Original Article

Prevalence of canine parvovirus and rotavirus with diarrhea in Western Taiwan

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Abstract

In this study, we investigated the prevalence of Diarrhea pathogens in pet dogs referred to as Canine Parvovirus (CPV) and Canine Rotavirus (CRV) in western Taiwan using conventional polymerase chain reaction (PCR). Fecal samples were collected from 240 dogs which have the symptoms of diarrhea examined by the veterinary hospitals from March 2015 to March 2017. The PCR sensitivity of total DNAs extracted from 0.1g fecal samples ranged from 10 ng to 100 ng. The prevalence of CPV and CRV infections were 23.3% (56/240) and 9.2% (22/240) respectively. The related analysis between prevalence rates and the epidemiological data of pet dogs were correlated with the age, season, area, vaccination and breed. The results showed that both the diseases have the highest occurrence in winter and spring, and the highest proportions might occur in puppies, suburbs and mixed-breed dogs. Non-vaccination dogs were the most prone to Canine Parvovirus Enteritis. When dog puppies were infected with CPV, the mortality rate was high. Since Canine Rotavirus is a zoonosis, the more human being is exposed to it, the higher its occurrence will be. This study has provided the clinical veterinarian the advanced ability of both the diseases diagnosis and crucial information for prevention and control of Canine Parvovirus and Rotavirus diseases in Taiwan and neighborhood countries.

Keywords: Canine, Parvovirus, PCR, Rotavirus, Taiwan

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Introduction

Canine parvovirus (CPV) is an emerging DNA virus that was first observed to cause disease in canines in 1978 and has since become an ubiquitous pathogen worldwide. Canine Parvovirus-2 (CPV-2) can infect all-aged dogs. Its two strains of CPV-2 and CPV-2b were separated in 1979 and 1984 respectively (Sokolow SH *et al*., 2005). Two distinct parvoviruses are now known to infect dogs—the pathogenic CPV-2 and CPV-1. CPV-2, the causative agent of acute hemorrhagic enteritis and myocarditis in dogs, is one of the most important pathogenic viruses with high morbidity (100%) and frequent mortality up to 10% in adult dogs and 91% in pups (S. Nandi *et al*.,2010; Miranda C, 2016; Kim HH, 2018; Kilian E, 2018). The disease condition has been complicated further due to emergence of a number of variants named CPV-2a, CPV-2b and CPV-2c over the years and involvement of domestic and wild canines (S. Nandi *et al*., 2010). CPV-2c (Glu426 variant) was found in Italy, Vietnam and Spain (Decaro N *et al*., 2006). Among those viruses, the sensitivity of the breeds to the parvovirus is higher, such as Norwegian Nasal, Black Labrador, Doberman pinscher and American Pit Bull Terrier, and the condition is more serious after infection. Most CPV infections are caused by the mixture of the two strains but the original strains**.** The virus is not the same virus originally found, but the general test could not tell the origin from others (Martella V *et al*., 2005). CRV is a zoonotic disease. The first case of CRV found in young children, which is a kind of common infectious diarrheal disease (Bishop RF *et al*., 1973; Bishop R , 2009; Kirkwood CD, 2017), is confirmed that it will also cause domestic diarrhea later, and found that many species of animals infected with CRV. G3P[3] and G3P[9] strains have been detected sporadically in humans (Matthijnssens J *et al*., 2011; Wang YH ,2013) and most of the disease is acute gastroenteritis. HCoV 229E and OC43 are the causes of the common cold which are now globally endemic in humans (J.S.M. Peiris, 2012).

In 1996, a CRV case of acute gastroenteritis found in an Italian child was suspected of being infected by rotavirus (Grazia SD *et al*., 2007). Because CRV is an epidemic and could scatter suddenly and widespread rapidly, dogs can be infected at all ages. The virus is spread by the faecal-oral route but airborne or droplet transmission has also been postulated. The virus is so infectious that even cured vitality and strong infectious and cured animals can continue to excrete virus for 3 weeks. The pollution of the feeding environment and water sources are likely to cause a cluster infection even the virus caused by a relatively low mortality (Ogilvie I *et al*., 2012).

CPV-2 has been incriminated as primary pathogens. CPV-1 and canine rotaviruses (CRV) can produce mild to inapparent illness in young pups (less than 8 weeks old), and their clinical significance is considered low (Jane E. *et al*., 2016). To provide the knowledge of prevention and control, we would like to use this survey to understand the prevalence of these two diseases in Taiwan in recent years.

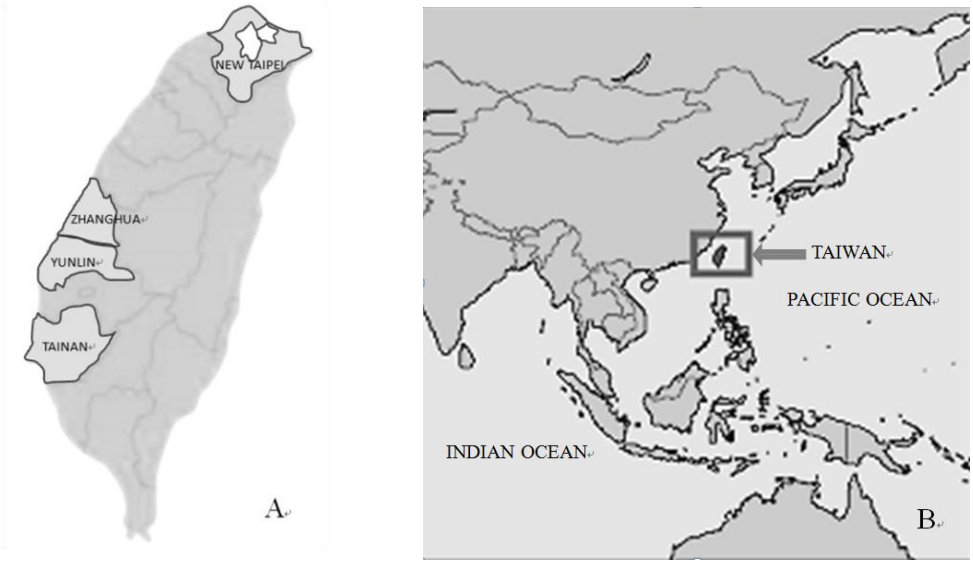


Figure 1 A: Sampling regions (New Taipei, Zhanghua, Yunlin, Tainan) at Western Taiwan.
B: Regional map of Taiwan (From the website of Ministry of Foreign Affairs of Taiwan).

Materials and Methods

Experiment Design: The study is planned for two years (spring, summer, autumn, winter two rounds) from March 2015 to March 2017, screened by animal hospitals in western Taiwan, to investigate 240 cases (one fecal sample by each dog) of suspected viral enteritis as a statistic to understand the main cause of viral enteritis and its infection relation with season, age, suburbs and urban areas, immune-protective effect of canine vaccine and dog species.The epidemiology of both canine parvovirus 2 (CPV2) and Canine coronavirus (CCoV) canine viral enteritis were investigated by molecular biology polymerase chain reaction (PCR) in combination with clinical diagnosis.

Samples: This study included 240 diarrhea dogs from animal hospitals of western Taiwan, including New Taipei, Zhanghua, Yunlin and Tainan (Fig 1). Dogs from 10 hospitals (New Taipei 3 hospitals, Zhanghua 2, Yunlin 2 and Tainan 3) are picked, with 24 dogs per

hospital. Fecal samples were collected directly upon defecation. To prevent contamination, 5–10 g of faeces was collected with a spoon and stored in a sterile plastic sampling tube. The samples were kept refrigerated and processed in the laboratory within 24 hr after collection.

Polymerase chain reaction assay: RNA and DNA was extracted from fecal using Corning Axygen AP-MN-BF-VNA-250 AxyPrep TM Body Fluid Viral DNA & RNA Purification Miniprep Kit (BIOKIT, Miaoli County, Taiwan). CPV uses the primer pair: **5′-**GAAGAGTGGTTGTAAATAATA**-3′, 5′-**CCTATATC ACCAAAGTTAGTAG**-3′.** In the case of rotavirus, RT-PCR was performed for each of the samples, using the M-MLV Reverse Transcriptase Rnase H-Kit (Bioman, USA.), and the specific primer was used for reverse transcription of RNA and polymerase chain reaction using the primer pair: 5′-TTGCCACCAATTCAAAAT AC-3′ and 5′-ATTTCGGACCATTTATAACC-3′ (Table 1). Conventional PCR was performed using 0.625 mM dNTPs, and the specific primers (Forward, Reverse) were respectively 0.4 µM, 5U Taq DNA Polymerase, 20 U RNase inhibitor and Buffer solution. PCR reaction program protocol was as follows: CPV, step 1: pre-denaturation at 94°C for 1 min; step 2: 94°C for 15 sec (denaturation), 52°C for 30 sec (annealing); Step 3: extension at 72°C for 2 min, performed for 40 cycles; step 4: Final extension at 68°C for 7 min. Rotavirus , step 1: pre-denaturation at 94°C for 1 min; step 2: 94°C for 15 sec (denaturation), 52°C for 30 sec (annealing) ; Step 3: extension at 72°C for 1 min, performed for 40 cycles ; step 4: Final extension PCR temperature was 72°C for 7 min. The process was repeated three times. (Varaporn Korchunjit *et al.*, 2014.) The primer sequences were designed and used in previous publications (Pletcher JM *et al.*,1979 ; Wang HC *et al.*, 2005). Table1. The electrophoresis of amplicons was achieved in a 1.5% agarose gel (stained with 0.5 mg/mL ethidium bromide) and visualized in a MS UVCI Image Capture visualized transilluminator (Major Science, USA).

Table 1 Sequences of PCR primers

Pathogens	Oligonucleotide sequence (5′-3′)	Expected size (bp)
CPV-2	**5′-**GAAGAGTGGTTGTAAATAATA**-3′**	416
	5′-CCTATATCACCAAAGTTAGTAG**-3′**	
Rotavirus	**5′-**TTG CCA CCA ATT CAA AAT AC**-3′**	234
	5′-ATT TCG GAC CAT TTA TAA CC**-3′**	

Viral PCR susceptibility test: DNA extracted from canine parvovirus (CPV) infection detected by PCR increases with a primer (Table 1), which shows that the minimum DNA amount of the target gene 416 bp can be detected by the primer as 10^{-3}ng (Fig 2A). Canine rotavirus (CRV) infected with the DNA of the detection of the target gene 234 bp. The minimum amount of DNA is 10^{-1} ng (Fig 2B).

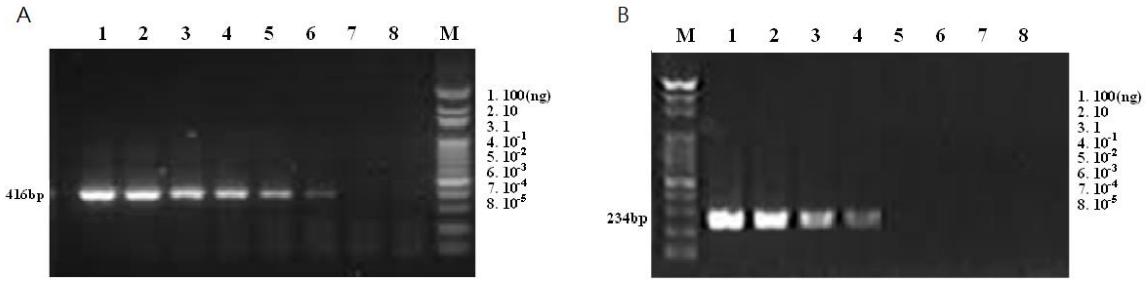


Figure 2 PCR susceptibility test target gene. A. Canine Parvovirus B. Canine Rotavirus

Results

Total prevalence rate: Fecal samples were collected from 240 dogs in different age, season, area, vaccination and breed. Prevalence of CPV and CRV are 23.3% (56/240), 9.2% (22/240) respectively (Table 2). There is 1.67% (4/240) co-infection with both rotavirus and parvovirus in this study, which occurs to 4 non-vaccination mixed-breed puppies in spring in suburbs.

Age distribution: Fecal samples were collected from 240 dogs , 60 dogs per age group. CPV: 1 month~1yr 60% (36/60), 1yr~6yrs 20% (12/60), 6~11yrs 6.7% (4/60), 11yrs-16 yrs 10% (6/60). CRV: 1 month~1yr 13.3% (8/60), 1~6yrs 6.7% (4/60), 6~11yrs 6.7% (4/60), 11~16yrs 10% (6/60). The prevalence of CPV was higher than that of CRV. Age is not a factor in the prevalence of CRV, while CPV showed significant difference in puppy of different ages.

Proportion of positive detection samples of all ages: CPV positive, prevalence is differentiated by age: 1 month to 1yr 64% (36/56), 1yr~6yrs 22% (12/56), 6yrs~11yrs 7% (4/56), 11yrs~16yrs 7% (4/56), showing that the puppy accounts for higher proportion of canine parvovirus. In CRV positive, prevalence is differentiated by age, 1 month to 1yr 37% (8/22), 1yr~6yrs 18% (4/22), 6yrs~11yrs 18% (4/22), 11~16yrs

27% (6/22), and the percentage of pups infect CRV was higher. In all age groups, CPV and CRV were the most prevalent puppies. In puppies, CPV is also higher than CRV.

Table 2 Enteritis prevalence rate caused by canine parvovirus and rotavirus in pet dogs.

Items	Kind of virus	Various prevalence rates				
Total	Parvovirus	23.3%(56/240)	-	-	-	-
	Rotavirus	9.2%(22/240)	-	-	-	-
Age	Parvovirus	1 month~1yr 60% (36/60)	1~ 6yrs 20% (12/60)	6~11yrs 6.7% (4/60)	11-16 yrs 10% (6/60)	-
	Rotavirus	1 month~1yr 13.3% (8/60)	1~6yrs 6.7% (4/60)	6~ 11yrs 6.7% (4/60)	11~16yrs 10% (6/60)	-
	Parvovirus*	1 month~1yr 64% (36/56)	1~6yrs 22% (12/56)	6~11yrs 7% (4/56)	11~16yrs 7% (4/56)	-
	Rotavirus*	1 month~1yr 37% (8/22)	1~6yrs 18% (4/22)	6~11yrs 18% (4/22)	11~16yrs 27% (6/22)	-
Season	Parvovirus	Spring 50% (30/60)	Summer 10% (6/60)	Autumn 3.3% (2/60)	Winter 30% (18/60)	-
	Rotavirus	Spring 10% (6/60)	Summer 3.3% (2/60)	Autumn 3.3% (2/60)	Winter 20% (12/60)	-
	Parvovirus*	Spring 54% (30/56)	Summer 11% (6/56)	Autumn 3% (2/56)	Winter 32% (18/56)	-
	Rotavirus*	Spring 27% (6/22)	Summer 7% (2/22)	Autumn 9% (2/22)	Winter 55% (12/22)	-
Area	Parvovirus	Suburbs 41.7% (40/96)	Urban 11.1% (16/144)	-	-	-
	Rotavirus	Suburbs 14.6% (14/96)	Urban 5.6% (8/144)	-	-	-
	Parvovirus*	Suburbs 71% (40/56)	Urban 29% (16/56)	-	-	-
	Rotavirus*	Suburbs 64% (14/22)	Urban 36% (8/22)	-	-	-
Vaccination	Parvovirus	Vaccination 3.8% (6/156)	-	-	-	-
		Non-vaccination 59.5% (50/84)	-	-	-	-
Breed	Parvovirus	Mixed 65% (36/56)	-	-	-	-
	Rotavirus	Mixed 55% (12/22)	-	-	-	-
	Parvovirus*	Mixed 33.3% (36/108)	Poodle 21.4% (12/56)	Maltese 11.1% (4/36)	Pomeranian 9.1% (2/22)	Other 11.1% (2/18)
	Rotavirus*	Mixed 11.1% (12/108)	Poodle 7.1% (4/56)	Maltese 5.6% (2/36)	Pomeranian 9.1% (2/22)	Other 11.1% (2/18)

**Proportion of positive samples.*

Season distribution: In 240 dogs sample, 60 dogs per season group, the prevalence of CPV is as follows, February-April (Spring) 50% (30/60), May-July (Summer) 10% (6/60), August-October (Autumn) 3.3% (2/60), November-January (Winter) 7.5% 30% (18/60), showing CPV is most prevalent in February-April (Spring). Prevalence of CRV in February-April (Spring) is 10% (6/60), May-July (Summer) 3.3% (2/60), August-October (Autumn) 3.3% (2/60), November-January (Winter) 20% (12/60). Prevalence of CRV showed the highest in November ~ January (Winter). CRV and CPV enteritis both in the spring and winter two-quarter is higher, CRV is higher in winter than in the spring, CPV enteritis is higher in spring than in winter.

Proportion of positive detection samples of all season: In CPV positive, prevalence was differentiated by season. February-April (Spring) is 54% (30/56), May-July (Summer) 11% (6/56), August-October (Autumn) 3% (2/56), November-January (Winter) 32% (18/56), It shows the prevalence in 2-4 months (spring) is the highest. In CRV positive, prevalence was differentiated by season. February-April (Spring) is 27% (6/22), May-July (Summer) 9% (2/22), August-October (Autumn) 9% (2/22), November-January (Winter) 55% (12/22). It shows prevalence in November-January (Winter) is highest.

Area distribution: In 240 dogs (96 in the suburbs, 144 in the urban area), CRV and CPV are detected in suburban and urban areas, with prevalence of CPV in the suburbs equal to 41.7% (40/96, higher), the urban

area 11.1% (16/144). CRV is 14.6% (14/96) higher in the suburbs and 5.6%(8/144) in urban area., CRV and CPV enteritis are the most in the suburbs. Prevalence of CPV is 71% (40/56) in the suburbs and 29% (16/56) in urban area. In CRV positive, prevalence is 64% (14/22) in the suburbs and 36% (8/22) in urban area.

The difference of vaccination or non-vaccination: The efficacy of the vaccine is observed, since the prevalence of 156 dogs with normal canine parvovirus vaccine is 3.8% (6/156) , and 59.5% (50/84) of the dogs with no vaccine canine parvovirus is more prone to the disease, showing the effectiveness of vaccine protection.

Breed distribution: Among the two kinds of virus positive, prevalence was differentiated by dog species, Prevalence of CPV : mixed-breed is 33.3% (36/108 higher), poodle 21.4% (12/56), Maltese 11.1% (4/36), Pomeranian 9.1% (2/22), and others accounted for 11.1% (2/18). Prevalence of CRV, mixed-breed is 11.1% (/12/108), poodle 7.1% (4/56), Maltese accounted 5.6% (2/36), and the other is 9.1% (2/22). Prevalence of CRV and CPV enteritis are higher in mixed breeds. In each dog species, the prevalence of CPV in mixed breeds of dogs accounted for 65% (36/56). Prevalence of CRV is higher in mixed breed 55% (12/22).

Conclusion: CPV and CRV enteritis were the highest among puppies, but age is not so much a factor in the case of CRV. Both CPV and CRV have the highest prevalence in spring and winter, among which the CRV was higher in winter than in spring, and the prevalence of CPV was higher in spring than in winter. Both CRV and CPV have the highest in the suburbs, with both CRV and CPV having the highest rates of mixed breeds. Among CPV prevalence, no vaccination is higher than the vaccination.

Discussion

In this study, experimental data show the majority of infectious canine enteritis was still occurring in puppies, which has been mentioned in the previous studies. Unvaccinated dogs of all ages and breeds are susceptible to infection. These may be due to the decline of maternal antibodies and the infection of susceptible adult dogs which were not vaccinated are at risk of contracting the virus. Likely only a few CPV enteritis cases are caused when a puppy vaccine-induced antibody titer is insufficient or an adult canine which vaccine antibody titer decline in the case of infection, but most are still protected by vaccination.

Purebred dog breeds should be more likely to suffer from CPV than other dog breeds, especially the Rottweiler, Black Labrador, Doberman Pinscher, American Pit Bull Terrier. (Decaro N *et al.*, 2012; Meggiolaro MN, 2017). What is mentioned above is different from our results : CPV and CRV enteritis both occurred in mixed breeds dogs. The results may be caused by the fact that most of the mixed breeds in the case have not been vaccinated or have not been vaccinated on time. Most of these mixed breeds are fed by the suburbs host, so the results of study clarified that the highest prevalence of canine parvoviral enteritis in suburbs.

In this study, that prevalence of CRV is shown to be the highest in the winter or spring season. However, the climate in southern Taiwan is similar to that of tropic area and exists lower prevalence. CPV does not have a specific seasonal prevalence, it causes CRV enteritis to be neglected easily because it will be diagnosed as indigestion or bacterial infection if the sick dog exists diarrhea symptoms, canine parvovirus, canine distemper, coronavirus disease, or coccidiosis will be excluded in general situation which should be associated with the immune decline because of the stress of cold or temperature changes of animals. Infection virus can be detected from some dogs feces only after a period of recovery, and it becomes an important recessive source of infection (Hoang M, 2019; Schoeman JP, 2013 ; Truyen U, 2006).

In this study, it shows that CRV exists mostly in the suburbs dogs and the mixed-breed ones which have not been mentioned in the previous studies. This study makes us know that the results are caused by the poor raised environment and mostly mixed-breed does in suburban areas. Unfortunately, another dog will be infected after drinking the contaminated water once a dog is infected with CRV, and the virus is discharged through the fecal and thus pollutes the environment and drinking water, and will combine with the bacterial enteritis easily to produce diarrhea symptoms severely as sent to the Vet., and taken samples. Therefore, this study finds that the mixed dogs in the suburbs account for higher prevalence of CRV.

There is 1.67% (4/240) co-infection with both rotavirus and parvovirus in this study, which occurs to 4 non-vaccination mixed-breed puppies in spring in suburbs. Then, the first report on circulating canine rotavirus in Mexico. Fifty samples from dogs with gastroenteritis were analyzed using polymerase chain reaction and reverse transcription polymerase chain reaction in order to identify parvovirus and rotavirus, respectively; 7% of dogs were infected with rotavirus exclusively, while 14% were co-infected with both rotavirus and parvovirus; clinical signs in co-infected dogs were more severe.(Ariadna Flores Ortega, 2017).

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