Occurrence of canine parvo virus -2 enteritis in indigenous and pure breeds of adult dogs: A shift in host susceptibility patterns?

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Abstract

Canine parvo virus-2 (CPV-2) causes acute haemorrhagic gastroenteritis (HGE), myocarditis and leukopenia in dogs. In the absence of careful therapy, it leads to a high mortality due to secondary bacterial complications and sepsis. Though adult dogs are being considered to be relatively resistant, change in virus strains, varying virulence and immune response with respect to age and breed warrants the need for an update in the susceptibility pattern of older breeds. The CPV-2 could be detected in 20.0% dogs of various breeds above one year old (1-9 years) showing the signs of HGE, by immunochromatography test (ICT). Dogs in the age group of 1-1.5 years (62.5%; (p=0.0075) and Rajapalayam breed (66.6 %; p=0.0065)) were found to more susceptible than other age and breeds. The mean lymphocyte count (12.9±1.53) was significantly low (p=0.0032) and the mean neutrophil count (83.3±1.63) was significantly high (p=0.0034) in PVE cases, when compared to the mean lymphocyte (21.7±2.09) and neutrophil counts (72.9±2.62) in other HGE cases. No significant difference (p>0.05) was observed in other haemato-biochemical values in PVE cases in comparison with other HGE cases. The mean neutrophil-lymphocyte ratio (NLR) was significantly high (p=0.0092) in PVE cases (7.65±1.08) when compared to other HGE cases (3.89±0.63). A reduction (p= 0.0123) in mean calcium level (8.9±0.24) with an increase (p=0.34) in mean alkaline phosphatase (ALP) (204.2±63.5) and phosphorous levels (10.04±5.15) were observed when compared to other HGE cases. Lymphocyte and absolute neutrophil count could be useful bio-markers in recognizing PVE cases among other HGE cases. Increasing frequency of PVE in older breeds raises the concern of doubtful native or adaptive immunity. A serosurveillance for CPV-2 antibodies in older dogs would help to assess the immune response against changing virus strains and understand the shift in susceptibility pattern of unvaccinated native and pure breeds in various geographical regions.

Key words: Adult breeds, CPV-2, Immunochromatography, Increasing incidence, Neutrophil-lymphocyte ratio, Prognostic markers

Introduction

Canine parvo virus -2 (CPV-2) of the genus *Carnivore protoparvo virus* (CPPV) is a highly contagious virus causing acute haemorrhagic gastroenteritis (HGE) in dogs both purebreds and mongrels (Kumar et al., 2023) worldwide, with a high case-fatality rate in unvaccinated puppies. Vaccination, though in practice, fails to completely prevent the disease as clinical cases are still being reported worldwide (Umar et al*,* 2024). It also infects lymphocytes causing acute lymphopenia and myocytes causing myocarditis in young puppies (Alzuheir et al*.*, 2024). Translocation of intestinal bacteria leads to endotoxaemia, systemic inflammatory response syndrome (SIRS), sepsis, multi organ dysfunction and death (Alves et al*.,* 2020; Sevim et al., 2025). The mortality rate in unvaccinated puppies can be as high as 91% (Temizkan et al., 2023). A recent survey on various outbreaks in India revealed the existence of an extensive array of genetic variants in India from 2010 to 2023 (Jyothi et al., 2024).

Since the virus affects the rapidly multiplying cells of growing puppies, the disease is reported to be more severe in puppies in the age group of 6 weeks to 6 months of age. Hence, the disease in acute form is rarely reported in adult dogs even when unvaccinated against CPV-2, owing to the development of acquired immunity by natural infection (Sayed-Ahmad et al*.*, 2020). The disease may also occur in mild or subclinical form (Decaro et al*.,* 2005) which is usually goes unnoticed due to the absence of characteristic haemorrhagic enteritis when presented for treatment.

Still, unvaccinated pure breeds above 1 year age are at the risk of entering into the 'Window of susceptibility and vulnerability' or acquiring re-infection owing to their genetic immunodeficiency or breakdown of immunity (native or adaptive) and this may lead to a more severe haemorrhagic gastro enteritis and death. Nevertheless, most of the available reports in India focussed on detection of CPV-2 in young dogs (below 6 or 12 months old) with HGE. Further, many older dogs with haemorrhagic diarrhoea are being misdiagnosed for other common haemorrhagic diseases caused by *Leptospira interrogans* serovars*, Babesia canis, Anaplasma platys, Ehrlichia canis* and *Campylobacter jejuni*, resulting in failure of treatment.

Hence, the study was undertaken to identify the epidemiological trends of parvo viral enteritis (PVE) in various adult dog breeds, compare the prognostic indicators with that of other HE cases, and complications by haemato-biochemical analysis.

Materials and Methods

Adult dogs of various breeds (n=50) presented with the history of anorexia, haemorrhagic diarrhoea and/ vomiting to the veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal in the year 2024 were taken up for diagnosis of parvo viral enteritis (Figure 1). All the dogs under investigation had either incomplete or no vaccination against CPV-2. Faecal swabs were collected from all the cases on the day of presentation and subjected to sandwich lateral flow assay based immunochromatography test (Pet X®, J &B Biotech Ltd, UK) for the on-spot diagnosis of CPV-2 virus. The swab was inserted into the provided assay buffer and agitated to get efficient sample extraction. Four drops of the treated sample was placed in to the sample hole 's' of the test device and the result was interpreted within 10 minutes. Development of red lines (faint or dark) both in control (C) and test (T) zones indicated positivity (Figure 2a). and the red line only in C zone indicated negativity (Figure 2b).

Epidemiological factors with respect to the incidence specific to age, sex and breed were (Table 1) were statistically analysed (Chi-square test). Whole blood and serum samples were subjected to haemato-biochemical analysis by using auto Veterinary hematology Analyzer (Rayto®, Version 2.4e, RT7600Vet, Rayto Life and Analytical Sciences Co., Ltd., China) and auto Biochemistry analyzer (A15 Biosystems SA, Spain). The results were compared with that of the dogs presented with haemorrhagic diarrhoea but negative for CPV-2 infection and statistically analysed by using student t-test (Table 2). Box-whisker plots were created to visualize the distribution of lymphocytes and neutrophils (prognostic indicators) in PVE cases and other haemorrhagic enteritis (HGE) cases (Figure 3). The Mann- Whitney test was subsequently applied to assess the statistical significance of difference between these groups. Neutrophil-lymphocyte ratio (NLR) and it's significance by Welch's test was calculated for both PVE and other HGE cases (Table 3), and box plots and bar charts (Figure 4) were drawn to understand the severity of disease in both groups.

Results and Discussion

The point-of-care assay, immunochromatography test (ICT) detected a positivity (Figure 2) of 20.0 per cent (n=10) for CPV-2 infection in adults in the age group of above 1 year and up to 9 years. The ICT was reported to be an useful technique in epidemiological and diagnostic studies (Khare et al., 2019; Sayed-Ahmed et al., 2020; Hemali et al*.*, 2022).

Fever, haemorrhagic diarrhoea, vomiting and dehydration were the common clinical signs reported in all the positive cases (Sayeed-Ahmad et al., 2021), as CPV-2 infects rapidly multiplying epithelial cells in gastro intestinal tract. The petechiae/ecchymoses (Figure 1) observed on ventral abdomen and face in one of the PVE

Table 1. Epidemiological determinants associated with the incidence of parvo viral enteritis in adult dogs (above 1 year old) in Namakkal district

Epidemiological Determinants in adults		HGE cases (n=50)	Test Positives (n=10)	Specific incidence rate	Statistical test (Chi-square, p value)
Age (in years)	1.0 -1.5 yr	08	05	62.5	χ^2=157812 (p=0.0075
	1.5- 2.0yr	10	0	0.0	
	2.0- 2.5 yr	15	04	26.6	
	2.5 -3.0yr	07	0	0.0	
	3.0-3.5 yr	03	01	33.3	
	3.5 -9.0 yr	07	0	0.0	
Sex	Male	49	10	98.0	χ^2=0 (p=1.0)
	Female	01	0	0.0	
Breed	Rajapalayam	03	02	66.6	pχ^2=16.1223 (p=0.0065)
	Spitz	07	04	57.1	
	Great Dane	02	01	50.0	
	Labrador retriever	04	01	25.0	
	Non -descriptive	19	02	10.5	
	Other Pure breeds (German shepherd, Dobermann and Rottweiler) and native breeds (Chippiparai and Kombai)	15	0	0.0	

Table 2. Haemato-biochemical changes in PVE and other HGE cases of above one year old

Parameters	PVE cases (Mean ± SE)	Other HGE cases (Mean ± SE)	P Value by t- test
Hb (g/dl)	15.72±1.23	14.7±1.29	0.48
PCV (%)	48.45±2.57	44.91±3.27	0.61
RBC (×10^6/µl)	7.01±0.33	6.445±0.44	0.21
WBC (×10^3/µl)	19.18±3.85	22.13±2.5	0.06
Neutrophils (%)	83.3±1.63	72.9±2.62	0.0034**
Lymphocytes (%)	12.9±1.53	21.7±2.09	0.0032**
Monocytes 9%)	3.8±0.29	5.1±092	0.12
Platelet (×10^5/µl)	369.8±24.04	430.2±59.45	0.37
Total protein (g/dl)	6.71±0.7	6.54±0.33	0.76
Albumin (g/dl)	2.94±0.2	3.01±0.27	0.95
Globulin (g/dl)	3.74±0.32	3.53±0.3	0.74
ALP (u/l)	204.2±63.5	118.5±42.12	0.34
ALT (u/l)	47.1±9.8	38.45±5.71	0.72
BUN (mg/dl)	22.3±4.46	17.08±3.06	0.40
Creatinine (mg/dl)	1.13±0.18	1.33±0.2	0.14
Calcium (mg/dl)	8.9±0.24	10.89±0.6	0.0123*
Phosphorous (mg/dl)	10.04±5.15	4.53±0.79	0.34
Glucose (mg/dl)	122.77±25.1	69.2±6.38	0.09
Sodium (meq/l)	140.3±3.19	133.46±5.18	0.33
Potassium (meq/l)	3.98±0.2	3.87±0.22	0.50
Chloride (meq/l)	109±3.14	102.2±4.1	0.27

P<0.05 - Significant at 5% level; P<0.01 - Significant at 1% level

Table 3. Neutrophil-lymphocyte ratio (NLR) in PVE and other HGE cases of above one year old

Case No	NLR in PVE cases	NLR in other HGE cases	Welch's test (p value)
1	5.47	2.54	3.0046 (p=0.0092)
2	6.23	5.47	
3	5.27	2.38	
4	4.33	2.52	
5	9.78	2.43	
6	9.78	6.38	
7	4.33	2.96	
8	6.46	4.00	
9	9.67	7.82	
10	15.17	2.39	
Mean±SE	**7.65±1.08**	**3.89±0.63**	

P<0.05 - Significant at 5% level; P<0.01 - Significant at 1% level

Fig 1. Adult dogs of various breeds (Great Dane, Rajapalayam and Labrador retriever) affected with canine parvo viral enteritis. Arrow marks indicate petechiae and ecchymoses on eyelids and ventral abdomen.

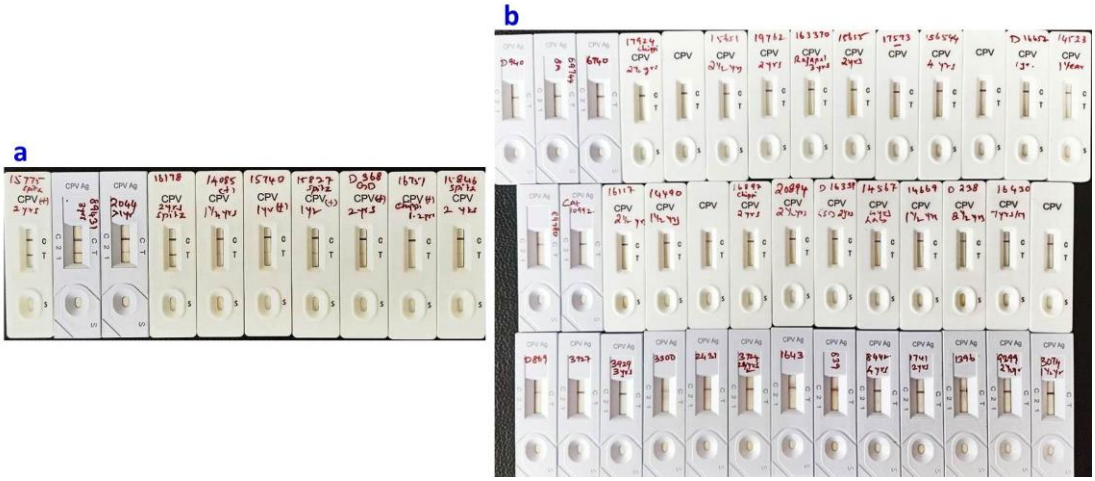

Fig 2. (a) Immunochromatography test showing positivity and (b) negativity for CPV-2 in faecal samples of adult dogs of various breeds

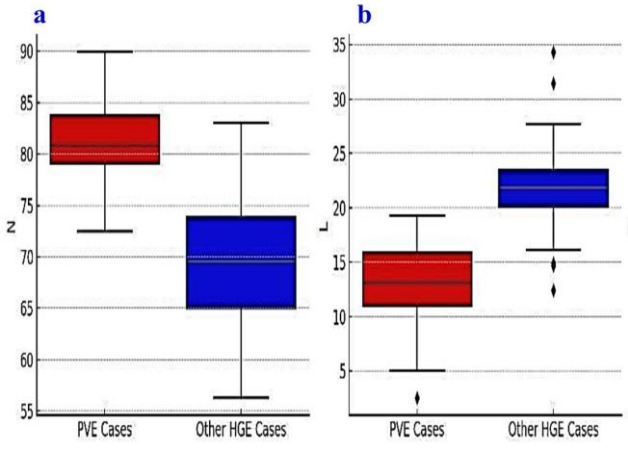

Fig 3. Box-Whisker plot analysis of (a) neutrophil and (b) lymphocyte counts in dogs of PVE and other HGE cases

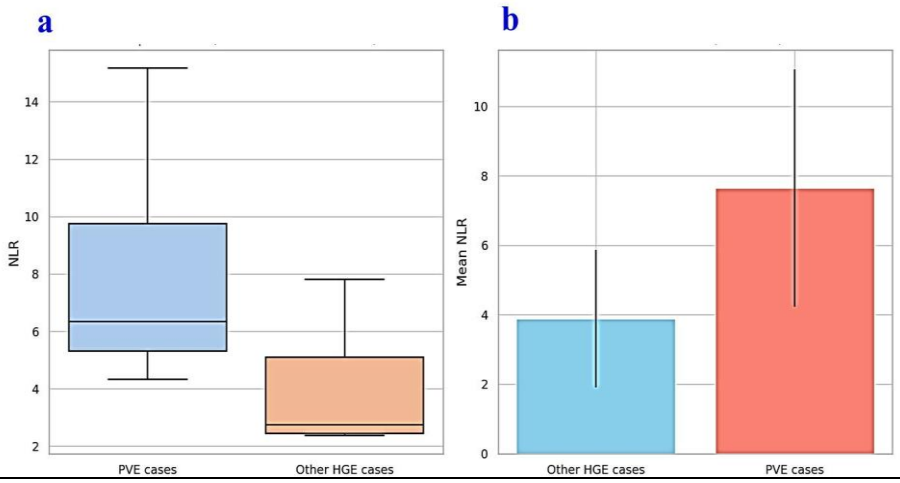

Fig 4. (a) Box-Whisker plot analysis of neutrophil -lymphocyte ratio (NLR) and **(b)** Bar chart showing mean NLR in dogs of PVE and other HGE cases

cases might be due to the systemic inflammatory response (SIRS) and disseminated intravascular coagulation (DIC) associated with hypercytokinemia (Paul et al., 2023; Sevim et al., 2025). All the PVE cases (n=10) survived after continuous treatment in the hospital. Out of 10 PVE cases, five had history of no vaccination and five had incomplete vaccination (with incomplete initial vaccination or with initial vaccination and without annual revaccination).

Among the breeds, the Rajapalayam was found to be the most susceptible native breed followed by pure breeds. In contrast, previous studies suggested a stronger resistance in native breed and mixed-breed dogs to CPV-2 than purebreds (Qi et al. 2020; Umar et al., 2024; Zhou et al., 2025). In India, available reports suggested increased susceptibility to PVE in puppies of pure breeds mainly, Labrador, Rottweiler, Dobermann, German shepherd and Spitz. The Rajapalayam breed is a conserved native breed in South India and puppies of this breed gained great demand because of it guarding behaviour (Raja et al., 2018). The positivity implied a significant association of the virus with the dog's utility (Kantere et al., 2021). Further investigation on pattern of disease incidence in indigenous breeds is essential for a strong conservation of the rare native breeds.

Higher incidence in the age group of 3.0-3.5 yr could be due to less number of HGE cases presented in this age group. Previously, several studies reported a higher incidence of PVE in dogs in the age group of 0-6 month old and preferably in non-descript dogs (Khare et al., 2019; Qi et al., 2020; Jayaprasad et al., 2025). In contrast to this report, in India, previously a very low incidence of 1.1% (Khare et al., 2019), 0.0% (Chethan et al., 2021) and 2.76% (Hemali et al., 2022) was recorded for CPV-2 in dogs of above 1-year-old presented with the signs of gastroenteritis.

The low incidence in all puppies above 4 month old could be associated with the acquired immunity due to natural infection in their later part of life (Khare et al., 2019; Sayed-Ahmad et al., 2020).The higher incidence in non-descript puppies reported in most of the studies was presumably due to the increased population density of non-descripts (Sayed-Ahmed et al., 2020) together with lack of awareness on vaccination (Chethan et al., 2021). The higher incidence in pure and native breeds observed in this study might be due to their low or poor acquired immunity.

Among sex, male was most susceptible than female, as most of the dogs presented for treatment belong to only male category and this finding was in accordance with that of previous studies (Khare et al., 2019). In contrast, Chethan et al. (2021) recorded a higher positivity in females than male dogs with PVE. Nevertheless, there is reported to be no sex predilection for CPV-2 infection in dogs (Greene, 2012). The incidence specific to age and breed was found to be significant ($p<0.01$) between groups within each host determinant.

The mean lymphocyte count, though within range, was significantly low in PVE cases ($p<0.01$) when compared to other HGE cases which showed lymphocytosis. Lymphocyte depletion in PVE occurs, resulting in immunosuppression, due to the virus affinity to bone marrow precursors, thymus atrophy (Decaro and Buonavoglia, 2020), cortisol release and protein-losing enteropathy (Sevim et al., 2025). The mean neutrophil count, though within range, was significantly high ($p<0.01$) when compared to that of other HGE cases with normal range of neutrophil count. The resultant leucocytosis could presumably be due to the absolute neutrophilia ($15.98x10^3/\mu l$) associated with the secondary bacterial complications resulting in sepsis (Ay et al., 2022), though the CPV-2 causes neutropenia in most of the cases (Behera et al., 2015). Hence, the absolute neutrophil count and lymphopenia are reported to be the prognostic indicators or biomarkers to predict the survivability of PVE cases (Zhou et al., 2025).

The mean NLR in PVE cases (7.65±1.08) was significantly high ($p<0.01$) when compared to that of other HGE cases (3.89±0.63), indicating the risk of sepsis in PVE cases. Previously, Munoz et al. (2022) recorded a significant increase in NLR (0.4-4.6) in puppies with PVE. The NLR is another biomarker or prognostic indicator that reflects the balance between innate and adaptive immunity. Persistent lymphopenia could also result from an elevation of neutrophils in sepsis (Munoz et al., 2022; Ay et al., 2022).

Elevated levels ($p>0.05$) of mean ALP (hepatopathy), hypocalcaemia and hyperphosphatemia could be associated with hepatic or renal hypoxia (pre-renal azotemia) in severely dehydrated cases of PVE (Shah et al., 2013). This effect could also be due to secondary bacterial infection that leads to change in cytokine response and systemic release of various inflammatory mediators with resultant organ damage (Paul et al., 2023).

Box and whisker plot analysis (Figure 3) indicated significantly higher neutrophil levels with significantly lower lymphocyte levels in PVE cases than other HGE cases. The differences were statistically validated by the Mann-Whitney U test, supporting their role as prognostic indicators in canine parvo viral enteritis. The median NLR in PVE group was notably elevated and several cases showed markedly high outliers, reflecting a strong SIRS. In contrast, the other HGE group had a more narrow distribution of NLR values with most cases clustering around lower values, indicating a milder systemic reaction. The bar chart also supports by showing a high mean NLR ($p<0.01$) in PVE group than other HGE group.

Conclusion

Dog breeds of above 1-4 years were under the 'window of susceptibility', raising the concern of native or adaptive immunity. Decreasing lymphocyte count, absolute neutrophilia and a high NLR were found to be useful biomarkers in early recognition of PVE cases and the risk of sepsis.

Conflicts of Interest: None

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