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Prevalence of canine parvoviral enteritis in vaccinated and unvaccinated puppies in and around Chennai, Tamil Nadu, India

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Abstract

A survey was conducted on parvoviral infection in dogs in and around Chennai, Tamil Nadu, India for a period of two years from January 2018 to December 2019. Fifty bloody diarrhetic faecal samples were collected from young pups suspected for canine parvovirus infection subjected to Polymerase Chain Reaction (PCR) assay for confirmation using stool DNA kit. The prevalence of canine parvoviral enteritis in the study area was 96 per cent. The prevalence of canine parvoviral enteritis was found to be higher in vaccinated exotic breed puppies (90 per cent) than vaccinated native breeds (40 per cent). The age wise prevalence of canine parvoviral enteritis showed no difference in both age groups of below two months (61.29 per cent in vaccinated puppies and 39.70 per cent in unvaccinated puppies) and above two months (57 per cent in vaccinated puppies and 42.10 per cent in non vaccinated puppies). No significant difference was noticed in both sexes in vaccinated and unvaccinated puppies. Though PCR results revealed no significance difference, vaccinated puppies showed higher incidence (60.41 per cent) than unvaccinated puppies. With regard to recovery rate, vaccinated puppies recorded higher percentages than unvaccinated puppies.

Keywords: dog, canine parvoviral enteritis, prevalence, PCR

Introduction

Dogs belonging to the family *Canidae* (canines) are considered as a part of human's family since ancient times. Because of their loyalty and unconditional love towards their owners, a vast majority of people around the world love them by enjoying their company in all walks of life. Like other animals, dogs are prone to many of the infectious diseases including bacterial, viral, fungal and parasitic diseases. Among various viral diseases, canine parvo viral enteritis is a highly contagious viral disease of great concern to the dog owners, practicing veterinarians and scientists due to its high morbidity and mortality rates. Although it affects all age groups, it affects mostly pups of age group between six to twenty weeks characterised by vomiting, bloody diarrhoea and severe leukopenia (Goddar, *et al.*, 2008)^[5]

The outbreaks of CPV have been reported from many countries including India. The prevalence study in India was first reported by Balu and Thangaraj in Madras (Balu and Thangaraj, 1981)^[2]. The pattern of disease experienced in a population is largely influenced by the susceptibility of host, vaccination, environmental conditions such as housing, hygiene, population density, and pathogenicity of the infectious agent (Behera, *et al.*, 2015)^[6]. Keeping in view of this, the present study was undertaken to find out the prevalence of canine parvoviral enteritis in vaccinated and non vaccinated puppies in and around Chennai, Tamil Nadu, India.

Materials and Methods

Fifty dogs showing signs of vomiting and bloody diarrhoea, irrespective of age, breeds and sex were chosen for the present study. These dogs were maintained either in houses as a part of family members or in shelters by the commercial dog breeders in and around Chennai. In addition, the dogs were presented to the Madras Veterinary College Teaching Hospital for disease diagnosis and treatment was also selected. Diarrhetic faecal samples were collected from suspected gastro enteritis puppies and stored in a sterile container containing Phosphate Buffered Saline (10% W/V) at -20 °C until further use. DNA from the fecal samples were isolated using QIAamp DNA stool mini kit (Qiagen, Hilten, Germany) according to

manufacturer's instructions.

The isolated DNA was subjected to PCR assay for detection of parvovirus infection. A reaction volume of 25 µl was prepared, consisting of 0.5 µl of each forward and reverse primer (sense, 5'-AAAGAGAGCCAGGAGAGGTA-3'; anti-sense, 5'-TTCTGACAGCAGGTTGACCA-3'), 0.5µl of 2 mM dNTPS (fermentas), 2.5 µl of Taq DNA buffers A 10X (Tris with 15 mM of MgCl₂) (Genei, Bangalore, India), 0.25 µl of Taq DNA polymerase (5U/ µl concentration, Genei, Bangalore) and finally 5 µl of lysate was added. Then the rest volume was adjusted to 25 µl by addition of nuclease-free water (Genei, Bangalore)

The reaction mixture was prepared in 200 µl PCR tubes. The amplification was performed in a thermocycler (Biorad) with a reaction condition comprised of an initial denaturation at

95 °C for 3 minutes, then 30 cycles of denaturation at 95 °C for 30 seconds, primer annealing at 55 °C for 30 seconds and extension at 72 °C for 30 seconds, a final extension at 72 °C for 5 minutes and hold on 4 °C for 10 minutes. The amplified PCR product were analysed on 1% agarose gel with the positive control and visualized under UV. The prevalence study was carried out in vaccinated and unvaccinated puppies with relation to breeds, age and sex on the basis of PCR results. The different breeds exotic dogs were used in this study viz German shepherd, Labrador Retriever, Doberman and beagle dog. These breeds were categorised into below 2 months and above 2 months age group. All the data with related to breed, age, sex and recovery rate were summarised in Table (1 - 2). Chi-square test was performed using Statistical Package for Social Science (SPSS).

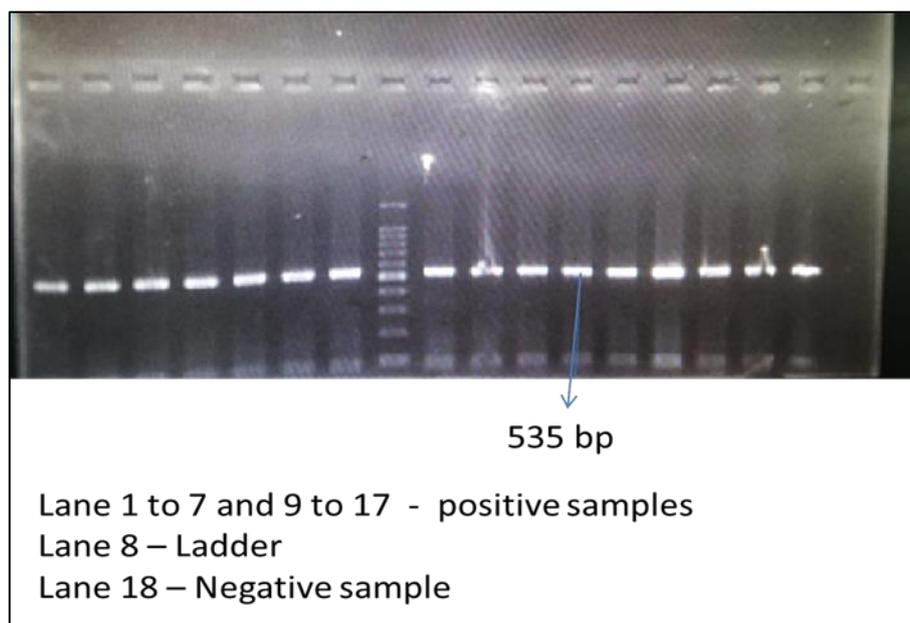
Table 1: Details of Breed, Age, Sex, Vaccination status and Survivability of dogs in and around Chennai

Serial number	Breed	Age	Sex	Vaccination status	PCR Result	Survivability
1	Native cross breed	62	Male	Not vaccinated	Negative	Died
2	Native cross breed	33	Male	vaccinated	Negative	Died
3	Native cross breed	35	Female	Not vaccinated	Positive	Died
4	Native cross breed	38	Female	Not vaccinated	Positive	Died
5	Native cross breed	48	Female	Not vaccinated	Positive	Died
6	Native cross breed	56	Female	Not vaccinated	Positive	Died
7	Native cross breed	60	Female	Not vaccinated	Positive	Died
8	Native cross breed	60	Female	Not vaccinated	Positive	Died
9	Native cross breed	180	Female	Not vaccinated	Positive	Died
10	Labrador retriever	90	Female	Not vaccinated	Positive	Died
11	Native cross breed	58	Male	Not vaccinated	Positive	Died
12	Native cross breed	60	Male	Not vaccinated	Positive	Died
13	Native cross breed	60	Male	Not vaccinated	Positive	Died
14	Native cross breed	65	Male	Not vaccinated	Positive	Died
15	Native cross breed	72	Male	Not vaccinated	Positive	Died
16	Native cross breed	84	Male	Not vaccinated	Positive	Died
17	Native cross breed	180	Male	Not vaccinated	Positive	Died
18	Labrador retriever	60	Male	Not vaccinated	Positive	Died
19	Native cross breed	54	Female	Not vaccinated	Positive	Recovered
20	Native cross breed	50	Male	Not vaccinated	Positive	Recovered
21	Native cross breed	90	Male	Not vaccinated	Positive	Recovered
22	Native cross breed	34	Female	vaccinated	Positive	Died
23	Pit bull	48	Female	vaccinated	Positive	Died
24	Native cross breed	56	Female	vaccinated	Positive	Died
25	Native cross breed	60	Female	vaccinated	Positive	Died
26	Labrador retriever	84	Female	vaccinated	Positive	Died
27	Beagle	90	Female	vaccinated	Positive	Died
28	German shepherd	90	Female	vaccinated	Positive	Died
29	Native cross breed	40	Male	vaccinated	Positive	Died
30	Native cross breed	45	Male	vaccinated	Positive	Died
31	Native cross breed	50	Male	vaccinated	Positive	Died
32	Labrador retriever	54	Male	vaccinated	Positive	Died
33	Native cross breed	55	Male	vaccinated	Positive	Died
34	German shepherd	55	Male	vaccinated	Positive	Died
35	Doberman	58	Male	vaccinated	Positive	Died
36	Labrador retriever	68	Male	vaccinated	Positive	Died
37	Labrador retriever	68	Male	vaccinated	Positive	Died
38	German shepherd	90	Male	vaccinated	Positive	Died
39	Native cross breed	52	Female	vaccinated	Positive	Recovered
40	Doberman	70	Female	vaccinated	Positive	Recovered
41	German shepherd	90	Female	vaccinated	Positive	Recovered
42	German shepherd	35	Male	vaccinated	Positive	Recovered
43	Labrador retriever	45	Male	vaccinated	Positive	Recovered
44	Doberman	52	Male	vaccinated	Positive	Recovered
45	Doberman	56	Male	vaccinated	Positive	Recovered
46	Doberman	58	Male	vaccinated	Positive	Recovered
47	Native cross breed	60	Male	vaccinated	Positive	Recovered
48	Native cross breed	65	Male	vaccinated	Positive	Recovered
49	Native cross breed	75	Male	vaccinated	Positive	Recovered

50	Doberman	120	Male	vaccinated	Positive	Recovered
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Table 2: Prevalence of canine parvoviral enteritis in puppies based on PCR assay-Breed, sex, age and survival status

Canine parvoviral enteritis in puppies							
		Vaccinated	Unvaccinated	Total	Chi-Square value	p-value	Significant level (<0.05)
PCR	Positive	29 (60.41%)	19(39.58%)	48	0.868	0.7682	Not significant
	Negative	1(50%)	1(50%)	2			
Breed	Native cross	11(39.28%)	17(60.71%)	28	12.547	0.000397	Significant
	Exotic	18(90%)	2(10%)	20			
Sex	Female	10 (52.63%)	9(47.36%)	19	0.797	0.371994	Not significant
	Male	19(65.51%)	10(34.48%)	29			
Age	Below 2 months	18 (60%)	12(40%)	30	0.0058	0.939254	Not significant
	Above 2 months	11(61.11%)	7(38.88%)	18			
Survivability	survivors	12 (80%)	3(20%)	15	3.4988	0.61412	Not significant
	nonsurvivors	17(51.51%)	16(48.48%)	33			

**Fig 1:** VP2 gene amplicon of CPV isolates from Chennai, Tamil Nadu, India

Results

The overall prevalence of canine parvoviral enteritis in the present study was found to be 60.41% in vaccinated puppies and 39.58% in unvaccinated puppies. Out of 50 faecal samples tested 48 (vaccinated -29 and unvaccinated -19) were positive by PCR for canine parvoviral enteritis (Fig 1). In addition, all the 48 puppies exhibited signs of parvoviral enteritis. Breed wise analysis of data showed a highest prevalence in vaccinated exotic breed (90%) when compared to the unvaccinated exotic breed (10%). Prevalence of canine parvoviral enteritis in vaccinated native cross breed was found to be (39.28%) while unvaccinated native cross breed was observed to be (60.71%) (Table 2). Out of 30 diarrheic faecal samples tested, 18 vaccinated puppies (60%) and 12 unvaccinated puppies (40%) from the age group of below 2 months were positive for canine parvoviral enteritis. In contrast, out of 18 faecal samples from the puppies belonging to the age group of more than 2 months, 11 vaccinated puppies (61.11%) and 7 unvaccinated puppies were positive for canine parvoviral enteritis (Table – 2). Between female and male, prevalence was not statistically significant ($P < 0.05$). Out of 19 females, 10 vaccinated females showed an incidence of 52.63% while the remaining 9 unvaccinated female recorded as 47.36%. Out of 29 male, 19 vaccinated males showed prevalence of 65.51% while 10 unvaccinated males recorded as 34.48% positive for canine parvoviral

enteritis. Thus, sex wise prevalence of canine parvoviral enteritis revealed higher in males than females. Out of 48 positive samples, 15 puppies were found to be recovered completely (12 vaccinated with 80% and 3 unvaccinated with 20%) and 33 puppies failed to recover (17 vaccinated with 51.51% and 16 unvaccinated with 48.48%). No significant difference was observed between survivors and non survivors in canine parvoviral enteritis.

Discussion

Vasantha Kumar (2011) [10] reported that an overall prevalence of 21.08% while Banja *et al.*, (2002) [3] recorded an higher prevalence rate of 53.4 per cent and Sanjukta *et al.*, (2011) [9] observed an incidence of 33.33 per cent. An increase in prevalence of canine parvoviral enteritis in the present study could be attributed to the cause of delay in vaccination of the pups and the pet owners might be unaware of the importance of vaccination. The present findings are in accordance with the observation of Sakar *et al.* (2005) [8] who also recorded similar findings and opined that in India canine parvoviral enteritis has got an emerging status, exotic breeds of German shepherd, Laborador, Doberman and Beagle dog are at higher risk of canine parvoviral enteritis whereas native and native cross breeds are less susceptible. Similarly Sanjukta *et al.* (2011) [9] reported the highest prevalence of canine parvoviral enteritis among Doberman (50%) followed

by German shepherd (41.1%) and least in Mongrel dogs (19.56%) as observed in the present study in which an increased incidence was recorded in exotic breed when compared to native breeds. The present findings are in agreement with that of Barush *et al.* (2004) [4] who also reported similar observations. The higher prevalence in younger pups could be due to presence of neutralizing maternal antibodies and rapid multiplication of canine parvovirus in intestinal mucosal epithelium in young pups (Banja, *et al.* 2002) [3]. Higher prevalence of canine parvoviral enteritis in puppies might be due to weaning of puppies at an early age and inadequate consumption of mother's milk which in turn leads to low maternal antibody. These findings are in accordance with previous report of Phukan, *et al.* (2010) [7]. In contrast, Banja *et al.* (2002) [3] reported no influence of sex on incidence of canine parvoviral enteritis. The high prevalence of canine parvoviral enteritis in male pups might be attributed to the increased exposure to canine parvovirus due to their behavior and selective preference of keeping males as pet by the pet owners (Anderson, 1980) [1].

The present study confirmed an increased incidence of recovery in vaccinated puppies than unvaccinated puppies. Recovery percentage is very low in canine parvoviral enteritis of unvaccinated puppies. These findings are similar to the previous reports of sarpong *et al.* (2017) [11].

Conclusions

Puppies of all age groups were found to be susceptible to CPV infection but puppies age less than two months were more susceptible than adults. Sex wise, both male and female puppies were affected while breed wise, both native cross breeds and exotic breeds were found to be susceptible to canine parvoviral enteritis. The percentage of infection was higher in vaccinated exotic breeds when compared to unvaccinated exotic breeds. Vaccination of young puppies with appropriate day along with maternal antibody titer is very important than treatment. Local variant of Canine Parvo Virus should be isolated for vaccine purpose to control canine parvoviral enteritis.

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