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Seroprevalence of parvovirus infections from vaccinated and unvaccinated dogs of Andhra Pradesh

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Abstract

The present study aimed to assess the presence of Canine parvovirus 2 (CPV-2) antibodies in both vaccinated and unvaccinated dogs. The canine parvovirus disease in dogs was monitored by the use of live attenuated and killed vaccines besides new generation vaccines were also being developed for the control strategy. A total of 542 serum samples from different regions of Andhra Pradesh were used for the study. The age, sex, breed, and vaccination status were noted. The seroprevalence of canine parvovirus infection was studied by employing the methods of indirect ELISA and haemagglutination inhibition (HI) assay. The CPV infection rate was recorded as 94.65% in vaccinated and 72.50% in unvaccinated by indirect ELISA. Haemagglutination inhibition assay could detect 56.10% in vaccinated and 16.42% in unvaccinated ones. The titres ranged from 419 to 2337. Vaccinated dogs had more antibodies than the unvaccinated ones thus indicating the presence of antibodies in all the dogs suggesting that CPV infection is ubiquitous and natural infection of CPV aids in boosting immunity.

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Introduction

Canine parvovirus 2 (CPV-2) evolved in 1978, and is one of the most dreadful viruses for pups and dogs exhibiting mortality up to 91% and 10%, respectively (Nandi 2019). CPV-2 is characterised by acute haemorrhagic enteritis with a foul odour and myocardial infection (Zienius et al. 2016). It is highly contagious and contact with the infected virions in

feces could lead to deaths often in healthy dogs (Nandi and Kumar 2010). Pups infected with CPV develop vaccination failure due to the interference of maternal antibodies (Meers et al 2007). CPV-2 is a DNA virus belonging to the *Protoparvovirus* genus of the *Parvoviridae* family. It constitutes nearly 5200 nucleotides packed in an icosahedral capsid (Cotmore et al., 2014) made of three viral proteins viz. VP1, VP2, and VP3. VP2 is immunodominant and responsible for tissue tropism and genetic alterations in this gene lead to the formation of new antigenic variants (Truyen 1995).

As of today the disease was reported in many countries and spread worldwide, despite several immunoprophylactic agents being developed as control strategies for the prevention of the disease. The most likely reason may be due to more number of unvaccinated dogs, interference of maternal antibodies in the process of active immunization, and the emergence of new variants CPV-2a, CPV-2b, CPV-2c, New CPV-2a, New CPV-2b, etc. (Fan et al 2016). There is a need to evaluate the circulating variants of CPV-2 and understand the evolution of the virus so as take measures in developing new vaccines incorporated with the antigenic variants prevailing to minimize the virus spread (Pinto et al 2012). Antigenic variations may interfere with the effectiveness of the vaccine as they were reports of the occurrence of disease in vaccinated dogs too (Decaro et al 2008).

Several reports were given on the occurrence of CPV infection of variants in India (Nandi (2019), OIE, Phukan (2004)). Though the dogs were vaccinated, outbreaks of CPV infection were reported frequently all over India. Most of the dogs receive minimal therapeutic care and were not vaccinated regularly. To assess the extent of CPV exposure in the dogs, the detection of antibodies from serum samples was carried out on both vaccinated and unvaccinated dogs from various districts of Andhra Pradesh.

Materials and Methods

Collection of sera samples

Five hundred and forty-two serum samples from both vaccinated (262 samples) and unvaccinated dogs (280) were collected from Super Speciality Veterinary Hospital, Vijayawada, Teaching Veterinary Clinical Complex, NTR CVSc., Gannavaram and CVSc., Tirupati and various Veterinary Polyclinics across different districts of Andhra Pradesh. The samples were collected during the period from July 2018 to May 2019. Two ml of blood was drawn from the dogs and placed into a sterile test tube and placed in a slanting position till the blood was clotted. The serum was separated and centrifuged and preserved at -20 C till further use.

Table 1. Summary of the dog samples with regard to sex, breed, and age.

Variable	Number	Vaccinated	Unvaccinated
Sex			
Male	273	152	121
Female	269	110	159
Breed			
Pure	123	98	25
Mixed	419	164	255
Age group (Years)			
1(0-2)	262	137	125
2(3-5)	163	78	85
3(6-8)	78	26	52
4(9-11)	24	15	9
5(≥12)	15	6	9

Detection of CPV antibody by Indirect ELISA

Canine Parvovirus antibodies in sera samples of vaccinated and unvaccinated were detected using Indirect ELISA. **ELISA Procedure:** The detection of antibody levels to CPV infection in the test serum samples was estimated by indirect ELISA kits purchased from B.V. European Veterinary Laboratory (KIT D1001-AB01, Cat ≠ D030918). The 96-well ELISA plates were washed thrice with 300 µl of wash solution. All the wells were coated with 100 µL of inactivated canine parvovirus antigen and the plate was incubated at 37°C for 75 min.Positive and negative controls in the kit were reconstituted prior to use. The positive control was reconstituted in 0.5 ml Aqua Bidest water and the negative control in 1.0 ml Aqua Bidest water. The positive and negative controls were diluted in the ratio 1:250 in ELISA buffer andplaced in the respective wells. All the test sera samples were diluted in the ratio of 1:250 with the ELISA buffer.

The plate coated with the antigen after the specified incubation period was emptied and washed thrice with 300 µL wash buffer. Blank, positive and negative controls which were diluted were placed in the first three wells. To the remaining wells, 100 µL of diluted sera samples in duplicate were added, sealed, and incubated at 37°C for 60 min. The wells were emptied and washed thrice with 300 µL wash buffer. Then100 µL of conjugated anti-species antibody was added and incubated at 37°C for 60 min. The plate was washed thrice with 300 µL wash buffer. Equal parts of substrate buffer A and buffer B were mixed with gentle shaking prior to use. To each well, 100 µl of the prepared substrate was added and the plate was covered and incubated for 20 min in a dark place. Finally, the reaction was stopped by adding 100 µl of stop solution, and the absorbency values were read at 450 nm in an ELISA reader. (Thermo,51119000, Germany).

Interpretation for each sample was done by calculating the S/P percentage (S/P %).

Samples presenting with an S/P ratio < 0.22 are negative indicating specific antibodies to parvovirus were not detected.

Samples presenting with an S/P ratio ≥ 0.22 are positive indicating specific antibodies to parvovirus were detected.

$$\frac{S}{P} = \frac{OD_{Sample} - \text{Mean value } OD_{Negative\ Control}}{\text{Mean value } OD_{Positive\ Control} - \text{Mean value } OD_{Negative\ Control}}$$

Detection of CPV antibodies by Haemagglutination Inhibition test.

Canine parvovirus antibodies in serum were also detected by HI as per OIE (2018) with slight modifications. Two-fold serial dilutions of 50 µl amounts of the test sera samples were made in 0.2M Sorenson's PBS of pH 7.0 in 96-well 'U' bottom microtitre plates. To each well 50 µl of 4 HA units of canine parvovirus was added and the plate was left for one hour at room temperature. Later, 50 µl of 0.8 percent pig erythrocytes was added to each well after gently mixing and RBCs are allowed to settle at 4°C for 1 hour. One well, added with 50 µl of 0.2 M Sorenson's PBS of pH 7.0 and 50 µl of 0.8 percent pig erythrocytes, served as RBC control. As a virus control, one well was added with 50 µl of 0.2M Sorenson's PBS of pH 7.0, 50 µl of canine parvovirus antigen, and 50 µl of 0.8 percent pig erythrocytes. The hyperimmune serum for CPV raised in rabbits was used as positive serum control. The highest dilution of the sample showing complete haemagglutination inhibition was considered as the haemagglutination inhibition titre. The validity of results was assessed against positive control serum. HI titres are regarded as positive if there is inhibition at a serum dilution of 1:16 against 4 HA units ($\log 2^4$) (Yang *et al.*, 2010).

Statistical Analysis

The results of the study were analysed using the SPSS (Statistical Package for the Social Sciences) software version 17.0 (SPSS Inc. Chicago, IL, USA). The Pearson Chi-square was used to assess the significance of Indirect ELISA and HI. P value less than 0.05 was considered statistically significant.

Test Sensitivity and Specificity

The test sensitivity and specificity of Indirect ELISA and HI were calculated as given by Wayne (2016).

$$\text{Test Sensitivity} = \frac{Screen\ positive}{Total\ Vaccinated}$$

$$\text{Test Specificity} = \frac{Screen\ negative}{Total\ Unvaccinated}$$

Results

A total of 542 sera samples were collected comprising 273 male and 269 female dogs. The majority of the dogs were of mixed breeds (419/542) while 123/542 were of pure breeds (including Pomeranian, Pug, Labrador, German shepherd, and Boxer). Most of the young age groups had the presence of antibodies in both vaccinated and unvaccinated sera samples.

Detection of CPV antibodies by Indirect ELISA

Out of 542 serum samples tested, 451(83.21%) detected canine parvovirus-specific antibodies. S/P ratio ≥ 0.22 were considered positive, and below 0.22 negative, for CPV antibodies. The vaccinated animals (262) showed higher antibody serum titres (94.65%) than the unvaccinated (280) (72.50%). The serum antibody titre against CPV was distributed from 419 to 2337.

Detection of CPV antibodies by Haemagglutination inhibition (HI) assay

HI assay was employed for the detection of CPV antibodies in the test sera samples. Serum samples with HI titres (<16) were considered as negative. Out of 542 sera samples tested, 193(35.60%) produced a HI 1 in 16 to 1 in 32. One hundred and forty-seven sera samples (56.10%) from the vaccinated group and 46 (16.42%) from the unvaccinated group could detect the CPV-specific antibodies, respectively.

Comparative assessment of CPV antibodies by Indirect ELISA and HI

Status	Total number of samples	Number of sera samples detected CPV-specific antibodies by Indirect ELISA	Number of sera samples detected CPV-specific antibodies by HI assay
Vaccinated	262	248	147
Unvaccinated	280	203	46
Total	542	451	193

Discussion

Canine parvovirus is one of the leading causes of enteritis in pups and dogs, sometimes leading to mortality. Thus, it is essential to investigate the presence of CPV antibodies in dogs. Most of the pups below 6 months or less than that were infected with CPV prior to the vaccination. Determining the level of CPV antibodies may be of use to know the level of maternally derived CPV-specific antibodies in pups and also the optimal moment of vaccination as the presence of maternal antibodies seems to be a major problem in the vaccination strategy against CPV.

In the present study, it was found that CPV antibodies were prevalent in Andhra Pradesh. Irrespective of vaccination anti - CPV antibodies were found in both vaccinated and unvaccinated dogs indicating that they were exposed to CPV infection. The findings were similar to the previous study of Zimbabwe and South Korea which also reported a high proportion of seroconversion in dogs. A total of 542 sera samples from dogs were screened, out of which 451 were found positive by Indirect ELISA (Vaccinated 248 and Unvaccinated 203) and 193 by haemagglutination inhibition assay (Vaccinated 147 and Unvaccinated 46). Indirect ELISA recorded 94.65% in vaccinated and 72.50% in unvaccinated dogs whereas HI detected 56.10% in vaccinated and 16.42 % in unvaccinated dogs. Of the 349 HI negative samples (titre < 16), 91 were also negative in the Indirect ELISA (titre < 100). Most of the remaining 258 serum samples showed relatively low antibody titres in the Indirect ELISA (titre < 419). Of the 193 HI-positive samples, all were positive in Indirect ELISA also. However maximum titre recorded in the Indirect ELISA was nearly 2337, thus the test titre values were 77% times higher than those found in the HI assay. It could be expected that low titres of sera in the Indirect ELISA would score negatively in the HI test. The probability of the Chi-Square test of independence is < 0.00001, hence there is a significant difference in the proportions of positives obtained by ELISA and HI. The variation in the detection of CPV antibodies by ELISA and HI from vaccinated and unvaccinated dogs was statistically significant ($P < 0.05$).

Yang *et al.* (2010) conducted a seroepidemiological survey of CPV-2a from stray dogs by employing the HI test. The incidence of stray dogs showing HI antibody titre above 1:5120 was estimated to be 26.2% and the positive rate tested for CPV-2a was nearly 93.8%.

Rimmelzwaan *et al.* (1990) also detected CPV-specific antibodies in dog sera samples by Indirect ELISA and compared them with the HI test. The Indirect ELISA especially proved to be more sensitive than the HI test. The higher sensitivity and specificity of ELISA as compared to the HI test and its ease of use, make it suitable for routine use in serology and diagnosis of CPV infection.

In the present study, high antibody level was seen in vaccinated dogs than in unvaccinated dogs, contrary to the observation made by Deka *et al.* (2015) who reported higher antibody serum titre 1.00 to 2.806 at dilution (log_{10}) in unvaccinated dogs (66.67%) than vaccinated (33.82%) by using Indirect ELISA. They suggested that natural infection boosted the acquired immunity thus higher antibody level was observed in unvaccinated dogs. Few vaccinated dogs had a poor antibody response against CPV due to the interference of maternal antibodies, immunity gap, inappropriate age at vaccination, lack of booster doses, and improper maintenance of cold chain. Phukan *et al.* (2004) also screened sera samples for CPV antibodies by Indirect ELISA and found 76 % of the samples were 99.99 to 100% positive.

Conclusion

Seroprevalence of CPV was done by Indirect ELISA and HI for a total of 542 sera samples. Indirect ELISA recorded 94.65% in vaccinated and 72.50% in unvaccinated dogs whereas HI detected 56.10% in vaccinated and 16.42 % in unvaccinated dogs. The variation in the detection of CPV antibodies by Indirect ELISA and HI from vaccinated and unvaccinated dogs was statistically significant ($P < 0.05$).

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