RESEARCH ARTICLE

Comparative Efficacy of Canine Parvovirus-2 Infection Diagnostic Tests Commonly Prevailing in India

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Abstract

Early and rapid field diagnosis of CPV-2 infection is essential for preventing disease transmission from infected to susceptible dogs. In the present study total 91 fecal samples and rectal swabs collected from a diarrheic and non-diarrheic dog from clinics located in and around Nagpur. Polymerase Chain Reaction, Rapid Antigen detection kit, and Hemagglutination test (HA) were able to detect CPV-2 in 41 (45.05%), 34 (37.36%) and 22 (24.17%) samples, respectively. The evaluation revealed that the PCR method was found to be most sensitive followed by antigen detection kit and HA field test. Sensitivity and specificity of antigen detection kit with respect to PCR method were found to be 78.08% and 96.00%, respectively. The finding suggests PCR with the highest sensitivity may be taken as a gold standard test of CPV-2 relative to other diagnostic tests. HA test could be employed for the preliminary screening in field because of its rapid response and low cost.

Keywords: Canine parvovirus-2, Gastroenteritis, HA, Rapid antigen detection kit, PCR.

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Introduction

Canine parvovirus-2 is one of the most pathogenic viruses belongs to family *Parvoviridae* (Murphy *et al*., 1999); it causes a highly contagious and fatal disease of dog especially of pups. It is responsible for causing diarrhea accompanied by severe hemorrhagic enteritis and myocarditis in dogs.

The virus is non-enveloped having 25 nm in diameter with icosahedral symmetry and its genome consist of single-stranded negative-sense DNA virus having a size of 5.2 kb in length (Murphy *et al*., 1999). CPV has three main antigenic variants viz. 2a, 2b, and 2c which are distributed among the dog population throughout the world. (Decaro *et al*., 2006). Out of these three variants, Canine parvovirus -2b causes highly infectious viral disease of dogs. It is spread by direct or indirect contact feces of dogs and results in a life-threatening infection (Appel *et al.,* 1979). Rapid diagnosis is essential for screening diarrheic puppies to prevent infection to other population. Several laboratory techniques, e.g. Electron microscopy, virus isolation, enzyme immunoassay, and amplification of viral DNA using the polymerase chain reaction are used for laboratory confirmation of clinical diagnosis, but their accuracy, speed, cost, and availability is questionable. Keeping in view the above facts, the present study was undertaken to compare the efficacy of commonly used laboratory tests viz Antigen detection kit, haemagglutination test and polymerase chain reaction for CPV diagnosis.

Materials and methods

Sample Collection and Preparation

Total 91 fecal samples/rectal swabs were collected from dog exhibiting clinical signs like vomition, anorexia, high temperature, depression, and gastroenteritis, hemorrhagic enteritis, from clinics located in and around Nagpur. Samples were collected by using sterile swab immersed in PBS and stored at -20°C for further processing. The samples were then centrifuged at 2500 rpm for 20 min at 40°C. The supernatant was separated and preserved at –20°C and was used for further laboratory analyses.

Hemagglutination Test

Blood was collected from healthy pigs in Alsever's solution at a ratio of 1:1. The centrifugation was carried out at 1500 rpm for 20 minutes and the collected RBCs were washed thrice in Phosphate buffered saline (PBS), pH 7.2. The cells were washed with normal saline and centrifuged at 3,000 rpm for 5 minutes. This was repeated two times, and the cells were constituted to a final concentration of 10% from which 1% was reconstituted for the HA test. HA titer of 64 and above was considered as positive for CPV antigen (Shashidhara and Kapil., 2009).

Antigen Detection Kit

Ubioquick VET Canine Parvovirus antigen rapid test kit was used to detect canine parvovirus antigen in a fecal sample.

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The sample was considered positive for the presence of CPV if two red bands appeared, one in the control line (C) and the other in the test line (T). The sample was considered negative for CPV if one red band appeared in the control line(C) with no apparent and in the test line (T) (Sundaran et *al*., 2015).

Polymerase Chain Reaction

DNA was extracted from the fecal samples by a commercial available Promega kit (USA), as per the manufacturer instructions and extracted DNAs were stored at –20°C. Detection of Parvovirus VP2 gene amplification using CPV-2 ab-F and CPV-2 ab-R set of primers was used and conditions were optimized (Sheikh *et al.,* 2017). The primer sequences and nucleotide position of oligonucleotide primers are shown in Table 1.

The extracted viral DNA was amplified by PCR assay using VP2 gene-specific primers. The reaction mixture consisted of 2 µL Template DNA, 10 µL 5X PCR buffer, 3 µL $MgCl_2$, 1 µL dNTPs, 1 µL Taq polymerase, 2 µL Forward Primer, 2 µL Reverse Primer, 29 µL Nuclease-free water to make the volume of 50 µl. All these ingredients mixed properly by vortexing. The PCR was programmed as Initial Denaturation at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, annealing at 50°C for 2 min, extension at 72°C for 2 min and a final extension at 72°C for 10 min. 5 µl of PCR product was then mixed with 3 µL of bromophenol blue (6X) and was run on gel electrophoresis and visualized by using UV transilluminator (Syngene G box, Uk).

Results and discussion

Out of 91 samples screened, 22 (24.17%) samples (Nine samples showed 64 HA titer, 6 samples showed HA titer 128, four samples showed 256 HA titer, two samples shows HA titer 512 and only one sample shows HA titer 1024.) were found positive by HA titer agglutinated pig RBC with titer ranging from 64-1024. By using UbioquickVET antigen detection kit. 37.36% (34/91) fecal samples were found positive and 41/91 (45.05%) by PCR. Our results shows that the PCR method was more sensitive than HA and antigen detection kit method.

In the present study, a very poor correlation between HA and PCR method was observed, many positive viral DNA remain in an HA negative samples. Sensitivity and specificity of antigen detection kit (UbioquickVET kit) to PCR were found to be 78.08% and 96% respectively. The results are in agreement with the findings of Vakili *et al.* (2014) who stated that PCR remnants more sensitive than test LFA. The necessity of expensive equipment and reagents restricts PCR use as a field level test (Mohyedini *et al.,* 2013).

Table1: Details of primers specific for VP2 gene of CPV used in PCR

Primer name	*Sequence primers (5'-3')*	*Product size*
CPV-2ab-F	GAAGAGTGGTTGTAAATAATT	681 bp
CPV-2ab-R	CCTATATAACCAAAGTTAGTAC	

Table 2: Sensitivity and specificity of antigen detection kit in relation to PCR for detection of Canine Parvovirus Antigen

		PCR				
Test		*Positive*	*Negative*	*Total*	*Sensitivity*	*Specificity*
Antigen detection kit	Positive	32	2	34	78.08%	96%
	Negative	9	48	57		
Total		41	50	91		

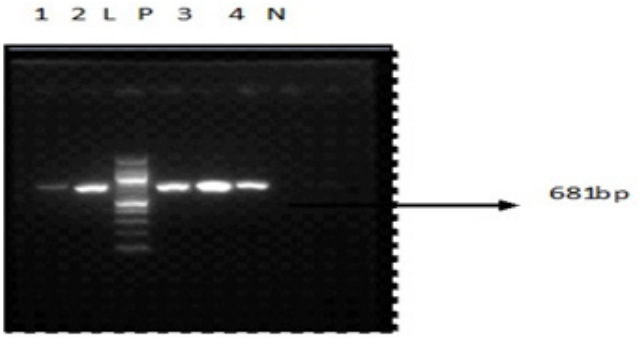

Fig. 1: Agar gel electrophoresis showing, the amplicon of 681 bp of canine parvovirus (CPV) positive samples using CPV forward and reverse primers L= 1000 bp DNA ladder, N= negative control, P = Control positive (inactivated vaccines). Lane 1-2 and 3-4, 681 bp PCR product of canine parvovirus (CPV) positive samples.

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