

Serological and Hemto-Biochemical Study of Canine Parvovirus and their Associated Risk Factor

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Abstract

The clinical microbiology and laboratory findings in puppies naturally infected with Canine Coronavirus (CCoV) and/or Canine Parvovirus (CPV) were compared with findings in uninfected puppies. Lymphopenia was the only parameter related to CCoV infection that was statistically significant, vomiting, anorexia, lethargy, hemorrhagic fluid diarrhea, leukopenia, lymphopenia, thrombocytopenia, hypoglycemia and hypoproteinemia were correlated with CPV infection.

Canine parvovirus is globally distributed disease of dogs, caused by canine parvovirus. This study was conducted to evaluate the sero-prevalence and their associated risk factors for canine parvovirus infection along with the changes in hematological and biochemical parameters in between seropositive and seronegative dogs. A total of 154 dogs affected from gastroenteritis were screened for this study, out of which 33 dogs were found to be affected with CPV infection. The data and risk factors were screened on SPSS by logistic regression model. The whole seroreactivity to Canine parvovirus infection was 20.77%. The associated risk factors of the disease occurrence were; age of dogs and unvaccination status of dogs. Hematological parameters of seropositive dogs showed significant changes ($p < 0.05$) in values of total erythrocyte count, total leukocyte count, hemoglobin and packed cell volume as compared to seronegative dogs. The biochemical parameters like Alanine Transaminase (ALT), Aspartate Amino Transferees (AST) and Alkaline Phosphatase (ALP) in seropositive dogs were significantly ($p < 0.05$) high. The study highlights that unvaccinated status, young age are found to be significant risk factors associated with the occurrence of CPV and has an effect on the hemato-biochemical parameters of dogs.

Keywords: Clinical microbiology; Parvovirus; Risk factor; Dogs; Hemtobiochemical parameters

Introduction

Canine parvovirus enteritis is a highly contagious and fatal disease caused by parvovirus type-2 affecting mainly intestinal tract and causing vomition, diarrhoea and fever. Acute enteritis is the most common manifestation of the disease and doberman, rottweiler and German Shepherd (GSD) dogs seem to be more susceptible to parvovirus infection than other breeds. Initial clinical signs are nonspecific, and include anorexia, depression, lethargy and fever. On later stages the typical signs include vomiting and small bowel diarrhea that can range from mucoid to hemorrhagic. Acute parvoviral enteritis can be seen in dogs of any breed, age or gender but puppies between 6 weeks and 6 months of age appear to be more susceptible. Factors that predispose to parvoviral infection in puppies are lack of protective immunity, intestinal parasites and overcrowded, unsanitary and stressful environmental conditions. Outbreaks of CPV have been reported from many countries including India. The prevalence study in India was first reported by balu and thangaraj in madras. The leukocyte count during CPV enteritis is generally characterized as significantly depressed, with a transient lymphopenia being the most consistent finding. The present study was an attempt to study the hematobiochemical alterations in canine parvovirus infection [1].

Canine Parvovirus (CPV) infection is a very contagious disease that affects dogs. (Dogs are susceptible to the highly contagious disease known as Canine Parvovirus (CPV) infection). This is widespread throughout the world and causes considerable morbidity and mortality in dogs, particularly in puppies. In 1967, this virus was firstly observed in those dogs, in which GIT and lungs problem were present. There are two varieties of canine parvovirus, known as CPV1 and CPV2. Most CPV-1-infected patients have no symptoms. Both domestic dogs and wild canids are afflicted by the most dangerous illness, CPV2. The two CPV2 strains are CPV-2a and CPV-2b. In the United States; outbreaks of unfamiliar enteric contagious diseases were reported in 1978. The main causing agent this outbreak was isolated and was considered as a new member of the parvoviridae family; it was named as CPV-type 2. Canine parvovirus spread rapidly in the

canine population because of the absence of previous insusceptibility [2]. During 1980 it was spread worldwide. The parvovirus genome is comprised of negative sense ssDNA (single-stranded) having 3 structural proteins VP1, VP2, and VP3 and there are 2 non-structural protein names, Non-Structural protein 1 (NS-1) and Non-Structural protein-2 (NS-2), develop through alternate hitch of viral messenger RNA. VP2 is an amino group terminated (NH₂) abbreviated from VP1 while Posttranslational Proteolytic Cleavage (PPC) of VP2 results in VP3. VP2 is the major element of capsid and VP3 present in only complete DNA-containing virions while empty particle doesn't contain VP3 protein. VP2 converts into VP3 by treatment of full particle with trypsin. Canine Parvovirus 2 (CPV-2) has icosahedral symmetry, non-enveloped with ssDNA genome, and 25 nm in diameter.

There are few existing studies that discuss the potential dangers of parvovirus infection in dog populations. Age, endoparasitism, unclean settings, and a lack of protective immunity have all been listed as significant risk factors for CPV. Numerous investigations revealed that, as predicted, unvaccinated animals had a higher risk of being CPV positive than vaccinated animals at young ages. Discovered that as age increased, the influence of vaccination status seemed to diminish. Breed, gender, and the time of year were also mentioned as risk factors for the development of CPV enteritis, with mixed breeds occasionally being mentioned as having a lower risk than pure breeds. Pinschers rottweilers, doberman, American pit bull terriers, German shepherd and English springer spaniels dogs were among the breeds having a noticeably elevated risk of developing CPV enteritis [3].

Significant alterations in packed cell volume, neutrophils, lymphocytes, and hemoglobin content were noted in CPV-infected dogs. A considerable decline in plasma glucose, total plasma protein, and the A: G ratio was discovered by biochemical analysis (albumin: globulin). Potassium and chloride levels were significantly lower than they should have been. BUN was seen to have significantly increased, as well as ALT (alanine aminotransferase). Our study's major objective is to look at potential risk factors for CPV infection and changes to the Hemato-biochemical parameters in naturally infected dogs [4].

Materials and Methods

The study was carried out in the university of veterinary and animal sciences ravi campus Pattoki, Pakistan, hospital from february to august 2019. It is situated on a globe map at 31°1'0N latitude and 73°50'60E longitude, and it is 186 meters (610 feet) in elevation. A total of 154 dogs were part of this study. The criteria for selection of dogs were irrespective of breed and gender. Ethical approval was not necessary. All of the animals included in this study were clinical cases and were examined, diagnosed and treated as per standard treatment and examination procedure. During the sample collection process,

information was gathered *via* a short questionnaire in order to analyze potential risk factors linked to the development of disease [5].

Selection standards for sampling

Fecal samples were taken from dogs presenting the same clinical signs of CPV-2 infection, including lethargy, vomiting, anorexia, weight loss and diarrhea [6].

Diagnosis

Rapid detection tools from Quacking Biotechnology were employed to get a confirmation diagnosis of the CPV-2 antigen. The procedure was followed exactly as the manufacturer had instructed [7].

Hemato-biochemical investigation

Blood was collected in EDTA-coated and plain vacutainers from twenty dogs (10 sero-negatives and 10 sero-positives). Hematological analysis was performed using the hematological analyzer to determine the Hemoglobin (Hb), Total Erythrocyte Count (TEC), platelet count, Total Leukocyte Count (TLC), and the Pack Cell Volume (PCV). Serum sample were analyzed for liver function test like Alanine Transaminase (ALT), Aspartate Aminotransferase (AST) and Alkaline Phosphatase (ALP) [8].

Statistical analysis

Thursfield method was used to find prevalence. For analysis of risk factors chisquare and univariable test were conducted and final logistic regression model was used to find out potential risk factors. For analysis of hemato-biochemical parameters between seropositive and seronegative dogs Student t-test was used. SPSS (version 20.00) were used for analysis of data and statistically p-values (<0.05) was considered significant [9].

Results

The study showed 20.77% (32/154) prevalence of CPV-2 in whole district of kasur. Initially four factors were screened by *Chi-square* and univariable model. Vaccination status of dogs, Breed, gender and age were analyzed to find out their association with the disease onset. The prevalence was statistically ($P>0.05$) non-significant in different breeds of dogs. However, higher prevalence was found in German shepherd dog (40%), Labradors (23.5%) followed by crossbreeds (13.7%) whereas pointers (13.3%), Rottweilers (8%) and boxers (1.5%) are less affected (Table 1). Gender of dogs were also found non-significant ($P>0.05$) factor with disease occurrence. However, male dogs (59.06%) had a greater frequency of CPV infection in comparison to the female dogs (41.94%) [10].

Table 1: Breed wise prevalence.

S.	Breed	Total
1	German Shepherd	40%
2	Labrador	23%
3	Rottweilers	8%
5	Mixed breed	13.7%
6	Boxer	1.5%
7	Pointer	13.3%

Un-vaccination status and age of the dogs were found as key risk factors ($P < 0.05$). The infection was more common in the 0-3 months age group (45.9%), followed by the 3-6 months age group (39.09%) and the 6-12 months age group (11.91%), having

a minor incidence (3.1%) in the age groups above 12 months (Table 2) [11].

Table 2: Age wise prevalence.

S. No	Age groups	total
1	0-12 weeks	45.9%
2	3-6 month	39.09%
3	6-12 month	11.91%
4	1-2 year	3.1%

A final regression model was created to identify any relevant risk variables for CPV. This model was initially applied to variables with $P < 0.2$ in the univariate analysis. The backward stepwise strategy was used to eliminate the non-significant

variables one at a time. Age of the dogs and lack of vaccination were the main factors towards disease dynamics (Table 3) [12].

Table 3: Final logistic regression model.

Variable	Response level	Wald statistics	Standard error	Significance	Odd ratios	95% CI	
						Lower	Upper
age	≥ 3 Months	9.106	1.13	0	30.144	3.301	286.074
	≥ 6 Months	14.679	1.153	0.002	95.223	9.859	921.773
	≥ 1 Year	1.68	0.55	0.194	2.043	0.546	5.1158
vaccination status	Un-vaccinated	0.03225	0.677	0.157	0.384	0.8228	0.386
	vaccinated	1.653	0.869	0.198	3.056	0.557	17.776

Hemato-biochemical parameters of seropositive and seronegative animal

Hematological examination showed that the hemoglobin concentration significantly decreased in seropositive dogs compared to seronegative dogs. The erythrocytes, packed cell volume, were significantly decreased in seropositive dogs compared to seronegative dogs. The leucocytes count, neutrophils and lymphocytes were significantly reduced in

seropositive dogs as compared to seronegative dogs. The platelets, monocytes, eosinophils and basophils did not differ significantly. Serum ALT, AST and alkaline phosphatase were significantly increased in seropositive dogs as compared to seronegative dogs (Table 4) [13].

Table 4: Haemato-biochemical analysis of seropositive dogs and seronegative dogs.

Parameters	Seropositive	Seronegative	P-value
WBC (10 ⁹ /l)	4.77 ± 0.76	9.60 ± 0.89	0
LYM (%)	10.00 ± 1.12	18.00 ± 1.10	0
NEUT (%)	30.0 ± 4.85	79.0 ± 4.60	0
RBC (10 ¹² /l)	4.98 ± 0.43	6.36 ± 0.24	0.017
Hb(g/dl)	10.19 ± 0.39	12.23 ± 0.40	0.003
PCV%	38.00 ± 1.62	45.0 ± 0.57	0.001
ALT(IU/l)	205.0 ± 14.55	159.0 ± 10.58	0.019
AST (IU/l)	135.0 ± 11.88	29.0 ± 11.15	0
ALP (IU/l)	201.0 ± 19.20	30.0 ± 19.43	0

Discussion

Sero-prevalence of CPV in dogs

Parvoviruses are the most important cause of enteritis disease in dogs. The whole prevalence of CPV infection found in this study (20.77%) was in correlation with the results and who reported prevalence 21.33%. In contrast to the results of present study, and investigated high prevalence in Jabalpur (45.30%) and Chhattisgarh (65.04%). Such high incidence might be due to prevalence of endemic infection in the population under study. Though, it is very difficult to elaborate these variations in the incidence of CPV because of different sample analysis methods and different study areas [14].

Risk factors associated with the occurrence of disease

The current study showed that the most affected breed is the German shepherd dog (40%) followed by Labradors (31.5%) and crossbreeds (13.7%), while pointers (13.5%), Rottweilers (8%) and boxers (1.5%) are less affected breeds. This was an agreement with previous studies made observed a higher incidence in German shepherd dog breeds. In Pakistan, people extremely like German shepherd dogs and this might be the key reason for the high prevalence of the infection in this particular breed. Medium and large breeds are more susceptible to acquiring the CPV infection [15]. The high rates in deshi breeds may be due to its high population, which puts them in close proximity and makes it easier for the infection to spread, or by the owners' poor adherence to the recommended vaccination schedule, which results from their lack of basic understanding. Secondly, the number of Rottweilers kept as pet animals in Pakistan is declining due to the high aggressive nature of this particular breed. Pug, boxer and pointer are very rarely kept by pet owners (Pet owners hardly ever keep pugs, boxers, or pointers.); therefore, this could be a major reason for the lower prevalence. Due to the fact that different geographic regions

have different breed population densities, no precise observations on breed vulnerability can be made. In current study, more cases of canine parvovirus infection were observed in the hard summer season. This correlated with the findings [16].

The highest occurrence of CPV infection was found in dogs aged 0 to 3 months *i.e.* 45.9%, followed by dogs aged 3 to 6 months age *i.e.* 39.09%, dogs aged 6 to 12 months *i.e.* 11.91%, and dogs older than 12 months *i.e.* 3.1%. These findings correlated with those who reported increased prevalence in dogs between the ages of 0 to 3 months. It has been reported earlier that CPV is reliant on the mitotic activity of myocardial and enteric cells which is at its peak in puppies. In above 1 year old, very few cases were reported which suggests that antibodies may have developed in adults as a result of vaccination schedules or mild virus exposure that led to the development of antibodies in the host, or some other factors that need to be explored. This study also showed that the prevalence of the disease is higher in males than in females. Also reported higher incidence among males. The high prevalence of CPV in male dogs might be due to selective preference of keeping male dogs by pet owners and also most of the admitted dogs were male [17].

Hemato-biochemical parameters of seropositive and seronegative animals

The canine parvovirus was found to reduce hemoglobin, white blood cells and neutrophils but increased packed cell volume in the current study. This observation was also made. That canine Parvovirus causes hematological changes in infected dogs. The cytotoxic action of the virus on the hematopoietic cells and bone marrow cells during the acute phase of the illness was thought to be the cause of leucopenia and neutropenia. Severe neutropenia in CPV enteritis can be attributed to the virus' active destruction of myoblasts in the bone marrow, but it can also be brought on by end toxemia, which may cause neutrophils to marinate, as well as a significant loss of

neutrophils through the intestinal wall. RBCs were found decreased significantly in seropositive dogs as compared to seronegative dogs. The seropositive dogs were showing symptoms of anemia manifested clinically by pale mucous membranes and general weakness. Seropositive dogs were founded to have high mean values of PCV as compared to seronegative dogs. Increased PCV levels observed due to severe dehydration and fluid losses through vomiting and diarrhea as reported by [18].

Conclusion

ALT and AST had observed a significantly increased in seropositive dogs as compared to seronegative dogs. Similar findings were also reported by. Inflammatory bowel disease and hepatic diseases had contributed to the rise of liver enzymes. The study emphasizes that unvaccinated status, young age are found to be significant risk factors associated with the occurrence of CPV and has an effect on the hemato-biochemical parameters of dogs.

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