*Original Research*

# Comparison of Three Tests for Diagnosis of Canine Parvovirus Infection in Himachal Pradesh

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**Abstract**

*The aim of present study is to evaluate the most sensitive and specific test for the diagnosis of canine parvovirus. For this three different tests like PCR, HA/HI and dot-ELISA were used to detect canine parvovirus infection. A total of 102 faecal samples were collected from the dogs having haemorrhagic gastroenteritis from different regions of Himachal Pradesh. The samples were tested by PCR, HA/HI and dot-ELISA diagnostic tests. Out of total 102 faecal samples collected from dogs having haemorrhagic gastroenteritis PCR detected 52 samples positive, dot-ELISA showed 49 samples positive s and HA/HI detected 41 samples positive. Therefore, percent positivity obtained by PCR was (50.98%), dot-ELISA was (48.03%) and HA/HI was (40.19%). Study concluded that PCR is the most important diagnostic test for the diagnosis of canine parvovirus and it is highly sensitive and specific test.*

**Key words:** Canine parvovirus, dot-ELISA, HA/HI, PCR

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## Introduction

Canine parvovirus is a highly contagious infectious agent that causes gastroenteritis in dogs. Canine parvovirus (CPV) is the most significant viral cause of acute haemorrhagic enteritis and myocarditis in puppies over the age of 3-4 months (Hoelzer and Parrish, 2010). CPV is a single stranded DNA virus and is a major pathogen of dogs. Over a period enteric form of disease has predominated and it persists as a major problem in breeding kennels, or where vaccination is widely practiced Sagazio *et al.* (1998). The original virus (CPV-2) was subsequently replaced by the new variants, CPV-2a, CPV-2b and CPV-2c. The presence of CPV in India has been confirmed by Ramadass and Khader (1982) among different variants of CPV most predominant type in Himachal Pradesh is CPV- 2b by Sharma *et al.* (2016). There are different test for the diagnosis of canine parvovirus like electron microscopy, virus isolation, PCR, HA/HI and

ELISA among all HA/HI, ELISA and PCR is the most important test as virus isolation and electron microscopy cannot be done in the routine process. Since no such study of comparison has been carried out in Himachal Pradesh earlier, so in this study a comparison of three important laboratorial tests i.e. HA/HI, PCR and dot-ELISA was done to find out the most specific and sensitive test for the diagnosis of canine parvovirus.

## Materials and Methods

### Ethical Approval

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

### Collection and Processing of Faecal Samples for Different Diagnostic Tests

A total of 102 faecal samples were taken from the dogs having haemorrhagic gastroenteritis from different regions of Himachal Pradesh. Faecal samples/rectal swabs were then 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 4ºC in a refrigerated centrifuge. The supernatant was thereafter collected, filtered through 0.22 µm filter and stored at -20°C till further use. DNA from a total 102 faecal samples was extracted using (Phenol-Chloroform-isoamyl alcohol) method. 102 DNA samples extracted from these faecal samples was subjected to (CPV-2ab specific PCR) for molecular studies from published primers by (Senda *et al.*, 1995).

Faecal sample from a healthy dog and confirmed negative by CPV-2ab PCR was used as negative control. A live CPV virus kindly provided by the Central Military Veterinary Laboratory (CMVL), Meerut, India was used as positive control. For the preparation of faecal antigen to be used in HA and dot-ELISA diagnostic tests, faecal samples were treated with 10µl of choloroform. The hyper immune serum was raised according to the method of Carmichael *et al.* (1980). Hyperimmune serum against CPV was raised in 8 months old male rabbits. Two rabbits were injected with inactivated canine vaccine Megavac-P (CPV containinig vaccine from Indian Immunologicals India). 1.0 ml of vaccine was inoculated into each rabbit by intradermal (i.d). and sub cutaneous (s.c.) routes at multiple sites using a 23 G needle. The first booster injection was given 4 weeks after 1st injection. Second booster injection was given after 4-6 weeks of the first booster injection. As detectable amount of antibody was produced after this injection blood was collected from the animals, serum separated and stored at -20oC for further use. HA/HI was done according to the method of Carmichael *et al.* (1980) in which 0.6% suspension of pig RBC's are used and 1:10 concentration of serum is used in HI after standardization. The dot ELISA was carried out as per the method of Waner *et al.* (2003). Two microlitres of the fecal antigen in PBS–Tween-20 (PBS-T) along with positive





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and negative control antigens were dotted on Nitrocellulose membrane (NCM) strip at 1 cm apart and were allowed to dry at room temperature for about 20 to 30 min. The antigen free sites on NCM strips were blocked by immersing in blocking buffer (1 per cent Bovine Serum Albumin in PBS-T) at 37oC for 30 min. The NCM strips were washed 3 times in PBS-Tween 20. The strips were then incubated with hyper immune serum raised in rabbits diluted 1:100 dilution in PBS-T and incubated at 37oC for 30 min. The optimal dilution of 1:100 was determined earlier by titration on positive samples with 1:50, 1:100, 1:150 and 1:200 of hyper immune serum. Following further 3 washings to remove unbound antibodies, the NCM strips were incubated at 37oC for 30 min. with goat raised-anti rabbit IgG HRPO conjugate (Sigma Immunochemical, St. Louis, USA) diluted to 1:1000 in PBS-Tween. The optimal dilution of 1:1000 was determined earlier by titration with 1:1000, 1:2000, 1:5000, 1:10000 dilutions on positive samples.

The strips were washed again in the manner described above and were dipped in substrate solution. The reagents were allowed to react for 30 seconds. The enzymatic reaction was stopped by washing the NCM in tap water and air dried before visual interpretation of the results. The appearance of brown spot at the site of antigen coating was considered as a positive reaction.

**Results and Discussions**

This was the first study in the Himachal Pradesh which provides a comparison of diagnostic abilities of three diagnostic test routinely used in the laboratory for the diagnosis of canine parvovirus in obtaining the prevalence of this infection.

**HA/HI Results**

This diagnostic test detected 41 samples positive out of 102 samples. Samples showing HA titre less than 1:32 were considered negative. An overall percent positivity of (40.19%) was obtained by this test. The overall prevalence of CPV infection obtained by HA/HI diagnostic tests by Reddy *et al*. (2015) was 33.17 percent which was very similar to our study. In the current study lower sensitivity of HA/HI was obtained which is against the study of Sherikar and Paranjape (1985), Dahiya and Kulkarni (2004) and Desario *et al.* (2005) who obtained a varying percentage of positivity from 56.1% to 71.0%. The lower sensitivity is due to the fact that most of CPV-2 strains lacking HA activity by Cavalli *et al.* (2001). Moreover, canine parvovirus can be detected by HA only after few days post infection by (Decaro *et al*., 2005; Desario *et al*., 2005).

**Dot-ELISA**

All the 102 samples were screened by dot-ELISA and 49 samples showed positive reaction. A percent positivity of 48% was obtained. Dot-ELISA was also carried out by Panneer *et al.* (2008) who obtained (50%) positivity by this test. The difference in the sensitivity of two test is due to the fact that the capacity





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of dot-ELISA test is high to detect early stage of infection undetectable by HA test as high amount of virus is required to determine a visible CPV-2 induced HA by Decaro *et al.* (2005). This is supported by the study that some samples which showed low HA titre of less than 1:32 showed positive reaction with Dot-ELISA.

**CPV Specific PCR Assay**

In this study DNA extracted by (phenol-chloroform-isoamyl alcohol) method from all the 102 faecal samples were subjected to CPV specific PCR assay targeting the VP2 gene of canine parvovirus. 52 samples showed positive results out of a total 102 samples. An overall percent positivity of (50%) was obtained by this study. A total of 38 out of 61 samples (62.29%) were found positive by Pandya *et al.* (2017). Similar findings were reported previously by Mohanraj *et al.* (2010) who got 66.23% positivity, Mukhopadhyay *et al.* (2012) who got 57.85% positivity and Miranda *et al.* (2016) who got 64.1% positivity.

**Table 1:** Comparison between diagnostic tests PCR, HA-HI, dot-ELISA

| | Positive Samples | Total Samples | % Prevalence |
|---|---|---|---|
| PCR | 52 | 102 | 50.98 % |
| HA-HI | 41 | | 40.19 % |
| Dot-ELISA | 49 | | 48.03 % |

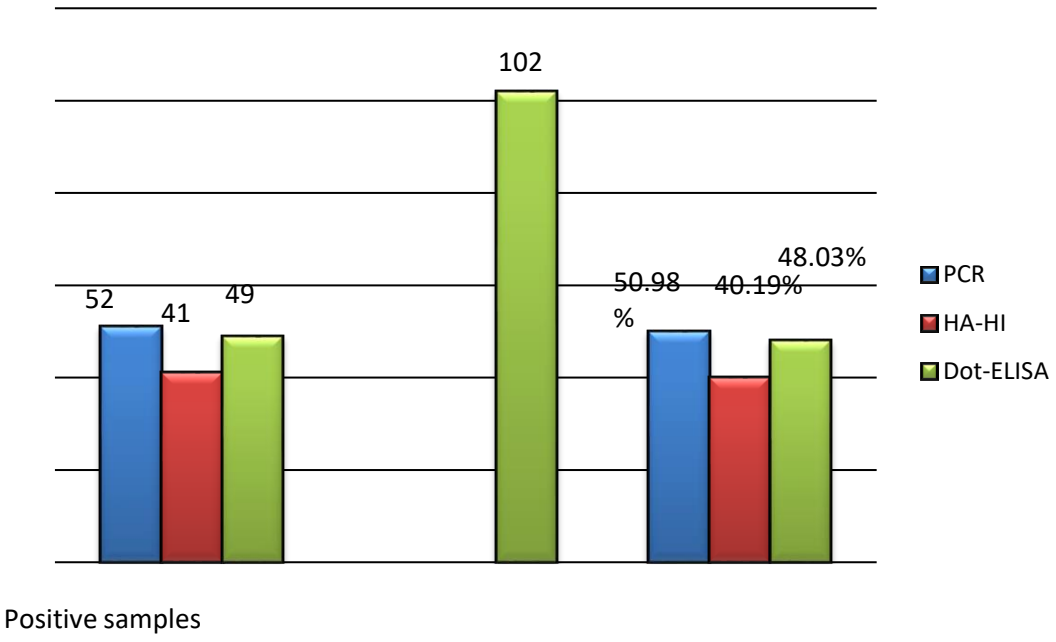


**Fig. 1:** Comparison between diagnostic tests PCR, HA-HI, dot-ELISA

A similar study of comparison between PCR, HA and ELISA has been done by Silva *et al.* (2013) in which CPV antigen was detected in 44/112 samples (39.3%) by EIA, 40/112 (35.7%) by HA and by PCR, 57/112 samples (50.9%) were found positive for CPV DNA. EIA, HA and PCR were able to detect all types of





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CPV, indicating that the genetic variations resulted from continuous evolution of CPV did not affect the ability of these tests based on antigen or genome detection by Decaro *et al.* (2010). Panneer *et al.* (2008) made a comparison between HA/HI and dot-ELISA he observed that dot-ELISA is better in comparison to HA/HI for calculating the overall prevalence of canine parvovirus. Similarly a comparison between HA and PCR was made by Ali *et al.* (2015) in his study he found that HA was able to detect CPV antigen in 35 samples out of 50 samples and PCR can detect 39 samples positive out of 50 samples and a percent positivity of 70% and 78% obtained by these two diagnostic tests respectively and one can use HA as a field level diagnostic tests where costly facilities of PCR are not available.

**Conclusion**

HA/HI is an important test it can detect all the acute infections. Dot-ELISA is more sensitive test than HA/HI. It is concluded in this study that some pups with suggestive clinical signs of CPV infection may not be positive for CPV with dot-ELISA or HA, so those samples should be tested by more sensitive and specific techniques such as PCR to improve the accuracy of CPV diagnosis. PCR is the most sensitive and specific test for CPV diagnosis.

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