Int.J.Curr.Microbiol.App.Sci (2020) *9*(5): 743-750

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 9 Number 5 (2020)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2020.905.082

Comparative Efficacy of Diagnostic Methodology for Detection of Canine Parvo Virus Faecal Antigen

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Keywords

Canine Parvovirus, ScanVet Parvo HA, Dog, PCR, Fecal samples

Article Info

Accepted:
05 April 2020
Available Online:
10 May 2020

A B S T R A C T

The study was conducted to compare diagnostic efficacy of two techniques viz. Scan Vet Parvo rapid faecal antigen detection kit and haemagglutination (HA) test with polymerized chain reaction (PCR). A total of 145 fecal samples from dogs showing similar clinical signs of parvovirus infection were collected aseptically from Veterinary Clinical Complex, Veterinary College, Navsari. The ScanVet Parvo was able to detect CPV antigen in 34 (68.00%) samples out of 50. The HA test found positive in 44(30.34%) samples, While using PCR, 63 samples (86.00%) were found positive out of 145 including 9 and 19 negative samples of ScanVet Parvo and HA test, respectively. However, both the test showed 100% specificity and sensitivity 79.07% (Scan Vet Parvo) and 69.84 % (HA) versus PCR. Thus, it is suggested that ScanVet Parvo could be employed for the preliminary screening in field condition and HA test in the laboratory. However, negative samples should further be confirmed through PCR.

Introduction

Canine Parvovirus is a highly fatal infectious disease of growing pups. It is highly contagious and represents one of the most common causes of acute hemorrhagic diarrhea in pet dogs below 6-8 months of age. Hence, early and quick diagnosis is really helpful in the saving the life of puppy. Virus is generally shed extensively for 7–12 days in vomitus and feces, but long-term excretion may also occur as well. The confirmative diagnosis can be made by immune-electron microscopy (IEM) or polymerase chain reaction (PCR) of fecal samples.

The sensitivity of IEM is believed to be relatively low due to large quantities of virus required for a positive test result. Further, the availability of instrument is also not feasible

at district level laboratories. Whereas PCR has been described as both sensitive and specific for the detection of CPV enteritis but it also required time and high cost (Ali *et al*., 2015). Thus, the present study carried out to find the efficacy of less time consuming and low cost diagnostic methodology was by HA and Scan Vet Parvo versus PCR.

Materials and Methods

The dogs presented at Veterinary Clinical Complex, Veterinary College (VVC), Navsari with similar or relevant clinical exhibitions of CPV *i.e*. vomition, diarrhea, dysentery, melena, dehydration and anorexia were considered for primary screening. The age group of 0-8 months was targeted for present study. The two faecal samples of each dog were aseptically collected using hi culture swab directly from rectum. Out of 145 samples, 50 were immediately used as per manufacturer's instructions for immunochromatography strip test provided with ScanVet Parvo Kit. The 'Scan Vet PARVO' India. The another feacal swabs of the same dogs were made bacteria free by filtration with 0.22 micron syringe filter and kept frozen state at minus 20^{o}C for HA and Polymerized Chain Reaction assay. Further, the present study was carried out on clinical cases; hence, ethical approval was not obtained.

Immunochromatography strip test

The faecal swab put into a sample tube containing 2ml of buffer. The buffer with swab was swirled and four drops of liquid were put into the specimen well (S). The test results were read within 5-10 minutes. One red-purple band appears in the control line with no apparent band in the test line (T) considered as negative sample (Figure 1) and two red-purple bands appear one in the control line (C) and other in the test line (T) noted as positive sample (Figure 1).

Haemagglutination (HA) test

The HA test was carried out using porcine RBC as per method suggested by Carmichael *et.al.*,(1980).The porcine blood was collected in the Alsever's solution in the ratio of 1:1 and allowed to stand for 24 hours in refrigerator. The PCV was collected after centrifugation and washed thrice with Phosphate Buffer Saline buffer (0.1 M PBS, pH -7.2). The suspension of 1% porcine RBC was made in 0.1 M PBS. The test carried out using 96 well V bottom microtitre plate, 50 µl of 0.1 M PBS (pH- 6.5) was dispensed in well A-1 and thereafter across the rows (1-12) and columns (A-G) using 10- 100 µl variable volume multichannel micropipette. Further, one vial of lyophilized virus vaccine was reconstituted with 1 ml of sterile PBS and used as positive control for HA. A 50µl of sample (faecal suspension) was put in to first well of microtitre plate, then it was serially diluted (1:2) across the row (1-9), so dilutions were created from 1:2 to 1:512 across through the first column. The 50 µl of PBS was dispensed all working wells and thereafter, 50 µl of 1% porcine RBC suspension was placed. The plate was agitated at 300 rpm for 5 min on ELISA plate shaker to ensure proper mixing of reactants. Then plate was put at 40^{o}C for 4 hours and formation of serrated edged mat and button were recorded as positive and negative results (figure 3), respectively. Accordingly titer was also calculated as reciprocal of the last well with agglutination.

Genomic DNA extraction

The genomic DNA of CPV from the faecal samples was extracted by phenol-chloroform method reported by Manojkumar *et al.*, (2011). The inhibitory substances samples was removed by treating with 200µL of

sodium dodecyl sulphate (SDS) and proteinase K with a final concentration of 1% and 250µg/mL, respectively and kept at 56˚C for 30 min. A volume of 200µL of Tris saturated phenol, chloroform and amyl alcohol (25:24:1) was added with 1:1 ratio of sample in an Eppendorf tube and mixed thoroughly. Then, it was centrifuged at 10,000 rpm for 5 min and the upper layer was collected in another Eppendorf tube. Further, its volume was estimated and its 1/10th volume of 3 M sodium acetate (pH 5.5) and 1 mL of chilled ethanol were added, mixed and kept at -20˚ C for overnight. The tubes were centrifuged at 12,000 rpm for 15 min. The supernant was discarded and the pellet was washed with 500µL of 70% ethanol, followed by centrifugation at 12,000 rpm for 2 min. The ethanol was discarded and pellet was dried in orbital chilling/heating block and resuspended in 25 µL nuclease free water. Finally, the purity of DNA was checked by microlitre spectrophotometer by taking 260/280 nm ratio. The sample was processed further only when 260/280 ratio was found between 1.6 to 1.8 and the concentration of DNA was 20ng/µL or above.

Details of primer

Two sets of primer pairs, P*ab* which detects CPV types 2*ab* and 2*b* designed for this study, described by (Pereira *et al*., 2000). Primer pair P*ab* sense (5´-AAGAGTGG TTGTA AATAATA-3´) and P*ab* anti-sense (5´-CCT ATATCACCAAAGTTAGTAG-3´) located, respectively, at 3025-3045 and 3685-3706 of the CPV genome, yields a 681 base pair (bp) product, while P*b* sense (5´-CTTTAACCT TCCTGTAACAG-3´) and P*b* anti-sense (5´-CATAGTTAAATTGGTT ATCT AC- 3´) located, respectively, at 4043-4062 and 4449-4470 yields a 427 bp product.

Statistical analysis

The sensitivity and specificity of 'ScanVet PARVO' rapid test and HA were calculated by MedCalc statistical software comparing the test result with result of PCR considering as gold standard using formula given by Samad *et al., (*1994). Further, Chi- square test for the same was calculated using Social Science Statistics Calculator software. The p <0.05 was considered as significant.

Results and Discussion

The ScanVet PARVO was able to detect CPV antigen in 34 (68.00%) samples out 50. All the suspected 50 samples were further examined through PCR assay. Out of 50 screened samples, 43 samples (86.00%) were found amplified and yielded the products at 681 and 427 bps including 9 ScanVet PARVO negative samples. Among the 43 PCR positive samples, 37 amplified products found to be of ~ 681 bps and 6 of ~ 427 bps. The samples amplified at 681 and 427 bps were positive for CPV infection of type 2a and type 2b, respectively (Figure 2). The significant difference could be observed between the two tests (*p* value : 0.032).

Similarly, 44 (30.34%) samples were found positive with different titer (Table 4) out of 145 screened samples through HA test. The more number of samples were found positive at the titer 1:64 (12), followed by 1:16 (10), 1:32 (8), 1:128 (6), 1:8(3). The highest titers were observed at 1:512 (02) and 1:256 (03). The significant difference observed between the two tests (*p* value: 0.020).

Sensitivity, specificity, prevalence, positive and negative predictive values and accuracy of *ScanVet PARVO* strip test and HA test (Table 3) in comparison with PCR (Table 1 and 2) were analyzed.

Canine Parvoviral infection is an important problem globally and locally because of increasing canine population day by day in India. The exhibited clinical signs of CPV are

confusing with other gastro-enteric diseases and hence rapid and early diagnosis of the disease has crucial role so far as treatment is concerned. Conventional methods such as Eletronmicroscopy and virus isolation are time-consuming and expensive, Whereas serological tests could detect the antibody, but fail to detect the acute infection. The widely used HA HI tests are simple, but are less sensitive and always require fresh porcine erythrocyte which was difficult to avail.

Hence, these tests are now replaced by molecular methods like PCR which is having high specificity and sensitivity than the conventional antigen or antibody-based methods (Sakulwira *et al*., 2001) However, the availability of expensive equipment and reagents for PCR restricts its use as a field level test. The most rapid method for diagnosing parvo viral infections in practice is Immunochromatography (IC) based canine fecal antigen test kits which are sensitive, simple, and rapid (Tinky *et al*., 2015). Whereas HA test could also be performed at first laboratory screening test.

In the present study, out of the 50 samples screened through ScanVet Parvo strip test, 34 (68.00%) were detected as true positive for CPV infection whereas 9 were found false negative through PCR. The nine PCR positive samples failed to give a positive result in strip test may be due to the requirement of large amount of viral antigen to produce a clearly visible band. This result is in agreement with Reddy *et al., (*2015) and Tinky *et al., (*2015) who reported that the quantity of viral particles can affect the IC test result which was observed to be one of the disadvantages of this test. Moreover, when compared to the HA assay, canine fecal antigen test kit showed higher sensitivity (79.07%) but lower in negative predictive value (probability that the disease is not present when the test is negative). Further, ScanVet PARVO strip test can detect the presence of parvovirus but will not be able to distinguish the type involved (Sutton *et al.,* 2015).

HA has been widely used for CPV screening due to the ease of implementation and low cost. However, 19 samples considered HA-negative were found to contain virus DNA. This discrepancy may be due to the presence of CPV strains lacking HA activity (Parrish *et al.,* 1988), or to the fact that high viral titers are required to produce HA and that specific antibodies in the intestinal lumen frequently sequestrate most of the CPV virions, thus preventing or reducing parvoviral binding to erythrocytes (Desario *et al.*, 2005).

When specificity of ScanVet Parvo strip test and HA test compared with PCR, it was observed to be 100.00% for both the test. Reddy *et al., (*2015), Tinky *et al.,* (2015), Silva *et al.,* (2013), Chollom *et al.,* (2012) obtained the similar results with immune - chromatography strip test.

Table.1 Comparison of PCR and ScanVet PARVO strip test

Test		**PCR (Standard test)**		
	Variables	**Positive**	**Negative**	**Total**
ScanVet Parvo	**Positive**	34	0	34
	Negative	9	7	16
	Total	43	7	**50**

Table.2 Comparison of PCR and Haemagglutination test

Test		PCR (Standard test)		
	Variables	**Positive**	**Negative**	**Total**
HA Test	**Positive**	44	0	44
	Negative	19	82	101
	Total	63	82	**145**

Table.3 Statistical values for tests

Parameters	Scan Vet Parvo		Haemagglutination test	
	Value	**95% Confidence Interval**	**Value**	**95% Confidence Interval**
Sensitivity	79.07%	63.96% to 89.96%	69.84%	56.98% to 80.77%
Specificity	100.00 %	59.04% to 100.00%	100.00 %	95.60% to 100.00%
Disease prevalence	86.00%	73.26% to 94.18%	43.45%	35.25% to 51.92%
Positive Predictive Value	100.00%	--	100.00%	--
Negative Predictive Value	43.75 %	30.32% to 58.17%	81.19 %	74.77% to 86.27%
Accuracy	82.00%	68.56% to 91.42%	86.90%	80.30% to 91.92%

Table.4 HA titres in positive faecal samples

HA titer	No. of positive faecal samples
1:8	03
1:16	10
1:32	08
1:64	12
1:128	06
1:256	03
1:512	02
Total	**44**

Figure.1 Scan Vet PARVO Strip Test

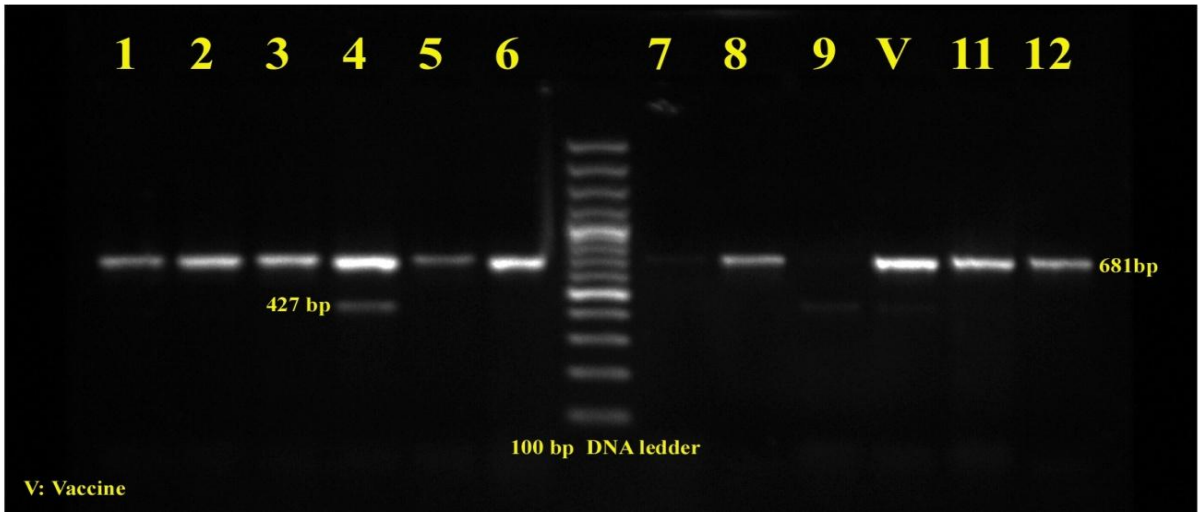

Figure.2 Amplified products at 681 bp and 427bp through PCR technique

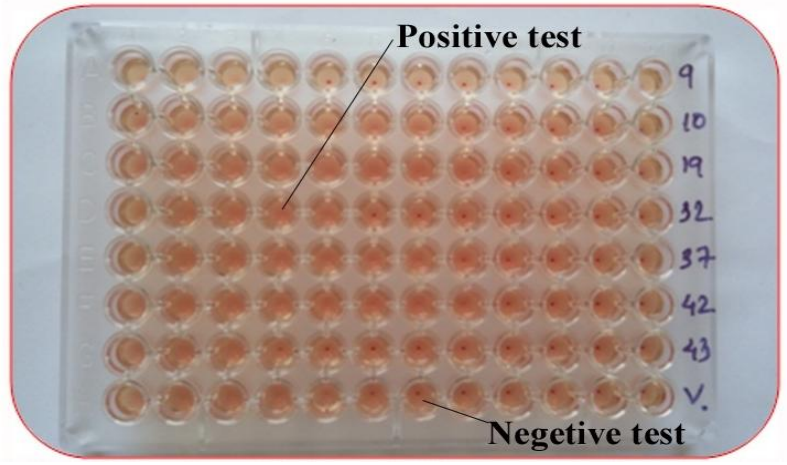

Figure.3 96 Well (bottom V Shaped) Haemagglutination test

But Cuong *et al.,* (2015) reported that rapid antigen detection test was 100 % specific and 100% sensitive to the PCR technique in their study which was contradictory to the present findings with unknown reasons or the sample could be collected from highly probable cases of CPV. The statistics of the present study revealed that Positive predictive value of both the test (probability that the disease is present when the test is positive) was 100% but HA test (86.90% %) proved better accuracy in the disease diagnosis than the Scan Vet Parvo strip test (82.00%).

Based on the present findings it can be concluded that 'ScanVet Parvo' strip test may be used as field test for the screening of CPV infections in routine veterinary practice while HA test can be helpful in preliminary confirmation of disease at laboratory set up at low cost. However, negative samples of both the tests should be further tested by more sensitive and specific technique such as PCR to improve the accuracy of CPV diagnosis.

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How to cite this article:

Mehta. S. A., S. V. Mavadiya, A. A. Vagh, S. M. Parmar, J. A. Vala and Patel. R. M. 2020. Comparative Efficacy of Diagnostic Methodology for Detection of Canine Parvo Virus Faecal Antigen. *Int.J.Curr.Microbiol.App.Sci*. 9(05): 743-750.
doi: https://doi.org/10.20546/ijcmas.2020.905.082