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Study of Different Risk Factors for Canine Parvovirus infection by Haemagglutination Assay

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ABSTRACT

Haemorrhagic gastroenteritis in canine is caused by different etiological agents like canine parvovirus, *E.coli, Salmonella, Campylobacter,* Coccidia and Giardia, among these agents canine parvovirus is the most important. Canine parvovirus binds to the sialic acid receptors which are present on the RBC's, so haemagglutination test is used to detect canine parvovirus. In this study a total (n=102) of faecal samples from canines having haemorrhagic gastroenteritis were taken. All the 102 samples were subjected to haemagglutination assay and the prevalence of CPV was studied. Host associated risk factors like age, sex, breed, vaccination and seasons responsible for occurrence of canine parvovirus infection are recorded. Out of the 102 samples haemagglutination assay detected 41 samples as positive and a percent positivity of (40.19%) was recorded by this diagnostic test. Age wise prevalence was (69.23%) in (0-6 month) age group which is more than (7-12 month) (13.33%) and more than a year group (5%). Sex wise prevalence was more in males (47.94%) than in females (20.78%). Breed wise prevalence was more in Labrador (78.57%) followed by Doberman Pinscher (62.50%) lower prevalence was reported in Pomerarian and German shephered breeds. Non vaccinated canines showed a high prevalence of (42.70%) and in vaccinated canines no disease prevalence was detected. In the season wise prevalence, spring season showed more prevalence (54.76%) followed by summer season which showed (37.5%) prevalence and the least prevalence of (11.11%) was recorded in the winter season. Study showed that Haemagglutination assay is a good diagnostic test for the study of canine parvovirus where modern facilities of molecular diagnosis and the costly faecal ELISA test kits are not available.

Keywords: Canine Parvovirus infection, Canines, HA-HI

Canine parvovirus infection is a highly contagious disease that causes severe gastroenteritis in canines.CPV-2, the causative agent of acute haemorrhagic enteritis and myocarditis in canines, is one of the most important pathogenic viruses with high morbidity (100%) and frequent mortality upto 91% in untreated pups (Bhat *et al.,* 2012). As large amounts of parvoviruses are shed in the faeces of infected canines, an infected canine sheds 35 million viral particles (35,000 times the typical infectious dose) per ounce of stool so a simple test that could detect these viruses straight from the faeces is highly recommended. There are many test for the diagnosis for canine parvovirus such as PCR, faecal ELISA and HA among all, haemagglutination assay is low in cost and can be used for detection of canine parvovirus without much efforts and skill, but it is generally affected by certain non specific factors in the faecal material thus resulting in non specific agglutination, so the specificity of the test can be enhanced by haemagglutination inhibition test. CPV recognizes cell surface N-glycolylneuramininc acid and these receptors are present on erythrocytes which causes haemagglutination of RBCs by parvovirus (Tonas *et al.,* 2013) that makes Haemagglutination assay as an important test for CPV diagnosis. HA test can be performed by employing erythrocyte from various species such as swine, sheep, goat, poultry and canines. Among the erythrocyte

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of different species, pig RBCs showed the characteristic haemagglutination. Erythrocyte from other species does not give specific haemagglutination and therefore porcine RBCs were used for diagnosis of CPV-2 in India (Dahiya *et al.,* 2004; Kumar *et al*., 2004 and Pandya *et al*., 2017). Similarly, in this study porcine RBC's are used for the haemagglutination assay and diagnosis of CPV-2 infection in pups.

MATERIALS AND METHODS

Ethical approval

The study was conducted following due approval by the institutional animal ethics committee of Dr. G.C.Negi College of veterinary and animal sciences, Palampur, Himachal Pradesh

Collection and processing of Faecal Samples

102 faecal samples were taken from the rectum of canines having history of haemorrhagic gastroenteritis from different places of Himachal Pradesh from (September 2011-June 2013). Samples were emulsified in 1 ml of 0.1 M PBS of pH 7.4 along with antibiotic antimycotic solution (10,000 units Penicillin, 10 mg streptomycin and 25 µg amphotericin B per ml in 0.9% normal saline). This emulsion was then centrifuged at 6000 rpm for 15 min at in a refrigerated centrifuge. The supernatant was there after collected, filtered through 0.22 µm filter and stored at -20°C till further use. Faecal sample from a known healthy canine and detected negative by the CPV specific PCR was simultaneously processed and used as a negative control. A live CPV virus kindly provided by the Central Military Veterinary Laboratory (CMVL), Meerut, India was used as positive control.

Preparation of faecal antigen

Ninety microlitre of processed fecal sample was taken and treated with 10 µl of chloroform and mixed well. After vigorous shaking and keeping it for 10 minutes at 4°C, the samples were centrifuged at 10000 rpm for 10 minutes. The supernatant fluid was collected and then tested for HA activity. Faecal samples were treated with chloroform to reduce non specific agglutination as per method of Carmichael and Binn (1981). Negative control was also processed like this.

HA test procedure

HA was done according to the method of (Carmichael *et al.,* 1980). The blood samples from pigs were collected from ear vein and mixed in alsever's solution and stored under refrigeration. The RBC's is washed thrice in sterile PBS and made into a stock solution. Different dilutions of pig RBC's (0.1 – 1%) were made in PBS for standardization of hemagglutination test. 50 µl of diluent (PBS pH-4) was added in all the wells of the V bottom microtitre plate across the rows for each sample. Serial two fold dilution of processed sample (faecal suspension) were then made by adding 50 µl of sample in the first well of the row and after thorough mixing, 50 µl was transferred to next well of the row. Each sample was diluted from 1:2 through 1:4,096. Thereafter, 50 µl of 0.6 per cent RBC suspension from pig were added to each row of the microtitre plate. The plates were mixed well then incubated for 4 hrs at 4°C and the results recorded. The HA test can be performed by incubating the plates at various temperature such as 4°C, 25 and 37°C and the best results were found at 4°C followed by at 25°C and least titre at 37°C (Kumar *et al.,* 2004) Positive agglutination was indicated by matt formation, and button formation indicated lack of agglutination. The titer was calculated as the reciprocal of the last well with agglutination.

HI test

The hyper immune serum was raised according to the method of (Carmichael *et al.,* 1980). Faecal samples showing positive results on HA were subjected to HI as per the method of (Carmichael *et al*., 1980). With this method false negative samples could not be screened this is used with HA to increase its specificity. 1:10 concentration of serum was used in this study. All the samples were tested by HI test as it can detect the samples showing low HA titres.

RESULTS AND DISCUSSION

This study was done with the objective of finding different predisposing factors for the occurence of canine parvovirus using haemagglutination assay from infected animals as

the molecular tests are time consuming and they require well furnished labs. All the 102 faecal samples collected in the study were subjected to haemagglutination assay and a total of 41 samples found to be positive out of 102 samples and a percent positivity of (40.19%) was recorded. A CPV specific PCR was also performed on the same samples which detected 52 samples positive out of 102 samples and a percent positivity of (50.98%) is recorded. Although the molecular detection is highly specific and sensitive but we can use the haemagglutination assay where a cheap source of diagnosis is required and no modern lab facilities are available.

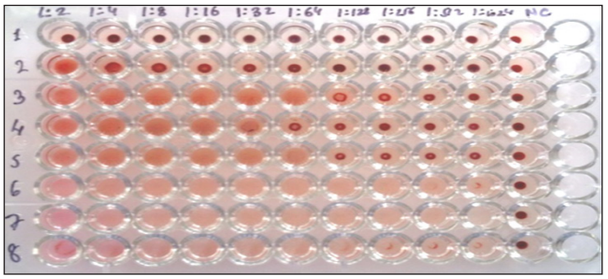

Fig. 1: Haemagglutination titer of faecal samples 1-8

Out of these 102 samples, 41 samples showed positive Haemagglutinating activities with HA titer ranging from 32-4096. Samples showing HA titer less than 1:32 were considered as negative for CPV. Based on the HA titre, the samples were divided into highly positive if the titre was found to be 1:64 or more, whereas the samples having HA titre of 1:32 were considered as weakly positive. Out of 102 samples, 41 samples showed HA titer of 1:32 and above, while three samples out of these 41 showed a high titer of 1:4096 in the HA test. Rest sixty one samples were found to be negative for HA activity. This finding corroborated with the findings of (Archana *et al*., 2009) who recorded prevalence of 45.30% and (Pandya *et al*., 2019) who recorded 50.82 % prevalence by HA. The overall prevalence of CPV infection in the study of (Reddy *et al*., 2015) was 33.17 percent by HA. Higher values i.e. 71.42% was reported earlier by (Kumar *et al*., 2004). The low to moderate prevalence of HA activity in our work is supported by the work of workers like (Panneer *et al.,* 2008) who reported 28.7 per cent HA positivity and (Parthiban *et al.,* 2011) who obtained a moderate 36.71 percent prevalence of CPV by HA. This could be attributed to the fact that presence of antibodies in the intestinal lumen of infected canines may bind CPV virions and thus prevent HA activity.

In this study PBS buffer is used. Apart from this, various buffer system can be used for HA test such as normal saline solution (0.9% NSS), phosphate buffer solution with BSA (15 mM PBS + 0.1% BSA) and phosphate buffer saline solution (PBSS) (15 mM PBS + 0.9% NSS) etc. The optimum results were obtained with PBS followed by PBS with BSA and PBSS in a pH range of 4–6 but the results of all three systems were comparable (Dahiya *et al.,* 2004; Kumar *et al*., 2004).

Table 1: Risk factors for the occurrence of CPV infection and Prevalence study by Haemagglutination assay

Risk factor	Number of HGE cases	Number of CPV cases	Prevalence
Age group			
(0-6 months)	52	36	69.23%
(7-12 months)	30	4	13.33%
More than 12 months	20	1	5%
Total	**102**	**41**	
Sex of the animal			
Male	73	35	47.94%
Female	29	6	20.68%
Total	**102**	**41**	
Breed of dog			
Rottweiler	20	11	55.00%
Doberman Pinscher	08	5	62.50%
Labrador	14	11	78.57%
German shepherd	17	2	11.76%
Pomerarian	05	—	—
Mixed	11	3	27.27%
Mongrel	27	9	33.33%
Total	**102**	**41**	
Vaccination status			
Vaccinated	6	0	0
Non-vaccinated	96	41	42.70%
Total	**102**	**41**	
Season			
Spring (Feb-March)	42	23	54.76%
Summer (April-June)	40	15	37.5%
Rainy (July-Oct.)	11	2	18.18%
Winter (Nov.-Jan.)	9	1	11.11%
Total	**102**	**41**	

In the (0-6 months) age group prevalence of disease was 69.23% and in the (7-12 months) age group prevalence of disease was 13.33% and in more than one year age group the prevalence was 5% our findings are similar to (Baruah *et al*., 2004) and (Reddy *et al.,* 2015). The higher incidence of CPV below 6 months might be due to the affinity of the virus for rapidly multiplying intestinal crypt cells in weaning pups with higher mitotic index due to changes in bacterial flora as well as in the diet due to weaning (Deka *et al*., 2013). In the present study prevalence was reduced with increase in age probably due to the acquired immune response.

In the sex wise distribution male canines have high prevalence of about (47.94%) and female canines have low prevalence of about (20.68%) our study is quite similar to (Reddy *et al*., 2015) who got higher prevalence of CPV in male canines (37.30%) than female canines (27.47%) however according to (Umar *et al.,* 2015) female canines were having high prevalence of about (49.4%) than the male (33.7%). This may be due to the fact that males with superior breeds are domesticated more by the people than the female canines.

In our study Labrador (78.57%) shows high prevalence of disease followed by Doberman (62.50%) and Rottweiler (55.00%), German Shephered (11.76%) showed lower prevalence. In this study mongrel showed a high prevalence of disease about (33.33%) which is different from the study of other workers (Reddy *et al*., 2015) and (Sanjukta *et al*., 2011) who recorded lower prevalence of disease in mongrels. It may be due to the lack of vaccination in these species and most of them were stray not domesticated and so they are in the habit of moving here and there and even in the hospital premises from where they can pick the infection easily.

In the vaccination wise distribution there is high prevalence of CPV (42.70%) in non vaccinated canines as compared to vaccinated canines where no case has been reported to be positive. In non-vaccinated canines the prevalence was higher in compared with vaccinated one. The finding was agreed with (Reddy *et al*., 2015) where unvaccinated puppies aged between six weeks and six months are at greatest risk of developing CPV infection. The higher prevalence of CPV infection in Non-vaccinated canines is due to Lack of protective immunity. Lower prevalence of CPV infection in vaccinated canines indicated that current vaccines conferred reasonably good protection (Cavalla *et al*., 2001).

The prevalence of CPV in the present study was 54.76% in the spring season and 37.5% in the summer season and 11.11% in winter season, our study is similar to the study of (Duijvestijna *et al*., 2016) who found more prevalence of CPV infection in spring season than winter season. (Umar *et al.,* 2015) recorded 62% prevalence in summer months and 38% in winter months. (Zhao *et al.,* 2016) reported that morbidity due to canine parvovirus in the summer season was more than winter season. This is due to the hardiness of virus to survive more in the summer season extremely cold temperatures and bleach (sodium hypochlorite) can kill the virus. CPV is usually killed or dormant over the winter season so most cases are in the warmer spring and summer months.

CONCLUSION

This study concluded that the Haemagglutination assay is an important test for the study of canine parvovirus although it should be combined with the modern PCR assay but there is slight difference in the percent positivity obtained by HA and PCR. This diagnostic assay is low of cost and does not require costly apparatus and modern machines.

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