uočene samo u jednom uzorku, koji je bio CaHV-1 pozitivan i na PCR testu. Filogenetska analiza je pokazala da postoji neprestana cirkulacija CaHV-1 na Tajlandu. U najvećem broju slučajeva, CaHV-1 je bio pratilac i ostalih virusa izazivača CIRDC (u 68,8% slučajeva). Međutim, CaHV-1 je mogao da se dokaže i kao samostalan uzročnik respiratornih smetnji. Iz tih razloga, brza postmortalna dijagnoza može da olakša ispitivanje oblika CaHV-1 infekcija, koje su po pravilu fatalne za novorođene životinje, a koje kod odraslih, jednom godišnje vakcinisanih pasa iziazivaju respiratorno obolenje.