Risk factors associated with canine parvovirus disease in dogs: A case-control study

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ABSTRACT

Canine parvovirus type-2 (CPV-2) is one of the most contagious viral agents causing acute enteritis in pups with high rate of morbidity and mortality. The study aimed to investigate the risk factors associated with canine parvovirus (CPV) occurrence in dogs in Ho Chi Minh City, Vietnam. A total number of 132 dogs less than six months of age were recruited in the study and split into two groups: 'CPV diseased dogs' included 44 (33.3%) dogs positive with canine parvovirus confirmed by rapid immuno-migration test, and 'normal dogs' consisted of 88 (66.7%) healthy dogs. Results indicated that the unvaccinated dogs had 11.76 times more risk of CPV infection than vaccinated dogs (OR = 11.76, $P < 0.001$). The risk of CPV in dogs raised with others at the same age was 5.01 times (OR = 5.01, $P = 0.027$) higher than those raised singly. The risk of CPV infection of dogs interacting with neighbour dogs was 3.13 times (OR = 3.13, $P = 0.028$) higher than the dogs having no contact with other dogs living nearby. Briefly, our study highlights potential factors for CPV disease prevention, especially CPV vaccination is the most important driver affecting the occurrence of parvovirus in young dogs.

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1. Introduction

In the 1970s, outbreaks of severe gastroenteritis caused by canine parvovirus type-2 (CPV-2) in dogs were first reported in Europe (1976) and the United States (1978) (Pollock & Coyne, 1993). The emergence of canine parvovirus disease in dogs have been currently spreading worldwide (Decaro & Buonavoglia, 2012). Gastroenteritis and myocarditis are recognized as two common clinical forms of CPV. While myocarditis is rarely found due to maternal effective immunization protecting pups in early period of life (Mohammed et al., 2008), gastroenteritis commonly occurs particularly in less than 6-month aged dogs with clinical signs including bloody foul smelling diarrhoea, vomiting and severe dehydration (Goddard & Leisewitz, 2010). The infection is transmitted by fecal-oral route through direct contact with contaminated feces or exposure to CPV fomites (Lamm & Rezabek, 2008). Canine parvovirus type-2 is highly contagious and stable under a variety of environmental conditions for months to years (Ling et al., 2012). The high mortality due to CPV was mostly found in unvaccinated dogs and up to 91% in cases without therapeutic intervention (Brady et al., 2012).

Previous studies around the world investigated the association between CPV infection and factors related to dogs' general information (i.e.,

age, gender, breed, vaccination status, etc.) (Mohammed et al., 2008; Naveenkumar et al., 2019; Sharma, 2019), dogs' clinical information (i.e., dehydration, rectal temperature, vomiting, diarrhoea, etc.) (Miranda et al., 2015), dog free-roaming access (Hsu et al., 2003), space and time of CPV infection (Brady et al., 2012). In Vietnam, since the first cases of CPV were observed in dogs in 1994 (unpublished data), there have been subsequent reports on parvovirus infection, which mostly focused on the evolution of antigenic CPV (Nakamura et al., 2004), incidence of parvovirus in dogs (Mai et al., 2018), molecular characterization of CPV (Quynh & Dai, 2014; Hoang et al., 2019; Vo et al., 2019). There is still little known about the factors regarding increased occurrence of CPV infection in dogs in Vietnam. Thus, our study aimed to investigate risk factors as potential drivers associated with canine parvovirus in dogs. The results of study will serve to increase awareness of dog owners in CPV prevention and support veterinarians in early treatment of CPV in dogs in Vietnam.

2. Materials and Methods

2.1. Study area and dogs' selection

The study was conducted at a veterinary clinic located in Thu Duc District, Ho Chi Minh city from January 2020 to June 2020. Dogs delivered to the clinic with typically CPV clinical suspicion (i.e., smelly haemorrhagic diarrhea, vomiting, rapid dehydration, lethargy and anorexia) (Oliveira et al., 2018) and confirmed using Rapid Immune-migration (Rapid CPV Ag Test Kit, Zhenrui Biotech Inc., Shenzhen, China) were included in a group named 'CPV diseased dogs'; this group was defined as cases in the study. Besides, another group named 'normal dogs', defined as controls, was healthy dogs carried for other services provided at clinic (i.e., vaccine injection, grooming, spa) with the criteria of normal vital signs (body temperature 38 - 39°C, heart rate 70 - 120 beats/min, respiratory rate 18 - 34 breaths/min) and healthy appearance (moist nose, pink gums, bright eyes without watering, smooth and supple skin, glossy coat, agile signs) (Bukowski & Aiello, 2011). The clinic had a service of weekly calling to dog owners to ask about pet's health condition; healthy dogs were defined in controls were those without any announcement of CPV disease signs after a week from the date dogs brought to the clinic. This criterion helped to define 'normal dogs' were truly healthy and had no infection of parvovirus since the incubation period of CPV in dogs is ranged from 3 - 7 days (Lamm & Rezabek, 2008).

2.2. Detect canine parvovirus by rapid immuno-migration

The test selection was relied on its absolute sensitivity (96.9%) and specificity (96.9%) in a good agreement with hemagglutination inhibition (HI) assay considered as 'gold standard' for detecting CPV in dogs (VETSCAN, 2018.). The principle of the rapid test is to detect specific epitopes of a soluble antigen of all strains of canine parvovirus shed in diseased dog's feces by using Rapid immuno-migration. Samples containing CPV antigen when put into contact with sensitized particles would create a complex which then migrates along a membrane before captured on a sensitized reaction zone where the cumulative concentration could result in a clearly visible purple band. Another purple band located in opposite side of the membrane on the kit is a control band to ensure the test was performed correctly.

2.3. Data collection

The dog owners in both groups of cases and controls were asked to participate in the study and approve providing their responses to structured questionnaire. The questions focused on groups of information could be potential risk factors affecting occurrence of parvovirus in dog including: (1) demographic characteristics of dog owners: age, gender; (2) dog-related information: age, gender, breed of dogs; and (3) dog-caring practice: CPV vaccination status, dog-keeping habits, raised with other dog/s, interaction with neighbour dog/s. Details of participant information sheet (PIS) and information consent form (ICF) have been read carefully and signed by all participants in study.

2.4. Statistical analyses

Risk factor analyses for outcome variable of CPV status (CPV diseased dogs/ normal dogs) were carried out by logistic regression. The explanatory variable investigated were: (1) dog's owner gender (male/female); (2) dog's owner age (years); (3) dog's gender (male/female); (4) dog's

age (months); (5) breed of dog (Vietnamese native/ foreign/ mixed); (6) CPV vaccination status (yes/no); (7) dog-keeping habit (mostly inside the house/ freely outside access/ restrictively outside access); (8) raising with other dog/s (same-aged dog/ different-aged dog/ no); and (9) interaction with neighbour dog/s (yes/no/unknown). Stepwise approach was applied to select a final model of multivariable model. Univariable models were screened and those with $P < 0.20$ were kept as a candidate for final model. Variables of multivariable model with $P < 0.05$ were considered as significant factors (risk/protective) associated with infection of parvovirus in dogs. All statistical analyses were done using R statistical software (The R, 2020).

3. Results and Discussion

3.1. The selection progress of study dogs

Over study period of 6 months, a total number of 44 cases of CPV infection were investigated by the rapid test. In 'normal dogs' group, a total number of 107 dogs participated in the study. However, since all 44 CPV infected dogs were all less than 6 months old, in order to avoid selection bias, we classified 'normal dogs' group and selected only 88 dogs (out of 107) with less than 6-month age as non-cases or controls for epidemiological analyses. The geographical locations of a total number of 132 study dogs (44 cases and 88 controls) are presented in Figure 1.

3.2. Descriptive characteristics of dog's owners and dog-related information

Demographic information of dog's owners and dog-related characteristics are described in Table 1. Out of 44 CPV infected dogs, the proportion of dogs injected CPV vaccine was 25.0% (11/44), whereas 81.8% (72/88) dogs in normal group had vaccination of CPV ($P = 0.005$). More than half of study dogs (50.8%) were foreign breed (i.e., Poodle, Dachshund, Pomeranian, Rockweiler, Cardigan Welsh Corgi, Pekingese, etc.), the remaining dogs were equal (∼25%) in Vietnamese native breed (Indochina dingo, Phu Quoc ridgeback) and mixed breed (Indochina dingo cross-bred with foreign breed); the highest morbidity of CPV infection was in Vietnamese native breed (40.9%), while in foreign and mixed breed the morbidity were 36.4% and 22.7% respectively ($P = 0.005$). Study dogs were mostly kept inside the house, the percentages were 54.6% and 64.8% in both CPV diseased and normal group; however, in case group the percentage of dogs freely outside access was relatively high, accounted for 22.7% (10/44) compared with small percentage 5.7% (5/88) in control group ($P = 0.01$). Dogs raised with the same-aged dogs had morbidity of parvovirus in case group (22.7%) higher than those in normal group (8.0%) ($P = 0.035$). Besides, history of interacting with neighbour dog/s between two groups was significantly different, 50.0% for case group and 33.0% for control group ($P = 0.014$).

3.3. Risk factor analyses

In the multivariable model, three variables of CPV vaccination status, raising with other dogs and interaction with neighbour dogs remained significant from the univariable models (Table 2). Unvaccinated dogs had 11.76 times more risk of getting parvovirus disease than vaccinated ones (OR = 11.76, $P < 0.001$). The risk of CPV infection in dogs raised with other same-aged dogs was 5.01 times (OR = 5.01, $P = 0.027$) higher than those raised singly. Dogs having interaction with neighbour dog/s had 3.13 times (OR = 3.13, P = 0.028) increased risk of getting CPV than dogs without any contact with other nearby dog/s. Variables of Breed and Dog-keeping habit became non-significant in final model since they were confounded by variable CPV Vaccination status (χ^2 Test, $P = 0.001$; Fisher's Test, $P = 0.002$).

3.4. Discussion

To our knowledge, this is the first study investing of factors associated with CPV infection in Vietnamese dogs. We conducted a case-control study with case group was identified using the rapid antigen detection test, and control group was defined based on vital and healthy signs at the time dogs delivered to the clinic and without any announcement of getting disease a week afterward. Control group was therefore appropriate and comparable to the case group in estimates of expected exposures in our study.

In our study, all participants were raising less than 6-month dogs in both groups. The selection bias was considered by exclusion of dogs higher than 6 months of age to get truly representative population from which the cases might have expo-

Table 1. Descriptive characteristics of dog's owners and dog-related information

Characteristics		CPV diseased dogs (n = 44)	Normal dogs (n = 88)	*P*-value
Owner's age* (Median [IQR])		35.0 [29.8-40.0]	33.5 [28.0-39.0]	0.542
Owner's gender** (%)	Male	23 (52.3%)	34 (38.6%)	0.192
	Female	21 (47.7%)	54 (61.4%)	
Dog's age** (%)	$\leq$ 3 months	30 (68.2%)	52 (59.1%)	0.409
	$>$ 3 months	14 (31.8%)	36 (40.9%)	
Dog's gender** (%)	Male	29 (65.9%)	50 (56.8%)	0.415
	Female	15 (34.1%)	38 (43.2%)	
Breed of dogs** (%)	Vietnamese native	18 (40.9%)	14 (15.9%)	0.005
	Foreign	16 (36.4%)	51 (58.0%)	
	Mixed	10 (22.7%)	23 (26.1%)	
CPV vaccination status** (%)	Yes	11 (25.0%)	72 (81.8%)	0.005
	No	33 (75.0%)	16 (18.2%)	
Dog-keeping habit*** (%)	Mostly inside the house	24 (54.6%)	57 (64.8%)	0.010
	Freely outside access	10 (22.7%)	5 (5.7%)	
	Restrictively outside access	10 (22.7%)	26 (29.5%)	
Raising with other dogs** (%)	Yes (same-aged dog/s)	10 (22.7%)	7 (8.0%)	0.035
	Yes (different-aged dog/s)	12 (27.3%)	21 (23.9%)	
	No	22 (50.0%)	60 (68.1%)	
Interaction with neighbour dog/s** (%)	Yes	22 (50.0%)	29 (33.0%)	0.014
	No	15 (34.1%)	57 (64.8%)	
	Unknown	7 (15.9%)	2 (2.2%)	

*Wilcoxon Test, **χ^2 Test, ***Fisher's Test.

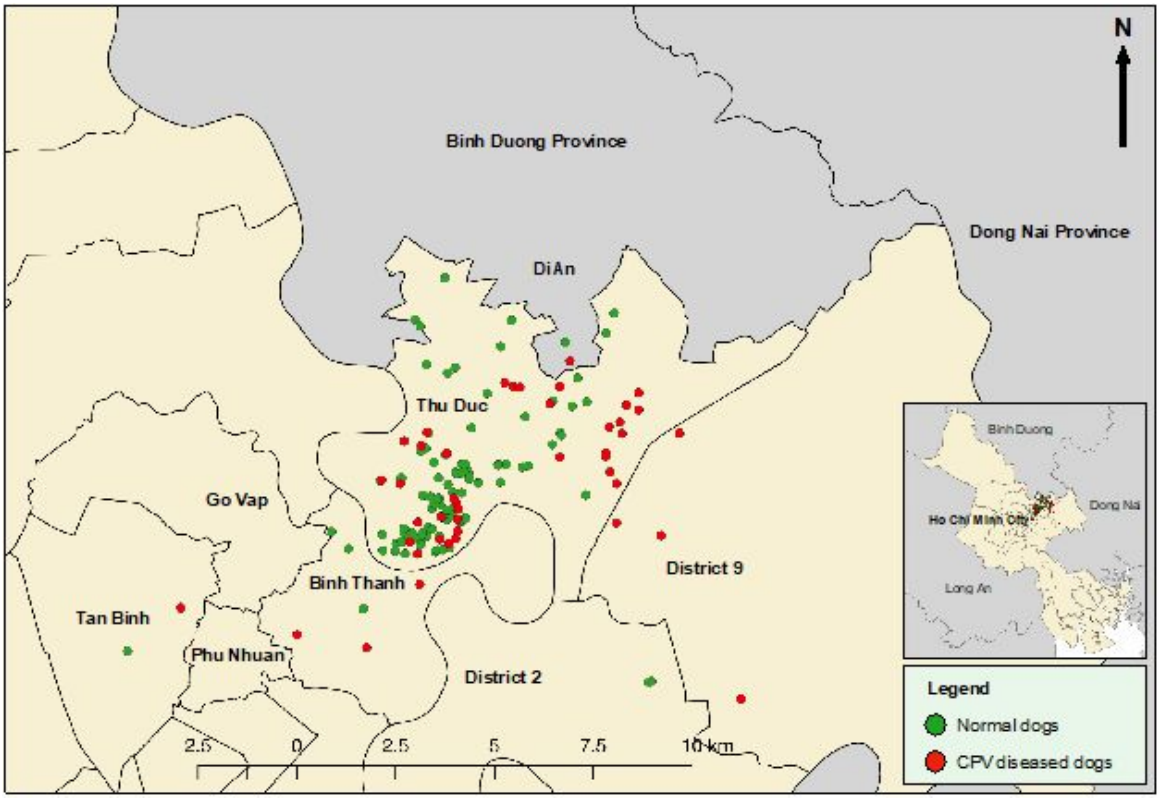

Figure 1. Map of study area in Ho Chi Minh City and participants' geographical locations. The geographical coordinates were collected on Google Map based on the exact addresses provided by participants. Vector layers of quantum geographic information system (QGIS 2.14) were applied to plot the coordinates on the map.

sure (Lamorfe, 2020), but still maintain the statistical power by getting more than one control for every case (the ratio of 2 controls: 1 case in our study) (Lewallen & Courtright, 1998). Besides, participants were raising dogs within duration of up to 6 months, the time was supposed not too long from their memories when they responded to the questionnaire. This helped to reduce recall bias, a common problem in case-control study (Boone et al., 2012).

Our finding indicated the critical importance of vaccination in CPV prevention in dogs. This was consistent with previous studies conducted in Portugal and Nigeria (Mohammed et al., 2008; Miranda et al., 2015) where unvaccinated dogs had a risk of infecting CPV higher than dogs injected CPV vaccine 2.00 and 25.14 times, respectively. In addition, exposure of naive dogs to the feces of CPV-2 infected animal or fomites was also important risk factors of CPV infection (Hsu et al., 2003). Our study found the increased incidence of CPV in dogs who had interactions with other neighbour dogs as well as lived with other same-aged dogs. Therefore, the recommendations for inhibition of dog free outside access and cleaning and disinfection of housing, bedding and other materials in contact with affected animals should be thoroughly performed on a regular basis (Lamm & Rezabek, 2008).

Generally, in Vietnam core vaccination of a multivalent modified-live viral (MLV) vaccine is recommended at 8^{th} - 10^{th} weeks of age, and an additional booster vaccination at 12^{th} - 14^{th} weeks, then a booster vaccination is administered in subsequent years. There were 11 cases of clinical illness in vaccinated dogs in our study with the age median of these dogs was 2.5 months [IQR 1.5-3.0] and all were the first vaccinations. There has been shown that overwhelming viral dose while antibodies yielded from only one shot of vaccination were inadequate to protect dogs (Lamm & Rezabek, 2008). In addition, the susceptibility for CPV infection in pups actually begins 2-3 weeks before the waning of maternal an-

Table 2. Logistic regression models investigating risk factors associated with occurrence of canine parvovirus in dogs

Variables	Univariable Model			Multivariable Model*		
	OR (95% CI)		*P*-value	OR (95% CI)		*P*-value
Breed (Baseline=Foreign)						
Vietnamese native	4.10	(1.67 - 10.00)	0.002			
Mixed breed	1.39	(0.54 - 3.52)	0.492			
CPV vaccination status (Baseline=Yes)						
No	13.50	(5.65 - 32.26)	< 0.001	11.76	(4.55 - 30.38)	< 0.001
Dog-keeping habit (Baseline = mostly inside the house)						
Freely outside access	4.75	(1.47 - 15.38)	0.009			
Restrictively outside access	0.91	(0.38 - 2.18)	0.839			
Raising with other dogs (Baseline = No)						
Yes - same aged dog/s	3.90	(1.32 - 11.50)	0.134	5.01	(1.20 - 20.98)	0.027
Yes - different aged dog/s	1.56	(0.68 - 3.69)	0.312	1.85	(0.60 - 5.70)	0.281
Interaction with neighbour dog/s (Baseline = No)						
Yes	2.88	(1.30 - 6.38)	0.009	3.13	(1.13 - 8.68)	0.028
Unknown	13.30	(2.50 - 70.74)	0.241	20.93	(2.91 - 75.72)	0.251

Legend: The tables illustrates only significant variables in univariable models, considered as candidates for multivariable model. *Model intercept = -2.923, SE = 0.526.

tibodies at 8-12 weeks of age (Nandi & Kumar, 2010), the neutralization of early viral vaccine by maternal antibodies could be a reason interpreting for these cases. Besides, the possibility of CPV infection with variant wide-type strains could be eliminated since the demonstration of cross-protection among virus types by multivalent modified-live viral vaccine were reported in previous studies (Truyen, 2006; Glover et al., 2012).

Our study had several limitations: We conducted convenience sampling relied on dogs delievered to the clinic within six months, spatial and temporal data were not fully collected to have a comprehensive description of risks associated with CPV in dogs. Since a lack of information about the time of vaccination in both study dogs and their mothers, the effects of vaccination on CPV disease in dogs, particularly vaccinated dogs, were unable to precisely interpret. However, to a certain extent, we believe that our findings can be extrapolated to other dog populations raised in other areas in Ho Chi Minh city, Vietnam.

4. Conclusions

Our findings highlight that improving the awareness in CPV vaccination and caring practice of dog owners are the crucial factors to reduce the risks of CPV infection in dogs. There is a need for follow-up studies on spatio-temporal epidemiology of CPV to have problem-solving approaches towards canine parvovirus, one of the most currently popular diseases in dogs in Vietnam.

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Conflict of interest declaration

The authors declare no conflict of interest.

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