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Research article

Cross-sectional investigation and risk factor analysis of community-acquired and hospital-associated canine viral infectious respiratory disease complex

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ABSTRACT

Canine infectious respiratory disease complex (CIRDC) is associated with multiple factors. The possible transmission source can be via community-acquired infection (CAI) or hospital-associated infection (HAI), but the variable factors within these two routes are not well described. This study aimed to (i) investigate a cross-sectional incidence of canine respiratory viruses, including influenza (CIV), parainfluenza, distemper (CDV), respiratory coronavirus (CRCoV), adenovirus-2, and herpesvirus, in respiratory-diseased dogs, and (ii) analyze the possibly related risk factors. In total 209 dogs with respiratory illness, consisting of 133 CAI and 76 HAI dogs, were studied. Both nasal and oropharyngeal swabs were sampled from each dog and subjected for CIRDC virus detection using multiplex PCRs. Common six viruses associated with CIRDC were detected in both groups with CIV and CRCoV being predominantly found. Only CDV was significantly more prevalent in CAI than HAI dogs. Multiple virus detections were found in 81.2% and 78.9% of CAI and HAI dogs, respectively. Co-detection of CIV and CRCoV was represented the highest proportion and most often found with other CIRD viruses. Moreover, the clinical severity level was notably related to the age of infected dogs, but not to the vaccination status, sex and transmission route. Since healthy or control dogs were not included in this study, the prevalence of the CIRD virus infections could not be assessed.

1. Introduction

Canine infectious respiratory disease complex (CIRDC) is a major cause of respiratory illness and morbidity [1]. It is a complex condition for disease occurrence, involving multifactorial etiologies. Overcrowding, stress, age and other underlying factors result in interference with the dog's immune response and play a role in disease predisposition [2, 3, 4, 5, 6]. Infectious agents serve as a key factor in promoting the severity of a clinical symptom. The CIRD viruses (CIRDVs) that are commonly associated with CIRDC are canine parainfluenza (CPIV), canine distemper (CDV), canine adenovirus type 2 (CAdV-2), canine herpesvirus 1 (CaHV-1), canine respiratory coronavirus (CRCoV), and canine influenza virus (CIV) [1, 7, 8, 9].

During the last decade, novel viruses, such as canine pneumovirus (CnPnV) [10, 11] and pantropic canine coronavirus [12], which induce respiratory problems in dogs, have also been discovered. In addition, canine bocavirus [13, 14], canine hepacivirus [15] and canine circovirus [16] have been detected in respiratory tract samples of dogs showing respiratory illness, but the pathogenic roles of those viruses are poorly determined. Although new viruses have emerged, the common CIRDVs are still important contributors to respiratory disease [17, 18]. Additionally, current core vaccines that are routinely used in dogs prevent some CIRDVs, such as CPIV, CDV, and CAdV-2, but not CaHV-1, CRCoV and CIV. This lack of a vaccine for CaHV-1, CRCoV, and CIV is likely to allow the spread of infection.

Hospital-associated infection (HAI) has recently been described as an "ever-present risk" [19]. The current literature includes reported outbreaks of CPIV and CaHV-1 in animal healthcare facilities, where these nosocomial infections worsened any ongoing disease and enhanced the morbidity and mortality rates. In addition, infections among communities are documented and serve as a source for disease dissemination. Community-acquired infections (CAIs) are those that are acquired without exposure to hospitals or with limited regular exposure with a health care center or both [20]. Currently, CAIs with CIRDVs are believed to have a concomitant effect in respiratory disease [1, 21, 22, 23, 24].

The identification of multiple-viral-induced CIRDC involves the

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simultaneous rapid nucleic acid-based detection, typically by the reverse transcription polymerase chain reaction (RT-PCR) for RNA viruses and PCR for DNA viruses. These detection methods have led to an increased and better realization of the prevalence of CIRDC worldwide [3, 25, 26]. However, whether the severity of the respiratory disease depends on the number of viral infections is equivocal. Although epidemiological evidence of CIRDC has been reported periodically, the classification among HAIs and CAIs has largely not been evaluated [7, 24].

A comprehensive understanding of the source of infection (HA or CA) and other associated risk factors may help guide the successful implementation of preventive strategies. To emphasize this point, this study focused on the associations between the incidence of all six common CIRDVs, source of infection and the possible related risk factors of the dog's age, sex and vaccine status. Moreover, the relationship between clinical severity and number of viral infections was also evaluated in respiratory-ill dogs during 2013–2016 in Thailand.

2. Materials and methods

2.1. Study design

The prospective cross-sectional study was conducted from March 2013 to April 2016. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant institutional guides on the care and use of laboratory animals (Chulalongkorn University Animal Care and Use Committee Approval, No. 1431005).

2.2. Study population

A total of 209 dogs with respiratory illness were included in the study. A nasal swab (NS) and an oropharyngeal swab (OS) were obtained from each dog. These dogs with respiratory illness were obtained from the animal hospitals and private clinics located at Central (n = 127), Eastern (n = 21), Southern (n = 49), and Western (n = 12) regions of Thailand. Any dog with respiratory problems associated with underlying cardiopulmonary disease, functional or anatomical airway disease and neoplasia, based on physical examination and radiographic investigation, or any dog with a history of vaccination within 2 weeks before showing respiratory clinical signs, was excluded from the study. The history of respiratory clinical presentation at hospital or clinic for at least 72 h after being admitted was recorded and used to categorize the dogs into the CAI and HAI dog groups. The CAI group was comprised of dogs with respiratory illness at first presentation at a hospital or a clinic and with no history of hospital or clinic admission within last 3 months, while the HAI group was comprised of likewise symptomatic dogs that showed respiratory problems within 72 h after admission to a hospital or clinic.

Respiratory signs of the dogs were recorded by the attending veterinarian on the date of sampling, as follows: 1, mild cough; 2, nasal discharge and cough; 3, nasal discharge and cough with depression; 4, nasal discharge and cough with depression and evidence of bronchopneumonia based on radiographs. Due to the fact that the different viral infections may show in either local and systemic diseases and result in difference of clinical severity [27], and the respiratory scores were further graded to clinical severity level for clinical screening [28, 29, 30]. The record was further graded for the clinical severity level as mild (score 1 and 2), moderate (score 3) and severe (score 4), as described previously [6, 7]. General signalment of age, breed, sex and vaccination history for CPIV, CAdV-2 and CDV was systemically recorded for further interpretation.

2.3. Sample collection

The NS samples were collected by gently inserting a sterile rayon tipped applicator (Puritan®, Puritan Medical Product, ME, U.S.A.) into the nostril to a one-third depth of the nasal passage, while the OS samples were collected by rolling the swab onto the soft palate. The swabs were immersed in 0.5 mL of 1% sterile phosphate buffered saline (PBS) and stored at -80 °C until assayed.

2.4. Viral nucleic acid extraction and reverse transcription

Viral nucleic acids were extracted from the NS or OS samples (above) using a Viral Nucleic Acid Extraction Kit II (GeneAid, Xizhi, Taiwan) according to the manufacturer's protocol, and then quantified and qualified using Nanodrop® Lite (Thermo Fisher Scientific Inc., Massachusetts, U.S.A.) at an absorbance of 260 and 280 nm. The extracted nucleic acid was divided into two aliquots, one for two-step RT-PCR for RNA viruses (CIV, CPIV, CDV, and CRCoV) and the other for direct PCR for DNA viruses (CAdV-2 and CaHV-1). For the first stage RT-PCR assay, 100 ng of total RNA was used as the template to generate complementary DNA (cDNA) using the Omniscript® Reverse Transcription Kit (Qiagen GmbH, Hilden, Germany). The cDNA and DNA were kept at -20 °C until used for subsequent PCR amplifications.

2.5. Multiplex RT-PCR/PCR for RNA/DNA virus detection

Both multiplex RT-PCR (for CIV, CPIV, CDV and CRCoV) and multiplex PCR (for CAdV-2 and CaHV-1) were performed as previously reported [26]. In addition, a first round of RT-PCR for CRCoV detection was conducted prior to performing the multiplex RT-PCR to augment the detection sensitivity. Specific oligonucleotide primers for CIV (M gene), CPIV (NP gene), CDV (NP gene), CRCoV (S gene), CAdV-1 (E3 gene), CaHV-1 (GB gene), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), as an internal control, were used. The PCR primer sequences, reaction mixture, assay conditions and amplicon visualization were performed as previously reported [26].

2.6. Statistical analysis

Descriptive analysis was used as the median for age. Data were evaluated using the SPSS statistical analyser version 22.0 software (IBM Corp, New York, U.S.A). Differentiation of the CAI and HAI groups was compared using Fisher's exact and χ^2 tests. Clinical severity level against the demographic of infected dogs was performed using χ^2 tests. Incidence of CIRDC viruses was calculated with a confidence interval (CI) of 95%. In these cases, a P-value of <0.05 was considered statistically significant. The associated respiratory score with respect to the CIRDCV detections was evaluated using χ^2 tests for individual infection, while logistic regression was evaluated in multiple infections. Multiple comparisons with a Bonferroni correction a significant P-value at 0.001 was considered statistically significant.

3. Results

3.1. Clinical data of study population

The 209 dogs from four regions of Thailand included in this study were categorized into 133 CAI and 76 HAI dogs based on the presentation of their respiratory symptoms and history of time of clinical presentation. Vaccination against CPIV, CDV, and CAdV-2 accounted for 32.3% (43/133) and 28.9% (22/76) dogs in the CAI and HAI groups, respectively. Unvaccinated CAI and HAI dogs were 56.4% (75/133) and 59.2% (45/76), respectively. No history of vaccination was recorded in 15 (11.3%) and 9 (11.8%) of the CAI and HAI dogs, respectively.

The CAI group was comprised of 58.6% (78/133) male and 39.8% (53/133) female dogs, while the HAI group consisted of 46.1% (35/76) male and 43.4% (33/76) female dogs. Sex was not documented in two CAI and eight HAI group dogs. The age of the dogs ranged from 1 month to 14 years in the CAI group (median; 7 months, 95% CI; 2.92–3.08) and from 0.5 month to 13 years in the HAI group (median; 7 months, 95% CI; 1.89–2.11). Shi Tsu was the major breed in both the CAI (48.2%) and HAI (39.1%) groups. Coughing served as a common respiratory sign in dogs

from both groups, accounting for 78.2% (CAI group) and 78.9% (HAI group) of the dogs, followed by nasal discharge, depression and bronchopneumonia as 66.2% (CAI) and 61.8% (HAI), 49.6% (CAI) and 53.9% (HAI), and 28.6% (CAI) and 30.3% (HAI), respectively.

3.2. Detection of respiratory viruses

Analysis by multiplex PCR assays revealed that most dogs in both groups were positive for at least one CIRDV (96.9% in CAI and 94.7% in HAI groups). Among the six common CIRDVs, CIV and CRCoV were commonly found in both groups (CIV; 57.9% for CAI, 60.5% for HAI and CRCoV; 62.4% for CAI, 59.2% for HAI), while CAdV-2 was the least frequently detected (8.3% for CAI and 11.8% for HAI). The populations of other viruses in the CAI and HAI groups were CPIV, CaHV-1, and CDV, respectively. The CAI dogs had no statistical significance to the HAI dogs in term of virus detections (Table 1).

Multiple virus infections were detected at similar levels in both groups, at 81.2% (108/133) of CAI dogs and 78.9% (60/76) of HAI dogs, where double viral detections were the most frequently found (Table 2). The frequency of multiple virus detections in both groups decreased with increasing numbers of viruses, with no infection with all six viruses being found in either the CAI or HAI dogs. There was no significant difference between the CAI and HAI dogs in terms of multiple virus infections.

When the variable demographic factors and single or multiple CIRDV detections were analyzed, the main population was male and puppy in both groups. The common respiratory problems in both groups were nasal discharge, cough and depression. However, there was no association between sex, age, vaccination status and respiratory signs for single or multiple CIRDV detections (Table 3). The variable demographic factors were also compared with the clinical severity level, revealing that most CIRDV-infected puppies had a greater severity compared with the other age groups. There was a statistically significant association between the age of dogs and clinical severity level ($P = 0.012$), with the exception of sex, vaccination status, type of affected dog and number of different CIRDVs detected (Table 4). The respiratory score was compared with CIRD agents detected in both the CAI and HAI groups (Table 5). Most CIRDV-positive dogs expressed a respiratory score of 3 or 4 in both the CAI and HAI groups, which accounted for 60.2% (80/133) and 61.8% (47/76) respectively. Moreover, double infection with CIV and CRCoV was predominantly detected in both groups with a statistical association ($P = 0.009$, Table 5).

4. Discussion

The CIRDC, a common respiratory disease complex in dogs, is associated with environmental factors, individual host susceptibility and infectious pathogens, which are primarily viruses [1, 31, 32, 33]. The CIRDVs are commonly detected in dogs with respiratory problems and are endemic in poor conditioned dogs, overcrowded shelters, hospitals and pet grooming centers [2, 3, 8, 19, 34]. Currently, the prevalence and types of HAIs are not well established compared to CAIs. The epidemiology of CIRDV-infected dogs has been reporting over many years but with contrasting results [3, 8, 24]. In this study, we focused on the incidence of CIRDV detections in terms of CAI and HAI, which are supposed to be an important factor of CIRDC infections. The HAI-diseases, especially respiratory tract infections, have been considered as a risk for nosocomial transmission, but they have not been investigated in clinically-infected dogs [19, 34]. In this study, all six common CIRDVs

Table 1
Detection of CIRDVs in respiratory ill dogs in the CAI and HAI groups using multiplex PCR assays.

CIRDV	CAI[a]	HAI[a]	*P*-value
CIV	57.9 (77/133) 49.4–65.9	60.5 (46/76) 49.3–70.8	0.701
CPIV	39.1 (52/133) 31.2–47.6	32.9 (25/76) 23.4–44.1	0.371
CDV	35.3 (47/133) 27.7–43.8	22.4 (17/76) 14.4–33.3	0.049
CRCoV	62.4 (83/133) 53.9–70.2	59.2 (45/76) 47.9–69.6	0.648
CAV-2	8.3 (11/133) 4.5–14.3	11.8 (9/76) 6.1–21.2	0.398
CHV	39.1 (52/133) 31.2–47.6	32.9% (25/76) 23.4–44.1	0.371

[a] Data are shown as the %, (number of infected dogs/total number assayed) and (below) the 95% CI limits.

Table 2
Detection of multiple CIRDV infections in dogs with respiratory illness in the CAI and HAI groups using multiplex PCR assays.

Infection	CAI dogs[a]	HAI dogs[a]	*P*-value
2 viruses	35.3 (47/133) 27.72–43.78	43.4 (33/76) 32.86–54.62	0.248
3 viruses	30.1 (40/133) 22.91–38.86	27.6 (21/76) 18.78–38.63	0.709
4 viruses	13.5 (18/133) 8.65–20.47	5.3 (4/76) 1.67–13.16	0.061
5 viruses	2.3 (3/133) 0.48–6.72	2.6% (2/76) 0.17–9.65	0.864
6 viruses	0	0	-

[a] Data are shown as the %, (number of infected dogs/total number assayed) and (below) the 95% CI limits.

Table 3
Characteristics of 209 dogs with respiratory illness compared between the numbers of different CIRDV infections, as detected using multiplex PCR assays.

Characteristic	Number of infections			*P*-value
	Single CIRD virus (n = 33) N (%) [95% CI]	Multiple CIRD viruses (n = 168) N (%) [95% CI]	Negative (n = 8) N (%) [95% CI]	
Sex				0.824
Male	18 (54.5) [40.7–73.6]	90 (53.6) [48.2–63.4]	5 (62.5) [30.4–86.5]	
Female	13 (39.4) [26.4–59.3]	71 (42.2) [36.7–51.8]	2 (25.0) [6.3–59.9]	
N/A	2 (6.1) [0.68–20.6]	7 (4.2) [1.9–8.5]	1 (12.5) [0.01–49.2]	
Age[a]				0.527
Puppy	11 (33.3) [19.7–50.5]	58 (34.5) [27.7–42.0]	7 (87.5) [40.1–93.7]	
Growing	8 (24.2) [13.0–42.3]	49 (29.2) [22.9–36.7]	1 (12.5) [0.11–49.2]	
Adult	9 (27.3) [14.9–44.4]	28 (16.7) [11.8–23.2]	0 (0)	
Senior	5 (15.2) [6.4–32.2]	33 (19.6) [14.4–26.5]	0 (0)	
Vaccination				0.949
Yes	10 (30.3) [19.8–52.7]	53 (31.6) [27.9–43.0]	2 (25.0) [6.3–59.9]	
No	19 (57.6) [47.3–80.2]	98 (58.3) [57.0–72.1]	3 (37.5) [13.5–69.6]	
N/A	4 (12.1) [4.2–27.9]	17 (10.1) [6.3–15.7]	3 (37.5) [13.5–69.6]	
Clinical sign				
Nasal discharge	27 (81.8) [65.3–91.8]	129 (76.8) [69.8–82.6]	8 (100.0) [62.7–100]	0.526
Cough	23 (69.7) [52.5–87.5]	107 (63.7) [56.2–70.6]	5 (62.5) [30.38–86.5]	0.509
Depression	18 (54.5) [68.6–93.8]	84 (51.5) [42.5–57.5]	5 (62.5) [30.38–86.5]	0.633
Bronchopneumonia	10 (30.3) [17.3–47.5]	46 (27.4) [21.8–34.6]	5 (62.5) [30.38–86.5]	0.732

N/A: no data available.
[a] Age of the infected dogs: puppy <3 months; growing >3 month–1 y; adult > 1–5 y; senior >5 y.

Table 4
Clinical severity levels of dogs with respiratory illness associated with possible variable factors.

Characteristic	Clinical severity level[a]			*P*-value
	Mild (n = 83) N (%) [95% CI]	Moderate (n = 75) N (%) [95% CI]	Severe (n = 51) N (%) [95% CI]	
Sex				0.775
Male	45 (54.2) [45.3–66.6]	43 (57.3) [48.2–70.3]	25 (49.0) [39.2–66.7]	
Female	35 (42.2) [33.9–55.3]	29 (38.7) [29.7–51.8]	22 (43.1) [33.3–60.8]	
N/A	3 (3.6) [0.8–10.5]	3 (4.0) [0.9–11.6]	4 (7.8) [2.6–19.0]	
Age				0.012*
Puppy	26 (31.3) [22.3–41.9]	21 (28.0) [19.1–39.1]	29 (57.0) [42.3–68.8]	
Growing	25 (30.1) [21.6–41.1]	24 (32.0) [17.4–34.6]	9 (17.6) [9.54–31.0]	
Adult	17 (20.5) [13.1–30.5]	11 (14.7) [6.4–19.5]	9 (17.6) [9.54–31.0]	
Senior	15 (18.1) [11.3–28.1]	19 (25.3) [12.9–28.9]	4 (7.8) [2.6–19.4]	
Vaccination				0.845
Yes	26 (31.3) [24.9–45.9]	22 (29.3) [23.1–45.4]	17 (33.3) [25.7–53.4]	
No	49 (59.1) [54.0–75.1]	44 (58.7) [54.6–76.9]	27 (53.0) [46.6–74.3]	
N/A	8 (9.6) [4.7–18.1]	9 (12.0) [6.2–21.5]	7 (13.7) [6.5–26.0]	
Type[b]				0.988
CAI	53 (63.9) [53.1–73.4]	48 (64.0) [52.7–73.9]	32 (62.7) [49.0–74.7]	
HAI	30 (36.1) [26.6–46.9]	27 (36.0) [26.0–47.3]	19 (37.3) [25.3–51.0]	
Number of infections				0.590
Negative infection	1 (1.2) [< 0.01–7.2]	2 (2.7) [< 0.01–9.8]	5 (9.8) [3.8–21.4]	
Single infection	13 (15.7) [9.3–25.1]	10 (13.3) [7.2–23.0]	10 (19.6) [10.8–32.7]	
Double infection	32 (38.6) [28.8–49.3]	30 (40.0) [29.7–51.3]	18 (35.3) [23.6–49.1]	
Triple infection	26 (31.3) [22.3–41.9]	22 (29.3) [20.2–40.5]	13 (25.5) [15.4–38.9]	
Quadruple infection	9 (10.8) [5.6–19.6]	9 (12.0) [6.2–21.5]	4 (7.8) [2.6–19.0]	
Quintuple infection	2 (2.4) [< 0.01–8.9]	2 (2.7) [< 0.01–9.8]	1 (2.0) [< 0.01–11.3]	

[a] Clinical severity level: mild (score 1–2), moderate (score 3), and severe (score 4).
[b] CAI: Community-acquired infection (n = 133); HAI: Hospital-associated infection (n = 76).
* Statistically significant.

were detected in both sample groups, suggesting that these viruses might either be present or disseminated in both environments. Moreover, we found that CRCoV and CIV had a higher detection rate in both the CAI and HAI dogs, while CPIV had a lower rate. This finding is in contrast to previous observations that found CPIV was the most commonly detected virus in CIRDC dogs [3, 7, 24]. Furthermore, it is surprising that 96.9% in CAI and 94.7% in HAI groups displayed at least one virus detection, which is unusual in previous observations for canine respiratory disease that revealed the low occurrence of CIRD viruses [4, 5, 7, 35], although this might be influenced by various factors, including the different geography, sample size, sample population, sampling protocols and also validated detection method.

The evidence of CDV, CPIV, CAdV-2, CaHV-1, and CIV circulation in Thailand has been documented previously [17, 18, 26, 36, 37], but not for CRCoV. This study, therefore, is the first report of CRCoV-infected dogs in Thailand. The most commonly detected viruses were CIV and CRCoV (>50%) in both the CAI and HAI groups, which is in contrast to previous reports in Asia [38] and Europe [3, 7, 24, 39].

Meanwhile, CIRDVs were detected in both the CAI and HAI dogs without any significant difference in the frequency of occurrence among the two groups, potentially suggesting that these viruses are already circulating in both sample groups. This is in accordance with a recent study that reported that there was no significant difference in CPIV, CAdV-2 and CRCoV infection levels between dogs from private households and those from shelters or kennels [3, 40].

In the present study, 81.2% (108/133) of CAI and 78.9% (60/76) of HAI dogs had multiple CIRDV infections, supporting the complex condition of the disease, which is often manifested as a co-infection rather than a single pathogen [2, 3, 24, 26, 38]. We also observed that the majority of multiple CIRDV infections were co-detected with either CIV, CRCoV, or both. However, this observation does not allow interpretation as to whether they are primary and/or secondary pathogens. Infection with CIV and CRCoV usually induces mild clinical symptoms by interfering with the respiratory defense mechanism and so leads to super-infections with other pathogens [1, 41, 42]. Moreover, co-infection of CIV and CRCoV may be synergistic and lead to severe tracheobronchitis [41].

There were no significant differences in sex, age and vaccination status of the dogs between single and multiple virus detections. However, the vaccinated dogs had a lower proportion of both single and multiple detections compared with the unvaccinated dogs. Interestingly, there was a significant difference in the CIRD-affected age groups when compared with the clinical severity level. CIRD-affected puppies had a more severe clinical level compared with the dogs in the other age groups. This could reflect their increased host susceptibility, such as from a premature immune response and being unvaccinated. Further study with large-scale sample populations and meta-analysis of clinical information is warranted.

Significant association between CIRD agents and clinical respiratory scores was not observed in co-detection with CIV and CRCoV. This co-detection represented the highest proportion and was most often found with other CIRDVs. Thus, CIV and/or CRCoV could be either primary or secondary agents that are commonly found as co-infections with the other pathogens. However, bacterial infections could not be ruled out in this study. Many investigations have revealed that either viral or bacterial co-infection leads to an increased severity, but some studies have revealed no significant differences in the clinical severity between single and multiple infections [24, 39, 43, 44].

Since healthy or control dogs were not included in this study, then the prevalence of the CIRDV infections could not be assessed. Furthermore, this study used upper airway swabs as the sampling method for virus detection with PCR. It has been widely shown that healthy dogs may also have positive PCR results for CIRDVs in upper airway samples. Thus, a positive viral PCR in an upper airway sample is not necessarily proof of a symptomatic infection but could represent only exposure to the pathogen. Thus, the result of CIRDV detection by PCR from respiratory swabs can only imply that they represented the CIRD pathogens that might be associated with respiratory problem. Moreover, since we only focused on six viral pathogens associated with CIRDC, then the role of other pathogens, including known bacterial pathogens, such as *Bordetella bronchoseptica* and *Mycoplasma* sp., and other novel viruses, such as CnPnV, that might be implicated remains unknown.

5. Conclusions

All six respiratory CIRDVs examined, and primarily CIV and CRCoV, were detected in both the CAI and HAI dogs. Co-infections with CIV and CRCoV were often detected with themselves or others in both study groups. No difference in the types of CIRD-associated viruses was found between the CAI and HAI groups, with the exception of the CDV being associated more with CAI dogs. The clinical severity of CIRDC infection was related to age of the affected dogs, but not for multiple infections, type of affected dogs (CAI and HAI), and vaccination status. Additionally, the variable respiratory signs and vaccination did not differ between

Table 5
Association of CIRD agents with the clinical respiratory score.

	CAI[a]				HAI[a]				Total	*P*-value
	Clinical respiratory score				Clinical respiratory score					
	1	2	3	4	1	2	3	4		
Negative	0/20	6.1% (2/33)	2.5% (1/40)	2.5% (1/40)	7.7% (1/13)	0/16	8.3% (2/24)	4.3% (1/23)	3.8% (8/209)	0.474
CDV	0/20	3.0% (1/33)	0/40	0/40	0/13	0/16	0/24	0/23	0.5% (1/209)	0.947
CaHV-1	0/20	12.1% (4/33)	5.0% (2/40)	10.0% (4/40)	0/13	0/16	4.2% (1/24)	4.3% (1/23)	5.7% (12/209)	0.580
CIV	5.0% (1/20)	3.0% (1/33)	5.0% (2/40)	5.0% (2/40)	7.7% (1/13)	6.3% (1/16)	0/24	4.3% (1/23)	4.3% (9/209)	0.662
CPIV	5.0% (1/20)	0/33	0/40	0/40	0/13	6.3% (1/16)	4.2% (1/24)	8.7% (2/23)	2.4% (5/209)	0.134
CRCoV	0/20	0/33	5.0% (2/40)	2.5% (1/40)	0/13	12.5% (2/16)	0/24	4.3% (1/23)	2.9% (6/209)	0.086
CAdV-2+CaHV-1	0/20	0/33	0/40	2.5% (1/40)	0/13	6.3% (1/16)	4.2% (1/24)	0/23	1.4% (3/209)	0.220
CDV + CAdV-2	0/20	0/33	0/40	0/40	0/13	0/16	0/24	4.3% (1/23)	0.5% (1/209)	0.058
CDV + CaHV-1	0/20	0/33	2.5% (1/40)	2.5% (1/40)	0/13	0/16	4.2% (1/24)	0/23	1.4% (3/209)	0.568
CDV + CRCoV	0/20	0/33	5.0% (2/40)	7.5% (3/40)	0/13	0/16	4.2% (1/24)	0/23	2.9% (6/209)	0.641
CIV + CDV	0/20	0/33	2.5% (1/40)	2.5% (1/40)	0/13	0/16	0/24	0/23	1.0% (2/209)	0.865
CIV + CaHV-1	5.0% (1/20)	0/33	0/40	5.0% (2/40)	7.7% (1/13)	6.3% (1/16)	8.3% (2/24)	0/23	3.3% (7/209)	0.282
CIV + CPIV	0/20	6.1% (2/33)	2.5% (1/40)	0/40	7.7% (1/13)	0/16	0/24	4.3% (1/23)	2.4% (5/209)	0.404
CIV + CRCoV	5.0% (1/20)	12.1% (4/33)	20.0% (8/40)	7.5% (3/40)	46.2% (6/13)	25.0% (4/16)	20.8% (5/24)	8.7% (2/23)	15.8% (33/209)	0.009*
CPIV + CDV	10.0% (2/20)	3.0% (1/33)	2.5% (1/40)	2.5% (1/40)	0/13	0/16	0/24	0/23	2.4% (5/209)	0.601
CPIV + CaHV-1	0/20	6.1% (2/33)	0/40	0/40	7.7% (1/13)	0/16	0/24	4.3% (1/23)	1.9% (4/209)	0.282
CPIV + CRCoV	15.0% (3/20)	0/33	0/40	2.5% (1/40)	0/13	0/16	0/24	4.3% (1/23)	2.4% (5/209)	0.642
CRCoV + CAdV-2	0/20	0/33	0/40	0/40	0/13	0/16	0/24	4.3% (1/23)	0.5% (1/209)	0.057
CRCoV + CaHV-1	0/20	3.0% (1/33)	2.5% (1/40)	5.0% (2/40)	0/13	0/16	0/24	4.3% (1/23)	2.4% (5/209)	0.592
CDV + CAdV-2+CaHV-1	5.0% (1/20)	0/33	0/40	0/40	7.7% (1/13)	0/16	0/24	0/23	1.0% (2/209)	0.077
CDV + CRCoV + CaHV-1	0/20	0/33	0/40	5.0% (2/40)	0/13	0/16	0/24	0/23	1.0% (2/209)	0.865
CIV + CDV + CaHV-1	5.0% (1/20)	0/33	2.5% (1/40)	0/40	0/13	6.3% (1/16)	4.2% (1/24)	0/23	1.9% (4/209)	0.399
CIV + CDV + CRCoV	5.0% (1/20)	3.0% (1/33)	5.0% (2/40)	10.0% (4/40)	7.7% (1/13)	6.3% (1/16)	4.2% (1/24)	0/23	5.3% (11/209)	0.677
CIV + CPIV + CAdV-2	0/20	3.0% (1/33)	2.5% (1/40)	0/40	0/13	0/16	0/24	0/23	1.0% (2/209)	0.865
CIV + CPIV + CDV	0/20	0/33	0/40	0/40	0/13	0/16	0/24	4.3% (1/23)	0.5% (1/209)	0.058
CIV + CPIV + CRCoV	5.0% (1/20)	6.1% (2/33)	2.5% (1/40)	10.0% (4/40)	0/13	12.5% (2/16)	8.3% (2/24)	8.7% (2/23)	6.7% (14/209)	0.563
CIV + CRCoV + CAdV-2	0/20	3.0% (1/33)	2.5% (1/40)	0/40	0/13	0/16	0/24	0/23	1.0% (2/209)	0.865
CIV + CRCoV + CaHV-1	10.0% (2/20)	6.1% (2/33)	0/40	5.0% (2/40)	0/13	6.3% (1/16)	0/24	13.0% (3/23)	4.8% (10/209)	0.146
CPIV + CDV + CAdV-2	0/20	0/33	5.0% (2/40)	0/40	0/13	0/16	4.2% (1/24)	0/23	1.4% (3/209)	0.568
CPIV + CDV + CaHV-1	0/20	3.0% (1/33)	2.5% (1/40)	0/40	0/13	0/16	0/24	4.3% (1/23)	1.4% (3/209)	0.542
CPIV + CRCoV + CAdV-2	0/20	0/33	0/40	0/40	0/13	0/16	4.2% (1/24)	0/23	0.5% (1/209)	0.069
CPIV + CRCoV + CaHV-1	5.0% (1/20)	6.1% (2/33)	2.5% (1/40)	2.5% (1/40)	0/13	0/16	4.2% (1/24)	0/23	2.9% (6/209)	0.641
CIV + CDV + CRCoV + CaHV-1	5.0% (1/20)	3.0% (1/33)	2.5% (1/40)	0/40	0/13	0/16	4.2% (1/24)	0/23	1.9% (4/209)	0.645
CIV + CPIV + CAdV-2+CaHV-1	0/20	0/33	2.5% (1/40)	0/40	0/13	0/16	0/24	0/23	0.5% (1/209)	0.947
CIV + CPIV + CDV + CRCoV	0/20	9.1% (3/33)	7.5% (3/40)	2.5% (1/40)	0/13	6.3% (1/16)	0/24	0/23	3.8% (8/209)	0.449
	0/20	0/33	0/40		0/13	0/16	0/24	0/23		0.947

(*continued on next page*)

Table 5 (*continued*)

	CAI[a]				HAI[a]				Total	*P*-value
	Clinical respiratory score				Clinical respiratory score					
	1	2	3	4	1	2	3	4		
CIV + CPIV + CRCoV + CAdV-2				2.5% (1/40)					0.5% (1/209)	
CIV + CPIV + CRCoV + CaHV-1	10.0% (2/20)	3.0% (1/33)	2.5% (1/40)	0/40	0/13	0/16	0/24	0/23	1.9% (4/209)	0.686
CPIV + CDV + CRCoV + CaHV-1	5.0% (1/20)	0/33	0/40	2.5% (1/40)	0/13	0/16	0/24	4.3% (1/23)	1.4% (3/209)	0.542
CPIV + CRCoV + CAdV-2+CaHV-1	0/20	0/33	0/40	0/40	0/13	0/16	4.2% (1/24)	0/23	0.5% (1/209)	0.375
CIV + CPIV + CDV + CRCoV+ CAdV-2	0/20	0/33	2.5% (1/40)	0/40	0/13	0/16	4.2% (1/24)	0/23	1.0% (2/209)	0.375
CIV + CPIV + CDV + CRCoV + CaHV-1	0/20	0/33	2.5% (1/40)	2.5% (1/40)	0/13	0/16	0/24	4.3% (1/23)	1.4% (3/209)	0.542
	9.6% (20/209)	15.8% (33/209)	19.1% (40/209)	19.1% (40/209)	6.2% (13/209)	7.7% (16/209)	11.5% (24/209)	11.0% (23/209)		

CIV, canine influenza; CPIV, canine parainfluenza; CDV, canine distemper; CRCoV, canine respiratory coronavirus; CAdV-2, canine adenovirus-2; CaHV-1, canine herpesvirus 1.

[a] Data are shown as the % (number of infected dogs/total number assayed).

[*] Multiple comparisons with a Bonferroni correction a significant P-value is in the region of 0.001.

single and multiple infections. The high detection rate of the CaHV-1, CRCoV, and CIV is evidence for the need for further consideration of a suitable vaccination regimen.

Declarations

Author contribution statement

Chutchai Piewbang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Anudep Rungsipipat, Yong Poovorawan: Conceived and designed the experiments; Analyzed and interpreted the data.

Somporn Techangamsuwan: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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