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RESEARCH ARTICLE
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Polymerase chain reaction based epidemiological investigation of canine parvoviral disease in dogs at Bareilly region

Jobin Thomas, Mithilesh Singh, T. K. Goswami, Suman Verma and Surendra Kumar Badasara

Department of Immunology, Indian Veterinary Research Institute, Bareilly, Uttar Pradesh, India.
Corresponding author: Mithilesh Singh, e-mail: drmithileshsingh@yahoo.com,
JT: jobss2k6@gmail.com, TKG: goswami.tapas@gmail.com, SV: suman0751@gmail.com, SKB: badsara.vet@gmail.com
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Abstract

Aim: The aim of this study was to screen the suspected samples by polymerase chain reaction (PCR) and epidemiological analysis of positive cases of canine parvovirus type2.

Materials and Methods: Fecal samples were collected from dogs suspected for canine parvovirus type 2 (CPV-2) and viral DNA was extracted. Primers were designed, and PCR was done with all extracted DNA samples. Age, sex and breed wise distribution of positive cases were analyzed.

Results: Out of a total 44 collected fecal samples, 23 were found to be positive for CPV-2 by developed PCR. The disease was found to be more common in Labrador male pups of 3-6 months of age. The percentage of positive cases in vaccinated dogs was found to be around 17.4%.

Conclusion: Almost half (52.3%) of total collected samples were found to be positive by PCR. However, number of field samples are needed to further validate this test and additionally sequence analysis needs to be done to ensure the prevalent field strain of CPV-2.

Keywords: Canine parvovirus type 2, fecal sample, polymerase chain reaction.

Introduction

Canine parvovirus type 2 (CPV-2) is a non-enveloped single-stranded DNA virus which is responsible for an acute and sometimes fatal enteritis in dogs [1]. It accounts for up to 26% of the mortality among all viral diseases of dogs [2]. The virus first appeared in 1978, probably arose from a very closely related virus in cats, the feline panleukopenia virus through a small number of mutations in the single capsid protein. Then, it has spread worldwide and being replaced by some new antigenic variants likes CPV-2a, CPV-2b and CPV-2c [3]. Currently, this is one of the important pathogenic viruses affecting dogs with high morbidity and mortality [4].

Parvoviral disease of dogs is mainly manifested as two forms, intestinal and myocardial form. Intestinal form is more common which is characterized by hemorrhagic gastroenteritis, and the less common myocardial form is characterized by myocarditis [5]. Several molecular and serological techniques are available for the detection of viral antigens and antibodies to the virus [5]. Vaccination is the most efficient strategy for the control of the disease.

The present study was undertaken with an objective to determine the epidemiology of CPV-2 infection by polymerase chain reaction (PCR) in Bareilly region.

Materials and Methods

Ethical approval

Not necessary as fecal samples were collected from clinical cases.

Sample collection

A total of 44 fecal samples were collected from dogs suspected of CPV-2 infection showing typical clinical signs such as foul smelling, hemorrhagic diarrhea and vomiting presented at Veterinary Polyclinic, Indian Veterinary Research Institute during the time period from October 2013 to April 2014.

Extraction of DNA from the fecal sample

The DNA was extracted from all 44 fecal samples collected from dogs using QIAamp DNA Mini Kit (Qiagen, Germany) as per the suggested protocol.

Primer designing

Specific primers were designed for the amplification of a part of VP2 (tVP2) gene of CPV-2 by comparing the published sequence of CPV-2 (Gene bank accession number EU377537.1, GI 166153875) using IDT Oligoanalyzer 3.1. PCR was standardized using the recombinant plasmid containing VP2 gene (KJ364525) obtained from immunology lab, IVRI. To standardize the optimum annealing temperature for proper amplification of tVP2 gene, gradient PCR was also done using the same positive control with annealing temperature ranging from 52°C to 64°C (Tm of CPV PF is 63.1°C and CPV PR is 54.4°C).

The sequences of the forward (CPV PF) and reverse (CPV PR) primers are given in Table-1.

Table-1: Primers used for amplification of tVP2 gene.

Target gene	Name of primer	Sequence (5'-….-3')	Length	PCR amplicon size
tVP2 gene	CPV-PF	ACTGCAGCGAATTCAGGTGATGAATTTGCTACAG	34	~564 bp
	CPV-PR	CTGACCTCGAGAGTATAGTTAATTCC	26	

PCR=Polymerase chain reaction, CPV=Canine parvovirus type

Polymerase chain reaction

Polymerase chain reaction was performed using DNA extracted from fecal samples with positive control as recombinant plasmid DNA (KJ364525) and negative control without any template DNA. PCR reaction was set up in a standard 25 µl reaction containing the following reagents using the standardized condition. The components used for PCR are given in Table-2. All the PCR ingredients were procured from Thermoscientific, USA.

PCR product was visualized using 1% agarose gel electrophoresis.

Analysis of positive cases

Sex, breed and age wise distribution of positive cases were analyzed.

Results

The PCR conditions were standardized for the designed primers CPV PF and CPV PR as follows:
Step 1: Initial denaturation - 95°C for 5 min.
Step 2: Denaturation - 95°C for 45 s.
Step 3: Annealing - 58°C for 45 s - 32 cycles.
Step 4: Elongation - 72°C for 1 min.
Step 5: Final elongation - 72°C for 10 min.

Out of 44 fecal isolated DNA samples, 23 were found to be positive by developed PCR as it revealed a single and uniform band of 564 bp size on 1% agarose gel (Figure-1). On the basis of analysis of the positive samples it is revealed that the disease was found to be more common in Labrador pups and 3-6 months male puppies are mostly affected. The age, sex and breed wise analyses of positive cases are given in Figures-2-4 respectively. Out of the total positive samples 17.4% of cases were from vaccinated dogs.

Discussion

Parvoviral disease is an economically important disease of domestic and wild carnivores, especially dogs [5] with two clinical forms-intestinal and myocardial. The main clinical symptom associated with the intestinal form of CPV-2 affected dogs is hemorrhagic gastroenteritis. It is difficult to precisely diagnose CPV-2 infection from the main clinical signs, such as vomiting and diarrhea because these symptoms are more or less common to other enteric diseases [6]. There are several methods for confirmatory diagnosis of parvoviral disease [7]. PCR technique is widely used as a tool for the diagnosis of canine parvoviral infection which provides rapid, sensitive and accurate diagnosis of the disease [8]. In the present study, PCR was developed by designing new primers considering all the critical residues contributes to strain differences so that every variant of CPV-2 can be detected. Primers were designed by considering the facts like absence of dimerization capability, absence of significant hairpin formation and lack of secondary priming sites in the template. Melting temperature and GC content (CPV PF - 44.1%, CPV PR - 42.3%) of the primers were in the normal range. Optimization

Table-2: Polymerase chain reaction.

Component (stock)	Amount
10×Taq buffer	2.5 µl
MgCl2 (1.5 mM)	1.5 µl
Forward primer, CPV-PF (10 pM/µl)	0.5 µl
Reverse primer, CPV-PR (10 pM/µl)	0.5 µl
dNTP mix (10mM)	0.5 µl
Taq DNA polymerase (0.5 U/µl)	0.5 µl
Nuclease free water	17.5 µl
DNA	1.5 µl
Total	25.0 µl

CPV=Canine parvovirus type

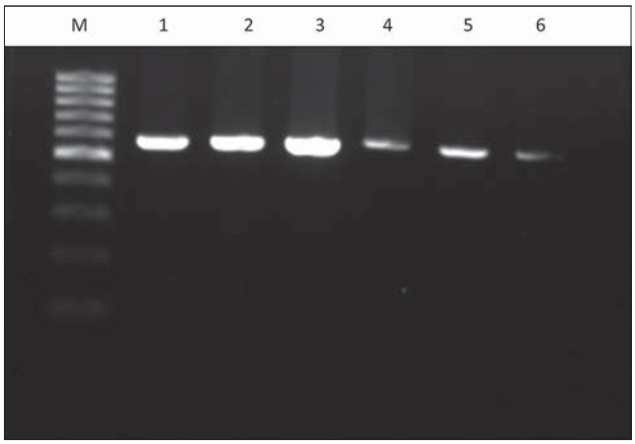

Figure-1: 1% agarose gel electrophoresis showing 564 bp size band from positive samples. Lane M: 100 bp plus ladder. Lane 1-6: Positive samples.

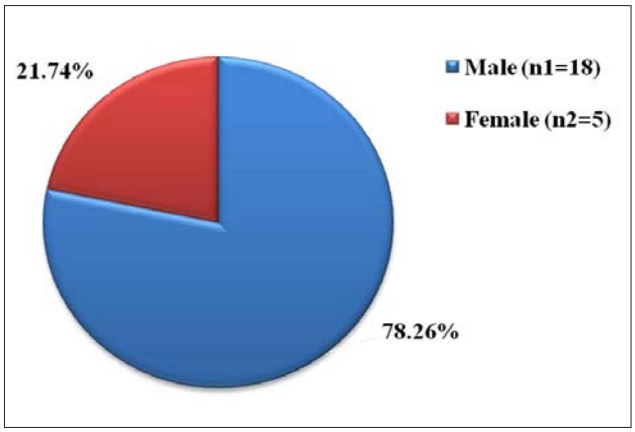

Figure-2: Sex wise analysis of positive samples.

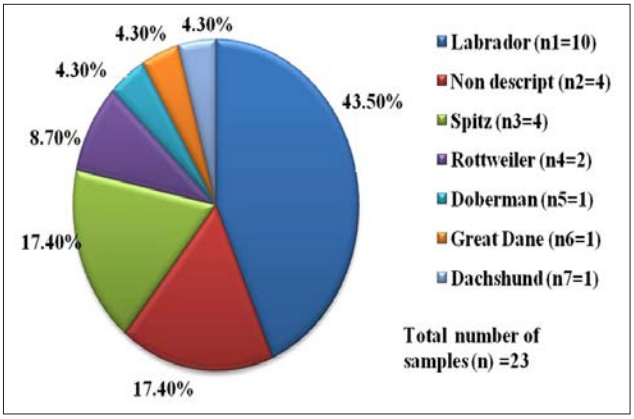

Figure-3: Breed wise analysis of positive samples.

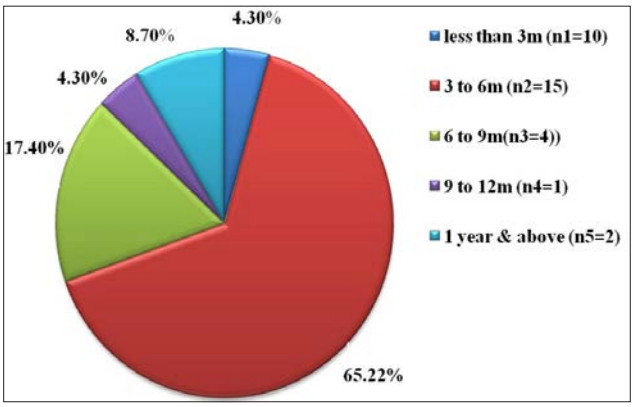

Figure-4: Age wise analysis of positive samples.

of annealing temperature was done by gradient PCR. The optimal annealing temperature depends upon the melting temperature of the primer-template hybrid. High annealing temperature result in the inefficient primer annealing, but very low annealing temperature may lead to a non-specific annealing. In the present study, a fairly good amplification of a specific product could be observed at an annealing temperature of 58°C corresponding to 564 bp size on 1% agarose gel. Thus, annealing temperature of developed PCR was optimized as 58°C.

PCR results indicated that 52.3% of dogs clinically showing the typical disease symptoms were found to be positive for CPV-2. The present finding revealed that the disease was more common in Labrador breed which is in contrary to the previous reports that German shepherd, Rottweiler and Doberman are more susceptible to CPV-2 [9]. However, in the current study most of dogs (around 39%) admitted in the hospital were Labrador puppies compared to other breeds like German shepherd, Rottweiler and Doberman. This may be the reason for relatively high percentage of positive cases observed in Labrador dogs. Younger dogs are generally protected from CPV-2 infection by maternally-derived immunity [5]. Susceptibility to CPV-2 infection often coincides with the time that puppies are separated from dams as the level of protective immunity, or maternal immunity declined [3]. In the current study, 62.51% of the cases were from 3 to 6 months aged puppies parallel to previous reports. In addition, it is found that disease was mainly observed in male dogs and reason may be due to the fact that more than 65% of admitted dogs were male. Even though, there is no sex prelidiction for canine parvovirus disease, it is speculated that males usually travel more than female and then they are more susceptible to field challenge [10]. There was no any biasness in the collection of samples in respect to breed, sex and age.

In parallel to the previous reports of vaccination failure [11], 17.4% of infected dogs in the current study were vaccinated against CPV-2. PCR positivity in recently vaccinated dogs may be due to the continuous excretion of the vaccine strain through feces up to 2-3 weeks following vaccination. However, there are many reasons for failure of vaccination. There are many reports that dogs may suffer from parvoviral enteritis even after practicing all the recommended guidelines of immunization [11]. One of the principal reasons behind the failure of vaccination is the persistence of interfering levels of maternal antibodies. The successful vaccination can only be achieved when maternal antibody levels decline to less than or equal to 1:10 [12]. Another cause of vaccination failure is the poor vaccine efficacy. There are some concerns that whether the CPV-2 based vaccines are effective against new antigenic variants [13]. In India, most of the vaccines marketed are based on the original CPV-2 isolated about 30 years ago. However, CPV-2a and 2b has recently outpaced the original CPV-2 incidence in dogs in most parts of the world [14] including India [15]. Hence, sequence analysis of CPV-2 isolates may throw some light on reasons behind vaccination failure.

Conclusion

PCR is a sensitive technique for diagnosing the paroviral disease. In the present study, 52.3% suspected cases were confirmed to be positive by PCR. Disease reported in vaccinated animals suggests that further studies are needed to explain the reasons behind vaccination failure. The emergence of new strains makes the vaccine efficacy questionable. So research studies should be focused on this aspect for the effective control, early diagnosis and proper treatment of the disease.

Authors' Contributions

This study was a part of M.V.Sc. thesis of the JT submitted to IVRI, Deemed University. JT, MS, SV and SKB have equally contributed in the sample collection and processing while MS and TKG contributed in manuscript preparation and editing.

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Competing Interests

The authors declare that they have no competing interests.

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