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Canine parvo viral enteritis in dogs: Diagnostic and therapeutic evaluation[#]

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Abstract

A total of 110 dogs presented to Teaching Veterinary Clinical Complex, CSKHPKV, Palampur (H.P.), India with the history of enteritis/gastroenteritis were screened for Canine Parvovirus (CPV). Out of these 110 dogs, 48 dogs were found positive for CPV by using Scanvet - a rapid antigen detection kit. All the 110 samples were subjected to Polymerase Chain Reaction (PCR) to check whether there were some cases that were false positive or false negative by Scanvet. Positive cases were, then, divided into 4 groups of 12 dogs each, with 4 different treatment regimens followed for each group. In addition to standard treatment protocol, our study tested effect of immunomodulators (Vitamin E & Selenium) and antiviral "Oseltamivir" on recovery of dogs. This type of study (using Oseltamivir and immunomodulators) was not done previously in the state and hence, our aim was to test whether this treatment increases survivability and recovery rate for dogs with Parvoviral enteritis in Himachal Pradesh. Groups containing immunomodulators were more efficacious than their counterparts without immunomodulators. Addition of Oseltamivir and immunomodulators to standard therapy resulted in reduced mortality, cessation of diarrhoea and vomiting earlier than routine therapy. Scanvet diagnostic kit was found to be a quick alternate method for diagnosis of Parvo Viral Enteritis. Overall, the group treated with antibiotic+ supportive+ symptomatic+ antiviral treatment along with immunomodulator displayed promising results.

Keywords: Dogs, parvo-virus, severe disease, treatment, diagnostic methods, antiviral

Canine parvovirus enteritis (PVE) is caused due to three mutations in canine parvovirus type 2. These are recognised as CPV2, family Parvoviridae and Genus Parvovirus. During the late 1970s, CPV-2 emerged as the leading cause of global outbreaks of acute canine enteritis (Battilani *et al.*, 2006; Decaro and Buonavoglia, 2012; Greene, 2012; Truyen, 2006). The CPV-2 caused the infections thereafter for almost a decade until it evolved itself into two new variants CPV-2a and CPV-2b (Carmichael, 2005; Martella *et al.*, 2006). Over the next few years, no new mutation was

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recorded worldwide. However, in the year 2000, a third variant CPV-2c was reported in Italy and is being found all around the world ever since except Australia (Buonavoglia *et al.*, 2001; Decaro *et al.*, 2006; Ntafis *et al.*, 2010). All of these variants are believed to contain similar pathogenicity which leads to identical clinical disease (Decaro and Buonavoglia, 2012; Markovich *et al.*, 2012). Overall, PVE is a highly contagious and fatal viral disease found in dogs of all ages (Goddard and Leisewitz, 2010; Sykes, 2014).

Although, intense clinical disease commonly occurs in puppies of age less than six months, adults with inadequate immunity may also be potentially affected (Kalli et al., 2010; Markovich et al., 2012). The disease manifests in two forms: cardiac and intestinal form. Schatzberg et al. (2003) suggested that the cardiac form is only seen in young puppies. Prittie (2004) reported that the intestinal form of disease is characterised by foul smelling, dark bloody faeces, severe vomiting, dehydration, anorexia, depression and can be fatal within 2 days of post infection in very acute cases. Although, a large no. of diagnostic tests viz. Haemagglutination test, ELISA, PCR are available, these are very costly, time consuming (Mosallanejad et al., 2008; Oh et al., 2006) and need expensive and elaborate laboratory set up. Rapid diagnostic tests offer promise in the cost effective and immediate diagnosis of this disease. However, the authenticity, validity and repeatability of newly developed tests like Scanvet Rapid Parvo Virus Detection kits needs further investigations.

Therapeutic management of parvo viral infection includes the conventional treatment comprising of the use of antibiotics in majority of cases along with supportive and symptomatic therapy (anti-emetics, fluids, electrolytes, intestinal protectants and analgesics). However, the results obtained are highly variable (Goddard and Leisewitz, 2010; Roy et al., 2010). Recently, one antiviral compound "Oseltamivir" originally developed to treat influenza infection in human is also being tried to treat parvo viral infection in dogs. Although several anecdotal reports have come out from practising veterinarians, especially

from North America indicating its beneficial effects, only two scientific studies (Savigny and Macintire, 2010; Schatzberg et al., 2003) have been conducted so far to validate the findings. Hence, more systematic studies are required to be undertaken to determine its efficacy. Immunomodulatory approaches using micronutrients, especially, Selenium, Vitamin E and Vitamin C as a non-specific way of maintaining adequate host defense against infection have been mentioned in the treatment of CPV infections and this is in line with Kandil and Zeina (2005). The present communication reports diagnosis and treatment of canine parvo viral infection in dogs in Palampur valley of H.P. using different treatment approaches in order to find appropriate treatment that can ameliorate morbidity and mortality. The main aim of this study was to investigate authenticity and validity of rapid diagnostic tests along with investigating efficiency of new methods of treatment using antiviral and immunomodulators.

Materials and methods

The present study was conducted on a total of 110 dogs presented to Teaching Veterinary Clinical Complex, College of Veterinary and Animal Science, Palampur, with the history of diarrhoea and vomiting. Every dog brought to the clinic was a private pet, and all the owners had given their consent for the treatment. The faecal samples of dogs were screened for parvo viral infection by using Scanvet Canine Parvo virus antigen detection kit developed by Intas Pharmaceuticals Ltd., Ahmedabad (India). The faecal samples were collected from suspected dogs with the help of sterile swab given in the kit by inserting into rectum and followed by the test as per manufacturer's instructions. All the 110 cases were further subjected to PCR in order to detect false positive or false negative by Scanvet kit. For PCR, faecal swabs of all dogs were made bacteria free by filtration with 0.22micron syringe filter and kept frozen at -20°C. PCR tests were conducted at Department of Veterinary Microbiology, COVAS, Palampur, H.P and further study was done on cases found positive with Scanvet Rapid antigen detection kit.

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Polymerase Chain Reaction

Genomic DNA of CPV-2 was extracted by phenol chloroform method from the faecal samples as per the standard method (Sambrook and Russell, 2011). The PCR was standardised for the primer set pCPV 2ab as reported by Pereira *et al.* (2000) with slight modifications (Table 11). The pCPV 2ab primer set amplify portion of VP1/VP2 gene of both CPV 2a and CPV 2b variants (3025 to 3706 nucleotide position of CPV genomic DNA) to yield a product size of 681 bp.

The reaction was optimised using 2.5 μ L of 10x Taq DNA polymerase buffer (100mM TrisHCl, pH 8.8, 500mM KCl, 0.8% Nonidet P40, 25mM MgCl₂₎, 200 μ M of each dNTP, 10 pmol of each forward and reverse primer (0.5 μ L each), 5 μ L of processed faecal sample and nuclease free water to make the volume to 25 μ L.

Therapeutic study

Positive cases as found with Scanvet kit were further included for various therapeutic trial groups. The ailing dogs were placed in 4 different therapeutic groups comprising of 12 animals in each group. The treatment regimens for each group are shown in Table 2.

Symptomatic and supportive therapy consisted of parenteral, anti-emetics,

hemostats, B- complex vitamins and fluids i.e., Ringer's lactate and dextrose normal saline. Minimum treatment duration with antimicrobial agent was 3 days in each of the group, extendable up to 5 days, if situation warranted.

All the 48 dogs found positive as per Scanvet kit were first divided into mid-stage and late-stage cases. Cases presented within 2 days of the development of diarrhoea/dysentery were labelled as mid-stage cases and cases presented after 2 days were labelled as latestage cases. Out of 48 cases, 16 cases were late-stage and 32 were mid-stage cases. These cases were further divided into 4 treatment regimen groups as per (Table 3).

Statistical analysis

The data obtained were subjected to statistical analysis by using computer software Instat from GraphPad software 2008, California Corporation, and as used by Bhat *et al.* (2013).

Table 3.	Group	formation	of	mid-stage	and
	late-sta	age cases			

Group	Mid-stage cases	Late-stage cases	
Group I	7	5	
Group II	7	5	
Group III	9	3	
Group IV	9	3	

Table 1. Primer sequence used for the amplification of VP1/VP2 gene of CPV

Sr. No.	Forward and Reverse Primers	Primer Sequence		Annealing temperature and product size
1.	pCPV-2ab (F)	5'-GAA GAG TGG TTG TAA ATA ATT-3' (21 mer)	3025-3045 to	55°C
2.	pCPV-2ab (R)	5'-CCT ATA TAA CCA AAG TTA GTA C-3' (22 mer)	3685-3706	681bp

Table 2. Treatment regimens

Group	Treatment regimen			
Group I	Treated with antibiotics with supportive and symptomatic therapy			
Group II	Treated with antibiotics and immunomodulators along with supportive and symptomatic therapy			
Group III	Treated with antiviral along with the use of antibiotic, symptomatic and supportive therapy			
Group IV	Treated with antiviral and immunomodulator along with using antibiotics, supportive and symptomatic therapy			

The mean values of different parameters between control and diseased group; control, pre and post treatment were compared at 1%, 5% and 10% level of significance using "t" test and "ANOVA".

Results and discussion

Notwithstanding the fact that 48 cases were found positive both by Scanvet kit and PCR, there were at least 8 such cases which have been found negative by Scanvet and positive by PCR. Figures 1 and 2 below show the results of antigen detection kit, whereas figure 3 shows the results of PCR.



Fig.1. Positive result of rapid antigen detection kit (Control and test bands are visible).



Fig. 2. Negative result of rapid antigen detection (Only control band is visible).

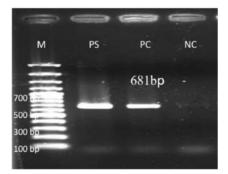


Fig. 3. PCR products: M 100 bp marker, PS positive sample, PC positive control, NC negative control

It means Scanvet kit showed 8 false negative cases (Table 4) that might be due to the requirement of large amount of viral antigen to produce a clearly visible band (Reddy *et al.*, 2015; Tinky *et al.*, 2015) who reported that the quantity of viral particles can affect the immunochromatographic (IC) test result which was observed to be one of the disadvantages of this test.

Therapeutic studies

The consistency, frequency and colour of faeces returned to normal within 3.10 ± 0.51 days of initial treatment in dogs of group IV which was the minimum amongst all the four treatment groups. Similarly, the vomiting stopped completely in minimum time of 3.08 ± 0.66 days in dogs of this group. Overall survival rate was also highest viz. 91.67% (11/12) in this group. Number of days taken for cessation of diarrhoea and vomiting was maximum in group I with value of 4.33 ± 0.88 and 4.25 ± 0.96 days respectively. The survivability was also noticed to be minimum i.e., 71.43% (5/7) in dogs of group I. It was worth noting that the survival rate was better in mid-stage cases as compared to late-stage cases in all the treatment groups (Table 5).

All the survived animals regained their normal appetite in 5-7 days of start of treatment and were active, alert and bright in appearance. The total erythrocytic count (TEC) increased significantly (p< 0.01) in all the four groups after institution of the treatment. Similarly, the total leucocytic count (TLC) also increased significantly (p<0.01) in all the groups. However, the increase was more pronounced in dogs of group III which were given Oseltamivir additionally and in dogs of group IV which were given immunomodulators with Oseltamivir and standard treatment. The pre-treatment mean values of haemoglobin and packed cell volume were recorded to be significantly (p<0.01)

Table 4. Comparison of Scanvet kit and PCR test strips

Test		PCR test kit			
lesi	Result	Positive	Negative	Total	
Scanvet kit	Positive	48	0	48	
	Negative	8	54	62	
	Total	56	54	110	

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lower in all the groups as compared to healthy dogs (Table 6). After institution of treatment, these values were further decreased but the decline was non-significant (p>0.05). There was a non-significant (p>0.05) increase in the mean values of plasma glucose, thus, showing slight improvement in dogs of all the groups. However, a significant increase (p<0.01) was found in blood glucose level of group IV after institution of treatment.

The total plasma protein was found to be lower in dogs of all the four groups even after initiation of the treatment. The blood urea nitrogen (BUN) which was significantly (p<0.01) higher in all the parvo virus affected dogs decreased significantly (p< 0.01) in all the treatment groups (Table 7). Plasma sodium concentration increased significantly (p<0.01) in dogs of all the groups, except group III which showed non-significant increase (p>0.05), thus showing improvement in all the four treatment groups. Plasma potassium concentration showed non-significant increase (p>0.05) whereas plasma chloride showed significant increase (p<0.01) in all four treatment groups indicating gradual improvement.

Number of animals scanned for the CPV is statistically a significant number to conclude that such kits can be a good alternate method for rapid and accurate diagnosis of parvo viral infection especially in field conditions where facilities to perform other tests like PCR and HA are non-existent.

We tried our best to put mid-stage and late-stage cases equally in all 4 treatment groups, so that we can remove any bias in our results. As the number of days taken for cessation of diarrhoea and vomiting were minimum in group IV along with maximum

 Table 5.
 Comparative efficacy and survivability in various treatment protocols for CPV infectious dogs

	Days taken for	Days taken for	Survivability		
Group	cessation of diarrhoea (Mean ± S.D)	cessation of vomiting (Mean ± S.D)	Mid-stage cases	Late-stage cases	Total survivability
Group I	4.33 ± 0.88	4.25 ± 0.96	71.43% (5/7)	40.00% (2/5)	58.33% (7/12)
Group II	3.66 ± 0.98	3.50 ± 0.67	85.71% (6/7)	60.00% (3/5)	75.00% (9/12)
Group III	3.83 ± 1.02	3.75 ± 0.86	88.89% (8/9)	66.67% (2/3)	83.33% (10/12)
Group IV	3.10 ± 0.51	3.08 ± 0.66	100% (9/9)	66.67% (2/3)	91.67% (11/12)

 Table 6.
 Effect of treatment on haematological parameters (Mean ± S.D.) (n=12) in CPV infectious dogs

Hb (g/dl)	Group I	Group II	Group III	Group IV		
Healthy group	12.41 ± 1.37 ^b	12.41 ± 1.37 [♭]	12.41 ± 1.37 [♭]	12.41 ± 1.37 ^b		
Pre-treatment value (0 h)	9.08 ± 1.50^{a}	9 ± 1.47ª	9 ± 1.47ª	9.08 ± 0.44^{a}		
Post-treatment value (72h)	8.25 ± 0.93^{a}	8.41 ± 0.81ª	8.50 ± 0.63^{a}	8.75 ± 0.75ª		
PCV (%)						
Healthy group	36 ± 4.24^{b}	36 ± 4.24^{b}	36 ± 4.24^{b}	36 ± 4.24 ^b		
Pre-treatment value (0 h)	27.58 ± 3.44 ^a	26.66 ± 4.61ª	26.83 ± 4.62^{a}	26.83 ± 4.62^{a}		
Post-treatment value (72 h)	25.75 ± 2.95ª	25.58 ± 2.93ª	24.75 ± 3.69^{a}	26.16 ± 3.15ª		
TEC (× 10 ⁶ /μl)						
Healthy group	5.73 ± 0.87 ^b					
Pre-treatment value (0 h)	4.55 ± 0.55^{a}	4.55 ± 0.53^{a}	4.37 ± 0.59^{a}	4.60 ± 0.61^{a}		
Post-treatment value (72 h)	5.60 ± 0.74 ^b	5.62 ± 0.74 ^b	5.73 ± 0.75 ^b	5.65 ± 0.64 ^b		
TLC (× 10 ³ /μl)						
Healthy group	9.33 ± 1.65 [♭]	9.33 ± 1.65 [♭]	9.33 ± 1.65 [♭]	9.33 ± 1.65 ^b		
Pre-treatment value (0 h)	5.60 ± 0.74^{a}	5.50 ± 1.09^{a}	4.81 ± 0.42^{a}	5.01 ± 0.45^{a}		
Post-treatment value (72 h)	11.48 ± 0.66°	11.79 ± 1.28 ^b	12.78 ± 0.66°	13.08 ± 0.48°		

Values with different superscripts in a column differ significantly (p<0.01)

Glucose (mg/dl)	Group I	Group II	Group III	Group IV	
Healthy group	105.58 ± 6.08 ^b				
Pre-treatment value (0 h)	81.08 ± 7.36ª	81.83 ± 6.87^{a}	78.41 ± 7.31ª	79.41 ± 6.31ª	
Post-treatment value (72 h)	84.08 ± 6.82^{a}	87.08 ± 4.27ª	79.25 ± 6.55^{a}	86.75 ± 6.57°	
Total Protein (g/dl)					
Healthy group	6.60 ± 0.59^{b}	6.60 ± 0.59^{b}	6.60 ± 0.59^{b}	6.60 ± 0.59^{b}	
Pre-treatment value (0 h)	4.86 ± 0.95^{a}	4.95 ± 0.88^{a}	4.78 ± 0.78^{a}	4.61 ± 0.67^{a}	
Post-treatment value (72 h)	4.60 ± 0.66^{a}	4.79 ± 0.77^{a}	4.45 ± 0.47^{a}	4.40 ± 0.42^{a}	
BUN (mg/dl)					
Healthy group	12.0 ± 2.0^{a}	12.0 ± 2.0^{a}	12.0 ± 2.0^{a}	12.0 ± 2.0^{a}	
Pre-treatment value (0 h)	25.91 ± 3.26 ^b	26.75 ± 4.80 ^b	25.08 ± 3.39b	26.58 ± 4.79 ^b	
Post-treatment value (72 h)	13.25 ± 1.65ª	12.83 ± 1.69ª	13.41 ± 1.50ª	11.83 ± 1.40ª	
Sodium (mmol/l)					
Healthy group	147.33 ± 4.63 ^b				
Pre-treatment value (0 h)	126.33 ± 4.37^{a}	126.33 ± 4.37ª	133.58 ± 5.74ª	128.66 ± 5.21ª	
Post-treatment value (72 h)	136.33 ± 4.09°	136.41 ± 4.12°	139.66 ± 4.51ª	140.83 ± 3.73°	
Potassium (mmol/l)					
Healthy group	4.15 ± 0.45^{a}	4.15 ± 0.45^{a}	4.15 ± 0.45^{a}	4.15 ± 0.45ª	
Pre-treatment value (0 h)	3.50 ± 0.29^{b}	3.55 ± 0.28 ^b	3.59 ± 0.25 ^b	3.61 ± 0.15ª	
Post-treatment value (72 h)	3.73 ± 0.15 ^b	3.76 ± 0.14 ^b	3.84 ± 0.10 ^b	3.89 ± 0.80^{a}	
Chloride (mmol/l)					
Healthy group	113.50 ± 2.93 ^b				
Pre-treatment value (0 h)	93.33 ± 2.99^{a}	93.25 ± 2.89^{a}	93.33 ± 2.99^{a}	94.16 ± 2.72^{a}	
Post-treatment value (72 h)	98.93 ± 2.79°	98.75 ± 2.59°	98.75 ± 2.59°	98.25 ± 2.14°	

 Table 7.
 Effect of treatment on blood biochemical profile (Mean ± S.D.) (n=12) in CPV infectious dogs

Values with different superscripts in a column differ significantly (p<0.05)

survivability, the treatment regimen of this group gave best results in our study. However, treatment regimen of group I showed least survivability among all four treatment groups and took maximum days for cessation of diarrhoea and vomiting. As the survival rate was better in mid-stage cases as compared to latestage cases in all the treatment groups, thus, it can be concluded that chances of recovery are lesser in advanced cases.

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A variety of treatment protocols have been suggested and adopted by various researchers to treat the parvo virus affected dogs (Chakrabarti, 2012; de Mari *et al.*, 2003; Roy *et al.*, 2010). However, wide variation has been noticed with regard to their efficacy. Many clinicians have advocated extensive use of symptomatic and supportive therapy, especially fluids for successful treatment of parvo virus infection as reported by Prittie (2004); however, Roy *et al.* (2010) have recommended the additional use of antibiotics (Ceftriaxone and Tazobactam) along with supportive therapy to obtain the better response. Navva et al. (2022) suggested supplementation of intravenous sodium bicarbonate in treatment of parvoviral enteritis. Savigny and Macintire (2010) carried out detailed investigation of the use of Oseltamivir to treat the parvo virus affected dogs. A significant weight loss during hospitalization as well as significant decrease in WBC count were noticed in control group as compared to treatment group and no major adverse effects were identified that could be associated with Oseltamivir administration (Savigny and Macintire, 2010). Our study showed higher survivability rates in Oseltamivir treated groups which has been shown in table 6.

Oseltamivir is an antiviral drug, basically, developed for the treatment of human influenza. It acts on the influenza (RNA) virus by way of inhibition of neuraminidase enzyme, thus preventing the virus replication in the body as reported by Gubareva *et al.* (2000). Parvo virus is a DNA virus and it does not rely upon

neuraminidase for its replication/pathogenicity and thus, Oseltamivir does not possess direct antiviral activity on this virus. There are some scientific studies especially one in North America that has shown beneficiary effect of the drug in parvo virus affected dogs reported by Savigny and Macintire (2010). This may be due to some indirect actions including inhibition of adherence, translocation and colonisation of secondary bacterial infections and thus, preventing the sepsis and endotoxaemia and systemic inflammatory response and eventual organ failure that is thought to be the main mechanism behind the mortality of CPV enteritis (Crawford and Sellon, 2010; Macintire and Douglass, 2006).

In the light of above therapeutic observations, it was inferred that addition Oseltamivir of (anti-viral drua) and immunomodulators (Selenium and Vitamin E) in the already prevailing conventional therapy (antibiotics + symptomatic and supportive therapy) resulted in higher recovery and survivability in parvo virus affected dogs. Total leucocvtic count increased significantly in the groups treated with Oseltamivir as well as Oseltamivir + immunomodulators. Thus, it can be inferred that addition of Oseltamivir as anti-viral, and Vitamin E and Selenium as immunomodulators provide better results as far as survivability and cessation of symptoms (diarrhea and vomiting) is concerned. It is further added that we have not applied any statistical test on survivability, and hence further studies are warranted to validate the above findings covering more animals and a wider geographical area.

Conclusion

Scanvet rapid antigen detection kit is a quick alternate method for diagnosis of parvo viral enteritis in field conditions where PCR can't be used. Above findings suggest that addition of Oseltamivir (anti-viral drug) and immunomodulators (Selenium and Vitamin E) in the already prevailing conventional therapy (antibiotics + symptomatic and supportive therapy) delivers higher recovery and survivability in parvo virus affected dogs. Thus, it can be concluded that addition of Oseltamivir and immunomodulars provide better results as far as survivability and cessation of symptoms is concerned.

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