INCIDENCE OF CANINE PARVOVIRUS INFECTION OF DOGS IN ANDHRA PRADESH

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The present study was conducted on the incidence studies of the canine parvovirus (CPV) infection from different parts of Andhra Pradesh, India. A total of three hundred and forty two (n=342) canine parvovirus suspected clinical faecal samples of different age group of dogs from both vaccinated and non-vaccinated animals were collected. The faecal samples were preliminarily screened by haemagglutination assay and further confirmation by employing polymerase chain reaction (PCR). Dogs of age group below four months were more susceptible for CPV infection at a higher rate (77.59%) and were predominantly seen in males. PCR technique was found to be more sensitive in confirming the CPV infection (68.42%) than haemagglutination assay (20.67%).

Key words: Canine parvovirus (CPV), Enteritis, Haemagglutination assay, Incidence, Polymerase chain reaction

Canine parvovirus enteritis was first reported in USA (Eugster and Nain, 1977), but the identification of the causative virus was first documented as CPV-2 in Canada in June, 1978 (Kelly, 1978; Appel *et al.*, 1979). Canine parvovirus infection is one of the devastating disease of viral etiology affecting all age group of dogs and most commonly its occurrence is seen in pups

below eight weeks age group causing nearly 90 percent mortality. In acute form of disease condition it is characterised by vomitions, haemorrhagic enteritis with foul odour, dehydration finally leading to death if associated with myocarditis. In the myocarditis form of CPV-2 infection, the clinical signs may or may not be evident and pups may die sudden without any signs

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(Meunier *et al.*, 1984). The affected dogs void the viral particles in their faeces thus acting as a main source of transmission in spreading of the disease. The virus is shed during the acute phase of enteric fever and survives at least for four months in faeces and environment (Carmichael and Binn, 1981). Several outbreaks of CPV infection in India was reported by Chinchkar *et al.* (2006) and in southern India by Vivek *et al.* (2013). Screening of Canine parvovirus infection from different districts of Andhra Pradesh was carried out in this study based on age, sex and vaccination status.

MATERIALS AND METHODS

Three hundred and forty two faecal swabs from the rectum of clinically suspected dogs were collected from different districts of Andhra Pradesh. The samples were collected during the months of June, 2017

to December, 2018. Details are given in Table 1.

Three hundred and twenty seven samples were of unvaccinated status against canine parvovirus infection and only three samples were reported of being vaccinated only once and no boosters were further given. Sterile swabs were used for the collection of the faecal sample. Sorensens phosphate buffer saline (0.2M) having pH 7.0 was used as the transport media. The faecal swabs were emulsified in 1 mL of Sorensens phosphate buffer saline and later transferred to 1.5 mL centrifuge vials and centrifuged at 6000 rpm for 15 min. at 4°C. All the faecal material gets settled at the bottom and supernatant was collected and preserved at -80°C until use for performing haemagglutination tests and polymerase chain reaction.

Table 1. Details of faecal samples of dog collected from different districts of Andhra Pradesh of various age groups and both sexes

Sl. No.	Place of Collection	Number of Samples
1	Srikakulam	14
2	Visakhapatnam	25
3	East Godavari (Kakinada, Rajamundry)	52
4	West Godavari (Tanuku, Eluru)	19
5	Krishna (Teaching Veterinary Clinical Complex and Super Speciality Veterinary Hospital)	54
6	Guntur	44
7	Nellore	51
8	Tirupati	26
9	Kadapa	14
10	Kurnool	43
Total		342

Haemagglutination assay was carried out as per Carmichael *et al.* (1980). Ninety microliter of the processed faecal sample was mixed with ten microliter of chloroform and placed at 4°C for 10 min. Later centrifugation was carried out at 10,000 rpm for 10 min and the supernatant obtained was used for haemagglutination assay using 0.8% pig erythrocytes in a 96 well U bottom microtitre plate.

The DNA extraction from the processed faecal sample was done by employing boiling method (Vieira *et al.*, 2008). 100 µL of supernatant was boiled at a temperature of 96°C for 10 min and immediate snap chilling in crushed ice (Uwatoko *et al.*, 1995). Centrifugation was performed at 13,200 rpm for 10 min at 4°C. The supernatant obtained was further diluted in distilled water at a ratio 1:5 prior to DNA amplification so as to decrease the faecal residues that may interfere in the polymerase chain reaction (Decaro *et al.*, 2006). The VP2 capsid protein is highly immunogenic and the primer pair pCPV-2ab primer Forward (5'-GAAGAGTGGTTGTAAATAATT-3') Reverse (5'-CCTATATAACCAAAGTTA GTAC-3') amplify portion of VP1/VP2 gene of both CPV-2a and CPV-2b variants (3025-3706 nucleotide position of CPV genomic DNA) and produce an amplicon size of 681bp (Senda *et al.*, 1995; Pereira *et al.*, 2000).

PCR was carried out as per the method described by Panda *et al.* (2009). Initial denaturation at 95°C for 5 min followed by 30 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 2 min, extension 72°C for 2 min and final extension 72°C for 4 min.

The amplified products were analysed on 1.5% agarose with ethidium bromide and performed gel electrophoresis in 1X tris acetate buffer and observed in a Gel Doc (Bio-rad, USA).

RESULTS

A total of 342 samples were screened by haemagglutination assay of which 71 (20.76%) samples had a HA titre varying from 1:32 to 1:512. Eighty one faecal samples had a titre ranging from 1:2 to 1:16 and remaining 213 could not haemagglutinate the swine RBC and were negative for HA test. The incidence of the disease was highest in dogs of age group 6 weeks to 4 months (77.59%), followed by 4 months - 1year (46.42%) and was comparatively lesser in adult dogs (Table 2).

Canine parvovirus enteritis was predominantly noticed in males (77.56 %) when compared to females (53.73%) and high incidence of CPV infection was recorded in non-vaccinated dogs than the vaccinated ones (Table 3).

Table 2. Occurrence of canine parvovirus in different age groups of dogs

S.No	Age group	Number of samples obtained	Positive by PCR	% of positivity by PCR
1	6 weeks - 4 months	241	187	77.59
2	4 months - 1 year	84	39	46.42
3	Above 1 year	17	6	35.29

Table 3. Details of occurrence of canine parvovirus based on sex and vaccination status of dogs

S.No	Status	Number of samples obtained		Positive by PCR	
		Male	Female	Male	Female
1	Vaccinated	2	1	2 (100%)	1 (100%)
2	Non- vaccinated	205	134	159 (77.56)	72(53.73%)

Table 4. Details of occurrence of canine parvovirus in different districts of Andhra Pradesh based on PCR Assay

S.No	District	Number of samples collected	No. of samples positive by PCR assay
1	Srikakulam	14	10 (71.42%)
2	Visakhapatnam	25	20 (83.33 %)
3	East Godavari (Kakinada, Rajamundry)	52	49 (94.23%)
4	West Godavari (Tanuku, Eluru)	19	10 (52.63%)
5	Krishna (Teaching Veterinary Clinical Complex and Super Specialty Veterinary Hospital)	54	39 (72.22 %)
6	Guntur	44	35 (79.54%)
7	Nellore	51	9 (17.64 %)
8	Tirupati	26	15 (57.69 %)
9	Kadapa	14	12 (85.71%)
10	Kurnool	43	35 (81.39%)
Total		342	234 (68.42%)

The occurrence of canine parvovirus in Andhra Pradesh was assayed by employing polymerase chain reaction. The infection was present in almost all the districts screened with highest percentage of infection in East Godavari (94.23%) and less percentage of infection in Nellore (17.64 %) (Table 4).

DISCUSSION

In the present study the incidence of the disease was reported in dogs of age group 6 weeks to 4 months (77.59%) at a higher range when compared to dogs of age group 4 months and above one year. Predisposing factors like increased intestinal epithelial

turnover due to change in the microflora, weaning process and declining maternal antibodies level may be the reason for increased incidence of CPV in young pups (Stepita *et al.*, 2013). Similar findings were reported by Mohanraj *et al.* (2010), Parthiban *et al.* (2010), Vivek *et al.* (2013) and Xu *et al.* (2013).

Canine parvovirus enteritis was predominantly noticed in males (77.77 %) when compared to females (54.07%) and high incidence of CPV infection was recorded in non-vaccinated dogs conferring that the vaccines currently in use were protecting the dogs from CPV infection

M – 100 bp ladder, Lane 1 to 14 - Positive samples from suspected clinical faecal samples, N- Negative faecal sample of healthy dog

Fig. 1. Screening of canine parvovirus infection of faecal samples of dogs by Polymerase chain reaction

(Touihri *et al.*, 2009; Nandi *et al.*, 2010). However, CPV infection was also reported in few cases of vaccinated dogs which may be due to other reasons of vaccine failure like improper maintenance of cold chain.

HA is a simple and rapid test in detecting the CPV infection. The viral HA titres usually range from 128 to 10,240 between post infection days of 4 and 7, and later the virions tend to cease in the faeces from seventh day onwards. The haemagglutinating activity has a lower sensitivity which might be due to the presence of antibodies binding to the virions in the lumen of intestine of infected dogs thus inhibiting haemagglutination assay. Initial preliminary screening of canine parvo infection by HA was economical but can detect only when high concentrations of virus is voided in the faeces. All infected dogs may not necessarily exhibit clinical manifestations but they may shed the virions in faeces only during the acute phase of enteric fever and show significant rise in the serum antibody titers (Stann *et al.*, 1984). HA has few disadvantages like continuous source of swine RBC and need to check specificity of low titre samples with haemagglutination inhibition assay.

Out of 342 samples, 234 (68.42%) samples yielded an amplification product size of 681bp. Polymerase chain reaction is considered as most sensitive in detecting the parvo virions as it can even detect low concentrations of virus in the sample. In the present study though the samples were

collected with clear clinical signs suggestive of CPV, HA test was able to detect only 71 (20.76%) samples and PCR proved to be highly sensitive with a percentage of 67.83. Similar conclusions were drawn by Akbar *et al.* (2015) where they also compared the efficacy of two most commonly used diagnostic tests for CPV i.e., haemagglutination assay (HA) and polymerase chain reaction (PCR). Fifty fecal samples from dogs suggestive of parvovirus enteritis were collected and on performing HA, the test was able to detect CPV antigen in 35 samples, 32 samples tested highly positive with titers >128, 3 tested weakly positive with titers ranging from 32 to 64 and 15 were negative (titers <16). Using PCR, 39 samples were found positive including 6 HA-negative samples. Thirty percent of dogs presenting bloody diarrhoea did not show infection by HA. It is concluded here that specificity and sensitivity of PCR detection is non-significantly higher ($P > 0.05$) than HA. These findings have confirmed that HA test could be employed for the preliminary screening of the agent in field because of its less cost and rapid results but negative results from HA tests of suspected cases should be confirmed through molecular methods. Several serological and molecular diagnostic assays were developed for quick confirmation of the CPV disease and they have been prompt, reliable and precise (Decaro *et al.*, 2005).

Incidence is high in animal shelters, pet stores, breeding kennels. Nosocomial infection plays a vital role in transmission of virus from infected to healthy dogs due to improper sanitization measures taken in the hospital premises in disposal of voided faeces containing parvovirus. All age group of dogs are susceptible to get the infection but predominantly the virus tends to grow in young growing puppies of age group 6 weeks to 4 months old.

CPV a sturdy virus, its successful control of the disease can be implemented with judicious use of the vaccines against CPV. Public awareness programmes should be implemented for vaccinations to pet animals

for protection against the disease as in the present study maximum incidence of disease was observed in non-vaccinated and irregularly vaccinated dogs. Proper sanitation and disinfection measures should be practised especially by the pet shop owners at places like kennels, breeding management areas to ensure free of CPV presence to stop the indiscriminate spread of the disease to healthy ones.

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