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Vivek Subramanian
Veterinary Assistant Surgeon, District Livestock Farm, Naduvoor, Thanjavur, Tamil Nadu, India

G Selvaraju
Professor and Head, Department of Veterinary Public Health and Epidemiology, Veterinary College and Research Institute, Namakkal. Tamil Nadu, India

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

KM Palanivel
Professor and Head, Department of Veterinary Preventive Medicine, Veterinary College and Research Institute, Namakkal. Tamil Nadu, India

Corresponding Author:
Vivek Subramanian
Veterinary Assistant Surgeon, District Livestock Farm, Naduvoor, Thanjavur, Tamil Nadu, India

Prevalence of canine parvo viral infection in domestic dogs by monoclonal antibody mediated faecal ELISA

Vivek Subramanian, G Selvaraju, S Saravanan and KM Palanivel

Abstract
The study was conducted to know the prevalence of canine parvovirus (CPV) infection in Namakkal region of Tamil Nadu, India. Totally 180 faecal samples, in which 120 and 60 faecal swabs were collected from clinically infected and apparently healthy dogs respectively, at Teaching Veterinary Clinical Complex, Veterinary College and Research Institute, Namakkal. Monoclonal antibody - mediated faecal Enzyme Linked Immuno Sorbent Assay (f- ELISA) test kits were used to detect the presence of parvovirus in faecal samples of dogs. Overall percentage of positivity for CPV infection in clinically infected and apparently healthy animals by faecal ELISA was 60 percent and 13.3 percent respectively. Highest percentage of prevalence (53.06%) was observed in 3 – 6 months age group. Pure bred domestic dogs were more prone to canine parvo infection (50.0%). Significantly, higher positivity was observed in males (50.68%) when compared with females (17.60%). As far as vaccination status and seasonal factors concerned, CPV infection was more prevalent in unvaccinated animals (59.32%) and south west monsoon season (57.2%).

Keywords: canine parvovirus, prevalence, f- ELISA, risk factors

Introduction
Dog has become indispensable in man's life as an embodiment of love and affection. Dogs have diversified utility ranging from tracking, hunting, and instruments of war, bomb detecting squad and healer of both physical and emotional problems of humans, detecting criminals and guiding blind people (Carmichael, 2003) [4]. In view of the need of dogs in these above aspects it is necessary to study the various diseases that are producing a high morbidity and case fatality rate. Among the predominantly occurring viral diseases of dogs, Canine parvovirus (CPV) infection is a highly infectious viral disease of dogs of great concern to pet owners, practicing veterinarians and scientists due to its high morbidity and mortality rates especially in young animals (Bargujar *et al*., 2011) [2].

Canine parvovirus disease occurs in two clinical forms – intestinal and myocardial. The main clinical symptom associated with the intestinal form of CPV-2 affected dogs is hemorrhagic gastroenteritis. It is difficult to precisely diagnose CPV-2 infections from the main clinical signs, such as vomiting and diarrhoea because these symptoms are more or less common to other enteric disease. Hence, a laboratory diagnosis of CPV is performed by demonstrating the presence of virus in faeces (Sakulwira *et al.*, 2001) [16]. An enzyme-linked immunosorbent assay (ELISA) system was developed for the rapid detection of canine parvovirus (CPV) or CPV antigen in dog faeces. Monoclonal antibodies were used to develop a double antibody enzyme-linked immunosorbent assay for the detection of canine parvovirus (CPV) antigen in faecal samples. The assay was specific for the hemagglutinating protein of CPV and detected as little as 1.5 ng of virus within a 15-minute incubation period. The use of monoclonal antibodies against 2 epitopes on the CPV antigen permitted the simultaneous addition of test sample and enzyme-conjugated antibody, thus considerably simplifying the manipulations required for the assay (Mildbrand *et al*., 1984) [11]. In this study monoclonal antibody mediated faecal ELISA kit was used to know the prevalence of canine parvo viral infection in Namakkal region of Tamil Nadu.

Material and Methods
A total of 180 faecal samples were collected from apparently healthy and clinically infected dog along with relevant epidemiological data at Teaching Veterinary Clinical Complex,

Veterinary College and Research Institute, Namakkal and presented in Table-1. Collected faecal samples were emulsified in 0.1M Phosphate buffer saline of pH 7.4 and centrifuged at 6000 rpm for 15 min at in a refrigerated centrifuge. The supernatant was collected and stored in -20◦c for faecal ELISA to detect the canine parvo virus. Six number of monoclonal antibody - mediated ELISA test kits (32 reactions per kit) were purchased from B.V. European Veterinary Laboratory, Netherlands to detect the presence of parvovirus in faecal samples of dogs (Plate -1). The kit contains, 4 x 8 well test strips (32 wells), 2 x buffer (green), 1 x conjugate (red), 1 x substrate A (White), 1 x substrate B (blue), 1 x positive control (yellow) and 1 x negative control (brown). Canine parvoviral antigen in faeces was detected by using monoclonal antibody mediated faecal ELISA Kit as per the protocol recommended by manufacturer.

Results and Discussion

All the collected 180 faecal samples were subjected to monoclonal antibody mediated faecal ELISA kit. Results were interrupted by comparing the colour of test samples wells with positive control and negative control substrate given in the faecal ELISA kit itself (Plate 2). Briefly, if the sample colour is dark blue, at least as blue as the positive control, the sample is scored as positive. But, if the sample colour is equally blue or less blue than the negative control, the sample is scored as negative Out of 180 samples 80 samples were found to be positive for canine parvovirus infection with per cent positivity of 44.44 by f-ELISA.

Enzyme linked Immunosorbent assay is common (Waner *et al*., 2003) [21], rapid (Nandi and Kumar, 2010) [13] and inexpensive (Chou *et al.*, 2013) [5] test in detection of parvovirus infection in dogs. In this study, prevalence of CPV infection by commercial faecal ELISA in dogs was 44.44 per cent in Namakkal. This is in agreement with Kumar *et al.* (2010) [10] who reported a prevalence of 45.30 per cent by ELISA in Uttar Pradesh.

Risk factors associated with prevalence of canine parvovirus infection are presented in table-1. Numerically higher positivity of 53.06 per cent was observed in the age group of 3-6 months followed by 0-3 months (45.33 per cent), 6-12 months (36.36 per cent) and above 12 months (33.33 per cent) without statistical significant between age groups. This is in agreement with Sakulwira *et al*., (2003) [17]; Thomas *et al.* (2014); Behera *et al.* (2015) [3] who reported that CPV infection was most commonly found between 3 and 6 months. Parthiban *et al.* (2010) [15] and Srinivas *et al.* (2013) [19] were also reported that prevalence of CPV infection found between 0 and 6 months. Higher prevalence in young animals might be due to increased intestinal epithelial turnover caused by the changes in microflora, diet and diminishing maternal antibody level which are the predisposing factors to CPV infection in pups as indicated by Decaro *et al.* (2004) [6]. Parrish *et al.* (1985) [14] were also indicated that canine transferrin receptor is expressed at high density on actively dividing cells and thus enhances the pathogenesis of parvovirus infection in young pups.

Similarly, higher positivity was observed in purebred (50.00 per cent) followed by non-descript (45.83 per cent) and crossbred (28.94 per cent) without statistical significance between breeds of dogs. Houstan *et al.* (1996) [7] and Wazir *et al.* (2013) [22] also reported that higher prevalence of canine parvovirus infection in purebred than other breeds. Higher positivity in purebred might be due to genetic predisposition. But, Archana *et al.* (2009) [1] and Behera *et al*. (2015) [3] also reported that highest prevalence of CPV infection in non-descript dogs when compared with pure breeds and it could be due to the lack of awareness of dog owners who usually pay less attention and care in terms of their vaccination, feeding, management and reporting the ailing dog at an early stage for the treatment.

Significantly (p<0.01) higher positivity was observed in males (50.68 per cent) when compared with females (17.60 per cent) between males and females. This is in accordance with the results of Archana *et al.* (2009) [1], Parthiban *et al.* (2010) [15], Islam *et al.* (2014) [8] and Behera *et al.* (2015) [3]. This might be due to travelling a long distance by males to mate or compete with other males over a bitch on heat. At the point of conglomeration they may become infected by the virus via contact with an infected bitch through sniffing and licking of the anal region which may be contaminated with infected faeces (Shima *et al*., 2014) [18].

Significantly (p<0.01) higher positivity was observed in clinically affected dogs (60.00 per cent) when compared with apparently healthy dogs (13.3 per cent). This is in agreement with Islam *et al*., (2014) [8] who reported that significantly higher prevalence of CPV was recorded in diarrhoeic dogs compared with those having no diarrhoea. He also stated that dogs with poor health condition were more vulnerable to canine parvovirus infection compared to those with normal health status.

Significantly (p<0.01) higher positivity was observed in unvaccinated dogs (59.32 per cent) and when compared with vaccinated dogs (16.12 per cent). This concordance with the finding of Parthiban *et al.* (2010) [15], Suartha *et al.* (2011) [20] and Shima *et al.* (2014) [18]. Higher numbers of CPV infection recorded in unvaccinated dogs most likely suggests that they lack protection from the virus, which in turn could be due to ignorance on the part of owner, high cost of vaccination, poor husbandry and faulty biosecurity practices (Muzaffar *et al*., 2006) [12].

Significantly (p<0.01) higher positivity was observed in Southwest monsoon season (57.20 per cent) followed by Northeast monsoon season (26.00 per cent) and winter season (20.00 per cent) between different season of Namakkal. This result is in agreement with Panneer *et al.* (2008) who found that the occurrence of more numbers of CPV infection during the month of June – August (South west monsoon). The possible reason for this may be stress due to change in weather, though earlier studies have indicated that during warm and humid conditions the number of CPV positive cases increases (Kaur *et al*., 2010) [9]. Present study indicated that significant predisposition exist in the sex, health and vaccination status and season.

Table 1: Prevalence of canine parvovirus infection based on f-ELISA

S. No.	Epidemiological factors	Categories	No of tested	No of positive by f-ELISA	Per cent positive	P value
1.	Health status	Apparently healthy	60	8	13.3	0.0001**
		Clinically affected	120	72	60.0	
2.	Age	0 – 3 months	75	34	45.33	0.353093 NS
		3 – 6 months	49	26	53.06	
		6 – 12 months	44	16	36.36	

		Above 12 months	12	4	33.33	
3.	Breed	Purebred	94	47	50	0.085925[NS]
		Crossbred	38	11	28.94	
		ND	48	22	45.83	
4.	Sex	Male	146	74	50.68	0.000791**
		Female	34	6	17.6	
5.	Season	Southwest monsoon	110	63	57.2	0.00123**
		Northeast monsoon	50	13	26.0	
		Winter	20	4	20.0	
		Summer	-	-		
6.	Vaccination status	Vaccinated*	62	10	16.12	0.0001**
		Unvaccinated	118	70	59.32	

[NS] – Not significant
** - Significant at 99% level

Plate 1: Monoclonal Antibody Mediated faecal ELISA kit for CPV antigen detection

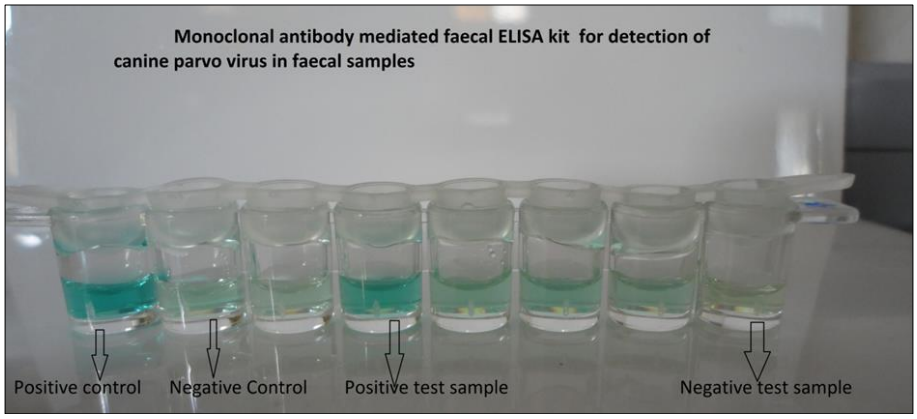

Plate 2: Results of CPV antigen detecting faecal ELISA kit

Conclusion

In this study, faecal ELISA is the rapid method of detection of canine parvo viral infection in the faecal samples and easy to perform within a period of one hour without any sophisticated instruments. It can be used in the canine medical practice for early diagnosis of canine parvoviral infection at initial stage.

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