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Jitendra Tanwar
Teaching Associate, Veterinary Clinical Complex, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

DK Bihani
Former Professor and Head, Department of Veterinary Clinical Medicine Ethics and Jurisprudence, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Sunita Choudhary
Assistant Professor Veterinary Clinical Medicine Ethics and Jurisprudence from College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Gaurav Jain
Teaching Associate, Veterinary University Training and Research Centre (Rajasthan University of Veterinary and Animal Sciences, Bikaner) Bakalia, Nagaur, Rajasthan, India

Anju Chahar
Professor and Head, Department of Epidemiology and Preventive Veterinary Medicine, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Corresponding Author:
Jitendra Tanwar
Teaching Associate, Veterinary Clinical Complex, College of Veterinary and Animal Science, Rajasthan University of Veterinary and Animal Sciences, Bikaner, Rajasthan, India

Prevalence of canine parvovirus infection in Bikaner (Rajasthan) by polymerase chain reaction

Jitendra Tanwar, DK Bihani, Sunita Choudhary, Gaurav Jain and Anju Chahar

Abstract
The present study was focused on prevalence of canine parvovirus infection in dogs of north western arid area Bikaner (Rajasthan). Prevalence rate was studied on the basis of age, breed and gender. A total of 100 dogs' of different age, breed and gender which were presented to the VCC, College of Veterinary and Animal Science, Bikaner showing signs of diarrhea (hemorrhagic or non- hemorrhagic) and vomiting were selected for the present study and fecal samples were collected for isolation of DNA using polymerase chain reaction. The overall prevalence of CPV infection was found to be 30 per cent by PCR. The prevalence of canine parvovirus infection was highest in male dogs (34.61%) and in less than 3 months of age (40.54%) dogs, Labrador breed has shown highest prevalence of 41.66% and non-vaccinated dogs showed 43.33% of prevalence.

Keywords: Canine, parvovirus, Bikaner, Labrador, diarrhea, PCR

Introduction

In present time, dogs are the most widely abundant terrestrial carnivores, they performs many roles for humans such as hunting, protection, aiding disabled people and therapeutics. Dogs are infected from many diseases (nutritional, bacterial, viral and parasitic) in their lifespan. Amongst viral infections, canine parvovirus (CPV) belongs to the family of relatives parvoviridae, subfamily parvovirinae and genus parvovirus has been determined to be a well-known pathogen of dogs and is accountable for approximately 27% of dog diarrheic cases (Sakulwira *et al.*, 2001) [17]. In dogs, infection because of canine parvovirus 2 (2a, 2b and 2c) (Greene and Decaro, 2012) [9] is characterized by clinical signs of fever, anorexia, lethargy, depression, vomiting, mucoid to hemorrhagic diarrhea, weakness and sometimes leukopenia broadly seen in puppies from 6 to 20 weeks old age of both gender and in all the breeds. Recently the polymerase chain reaction (PCR) technique has been increasingly used as a tool for the diagnosis of canine parvoviral infection having high degree of sensitivity and specificity in detecting CPV in fecal sample (Agnihotri *et al.,* 2017) [1].

Material and Method

Sample collection

A total of 100 dogs of different gender, age, breed presented to the VCC, College of Veterinary and Animal Science, Bikaner showing signs of diarrhea (hemorrhagic or non-hemorrhagic) and vomiting were selected for this study and fecal samples were collected with sterile rectal swab in phosphate buffer saline (pH=7.2) and preserved at -20˚C for polymerase chain reaction.

Data were recorded regarding age, breed and gender of the animal.

Sample preparation and DNA isolation

DNA was manually isolated by Phenol-Chloroform Isoamyl (PCI) method by Barker (1998) [3]. The fecal suspensions with PBS had been processed by centrifuging at 1500 rpm for 10 min at 4 °C. Then supernatants have been collected in 2 ml series tube. The supernatant (500 µl) was boiled for 10 min at 100 °C to inactivate the polymerase chain reaction (PCR) inhibitors and chilled on ice. After boiling the sample was centrifuged at 1500 rpm for 5 min and the collected supernatant was used as a source of DNA template for PCR. The lysate was stored at −20 °C until further use.

Amplification of canine parvovirus (VP2 gene) in feces of dogs by polymerase chain reaction

The DNA extracts also were tested by a VP2 gene of CPV PCR assay, following the method developed by Pereira *et al.* (2000) [16]. VP2 partial gene of CPV amplified by conventional PCR by the using already published primer 555-Forward- AGGAAGATATCCAGAAGGA and 555-Reverse-GGTGCTAGTTGATATGTAATAAACA (Buonavoglia *et al.,* 2001) [6] supplied by Xcelris Ltd (Table-1).

The primers amplified portion of VP2 gene of CPV to yield a product size of 583bp. The reaction mixture for PCR was prepared in 200µl thin walled PCR tubes (Table-3). The master mix was prepared for each sample by adding 20 µl of Nuclease Free Water (NFW), 25 µl PCR master mixture, 1 µl of forward and 1 µl reverse primers each and the template DNA (3 µl) was separately added in the tubes. The 50 µl reaction mixture was kept for amplification in programmed thermal cycler. The PCR amplification conditions were as follows: initial denaturing at 95 ℃ for 5 min followed by 36 cycles of 95 ℃ for 30 s, annealing 55 ℃ for 30 s, and extending at 72 ℃ for 30 s, with a final extension at 72 ℃ for 10 min.

After the amplification, the PCR product was stored at -20˚c for further analysis. The amplified PCR products were analyzed on 1% agarose gel (Lonza, USA) visualized under UV transilluminator (Alpha Innotech, USA) as per the procedure described by Sambrook and Russel (2001) [18].

Table 1: Primer sequence and position of Oligonucleotide used for the amplification of VP2 gene of CPV

Primer	Primer Sequence	Sense	Position	Amplicon Size
555-for[b]	CAGGAAGATATCCAGAAGGA (20mer)	+	4003-4022	583bp
555-rev[b]	GGTGCTAGTTGATATGTAATAAACA (25mer)	_	4561-4585	

Table 2: PCR reaction condition

PCR Steps	Temperature	Time
Initial denaturation	95℃	5 min
Final denaturation	95℃	30 seconds
Annealing	55℃	30 seconds
Initial extension	72℃	30 seconds
Final extension	72℃	10 min

Table 3: PCR components for each reaction

S. No.	PCR Components	Quantity
1.	2x PCR assay buffer MgCl2 (4mM), dNTP (0.4mM),Taq DNA polymerase(0.05UµL)	25 µl
2.	Primer-F	1 µl
3.	Primer-R	1 µl
4.	Template DNA	3 µl
5.	Nuclease free water	20 µl
6.	Total	50 µl

Result and Discussion

The PCR screening of 100 suspected fecal samples revealed 30 samples as positive for canine parvovirus showing band size of 583 bp (Fig.1). The prevalence of canine parvoviral infection was 30% in cases presented to VCC, CVAS, Bikaner. The prevalence study was calculated with respect to total positive 30% (Table-4). The present aspect is in agreement with previous findings of Islam *et al.* (2014) [11], Sen *et al.* (2016) and Navarro *et al.* (2017) [15] and that they stated a usual prevalence of canine parvoviral infection as 30%, 30% and 24% respectively.

The occurrence of CPV infection highest in Labrador with 41.66% (10 out of 24) followed by in Pitbull 40% (8 out of 20), Rottweiler 33.33% (2 out of 6), German Shepherd 30% (6 out of 20), non-descriptive 16.66% (2 out of 12), Dalmation 12.50% (1 out of 8) and Sptiz 10% (1 out of 10) breed of dogs. Similar research had been executed by Haque and Tayyaba (2011) [10]; Kumar *et al.* (2011) [12]; Singh *et al.* (2013) [24] and Bhargavi *et al.* (2017) [4] and those employees stated that among pedigree puppies, Doberman, German Shepherd, Labrador and Rottweiler are high hazard breeds for parvovirus gastroenteritis. Labrador retriever, German Shepherd and Doberman Pinscher are the common breeds of puppies kept as pet in this location.

The gender wise prevalence revealed high occurrence i.e. 34.61% (18 out of 52) in male as compared with 25% (12 out of 48) in female. Previous studies on prevalence of CPV in male and female dogs (Bhatt *et al.,* 2015; Sen *et al.,* 2016; Bhargavi *et al.*, 2017 and Folitse *et al.,* 2017) [5, 4, 8] found results similar to the present study. They found that prevalence of parvovirus infection was higher 68.88%, 24.13%, 68.75% and 61.54% in males than females.

The age wise prevalence of CPV infection revealed the prevalence in the dogs <3 months of age was 40.54% (15 out of 37 examined dogs), followed by in 3-6 months of age 28.88% (13 out of 45 dogs), than in 6-9 months of age 12.5% (1 out of 8) and least in >9 month of age 10% (1 out of 10). Thus the highest prevalence was reported in the age group of less than three months. Similarly, highest prevalence were observed in 0-3 month pups or pups below 6 months age earlier by Apte (2016), Bhargavi *et al.* (2017) [4] and Foiltse *et al.* (2017) and prevalence rates recorded were 77.08%, 78.57%, and 65.67% respectively. High prevalence of CPV infection in young pups might be because of rapid and high epithelial cell turnover rate of the intestines with higher mitotic index of the enterocytes of intestinal crypts and in younger puppies (<8 weeks) there is higher intestinal mitotic activity than in older pups (Macartney *et al.,*1984) [13].

CPV infection was more prevalent in non-vaccinated dogs 43.33% (26 out of 60) compared to vaccinated dogs 10% (4 out of 40). Deka *et al.* (2013) [7], Apte (2016) and Folitse *et al.* (2017) [8] found similar results with highest prevalence of CPV in non-vaccinated dogs. This study indicates that non-vaccinated dogs are at high risk of parvovirus infection; this might be due to ignorance of the owners, high costs of vaccines, poor husbandry practices (Muzaffar *et al.,* 2006) [14].

Table 4: Overall prevalence of canine parvovirus infection by PCR

Total number of suspected cases	Positive by Polymerase chain reaction (PCR)	Prevalence (%)
100	30	30%

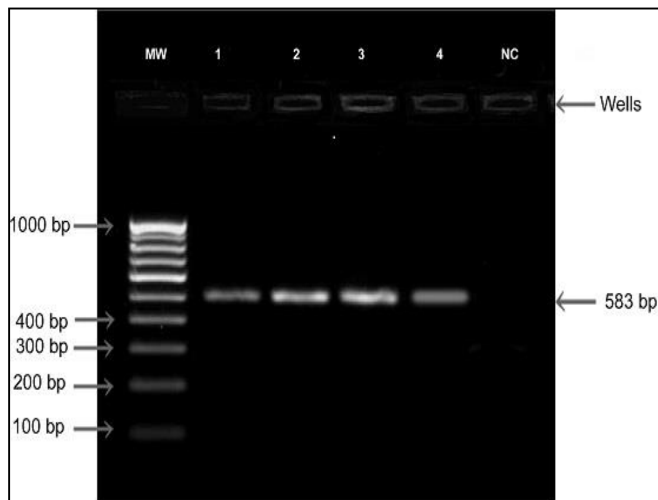

Fig 1: Polymerase chain reaction amplification product (VP2 gene) Legends: MW: Molecular weight marker, Wells: Amplicons (583 bp), NC: Negative control

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