RESEARCH ARTICLE

Assessment of Efficacy of Faecal Antigen Detection Kit and Occurrence of Sepsis in Canine Parvovirus Enteritis in Dogs

Divya Chauhan[1], Ashish Srivastava[1]*, Ajay Pratap Singh[2], Mukesh Kumar Srivastava[1], Med Ram Verma[3]

Abstract

Canine parvovirus (CPV) enteritis is one of the most common life-threatening diseases in dogs. Immuno-suppression and intestinal barrier disruption predispose affected dogs for sepsis and make them a suitable population to study sepsis. The present study focuses on the diagnostic efficacy of faecal antigen test kit and on the occurrence of sepsis in canine parvovirus enteritis along with its association with morbidity and mortality. Tentative diagnosis for CPV was based on clinical signs and haematology, confirmation was done by Snap® parvo antigen test kit and PCR using faecal samples. Total 29 dogs between 6 weeks to 1 year of age were included comprising 6 healthy and 23 non-vaccinated CPV positive dogs. Efficacy of diagnosing CPV via faecal antigen test kit was found to be 69.50%, while PCR showed 100% efficacy. The overall occurrence of Systemic Inflammatory Response Syndrome (SIRS) on the day of presentation in CPV dogs was 60.80% and survivability with SIRS was 71.43%. Blood culture revealed *Staphylococcus* spp. This study concludes that faecal antigen test kit gives rapid result with minimum labour and cost, but might give false negative results, and identification of sepsis at early stage might help the clinician in shifting the patient to more aggressive therapy.

Key words: Antigen detection kit, Dogs, Parvovirus, Sepsis, SIRS

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Introduction

Canine parvovirus most commonly affects puppies less than 6 months of age, but immunocompromised adults also suffer often (Marcovich *et al.,* 2012). It is one of the leading causes of morbidity and mortality in dogs globally (Goddard and Leisewitz, 2010). Canine parvovirus is associated with immuno-suppression and intestinal barrier disruption in the affected dogs. This predisposes the animals suffering from the disease to secondary bacterial infections and release of inflammatory mediators contributing to the progression of Systemic Inflammatory Response Syndrome (SIRS) and perpetuation of bacterial translocation cycle leading to sepsis (Krentz and Allen, 2017). Progression of sepsis in these patients causes multiple organ dysfunction syndrome and death (Alves *et al*., 2020). High incidence of sepsis and SIRS in puppies with canine parvovirus enteritis makes it a suitable sepsis model (Otto, 2007).

Diagnosis of canine parvovirus is based on signalment, history, clinical signs, symptoms and haematological examination. Etiology can be established with a high degree of certainty by DNA based serological tests like Polymerase Chain reaction (PCR), haemagglutination, immunofluorescence, immunochromatography tests, detection of specific antibodies, and ELISA (Sanekata *et al.,* 1996; Esfandiari and Klingeborn, 2000). PCR is considered to be the most reliable method for diagnosis owing to its greater accuracy (Nandi *et al.,* 2010). The present study focuses on the diagnostic efficacy of faecal antigen test kit and occurrence of sepsis in canine parvovirus affected dogs and its association with morbidity and mortality.

[1]Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, UP Veterinary University, Mathura, Uttar Pradesh-281001, India

[2]Department of Veterinary Microbiology, College of Veterinary Science and Animal Husbandry, UP Veterinary University, Mathura, Uttar Pradesh-281001, India

[3]Division of Design of Experiments, Indian Agricultural Statistics Research Institute, Library Avenue, Pusa, New Delhi-110012, India

Corresponding Author: Ashish Srivastava, Assistant Professor, Department of Veterinary Medicine, College of Veterinary Science and Animal Husbandry, UP Veterinary University, Mathura, Uttar Pradesh-281001, India. e-mail: ashishvetmed@gmail.com

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Materials and Methods

The animals enrolled in this study were canine parvovirus affected dogs presented to Veterinary Clinical Complex, DUVASU, Mathura (India) from December 2021 to June 2022 with no history of vaccination and between 6 weeks to 1 year of age, irrespective of their sex. The study was approved by Institutional Animal Ethics Committee under project number IAEC/21/11. Total 29 dogs were included in the study comprising 6 healthy and 23 non-vaccinated canine parvovirus (CPV) positive dogs. Blood samples were collected

on the day of presentation of dogs in EDTA vacutainers for the assessment of total leucocyte count. Out of all CPV positive dogs included, 4 dogs died even after receiving therapy.

Dogs suspected to be suffering from canine parvovirus were tentatively selected based on clinical signs of anorexia, vomiting, foul-smelling bloody diarrhoea, lethargy, dehydration, history of no vaccination and haematology depicting leucopenia due to depletion of rapidly dividing cells of lymphoid tissue, destruction of bone marrow precursors and increased demands of inflamed intestinal tract. Confirmation was done by using faecal samples on the basis of faecal antigen detection by the IDEXX SNAP®Parvo antigen test kit as per manufacturer's instructions and PCR for identification of viral DNA. Animals, who did not fulfill the inclusion criteria or had concomitant diseases capable of causing gastrointestinal signs were excluded. Dogs having similar clinical symptoms with faecal examination revealing presence of parasitic eggs were also excluded. All selected diseased dogs were treated with symptomatic and supportive therapy including intravenous fluid, antibiotic, antacid and antiemetic.

PCR Identification of Viral DNA

Fresh faecal samples were homogenized in phosphate buffer saline in 1:10 ratio followed by centrifugation at 2,500 rpm (g) for 5 min to remove any coarse debris. The supernatant was stored at-80°C for further processing. Stored faecal samples were thawed and filtered through 0.22 µ syringe filter and 200 µL of the filtrate was transferred to fresh micro-centrifuge tube for DNA extraction. DNA extraction was done using QIAamp®Fast DNAStool MiniKit (Qiagen, India) as per the manufacturer's instructions. Custom-synthesized oligonucleotide primers, namely forward primer (pCPV-RT) 5′ CAT TGG GCT TAC CAC CAT TT-3′ and reverse primer (pCPV-RT) 5′ CCA ACC TCA GCT GGT CTC AT-3′, derived from positions 3131-3155 to 3276-3295 of the VP1/VP2 gene of Canine Parvovirus 2, as described by Nandi *et al.* (2009), were utilized in the study.

The reagents were thawed before use. PCR reaction mixture for VP1/VP2 of canine parvovirus consisted of 7.5 µL of nuclease free water, 0.5 µL $MgCl_2$, 12.5 µL Dream Taq Green PCR (K1082) master mix, 1.0 µL each of forward primer (pCPV-RT) and reverse primer (pCPV RT) dispensed in a 0.2 mL PCR tube. Template DNA (3.0 µL) and taq polymerase were added individually in each tube in the last. The PCR components were mixed and spun shortly. PCR reaction was performed in thermocycler (Thermo Fisher Scientific). The thermal cycling conditions for *VP1/VP2* gene of canine parvovirus consisted of initial denaturation at 94 °C for 3 min, 30 cycles of denaturation at 94 °C for 30 sec, annealing at 52 °C for 1 min, extension at 72 °C for 30 sec and a final extension at 72 °C for 5 min. The resulting PCR products were analyzed on agarose gel (1.5%) electrophoresis as described by Sambrook and Russel (2001). The agarose gel (1.5%) was prepared in 1X TAE buffer and 0.5 µg/mL ethidium bromide was added. Ready-to-use 50 bp DNA Ladder (Generuler, Thermofisher) was run along with samples in separate well. The amplification products were electrophoresed for 50-60 min at 70 V. The gel was then visualized under Gel documentation system (Uvitek, United Kingdom).

Assessment of Occurrence of Sepsis in Parvovirus Affected Dogs

Invasion of infection triggers Systemic inflammatory response syndrome (SIRS). For SIRS diagnosis, at least two of the following four criteria were used to be met including temperature <100.04 °F or >102.92°F, heart rate >140 bpm, respiratory rate >30 breaths/min, White Blood Cell count < 6000 or >16,000 cells/µL (Alves *et al.*, 2020).

Blood culture was performed to identify the bacteria involved in and contributing to sepsis. 1 mL of fresh blood samples were collected in heparinized vacutainers with the help of a vacutainer adapter after sterilization of the collection site with an alcohol swab on day 0 before the administration of antibiotics for microbiological examination. The samples were inoculated in buffered peptone water for overnight growth and incubated overnight at 37°C in a bacteriological incubator (Scintech, India) for pre-enrichment of the sample. The growth on buffered peptone water was streaked on MacConkey lactose agar and Mannitol salt agar media (Himedia, India) and incubated overnight at 37°C for isolation of bacteria. Gram's staining was performed as per the standard protocol. Biochemical characterization was done by catalase and oxidase test as per standard methods.

Results and Discussion

The comparative efficacy of antigen test and PCR assessed revealed that the efficacy of faecal antigen test kit in diagnosing the disease was found to be 69.50% (16/23), while PCR showed 100% (23/23) efficacy through agarose gel electrophoresis of amplified product of 160 bp CPV primer. Six faecal samples from healthy dogs were also tested via Snap® Parvo antigen test kit and all 6 were found negative. This indicates that faecal antigen test kit does not give false positive result. False negative results by faecal antigen kit might be due to requirement of high viral load in faecal sample to test positive. It also indicates a greater accuracy of PCR in diagnosing the disease because PCR allows identification and amplification of very small amount of viral DNA present in the sample which makes it a highly sensitive and gold standard test.

Parameters such as temperature, heart rate, respiratory rate and total leucocyte count of individual parvovirus affected dogs were recorded on the day of presentation and SIRS was diagnosed on fulfilling the criteria outlined by Alves *et al.* (2020). The values of the parameters of individual CPV-positive dogs fulfilling the SIRS criteria, who survived and did not survive, are mentioned in Table 1, and dogs, who did not fulfill the SIRS criteria are mentioned in Table 2. All dogs who did not fulfill the SIRS criteria survived.

Table 1: Parameters of individual CPV-positive dogs fulfilling the SIRS criteria

Animals who fulfilled SIRS criteria		Unit - (degree Fahrenheit)	(bpm)	per minute	unit - (Thousand/microliter)
1	SIRS Survivors	98.4	96	36	5.3
2		99.5	160	30	9.5
3		101.9	160	35	7.7
4		98.3	174	24	11.7
5		98.2	112	24	4.9
6		102.6	192	32	6.4
7		97.4	184	28	7.9
8		99.8	150	28	10
9		100.9	134	16	5.4
10		101.5	132	38	8
	Mean ±SE	**99.85±0.567**	**149.4 ±9.79**	**29.10±2.10**	**7.68±0.70**
11	SIRS non-survivors	102.9	165	20	0.18
12		100.9	160	32	1.9
13		100.4	152	24	1.5
14		103.1	180	28	0.91
	Mean ±SE	**101.8±0.68**	**164.2±5.89**	**26.0±2.58**	**1.1±0.37**

Table 2: Parameters of individual CPV-positive dogs, who did not fulfill the SIRS criteria

Animals who did not fulfil SIRS criteria	Temperature	Heart rate	Respiration rate	Total leucocyte count
15	101.8	184	24	12.4
16	101.1	130	29	10.3
17	100.4	116	16	4.5
18	101.2	120	28	5.9
19	101.5	170	26	10
20	102.1	150	30	5.6
21	97.7	110	24	11.8
22	101.1	165	28	9.1
23	101.5	82	28	5.4
Mean ± SE	**100.93±0.434**	**136.33±11.04**	**25.88±1.41**	**8.33±1.00**

Table 3: Survivability and mortality in SIRS positive and SIRS negative dogs with treatment

Number of CPV positive dogs taken for study (23)	SIRS status	Number (%) of cases	Survivors	Non- survivors	Per cent Survivability	Per cent Mortality
	SIRS positive	14 (60.8%)	10	4	71.43	28.57
	SIRS negative	9 (39.2%)	9	0	100	00

Out of 23 CPV-positive dogs, 14 dogs were SIRS positive. Hence, the overall occurrence of SIRS was 60.80%, which is in near agreement with the findings of Alves *et al.* (2020), who recorded 65.20% occurrence. Out of these 14 dogs, 10 dogs survived and 4 dogs died even after receiving therapy with a survivability of 71.43% and mortality of 4/14 (28.57%), 9 dogs were found negative for SIRS and did not undergo mortality (Table 3). This indicates that SIRS can be considered as an important criterion in predicting the prognosis of the disease.

Alves *et al.* (2020) also recorded 76.50% survivability and 23.40% mortality, which was almost similar to our findings. The occurrence of sepsis in CPV-positive dogs is due to intestinal bacterial translocation and severe

immuno-suppression (Alves *et al.,* 2020). In addition, cellular destruction, intestinal hypomotility, dysbiosis and tissue necrosis are additional factors contributing towards development of sepsis (Mylonakis *et al.,* 2016). Release of inflammatory mediators together with the progression of SIRS contributes to bacterial translocation cycle through the damaged intestinal barrier and leads to sepsis (Krentz and Allen, 2017).

Blood culture was conducted on the day of presentation and prior to the initiation of treatment. Buffered peptone water showed turbidity after incubation overnight at 37°C. Mannitol salt agar (MSA) cultural characteristics were observed after incubation at 35-37°C for 18-72 h. No growth was however seen on MacConkey lactose agar. Gram-positive cocci arranged in a characteristic 'bunch of grapes' pattern were seen on MSA. Catalase-positive cultures produced oxygen and bubbling effervescence. Oxidase test did not show any colour development and the test was considered as negative. The above results reveal that *Staphylococcus* spp. was responsible for sepsis on day 0. Sunghan *et al.* (2019) also reported that the most common bacteria isolated on day 0 of infection was coagulase negative Staphylococcus which is considered as a major nosocomial pathogen as a result of intravenous catheter placement along with enterococcus. It is reported to have a major contribution towards mortality and therapeutic expenditure (Lobetti *et al.,* 2002; Moses *et al.,* 2012).

Conclusion

It can be concluded that faecal antigen test is user friendly and provides rapid result with minimal effort and cost, unlike PCR, but it has a major limitation in the form of false negative results. Clinician's experience accompanied by haematologic changes is fair enough for diagnosing the disease. Efforts should also be done to screen the dogs having signs suggestive of parvovirus enteritis for SIRS on the day of presentation which might help in prognosticating the outcome as SIRS positive animals have fairly high risk of mortality. Such animals can be shifted to more aggressive therapy including colloids, vasopressor agents and oxygen supplementation based on the requirement to reduce chances of mortality.

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