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ABSTRACT

The present research was undertaken to study the prevalence of CPV infection in and around Patna. A total of 600 serum collected from bloody diarrheic dogs were collected and screened for CPV antibody titre by using HA, HI, ELISA and Cardiac Troponin-I tests. 600 serum samples showed 225 sample to be positive in present investigation (37.50%) HA test, 325 samples out of 600 samples were found positive in present investigation (54.16%) HI test, out of 600 serum sample 394 sample (65.66%) were found positive in ELISA test and out of 600 serums sample 550 sample (91.66%) were found positive in Cardiac Troponin-I test. In present study, the overall prevalence of CPV was 65.66 % in and around Patna. Breed wise prevalence highest in non-descriptive (46.66 %) followed by German shepherd (24.21 %) and (Labrador (13.00 %) respectively. Biochemical parameters showed that a significant increase alkaline phosphatase whereas significantly decrease was noticed in total serum protein, albumin, globulin and AG ratio. In present therapeutics studies all three groups. Intravenous administration of immune plasma in Group B improve survival, reduce vomiting and diarrhea in dogs. Albumin level likely to normalized, after amino acid infusion in Group C. The amino acid infusion in Group C was found to be most effective for treatment of CPV.

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ABBREVIATION

%	-	Per Cent
>	-	Greater Than
<	-	Less Than
°C	-	Degree Celsius
µg	-	Microgram
µl	-	Microlitre
AGPT	-	Agar gel precipitation Test
CPV	-	Canine parvovirus
bp	-	base pair
CD	-	Cluster differentiation
CO_2	-	Carbon Dioxide
CPE	-	Cytopathic Effect
CSO	-	Central Stastical Organization
dNTP	-	deoxy nucleotide phosphate
EDTA	-	Ethylene diamine tetra chloro acetic acid
ELISA	-	Enzyme Linked Immuno Sorbent Assay
EMEM	-	Eagle‘s Minimum Essential Medium
et al.	-	et alii
Fig.	-	Figure
GM	-	Growth medium
HA	-	Hemagglutination Test
HI	-	Hemagglutination Inhibition Test
H&E	-	hematoxylin and eosin

HBSS	-	Hank‘s balanced salt solution
HRPO	-	Horse Radish Peroxidase
IgG	-	immunoglobulin G
mg	-	Milligram
MHC	-	Major Histocompatibility Complex
ml	-	Millilitre
MM	-	Maintenance Medium
mM	-	millimolar
NaHCO3	-	Sodium bicarbonate
nm	-	Nanometer
OD	-	Optical Density
ORF	-	Open reading Frame
PBS	-	Phosphate Buffer Saline
pM	-	picomolar
REA	-	restriction endonuclease analysis
SE	-	Standard Error
SRBC	-	Sheep red blood cell
Tn-I	-	Troponin-I
v/v	-	Volume by Volume
w/v	-	Weight by Volume

CERTIFICATE-I

This is to certify that the thesis entitled, **"Studies on canine parvovirus infection in dogs and its therapeutic modalities''** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Veterinary Science in the discipline of Veterinary Medicine** of the faculty of Post-Graduate Studies, Bihar Animal Sciences University, Patna, Bihar is the bonafide research work carried out by **Dr. Rajiv Kumar** Son/daughter of Shri **Arun Kumar Singh & Lalita Devi** under my supervision and that no part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been fully acknowledged.

Dr. Bipin Kumar
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CERTIFICATE-II

This is to certify that the thesis entitled, **"Studies on canine parvovirus infection in dogs and its therapeutic modalities''** submitted by **Dr. Rajiv Kumar, Admission No-BVC/M/VCM/001/2017-18**, **Son**/daughter of Shri **Arun Kumar Singh** to the Bihar Animal Sciences University, Patna, Bihar in partial fulfilment of the requirements for the degree of **Master of Veterinary Science** in the discipline of **Veterinary Medicine** has been approved by the Advisory Committee after an oral examination of the student in collaboration with an External Examiner.

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1 INTRODUCTION

Canine Parvovirus infection is highly fatal infectious disease and caused by type-2 canine parvovirus characterized by gastroenteritis or hemorrhagic gastroenteritis with vomition and fever. The first report Canine parvovirus infection was in USA (Eugster & Nain, 1977). Canine parvovirus infection is of two forms (CPV-1and CPV-2). The virus was classified on the basis of position of amino acid. After the first report of the disease in India, prevalence of disease has been found increasing during recent past (Banja *et al.,* 2002a) in vaccinated dogs.

The canine parvovirus causative agent belongs to genus *Parvovirus* and *Parvoviridae* family (Berns 1990, and Touihri *et al.,* 2009). The genome is single stranded DNA having size of 5.2Kb (Perez *et al.,* 2007). The Canine parvovirus infection causes mortality up to 10% and a high morbidity up to 100% (Appel *et al.,*1978). Maximum prevalence of canine parvo virus infection is seen young puppies between 1-4 months of age and less prevalence is recorded in adult dogs. Most adult dogs are immune to the disease either via natural infection or immunization.

The study on the prevalence of this disease in India was first reported by Balu and Thangaraj in 1981 in Madras. Its transmission from infected to susceptible dogs is mainly by the fecal, oral route but dog can also become infected from virus present on fomites such as shoes, clothing, food bowls and other utensils (Pollock, 1982a; Carmichael, 1994; Decaro *et al.,* 2005b). The incubation period of canine parvovirus infection in dogs is 4-5 days. Indirect transmission of the virus occurs during adverse environmental conditions and infection are also reported through insect and rodents vectors.

Predisposing factors for canine parvo virus infection in puppies like certain breeds are at increased risk namely Doberman, Pinscher, Labrador retriever and German shepherd (Houston *et al.,* 1996). Lack of appropriate vaccination protocol has been reported with a higher incidence of clinical disease occurring between July and

September than during the others months. Reports of all studies suggest increased susceptibility of parvo virus infection in puppies less than 6 months of age.

Clinical signs associated with canine parvo virus infection include anorexia, vomiting, foul smelling diarrhoea, depression and fever. Most affected puppies begin vomiting and develop small bowel diarrhoea within 24-48 hours of initial clinical sign (Kramer *et al.,* 1980). Large amount fluid and protein losses through the gastrointestinal tract result in severe dehydration, hypo-albuminemia and hypo- volemic shock.

There is slight rise of temperature in the initial stage of the disease but gradually turn to sub-normal level with advancement of vomiting and diarrhoea. There is no consistent character of the stool. It may be watery yellow in colour or tinged with frank blood. The course of illness is also highly variable depending on the infectious dose of the virus. Clinical sign usually develops from 3 to 5 days infection and typically persist for 5-7 days (Kramer *et al.,* 1980). The mortality and morbidity rates vary according to the age of the animals, the severity of challenges and the presence of undercurrent disease problem. Puppies can die suddenly of shock as early as 2 days into the illness (Stann *et al.,* 1984).

A presumptive diagnosis of canine parvo virus infection can be made based on the clinical sign such as depression, vomiting, diarrhoea, anorexia and fever. The diagnosis can also be done on the basis of fever, loss of appetite, dehydration, smelly bloody diarrhoea. Diagnosis can be performed in any dog with known exposure to parvo virus within the preceding 14 days of developing diarrhoea.

In the GI tract heaviest damage is caused by CPV-2 virus causing bloody diarrhea & secondary bacterial infections due to immune suppression. The virus kills the host in one of the two ways- diarrhea & vomition, which lead to extreme fluid loss and dehydration culminated with shock and death.

The second form of CPV-2 infection is cardiac syndrome or myocarditis which can affect puppies under 3 months of age (Appel, *et al.,* 1979) leading to the sudden death in young pups usually about 4 weeks of age (Mochizuki *et al.,* 1996). Acute heart failure with respiratory distress occurs in pups between 4 & 8 weeks of age. Sub-acute heart failure occurs in older pups usually 8 weeks or above.

The diagnostic tests include HA (Hemagglutination) (Kumar *et al.,* 2003). Enzyme Linked Immunosorbent Assay (ELISA) (Mohan *et al.,* 1993). Hemagglutination inhibition (HI) test has been documented in dog with CPV enteritis (Ok *et al.,* 2000).

The initial fluid of choice is a balanced electrolyte solution (i.e. Ringer's lactate solution). Once perfusion has been restored, the intravenous fluid rate is typically decreased to 4-10 ml/kg/hr. Balanced electrolyte solution, isotonic to blood is the initial fluid of choice. Puppies with ongoing anorexia, vomiting and diarrhoea are prone to the development of hypokalemia which can result in profound muscle weakness/paralysis, gastrointestinal ileus, cardiac arrhythmias and polyuria. Potassium chloride is added to the fluid as needed to prevent the development or worsening of hypokalemia. Plasma transfusion has been recommended as an adjunctive treatment for CPV enteritis for its ability to provide albumin, immunoglobulin and serum protease inhibitors that may help to neutralize circulating virus and diminish the accompanying systemic inflammatory response (Macintire DK, 1997).

Prevention of CPV infection is assurance of strong individual dog immunity. Serum antibody titre is correlated with immunity. Sero negative dogs are susceptible. Dogs with high antibody titre do not develop active infection or contribute to spread of virus (Pollock *et al.,* 1993).

Although a lot of work has been conducted in parvo virus infection, pertaining to their epidemiology, diagnosis, treatment and control, but still there is high mortality in clinical cases. There is doesn't of documented information in Bihar regarding the parvo virus infection in dogs. Considering the paucity in scientific informations about CPV, present study is undertaken with following objectives:

1. To ascertain the prevalence of canine parvo virus infection in dogs in and around Patna.
2. To assess the efficacy of canine parvo virus vaccine and evaluate antibody titre in vaccinated dogs.
3. To evolve an effective therapeutic module for canine parvo virus infection in dogs.

2 REVIEW OF LITERATURE

2.0 DISEASE HISTORY

Canine parvo virus enteritis was first reported in USA (Eugster and Nain, 1977), but the identification of the causative virus was first documented as CPV-2 in Canada in June 1978.

Canine parvo virus-2, the causative agent of acute hemorrhagic enteritis and myocarditis in dogs is one of the most important pathogenic viruses of canines. It is highly contagious and often fatal disease with high morbidity (100%) and frequent mortality up to 10 % (Appel *et al.*, 1978).

Serological analysis has since revealed that the first positive sera were collected in 1976 (Koptopoulos et *al.,* 1986) and there after fatal cases of enteric disease occurred with increasing frequency (Thomson, 1980).

CPV was designated type-2 to distinguish it from a previously recognized parvovirus of dog known as Minute Virus of Canines (Binn *et al.,* 1980).

A study conducted on the pathogenicity and seroprevalence of CPV-I in dogs revealed pneumonia, myocarditis and enteritis in young pups and trans placental infection in pregnant dams with embryo resorption and foetal death. On the contrary CPV-2 produces two prominent clinical form of disease i.e. enteritis with vomition and diarrhea in dogs of all ages. Myocarditis and subsequent heart failure in pups of less than 3 months of age was reported. (Apple *et al.,* 1979)

Like many other viruses, CPV-2 genetic evolution has given rise to new antigenic types (Parrish *et al.,* 1991). Shortly after the emergence of CPV-2, CPV-2a and CPV-2b arose and two variant strains now have completely replaced CPV-2 in circulation around the world. (Truyen *et al.,* 1996)

2.1 DISEASE INCIDENCE

2.1.1 GLOBAL

Appel *et al.,* (1978) for the first time reported hemorrhagic gastroenteritis due to parvovirus in pandemic form among canine population USA.

Thomson & Gagnon (1978) recorded deaths in dog's aged 7 weeks to 11 years in gastroenteritis associated with parvovirus like agent in Ontario, Canada. There after Thomson (1980) reported fatal cases of enteric disease in Ontario with increasing frequency. Initially the prevalence of CPV-2 infection in Ontario was monitored through autopsy examination of affected animals (Carman & Povey, 1984) with an impression that canine parvovirus morbidity was low and case fatality was high a was suggested by (Eugster *et al.,* 1998 and Nelson *et al.,* 1979).

Black *et al.,* (1979) reported high morbidity and about 50% mortality in CPV infection among dogs between two weeks and one year of age in USA.

Valicek *et al.,* (1981) demonstrated parvovirus like particles from dogs during an outbreak of hemorrhagic enteritis in puppies and older dogs with a recorded morbidity and mortality rates of 100% and 20 to 50% respectively in Czechoslovakia.

Jones *et al.,* (1982) conducted a longitudinal serological survey of CPV infection from serum samples of 106 healthy dogs in New Zealand. Approximately 23% of sample showed high titres more than 1:320 indicatives of CPV infection.

Pollock, 1982a; Carmichael, 1994; Decaro *et al*., 2005b CPV-2 is a highly contagious virus. Its transmission from infected to susceptible dogs takes place mainly by the faecal-oral route, but dogs can also become infected from virus present on fomites such as shoes, clothing, the hands of humans, food bowls and other utensils.

Stann *et al.,* (1984) detected parvovirus in 25% (40/161) of pound dogs that develop diarrhea while being used in research in Washington, USA. They observed that all infected dogs might not necessarily exhibit clinical manifestation but they might shed virus in faeces during the acute phase of enteric fever & show significant rise in the serum antibody titres.

Mason *et al.,* (1987) examined 1100 unvaccinated Beagles in USA and reported a tendency for outbreaks of CPV enteritis to occur in the autumn and early spring season with an inverse correlation between age and ability to acquire a titre to CPV-2 in exposed but clinically normal dogs. They also observed that dogs less than six months of age were primarily affected with CPV enteritis.

Houston *et al.,* (1996) reported that Rottweiler's, American Pit Bull terriers, Doberman pinschers and German shepherd dogs were greater risk, while toy poodles and cocker spaniels were at lower risk of developing CPV enteritis compared with mixed breed dogs. They observed that highest incidence (210 of 283 CPV enteritis case) of CPV enteritis was in dogs between 7 weeks and 14 years olds. Sexually intact dogs above six months of age were at four times greater risk than spayed or neutered dogs and intact males were twice as likely to be admitted with CPV enteritis in July, August and September compared to other months and non-vaccinated dogs were 12.7 times more likely to be admitted with CPV enteritis in Canada.

Truyen *et al.,* (1996) unlikely the original CPV-2 the newer variants were not restricted to infecting only members of Canidae. Some sub- strains are able to infect cats in vivo. While others cannot manage this but can infect feline cells in tissue culture.

Perez *et al.,* (2007) the different antigenic of CPV-2 are prevalent in varying proportion in different countries. The CPV-2a was found to be the prevalent antigenic type in South America.

Hong *et al.,* and Kapil *et al.,* (2007) reported that there is no evidence that CPV-2c is a more serious threat to either shelter or owned dogs than the other CPV strains. It is not possible to distinguish CPV-2c from CPV-2b or 2a isolates based on clinical signs. CPV-2c causes similar clinical signs as the previously known strains including mucoid or haemorrhagic diarrhoea, leukopenia and lymphopenia.

Joao Vieira *et al.,* (2008) although a few reports suggest that CPV-2c may cause more severe clinical sign and mortality particularly in adult dogs than type 2a and 2b others describe less severe disease and lower mortality rates in CPV-2c infected dogs.

Zhang *et al.,* (2010) carried out a molecular epidemiological in study of canine parvovirus strains circulating in China and reported that CPV-2b being the most

prevalent strain of CPV in China followed by a very low incidence of CPV-2a. They showed no CPV-2c and CPV-2a circulating in China in their study.

Filipov *et al.,* (2011) studied the canine parvovirus epidemiology in Bulgaria and found that CPV-2a variant was prevalent in Bulgaria and for the first time occurrence of CPV-2c was traced out using a rapid test based on detection of CPV antigens and a real-time polymerase chain reaction (RT-PCR) for detection of viral DNA.

Perez *et al.,* (2012) studied recent spreading of a divergent canine parvovirus type 2a (CPV-2a) strain in a CPV-2c homogenous population and found that in 2010, an unexpected epidemiological change occurred in Uruguayan as a consequence of the appearance of a novel CPV-2a strain. This variant rapidly spread through Uruguayan dog population and was detected in 38% cases.CPV-2 sequences were identical in all field viruses analysed and in addition to the 426 as residue, the sequences showed amino acid sub situations (267,324,440) not observed in the Uruguayan CPV-2c. This data suggests that the CPV-2a variant recently emerged in Uruguay and underwent clonal expansion.

Mohyedini *et al.,* (2013) reported that the mortality rate of the canine parvovirus was 25-30% in previous studies. In dogs that have been not treated aggressively, mortality rate was found to reach to 91 per cent.

Chinchkar *et al.,* (2014) reported that the interference from maternal antibodies and low antibody titre were the factors responsible for vaccine failure. Antigenic variations between available vaccine strains and the prevalent virus types were also indicated as possible reasons for vaccine failure.

Lin *et al.,* (2014) studied genetic characterization of type 2a canine parvoviruses from Taiwan. Their study revealed the emergence of an Ile321mutation in VP2.They stated that their VP2 sequence data revealed that both CPV-2a and 2b types were currently prevalent CPV 2 field strains circulating in Taiwan and unique Ile324 VP2 mutation was found in Taiwanese CPV 2a isolates and recent Asian isolates. CPV 2c was not observed in their study.

Pedro *et al.,* (2014) studied molecular and serological surveillance of canine enteric viruses in stray dogs from Vila do Maio, Cape Verde. Out of 53 samples 23 were positive for canine parvovirus DNA. Antibodies against canine parvovirus were detected

in 71.6% blood samples. They concluded that virus transmission and spreading occurs easily in large dog populations leading to high mortality rates particularly in unvaccinated susceptible animals.

2.1.2 INDIA

Balu and Thangaraj (1981) recorded prevalence of Parvoviral haemorrhagic gastroenteritis among dogs in and around Madras city.

Ramadass and Khader (1982) reported an incidence of 51.7 percent (45/87) CPV infection among dogs with symptoms of haemorrhagic enteritis in Madras city. The infection was prevalent equally in pure-bred, cross-bred and non- descript dogs with a majority of cases (26/45) occurring in dogs below six months of age.

Rao *et al.,* (1983) reported an outbreak of acute haemorrhagic enteritis suggestive of parvovirus infection in 292 dogs, especially less than nine months of age during June to August 1980 in Madras.

Sherikar and Paranjape (1985) examined 100 dogs and found 72 CPV positive cases during an outbreak of Parvoviral enteritis among dogs in and around Bombay city in 1981. No specific seasonal variation, breed, or age correlation was observed during the survey.

Narasimhaswamy (1988) reported an incidence of 34.7 per cent (24/69) CPV infection among dogs with signs of gastroenteritis in Bangalore.

Rao (1988) reported an incidence of 39.5 per cent of (15/39) Parvoviral enteritis among dogs in Bangalore.

Mohan *et al.,* (1992) noticed 78 positive cases of CPV infection from a total of 85 faecal specimens obtained from suspected dogs in Ludhiana, Punjab.

Saseendranath *et al.,* (1992) reported an incidence of 28.0 per cent (44/158) CPV infection in puppies between six and twelve weeks of age with symptoms of gastroenteritis in Madras between June and July, 1991.

Gunaseelan *et al.,* (1993) recorded a disease occurrence of 45.2 per cent (19/42) infection in dogs showing signs of persistent vomition and haemorrhagic enteritis in Madras.

Rai *et al.,* (1993) diagnosed 60 cases with clinical signs of vomition and diarrhoea (often haemorrhagic) as canine Parvoviral gastroenteritis in Ludhiana, Punjab.

Meerarani *et al.,* (1996) in Madras analysed 270 faecal samples from dogs with signs of enteritis by employing the PCR assay for studying its sensitivity and early detection of canine parvovirus infection in faecal samples and found that 72.9 per cent (197) dogs were positive for CPV by PCR assay as against 61.1 per cent (165) positive by HA test.

Udupa and Sastry (1996) studied the prevalence of CPV infection in stray and pet dogs in Bangalore which were 91.0 (131/144) and 28.6 per cent (20/70) respectively. They had recorded a higher prevalence rate of 71.4 per cent (40/133) in dogs between seven and nine months of age.

Subhashini *et al.,* (1997) recorded 56.6 per cent (68/120) incidence rate of CPV infection among dogs with clinical signs of enteritis and vomiting in Madras.

Joshi *et al.,* (1998) isolated CPV in 19.7 per cent (30/152) dogs with clinical signs of vomiting and enteric disease in Pant Nagar.

Deepa *et al.,* (2000) studied the serological survey on canine parvovirus infection in Kerala and conducted diagnostic tests like agar gel immune diffusion test (AGID), counter immune electrophoresis (CIEP) and Dot- ELISA for detection of antibodies to canine parvovirus. They concluded that Dot -ELISA was found to be more sensitive (71.3%) than AGID (10.9%) and CIEP (11.2%) for the detection of antibodies to CPV.

Banja *et al.,* (2002) conducted a comparative study of different laboratory tests for diagnosis of parvo virus and corona virus infection in dogs in Orissa. In the study faecal samples of suspected pet dogs were examined by dot-ELISA and HA test for parvo virus. The sensitivity and specificity respectively were 92.4 and 96.1 per cent for HA test.

Phukan *et al.,* (2004) conducted a study on the occurrence of canine parvovirus infection in and around Guwahati employing sandwich ELISA and indirect ELISA for detection of the antigen from the faecal samples and antibodies from the serum samples respectively. He recorded 72.22% positive cases in indirect ELISA (for CPV antibody) and 66.66% on sandwich ELISA (for presence of antigen).

Biswas *et al.,* (2006) made a prevalence study of canine parvovirus infection in and around Kolkata by early detection of the causative agents by polymerase chain reaction. During their investigation no sex variation was noticed. Dogs, of the age group of 0-6 months were mostly susceptible with highest mortality rate followed by 6-12 months and 12 and above months of age and highest occurrence was noticed during summer followed by rainy season and winter.

Panda *et al.,* (2009) studied antigenic characterization of Canine parvovirus by polymerase chain reaction. They found that out of 56 cases studied, 3 samples were positive with primer pair CPV-2 and 30 were positive with primer pair CPV-2ab and out of these 27 were positive for CPV-2b. They concluded that CPV-2b was the major antigenic type prevalent in the region in addition to smaller proportion of CPV-2a and CPV-2b circulating in field condition.

Savi *et al.,* (2009) studied genotyping of field strains of canine parvovirus in Haryana using PCR and RFLP. The PCR-RFLP indicated that all the field virus strains detected in the study were either CPV-2a or CPV-2b type which differed from vaccine strain (CPV-2) currently in use. The study demonstrated the potential application of RFLP to differentiate the CPV-2 antigenic variants. The presence of CPV even in vaccinated dogs, possibly suggested the vaccination failure.

Tajpara *et al.,* (2009) studied the incidence of canine parvovirus in diarrhoeic dogs by polymerase chain reaction. The incidence rate was higher in dogs below 6 months of age (30.30%) than those above one year of age (15.60%), Sex-wise incidence was higher in male dogs (25.60) than female dogs (19.23%). Unvaccinated dogs showed significantly higher incidence (31.40%) than the vaccinated dogs (13.33%). The incidences of CPV infection were found to occur more in summer months (27.77%) than in winter months (17.24%).

Manoj Kumar *et al.,* (2010) studied molecular cloning and restriction endonuclease analysis of Canine Parvovirus DNA amplified by polymerase chain reaction and concluded that PCR along with RE analysis of amplicons can be employed successfully for detection and characterization of CPV which in turn would help in proper and effective management and control of the disease.

Chinchkar *et al.,* (2014) conducted a study to characterize the field isolates of Canine parvovirus (CPV). The *in vitro* cross neutralization assay was performed against the vaccinated dog sera which revealed that the sera of original antigenic type CPV 2 can neutralize the antigenic variants 2a and 2b effectively. Thus they concluded that the vaccines containing CPV type 2 viruses can be used to immunize the dogs against the prevalent CPV 2a and CPV 2b infection.

Thomas *et al.,* (2014) carried out polymerase chain reaction based epidemiological investigation of canine parvoviral disease in dogs in Bareilly region. Almost half (52.3%) of total collected samples were found to be positive by PCR.

Kaur *et al.,* (2015) carried out study to isolate Canine parvovirus (CPV)from suspected dogs on Madin Darby Canine Kidney (MDCK) cell line and its confirmation by polymerase chain reaction (PCR) and nested PCR (NPCR). Further, CVP2 gene of the CPV isolates was amplified and sequenced to determine prevailing antigenic type.

Kaur *et al.,* (2015a) studied prevalence of Canine parvovirus in dogs in Ludhiana, Punjab. The prevalence using PCR was 11% and by nested PCR 50%. Nested PCR was found to be more sensitive than conventional PCR.

2.2 FACTORS AFFECTING THE DISEASE PREVALENCE

2.2.1 AGE, SEX AND BREED

Houston *et al.,* (1996) studied risk factors associated with parvovirus enteritis in 283 cases (1982-1991) of dogs. They opinioned that dogs more than 6 months old were twice prone to develop CPV enteritis.

Deepa *et al.,* (2000) studied sero-prevalence of CPV in dogs in Kerala and reported it to be 71%. Prevalence was 85% in dogs aged 0-6 months, 46% in dogs aged 6-12 months and 69.2% in dogs aged more than 12 months while breed wise prevalence

was reported to be highest in German shepherd. The study also indicated that influence of sex on prevalence of CPV was not significant.

Banja *et al.,* (2002a) reported a higher prevalence (53.4%) of CPV in Bhubaneswar city) and in various parts of Uttarakhand, Uttar Pradesh and Manipur (33.33%) states was recorded (Sanjukta *et al.,* 2011). The reason for the higher prevalence of CPV infection could be either due to delay in vaccination or the dog owners might be unaware of the importance of vaccination.

Puscasu *et al.,* (2002) reported the incidence of CPV infection to be 7.83% and the 80%cases were due to CPV -2 and the susceptibility varied with the age (1 to 5 months) and breed. Besides these, the overall incidence recorded was 7.83%.

Sakulwira *et al.,* (2003) conducted PCR and RT-PCR in faecal samples of 70 dogs suffering from gastroenteritis and reported high prevalence of CPV infection in 3-6 month old dogs.

Tajpara *et al.,* (2003) examined 65 faecal samples from dogs showing signs of diarrhoea by sterile rectal swabs for the detection of CPV infection by PCR and reported prevalence of CPV infection in 23.07% samples. Local breeds were found to be marginally more susceptible than exotic breeds as 26.66%, 25%, 11.76% and 27.23% of Doberman, Alsatian, Pomeranian and local breed dogs respectively were found to carry CPV infections. Prevalence in dogs below six months of age and above 1 year of age was reported to be 31.25% and 26.31% respectively. However, none of the dogs between six months to one year of age was found positive for CPV. Sex wise, male dogs (25.60%) were more affected than females (19.23%). Season wise incidence study revealed higher rate of CPV infected dogs in summer months (March to June) than winter months (December to February). Unvaccinated dogs showed significantly higher incidence (31.40%) of CPV infection than vaccinated dogs (13.33%).

Castro *et al.,* (2007) screened 341 faecal samples collected from puppies up to 6-month age with gastroenteritis at Rio de Janerio city, Brazil and CPV infection was conferred by Hemagglutination/ hemagglutination inhibition test, enzyme immunoassay, virus isolation in cell culture or polymerase chain reaction. They found 157 samples were positive for CPV. No correlation among sex, breed or age with the occurrence of CPV infection was observed.

Mosallanejad *et al.,* (2008) showed the prevalence of canine parvovirus in faecal samples of 78 diarrhoeic dogs referred to Veterinary Hospital of Ahwaz, Khouzestan, Iran. The confirmation of (CPV) was done by Immuno chromatography assay. Their study revealed that the infection to be more prevalent in dogs less than 6 months (21.95%) and in breed of Terriers (26.31%) and German shepherd (21%) but there was no significant difference between different sex, age groups and breeds.

Sagar *et al.,* (2008 a) investigated 123 dogs of Chhattisgarh suffered from parvoviral enteritis as diagnosed by clinical signs and confirmed by PCR test. Age wise prevalence gradually decreased with advancement of age. German shepherd, Labrador and Spitz dogs were more susceptible. The cases were highest during monsoon followed by post monsoon and prevalence was highest in dogs getting vegetarian food rather than those receiving non-vegetarian food.

Archana *et al.,* (2010) examined a total of 128 faecal samples of dogs suspected of Canine parvovirus infection by Hemagglutination (HA) and Hemagglutination inhibition (HI) tests. The prevalence of canine Parvovirus infection was reported to be 45.30%. The age wise prevalence was maximum (70.2%) in dogs up to 6 months, while breed wise it was maximum (56.90%) in non-descript dogs and sex wise prevalence revealed no significant difference ($p \leq 0.05$) in male (45.9%) and female (43.3%).

Roy *et al.,* (2010) reported that the overall prevalence of CPV infection in Chhattisgarh state was 65.04%, which was almost equally distributed among male and female. Prevalence was found to be highest between age group of 0-3 months. Some breeds like German Shepherded, Labrador and Spitz were more susceptible. Prevalence was more in dogs with vegetarian food rather than non-vegetarian food.

Yang *et al.,* (2010) conducted a sero-epidemiological survey of CPV-2 in stray dogs in South Korea between 2006 and 2007. A total of 405 canine sera were screened for the presence of antibodies against CPV-2 using a hemagglutination inhibition (HI) assay. The prevalence was reported to be 26.2%. No significant difference in the sero-positive rate was found between male and female dogs.

Kumar *et al.,* (2011) examined 140 dogs for sero-prevalence of Canine parvovirus infection in and around Indore region. The overall seroprevalence of CPV was

50.71%. They found that age, sex and vaccination study did not make a significant difference in the sero-prevalence rate.

Wazir *et al.,* (2011) reported prevalence of CPV infection in 1148 dogs in and around Jammu district. Overall prevalence of CPV was found to be 6.93%. Prevalence was higher in dogs of 3-6 months of age followed by 0-3 months and above 6 months of age. Male dogs had higher prevalence as compared to female dogs. Non-descriptive breed showed higher prevalence followed by Labrador, German shepherd, Pomerrian, Doberman and Bakerwali.

Mukhopadhyay *et al.,* (2012) conducted survey on 140 faecal samples collected from suspected dogs, 77 faecal samples (55%) were detected positive by PCR assay. The disease was predominantly noticed among pups of 0-6 months of age (74%) followed by 6-12 months of age (28%) and above 12 months of age (5.2%). Majority of the dog suffered were not vaccinated (75.3%) although 24.7% of the dogs were vaccinated at least once. Male dogs (68.8%) seemed to have suffered more in comparison to females (31.2%).

Dongre *et al.,* (2013 a) screened 100 dogs for incidence of CPV by standard ready kits and found highest (53%) among all enteral infection dogs. The incidence of CPV was highest (96.23%) in young dogs of age group 0-6 months followed by 3.77% in dogs of 6-12 months of age and no dogs were found positive for CPV more than 12 months of age. Male dog showed higher incidence (60.38%) as compare to female (39.62%). The German shepherd breed of dogs had highest incidence (60.38%), followed by Labrador (20.75%), Pomerian (9.43%), non-descript dogs (5.66%) and lowest in Great Dane dogs (3.77%).

Behera *et al.,* (2015) conducted epidemiological study of Canine parvovirus infection in dogs in and around Bhubaneswar, Orissa between Decembers 2012to March 2013. Age-wise prevalence study revealed the infection being more in the age group of 3-6 months (41.37%), followed by equal incidences of 27.59%in 1-3 months and 6-12 months age group, and a low incidence in age groups above 12 months (3.45%). The incidence was predominantly higher in males (86.21%) than females (13.79%).

2.2.2 VACCINATED AND UN VACCINATED

Deepa and Saseendranath (2002) reported vaccination failures in dogs vaccinated with killed or live virus vaccines. The reason for the failure of immune response to vaccine could be due to inadequate repeated boosters of killed vaccine as reported by Hoskins (2006) or due to newly emerging CPV strains (Decaro *et al.*, 2008).

2.2.3 CLINICAL SIGNS

Ramprabhu *et al.,* (2002) observed the clinical symptoms of hemorrhagic gastroenteritis and found that there was initial rise of temperature which declined to subnormal later. Inappetance, polydipsia, froathy yellow vomitus, retching and restlessness were also seen later. The faeces was brownish, semisolid admixed with excess mucus and later become foetid and fluidy.

Tajpara (2003) clinically characterized the CPV infection by severe vomiting and diarrhoea leading to fatal dehydration as well as myocarditis particularly in young pups.

Biswas *et al.,* (2005) selected 103 dogs suffering from Parvoviral gastroenteritis and diagnosed by PCR. They observed that the anorexia and dullness were the most predominant clinical signs followed by the vomition which was either hemorrhagic (43.69%) or non-hemorrhagic (56.31%). The hemorrhagic diarrhoea was noticed in the maximum number of cases (91.26%) than that of non-hemorrhagic diarrhoea cases (8.70%). Dullness was observed in 65.05% cases whereas 72.82% dogs showed moderate to marked dehydration. Increased body temperature (104-106 °F) was seen in 22.33% of dogs while, 63.11% dogs had subnormal body temperature.

Kaur *et al*., (2005) reported that the clinical signs of CPV were variable depending on the stage of presentation of dogs. However, in general anorexia, vomition and hemorrhagic diarrhoea were main clinical signs.

Castro *et al.,* (2007) showed the classical clinical signs of vomiting, anorexia, lethargy and hemorrhagic fluid diarrhoea in 69% of the CPV positive and 60% of CPV negative puppies. For the remaining 121 puppies, the association of clinical signs was variable. The comparison of the clinical signs observed in CPV-positive and CPV-negative puppies showed no difference ($p > 0.05$).

Sagar *et al.,* (2008 a) investigated 123 dogs of Chhattisgarh and they found the clinical symptoms of vomition, blood stain diarrhoea, rise of temperature and dehydration.

Bodhke *et al.,* (2010) conducted a study on 18 dogs and observed the symptoms of anorexia, vomiting and foul smelling hemorrhagic diarrhoea.

Singh *et al.,* (2011) studied 50 unvaccinated dogs and they observed the clinical signs of anorexia, vomiting and hemorrhagic diarrhoea.

Bastan *et al.,* (2013) showed the clinical sign of anorexia, lethargy, bloody diarrhoea, vomiting, moderate or severe dehydration, tachycardia and hypothermia.

Dongre *et al.,* (2013 b) observed the clinical signs of diarrhoea and vomition, with or without blood, in appetence, anaemia, fever and dehydration.

Thomas *et al.,* (2014) reported that canine parvovirus accounts for up to 26 % mortality among all viral diseases of dogs.

2.3 SERUM BIOCHEMICAL PREVALENCE STUDIES OF CANINE PARVOVIRUS

Macintire *et al.,* (1997) reported there is a significant increase in alkaline phosphatase (352.92 IU/L) and alanine transaminase (254.8 IU/L) which may occur as a result of hepatic hypoxia secondary to severe hypovolemia or the absorption of toxic substances due to loss of the gut barrier. Elevated alkaline phosphatase activity can also be associated with young age.

Weiss *et al.,* (1999) study and this correlates with Vomiting, dehydration, haemorrhagic enteritis, anaemia, dehydration, leukopenia, hypoglycaemia, increased ALT and ALP were the common findings.

Kaneko *et al.,* (2000) recorded the normal reference values of albumin globulin ratio to range between 0.59-1 in dogs.

Otto *et al.,* (2000) studies an endotoxin or cytokine mediated procoagulant effect on endothelial cells, loss of the natural anticoagulant and anti-thrombin (AT) through the gastrointestinal tract along with albumin consumption of AT as a result on

endotoxin-mediated activation of coagulation and hyper fibrinogenemia are postulated as contributing to the hypercoagulable state with CPV infection.

Mazzaferro *et al.,* (2002) recommended plasma infusion to raise the plasma albumin to 2.0–2.5 g/dl and the preferential administration of non-protein synthetic colloids to maintain the plasma COP between 13 and 20 mmHg. Protein loss through the gastrointestinal tract may result in hypo albuminemia which has been associated with feeding in tolerance multiple organ dysfunctions and increased mortality.

Nappert *et al.,* (2002) recorded biochemical changes including hypoproteinemia, hypo albuminemia, hypocalcaemia, hypokalaemia and hyponatremia in CPV infected dogs were observed anorexia, vomiting and diarrhoea which lead to the losses of fluid and electrolytes.

Ramprabhu *et al.,* (2002) reported a decrease in mean haemoglobin value in dogs affected with haemorrhagic gastroenteritis. There is a significant decrease in PCV (26.4 %) which may be due to excess dehydration caused by the vomiting and diarrhoeic fluids.

Dharmadheeran *et al.,* (2003) conducted clinical, hematological and biochemical studies in pups, experimentally infected with canine parvovirus infection. Pups showed a significant decline in PCV with slight decline in Hb. level. Significant decrease in TLC and variation in differential count were also observed. The values of AST and ALT showed significant increase whereas the levels of total protein, Na and K did not vary significantly.

Sharma *et al.,* (2003) examined thirty-seven dogs suffering from gastroenteritis. Hematological examination revealed significant increase in hematocrit value with neutrophilia and lymphopenia. The affected dogs had significant decrease in glucose, total protein, potassium, chloride and calcium concentrations with elevated BUN.

Biswas *et al.,* (2005) reported decreased haemoglobin and total erythrocyte count in CPV affected dogs. They also reported significantly lower level of protein, albumin and globulin in CPV infected dogs.

Kaur *et al.,* (2005) conducted study on 44 dogs with the history of hemorrhagic enteritis. The Canine parvovirus was diagnosed and confirmed by ELISA test. The mean Hb, TEC and PCV were reduced whereas TLC value were within the normal range.

Masare *et al.,* (2006) observed significant fall in Hb, PCV, TEC and TLC and also reported significant hyponatraemia, hyperalbuminemia, hyperglobinemia and increased blood urea nitrogen in CPV infection.

Baruah *et al.,* (2007) studied clinic-biochemical profile in canine parvovirus infection. A significant decrease of total serum protein was recorded and significant increase of SGOT was noticed. There was also increased activity of CPK and LDH, the level of serum Na and K was decreased in all the CPV infected cases.

Bhutia and Rao (2008) conducted haemato-biochemical study on 36 dogs critically ill with Parvovirus hemorrhagic enteritis. On haematological examination they showed decrease in glucose level.

Mosallanejad *et al.,* (2008) conducted Complete blood count on 78 diarrhoeic dogs referred to Veterinary Hospital of Ahwaz, Khouzestan, Iran. They showed that most infected dogs had leucopaenia, lymphopaenia and neutropaenia.

Sagar *et al.,* (2008 b) screening of 27 faecal samples of dogs showing symptoms of diarrhoea and vomiting and diagnosis of CPV was confirmed by polymerase chain reaction (PCR). Blood picture of affected dogs showed neutrophilia and lymphopenia. There were no significant differences in eosinophil, monocyte and basophil percentage. The affected dogs showed lower mean values of haemoglobin, packed cell volume, total erythrocyte count and total leukocyte count. There was decrease in mean blood glucose, Total serum protein, albumin and globulin whereas mean values of A/G ratio also increased. CBC and serum chemistry revealed a leukopenia (specifically neutropenia), an increased alkaline phosphatase, hyponatremia, hypokalemia and hypochloremia.

Kalli *et al.,* (2010) associated with CPV results in destruction of intestinal villi and disruption of gastrointestinal mucosal barrier which often develop a severe protein losing enteropathy.

Roy *et al.,* (2010) conducted haemato- biochemical study in dogs suffering from canine parvo virus. There was decrease in haemoglobin, PCV, total erythrocyte count, lymphocyte and increase in neutrophils. They also reported decrease in serum glucose, total serum protein, albumin, globulin, and an increase in A: G ratio.

Bastan *et al*., (2013) conducted the study on 59 dogs showing symptom of hemorrhagic gastroenteritis at Turkey and confirmation of CPV was done by PCR method. They observed that there was significantly lower, lymphocyte, monocyte and granulocyte counts but had significantly higher serum urea and creatinine concentrations.

Dongre *et al.,* (2013 c) screened 100 dogs for incidence of CPV by standard ready kits. Out of 53 positive dogs for CPV, 12 dogs were studied to know the haematological changes on 0, 3 and 7 of treatment regimen. The haemoglobin concentration, packed cell volume, total erythrocyte count decreased significantly and there was leucopaenia with neutrphilia and lymphopaenia was recorded.

2.4 THERAPEUTIC MEASURES

Randhawa et *al.,* (1997) treated a dog suffering from Isosporiasis, showing signs of diarrhoea, with combination of Sulphadimidine (100 mg / kg for three days) and Furadantin (20 mg / kg for five days) along with a supportive therapy. Dramatic improvement in the clinical condition occurred.

Roy *et al.,* (1999) diagnosed a case of parasitic diarrhoea (Diphyllobothriasis) in a six year old male Spitz dog. Past history revealed the treatment with Fenbendazole @ 50 mg/ kg b. wt. for the three consecutive days with no improvement. Treatment with Praziquantel@ 5 mg / kg body wt. along with supportive therapy proved successful.

Shinde *et al.,* (2000) conducted therapeutic study on 78 dogs of either sex suffering from gastroenteritis by dividing them into four groups. Group-I and II were treated with injection ciprofloxacin (5 mg/kg, l AO and enrofloxacin (5 mg/kg, *IM)* for 2 -3 days. Supportive therapy consisted of Ringer's lactate, inj. ascorbic acid (2 ml, l AO, Inj. Belamyl (up to 1 ml, I/M) and astringent mixture (2 gm., orally). In group-III, inj. gentamicin (4mg/kg, l AO was used along with supportive therapy as in group-I and II except D.N.S. in place of Ringer's lactate. In group-V which was of parasitic gastroenteritis, Panacur (30mg/kg, oral) along with gentamicin, inj. Belamyl and D.N.S.

(5%) were used. Complete recovery was observed in 2-3 days in group-I and II, in 3-4 days in group-III and 3-5 days in group-IV. They concluded that 2-3 days treatment with ciprofloxacin or enrofloxacin with additional supportive therapy are the most effective therapeutic agent in early recovery against clinical gastroenteritis in dogs.

Sriram and Jayathangaraj (2000) treated a one year old Great Dane dog for in appetence and diarrhoea together with occasional vomiting, with Ringers lactate solution parentally along with gentamicin. Subsequent to absence of response to therapy ciprofloxacin was later administered @ 8 mg/kg body wt. by slow I/V route, twice a day for five days; the animal recovered uneventfully together with the improvement of appetite.

Baruah *et al.,* (2005) studied therapeutic management of canine parvovirus infection. Ringers lactate solution @ 5-10 ml/Kg b. wt. for 3 days followed by 5% dextrose saline for a period of another 3 days were given. Cefotaxime was injected '@ l ml/kg b.wt. For 5 days and streptochrome given @ 0.1 to 0.2 mg/kg b. wt. I/M for 2 days. Conciplex was injected @ 2ml I/M at every alternate day. Reglan was injected @ 1 ml/kg b.wt. I/M for 3 days. It was observed that out of 23 dogs with CPV infection, 18 dogs recovered completely within 3 days and remaining 5 dogs recovered completely within 5 days. The study indicated that the treatment comprising of ringer's lactate followed by dextrose saline, Cefotaxime, streptochrome and Conciplex injection was effective.

Kaur *et al.,* (2005) treated 44 dogs suffering from haemorrhagic gastroenteritis. The routine symptomatic treatment comprising of ringer's lactate, broad spectrum antibiotics, antiemetic and multivitamins was given to all the affected dogs along with restriction of oral diet. The study indicated that the symptomatic treatment could save 100 per cent cases of canine parvovirus, 91.6 per cent of mixed infection and 95.4 per cent cases of haemorrhagic gastroenteritis. Cases suffering from canine corona virus infection recovered within 3-5 days.

Saxena *et al.,* (2006) conducted therapeutic management in 12 dogs suffering from gastroenteritis, I ringers lacate @ 20 ml/kg b wt. l A while in group II along with ringer’s lactate, lignocaine (2%) was given as a constant infusion @ 50mg/kg/min over a period of 2 hours. In group I, five animals recovered after 96 hr. and one animal recovered after 72 hr. In group II, one animal showed recovery after 24 hr. whereas four

animals recovered fully in 48 hr. and one animal showed recovery after 72 hr. The study indicated that lignocaine has an additional beneficial effect of recovery of gastrointestinal cases attributed to relief from pain felt in enteritis cases.

Dutta *et al.,* (2007) conducted therapeutic management of bacterial diarrhoea in dogs. The dogs diagnosed as cases of bacterial diarrhoea were equally divided into 3 groups. In group 1, 2, and 3 enrofloxacin @ 5mg/kg b.wt. Orally, gentamicin @ 4mg/kg b.wt. I/M and streptomycin @ 20mg/kg b.wt. I/M were given along with supportive therapy consisting of ringer's lactate solution @ 50-100 ml/kg b.wt. IV, multivitamin preparation @ 2ml/animal along with saline drip and metoclopramide hydrochloride @ 1 mg/kg b.wt. I/M. all the dogs of group-I recovered within 2 days and the vomition disappeared on the first day of treatment. In group 2, 7 dogs recovered within 2 days and the rest 3 on the third day. In group 3, 5 dogs recovered on second day, 4 on the third day and remaining one on fourth day of treatment. From the treatment trial, enrofloxacin, gentamicin and streptomycin were suggested in order of preference along with supportive therapy for treatment of bacterial diarrhoea.

Roy *et al.* (2010) conducted therapeutic study on dogs suffering from canine parvoviral (CPV) infection. They reported that pups treated with Cefriaxone and Tazobactum along with supportive therapy gave better response as compared to pups treated with Cefriaxone along with supportive therapy.

2.5 IDENTIFICATION

2.5.1 HAEMAGGLUTINATION (HA)

Studdert *et al.,* (1983) demonstrated CPV in 56 of 188 (30%) samples collected from dogs suspected for canine parvovirus by HA test.

Ezeokoli *et al.,* (1985) diagnosed CPV in 7 of 11 dogs with diarrhoea and suggested that hemagglutinating activity of the virus persisted in faeces for up to 22 days after onset of diarrhoea.

Sherikar and Paranjape (1985) diagnosed CPV infection in faecal samples of 71 dogs out of 100 dogs by HA test and opined the HA test in combination with HI test to be rapid and reliable.

Mohan *et al.,* (1992) conducted a micro titre plate HA test on 85 faecal samples obtained from clinical cases of CPV infection. Hemagglutination titres ranging from 1:40 and 1:20480 with majority between 1:160 and 120480: and inhibition of HA activity was seen in all cases by the use of a specific canine parvovirus antiserum.

Rai *et al.,* (1994) used HA test on 26 faecal samples from clinical cases of CPV haemorrhagic gastroenteritis and found that 22 samples had titres ranging from 1:40 to 1:20480. Most of these with HA activity of 320 to 1:20480 were collected 3-6 days after onset of illness. He opined that the faecal HA and HI tests were found to be specific rapid and in expensive.

Banja *et al.,* (2002) had conducted a comparative study for detection of CPV from the faecal materials of dogs in and around Bhubaneswar, using Dot- ELISA and HA. They opined that compared to the Dot ELISA, the sensitivity and specificity of HA were 92.4% and 96.1% respectively. The study indicated that the HA is as sensitive as ELISA for diagnosis of CPV infection.

Kumar *et al.,* (2003a) standardized the HA test using erythrocytes of different species to analyse the effect of different incubation temperature, buffers 7 pH on HA reaction. Fifteen faecal samples from clinical cases were collected. Out of 15, 10 samples agglutinated porcine erythrocytes. Best result (HA titre - 2048) was obtained at 4˚C followed by incubation temperature of 23˚C-26˚C and least HA titre (256) was obtained at 37˚C. However highest HA titre was obtained using PBS, PBSS and PBS with 0.1% BSA as diluents in the pH range of 4-6. Also specificity was ascertained by hemagglutination inhibition (HI) test and a HI titre of 3120 was obtained.

Muzaffar *et al.,* (2006) isolated CPV from 300 faecal samples of parvo suspected pet dogs in and around Lahore and presence of the virus in the samples was confirmed using HA test in rhesus monkey erythrocytes. They also collected blood from various animals including chicken, rabbit, dog and cat in order to check the hemagglutination potential of the causative agent to the 1% washed erythrocytes of various animal species. However, it was observed for the first time that the virus is capable to agglutinate chicken erythrocytes. These erythrocytes showed results of HA within 25 minutes.

Kapil *et al.* (2007) performed HA test to quantify CPV, the cell culture propagated CPV isolates were diluted twofold in PBS (pH 7.2) in V-bottom plates. For detection of CPV, about 0.5% swine erythrocytes in PBS with 1% foetal calf serum were added. After incubation for 4 h at 8°C, the hemagglutination titres were calculated as the reciprocals of the highest dilution showing complete matting.

Chinchkar *et al.,* (2014) reported that all the 45 samples were subjected to HA test using porcine RBC's after every passage on A-72 cell line. Sample producing distinct CPE in cell culture also showed ≥4 HA units per 50µl of tissue culture harvest on first passage. The HA and HI tests are routinely used methods for identification of CPV. The HI test is considered to be the confirmatory test for identification of CPV virus and it has been used extensively by many investigators.

2.6 SEROPREVALENCE STUDY OF CANINE PARVOVIRUS

2.6.1 HAEMAGGLUTINATION INHIBITION (HI)

Caraman & Povey (1984) made a sero prevalence study of canine parvovirus-2 in a selected sample of the canine population in Ontario, using micro titre hemagglutination inhibition test. No antibody was detected in samples collected in 1976 or 1977. The first positive sera were obtained in January 1978. By the end of 1978 antibodies to canine parvovirus-2 were wide spread in Ontario dogs and in 1980, 683 of 2191 dogs (31.2%) had antibody. This was before wide spread vaccination was being practised and indicated canine parvovirus-2 infection occurred frequently. The range of antibody titre was evaluated to designate the severity of infection. Low (8<32), moderate (≤ 32<256) or high (≤ 256).

Ok *et al.,* (2000) conducted a comparative study on diagnostic importance of ELISA & hemagglutination inhibition (HI) test in canine parvoviral infection of dogs. Faeces and serum samples were collected from unvaccinated dogs (n=15) affected with typical symptoms of canine parvoviral infection, admitted to the Clinic of Internal Medicine, Faculty of Veterinary Medicine, University of Selcuk. The samples were subjected to ELISA and HI test. HI titre of 256 or above was accepted as positive. It was concluded that efficacy of HI was 100 percent correlated with that of ELISA. This also indicated the simultaneous presence of the antigen and antibodies in the same animal. Both the tests are equally valuable as diagnostic tools.

Pereira *et al.,* (2008) reported antigenic characterization of Brazilian isolates of canine parvovirus employing hemagglutination inhibition test with a panel of monoclonal antibodies. 29 faecal samples collected from symptomatic dogs from 1980-86 and from 1990-95 were subjected to HI test using monoclonal antibodies. The result showed a strong predominance of the antigenic type-2a, indicating that the CPV epizooty in Brazil followed the same pattern observed in European and Asian countries.

Yang *et al.,* (2010) used HI test as a screening tool for serological survey of CPV-2a in stray dogs in South- Korea. HI test was performed in 96-well micro plate as described by Carmichael *et al.* (1980) with slight modification to remove nonspecific inhibitors. The positive rate in stray dogs tested for CPV-2a was 93.8 per cent, basing on the HI titre ≥ 1:10 of serum samples which were considered positive.

2.6.2 ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)

Teramoto *et al.,* (1984) used monoclonal antibodies to the CPV hemagglutination protein to examine dog faecal samples by ELISA. Highest correlation was between the ELISA and HA tests, with 94.4 per cent (67/71) of Samples showing agreement. He also opined that ELISA could be used as a sensitive and specific diagnostic assay for CPV infection.

Mohan *et al.,* (1993) used a parvovirus ELISA test kit on faecal samples for diagnosis of CPV. Out of 42 samples 19, 14, 6, 1 and 2 were found strongly positive, positive, weakly positive, doubtful and negative respectively. The ELISA and HA were in good agreement. He opined that the method was rapid, simple, sensitive and suitable for routine diagnosis.

Drane *et al.,* (1994) developed a CPV ELISA detection kit to be used as a rapid test for the detection of CPV antigen in canine faecal samples. The CPV ELISA had a sensitivity of 87.0 per cent and a specificity of 100 percent compared with 87.0 and 63.0 per cent for the HA respectively. He recorded visible result in less than 15 minutes.

Naveh *et al.,* (1995) studied on Immuno blot based enzyme-linked Immunosorbent assay (ELISA) method to detect CPV antibodies in sera of pregnant bitches and their offspring to study the response of pups to vaccination. With an easily accessible procedure for CPV antibody determination should be able to gauge the

response of pups after vaccination rapid self-contained Immuno blot ELISA test kit for the evaluation of antibody to canine parvovirus in dogs.

Waner *et al.,* (1996) assessment of maternal antibody decay and response to canine parvovirus vaccination using a clinic-based enzyme linked Immuno sorbent assay.

Biswas *et al.,* (2006) reported the incidence of CPV infection of dogs in India and its diagnosis HA, ELISA and PCR followed by RE analysis would be a valuable addition and considered to be the highly sensitive, specific and rapid to provide accurate diagnosis.

Sharma *et al.,* (2016) reported the first detection of CPV-2b in dogs with haemorrhagic gastroenteritis in Himachal Pradesh and absence of other antigenic types of CPV. Further CPV-specific PCR assay can be used for rapid confirmation of circulating virus strains under field conditions.

2.6.3 CARDIAC TROPONIN-I

Teramoto *et al.,* (1984) conducted a study for comparison of ELISA, DNA hybridization, Hemagglutination & electron microscopy for detection of canine Parvovirus infection. Canine faecal samples were analysed by enzyme-linked immune sorbent assays (ELISA) by using monoclonal antibodies to the canine parvovirus hemagglutinating protein. These data were compared with results obtained with DNA hybridization assays, hemagglutination assays, and electron microscopy. The highest correlation was observed between the ELISA and the hemagglutination tests with 94.4% of samples showing agreement. Lower correlation was obtained between ELISA and DNA hybridization tests (73.3%). Correlation between ELISA and electron microscopy was 60.9%.

Mohan *et al.,* (1993) used a parvovirus ELISA test kit on faecal samples for diagnosis of CPV. Out of 42 samples 19, 14, 6, 1 and 2 were found strongly positive, positive, weakly positive, doubtful and negative respectively. The ELISA and HA were in good agreement. He opined that the method was rapid, simple, sensitive and suitable for routine diagnosis.

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Duque-Garcia *et al.,* (2017) performed prevalence and molecular epidemiology of Canine parvovirus 2 in diarrheic dogs in a total of 70.42% of the individuals was confirmed positive for CPV-2. Statistically differences were found in the presentation of CPV-2 between the evaluated regions. Phylogenetic analyses confirmed the presence of the antigenic variants CPV-2a/2b.

3 MATERIALS AND METHODS

Aim of study

The present research was designed to evaluate antibody titre in vaccinated dogs and effective therapeutic module for canine parvo virus.

Place of work

The proposed research was carried out at the Department of Veterinary Medicine, BVC, Patna. The research was performed on clinical case of canine parvo virus infection various parameters such as alkaline phosphatase, total serum protein, albumin, globulin and AG ratio, were included in the research. Serological and immunological parameters such as HA, HI, ELISA and Cardiac Troponin-I analysis were performed at the laboratory of TVCC, BVC, Patna, India.

3.1 SELECTION OF ANIMALS

All the procedure of the investigation was approved by the local institutional ethical committee. This study was conducted on 600 dogs presented at OPD of veterinary medicine, BVC, Patna from September 2018 to July 2019 of different breeds, ages, sex, vaccinated and un-vaccinated dogs. The 24 dogs were selected on the basis of clinical symptoms such as acute vomition and dysentery.

3.2 EXAMINATION OF THE ANIMALS

The history of the animals was collected with respect to the immunization status especially the date of vaccination against canine parvo virus, duration of present illness, presence of blood in the vomitus, colour of the faeces and appetite.

Clinical investigation included rectal temperature, heart rate and respiration rate. Visible mucus membrane viz. conjunctival mucous membrane was examined and dehydration status was ascertained by state of dryness of nostrils and skin tenting time. Behaviour and physiological condition of body were also observed during the clinical examination.

Assessment of degree of dehydration was done as per the clinical signs described by Chakrabarti (2009) which are presented in table.

Table 3.2.1 Assessment of degree of dehydration on the basis of clinical sign

Observation	Degree of dehydration			
	Mild (4-6%)	Moderate (6-8%)	Severe (8-10%)	Sock (10-12%)
Skin fold test (tenting of skin)	absent	2-4 sec.	6-10 sec.	20-45 sec.
Sunken eyes	Not sunken.	Scarcely visible.	Pronounced	More pronounced
PCV %	40-45 %	50 %	55 %	60 %
Fluid required (ml/kg.b.wt.)	15-25	30-35	50-80	80-120

3.3 COLLECTION OF SAMPLES

3.3.1 SAMPLE COLLECTION

5ml of blood collected from the saphenous vein of suspected dog with the help of 5ml sterile syringe. 1.0ml blood transferred into EDTA vial for Hemagglutination test (HA). 4ml blood was transferred to centrifuge tube and kept at room temperature without any disturbance for two hours to allow the serum to effuse**.** The serum was separated by centrifugation at 3000 rmp for 15 minutes. The serum was stored at -20^{0}C until use for Total Serum Protein (TSP), Alkaline Phosphatase (ALP), Albumin, Globulin, AG Ratio, Hemagglutination Inhibition Test (HI), Enzyme Linked Immunosorbent Assay (ELISA) and cardiac Troponin-I.

3.4 BIOCHEMICAL ESTIMATION

The following biochemical estimations were carried out viz. total serum protein (TSP), alkaline phosphatase (ALP), Albumin: globulin ratio (A: G). biochemical estimated by using various kits.

3.4.1 Total Serum Protein

Estimation of Total Serum Protein was carried out from serum by direct biuret method (Doumas and Watson, 1971) using a total protein kits (Coral clinical systems Goa, India). The values were expressed in g/dl.

3.4.2 Alkaline phosphatase

Alkaline phosphatase estimated from serum by Mod. Kind & King's Methods using alkaline phosphatase kits (Coral clinical systems Goa, India). The values were expressed in KA units.

3.4.3 Albumin

Albumin was estimated from serum by bromocresol green methodology using an albumin kit (Coral clinical systems Goa, India). The values were expressed in g/dl.

3.4.4 Globulin

Globulin was estimated from serum by method total protein kit and the values were expressed in g/dl.

3.4.5 AG ratio (A: G)

Albumin**:** Globulin ratio (A: G) was calculated as the ratio of the albumin (g/dl) and globulin (g/dl). (Coral Clinical Systems Goa, India).

3.5. HAEMAGGLUTINATION TEST (HA)

3.5.1. Preparation of 1% suspension of sheep RBC

5 ml blood was collected from Sheep for estimation of Hemagglutination Test. Centrifuged 5 minutes at 3000 rmp. Then supernant was discarded. Same amount of NS was dissolved in SRBC. Again centrifuge for 5 minutes at 3000 rmp for 3 times. Finally, 0.1 ml SRBC suspension with adding 0.9 ml NS was prepared and then preserved at -20^{0}C. 1% SRBC suspension was prepared in PBS to be used in the HA test.

Hemagglutination test was performed as per the method of Konishi *et al.*, (1975) in 96 well micro titer plates.

3.5.2. Procedure

Using micro titer plates place two fold serial dilutions 50µl of test serum sample was made starting from initial 1:20 to 1:1280 dilution in PBS solution into each well of rows 1 and 3. Added 50µl of infected serum to the first well, mixed and transferred 50µl ml to the next well in rows 1 to 3 then Added 50µl 1 % suspension of SRBC in row 1 to 3. Incubated for 30 minutes at 37^0 C and then examined the plate in wells SRBC button as end point titer.

3.6. Hemagglutination Inhibition test (HI)

Hemagglutination inhibition test was performed as per the method of Johnson (1967) in 96 wells micro titer plates.

3.6.1. Procedure

Two-fold dilution of 25µl of test serum was made starting from initial 1:2 dilutions in PBS in 96 wells micro titer plates 1 to 3 rows. Added 25µl of 4HA unit antigen in 1 to 3 rows. Incubated for 30 minutes at 37^0 C. Then added 50 µl 1 % suspensions of SRBC. Control wells were included with positive control antigen, positive control serum and RBC control in a separated row of micro titer plates. Incubated for 10 minutes at 37^0 C. Examined the plate in wells button as end point titer.

3.7 ENZYME LINKED IMMUNOSORBANT ASSAY (ELISA)

ELISA was carried out in the present study according to the method of Teramoto *et al.,* (1984). Here antigen reagent of canine parvovirus ELISA Kit (Bioassay Technology laboratory) 1008 Junjiang international bldg. 228 Ningguo Rd. Yangpu Dist. Shanghai. China was used.

3.7.1. ASSAY PRINCIPAL

The micro titre plate was pre-coated with a target antigen. Positive/negative controls or samples are added to the wells and incubate. Antibodies in the samples bind to the antigens. Unbound antibody is washed away during washing steps. Avidin-Horseradish peroxidise (HRP) conjugated was then added and incubated. Unbound avidin-HRP was washed away during a washing step. Substrate solution was then added for color development. The reaction was stopped by addition of acidic stop solution and color changes into yellow that can be measured at 450 nm.

3.7.3 Procedure

I. The assay was performed at room temperature
II. Set a blank well without any solution.
III. Added 50µl negative control to each of the negative controls wells and 50 µl positive controls to each of the positive control wells.
IV. Cover the plate with a sealer and incubate for 30 minutes.
V. Dilute sample with sample diluent and mixed well.
VI. Cover with a plate sealer and incubate for 30 minutes at 37^0 C.
VII. Wash five times with 350 µl wash buffer manually.
VIII. Invert the plate each time and decant the contents; hit 4-5 times on absorbent materials to complete remove liquid. For automated washing, aspirate all wells and wash 5 times with wash buffer overfilling wells with wash buffer. Blot the plate on absorbent materials.
IX. Added 50 µl HRP to each well and incubate for 30 minutes and cover the pate with a sealer.

X. Remove the sealer and wash again five times.

XI. Added 50 µl substrate solutions A to each well and then added 50 µl substrate solutions B to each well. Incubate plate covered with a new sealer for 10 minutes at 37^0 C in the dark.

XII. Added 50 µl stop solution to each well, the blue color will change into yellow immediately.

XIII. Determine the optical density of each well immediately using microplate reader set 450 nm within 15 minutes after adding the stop solution.

3.7.4. Calculation of results

$$OD_{blanck} \leq 1.00$$

$$OD_{positive} \geq 1.00$$

$$OD_{negative} \leq 1.00$$

3.8 CARDIAC TROPONIN- I TEST

Cardiac Troponin-I was performed as per methodology laid down by Mohan *et al.*, (1993). Here antigen reagent canine parvovirus ELISA Kit (Bioassay Technology laboratory) 1008 Junjiang international bldg. 228 Ningguo Rd. Yangpu Dist. Shanghai. China was used.

This kit is an Enzyme Linked Immunosorbent Assay. The plate was pre-coated with Cardiac Tn-I antibody. Tn-I present in the sample was added and binds to antibodies coasted on the wells. And the biotinylated Cardiac Tn-I antibody is added and bind to Tn-I in the sample. Then Streptavidin-HRP was added and bind to the biotinylated Cardiac Tn-I antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added for color development in proportion to the amount of Cardiac Tn-I. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

3.8.1. PROCEDURE

I. The assay was performed at room temperature.

II. Added 50µl standard to standard wells.

III. Added 40µl sample to sample wells and then added 10µl anti- Tn- I antibody to sample wells and then added 50µl Streptavidin-HRP to sample well and standard wells Mixed well.

IV. Cover with a plate sealer and incubate for 60 minutes at 37^0 C.

V. Wash five times with 350µl wash buffer manually.

VI. Invert the plate each time and decant the contents; hit 4-5 times on absorbent materials to complete remove liquid. Remove the sealer and wash again five times.

VII. Added 50µl substrate solutions A to each well and then added 50µl substrate solutions B to each well. Incubate plate covered with a new sealer for 10 minutes at 37^0 C in the dark.

VIII. Added 50µl stop solution to each well, the blue color will change into yellow immediately.

IX. Determined the optical density of each well immediately using Microplate reader set 450 nm within 10 minutes after adding the stop solution.

3.9 THERAPEUTICS MODULES

3.9.1 Canine parvo virus infection

All the canine parvo virus positive case, are randomly categorized into three group viz. group A (vaccinated dogs), group B (un-vaccinated dogs), group C (un-known vaccinated dogs). Each group comprised of 6 dogs. 6 apparently healthy dogs were grouped in D-group.

3.9.2 THERAPEUTIC AGENTS EMPLOYED TO TREATMENT OF CANINE PARVO VIRUS

Table 3.9.2 Therapeutic agents employed to treatment of canine parvo virus

Group	No. of Dogs	Degree of dehydration %	Treatment
A	6	4	Ringer lactate (RL) @ 30-40 ml/kg b.wt., BID I/V DNS @ 20-30 ml/kg b.wt., BID Antibiotic-(Cefriaxone + Tazobactum @ 15-25 mg/kg b.wt. BID Ranitidine @ 2-4 mg/kg Botropose @ 0.5-1ml SOS B-Complex @ 1ml I/V Antiemetic- Ondem @ 1 ml I/M
B	6	8-10	Ringer lactate (RL) @ 30-40 ml/kg b.wt., BID I/V DNS @ 20-30 ml/kg b.wt., BID Antibiotic-(Cefriaxone + Tazobactum @ 15-25 mg/kg b.wt. BID Ranitidine @ 2-4 mg/kg Botropose @ 0.5-1ml SOS B-Complex @ 1ml I/V Antiemetic- Ondem @ 1 ml I/M Immune plasma therapy @2.5ml/kg
C	6	10-12	Ringer lactate (RL) @ 30-40 ml/kg b.wt., BID I/V DNS @ 20-30 ml/kg b.wt., BID Antibiotic-(Cefriaxone + Tazobactum @ 15-25 mg/kg b.wt. BID Ranitidine @ 2-4 mg/kg Botropose @ 0.5-1ml SOS B-Complex @ 1ml I/V Antiemetic- Ondem @ 1 ml I/M Amino acid therapy@1.2 ml/kg/h
D(CONTROL)	6		No treatment

Intravenous administration of immune plasma in Group B was compared with group A and group C. There were not effective in ameliorating clinical signs, reducing vomiting and diarrhea in dogs, reducing viremia and hastening hematological parameters.

3.10 Statistical Analysis

Data obtained was analyzed by standard statistical methods one-way ANOVA as per Snedecor & Cochran (2004).

4 RESULTS & DISCUSSION

The present study was carried out during September 2018 to July 2019 on 600 dogs of different age, breed and sex brought to the OPD of veterinary medicine, BVC, Patna.

Out of 600 dogs 206 dogs were suffering from gastroenteritis. Three hundred ninety-four (394) cases were confirmed as canine parvo virus by ELISA parvo kit.

4.1 PREVELANCE OF CPV INFECTION IN AROUND PATNA

The overall prevalence of canine parvovirus infection in total cases presented to TVCC, BVC, Patna was found to be 65.66 %.

Similar finding was reported by Banja *et al.*, (2002a) and Sanjukta *et al.*, (2011) who recorded higher prevalence (53.4%) of CPV in Bhubaneswar city and in various parts of Uttarakhand, Uttar Pradesh and Manipur (33.33%).

The reason for the higher prevalence of CPV could be either due to delay in vaccination or the dog owners might be unaware of the importance of vaccination.

Table 4.1. Prevalence of CPV infection in around Patna

TOTAL NO. OF DOGS EXAMINED	NO.OF DOGS FOUND POSITIVE	PREVALENCE (%)
600	394	65.66

4.1.2 AGE WISE PREVELANCE OF CPV

The age wise prevalence of CPV infection in dogs between 0-4 months of age was found to be 45.00% (72 out of 160 examined dogs), followed by 4-7 months of age (30.23%, 52 out of 172 dogs), 7-12 months of age (23.72%, 42 out of 177 dogs) and 1 years of above age (4.39%, 4 out of 91 dogs). Thus the maximum prevalence was

reported in age groups of 0-4 months. Similar observation was made by Roy *et al.,* (2010), Mukhpadhyay *et al.,* (2012), and Dongre *et al.,* (2013a). Higher prevalence of CPV in 0-4 months age was probably because of close affinity of virus with rapidly dividing cells of the intestine, which decline with the increase of age (Banja *et al.,* 2002).

Table 4.1.2. Age wise prevalence of CPV infection

S. no.	Age group	No. of dogs examined	No. of dogs found positive	Prevalence (%)
1	0-4 months	160	72	45.00
2	4-7 months	172	52	30.23
3	7-12 months	177	42	23.72
4	Above 1 years	91	4	4.39

4.1.3 SEX WISE PREVALENCE OF CPV

600 dogs were examined, the sex wise prevalence of CPV was found to be 31.04 % (104 out of 335 dogs) in male dogs as compared with 24.90 % (66 out of 265 dogs) in female dogs**.**

Table 4.1.3. Sex wise prevalence of CPV

Sex	No. of dogs examined	No. of dogs found positive	Prevalence (%)
Male	335	104	31.04
Female	265	66	24.90

These observations are in accordance with the finding of Deepa *et al.,* (2000), Castro *et al.,* (2007), Mosallanejabad *et al.,* (2008), Archana *et al.,* (2010), Yang *et al.,* (2010), Kumar *et al.,* (2011), Mukhpadhyay *et al.,* (2012), and Dongre *et al.,* (2013a) who reported significantly higher prevalence in males as compared with females.

CPV in male dogs might be attributed to more chances of exposer to infection due to their behaviour male as pets.

4.1.4 BREED WISE PREVALENCE OF CANINE PARVO VIRUS INFECTION

During the present study the dogs of various breeds were examined for canine parvo virus infection including Labrador, German shepherd and non- descript dogs. It was found that 46.66 % of parvo virus infection was found in non-descript dogs followed by 24.21 % in German shepherd and 13.00 % in Labrador.

Table 4.1.4 Breed wise prevalence of canine parvo virus infection

S. No	Breed of dogs	No. of dogs examined	No. of dogs found positive	Prevalence (%)
1	Non-descript	210	98	46.66
2	Labrador	200	26	13.00
3	German shepherd	190	46	24.21

Higher prevalence in non-descript dogs was also reported by Tajpar *et al.,* (2003), Archana *et al.,* (2010), and Wazir *et al.,* (2011), while Deepa *et al.,* (2000), Sagar *et al.,* (2008a), Roy *et al.,* (2010), however Dongre *et al.,* (2013a) reported higher prevalence in German shepherd breed of dogs. Which is in contrast to my findings. Higher prevalence in non-descript dogs in the present study may be due to ignorance of owners for following proper vaccination schedule in pups as well as dams. While the professional breeders follow proper vaccination schedule against CPV.

4.2 PREVALENCE OF CANINE PARVO VIRUS INFECTION IN VACCINATED, UN- VACCINAED DOGS AND UNKNOWN VACCINATED DOGS

In the present study out of 60 serum sample collected from vaccinated dogs 10 were found positive for CPV antigen whereas out of 60 serum sample collected from un-vaccinated dogs 30 were positive for CPV antigen whereas 60 samples collected from un known vaccinated dogs 25 were positive from CPV corresponding to 16.60%, 50% and 41.66% of CPV prevalence.

Table 4.2 CPV infection in vaccinated, un- vaccinated dogs and Un-known vaccinated dogs

Parameter	No. of dogs tested	Positive	Percentage %
Vaccinated dogs	60	10	16.66
Un-vaccinated dogs	60	30	50.00
Un known vaccinated dogs	60	25	41.66

Among vaccinated dogs 10 dog were positive for CPV infection because they missed booster dose of vaccine. Similar justification was made by Deepa and Saseendranath (2002), Hoskins (2006) and Decaro *et al.,* (2008) who reported vaccine failure in dogs vaccinated with killed / live virus or missing of booster dose of vaccines.

4.3 OBSERVATION ON INFECTED AND HEALTHY CONTROL ANIMALS

Twenty-four apparently infected and healthy dogs irrespective of age, sex and breed were taken randomly to severe as healthy control group. All the dogs taken as infected and control group were inspected and examined. They were clinically healthy, active, alert and had normal appetite, defecation and urination. While clinical observation of CPV affected dogs showed vomation, anorexia, hemorrhagic diarrhoea, paleness of mucous membrane, dullness, depression and restlessness.

The mean values of various vital parameters such as healthy control group were rectal temperature; heart rate and respiration rate were 101.40 ± 0.15^0F, 83.00 ± 2.50 per min. and 34.75 ± 2.28 per min respectively. While in CPV affected dogs it were 102.85±0.25, 90.00±2.50/minutes and 40.50±2.50/minutes respectively. Thus in the CPV affected dogs the value of heart rate and respiration rate were significantly higher than that of healthy control group.

The variation in the body temperature maybe associated with increased temperature as a result of viremia in initial stage and sub normal temperature in the severe fluid and electrolyte losses.

CPV infection may occur as canine parvo virus rapidly multiply in the germinal epithelium of the intestinal crypts, causing epithelial destruction and villous collapse.

The dehydration occurs due to persistent vomition and diarrhoea which results in severe fluid and electrolyte losses.

Table 4.3 Clinical observation in Infected and normal dogs (n=24).

S.No	Parameters	Infected dogs Values (Mean ± S.E.)	Healthy dogs Values (Mean ± S.E.)
1.	Rectal temperature (^{0}F)	102.85±0.25	101.40±0.15
2.	Heart rate (per min.)	90.00±2.50	83.00±2.50
3.	Respiration rate (per min.)	40.50±2.50	34.75±2.28
4.	Conjunctival mucus membrane	pale	pink
5.	Behaviour	Non Active and lazy	Active and alert
6.	Body physiology	Appetite, Water intake, Defecation And Urination	Normal appetite, Water intake, Defecation and Urination.

These finding in CPV infected dogs corroborate with findings of Ramprabhu *et al.,* (2002), Tajpara *et al.,* (2003), Biswas *et al.,* (2005), Castro *et al.,* (2007), Sagar *et al.,* (2008a), Bodhke *et al.,* (2011), Singh *et al.,* (2011), Bastan *et al.,* (2013) and Dongre *et al.,* (2013b).

4.4 BIOCHEMICAL ALTERATIONS

4.4.1 ALKALINE PHOSPHATASE (ALP)

Variation in Alkaline phosphatase values of different group are depicted in table. The ALP value of infected groups were significantly ($p \leq 0.05$) higher in comparison to healthy control group D. The mean values of infected group A, B and C were 197.27±16.80, 276.65±20.00 and 256.07±14.73 respectively which were high in comparison to healthy group 170.60±36.

Table 4.4.1. Mean±SE Alkaline Phosphatase (IU/L)

Group	Pre treatment	Post treatment
A	197.27^{bc} ±16.80	173.08^{b} ±7.76
B	276.65^{c} ±20.00	191.46^{c} ±5.16
C	256.07^{c} ±14.73	197.97^{bc} ±5.37
D (control)	170.60^{a} ±0.36	170.60^{a} ±0.36

*different superscript differs significantly ($p<0.05$)

Significantly increase in ALP 276.65±20.00 IU/L which may occur as a result of hepatic hypoxia secondary to severe hypovolemia or the absorption of toxic substances due to loss of the gut barrier. Increase value of ALP in dogs having hepatic dysfunction.

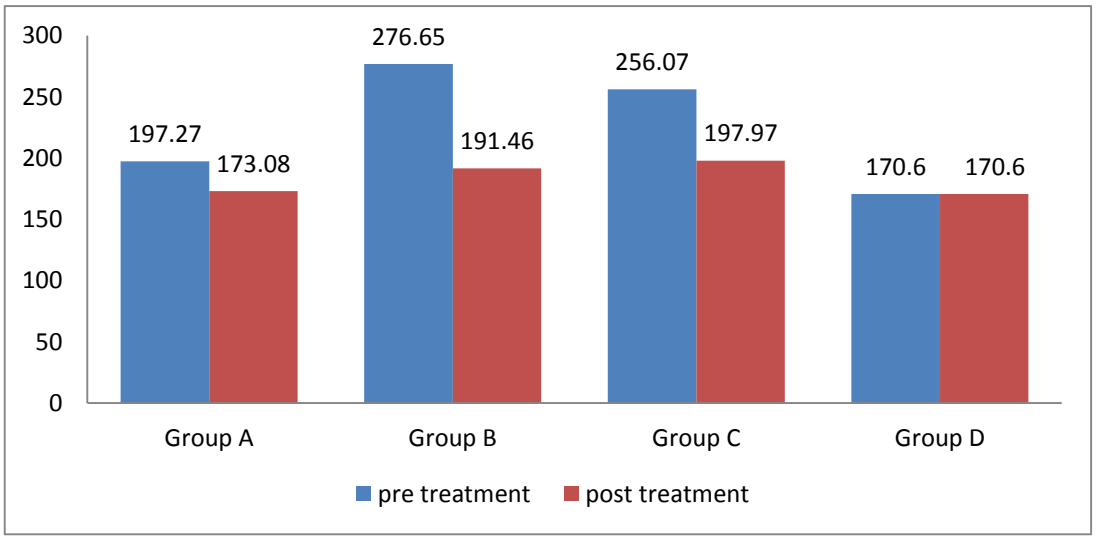

Fig.4.4.1 Alkaline Phosphatase (IU/L)

After treatment marked reduction in ALP were found in groups A, B and C and the mean value of ALP 173.08±7.76, 191.46±5.16 and 197.97±5.37 for these groups vary significant ($p \leq 0.05$) comparing with healthy groups showing positive response in different treatment groups.

Macintire *et al.*, (1997), Weiss *et al.*, (1999) and Baruah *et al.*, (2007) findings shows increase in ALP (352.92 IU/L) which may occur as a result of hepatic hypoxia secondary to severe hypovolemia or the absorption of toxic substances due to loss of the gut barrier.

Table 4.4.2. Mean±SE Total Serum Protein (g/dl)

Group	Pre treatment	Post treatment
A	$5.28^{a} \pm 0.10$	$7.13^{b} \pm 0.02$
B	$5.58^{ab} \pm 0.09$	$7.25^{b} \pm 0.07$
C	$5.75^{b} \pm 0.13$	$7.25^{b} \pm 0.05$
D (control)	$6.50^{b} \pm 0.10$	$6.50^{b} \pm 0.10$

*different superscript differs significantly ($p<0.05$)

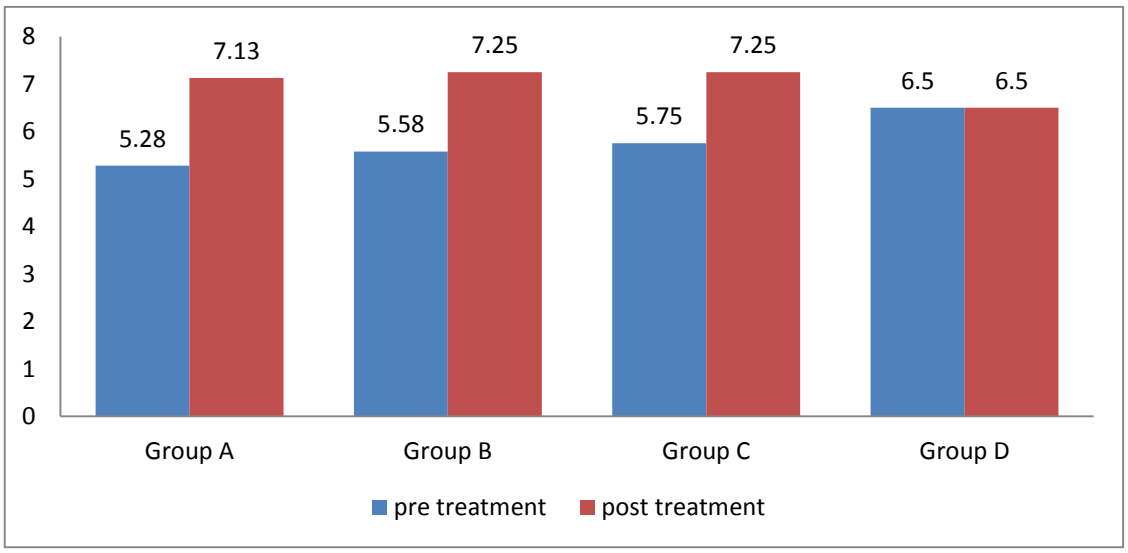

Fig. 4.4.2 Total Serum Protein (g/dl)

The total serum protein (g/dl) mean value of pre-treatment of infected groups A, B, and C was 5.28±0.10, 5.58±0.09 and 5.75±0.13 g/dl respectively, which was significantly lower ($p \leq 0.05$) than the control group.

After treatment the mean value TSP (g/dl) group A, B, and C were 7.13±0.02, 7.25±0.07, 7.25±0.05 respectively. Although was higher than the control group but it was non-significant ($p \leq 0.05$).

There was hypoproteinemia in this study which is findings of supported by Ramprabhu *et al.*, (2002) and Dharmadheeran *et al.*, (2003). Hypoproteinemia might be due to the leakage of serum protein through damaged capillaries of the villi of intestine and also due to less absorption through the damaged villi (Biswas *et al*., 2005).

Table 4.4.3 Mean±SE Albumin (g/dl)

Group	Pre treatment	Post treatment
A	2.38 ^{a} ±0.13	3.65 ^{ab} ±0.01
B	2.39 ^{a} ±0.07	3.71 ^{b} ±0.05
C	2.40 ^{a} ±0.06	3.72 ^{b} ±0.05
D(control)	3.51 ^{b} ±0.82	3.51 ^{ab} ±0.82

*different superscript differs significantly ($p<0.05$)

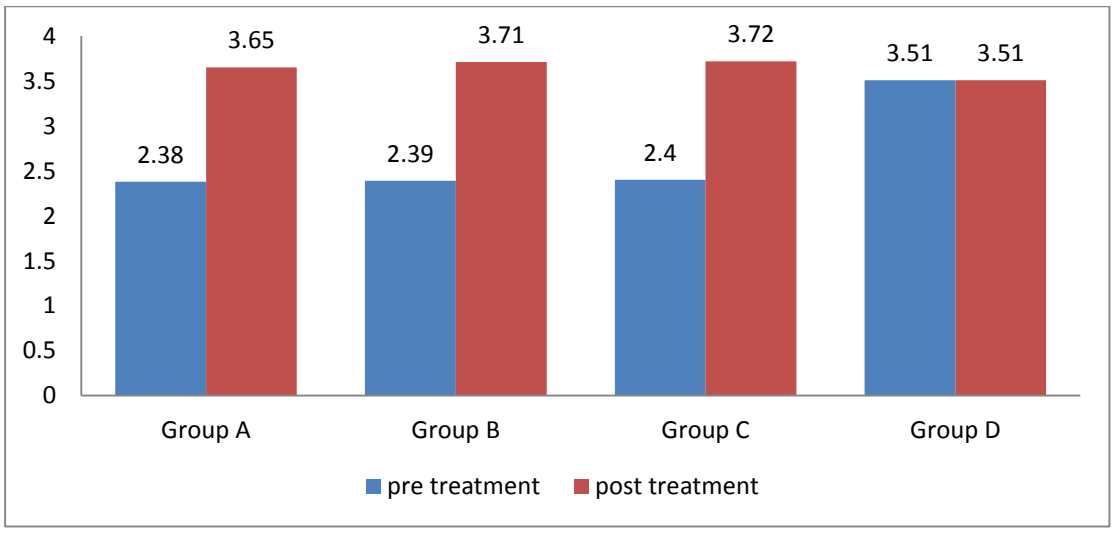

Fig.4.4.3 Albumin (g/dl)

The mean serum albumin levels of dogs infected with parvovirus group A, B, and C was 2.38±0.13, 2.39 ±0.07 and 2.40 ±0.06 respectively, which was significantly($p \leq 0.05$) lower than healthy control groups.

After treatment the mean value of Albumin (g/dl) in group A, B, and C were 3.65 ±0.01, 3.71 ±0.05 and 3.72 ±0.05 g/dl respectively non-significant but higher value in all treatment groups were observed in comparison to healthy control group.

Similar justification was given by Mazzaferro *et al.*, (2002), Nappert *et al.*, (2002), Baruah *et al.*, (2007) and Sagar *et al.*, (2008b) who reported that hypoalbumeninia is more specific in canine parvovirus due to protein loss through the gastrointestinal tract. Hypo albuminemia in CPV infected dogs were attributed to anorexia, vomiting and diarrhoea which lead to the losses of fluid and electrolytes.

Table 4.4.4. Mean±SE Globulin (g/dl)

Group	Pre treatment	Post treatment
A	2.90 ^{a} ±0.06	3.48 ^{b} ±0.03
B	3.18 ^{ab} ±0.13	3.53 ^{b} ±0.02
C	3.35 ^{b} ±0.12	3.46 ^{b} ±0.13
D (control)	3.33 ^{b} ±0.07	3.33 ^{b} ±0.07

*different superscript differs significantly ($p<0.05$)

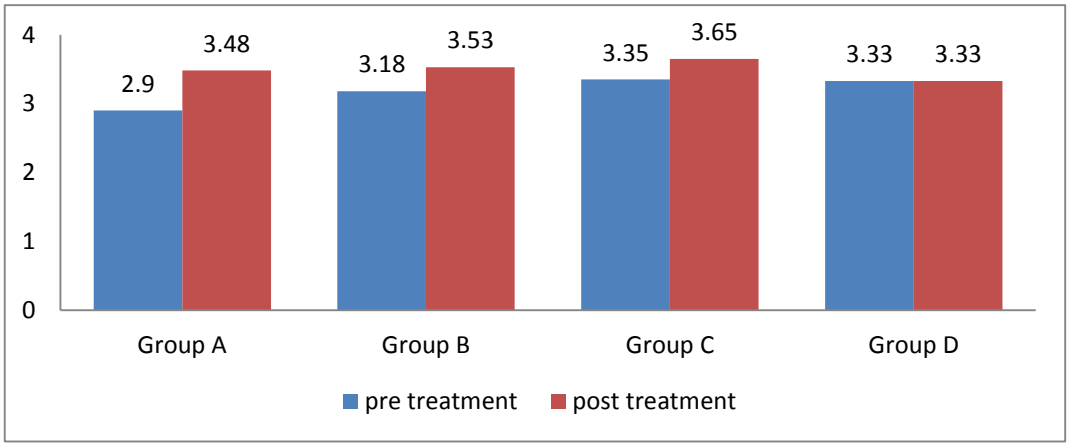

Fig. 4.4.4 Globulin (g/dl)

The mean serum globulin levels of dogs infected with parvovirus group A, B, and C was 2.90±0.06, 3.18±0.13and 3.35±0.12 respectively, which was significantly ($p \leq 0.05$) lower than healthy control groups.

After treatment the mean value of globulin (g/dl) in group A, B, and C were 3.48±0.03, 3.53 ±0.02 and 3.46±0.13 respectively. However, non-significant ($p \geq 0.05$) increases was noticed as compared to control group.

The present findings are in agreement with Biswas *et al.*, (2005), Baruah *et al.*, (2007) and Sagar *et al.*, (2008b), who reported significantly lower level of protein, albumin and globulin in CPV infected dogs. Hypoglobulinemia is more common in myocardial and occasionally the gastroenteritis form of disease.

Table 4.4.5. Mean±SE AG ratio (g/dl)

Group	Pre treatment	Post treatment
A	0.82^{a} ±0.05	1.04^{b} ±0.01
B	0.75^{a} ±0.05	1.04^{b} ±0.01
C	0.71^{a} ±0.03	1.16^{b} ±0.06
D (control)	1.05^{b} ±0.03	1.05^{b} ±0.03

*different superscript differs significantly ($p<0.05$)

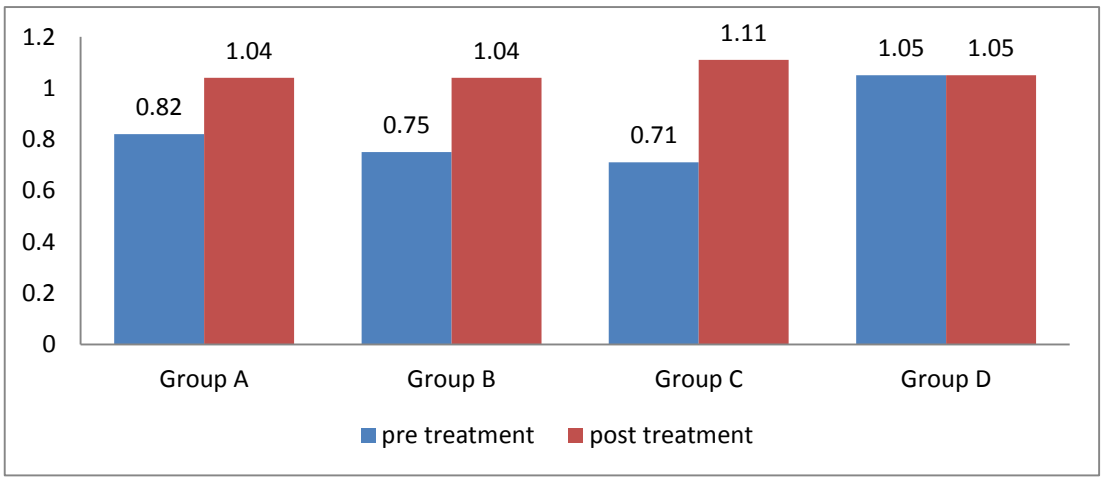

Fig. 4.4.5 AG ratio (g/dl)

The mean serum A/G ratio levels of dogs infected with parvovirus group A, B, and C was 0.82±0.05, 0.75±0.05 and 0.71±0.03 respectively, which was significantly ($p \leq 0.05$) lower than control groups.

After treatment the mean value of A/G ratio (g/dl) in group A, B, and C were 1.04±0.01, 1.04±0.01 and 1.16±0.06 respectively. However, non-significant ($p \geq 0.05$) increases was noticed as compared to control group.

The present findings are in agreement with Ramprabhu *et al.*, (2002), Sharma *et al*., (2003), Biswas *et al.,* (2005), Baruah *et al.,* (2007) and Sagar *et al.*, (2008b) who also reported the decrease in albumin and increase in globulin in hepatobiliary disorders lead to decrease in Albumin: Globulin (A: G) ratio.

4.5 EVALUATION OF ANTIBODY TITRE IN VACCINATED DOGS

4.5.1 Hemagglutination Assay (HA)

Hemagglutination (HA) test was performed using 1% suspension of SRBC of the infected CPV serum samples. In 600 serum samples 225 sample were positive and 375 sample negative with varying HA titre ranging from 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, and 1:1280 respectively. In the present investigation 37.50% cases were found positive for parvo virus infection through HA test.

Table 4.5.1. Screening of serum samples by Hemagglutination test

No. Of serum sample screened	No. Of sample found positive	HA titre						
		1:20	1:40	1:80	1:160	1:320	1:640	1:1280
600	225 37.50 %	50 8.33%	25 4.17%	50 8.33%	25 4.17%	50 8.33%	25 4.17%	0

HA titre of ≤ 1:10 in the present study was taken negative. Similar observation were of Mohan *et al.,* (1992), Kumar *et al.,* (2003a) who have use 1% SRBC

suspension and PBS in the pH range 7.2-7.4 to quantify the serum samples. Kumar *et al.,* (2003a) have also considered HA titre of <1:8 to be negative.

4.5.2 HEMAGGLUTINATION INHIBITION TEST (HI)

600 serum samples were collected from the dogs showing the symptoms of hemorrhagic gastroenteritis. The Hemagglutination inhibition test was performed with a titre 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048. 4HA unit was prepared. HI test performed by using 1 % suspension of SRBC. 325 samples out of 600 samples were found positive. HI titres of positive sample ranged from 1:64 to 1:2048. Out of positive samples, 75(21.43%), 25(7.14%), 100(28.56%), 25(7.14%), 75(21.43%), and 25(7.14%), sample showed HI titre 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048 respectively.

Table 4.5.2. Screening of serum sample by Hemagglutination inhibition test

No. Of serum sample screened	No. Of sample found positive	HI titre					
		1 : 64	1:128	1:256	1:512	1:1024	1:2048
600	325 54.16 %	75 21.43%	25 7.14%	100 28.56%	25 7.14%	75 21.43%	25 7.14%

Carman & Povey, (1984) expressed a titre range of 1:8<1:32, ≥1:32, <1:256 and ≥ 1:256 as low, moderate and high respectively CPV infection. Ok *et al.,* (2000) had accepted the HI titre of 1:256 or above as positive. While Yang *et al.,* (2010) conducted of CPV-2a in stray dog in South- Korea taking HI titre of ≥ 1:10 as positive samples. Present finding HI titre rang of 1:64 to 1:2048 of positive.

4.5.3 ENZYME LINKED IMMUNO SORBENT ASSAY (ELISA)

A total of 600 samples collected from vaccinated and non-vaccinated dogs were screened for canine parvo virus with ELISA test. ELISA a cut off value was determined by comparing mean OD value of positive and negative serum. The reading

was taken BIO RAD iMark^{TM} Microplate Reader at 450 nm. ELISA cut off OD value of ≤ 0.135 was taken. Out of 600 serums sample 394 sample (65.66%) were found positive**.**

Similar observation was reported by Teramoto *et al.,* (1984), Mohan *et al.,* (1993), Naveh *et al.,* (1995), Waner *et al.,* (1996), Biswas *et al.,* (2006), and Sharma *et al.,* (2016) who found titrating monoclonal antibodies to cpv-2**.**

4.5.4 CARDIAC TROPONIN-I

A total of 24 samples collected from vaccinated and non-vaccinated dogs were screened for canine parvo virus with Cardiac Troponin-I. Cardiac Troponin-I a cut off value was determined by comparing mean OD value of positive and negative serum. Cardiac Troponin-I cut off OD value of ≤ 0.130 was taken. Out of 600 serums sample 550 sample (91.66%) were found positive.

The plate was pre-coated with cardiac Tn-I antibody. Tn-I present in the sample was added and binds to antibodies coasted on the wells. And the biotinylated cardiac Tn-I antibody was added and bind to Tn-I in the samples. Then Streptavidin-HRP is added and bind to the biotinylated cardiac Tn-I antibody. After incubation unbound Streptavidin-HRP was washed away during a washing step. Substrate solution was then added and color development in proportion to the amount of cardiac Tn-I. The reaction was terminated by addition of acidic stop solution and absorbance was measured at 450 nm.

Similar observation were made by Teramoto *et al.,* (1984), Mohan *et al.,* (1993), Naveh *et al.,* (1995), Waner *et al.,* (1996), Biswas *et al.,* (2006), and Sharma *et al.,* (2016) who found titrating monoclonal antibodies to CPV-2 and comparing the result with cDNA hybridization results.

4.6 COMPARISON OF DIFFERENT SEROLOGICAL TESTS

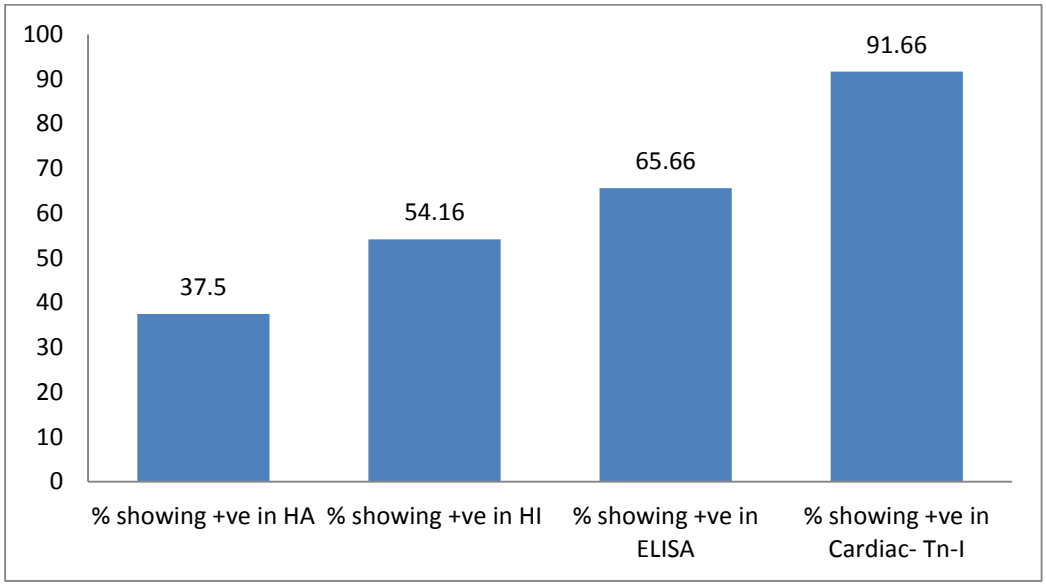

Fig. no. 4.6 comparison of different serological tests

4.7 OD VALUE OF ELISA AND Tn-I TITRES

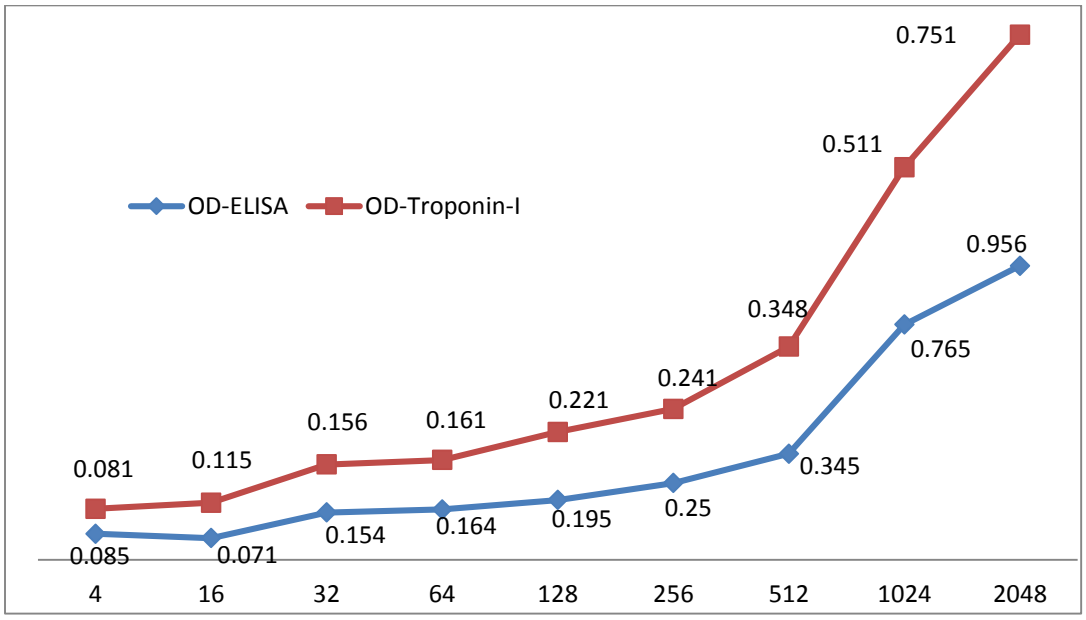

Fig. no. 4.7 OD value of ELISA and Tn-I titres

4.8 ASSESSMENT OF EFFECTIVE THEREAPEUTIC MODULE

In present therapeutic studies in Group A administration of RL@30-40 ml/kg IV, DNS@20-30 ml/kg IV, Antibiotic (cefriaxone + Tazobactum) @15-25 mg/kg BID IV, Ranitidine@2-4 mg/kg OD IV, Botropose@0.5-1ml SOS IV, CB12@1ml IV and Antiemetic@ 1mg/kg IV. 3 out of 6 dogs (50%) showed complete recovery from dehydration and other clinical signs on day 4 post treatments, while remaining 3 dogs (50%) recovered on day 5 post treatments. In CPV infection due to severe diarrhoea and vomiting, electrolytes are lost in high quantity in the faeces and vomitus and total body sodium, chloride and potassium are depleted (Ramprabhu, 2002). In the ionized form, calcium is essential for the functional mechanism of the clotting of blood, normal cardiac function, and regulation of neuromuscular irritability (Sutin and Paul, 2012).

In group B administration of Immune plasma@2.5 ml/kg and supportive therapy 4 dogs (66.66%) recovered completely from dehydration and other clinical signs on day 3 post treatment, while remaining dogs (33.33%) recovered on day 4 post treatment. Immune plasma therapy proves beneficial, especially when the total protein levels drop below 3.5 g/dl. A plasma transfusion serves to both help in restoration of plasma oncotic pressure and to provide a source of immunoglobulins (Mazzaferro *et al.,* 2002).

In group C administration of Amino acid@1.2 ml/kg/h and supportive therapy 5 dogs (83.33%) recovered completely from dehydration and other clinical sign on day 2 post treatment, while remaining dogs (16.66%) recovered on day 3 post treatment. administration of amino acids increases the plasma insulin concentration (Yamaoka *et al.,* 2006).

Thus in the present study among various treatment groups, group C (Amino acid +Supportive therapy) was found to be superior as all six (6) dogs recovered day 3 post treatment. However, the groups B and groups A showed comparable improvement on day 4 post treatment and day 5 post treatment respectively, hence amongst these three groups of group C was considered as superior than group B and group A on the basis of overall percentage recovery and improvement of number of dogs.

PLATES

Plate 1. CPV infected 5 months pup

Plate 2. CPV infected pup

Plate 3. Collection blood from CPV infected dog

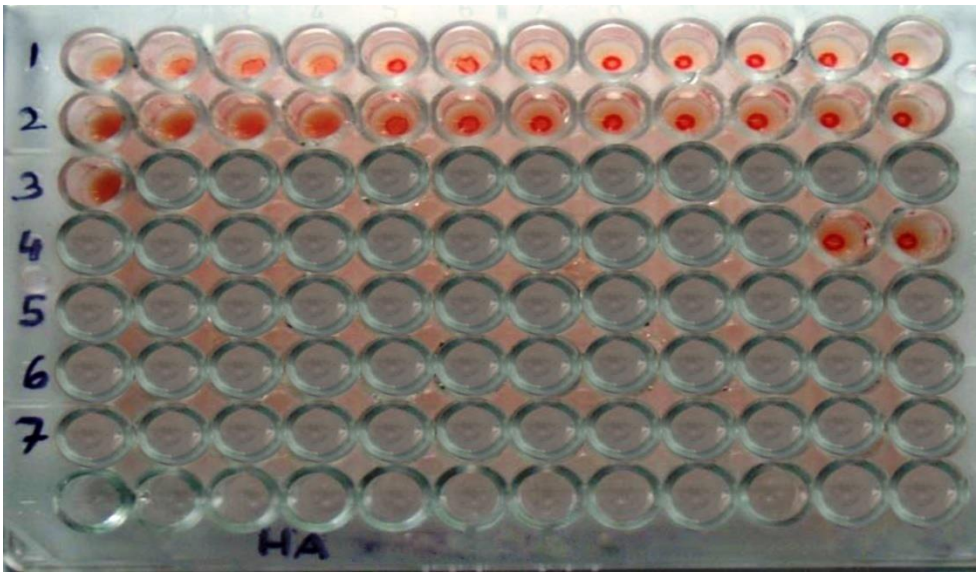

Plate 4. Hemagglutination Test of serum sample using 1% SRBC suspension

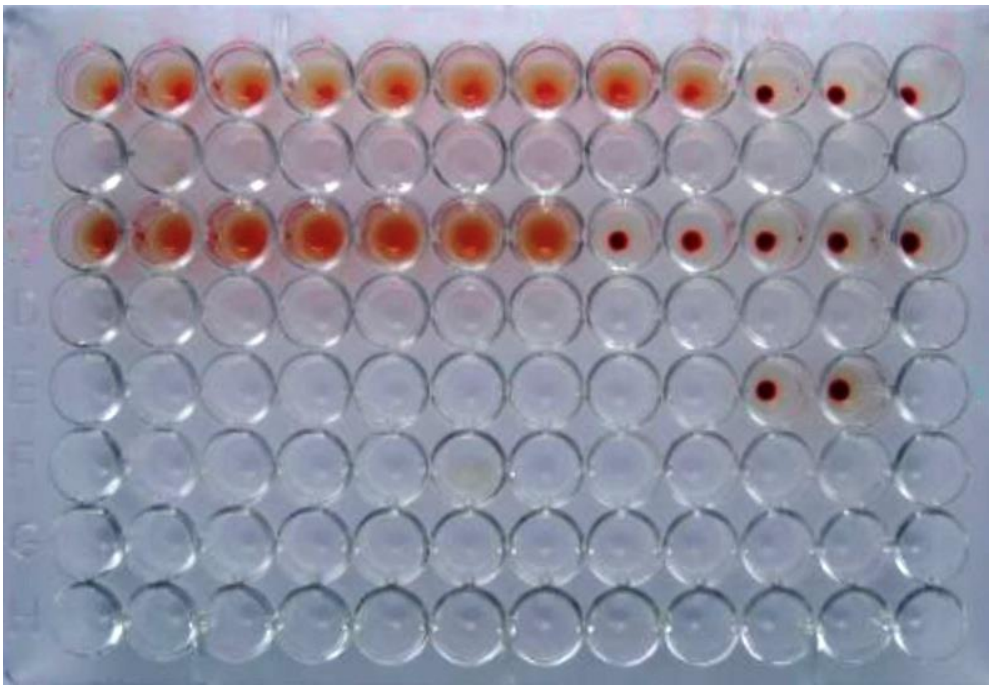

Plate 5. Hemagglutination inhibition test using serum sample with SRBC suspension.

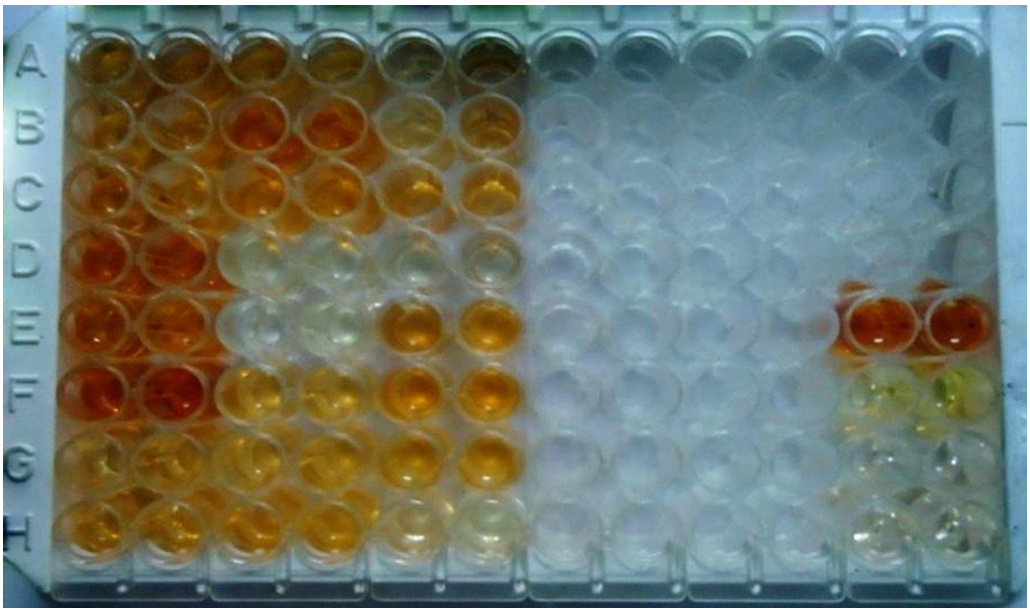

Plate 6. ELISA Test using serum sample yellow color are positive.

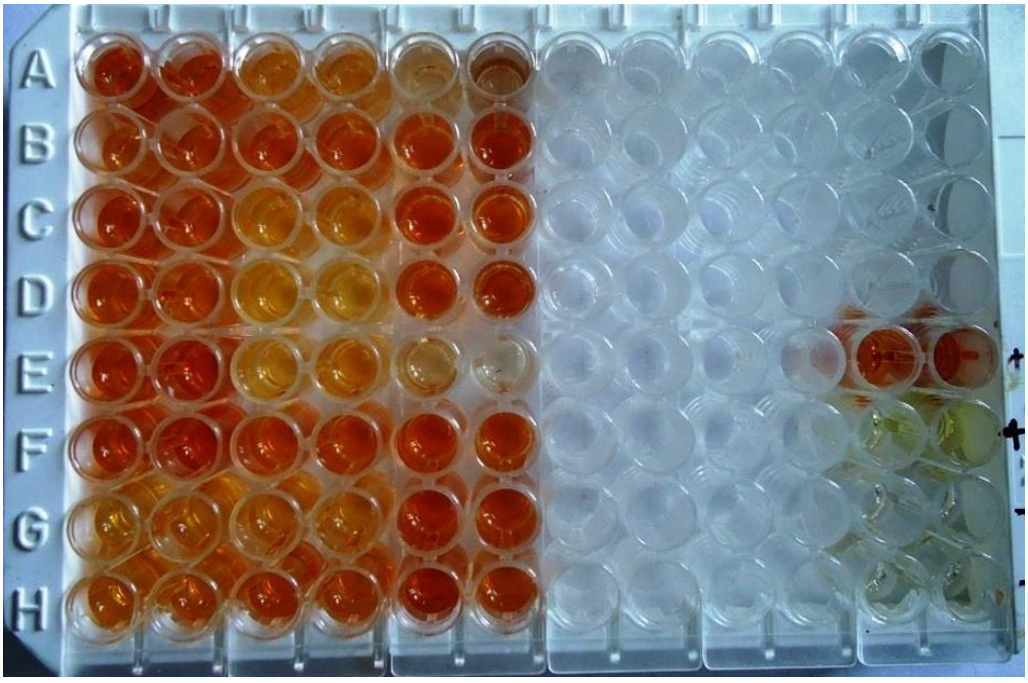

Plate 7. Cardiac Troponin-I test using serum sample yellow color are positive

Plate 8. Microplate reader

5 SUMMARY AND CONCLUSIONS

Canine Parvovirus infection is highly fatal infectious disease caused by type-2 canine parvovirus and characterized by hemorrhagic gastroenteritis with vomition and fever. The present research was undertaken to study the prevalence of CPV infection in and around Patna. A total of 600 serum samples were collected from bloody diarrheic dogs and screened for CPV antibody titre by using HA, HI, ELISA and Cardiac Troponin-I tests and canine parvovirus ELISA Kit (Bioassay Technology laboratory). The biochemical changes and therapeutic measures were also the results in infected cases.

From the total 600 serum sample Hemagglutination (HA) test was performed using 1% suspension SRBC. 600 serum samples showed that 225 sample were positive and 375 samples were negative. The HA titre in infected animals ranged from 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, and 1:1280 respectively.

The Hemagglutination inhibition test showed a titre of 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048. 4HA unit was prepared. HI test performed by using 1 % suspension of SRBC. In HI test 325 samples out of 600 samples were found positive. HI titres of positive sample ranged from 1:64 to 1:2048. Out of positive samples, 75(21.43%), 25(7.14%), 100(28.56%), 25(7.14%), 75(21.43%), and 25(7.14%), samples showed HI titre of 1:64, 1:128, 1:256, 1:512, 1:1024 and 1:2048 respectively.

In ELISA test, a cut off value was determined by comparing mean OD value of positive and negative serum. The reading was taken BIO RAD iMark^{TM} Microplate Reader at 450 nm. ELISA cut off OD value of $\leq$ 0.135 was taken. Out of 600 serum sample 394 sample (65.66%) were found positive.

In Cardiac Troponin-I test, a cut off value was determined by comparing mean OD value of positive and negative serum. Cardiac Troponin-I cut off OD value of $\leq$ 0.130 was taken. Out of 600 serums sample 550 sample (91.66%) were found positive.

In present study, the overall prevalence of CPV was 65.66 % in and around Patna. Breed wise examined of data indicated highest in non- descriptive (46.66 %) followed by German shepherd (24.21 %) and Labrador (13.00 %) respectively.

In present study, higher prevalence was observed in un-vaccinated (50%) dogs than vaccinated (16.66 %). It indicates that even immunized dogs were susceptible for CPV infection due to new antigenic variant.

In present study biochemical parameters showed that there a significant increases alkaline phosphatase total serum protein, albumin, globulin and AG ratio.

In present therapeutic studies in Group A consisting of administration of RL@30-40 ml/kg IV, DNS@20-30 ml/kg IV, Antibiotic (Cefriaxone + Tazobactum) @15-25 mg/kg BID IV, Ranitidine@2-4 mg/kg OD IV, Botropose@0.5-1ml SOS IV, CB12@1ml IV and Antiemetic@ 1mg/kg IV showed that 3 out of 6 dogs (50%) showed complete recovery from dehydration and other clinical signs on day 4 post treatments, while remaining 3 dogs (50%) recovered on day 5 post treatments.

In group B, the administration of Immune plasma@2.5 ml/kg and supportive therapy 4 dogs (66.66%) recovered completely from dehydration and other clinical signs on day 3 post treatment, while remaining dogs (33.33%) recovered on day 4 post treatment. Immune plasma therapy proved beneficial, especially when the total protein levels drop below 3.5 g/dl.

In group C, administration of Amino acid@ 1.2 ml/k g/h and supportive therapy, 5 dogs (83.33%) recovered completely from dehydration and other clinical sign on day 2 post treatment, while remaining dogs (16.66%) recovered on day 3 post treatment. administration of amino acids increases the plasma concentration.

Thus the present study shows among various treatment groups, group C (Amino acid +Supportive therapy) was found to be superior as all six (6) dogs recovered day 3 post treatment. However, the groups B and groups A showed comparable improvement on day 4 post treatment and day 5 post treatment respectively, hence amongst these three groups, group C was considered as superior than group B and group A on the basis of overall percentage recovery and improvement number of dogs. The results can be concluded as:

I. Prevalence of canine parvo virus infection in dogs in total cases presented to TVCC, BVC, Patna was found to be 65.66%.

II. Efficacy of diagnosis of canine parvo virus vaccine evaluated through antibody titre in vaccinated dogs was HA (37.50%), HI (54.16%), ELISA (65.66%) respectively.

III. Cardiac Troponin-I marker was elevated in dogs affected with CPV (91.66%)

IV. Biochemical parameters showed that a significant increase alkaline phosphatase whereas significantly decrease was noticed in total serum protein, albumin, globulin and AG ratio.

V. Immune plasma administration was group B leads decrease vomition & diarrhoea moreover viremia has also been observed with fast recovery.

VI. Albumin level was found to be normal after treatment with amino acid as in Group C.

VII. Among the four three groups, amino acid treated group (Group C) was found to be most effective for treatment of CPV infection.

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1. Phosphate buffer saline 1x

Ingredients	**gm/l**
Sodium chloride	8 gm
Disodium hydrogen phosphate	1.44 gm
Potassium chloride	0.2 gm
Potassium dihydrogen phosphate	0.24 gm
Distilled water	800 ml

Add distilled water to a total volume of 1 liter

8 Brief Resume Of The Student

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S.No.	Name of the exam/degree	School/College	Board/ University	Year of passing	Percentage
1.	Matrics	Banarsi High School Saidpur, Khagaria	BSEB, Patna	2002	66.42%
2.	Intermediate	SKB College Maranga, Purnea	BIEC Patna	2005	54%
3.	B.V.Sc. & A.H.	Bihar Veterinary College, Patna	BAU Sabour, Bhagalpur	2017	71.46%
4.	M.V.Sc.	Bihar Veterinary College, Patna	BASU Patna	2019	80.45%

Title of Thesis : **Studies on canine parvovirus infection in dogs and its therapeutic modalities.**

Scientific Popular Articles: 4

Membership in Professional Societies:

1. Member of Veterinary Council of India.
2. Member of Bihar State Veterinary Council.

Signature

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Date:

Place: B.V.C, PATNA-14 **(*Rajiv Kumar*)**