

## Prevalence of Canine Influenza Infection in Pet Dogs and Canine Parvovirus Infection in Street Dogs of Bangladesh

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**Abstract:** A cross-sectional study was conducted to investigate the prevalence of canine influenza (CI) infection in pet dogs and canine parvovirus (CPV) infection in street dogs of different age and sex by collecting rectal and nasal swab samples from three districts, Dhaka, Mymensingh and Sirajgonj, in Bangladesh using a RapiGEN<sup>®</sup> Canine Influenza Virus Ag Test kit and RapiGEN<sup>®</sup> Canine Parvovirus Ag Test Kit. Out of 114 rectal swabs and 115 nasal swab samples, the overall prevalence of CI and CPV was found to be 11.30% and 32.45%, respectively. The prevalence of parvovirus infection was found to be significantly higher in puppies and dogs 6 months of age (50.0%) than those > 24 months of age ( $p=0.005$ ). The prevalence was also higher in males (34.42%) than females (30.18%). The prevalence of CI was higher (30.43%) in dogs up to 6 months of age ( $p=0.011$ ) than 6-12 month (7.93%) and 12-18 month (6.66%) old dogs. Moreover, the prevalence of CI was found to be higher in males (16.10%) than females (5.66%). The prevalence of CPV infection also varied significantly in different study areas ( $p=0.0029$ ), with 12.72%, 12.5% and 7.14% of dogs found to be CI positive in Dhaka, Mymensingh and Sirajgonj, respectively. Overall, the highest prevalence of CI was found in local breeds (6.08%) followed by German shepherds and Keeshonds (1.73%), and Bloodhounds and Terriers (0.86%). Additionally, there were more positive CI found in unvaccinated dogs (14.81%) than vaccinated (2.94%) dogs. Dogs with flu-like symptoms were more positive (19.23%) for CI relative to those without flu-like symptoms (4.76%) samples. Overall, the results of this study indicate that canine vaccination should be initiated to prevent the occurrence of diseases and that regular monitoring should be continued in Bangladesh.

**Key words:** canine influenza, canine parvovirus, dogs, RapiGEN<sup>®</sup> Ag Test Kit, prevalence.

### Introduction

Canine influenza (CI), which is also known as Dog Flu, is a very contagious respiratory disease that occurs in canine populations in response to varieties of influenza virus A known as Canine Influenza Viruses (CIV); namely, equine influenza virus H<sub>3</sub>N<sub>8</sub>, which was discovered in 2004, and avian origin H<sub>3</sub>N<sub>2</sub>, which was identified in 2006 (Clark, 2005, Payungporn et al., 2008). Although these varieties were recently discovered, but the antibody against influenza A virus was first found in 1980 in Hong Kong (Houser and Heuschele, 1980). The disease was considered endemic, or native, to New York, New Jersey, Florida and the Colorado-Wyoming border area (Dalziel et al., 2014) and can rapidly transmit between individual dogs, but higher mortality rates may be seen in young, old or debilitated animals (Dubovi, 2010). Although this disease has high morbidity, it has low mortality (de Morais, 2006). To date, two different Influenza-A dog flu viruses, H<sub>3</sub>N<sub>8</sub> virus and H<sub>3</sub>N<sub>2</sub> have been identified. These viruses are usually spread through the air via coughing and

sneezing or by touching contaminated surfaces and then touching the mouth or eyes (Brankston et al., 2007).

Although H<sub>3</sub>N<sub>8</sub> CI was first reported in racing greyhounds, all breeds are now considered to be susceptible. The greatest risk of infection is among dogs that reside in kennels or are exposed to transient groups of dogs, as in animal shelters or dog day care facilities. Infected dogs from these high risk populations may introduce the virus into new areas (Barrell et al., 2010, Ramirez-Martinez et al., 2013).

Canine parvovirus (CPV) enteritis, which is one of the most common infectious diseases, is an important viral cause of diarrhea in dogs. CPV infection is a relatively new disease that initially emerged in 1978 in a native canine population and spread rapidly with high morbidity (100%) and mortality (10%) (Shackelton et al., 2005; Schoeman et al., 2013). CPV enteritis is a highly contagious disease spread by the fecal material of affected animals that can vary in severity from mild to over 90% fatal if untreated or not properly treated (Parrish, 1995). This virus causes vomiting, diarrhea (which is often bloody), lethargy (depression), fever, and life threatening dehydration.

Domestic and wild canines are usually affected (Nandi and Kumar, 2010) by CPV infection. Dogs at highest risk for infection are unvaccinated puppies or those that have not yet

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completed their vaccine series. CPV infection is most common in puppies between 6 weeks and 6 months of age (Pollock and Coyne, 1993). Diagnosis of CIV and CPV infection includes physical examination, blood tests, virus isolation, chest radiographs, ELISA, PCR and appropriate clinical signs, and a rapid test by a Directigen™ Flu A antigen detection kit (Desario *et al.*, 2005) or through detection of anti-CPV antibodies in the blood serum. Diagnostic techniques such as PCR have higher sensitivity and specificity than other methods of viral antigen determination in feces (Greene and Decaro, 2012). Reverse transcription polymerase chain reaction (RT-PCR) and the hemagglutination inhibition (HI) assay are tests of choice for serological diagnosis of infections in animals (Anderson *et al.*, 2012). Use of blood can also provide an estimation of viral load, which can help distinguish vaccination from natural infection (Veir *et al.*, 2009).

The best approach for diagnosis of CIV is collection of nasal swabs and serum samples. Conventional methods such as clinical signs of diagnosis are being utilized in Bangladesh for the diagnosis of CI and CPV infection. However, these techniques have been reported to be of less diagnostic value as such their diagnostic tests may have lost of limitation for confirmatory detection of CPV and CIV infection from field and laboratory samples. Clinical signs generally appear 2–7 days after the exposure (Ettinger *et al.*, 1995).

Therefore, this study was conducted to estimate the prevalence of CIV and CPV infection in pet and street dogs using a rapid antigen detection kit.

## Materials and Methods

### Study period and area

This research was conducted in the laboratory of the Department of Medicine, Bangladesh Agricultural University, Mymensingh, Bangladesh during the period of January 2015 to November 2015.

### Brief description of the experimental design

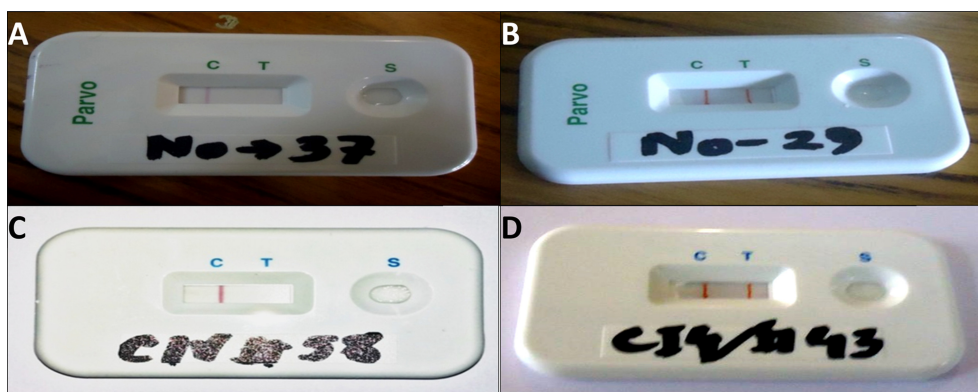
A total of 115 pet dogs nasal swab samples were collected from different places in three districts, Dhaka, Mymensingh and Sirajgonj, and a total of 114 rectal swab were collected from stray dogs in different zones of the Municipal Corporation of Mymensingh District in Bangladesh during the muni-

cipality's dog control program. Rectal swab samples were collected before the dogs were euthanatized by the municipal authority, while for nasal swab collection dogs were restrained by owners and hospital staff. The dogs studied were of different ages and genders with no history of vaccination against CIV and CPV infection. Tests were conducted using a rapid RapiGen® Canine Influenza Virus Ag Test Kit and a RapiGEN® Canine Parvovirus Ag Test Kit (RapiGEN Inc., South Korea, 2004) according to the manufacturer's instructions. The kit is a chromatographic immunoassay for the qualitative detection of CIV in canine nasal swabs and CPV antigen in canine feces. Clinical signs including the presence of nasal secretions and other flu like signs (if any), as well as age, sex and breed were recorded carefully. It was not possible to determine the diarrheic status of the selected dogs. The age of the dog was determined by asking the owner and examining the teeth. Health status of the dog was determined by close inspection.

Nasal swab and fecal swab samples were collected with the stick of the collection device or swab. Care was taken to avoid heavy contamination of the stick (swab) with extraneous material. The stick (swab) was inserted into extraction buffer bottle and the top portion securely fitted onto the base. The bottle was then agitated for 10 seconds by shaking vigorously to ensure good sample extraction, after which the test device was removed from its sealed pouch by tearing along the notch and snapped into the collection device. Next, 3–4 drops of extracted samples were dispensed into the sample wells by squeezing. The sample was allowed to stand for 10 minutes; however, results were obtained within 5 minutes. The test results were recorded by observing the color band by the naked eye.

### Interpretation of the test

Results of the tests were interpreted within 10 minutes by visual observation to determine if there was a single band for negative controls or a double band (one control and one treatment) for positive tests. A color band appeared in the left section of the results window to show that the test is working properly (C). The right section of the results window indicates the test results. If another color band appeared in the right section of the results window, this band is the test band (T). The presence of only one band within the results



**Fig 1.** Detection results for CPV and CIV kits. A, negative for CPV; B, positive for CPV; C, negative for CIV; D, positive for CIV.

window indicates a negative result. The presence of two color bands (T and C) within the results window, regardless of which band appeared first, indicates a positive result. If the purple color band was not visible within the result window after performing the test, the result was considered invalid (Esfandiari and Klingeborn, 2000).

#### Negative

One red/purple band appeared in the control line (c) with no apparent band in the test line (T), considered negative for CPV and CIV (Fig 1A and C).

#### Positive

Two red/purple bands appeared in the control line (c) and in the test line (T), considered positive for CPV and CIV (Fig 1B and D).

#### Invalid

No red/purple band appeared in the control line (C) or a band appeared in the test line (T) but not in the control line (C), then the test was invalid. There were no invalid results

found in this study.

#### Statistical analysis

Statistical analyses were conducted using the Statistical Package for Social Science (SPSS). Groups were compared by Chi-squared tests and a *p*-value of  $\leq 0.05$  was considered statistically significant. Prevalence was calculated according to Crichton (1995).

## Results

#### Results of CIV screening of pet dogs

A total of 115 (N = 115) nasal swab samples were collected from pet dogs from three districts and tested for the presence of CIV. The results are summarized in Table 1. More samples collected from Dhaka (n = 55) were CIV positive (12.72%) than from Mymensingh (12.50%, n = 32) and Sirajgonj (7.14%, n = 28), which was likely because there were more pet dogs in Dhaka. Evaluation of the total sample group revealed that 9.01% samples from Mymensingh were from the Bangladesh Agricultural University (BAU) campus

**Table 1.** Prevalence and associated risk factors of CIV infection in pet dogs

Variables	Category level	Number of samples tested	Number of positive	Positive rates (%)	Overall prevalence (%)	P-value	
Age	Up to 6 months	23	7	30.43	6.08	0.011**	
	6 < 12 months	63	5	7.93	4.34		
	12 < 18 months	15	1	6.66	0.86		
	18 months and older	14	0	0	0		
Sex	Male	62	10	16.12	8.69	0.077	
	Female	53	3	5.66	2.60		
Vaccinated	Yes	34	1	2.94	0.86	0.067	
	No	81	12	14.81	10.43		
Presence of flu-like signs	Yes	52	10	19.23	8.69	0.015**	
	No	63	3	4.76	2.60		
Breed	German Shepherd	18	2	11.11	1.73	0.854	
	Hound	8	1	12.50	0.86		
	Keeshond	9	2	22.22	1.73		
	Labrador	4	0	0	0		
	Local	59	7	11.86	6.08		
	Pomeranian	8	0	0	0		
	Terrier	9	1	11.11	0.86		
Area	BAU campus	11	1	9.01	0.86	0.960	
	Mymensingh	Kewatkhali	14	2	14.28		1.73
		Bolashpur	7	1	14.28		0.86
	Dhaka	CVH	55	7	12.72		6.08
		Betobari	8	1	12.50		0.86
	Sirajgonj	Solop	8	0	0		0
		Ullapara	12	1	8.33		0.86
Total		115	13	11.30	11.30		

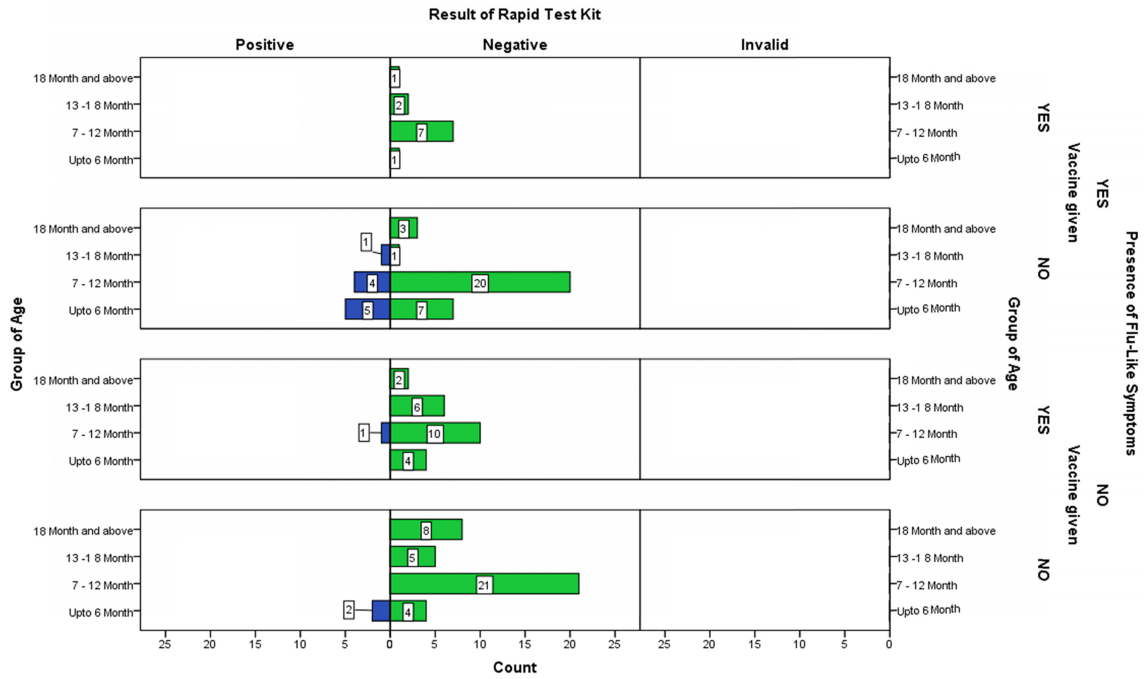


Fig 2. Prevalence of CI relative to age group, presence or absence of flu and vaccinated or unvaccinated dogs.

Table 2. Prevalence and associated risk factors of CPV infection in street dogs

Variables	Category level	Number of samples tested	Number of positive	Positive rates (%)	Overall prevalence (%)	P-value
Age	1-6 months	24	14	50.00	12.28	0.005**
	7-12 months	60	13	21.66	11.40	
	13-24 months	23	9	39.13	7.89	
	More than 24 months	7	1	14.28	0.87	
Sex	Male	61	21	34.42	18.42	0.780
	Female	53	16	30.18	14.03	
Health	Poor	70	30	42.85	26.31	0.004*
	Normal	44	7	15.90	6.14	
Area (Mymensingh)	Town Hall	7	2	28.57	1.75	0.0029**
	Akua	17	5	29.41	4.38	
	Boundary Road	8	3	37.50	2.63	
	Amlapara	11	4	36.36	3.50	
	Charpara	4	0	0	0	
	Mintu College Road	9	4	44.44	3.50	
	R. K. Mission Road	25	8	32.00	7.01	
	Senbari Road	13	3	23.07	2.63	
	Bau Campus Mymensingh	20	8	40.00	7.01	
Total		114	37	32.45	32.45	

(n = 11), but the prevalence of CI was highest (14.28%) in Kewatkali (n = 14) and Bolashpur (n = 7), whereas 12.72% of samples were found to be positive in CVH (n = 55), 12.50% in Betobari (n = 8), and 8.33% in Ullapara (n = 12). In this study, samples were divided into four age groups. Overall, dogs aged up to 6 months (n = 23) showed more pos-

itive results (30.43%) than those aged 6-12 months (n = 63; 7.93%) and 12-18 months (n = 15; 6.66%). No dogs aged above 18 months (n = 14) were found to be positive. Additionally, more males were positive (n = 62; 16.10%) for CIV than females (n = 53; 5.66%). Furthermore, 52 dogs had flu-like signs and 63 did not. Additionally, more dogs with flu-

like signs were positive for CI (19.23%) than those without flu-like signs (4.76%), and more unvaccinated dogs were positive (14.81%) than vaccinated dogs (2.94%). Overall, 11.11% of CIV positive dogs were German shepherd and Terrier breeds, while 11.86% were local breeds and 12.50% were Bloodhound breeds. The highest number (22.22%) of CIV positive dogs were Keeshonds.

#### **Overall distribution of CIV in accordance with age group, presence or absence of flu and vaccinated or unvaccinated dogs**

From a total of 115 samples, the prevalence of CI in dogs having flu-like symptoms with no history of vaccination at the age of up to 6 months was 9.62%, followed by 7.69% in dogs aged 7-12 months and 1.92% in the 13-18 months old group. For dogs with no flu-like symptoms and non-vaccinated dogs, those aged up to 6 months had 3.17% prevalence of CI, while this value was 1.58% in vaccinated dogs with no flu-like symptoms (Fig 2). Based on the total sample group, animals 0-12 months of age with flu like symptoms and no history of vaccination showed more positive results than other animals.

#### **Overall prevalence of CPV infection in stray dogs**

A total of 114 rectal swabs were tested for the presence of CPV-2 antigen. Among them, 37 showed positive results, indicating an overall CPV infection of 32.45% (Table 2).

#### **Age, sex, health status and area wise prevalence of CPV infection in street dogs**

The prevalence of CPV infection was significantly higher in puppies aged 1-6 months ( $n = 24$ ; 50.0%) than in other age groups (Table 2), while it was lower in older dogs more than 24 months of age ( $n = 7$ ; 14.28%;  $p < 0.005$ ). The prevalence of canine parvovirus infection in dogs was 34.42% (21 out of 61) in males and 30.18% (16 out of 53) in females (Table 2). However, the sex wise prevalence of CPV infection did not differ significantly. The prevalence of CPV infection was higher (42.85%) in dogs with poor health status than those with normal health (15.90;  $p = 0.004$ ). The area wise distribution of CPV infection is presented in (Table 2). The prevalence was higher in dogs from Mintu College Road (44.44%), and no positive reaction was found in dogs of Charpara.

## **Discussion**

The overall prevalence of CI infection in pet dog was 0.86% in the BAU campus, Betobari, Ullapara and Bolashpur areas, 1.73% in Kewatkhal, 6.08% in CVH and 0% in Solop. Based on nasal swabs, Pecoraro *et al.*, (2014) found 4.4%, 4.7%, 3.2%, 1.2% and 0% of dogs in New York, Colorado, South Carolina, Florida, and California and Texas shelter dogs were positive for CIV, respectively. Additionally, the prevalence of CI was found to be higher in dogs aged up to 6 months (6.08%) than in those aged 6-12 months (4.34%) and 12-18 months (0.86%). No dogs older than 18 months were found to be positive in the present study. Conversely, Zhao *et al.* (2011) reported a prevalence of CIV of 4.87% (HI), 6.19% (ELISA) and 7.41% among dogs of dif-

ferent ages, with a high prevalence in pet dogs of 1 to 3 years old, but low prevalence in pet dogs  $\leq 1$  year. Another study by Youzbashi *et al.* (1996) identified antibodies against influenza virus in 50.6% of dogs aged one month. Overall, these findings demonstrate that CIV infection can affect dogs of any age, but that young dogs are most susceptible. In this study, males were found to be positive 2.84 times more often (16.1%) than females (5.66%), whereas Zhao *et al.* (2011) found the prevalence in male dogs to be 7.78% and that in females to be 5.21%. These findings demonstrated that canine influenza virus infection is prevalent in male pet dogs. Canine influenza or dog flu is a contagious viral respiratory disease in dogs that can affect any canine species or breeds. In this study, 115 samples of pet dogs, 1.73% were positive for CIV in German shepherds and Keeshonds, while there were 0.86% in Bloodhounds and Terriers breeds. However, the highest number of CIV positive dogs (6.08%) was found in local breeds (Table 1). Dubovi (2010) mentioned that dogs of any breed or age were susceptible to infection, but that it is likely that some dogs that have recovered from infection retain limited immunity to re-infection. The current study revealed a significant relationship between the presence of flu-like symptoms and CIV infection. Specifically, this value was 19.23% in the flu presence group, which was 4.03% higher than those with no history of flu; however, Kang *et al.* (2013) mentioned that canine H<sub>3</sub>N<sub>2</sub> influenza virus isolated from pet dogs showed severe respiratory signs and other clinical symptoms such as fever, reduced body weight, and interstitial pneumonia. In another study, Song *et al.* (2009) reported that CIV susceptible dogs showed elevated rectal temperatures, severe necrotizing tracheobronchitis and bronchioalveolitis.

CPV infection in street dogs in Mymensingh was found to be significantly higher in puppies aged 1-6 months. Similar reports were also made by Parthiban *et al.* (2010a). The prevalence of CPV infection was relatively high (50%) in dogs aged 1-6 months and 21.66% in those aged 7-12 months, which is much higher (16.8-18.3%) than the values reported by Grigonis *et al.* (2002), but lower (83.8%) than those reported by Parthiban *et al.* (2010a). Specifically, the prevalence of CPV infection in dogs aged 1-6 months was found to be 1.27 times higher than that of dogs aged 13-24 months and 3.5 times higher than those more than 24 months old. These results showed that CPV infection was a common problem in street dogs in Mymensingh. Islam *et al.* (2014) also reported a similar prevalence of CPV infection in street dogs in Bangladesh. In the present study, the prevalence of canine parvovirus was found to be relatively higher in males (34.42%) than in females (30.18%). Similar findings were also reported by other authors (Parthiban *et al.*, 2010b; Islam *et al.*, 2014). There are currently a variety of methods available for the detection of CIV and CPV, including HA, HI, EM, PCR, RT-PCR, and ELISA. While most of these tests are time consuming and require specific equipment, the RapiGen<sup>®</sup> Canine Influenza Virus Ag Test kit and RapiGEN<sup>®</sup> Canine Parvovirus Ag Test Kit is one of the most reliable and rapid one-step tests based on the immunochromatographic assay of CIV and CPV antigen in canine nasal swab and feces. The test requires only 5-10 minutes to complete and

CIV and CPV antigen detection make this technique a useful tool for CIV and CPV diagnosis. Additionally, each of the general aspects of the test system evaluated was easy to perform and the results were easy to read.

## Conclusions

This study was conducted to estimate the prevalence of CIV and CPV antigen detection from field samples of dogs in Bangladesh. Over 229 (115+114) samples were finalized of which 13 dogs tested positive by screening test for CI and 37 dogs tested positive by screening for CPV. CIV infection in the pet dogs of Dhaka, Mymensingh and Sirajgonj was initially prevalent (11.30%) and CPV infection in street dogs of Mymensingh was highly prevalent (32.45%). Influenza viruses of assorted varieties have been the subject of concern for humans, wildlife, and domestic animals for many decades. However, CI or canine flu is a relatively new disease caused by canine influenza viruses of different strains. All dogs are susceptible to this condition, and there is no natural immunity. CPV is a highly contagious virus infecting members of the canine family that invades and destroys rapidly growing cells in the intestine, bone marrow and lymphoid tissue, resulting in nausea, vomiting and severe hemorrhagic (bloody) diarrhea. Invasion of the bone marrow cells causes a decrease in white blood cell count that leads to increased susceptibility to bacterial infections and occasionally a shock like condition known as endotoxemia. This disease can vary from mild to fatal if not properly treated; however, it is preventable, so vaccination should be initiated to prevent its occurrence and regular monitoring should be continued.

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