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Saravanan S
Associate Professor, Department of Veterinary Medicine, Veterinary College and Research Institute, Tirunelveli, Tamil Nadu, India

Ramprabhu R
Professor and Head, Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli, Tamil Nadu, India

Mohanapriya T
Assistant Professor, Department of Veterinary Pathology, Veterinary College and Research Institute, Tirunelveli, Tamil Nadu, India

Chitra R
Assistant Professor, Department of Animal Husbandry Statistics and Computer Applications, Veterinary College and Research Institute, Namakkal, Tamil Nadu, India

Ponnu Swamy KK
Professor and Head, Department of Veterinary Medicine, Veterinary College and Research Institute, Tirunelveli, Tamil Nadu, India

Corresponding Author:
Saravanan S
Associate Professor, Department of Veterinary Medicine, Veterinary College and Research Institute, Tirunelveli, Tamil Nadu, India

Retrospective study on risk factors and haemato-biochemical alterations associated with canine parvo viral enteritis

Saravanan S, Ramprabhu R, Mohanapriya T, Chitra R and Ponnu Swamy KK

Abstract
Among enteritis causing diseases of dogs, canine parvo viral enteritis still remains a common and important cause of morbidity and mortality especially in young ones. Clinical cases brought to Veterinary Clinical Complex, Tirunelveli were studied for the clinical signs and the pattern of the disease with respect to age, sex, breed and season. Co-infection of CPV enteritis with intestinal parasites was identified by faecal examination and *Ancylostomum caninum* was found to be the predominant one. Haematological analysis revealed changes like anaemia, lymphopenia, thrombocytopenia and neutrophilia in CPV affected dogs. Serum biochemical analysis revealed elevated levels of blood urea nitrogen, creatinine, alanine transaminase, aspartate transaminase and alkaline phophatase, and reduction in the levels of total protein, albumin, calcium, phosphorous, glucose, sodium and potassium in CPV affected dogs.

Keywords: CPV enteritis, helminthic infection, haemato-biochemical analysis, risk factors

1. Introduction
The dog population in India is estimated to be 25 million and 17% of households were reported to own a pet [1]. The affluent middle class in India is increasingly accustomed to Western culture with changing attitudes towards pet animal ownership, demanding a need for thorough understanding and knowledge on canine diseases.
Enteritis is the most common clinical condition among the various gastro intestinal disturbances, encountered in dogs [2]. Dogs are susceptible to the viral diseases like canine parvoviral infection, canine distemper, corona virus infection, canine hepatitis, canine parainfluenza and rabies [3] and among these canine parvo viral enteritis cause high morbidity and mortality in unvaccinated puppies. Canine parvovirus type 2 (CPV-2) (Family: Parvoviridae) is a small, non-enveloped ss-DNA virus (ICTV 2016). The virus shows genomic substitution rates, consequent of which the original CPV2 gave rise to the antigenic variant CPV 2a, CPV-2b and CPV-2c, which necessitates periodic update of virus type for selection of appropriate vaccine strain [4].
The virus affects mainly young dogs primarily less than one year-old and is frequently associated with hemorrhagic gastroenteritis [5] and severe leukopenia in dogs of all age, and myocarditis with resultant heart failure in pups of below 3 months age [6]. Death may ensue due to severe dehydration or complication by secondary invaders like *Escherichia coli* leading to septicaemia and disseminated intravascular coagulation [7]. The information on the occurrence of canine parvo viral enteritis in Tirunelveli locality is not available. Hence, this paper reports a retrospective study on the distribution of canine parvo viral enteritis in dogs with respect to host specific risk factors and season, in association with concurrent parasitism and haemato-biochemical changes in affected cases.

2. Materials and Methods
Retrospective data were collected from a cross section of dog population (n=1400) manifesting clinical signs suggestive of parvo viral enteritis irrespective of their breed, age and sex brought to Veterinary Clinical Complex, Veterinary College and Research Institute, Tirunelveli from the period April, 2018 to March, 2019.

Majority of the dogs had a history of no vaccination against the CPV-2 or single vaccination without booster doses. The epidemiological factors and temporal pattern associated with the disease are statistically analysed and represented in Table 1, Figure 1 and 2.

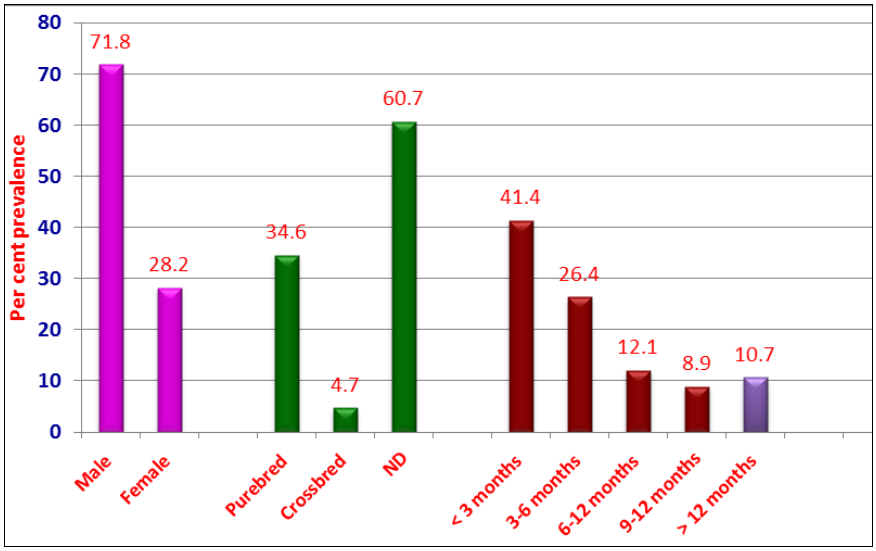

Fig 1: Host factors associated with the occurrence of canine parvo viral enteritis in Tirunelveli district

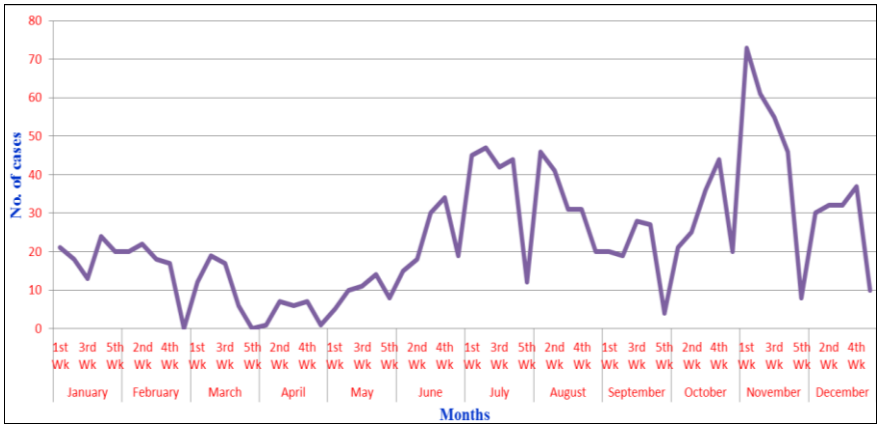

Fig 2: Temporal pattern of canine parvo viral enteritis in Tirunelveli district

Table 1: Epidemiological determinants associated with the occurrence of canine parvo viral enteritis in Tirunelveli district

Epidemiological determinants		Total (n=1400)	Per cent positives	P Value
Age	0-3 months	580	41.4	<0.001
	4-6 months	370	26.4	
	7-9 months	170	12.1	
	10-12 months	124	8.9	
	Above 1 year	150	10.7	
Sex	Male	1005	71.8	<0.001
	Female	395	28.2	
Breed	Rottweiler	19	1.4	<0.001
	Dobermann	38	2.7	
	GSD	57	0.0	
	Laborador retriever	71	5.1	
	Chippiparai	257	18.4	
	Others / Non -descriptive	958	68.4	
Season	Southwest monsoon	245	17.5	<0.001
	Northeast monsoon	452	32.28	
	Winter	543	38.78	
	Summer	150	10.71	

About 5 ml of blood was collected from cephalic vein or saphenous vein of which 2 ml of blood was transferred into a sterile vial containing 10 per cent EDTA for haemogram and 3 ml was transferred to clot activator vial for serum following instituional guidelines. Haematological parameters *viz.*, haemoglobin (Hb), packed cell volume (PCV), total erythrocyte count (TEC), total leukocyte count (TLC), differential leukocyte count (DLC) and platelet count were studied in apparently healthy dogs (controls, n=10) and parvo viral enteritis cases (n=25). Serum biochemical parameters

like blood urea nitrogen (BUN), total protein, albumin, alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphotase (ALP), potassium, sodium, chloride and glucose were estimated by automated analyser in apparently healthy dogs (controls, n=10) and parvo viral enteritis cases (n=21). Haemato-biochemical parameters as shown in Table 2 were analysed by statistical analysis (Mann-Whitney U test and Student's 't' test) by using SPSS software.

Fresh faecal samples were randomly collected from 313 cases irrespective of their deworming status, subjected to centrifugal sedimentation and the sediment was examined microscopically for the presence of helminthic eggs which were identified based on morphological characters [8].

3. Results and Discussion

The cases presented to the VCC, Tirunelveli were diagnosed based on the characteristic clinical signs like fever, vomiting and haemorrhagic diarrhoea and dehydration. Affected dogs presented the stools with watery or pasty stools of reddish, brownish or orange- brown colour and offensive odour as shown in Figure 3. The clinical sigs observed are corroborative of Uzuegbu [9] and De Oliveira *et al.* [5].

Fig 3: A Dobermann breed showing haemorrhagic diarrohoea

Table 2: Haemato-biochemical values with mean ± SD associated with the cases of parvo viral enteritis in Tirunelveli district

Parameters	CPV enteritis cases	Controls	P Value
Haematological values (Mean ± SE)			
Hb (g/dl)	16.90 ±0.93	17.39 ±0.38	0.897
PCV (%)	39.41 ±2.39	45.92 ±1.97	0.080
RBC (×10^6/ul)	6.39 ±0.41	6.79 ±0.32	0.787
WBC (×10^3/ul)	21888.00 ± 2997.82	16140.00 ± 3533.40	0.159
Platelet (×10^5/ul)	242000.00 ±25469.00	269900.00 ±30506.99	0.373
Neutrophils (%)	77.32 ±2.85	76.20 ±1.68	0.322
Lymphocytes (%)	14.84 ±2.40	18.90 ±2.04	0.107
Monocytes (%)	0.96 ±0.23	1.50 ±0.52	0.465
Eosinophils (%)	5.80 ±1.16	3.40 ±0.88	0.271
Basophils (%)	0.00 ±0.00	0.00 ±0.00	
Biochemical values (Mean ± SE)			
BUN (mg/dl)	128.18 ± 55.68	27.37 ± 2.55	0.124
Creatinine (mg/dl)	2.13 ± 0.90	1.12 ± 0.18	0.857
Total protein (g/dl)	6.81 ± 0.33	8.14 ± 0.44	0.029
Albumin (g/dl)	2.52 ± 0.17	2.93 ± 0.12	0.042
ALT (u/l)	44.47 ± 4.34	62.70 ± 10.31	0.201
AST (u/l)	58.20 ± 8.68	54.50 ± 5.83	0.711
ALP (u/l)	383.44 ± 158.67	81.99 ± 13.33	0.136
Calcium (mg/dl)	10.20 ± 0.69	11.00 ± 0.48	0.368
Phosphorous (mg/dl)	4.87 ± 0.73	4.73 ± 0.53	0.810
Glucose (mg/dl)	113.75 ± 17.29	127.30 ± 8.88	0.064
Sodium (meq/l)	143.09 ± 6.80	131.23 ± 5.19	0.430
Potassium (meq/l)	9.15 ± 5.23	3.58 ± 0.17	0.107

3.1 Host determinants

Statistical analysis revealed a highly significant difference ($P< 0.001$) between the different age groups, sex, breeds and seasons with respect to the incidence of the disease. In age-wise distribution of CPV, high incidence could be recorded in dogs of 0-3 month age group (41.4%) followed by 4-6 months (26.4%), 7-9 months (12.1%), above 1 year (10.7%) and 10-12 months (8.9%). This finding is in agreement with that of Ali *et al.* [10] and Behera *et al.* [11] who also recorded a high prevalence in dogs of less than 5-6 months age groups. The high incidence in dogs less than 3 months could be attributed to the affinity of the virus for rapidly multiplying intestinal crypt cells in weaning pups with higher mitotic index in association with waning of maternal antibody titres [12]. Though dogs of above 1 year are is uncommon due to exposure to natural infection, the susceptibility in this study might be due to lack of protective antibody titre. This finding concurs with that of De Oliveira *et al.* [5] who reported a severe disease in adults.

In breed-wise distribution of CPV, non-descriptive and other cross breeds (68.4%) were highly susceptible followed by the local breed, Chippiparai (18.4%) as the this breed is more commonly presented than any other breed. Similarly, Behera *et al.* [11] also reported a high prevalence in non- descript dogs. Among the exotic breeds, Labrador retriever and German shepherd (5.1%) and Laborador retriever (4.1%) were found to be more susceptible [11, 10].

In sex-wise distribution, males (71.8%) were mostly susceptible to parvo viral enteritis than females (28.2%). This finding corroborated with Thomas *et al.* [13] and this could be due to probability of exposure associated with selective preference of keeping male dogs by pet owners. However, there was no influence of sex on the incidence of CPV [14].

a. Seasonal factor

In season-wise distribution, parvo viral enteritis cases were recorded high during north-east monsoon (38.78%) followed by south-west monsoon (32.28%), summer (17.5%) and winter (10.71%). Previously, Kokila Priya *et al.* [15] also recorded a high prevalence of CPV-2 during monsoon. However, seasonal prevalence of the disease is subject to considerable geographic variation [16].

b. Concurrent helminthic infections

In parvo viral enteritis cases, co-incidence of helminthic infection (as shown in Figure 4a, 4b, 4c, 4d and 4e) by *Ancylostomum caninum* (11.8%) was recorded followed by *Toxocara canis* (2.23%) and *Taenia* spp. (0.95%). The increase in severity of CPV enteritis in some cases might be due to the concurrent infections by the intestinal parasites [17]. Previously, co-infection by ancylostomoes in CPV-2 infection was also recorded by Uzuegbu [9] and Saravanan and Palanivel [18].

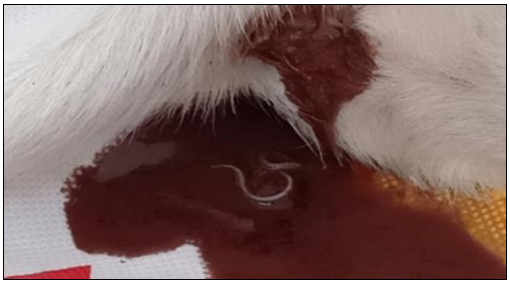

Fig 4a: A Spitz breed showing haemorrhagic diarrohoea with expulsion of T. canis

Fig 4b: Segments of *Taenia sp.* expelled out in vomitus of a non-descript dog

Fig 4c: Egg of *A. caninum* identified by microscopic examination of the stools of a CPV affected case (40×)

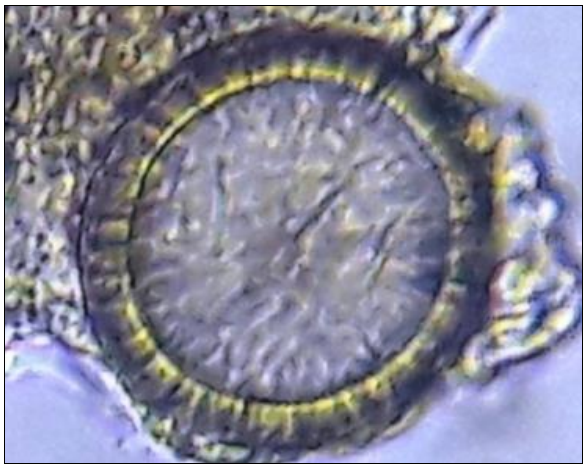

Fig 4d: Egg of *Taenia sp*. identified by microscopic examination of the stools of a CPV affected case (40×)

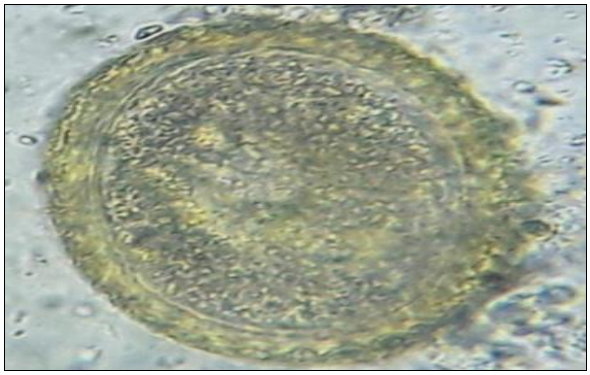

Fig 4e: Egg of *T. canis* identified by microscopic examination of the stools of a CPV affected case (40×)

4. Haematological analysis

Statistical analysis of haematological values as shown in Table 2 revealed no significant decrease (P>0.05) in the values (mean ± SE) of Hb, PCV, TEC, TLC, platelet counts and differential leukocyte counts in CPV enteritis cases when compared to control group. However, there was a reduction in haemoglobin levels in 48.0% and PCV level in 36.0%, anaemia in 24.0%, leukocytosis in 60.0%, thrombocytopaenia in 28.0%, neutrophilia in 84.0%, lymphopenia in 52.0%, monocytosis in 20.0% and eosinophilia in 20.0% of the clinical cases of canine parvo viral enteritis. Reduction in Hb and PCV levels, and anaemia could be associated with haemorrhagic enteritis. Similar findings were also recorded by Moneesh Thakur and Radhika Thakur[17]. Thrombocytopenia and lymphopaenia in this study could be attributed to the destruction of hematopoietic progenitor cells in the bone marrow and other lymphoproliferative organs [19]. Neutrophilia and leuckocytosis observed in this study could be due to secondary bacterial infection in the damaged intestinal tract and reactive leuckocytosis resulting in myeloid hyperplacia. However, normal leukocyte count in some cases, might be attributed to concomitant lymphopenia and neutrophilia. Similar findings were also recorded by [20].

5. Biochemical analysis

Statistical analysis of biochemical values as shown in Table 2 revealed a significant decrease ($P< 0.05$) in the values (mean ± SE) of total protein and albumin, whereas, no significant decrease (P>0.05) in the values (mean ± SE) of calcium, phosphorus, glucose, sodium and potassium levels in CPV enteritis cases when compared to control group. Statistically, no significant increase (P>0.05) in the values (mean ± SE) of BUN, creatinine, ALT, AST and ALP in CPV enteritis cases when compared to control group. However, there was an increase in the levels of BUN in 52.1%, creatinine in 14.29%, ALT in 14.3%, AST in 28.6% and ALP in 38.1%, and decreased levels of albumin in 61.0%, total protein in 15.05%, calcium in 23.8%, phosphorus in 9.5%, glucose in 9.5%, sodium in 42.5% and potassium in 33.3% of the clinical cases of canine parvo viral enteritis.

Increased levels of ALP and ALT could be associated with hepatic hypoxia secondary to severe hypovolemia or the absorption of toxic substances due to loss of the gut barrier [21]. Increased BUN level could be due to prerenal azotemia associated with low glomerular filtration rate and similar observation was made by Shah *et al.* [22]. Hypoalbunemia, hypoproteinemia, hypocalcaemia and hypophosphatemia might be as a result of vomiting, protein-losing enteropathy, intestinal haemorrhage and SIRS-mediated vascular permeability. Similar observations were made by Schoeman *et al.* [23]. Hypoglycaemia might be due to loss of appetite loss of nutrients associated with enteritis and decreased blood supply to liver affecting glucose metabolism. Hyponatremia and hypokalemia could be due to the loss of sodium and potassium in severe vomition and diarrhoea [20].

6. Conclusion

Canine parvoviral enteritis caused is an acute and highly contagious disease with high morbidity and mortality especially in unvaccinated dogs which are at high risk or 'window of vulnerability'. In terms of epidemiological factors, male dogs of less than 6 months age and non-descript group were found to be more susceptible. Incidence was found to be high during monsoon period that could favour the

survival of the CPV-2 virus in the environment. Concurrent infection by intestinal helminths could greatly increase the severity of the disease with fatal outcome. In affected dogs, significant haematological and biochemical alterations may occur depending on the severity of the disease, concurrent parasitic infection and secondary bacterial infection. Hence, regular deworming and timely vaccination of young pups is absolutely essential to prevent the attack of this serious disease.

7. Acknowledgement

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