RESEARCH ARTICLE

Prevalence of Canine Parvo Viral Infection in Dogs in and around Junagadh District of Gujarat State

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Abstract

A detailed study was conducted from October-2022 to March-2023 at the Veterinary Clinical Complex, Kamdhenu University, Junagadh, Gujarat involving screening of 817 dogs for presence of canine parvo viral infection via Rapid test kit and PCR. The prevalence of canine parvo virus in dogs was recorded as 4.77 % (39/817). Higher prevalence of parvovirus infection was recorded in dogs with age <3 months (53.84%). It was high in male dogs (56.41%) and in the month of December (38.46%). The prevalence was also found higher in non-descript breed (30.76%), in non-dewormed dogs (61.53%) and in non-vaccinated dogs (92.30%) compared to their counterparts. The findings highlight the influence of various factors on the prevalence of CPV and the level of awareness among pet owners of the region regarding prevention of this disease in their pets.

Key words: Dogs, Gastro-enteritis, Parvo virus, PCR, Prevalence, Vaccination.

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Introduction

In India, pet population is increasing day by day, especially dogs are almost 95% of total pet population followed by cats, rabbits, squirrels, tortoise, love birds and other small animals (Young, 1985). Dogs are susceptible to the contagious and infectious canine parvovirus (CPV) infection. This disease is caused by canine parvo virus-2 (CPV-2). Canine Parvo Virus-2 is a single-stranded DNA virus, and it is a mystery to science since it spreads quickly among susceptible canine populations and has a high pathogenicity to the canines (Haque and Tayyaba, 2011). CPV-2 was first recognized in 1977 and since then it has been considered as enteric pathogen of canine throughout world (Appel *et al*., 1979). After being discovered in 1979, CPV-2a swiftly replaced all other CPV types. Later, CPV-2b was identified in dog populations. Italy reported a novel antigenic type (CPV-2c) in 2001 (Buonavoglia *et al*., 2001). The Viral Protein 2 (VP2) epitope A has a critical amino acid at position 426 that is the primary antigenic variation between CPV-2 variants. Aspartic acid, asparagine, and glutamic acid are present at this position in CPV-2a, CPV-2b, and CPV-2c, respectively (Calderon *et al*. 2011; Navarro *et al*. 2017; Kapiya *et al*. 2019). This variant contributes to viral pathogenicity, vaccine failure, 2 virus host range and the sensitivity of diagnostic tests (Decaro and Buonavoglia, 2012).

Various studies reported the incidence of parvovirus infection in dogs in the range of 6.93% to 65.04% (Khare *et al*., 2019). Dogs of all age groups may contract the disease, but pups under three months are most vulnerable to this disease (Behera *et al*., 2015). There are no dog breeds that are immune to parvo virus infection. But the pure breeds like, German Shepherd, German Spitz, Doberman Pinscher, Daschund, Dalmatian, Labrador, Saint Bernard, Rottweiler and Great Dane are highly susceptible to this disease (Haque and Tayyaba, 2011). CPV disease is characterized clinically by enteritis, leukopenia, nausea, vomiting, depression and myocarditis in puppies over the age of 2 months (Decaro *et al*., 2007; Kang *et al*., 2008). This communication reports the prevalence of canine parvo viral infection in dogs in and around Junagadh district of Gujarat State.

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Materials and Methods

A total of 817 dog presented to Veterinary Clinical Complex, Veterinary College, Junagadh (India) from October 2022 to March 2023 were screened for Canine parvovirus infection. Dogs showing chief complaint of anorexia, foul-smelling diarrhoea, vomition, dehydration, depression and suspected for canine parvovirus infection were further subjected to clinical examination, screening by using Rapid CPV Ag Test Kit and confirmed by polymerase chain reaction.

Data related to the prevalence of parvovirus infection in the dog population was examined. Information was collected regarding age (<3 months, 3-6 months, 6-9 months, and >9 months), breed, sex, season, months, and immune state [vaccinated (full and incomplete) and non-vaccinated]. At the time of presentation, aseptically collected faecal samples from suspected dogs were placed in a sterile swab container, and were kept at - 20°C by adding 10% Phosphate Buffer Saline solution (PBS) with the aim to detect the CPV virus using Polymerase Chain Reaction (PCR).

PCR

Using the DNASure tissue micro kit, the genomic DNA of CPV-2 was extracted from the stool samples. The primers used for PCR were as detailed in Table 1. The composition of 25 µL reaction mixtures for PCR consisted of 2 X PCR Master mix 12.5 µL, DNA template 3 µL, Forward & Reverse primer 1 µL each, and Nuclease free water 7.5 µL. The PCR cyclic conditions involved initial denaturation at 94°C for 5 min, followed by 35 cycles each of denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec and extension at 72°C for 45 sec, and then final extension at 72°C for 3 min.

The data so collected was analyzed by Chi-square test using GraphPad prism 9.0 software. $p<0.05$ was considered statistically significant.

Results and Discussion

Overall Prevalence

Among 817 dogs in the study, 50 displayed strong clinical signs of parvo virus infection. Faecal swabs from these symptomatic dogs were analyzed using PCR and a canine parvo antigen test kit, revealing 39 PCR-positive and 32 antigen kit-positive samples. Thus in this study, the overall prevalence of parvo virus infection among the suspected dogs was determined to be 4.77 (39/817) %. On the basis of PCR, the prevalence was 78.00 % (39/50) and through the canine parvo antigen test kit it was 64.00 % (32/50). The rapid antigen test kit for parvovirus may be less reliable than PCR due to lower sensitivity, lower specificity, potential for genetic variation, and dependence on sample quality. PCR is considered more reliable for accurate detection of parvovirus infection due to its higher sensitivity and specificity.

Sayed-Ahmed *et al*. (2020) and Abhiram *et al*. (2023) have examined the prevalence of canine parvo virus (CPV) infection in dogs, and reported the prevalence rates of 59.70%, and 68.29%, respectively. These studies and present findings offered insights into CPV prevalence across populations and regions, influenced by factors like demographics, location, and methodology. Collectively, they underscore importance of Parvo virus globally, varying in intensity. The findings stress the ongoing requirement for effective management to curb the frequency of this disease. Kalavadiya (2018) and Khadse *et al*. (2023) recorded 40% and 20% prevalence by rapid test, and 40%, and 36%, by using PCR, respectively. These studies highlight the coherence of results from both diagnostic methods with our current trend, though the prevalence rates documented are quite lower than the present observations.

Gender-wise Prevalence

The gender-specific prevalence of CPV exhibited no statistically significant difference; nevertheless, it is worth noting that the prevalence in male dogs (56.41%) surpassed that in female dogs (43.58%) in terms of positive cases. The present finding corroborated with reports of Geetha and Selvaraju (2021) and Badwaik *et al.* (2022). The higher prevalence of CPV in male dogs could be attributed to higher male dogs reported during our study as well as their more active and playful nature having more outdoor activity this contracting other dogs. Additionally, the preference of pet owners to keep male dogs might contribute to this disparity.

Age-wise Prevalence

Age wise prevalence of CPV is presented in Table 2. It was also statistically non-significant between age-groups, though apparently it was quite high in <3 months old puppies (53.84%), and decreased with advancing age showing only 5.12 % prevalence in >9 months old dogs. Similarly, Foiltse *et al.* (2018), Khare *et al.* (2019), and Badwaik *et al.* (2022) consistently showed higher CPV prevalence in puppies aged 0-3 months or under 6 months. These studies collectively highlighted the vulnerability of young puppies to CPV infection. The higher prevalence of canine parvo virus (CPV) infection in young puppies can be attributed to several factors, such as less developed immune system and a strong affinity of this virus for rapidly dividing cells in the body, such as the intestinal crypts and bone marrow.

Table 1: Primer used for amplification of target VP2 gene fragment of viral DNA

Target gene	Primer sequence (5'to 3')		Size of amplicon	Reference
VP2 gene of canine parvo virus	F	TCCAGCAGCTATGAGATC	747 bp	Sakulwira *et al*. (2003)
	R	GATCTGTTGGTAGCAATAC		

During the weaning process, changes in diet can lead to alterations in the bacterial flora, resulting in a higher mitotic index of enterocytes in the intestinal crypts. This increase in cell division makes puppies more vulnerable to CPV infection. Furthermore, the decline of maternal antibody levels after approximately four weeks of age leaves puppies with limited protection against CPV. Finally, the incomplete or limited vaccination status of young puppies further contributes to their susceptibility to CPV. Vaccination plays a crucial role in providing immunity against CPV.

Breed-wise Prevalence

Breed wise prevalence of CPV presented in Table 3 revealed that it varied non-significant among different dog breeds, though it was quite high in Non-descript, German Shepherd and Labrador retriever as compared to other pure breeds. Our findings aligned with the research conducted by Khare *et al*. (2019), Sayed-Ahmed *et al*. (2020) and Geetha and Selvaraju (2021). The higher prevalence of CPV infection observed in certain breeds can be attributed to factors such as higher population density and poor adherence to vaccination schedules due to a lack of awareness or concerns among pet owners, as noted by Khare *et al*. (2019).

The predisposition of a disease in specific dog breeds is influenced by various factors, including the preferences of pet owners for certain breeds and the population of those breeds in a particular geographical area. These factors can make it challenging to establish a definitive diagnosis or draw a valid conclusion about breed predisposition for a specific disease. Non-compliance with the vaccination schedule by owners of non-descript breed dogs is another possible contributing factor to the higher prevalence of infection in this group.

Month-wise Prevalence

The chi-square test for month-wise prevalence of CPV was statistically non-significant, though the prevalence was quite high in December-January than in October-November and March among the total cases studied (Table 4). This may be due to almost similar prevalence noted in all months based on the positive cases found among the cases tested. Mehta *et al*. (2017) reported a greater number of cases in winter season (58.67%) followed by summer (37.50%) and monsoon (18.42 %) season. Kalavadiya (2018) and Parikh (2022) also reported higher cases in winter, particularly in December-January, followed by summer (36.36%). Like ours, a lower prevalence of CPV in October was observed by Badwaik *et al*. (2022). Infection rates for the disease are proven to be higher in spring and autumn due to increased outdoor activities of dogs and owners. These activities raise the exposure time, making dogs more prone to contact the disease. The virus's stability in colder temperatures adds to this, while indoor proximity in winter amplifies transmission risk. The colder weather can aid parvovirus survival, increasing exposure

Table 2: Age wise prevalence of canine parvovirus infection

Age	No. of sample	Positive by PCR	Prevalence (%)	χ2	P
<3 months	26	21(80.76 %)	53.84 %		
3-6 months	13	11(84.61 %)	28.20 %		
6-9 months	06	05(83.33 %)	12.82 %	4.755	0.190
>9 months	05	02(40 %)	5.12 %		
Total	**50**	**39**			

p>0.05, non-significant

Table 3: Breed wise prevalence of canine parvovirus infection

Breeds	No. of sample	Positive by PCR	Prevalence (%)	χ2	P
Pomeranian	02	01 (50 %)	2.56%		
Siberian Husky	02	01(50 %)	2.56 %		
Saint Bernard	02	02(100 %)	5.12 %		
Shih tzu	02	01(50 %)	2.56 %		
Golden retriever	01	00(00 %)	0.0 %		
Spitz	02	01(50 %)	2.56 %	7.770	0.651
Rottweiler	02	01(50 %)	2.56 %		
Doberman	04	03(75 %)	7.69 %		
German Shepherd	10	08(80 %)	20.51 %		
Labrador retriever	08	07(87.5%)	17.94 %		
Non-Descript	15	12(80 %)	30.76 %		
Total	**50**	**39**			

non-significant p>0.05

Table 4: Month-wise prevalence of canine parvovirus infection

Months	No. of sample	Positive by PCR	Prevalence (%)	χ2	P
October	2	1 (50.0%)	2.56 %		
November	5	3 (60.0%)	7.69 %		
December	18	15 (83.3%)	38.46 %		
January	14	12 (85.7%)	30.77 %	2.893	0.724
February	7	5 (71.4%)	12.82 %		
March	4	3 (75.0%)	7.69 %		
Total	**50**	**39**	**100%**		

p>0.05 non-significant

Table 5: Vaccination status wise prevalence of canine parvovirus infection

Vaccination status	No. of sample	Positive by PCR	Prevalence (%)	χ2	P
Vaccinated	5	1(20 %)	2.56 %		
Incomplete vaccinated	5	2(40 %)	5.12 %	17.37	0.0002
Non-vaccinated	40	36(90 %)	92.30 %		
Total	**50**	**39**			

p<0.01, highly significant

chances. Puppies born in November and December, around 1 to 4 months old during winter, are especially vulnerable to parvovirus infections.

Prevalence on Basis of Vaccination and Deworming Status

Prevalence of CPV on the basis of vaccination was highly significant as majority of infected dogs were non-vaccinated (Table 5). However, the dewormed dogs were non-significantly less infected (38.46%, 15/22) than non-dewormed (61.53%, 24/38). Tanwar *et al*. (2020), Geetha and Selvaraju (2021) and Abhiram *et al*. (2023) consistently reported a higher prevalence of canine parvovirus infection ranging from 43.33% to 97.14% among non-vaccinated dogs. The increased prevalence of CPV infection in non-vaccinated dogs can be attributed to the absence of protective immunity conferred by vaccination. Despite vaccination, parvovirus infections can still occur due to factors like vaccine failure, incomplete vaccination schedules, immune system challenges, new virus strains, and exposure to a high viral load. Non-dewormed dogs are at a higher risk of developing parvovirus infection due to several factors. Deworming helps control and prevent intestinal parasites, including worms, which can weaken the immune system. When a dog's immune system is compromised, it becomes more susceptible to various infections, including parvovirus.

Conclusions

Overall prevalence of Canine parvo viral infection was 4.77% in and around Junagadh region. Higher prevalence of parvovirus infection was recorded in dogs with age <3 months (53.84%), it was higher in male dogs (56.41%), in December (38.46%), in non-descript breed (30.76%) and in non-vaccinated dogs (92.30%) over their counter parts.

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