

PREVALENCE AND ASSOCIATED RISK FACTORS OF CANINE PARVOVIRUS AND CANINE INFLUENZA VIRUS INFECTIONS IN PET DOGS IN DHAKA DISTRICT OF BANGLADESH

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ABSTRACT

Background: There are approximately 1.6 million dogs in Bangladesh and almost 83% of these dogs live on the street and accordingly 17.0% dog population are kept as pet mostly in the metropolitan cities with major population in Dhaka, Chittogram and Sylhet in Bangladesh. Some promiscuous research findings on Canine parvovirus enteritis (PVE) and Canine influenza virus (CIV) have been reported in inland literature.

Objective: To determine the prevalence and associated risk factors of canine parvovirus and canine influenza virus infections in dogs supported with brief review for future direction of research and prevention

Materials and Methods: A cross sectional study was conducted on total of 173 pet dogs for the prevalence of CIV and 70 dogs for CPV infections of different breed, age and gender by collecting nasal swab samples for CIV and rectal swabs for CPV infection. Each of the collected nasal swabs was tested by RapiGen[®] Canine influenza Ag test kit and rectal swab samples with RapiGen[®] Canine parvovirus Ag test kit (RapiGen INC., South Korea, 2012). Chi-square test was used to detect the significance of risk factors of the infections in dogs.

Results: All the 173 nasal swabs of pet dogs collected from different thanas of the Dhaka district showed negative with RapiGen[®] CIV Ag test kit test. Out of four published reports on the prevalence of CIV infection in dogs, of which two reports showed 10.71 to 13.33% prevalence rate of CIV whereas two reports (including this one) showed negative result with the same test. An overall 7.14% prevalence of CPV infection in pet dogs was recorded in this study. The prevalence of CPV in relation to breed was found 22.22% in German shepherd and 2.86% in Labrador whereas local, Bull mastiff and Samoyed breeds found negative for CPV infection. The higher prevalence of CPV infection was recorded in puppies up to six months of age (14.81%) than in growing dogs aged between >6 to 12 months (7.14%) whereas adult (>1 to 2 years) and older (> 2 years) dogs found negative to this infection. Comparatively higher prevalence of CPV infection was detected in male (8.33%) than in female (5.88%) dogs. No CPV infection was recorded in vaccinated dogs, whereas 19.23% unvaccinated dogs affected with this infection. All the rectal swab samples of apparently healthy dogs (no sign of diarrhea) showed negative to CPV infection, whereas 25.0% dogs with diarrhea sign found positive to CPV infection. Review of inland literature reveals that out of nine articles published on CPV infection of which RapiGen[®] CPV Ag test kit has been used in four, PCR in one and clinical method of diagnosis in four articles, whereas only RapiGen[®] CIV Ag test kit has been used for the diagnosis of CIV infection.

Conclusion: The prevalence of CPE associated with diarrhea in 7.14% pet dogs has been recognized in this investigation with supports of earlier reports whereas the prevalence of CIV in pet dogs varied widely from negative to 13.33% prevalence in dogs. Age and vaccination of dogs have been recognized as primary risk factors which should be considered in planning a control program whereas others factors like breeds, season, geographical areas can be considered as secondary risk factors varied widely in different reports and countries. Comparative evaluation of different diagnostic tests to find out the 'gold standard' and vaccination against CPI in puppies may be suggested to control this disease in dogs.

Keywords: Prevalence, Risk factors, CIV, CPV, Dogs, Breeds, Dhaka district, RapiGen[®] Ag test kit, Brief review

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INTRODUCTION

The importance of dogs as pet has been well recognized in the family and society worldwide including Bangladesh.^{1,2} There are 1.6 million dogs in Bangladesh and almost 83% of these dogs live on the streets.³ The mean density of the stray dog population in Bangladesh has been estimated as 15 dogs / km².⁴ The population of the pet dogs has not yet been estimated in Bangladesh but many people in all the cities of Bangladesh are kept dogs as a pet animal abundantly in Dhaka, Chottogram and Sylhet cities. It appears that the pet dog rearing is getting popularity in urban than rural areas day by day. German shepherd, Doberman, Labrador, Rottweiler, German spitz, Puddle, Samoyed, cross and local dogs are available breeds of dogs in Bangladesh.^{2,5,6} Overall prevalence of clinical diseases of dogs including 2.33%⁵ and 3.55%⁶ clinical prevalence of CPE have been reported from Bangladesh. Canine influenza is an emerging contagious respiratory infection of dogs associated with high morbidity (close to 100%) but low mortality (< 8%). The sub-clinical prevalence (0-13.33%) of CIV infection in dogs based on the detection on the nasal swabs has been reported from Bangladesh.⁷⁻⁹ The CPE is one of the most important causes of morbidity and mortality in dogs younger than six months of age worldwide.¹⁰ It is caused by a single-stranded DNA virus known as Canine parvovirus belongs to the genus *Protoparvovirus* and family *Parvoviridae* that infects rapidly dividing cells of the gastro-intestinal tract, bone marrow, lymphoid tissue and cardiac myocytes.¹¹ The viruses of the family *Parvoviridae* are also found in other wild mammals including wild mink and the genetic variation from other wild life may have also played a role in the evolution of CPV-1 and CPV-2. Since the first isolation of CPV-1 and CPV-2, a genetic drift has resulted in two variants (CPV-2a and CPV-2b), followed by third variant (CPV-2c).^{10,11} All these three strains (CPV-2a, CPV-2b & CPV-2c) have a broader host range compared to the original CPV-2 strain and may cause naturally prevalent disease identical to feline panleukopenia in cats.¹⁰ Dogs of any age and breed can be infected with parvovirus but puppies between the ages of 6 and 16 weeks seem to be the most susceptible.¹⁰ The CPV infection is usually transmitted via fecal-oral route through contact with infected feces or contaminated surface of infected dogs to susceptible dogs. There are two forms of the disease has been reported, the enteric form characterized by acute fever, lethargy, anorexia, vomiting and bloody diarrhea, and cardiac form that scarcely reported in neonates with cardiac and respiratory failure associated with in utero infection.¹² The winter season, young age, local breed, non-vaccination status and male dogs have been reported as the significant risk factors for CPE at hospital clinical cases in Bangladesh.¹³ Age, gender, breeds, season, quantity and virulence of virus and carrier status of other diseases and parasites have been reported as major risk factors. A clinical dog with signs of severe vomiting, diarrhea, foul smelly dark feces and fever or hypothermia along with the duration of illness of 3 to 7 days defined as a CPE case.¹⁴ This virus infection causes high morbidity (100%) and 10% mortality in adult and 90% in pups.¹⁵ The proportionate 13.9 to 42% prevalence of CPV infection in dogs of Bangladesh has been reported.^{7,16-18} Despite development and administration of vaccination against CPV-2 strains are practiced, the disease is still one of the considerable veterinary and economic important.¹¹ This paper describes the prevalence and associated risk factors of CPV and CIV infection in pet dogs in Dhaka district in Bangladesh.

MATERIALS AND METHODS

The samples from randomly selected dogs were collected from six thanas of Dhaka district which includes Savar, Mirpur, Uttara, Cantonment, Gulshan and New Market. The research work was conducted in the laboratory of the Department of Medicine, Bangladesh Agricultural University (BAU), Mymensingh and Veterinary Diagnostic Laboratory of Remount Veterinary and Farm Depot, Savar Cantonment during the period of January to May 2017.

A cross sectional study was conducted on total of 173 pet dogs for CIV and 70 dogs for CPV infection of different age and sex by collecting nasal swab samples for CIV and rectal swabs for CPV. The diagnosis was performed by RapiGen[®] Canine Ag test kit (RapiGen INC., South Korea, 2012). Out of 70 pet dogs tested for CPV infection, of which 35 were Labrador, 18 were German shepherd (Photo 1), 02 were Samoyed, 02 were Bull Mastiff and 13 were local breed. Dogs studied were status of without history of vaccination against CIV and with or without the history of vaccination against CPV infection. The clinical signs specially the presence of nasal secretion and other flu like signs in case of CIV, whereas diarrhea, vomiting in case of CPV infection (if any) as well as age, sex, and breed were recorded carefully. The age of the dog was determined by checking the history sheet maintained by various organizations or through the questionnaire asked to the owner and by examination of teeth.¹⁹ The nasal swab (Photo 2) samples for CIV and rectal swab (Photo 3) for CPV infection were collected from the dog with cotton swab. These dogs were restrained mostly by the representatives of concern organizations and owners. These Thanas (areas) in Dhaka district were selected because these areas are having large number of pet dog population at organizational and also at private levels. Besides this, some of these areas are also having horse population which is thought to be a source of canine influenza. After collection, samples were transferred aseptically in separate sterile plastic vial and kept in the ice carrier with ice pack and then tested in the laboratory. Then, tests were carried out following manufacturer's instruction.



Photo 1. A German shepherd dog used for sample collection to detect CIV and CPV infections



Photo 2. Collection of nasal swab from a dog to detect CIV infection



Photo 3. Collection of rectal swab from a dog to detect CPV infection

Interpretation of the results

Result was read after 5 to 10 minutes with naked eye. The presence of one purple/red band control line within the result window indicates a negative result. The appearance of two red or

purple bands (C or T) within the result window, no matter which band appears first, indicates a positive result. If the control band fails to appear within the result window, the result was considered invalid.

Statistical analysis

Data of risk factors were analyzed by using standard procedure of Chi-square test for significance.

RESULTS

The nasal swab samples collected from 173 pet dogs from six thanas of Dhaka district showed negative result with RapiGen® CIV Ag test kit, whereas rectal samples of 70 dogs tested with RapiGen® CPV Ag test kit showed 7.14% (5/70) positive for CPV infection. The variation of the test results between two diseases of dogs encourages the authors to analyze all the available inland reports on these aspects (Table 1). It appears from Table 1 that a total of 2068 dogs have been tested in 11 studies during the period from 2010 to 2019, of which an overall 15.28% had CPV infection, whereas out of 533 samples tested in four reports, of which positive test result showed in 50% (two reports) and 50% (two reports) negative results with an overall 5.63% prevalence of CIV infection in Bangladesh.

SN	District/ Sites	Breed	No. of dogs	Types of samples	Tested for:	Positive No. (%)	Test used	Year of work	Ref
01.	BAU, M	Stray dogs	30	Rectal swab	CPV	09 (30.00)	RCP	2010	16
02.	DMFC	Stray dogs	50	Rectal swab	CPV	15 (30.0)	RCP	2014	7
03.	DMFC	Pet dogs	50	Rectal swab	CPV	11 (22.0)	RCP	2014	7
04.	M	Street dogs	114	Rectal swab	CPV	37 (32.00)	RCP	2015	20
05.	DMS	L + E	114	Rectal swab	CPV	37 (32.45)	RCP	2015	8
06.	CMA	Local	54	Rectal swab	CPV	06 (11.11)	PCR	2019	21
07.	CMA	Exotic	46	Rectal swab	CPV	14 (30.43)	PCR	2019	21
08.	CMA	L + E	100	Rectal swab	CPV	20 (20.00)*	PCR	2019	21
09.	TVHC	-	568	AVHR	CPV	33 (05.80)	Clinical	2013-18	13
10.	CVH	Multiple	197	AVHR	CPV	07 (03.55)	Clinical	2018-19	6
11.	CVH	L + E	80	-	CPV	25 (31.20)	Clinical	2015	22
12.	CVASU	L + E	50	-	CPV	21 (42.00)	Clinical	2017	22
13.	Dhaka!	Cross + E	545	Rectal swab	CPV	76 (13.94)	CI+RCP	2016-17	18
14.	Dhaka!!	L + E	070	Rectal swab	CPV	05 (07.14)	RCP	2017	**
	Overall	-	2068	-	CPV	316 (15.28)	-	2010-2019	
01.	DMFC	Stray dogs	50	Nasal swab	CIV	0	RCI	2014	7
02.	DMFC	Pet dogs	50	Nasal swab	CIV	0	RCI	2014	7
03.	DMS	Local	59	Nasal swab	CIV	7 (11.86)	RCI	2015	8
04.	DMS	Exotic	56	Nasal swab	CIV	6 (10.71)	RCI	2015	8
05.	DMS	Pet dogs	115	Nasal swab	CIV	13 (11.30)	RCI	2015	8
06.	M	Stray dogs	30	Nasal swab	CIV	04 (13.33)	RCI	2010	9
07.	Dhaka!!	L + E	173	Nasal swab	CIV	0	RCI	2017	**
	Overall		533	-	CIV	30 (05.63)	-	2010-2017	

Prevalence and risk factors of CPV and CIV in dogs

D = Dhaka M = Mymensingh F = Feni C= Chittagong S = Sirajgonj L + E = Local + Exotic

RCP = RapiGen canine parvovirus Ag test kit RCI = RapiGen canine influenza Ag test kit

CMA = Chattogram metropolitan area TVHC = Teaching Veterinary Hospital, CVASU

PCR = Polymerase chain reaction by using amplification refractory mutation system-

AVHR = Abstracted veterinary hospital record

CVH = Central Veterinary Hospital, Dhaka Dhaka! = Dr. Sagir's Pet Clinics and Research Centre, Dhaka

Dhaka!! = Five thana (Savar, New Market, Cantonment, Uttara & Gulshan)

*All the three variants, CPV2a, CPV2b and CPV2c reported in clinically sick dogs, of which CPV2c showed higher prevalence than other variants, whereas all the apparently healthy dogs tested reported molecularly negative.

**Results of this study

Overall 7.14% prevalence of CPV infection was recorded in pet dogs in the districts of Dhaka. Dog samples were collected from five thanas in the district of Dhaka but positive cases (33.33%) were recorded only in the Uttara thana (Table 2). Among these, 04 (22.22%) puppies of German shepherd and 1(2.86%) puppy of Labrador were found positive to CPV. In case of breed, the chi-square statistic was 5.2173 and the p-value was 0.022364 which was significant at (p <0.05) and there is strong relationship between breed and CPV infection (Table 2).

Table 2. Prevalence and risk factors of Canine parvovirus infection in pet dogs in Dhaka					
SNVariable	Category level	No. of samples tested	Positive No. (%)	p-value	Chi-square value
1. Thanas	Savar	27	0	0.022364	5.2173
	New market	07	0		
	Cantonment	02	0		
	Uttara	15	5 (33.33)		
	Gulshan	19	0		
2. Breed	German shepherd	18	4 (22.22)	0.476538	0.5068
	Labrador	35	1 (02.86)		
	Bull mastiff	02	0		
	Samoyed	02	0		
	Local	13	0		
3. Age	Up to 6 months	27	4 (14.81)	0.690661	0.1584
	>6 months to 1 year	14	1 (07.14)		
	> 1 to 2 years	22	0		
	> 2 years	07	0		
4. Gender	Male	36	3 (08.83)	0.690661	0.1584
	Female	34	2 (05.88)		
5. Diarrhea	Present	20	5 (25.0)	0.690661	0.1584
	Absent	50	0		
6. Vaccination	Yes	44	0	0.690661	0.1584
	No	26	5 (19.23)		
Overall		70	5 (07.14)		

Of the 70 pet dogs examined for CPV infection, of which 27 were up to 06 months of age, 14 were > 6 to 12 months, 22 were one year to two years, 07 were above 2 years of age where 04 dogs (14.81%) were found positive in puppies aged up to 06 months and 01(7.14%) dog positive in age group between > 06 months to 1 year of age. The chi-square statistic was found 0.5068 and p value 0.476538 which was not significant at ($p < 0.05$). Gender-wise prevalence of CPV infection in pet dogs showed that the higher prevalence of 8.33% CPV infection was recorded in male dogs than 5.88% in female dogs. The chi-square statistic was found 0.1584 and p value was 0.690661 which was not significant at ($p < 0.05$). Out of 70 dogs, 20 had diarrhea symptoms, of which 5 dogs (25.0%) were found positive and rest 50 apparently healthy dogs were found negative to CPV infection test (Table 2). In case of vaccination status, 44 dogs were vaccinated against CPV and all were negative (0%) whereas 26 tested dogs were not vaccinated against CPV of which 5 (19.23%) dogs were found positive to CPV infection (Table 2).

DISCUSSION

The severe clinical CPE typically occurs in dogs younger than six months of age however, adult dogs with insufficient immunity may potentially be affected. The incubation period of CPE following natural or experimental exposure ranges from 4 to 14 days and virus shedding starts a few days prior to the occurrence of clinical signs, progressively declining 3 to 4 weeks post-exposure.¹⁰ Within three days of exposure with CPV-2, dogs can shed virus in their feces with peak shedding occurring 4 to 7 days after infection. Accurate detection of viral shedding and infection is paramount to helping decrease spread of infection in veterinary hospitals, shelters and breeding kennels by isolating infectious animals because clinical signs are similar in dogs that test positive or negative by fecal ELISA.¹¹ Sub-clinical infection in wild, stray and domestic dogs that shed CPV in their feces can represent a significant potential source of infection to other dogs. Young puppies that are born to and allowed to nurse colostrum from vaccinated bitches have maternal derived antibodies (MDA) as passive immunity. As MDA levels start to decrease at 8 to 12 weeks of age, neonates are at risk of infection. Earlier decreases in MDA can occur if MDA level is low. MDA can interfere with vaccine-induced virus antigen, particularly between 49 and 69 days of age.¹¹ Vaccination failure of CPE has been documented in both young and adult dogs.^{23,24} The ELISA, immunomigration assay and immunochromatography assay are commonly applied in fecal or rectal swab material to detect viral antigen of the CPE in dogs. However, PCR and immune-electron microscopy have been utilized as gold standard tests.²⁵

An overall 7.14% prevalence of CPV infection in dogs of five Thana (Savar, Mirpur, New Market, Cantonment, Uttara and Gulshan) in Dhaka district has been recorded in this study. The positive cases of CPV infection (33.33%; $n = 15$) recorded only in dogs of Uttara Thana. However, the overall 7.14% CPV infection recorded in this study which compares well with 5.8% reported as clinical cases based on hospital records in Chottogram¹³ but comparatively higher prevalence rates of 13.9% and 31.2%²² in Dhaka, 30% in Mymensingh¹⁶ and 42% in Chottogram have also been reported in earlier studies from Bangladesh.^{7,17,18} These variations of the prevalence of CPV infection in different reports might be due to clinical and sub-clinical

cases, age and breeds of dogs, feeding and management practices, vaccinated and unvaccinated status of dogs, seasons and geographical locations and diagnostic methods and tests used.

The rectal swabs of different age groups of dogs tested for CPV showed higher infection in puppies (14.81%) up to 6 months in comparison to young dogs aged between > 6 months to one year old (7.14%), whereas dogs above one year old showed negative to CPV infection. These results support the earlier reports^{7,20} who also have reported highest CPV infection of 58.3% and 28.0% in dogs up to six months of age, respectively but contradict the 11.11% prevalence of CPV infection in dogs over 18 months age group reported from Bangladesh.⁷ Significantly higher prevalence of CPE has been reported in puppies aged between 1 to 3 months (48.7%) in compare with 4 to 6 months (17.2%) and over six months (8.3%) old dogs.²² However, puppies (≤ 6 months) have a significantly higher risk of parvovirus enteritis.¹³ Puppies are very susceptible to CPV infection due to presence of low level of MDA, however, vaccinations administered too early can interfere with MDA and result in a puppy being more susceptible to infection.¹¹ The negative results of all the tested adult dogs to CPV infection are in conformity with the earlier reports¹⁰ and it has been suggested that the adult dogs are generally resistant to CPV infection might be due to age resistant associated with either naturally exposed in early life or vaccinated at early stages.

Five available breeds of dogs tested for CPV infection of which only German shepherd (22.22%; n = 4) and Labrador (2.86%; n = 1) found positive with negative results in Bull Mastiff, Samoyed and local dog. The finding of higher prevalence rate of CPV in German Shepherd dogs supports the earlier reports of higher CPV infection in this breed of dogs.²⁶ The negative result on CPV infection in local dogs recorded in this study contradicts the earlier inland reports of 30.0%,⁷ 50.0%,²² 31.3%¹³ and 11.1%²¹ prevalence of CPV infection in local dogs of Bangladesh. Some inland literatures support that the local breeds of dogs are at more risk of CPE than exotic breeds, whereas some western reports disagree with it and reported higher risk in exotic breeds.^{27,28} Therefore, the highly contradiction in the literature on the comparative susceptibility of different breeds of dogs to CPE exists need further research to solve this contradiction. However, breed predisposition and seasonal prevalence of the disease are subject to considerable geographical variation.^{10,28,29}

Insignificantly higher prevalence of CPV infection in male (8.33%) than female (5.88%) dogs is in conformity with the earlier report^{16,20,22} who reported 37.5%, 34.4% and 36.3% CPV infection in male and 21.4%, 30.2% and 25.0% in female dogs, respectively. However, these findings contradict with the report¹⁷ who reported higher prevalence of CPV infection in female (45.45%) than male (29.29%) dogs.

None of the vaccinated dogs against CPE found positive to infection, whereas 19.23% unvaccinated dogs affected with CPV infection. Efficacy of vaccination has also influenced the earlier reports where 8.33% vaccinated dogs and 60.0% unvaccinated dogs developed CPV infection.²²

CONCLUSIONS

The CPV and CIV are highly contagious virus diseases of dogs worldwide including Bangladesh. It reveals from the results of this study that the CPV infection is clinically

important in dogs. Dogs of all ages are susceptible to CPV infection but puppies less than 6 months age are more susceptible than adult dogs. All breeds of dogs are susceptible to CPV but this study recorded CPV in German shepherd and Labrador breeds of dogs. CPV infection has only recorded in non-vaccinated dogs but vaccinated dogs found immune to this disease. Therefore, the identification of the potential risk factors associated with the CPE may be helpful to construct the ideal preventive measures of the disease in dog population.

ETHICAL APPROVAL

This study includes nasal and rectal swab samples of dogs did not involve any invasive techniques, accordingly it was supposed to not require any institutional ethical approval. However, the consent of all the selected dog owners was taken before collecting of samples.

CONFLICTS OF INTEREST

The authors declare no conflict of interest

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Prevalence and risk factors of CPV and CIV in dogs

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