SEROPREVALENCE AND DIAGNOSIS OF CANINE PARVOVIRAL INFECTION

By
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THESIS

Submitted in partial fulfilment of the requirement for the degree

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Kerala Agricultural University

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COLLEGE OF VETERINARY AND ANIMAL SCIENCES
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DECLARATION

I hereby declare that this thesis entitled "**SEROPREVALENCE AND DIAGNOSIS OF CANINE PARVOVIRAL INFECTION**" is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award to me of any degree, diploma, associateship, fellowship or other similar title, of any other University or Society.

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CERTIFICATE

Certified that this thesis entitled "**SEROPREVALENCE AND DIAGNOSIS OF CANINE PARVOVIRAL INFECTION**" is a record of research work done independently by **Miss. Deepa, P.M.**, under my guidance and supervision and that it has not previously formed the basis for the award of any degree, fellowship or associateship to her.

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INTRODUCTION

Dogs have formed a part of human civilization from times immemorial. Over the years, dog rearing has increased tremendously especially in urban and semi-urban areas. Therefore health cover to dogs has assumed great importance.

Diseases of dogs have always been a cause of great concern for millions of pet owners and veterinarians all over the world. Nevertheless, viruses as infectious agents in dogs, gained no less attention of scientists working on the subject.

The most dramatic change that occurred in veterinary gastroenterology in the decade of the 1980s was the emergence of viral enteritis as a common clinical problem. Prior to 1978, contagious enteritis was virtually unknown in dogs. Then beginning of 1978 and peaking in 1979 and 1980 clinicians began to encounter outbreaks of severe gastroenteritis among groups of dogs. The epidemiological profile was clearly one of the infectious agents with a relatively short incubation period.

The emergence of canine parvovirus (CPV) as a panzootic disease in dogs by about the mid 1978 was a phenomenon in

veterinary medicine, seemingly without precedence and has become an issue of great concern to pet owners, practicing veterinarians and scientists alike.

Although Binn *et al.* (1970) isolated parvovirus like agent from apparently healthy dogs, the importance of CPV as a pathogen for dogs has become evident only since 1977 (Eugster and Nairn, 1977).

Subclinically infected animals play a significant role in the epidemiologic chain. Serological evidence has suggested that the majority of infections are mild or subclinical. Since even subclinically affected dogs shed substantial quantities of the virus, these inapparent infections are important sources of contagion for dogs in kennels, shows, human shelters, and veterinary hospitals. Subclinical infection is also prevalent in stray dogs and wild canidae and feral dogs, and coyotes, wild red foxes, blue foxes, jackals, wolves, mink and bush dogs are important reservoirs of infection. Lenghaus (1980) suggested that the majority of domestic urban dogs had serological evidence of exposure to CPV infection and manifestation of subclinical diseases. Existence of endemic parvovirus cycles among wild canids and in large breeding colonies (Thomas *et al.*, 1984) and the persistence of the virus in the environment for long periods (5-7 months) make the eradication virtually impossible.

Eventhough the use of effective vaccines, has reduced the incidence of canine parvoviral infection among domestic dogs, outbreaks of canine parvoviral enteritis among vaccinated dogs have been reported occasionally.

Because of its rapid spread among susceptible population, and its panzootic nature, with high morbidity and mortality rate, the virus could evoke significant damage to the health of world dog population. Thus it becomes evident that an early and accurate diagnosis is absolutely essential for the proper treatment and control of the disease.

The diagnosis of fulminant cases of CPV is relatively easy, but less severe cases, which are more common, pose a diagnostic dilemma. In most instances it is impossible to differentiate CPV from myriad of other causes of acute enteritis in dogs on the basis of clinical signs alone.

The canine parvovirus infection has proved fatal in recent past in India, but being an emerging disease, requisite knowledge on the pathogenesis, pathology and diagnostic tests has not been adequately documented in our country. There is dearth of knowledge on different diagnostic tests and their application for the detection of parvovirus infection especially in India.

The present study was conducted with the following objectives.

* To study the prevalence of canine parvoviral (CPV) infections in dogs in Kerala by demonstrating canine parvoviral antigen in faeces and canine parvoviral antibodies in sera of dogs.

* To compare the sensitivity of different serological tests for early detection of antigen and antibodies against CPV.

Review of Literature

Chapter-II

REVIEW OF LITERATURE

2.1 History

Although initially a parvovirus like agent was isolated from faeces of apparently healthy dogs (Binn *et al.*, 1970), it was only since 1977 that canine parvovirus infection had become important in small animal medicine.

Eugster and Nairn (1977) found parvovirus in the faeces of puppies with diarrhoea in the United States, but unfortunately the virus they detected was not successfully propagated and its relationship to subsequent parvoviral isolates was uncertain.

The world wide epizootics in 1978 of a newly recognized infectious disease entity for dogs in which acute gastroenteritis with diarrhoea and myocarditis were the most prominent clinical signs was identified to be due to canine parvovirus (Appel *et al.*, 1979; Gagnon and Povey, 1979).

Studies on seroprevalence in Belgium (Schwers *et al.*, 1979), the Netherlands (Osterhaus *et al.*, 1980) and France (Chappius and Toma, 1980) indicated canine exposure to the virus in these countries as early as 1976.

2.2 Incidence

2.2.1 Global

Subsequent to identification of CPV in mid 1978, the disease was reported from several countries in the world.

Canine parvoviral enteritis had occurred in pandemic proportions in USA (Appel *et al.*, 1978; Eugster *et al.*, 1978). Thomson and Gagnon (1978) reported the disease entity in Canada, Jefferies and Blakemore (1979) in United Kingdom and Misciatteli *et al.* (1979) in Italy.

Johnson and Spradbrow (1979) reported the incidence of canine parvoviral enteritis simultaneously in two widely separated areas of Australia and isolated parvovirus from kidneys of moribund dogs.

Moraillon *et al.* (1979) reported a serious epidemic of CPV infection in dogs in France.

Schwers *et al.* (1979) reported the first clinical case of CPV infection in Belgium. Eventhough only 5.3 per cent of dogs showed CPV antibodies by the HI test during 1976-77, seroprevalence was increased to 36 per cent during 1979.

Walker *et al.* (1979) reported the first incidence of CPV infection in Australia in 1978 and suggested that it is a new

disease and not just the discovery of the cause of a disease that had been known clinically for many years.

Bergmann *et al.* (1980) observed an outbreak of canine parvoviral enteritis for the first time in Germany.

Carmichael *et al.* (1980) found that the first seropositive sample to CPV infection had appeared in USA in June 1978.

Evermann *et al.* (1980) observed an outbreak of canine parvoviral enteritis among a captive coyotes population in USA.

Helfer-Backer *et al.* (1980) found that positive titres (22.2%) to CPV infection were the same in both populations of families dogs and kennel dogs in Washington state.

Hornedo (1980) reported an outbreak of CPV infection with high morbidity and mortality in Mexico in 1980, affecting mostly puppies less than six months of age.

Lenghaus (1980) suggested that the majority of domestic urban dogs had serological evidence of exposure to CPV infection and manifestation of subclinical disease and also suggested that the high antibody titre in non-descript dogs attributed to constant natural exposures.

Mann *et al.* (1980) reported an outbreak of CPV infection affecting 14.2 per cent of South American canids at the National Zoological Park's, Department of Conservation and 20 per cent of canids that remained healthy had high HI titres to CPV before episodes of illness, indicating earlier subclinical exposure.

Merickel *et al.* (1980) observed an outbreak of acute canine parvoviral enteritis over 2 months in a closed research colony with morbidity and mortality 36 per cent and 12 per cent respectively.

Niemand *et al.* (1980) observed an outbreak of CPV infection in the Mannheim area between 1979 and 1980 with a mortality rate of 35.4% in Germany.

Olson *et al.* (1980) reported that the occurrence of CPV infection more than quadrupled in November and December 1979, but returned to normal in February 1980 in Sweden. The fall in prevalence was due to the increase in the number of dogs with the specific antibodies and also due to extensive vaccination.

Perl *et al.* (1980) reported the first incidence of CPV infection in Israel in 1980 where puppies aged 3 to 5 months were mainly affected.

Walker *et al.* (1980) showed that the first seropositive sample was collected in May 1978 in New South Wales, Australia and the number of serologically positive dogs has increased from 19 in 1978 to 90 in 1979.

Wierup (1980) reported an epidemic of CPV infection for the first time in Sweden in October 1979. Peak incidence was in December and only sporadic cases occurred after April 1980.

Azetaka *et al.* (1981) reported that the first seropositive sample to CPV infection was detected in January 1979 in Japan.

Binn *et al.* (1981) showed higher HI antibody titres in convalescent serum samples of 78 per cent of affected dogs and in 83 per cent of apparently healthy dogs.

Boros and Bartha (1981) observed that 93 out of 226 (41.1 per cent) dog carcasses examined in the summer of 1980 in Hungary, had typical gross and microscopic lesions of parvoviral enteritis.

Hinaidy (1981) observed that during 1980, 27.4 per cent of dogs showed canine parvoviral enteritis in Austria by HA and FAT.

Panjevic *et al.* (1981) reported the incidence of canine parvoviral enteritis for the first time in Belgrade area in Yugoslavia during 1980.

Valicek *et al.* (1981) reported an outbreak of CPV infection in dogs of all ages in Czechoslavokia in 1981 with 100 per cent morbidity and the mortality varied from 20 to 50 per cent.

Voros *et al.* (1981) reported an epidemic of acute canine parvoviral enteritis in Hungary in 1980.

Bucci *et al.* (1982) reported an outbreak of CPV infection for the first time in Egypt in 1981.

Bund and Laohasurayothin (1982) demonstrated antibodies to CPV in 95.4 per cent dog sera samples from West Berlin by HI test.

Chew-Lim *et al.* (1982) observed high titres of CPV antibodies in 75.5 per cent of 225 sera samples of unvaccinated dogs above 6 months of age between 1981 to 1982, in Singapore.

Hirasawa *et al.* (1982) detected CPV infection for the first time in Japan in 1979.

A longitudinal serological survey of CPV infection was conducted in New Zealand between 1974 to 1980 and the first seropositive titre (>1:320) was demonstrated in October 1979. The first confirmed clinical case of CPE had occurred in July 1979. In a serosurvey conducted between 1980 and 1981, 23 per cent of unvaccinated dogs showed HI titres >1:320 indicating previous CPV infection (Jones *et al.*, 1982).

Seroepidemiological survey on CPV infection in Japan from 1978 to 1979 showed that 16.7 per cent of dogs were seropositive, although none of the positive dogs had shown clinical signs of CPV infection (Mohri *et al.*, 1982).

Neuvonen *et al.* (1982) reported an outbreak of CPV infection among young raccoon dogs in Finland during 1980.

Sabine *et al.* (1982) reported that 60 per cent of urban dogs in sydney and 30 per cent of dogs from rural areas of Australia were exhibiting high levels of antibodies without evidence of clinical disease.

A serosurvey conducted among wild carnivores in Ontario, showed antibodies to CPV in 79.2 per cent red foxes, 22.3 per cent of wild raccoons, 1.3 per cent of wild skunks and in 6 of 7 coyotes (Barker *et al.*, 1983).

Serological investigations on the spread of CPV infection in Germany revealed that all samples were negative to CPV antibodies in whereas 1974/75, 67.7 per cent of samples were positive, during 1980/81 suggesting that canine parvovirus probably entered Germany in 1979 (Klunker *et al.*, 1983).

Serum antibodies to CPV were detected by indirect immunofluorescence in 63 per cent of healthy dogs in Germany in 1982, which was a higher proportion than that found in 1980 (34.5 per cent) (Maess *et al.*, 1983).

Wierup (1983) observed that prevalence of CPV infection was highest when the density of susceptible population exceeded 12/km² and ceased with the density of 6/km².

Carman and Povey (1984) observed that the first seropositive sample to CPV infection appeared during 1978 in Ontario, and in 1980, 28.6 per cent were seropositive. The infection was mainly subclinical.

Celer and Hejlicek (1984) observed the highest incidence of CPV infection among dogs in urban areas.

Cui *et al.* (1984) reported an outbreak of canine parvoviral enteritis in Shenyoung District, China in 1983.

Serological titres suggestive of recent CPV infection were found in upto 50 per cent of dogs tested, although the incidence of clinical illness was much less (Greene, 1984).

Hernandez *et al.* (1984) reported an outbreak of CPV infection for the first time in Costa Rica in 1981. But this outbreak was not serious compared to the reports in other countries.

Thomas *et al.* (1984) reported that there was no evidence of CPV infection among wild coyotes before 1979, after which seroprevalence rapidly increased to >70 per cent at all sites by 1982, suggesting that the onset of CPV-2 prevalence in free-ranging coyotes coincided with the recognition of the clinical disease and the seroprevalence in domestic dogs in the USA.

Veijalainen *et al.* (1984) reported the occurrence of CPV infection in blue foxes in Finland with serum antibody titres ranging between 640 and 2560.

A serosurvey among stray dogs in Franklin Country, Ohio in 1979 showed that all were seronegative, while in 1981, 69.2 per cent were seropositive. Of the total faecal samples examined, 12.4 per cent contained the virus, 76 per cent of which were seropositive (Gordan and Angrick, 1985).

Manson *et al.* (1985) observed an outbreak of CPV infection in a closed unvaccinated Beagle colony where dogs below 52 days of age did not show clinical signs indicating milk-borne immunity.

Hara *et al.* (1986) showed that HI titres of infected dogs kept in close confinment were over 1:10240, while privately owned dogs had low levels. In a dog pound, 56.4 per cent of dogs showed subclinical infection.

Ishikawa *et al.* (1986) conducted a serologic survey among laboratory Beagle dogs during 1976 to 1977 and found that titres were ranging from <1:16 to 1:32 and from 1980 to 1982 the titres ranged between <1:16 to >1:8192 indicating that the ratio of antibody positive dogs was higher than that in previous years.

Koptopoulos *et al.* (1986) conducted a retrospective survey of CPV infection in dogs by HI and SN test between 1969 and 1981 in Greece and found that all samples collected during 1969-70 were negative, while 3 out of 28 samples collected during 1974 were positive and 12.3 per cent were positive during 1979-81, suggesting that the positive reaction in 1974 were due to exposure to a related parvovirus from another species.

Rogers (1987) reported that CPV infection is the most frequently encountered viral infection of urban dogs with more than 2500 cases/annum.

Olson *et al.* (1988) in Sweden have reported that there is no significant difference in the HI titres between vaccinated and unvaccinated dogs of more than 12 months of age.

Schwendenwein *et al.* (1988) reported the incidence of CPV infection in 47 per cent of dogs with acute gastroenteritis, in Australia in 1985, 30 per cent in 1986, and 36 per cent in 1987, out of which 77, 69 and 27 per cent dogs died respectively.

Nicolae *et al.* (1990) investigated CPV infection both in an open kennel and closed experimental Beagle colony and found that the Beagle colony was completely free of infection, but more than half of the samples from the open kennel was positive.

Difruscia *et al.* (1991) conducted a study among diarrhoeic and normal dogs and found that 42.5 per cent of the diarrhoeic dogs were positive for CPV serologically and 23.7 per cent virologically. Of the normal dogs, 30 per cent were positive serologically and 10 per cent virologically.

England and Allen (1991) found that CPV antibody titres were similar in vaccinated and unvaccinated control groups, but this was due to incomplete partitioning of floor level, which allowed contact with vaccine virus excreted by the vaccinated dogs.

In a serosurvey among wild coyotes in USA from 1985 to 1988 showed that prevalence of antibody to CPV was 71 per cent and it did not differ among years, either between sexes or with age (Gese *et al.*, 1991).

In a serosurvey among blue fox vixens in Norway using HI test, 28 per cent vixens were found to be positive (Indrebo and Hyllseth, 1992).

Lu *et al.* (1992) reported that 70 per cent of dogs had positive HI titre against CPV infection in Taiwan during 1979-1982.

Alexander *et al.* (1994) observed that the prevalence of antibody to CPV-2 was 34 per cent among free ranging Jackals in Kenya between 1987 to 1988.

Mech *et al.* (1995) reported that CPV infection had little effect on a wolf population size, while the infection was endemic during 1979-90. Prevalence of CPV antibody in adult wolves increased to 87 per cent in 1993 and suggested that

winter wolf population will decline when the CPV prevalence among adult consistently exceeded 76 per cent.

Seroprevalence of CPV antibodies in breeding foxes and mink in Poland during 1993-94 showed that 28.7 per cent of foxes and 54.5 per cent of mink were positive (Mizak and Gorski, 1996).

2.2.2 India

Disease resembling CPV infection occurred since 1980 and proved fatal in many parts of India. An outbreak of a devastating gastroenteritis had been reported in canines by clinical evidence at Madras that closely resembled CPV (Balu and Thangaraj, 1981; Appaji Rao *et al.*, 1983). However, Ramadass and Khader (1982) reported the first confirmation of the etiological agent in India in 1982 from Madras.

Sherikar and Paranjape (1985) reported an outbreak of parvoviral enteritis in dogs in and around Bombay city in August 1981. High mortality (63.7 per cent) was observed among unvaccinated dogs. However, deaths (45 per cent) amongst the vaccinated dogs were attributed to faulty or improper time of vaccination.

Aiyappa (1986) detected the CPV specific antibody titres ranging from 1:32 to 1:512 in 21 per cent of sera samples from adult dogs and six out of 14 from pups in Bangalore.

Narasimhaswamy (1988) demonstrated CPV haemagglutinating antigen in 34.7 per cent of faecal samples from dogs with clinical signs of gastroenteritis in Bangalore.

Gunaseelan (1993) observed that the seropositivity among the vaccinates of both known breeds and non-descripts was significantly higher ($P<0.01$) when compared to non-vaccinates.

In the first serosurvey in India, Rajesh Mohan *et al.* (1993) using HI test detected 33 per cent seropositive samples for CPV infection in Punjab.

Seroprevalence of CPV infection in stray and pet dogs showed that 91 per cent of stray dogs and 28.6 per cent of pet dogs screened, were positive. Among HI positive stray dogs 61.1 per cent had high titres ranging from 1:320 to 1:2560, which was attributed to the possible repeat exposure to infection (Udupa and Sastry, 1996).

2.2.3 Kerala

Sulochana *et al.* (1987) reported an outbreak of canine parvoviral enteritis among dogs in Kerala particularly in Trichur and Ernakulam districts during June to August 1986,

and mortality was about 26 per cent. Out of the 15 apparently healthy animals nine had titres ranging from 64 to 1024. Since vaccination against this disease was not being practised at that time, it might be due to previous exposure to this virus.

2.3 Vaccine failure

Janssen *et al.* (1982) reported the occurrence of CPV infection in ten of 17 vaccinated juvenile bush dogs between 5 and 19 weeks old suggesting that maternal antibodies might have interfere with immunization.

Krakowka *et al.* (1982) observed distemper encephalitis in CPV vaccinated dogs suggesting that CPV alter the canine immune response and may be responsible for vaccination failures in dogs.

Ramadass *et al.* (1983a) reported CPV infection in seven of 10 dogs which had been immunized with live distemper virus vaccines indicating that vaccination might have reduced the immune response to parvoviral infection.

Greene (1984) suggested that the vaccination faliure may be attributed to inherent host factors, difficulties with the vaccine or errors made in the process of administration.

Brenner *et al.* (1989) showed a mortality rate of 62.5 per cent of dogs vaccinated with a live modified parvovirus vaccine, 9.1 per cent of dogs vaccinated with a killed parvovirus vaccine and 30.5 per cent of non-vaccinated dogs suggesting that live modified parvovirus vaccine might have immunosuppressive properties.

Dahlgaard (1989) investigated antibody content for CPV in vaccinated dogs and found that 25 per cent were negative, which explains why parvovirus can be the cause of gastroenteritis in such dogs.

2.4 Epidemiology

2.4.1 Natural host range

Appel *et al.* (1980) failed to reveal any CPV antibody in serum samples collected from people who were in contact with dogs suffering from CPV infection. Sera from more than 300 persons in close association with dogs suffering from CPV infection failed to reveal any HI antibodies to this virus (Carmichael and Joubert, 1980). Similar were the findings of Binn *et al.* (1981) where 19 laboratory personnel who worked with CPV affected dogs did not develop any antibodies and this group included 4 with concurrent diarrhoeal diseases.

Mann *et al.* (1980) reported the incidence of CPV infections in South American wild canids like bush dogs, crab eating foxes and maned wolves.

Domestic cats without antibody to feline panleukopenia virus were found to be susceptible to experimental infection with CPV without any apparent disease (Osterhaus *et al.*, 1980).

Carmichael and Binn (1981) reported that besides domestic dogs, most, if not all members of the genus canis are susceptible to CPV.

Appel and Parrish (1982) observed that raccoons were not susceptible to experimental CPV infection.

Neuvonen *et al.* (1982) showed that both raccoon dogs and blue foxes were susceptible to experimental CPV infection.

The natural host of FPV, MEV and CPV are the cat, mink and dog respectively (Siegl *et al.*, 1985).

Truyen and Parrish (1992) pointed out that canine parvovirus (CPV) have complex and overlapping host ranges and that distinct tissue tropisms exist in the homologus and heterologus hosts.

Truyen *et al.* (1996) observed that CPV type 2a and 2b replicate efficiently in cats, but CPV-2 does not replicate in cats indicating that cats can be naturally infected with CPV 2a or 2b.

2.4.2 Age

Eugster *et al.* (1978) observed that dogs of any breed, sex and age groups are affected with CPV enteritis.

Fluckiger (1980) reported that the occurrence of CPV infection were more than 80 per cent in dogs less than 18 months old and more than 50 per cent were in dogs at 3 months or younger.

Merickel *et al.* (1980) observed that weaned puppies are more susceptible to CPV infection than nursing puppies or adult dogs.

Mulvey *et al.* (1980) reported an acute parvovirus-induced myocarditis in 52 per cent of weanling pups between 4-8 weeks of age.

Smith *et al.* (1980) found that 67 per cent of seropositive dogs were less than six months of age and 87 per cent of clinical cases of CPE were associated with this age groups.

Woods *et al.* (1980) observed that eventhough dogs of all ages are susceptible, mortality rate was highest in 8-16 week old puppies.

Meunier *et al.* (1981) reported highest mortality due to CPV infection in 9 to 12 weeks old puppies, coinciding with decline in maternal antibody. Ramadass and Khader (1982) observed that majority of CPV cases were below six months of age.

Hammond and Timoney (1983) observed that half of the total number of faecal samples from dogs with gastroenteritis were from dogs less than six months of age with over 50 per cent of these positive for CPV.

Studdert *et al.* (1983) emphasized that CPV enteritis is a disease of pups less than six months of age.

An inverse relationship between antibody titres and age was observed (Jaroslow *et al.*, 1974; Schultz, 1984) and a stressful event in aging dogs may allow CPV infection to become established because of their already lowered immune capability (Mason *et al.*, 1987).

Mason *et al.* (1987) reported that outbreaks occurred in dogs less than six months of age with 74 per cent morbidity and 42 per cent mortality.

Rogers (1987) reported that young unvaccinated dogs between 12 and 16 week old are commonly affected with CPV infection.

Sulochana *et al.* (1987) observed the majority of CPV infection in pups below 8 months of age, eventhough dogs of all ages were affected.

Ernst *et al.* (1988) reported that the risk for CPV infection in dogs younger than six months was significantly greater than other age groups.

Sherikar *et al.* (1989) reported that adults seemed to be more affected than pups.

Gunaseelan (1993) observed that dogs below 6 months of age showed higher positivity to CPV, followed by dogs above 12 months of age.

Observations on different age groups indicated that 71.4 per cent of dogs in the age group of 7.9 months were infected with CPV infection (Udupa and Sastry, 1996). Similar observation has been made by Parrish *et al.* (1980) in which they have found CPV infection in 91.6 per cent of dogs older than six months.

2.4.3 Breed

Doberman Pincshers and Rottweilers were thought to be at increased risk of CPV enteritis (Rowley, 1981).

Ramadass and Khader (1982) reported that all breeds are equally susceptible to CPV infection.

Glickman *et al.* (1985) claimed that Doberman Pinschers, Rottweilers, and English Springer Spaniels were significantly susceptible to CPV enteritis.

Sherikar and Paranjape (1985) observed no significant susceptibility difference between the various breeds, though Doberman and Pomeranian breeds were most commonly affected.

Rogers (1987) reported an increased incidence of CPV infection in Rottweilers, Doberman Pinschers, and German shepherd dogs.

Ernst et al. (1988) reported that setters and pointers are more susceptible to CPV infection.

Gunaseelan (1993) observed that the prevalence of CPV infection was significantly higher ($P<0.05$) in breeds other than Doberman, German Shephered and non-descripts.

Mizak and Mizak (1994) observed that 85.9 per cent of German Shepherd dogs had CPV antibody titres ranging from 10 to 20480 and 33.3 per cent had titres ≥1280 suggesting natural infection.

Houston *et al.* (1996) reported that Rottweilers, American pit bull terriers, Doberman Pinshers, and German shepherd dogs were at greater risk and toy poodles and cocker spaniels were at lower risk of developing CPV enteritis.

Prevalence of CPV infection in different breeds indicated no specific breed predisposition to this infection (Udupa and Sastry, 1996).

2.4.4 Sex

Fluckiger (1980) observed more occurrence of CPV infection among males than in females.

Prange *et al.* (1982) reported that males account for 72 per cent total deaths due to CPV infection.

Ramadass and Khader (1982) reported that both sexes were equally susceptible to CPV infection.

Glickman *et al.* (1985) further observed that male Doberman and Rottweilers of six months of age and adult female English Springer spaniels were more susceptible.

The influence of sex on the incidence of CPV infection was not significant (Rogers, 1987; Ernst *et al.*, 1988; Udupa and Sastry, 1997).

Gunaseelan (1993) reported that the sex-wise prevalence rate within each breed was not found to be significant.

Houston *et al.* (1996) observed that sexually intact dogs >6 months of age were at 4 times greater risk than spayed or neutured dogs and intact males >6 months were twice as likely as sexually intact females to develop CPV enteritis.

2.4.5 Season

Kelly (1978) in Australia reported an outbreak of CPV in the months of August, September, October and March through May.

Hammond and Timoney (1983) while screening faecal samples from an epidemic of gastroenteritis in dogs in USA by EM found 48 per cent CPV positive in the samples collected during August and September.

Horner (1983) reported the peak incidence of CPV infection over spring and summer months from October to March in New Zealand. This probably reflects breeding cycles and also greater movement of animals to shows and boarding kennels.

Studdert *et al.* (1983) found peak positivity of CPV in August, September and October in USA.

Sherikar and Paranjape (1985) in Bombay observed that the incidence of CPV infection varied from month to month and the seasonal influence on the occurrence of CPV was not significant.

Ernst *et al.* (1987) in chile observed peak seasonality of CPV infection from January to March and suggested that climatic variants accounted for 22.5 per cent of the variation of disease prevalence and regression coefficient indicated that an increasing occurrence of cases during periods of high temperature and humidity.

Mason *et al.* (1987) while surveying the epidemiology of parvovirus enteritis over an extended period of seven years found that in five of seven years, outbreaks occurred in August, September or October, while in two of the seven years, outbreak occurred in March or April in USA.

The possible explanation for these time peaks would be a corresponding increase in susceptible population either through whelpings and weaned puppies (Greene, 1984).

Ernst *et al.* (1988) observed that there was a significantly lower risk for CPV infection during May, June, July and September in Chile.

Gunaseelan (1993) reported that the incidence of CPV infection were higher during April (5.6 per cent), June (2.8 per cent) and July (6 per cent) in Madras.

Houston *et al.* (1996) in Canada reported that dogs were more susceptible to CPV infection during July, August and September compared to rest of the year.

2.5 Diagnosis

Sabine *et al.* (1982) found through their survey that 31 per cent of practicing veterinarians in Australia and New Zealand used clinical signs as the sole method of diagnosis and during severe outbreaks, the information obtained was reasonably accurate.

2.5.1 Clinical signs

Infection with CPV may be manifested by myocarditis or gastroenteritis in susceptible dogs of any breed, sex and age groups (Fugster *et al.*, 1978; Black *et al.*, 1979).

The main clinical signs in CPV infection were vomiting, diarrhoea and dehydration. The vomitus was usually grayish

white and watery and the feces were usually first grayish or yellowish, then 1 to 2 days later contained various amounts of unclotted or partially clotted blood. Respiratory tract infection and mucopurulent nasal discharge were also observed (Eugster *et al.*, 1978).

Kelly and Atwell (1979) and Ingh *et al.* (1980) reported myocarditis syndrome in CPV infection with sudden death in 3-10 week old puppies.

Pollock and Carmichael (1979) recorded clinical signs in CPV infection as enlargement of lymphnodes, blood in the faeces, development of small vesicles in the mouth which may rupture and leave ulcers.

Rensburg *et al.* (1979) observed the occurrence of both gastroenteritis syndrome and myocarditis due to CPV infection in the same puppies.

An experimental inoculation of dogs carried out by Eugster (1980) showed that the susceptibility to CPV infection and the severity of the clinical manifestation of the disease were closely related to the immune status and responsiveness of individual animals.

The clinical manifestation of CPV infection within a group of puppies varieds considerably from an asymptomatic or

transient dullness and anorexia to an acute haemorrhagic gastroenteritis (Harcourt *et al.*, 1980).

Jedliczka (1980) observed the main symptoms in CPV were apathy, anorexia, vomiting, haemorrhagic diarrhoea, dehydration, tachycardia, weak pulse rate, moderate fever and showed leucopenia with less than 4000 leukocytes/mm^3 with mortality rate of 57.7 per cent.

Robinson *et al.* (1980) observed that puppies affected with CPV myocarditis showed signs of acute heart failure, rapid weak pulse and dysponea followed by sudden death.

Meunier *et al.* (1981) observed that clinical signs were variable and included fever, depression, dehydration, loose mucoid stool and watery diarrhoea with occasional vomiting and/or haematochezia. An increased incidence of respiratory disease was noted in affected dogs.

Panjevic *et al.* (1981) observed that after 2-5 days of incubation, infected dogs showed high temperature, great thirst, inappetence, red vomitus, and haemorrhagic diarrhoea Mortality was high (10-50%).

Voros *et al.* (1981) reported an acute form of CPV infection in dogs aged two months to 3 years, lasting 6-7 days showing clinical signs of severe anorexia, weakness, moderate

or high fever, recurrent vomiting, abdominal pain, watery and foul smelling blood stained diarrhoea, and between the second and fifth days severe leukopenia was observed.

Prange *et al.* (1982) observed clinical signs of CPV infection included feebleness, inappetance, persistent vomiting, and diarrhoea, sometimes haemorrhagic, morbidity rate of 2.5 per cent and mortality rate of 14.6 per cent.

Johnson and Castro (1984) isolated CPV from brain of a puppy, 7.5 week old which showed clinical signs like sudden onset of circling and blindness by FAT.

Thompson *et al.* (1985) observed that CPV infection has no effect on reproductive disorders and an attempt to isolate parvovirus from aborted fetuses, vaginal swabs and semen samples were unsuccessful.

Rajesh Mohan *et al.* (1993) showed that anorexia, depression and dehydration were common initial signs of CPV infection followed by vomiting and diarrhoeawithin 6-36 hours. In 92 per cent of the cases faeces contained blood. High body temperatures were observed in 26.7 per cent cases, convulsions in 2 cases, and oral mucosal lesions in one case.

2.5.2 Laboratory diagnosis

Though clinical signs supported a diagnosis of CPV enteritis in approximately 30 per cent of cases (Studdert *et al.*, 1983), virus detection or isolation is the most specific means of confirming parvoviral infection. Though large amount of the virus is present in the faeces, detectable parvovirus regardless of the methods used, is present only upto a short period after illness, which corresponds to two to three days after onset of clinical signs. Faecal submission within the first three days of clinical illness is frequently necessary to be of value in confirming a diagnosis (Carmichael and Binn, 1981; McCandlish *et al.*, 1981).

2.5.2.1 Agar gel immunodiffusion test (AGID)

Ramadass and Khader (1982) reported that a total of 45 out of 87 dogs (51.7%) with symptoms of haemorrhagic enteritis were found to be positive for CPV infection by using AGID. AGID test was found to be as sensitive as FAT. They examined salivary samples from these dogs by using AGID and observed a few samples to show positive precipitation lines.

Rao *et al.* (1983) confirmed six cases of concurrent infections with canine distemper and canine parvoviral infections by AGID.

Saseendranath *et al.* (1992) reported that 44 out of 158 (28 per cent) faecal samples from puppies aged 6-12 weeks with clinical signs of gastroenteritis were positive to CPV infection by agar gel immunodiffusion test.

Out of 249 faecal samples collected from dogs with symptom of haemorrhagic enteritis and screened by using AGID, 11.2 per cent were found to be positive to CPV infection (Gunaseelan, 1993).

Gunaseelan *et al.* (1993) developed a rapid agar gel immunodiffusion test using concentrated anti-canine parvovirus hyperimmune serum to detect canine parvovirus in faeces within a period of 3 to 4 hours. They found that 19 out of 42 faecal samples (45.2%) were positive. Though there was 100 per cent correlation with the test using unconcentrated anti-canine parvorirus serum, the time taken was very short when concentrated serum was used.

2.5.2.2 Counter immunoelectrophoresis (CIEP)

Schwers *et al.* (1980) used counter immunoelectro *osmophoretic technique for the detection of CPV antigen and* antibody from dogs with gastroenteritis and found that 52 per cent were positive for CPV antigen. On comparing CIEP and HI, they observed a clear precipitating band for all serum samples in which the HI titres were above 40.

Toma *et al.* (1982) conducted a survey for CPV antibodies in human beings using CIEP test and all samples were found to be negative indicating that parvovirus show little infectivity for man.

Schwers *et al.* (1983) demonstrated antibodies to CPV infection in four of the 142 (2.8 per cent) fox serum samples by using counter immunoelectroosmophoresis.

Zuffa and Rejholcova (1988) used counter immunoelectrophoresis (CIEP) for the detection of Aleutian disease antibodies in mink and found that 75.9 per cent were seropositive.

Serum samples of 112 sows, 53 sows with reproductive disorders, 61 gilts, 10 boars, 20 piglets that had not been given colostrum and 33 aborted swine fetuses were tested by CIEP and 100, 84.9, 63.9, 100, 40 and 27.3 per cent respectively were positive for parvoviral antibodies, suggesting that CIEP was sensitive in 81.3 per cent, whereas HI test was sensitive in 75.5 per cent only (Gaitamonova, 1990).

Ganesan *et al.* (1990) reported that when 900 faecal samples from dogs with enteritis were screened by using counter immunoelectrophoresis, 26 gave positive reactions for

corona viral antigen and 5 per cent of the dogs had mixed infections with canine parvovirus.

Salomskas *et al.* (1991) observed that 3.77 per cent of faecal samples from calves showed positive reaction for rotavirus by using CIEP test.

Saseendranath *et al.* (1992) reported that 44 out of 158 (28 per cent) faecal samples from puppies aged between 6 to 12 weeks with clinical signs of gastroenteritis were positive to CPV infection by using counter immunoelectrophoresis.

2.5.2.3 Haemagglutination test

CPV agglutinates pig and rhesus monkey RBC at 4°C and 25°C, but not at 37°C (Appel *et al.*, 1979; Burtonboy *et al.*, 1979).

The haemagglutination (HA) activity of CPV was utilized diagnostically in estimating the amounts of viral haemagglutinin in faecal samples and suggested that a HA titre of 64 and above were taken as positive (Carmichael *et al.*, 1980).

Carmichael *et al.* (1980) observed that the faecal HA titres of CPV ranged from 320 to 10, 240 between four and seven days post-infection or when signs of enteritis commenced and HA ceased, generally between days seven and nine post

infection. The specificity of the HA can be determined by simultaneous testing of samples by HI test with a CPV reference antiserum (Carmichael *et al.*, 1980; Mochizuki *et al.*, 1989).

A modification of HA test, was done, wherein CPV antigen in faecal samples were absorbed on to pig erythrocytes at 4°C; the antigen eluted from the cells at 37°C and the elute was then tested for HA activity. This was to remove any non-specific HA activity (Osterhaus *et al.*, 1980).

Mathys *et al.* (1983) observed that chloroform treatment of faecal samples had no influence on HA titres above 32 and also found that by using formalin treated rhesus macaque erythrocytes for HA test for the detection of CPV in faeces made no adverse influence on the HA sensitivity or specificity. Further, it extends the useful life of the stored erythrocytes.

Carmichael *et al.* (1980) observed that CPV strongly agglutinate porcine RBC and rhesus macaque RBC at the optimal pH of 7.2 and also found an inverse relationship between the porcine erythrocyte concentration and CPV HA titres. With each 0.5 per cent increase in erythrocyte concentration, there was an approximate two fold reduction in HA titre.

McCandlish *et al.* (1981) suggested that the simplest and quickest test for CPV detection is to check faecal sample for viral haemagglutinin using a suspension of pig erythrocytes.

The most simple, sensitive and rapid method of detecting CP virus was haemagglutination, which was twice as sensitive as virus isolation and eight times as sensitive as electronmicroscopy (Studdert *et al.*, 1983).

Celer (1984) reported that CPV agglutinate erythrocytes of pig and macaca at 4°C, but not those of cat, horse, cattle, sheep, rabbit and guinea pig.

Janthur and Kokkles (1984) used supernatant fluids from chloroform extracted suspensions of faeces as antigen in the HA test using piglet erythrocytes and found that HA titres were ranging from 1:40-1:40960.

Sherikar and Paranjape (1985) investigated an outbreak of parvoviral enteritis in dogs and found that 71 per cent and 72 per cent faecal samples were positive by HA and HI test with titres ranging from 4 to 2048 and 8 to 2048 respectively.

Komalafe (1985) detected CPV in the faeces of dogs six months after recovery from natural CPV infection indicating a carrier state in CPV infection.

Aiyappa (1986) and Narasimhaswamy (1988) used faecal HA test for demonstration of CPV in clinical cases of gastroenteritis and found that 20 per cent and 34.8 per cent were positive with HA titres ranging from 1:64 to 1:8192 and 1:32 to 1:16384 respectively.

Senda *et al.* (1986) in modifying the HA test found that by using two buffers, one alkaline (Borate buffered saline) and one acid (virus adjusting diluent) CPV strains agglutinated wider range of erythrocytes than had been previously reported and HA titres were observed at 37°C. They claimed the procedure as inconvenient and premixing of these two buffers resulted in lowering the CPV HA titres to the extent of eight fold.

Sulochana *et al.* (1987) showed that in an outbreak of CPV infection in dogs, 75.5 per cent were positive by HA test with HA titres ranging from 40-5020.

Sherikar *et al.* (1989) found that faecal HA and HI test were more rapid and economic than serum haemagglutination inhibition test, EM and FAT.

Szaniszlo and Horvath (1989) demonstrated CPV in faeces by using HA test and 79.5 per cent showed positive reaction with titres ranging from 1:160 to 1:10240, but no relation was found between titre and severity of illness.

Mohan *et al.* (1992) showed that 91.7 per cent of dogs with clinical signs of CPV infection were positive by HA test with titres ranging from 1:40 to 1:20480 and majority between 1:160 and 1:20480.

Gunaseelan (1993) reported that 18.1 per cent faecal samples were positive to CPV infection by HA test with titres ranging from 64 to 2048 and also suggested that chloroform treatment of faecal samples did not significantly alter the HA titres of samples, signifying the absence of nonspecific haemagglutinins.

Gunaseelan (1993) compared AGID, FAT and HA for detection of CPV in faeces and found highest positive correlation between FAT and HA (84.4%), while relationship between AGID and FAT, HA and AGID was of decreasing order.

Rai *et al.* (1994) observed that 85 per cent of faecal samples from clinical cases of haemorrhagic gastroenteritis were positive with titres ranging from 40 to 20480 and found that majority of these with HA activity of 320 to 20480 were collected 3 to 6 days after the onset of clinical illness.

Le-Than-Hai *et al.* (1995) investigated the relationship between excretion of CPV virus and immune status in dogs and found that out of 39 per cent of 64 healthy, under 1 year old dogs with HI titres from 1:128 to 1:572 and HA titres from

1:128 to 1:8192, 10.9 per cent of dogs excreted virus in their faeces, whereas 47 per cent of 71 dogs over 1 year old with HI titres from 1:128 to 1:2048 and HA titres from 1:128 to 1:4069, none excreted the virus in their faeces. Dogs with clinical signs of canine parvovirosis had HA titres of 1:128 to 1:16384 and HI titres of 1:128 to 1:256.

In comparing Latex agglutination test (LA), HA and EM for detection of CPV in faeces, Sanekata *et al.* (1995) found that LA and EM had identical sensitivities, but HA was 16 times more sensitive.

Udupa and Sastry (1996) observed that 15.3 per cent of stray dogs and 10 per cent of pet dogs were shedding the CPV virus in faeces with HA titres varying from 1:64 to 1:4096 and 1:64 to 1:16384 respectively.

Udupa and Sastry (1997) found that 60.5 per cent of dogs with clinical gastroenteritis were positive to CPV infection by serum HI test and of these 59.7 per cent of dogs had positive faecal HA titres with titres ranging from 1:64 to 1:262144.

2.5.2.4 Haemaglutination inhibition test

Pollock and Carmichael (1979) and Appel *et al.* (1980) observed significant amounts of CPV antibodies in the serum of

affected dogs, even five days following infection and recommended HI test for routine serological diagnosis of CPV infection.

Carmichael *et al.* (1980) considered HI titres of >320 and SN titre of >90 positive.

CPV antibodies in sera were reported to persist at high levels for at least one year (Carmichael *et al.*, 1980).

A reciprocal HI titre of >80 is considered by some laboratories sufficient enough to protect against virulent CPV infection (Pollock and Carmichael, 1982, Olson *et al.*, 1988). Udupa and Sastry (1996) suggested that a HI titre of 80 or more can be regarded as positive.

Walker *et al.* (1980) reported that a HI titre of 256 or more should be regarded as positive.

Azetaka *et al.* (1981) in an experimental inoculation studies found that, SN antibody was parallel with HI antibody in the time of appearance, development pattern and titre. They suggested that a HI titre of 128 or more can be regarded as positive.

An outbreak of CPV infection among laboratory Beagles showed that 78 per cent of the affected dogs and 83 per cent

of apparently healthy dogs were positive to CPV antibodies (Binn *et al.*, 1981).

Mohri *et al.* (1982) found that 16.7 per cent serum samples from stray dogs were positive to CPV infection by HI test.

HI assay requires initial absorption of serum to remove nonspecific haemagglutinins and inhibitors, which can reduce specific HI titre by upto four fold (Carmichael *et al.*, 1983).

Janthur and Kokkles (1984) conducted HI test using an infective faecal extract and piglet RBC and found that HI titres were ranging between 1:40 to 1:1280.

In an experimental infection, Ma Cartney (1984) detected circulating antibodies at day 5 after inoculation and the level increases rapidly with titres at 1024 or greater in all sera collected on or after day 7.

Olson *et al.* (1988) found that in nonvaccinated dogs <12 months old, 50.7 per cent of dogs had titres ≥1:10, in vaccinated dogs <12 months old only 11.5 per cent had titres ≥1:80 and in vaccinated adult dogs >12 months old, <50 per cent had titres ≥1:80, regardless of the time after vaccination and there was no significant difference in titres between vaccinated and non vaccinated dogs.

Mohan *et al.* (1992) reported that 87.6 per cent of serum samples showed positive reaction to CPV with titres varying from 1:40 to 1:2560.

Gorski *et al.* (1993) found that non-specific thermostable inhibitors of haemagglutination of CPV can be efficiently removed from serum samples by the absorption with 25 per cent Kaolin suspension, with which the sensitivity of HI can be increased.

Gunaseelan (1993) observed that seropositivity to CPV infection among vaccinates (82.1 per cent) was significantly higher ($P \geq 0.01$) when compared to non-vaccinates (65.3 per cent).

Rai *et al.* (1994) reported that 33 out of 36 (91.6 per cent) serum samples from clinical cases of CPV infection between 4 to 10 days were positive with titres varying from 40 to 1280.

Udupa and Sastry (1997) showed that 75.3 per cent dogs with clinical cases of gastroenteritis had positive serum HI titres ranging between 1:80 to 1:20480 and suggested that serum test was more sensitive than faecal HA test.

2.5.2.5 ELISA

Rice *et al.* (1982) compared systemic and local immunity in dogs with canine parvoviral enteritis and found that dogs with high levels of CPV coproantibodies had high serum antibody levels measured by HI and ELISA and low viral HA titres in faeces. Dogs with no coproantibody had high viral titres in the faeces.

Teramoto *et al.* (1984) compared ELISA, DNA hybridization, HA and EM for CPV detection in faeces, found highest correlation between ELISA ad HA (94.4 per cent). The study indicated ELISA to be a sensitive and specific diagnostic assay for CPV infection.

But Herbst *et al.* (1986) reported that ELISA is not as sensitive as EM, but it is simpler and quicker.

Fiscus *et al.* (1985) developed a rapid enzyme linked immunosorbent assay (ELISA) to detect the presence of anti CPV antibodies in dog serum using peroxidase conjugated monoclonal anti-canine immunoglobulins (IgG). They found that the results correlated well with SN and HI titres. Sera with SN titres <1:4, HI titre <1:10 had 87.9 per cent correlation with ELISA, while sera with SN titres of >1:64 or HI >1:80 had 94.4 per cent correlation. Since SN and HI assays have inherent

variables, the ELISA is useful for monitoring dogs for the presence of CPV antibodies.

Florent (1986) found that 28 dogs with a HI titre for CPV antibodies were strongly positive for CPV IgG by ELISA, but negative for CPV antibodies of the IgM class, thereby indicating that the HI test can be conveniently used for the serological assay for CPV antibodies.

Ei-Sanousi-A (1990) developed dot enzyme immunoassay for the direct detection of canine parvovirus in faeces and organs of diseased as well as those of dead, aborted and/or stillborn puppies. The assay was more sensitive compared with the standard solid phase ELISA, simple to perform and was recommended as a rapid technique for diagnosing CPV infection.

Rimmelzwaan *et al.* (1990) evaluated two different ELISA system (indirect and competitive ELISA) using monoclonal antibodies for the detection of serum antibodies to CPV against HI test and found that the indirect ELISA was about 2-10 times more sensitive than HI test, however the specificity of those systems might have been higher because of the use of the CPV specific monoclonal antibodies.

Drane *et al.* (1994) compared ELISA and HA test for the detection of CPV antigen and found that ELISA had a sensitivity of 87 per cent and specificity of 100 per cent

compared with 87 and 63 per cent respectively for the HA test. The poor specificity of HA results in low predictive value of 51 per cent compared to 100 per cent for the CPV ELISA.

Hara *et al.* (1994) comparing indirect ELISA and HI test demonstrated the higher sensitivity of the ELISA, with its titres showing a good correlation with the HI test.

2.5.2.6 Electronmicroscopy

Examination of faeces, intestinal contents and affected tissues by electron microscopy (EM) had been widely used to detect CPV particles (Eugster *et al.*, 1978).

Arens and Krauss (1980) detected parvovirus in 57.7 per cent of dogs with acute gastroenteritis and also in some convalescent and incontact animals, which had not shown signs of disease by immuno electronmicroscopy.

Osterhaus *et al.* (1980) demonstrated parvovirus like particles in the faeces of 13 dogs in an outbreak of contagious diarrhoea by negative contrast electronmicroscopy.

Carmichael *et al.* (1980) observed that several cases which were negative by EM were found to be positive by HA test and he compared EM, faecal HA, virus isolation and HI test on serum samples and found that samples with faecal HA titres

>2048 were positive by EM, virus isolation and by HI test with titres >640.

Hoffmann *et al.* (1980) conducted EM examination of faeces from dogs with acute gastrointestinal symptoms and 78 per cent showed parvovirus, and 8.6 per cent corona virus.

Lenihan *et al.* (1980) demonstrated parvovirus particles approximately 20 nm in diameter in the heart tissue from six puppies aged 2 to 6 weeks which died due to parvoviral myocarditis and suggested that electronmicroscopic examination of negatively stained cardiac tissue may be useful for rapid diagnosis of CPV myocarditis.

Roseto *et al.* (1980) conducted EM study on dog faeces collected at random and parvovirus were found in 23.2 per cent, coronavirus in 12.5 per cent, rotavirus in 3.5 per cent and both corona and parvovirus in 8.9 per cent samples.

Williams (1980) showed Astrovirus-like, coronavirus-like and parvovirus-like particles by EM of diarrhoeal faeces of pups and suggested that coronavirus and parvovirus are recognized agents of canine viral enteritis, eventhough astrovirus has not been previously reported in dogs.

Valicek *et al.* (1981) observed that six of 39 samples from diarrhoeic dogs were CPV positive, eight of 39 samples

were positive to rotavirus, and out of these 2 samples were also positive to both parvovirus and rotavirus.

Danner and Weber (1983) showed that 50 out of 115 (43.4 per cent) faecal and intestinal samples were positive to CPV by IEM, whereas 30 samples (26 per cent) were positive by HA test.

An electron microscopic examination of faecal and intestinal contents from dogs with clinical signs of gastroenteritis showed that CPV was the major virus identified (48%). In addition to CPV, three cases of coronavirus, two rota virus like cases, one astrovirus like cases and three dual infection with CPV and rota or CPV and astro-like virus were confirmed. They compared direct EM and IEM and found that 10 per cent samples were positive by EM, whereas 54 per cent were positive by IEM. Thus, the adoption of IEM facilitated identification of CPV and greatly increased the sensitivity of the technique (Hammond and Timoney, 1983).

Mochizuki *et al.* (1984) demonstrated CPV infection in 11 out of 65 (16.9 per cent) dogs by IEM and HA test.

Sherikar and Paranjape (1985) found that 15 out of the 20 faecal samples revealed parvo-virus like particles about 18-20 nm in diameter by EM and some of these particles were empty.

Herbst *et al.* (1987) reported that 38 per cent samples from dogs with diarrhoea were positive to CPV, 6 per cent to coronavirus, 0.7 per cent to both corona and parvovirus 1.1 per cent to corona vrius like particles, 1 per cent to picorna virus like particles and 0.7 per cent to rotavirus.

Biermann *et al.* (1991) conducted EM examination of faecal samples from enteritis cases in domestic dogs and cats, and parvovirus was found in 18.5 per cent of canine and 8.5 per cent of feline samples.

Vieler and Herbst (1995) detected CPV in 17.2 per cent of faecal samples by EM.

2.5.2.7 Polymerase chain reaction

Mochizuki *et al.* (1995) compared PCR, virus isolation and haemagglutination for detection of CPV in faecal samples and found that PCR was as sensitive as virus isolation and was more sensitive than HA test.

Subhashini *et al.* (1997) compared PCR, latex agglutination test (LAT) and improved haemagglutination assay for detection of CPV in faecal samples of dogs with clinical signs of gastroenteritis and found that the correlation between PCR and HA was 96.7 per cent, whereas that between LAT and HA was 82.1 per cent. They suggested that PCR assay is

highly sensitive and specific and is superior to LAT for detecting CPV infections.

2.5.2.8 Coagglutination test

Singh and Abhilasha (1998) developed coagglutination test (COAT) for the rapid diagnosis of CPV infection, compared COAT and AGID, and found that AGID is slightly less sensitive than COAT. In comparing COAT and HA, HA is more sensitive than COAT, besides COAT has an advantage over HA, as the COAT reagent can be stored for a considerably longer period. Thus, COAT can be considered as simple, rapid, sensitive and specific, and can be recommended as field test for diagnosis of CPV infection.

Materials and Methods

Chapter-III

MATERIALS AND METHODS

The study was carried out in the Department of Preventive Medicine, College of Veterinary and Animal Sciences, Mannuthy during 1997-98.

3.1 Materials

3.1.1 Glasswares and reagents

In this study, Borosil brand of glasswares, Laxbro plastics and analytical or guaranteed reagent grade chemicals were used.

The materials were processed using standard methods (Hoskins, 1967) and sterilized either in hot air oven or autoclaved depending upon the materials to be sterilized.

3.1.2 Collection and preservation of test faecal samples

A total of 57 faecal samples were collected from dogs of various breeds and of both sexes, showing signs of persistent vomiting and haemorrhagic enteritis, suggestive of canine parvoviral infection, over a period of 18 months. Collection of samples was made with sterile rectal swabs and was immersed in sterile phosphate buffered saline (PBS, pH 7.2).

The samples were then centrifuged at 5000 rpm for 20 min in a refrigerator centrifuge at 4°C (Remi, C-24). The supernatents of the samples were treated with 1/10 volume of chloroform. After vigorous shaking, the samples were kept for 10 minutes undisturbed and centrifuged at 1500 rpm for 15 minutes. The supernatents of the samples were frozen at -20°C till use.

3.1.3 Test sera samples

Blood from three hundred and forty nine dogs of various ages with known vaccination history were collected from all over Kerala. Serum was separated and suitable aliquots were stored at -20°C. The sera samples were screened for the presence of antibodies against canine parvovirus by the serological tests, AGID, CIEP, HI and Dot-ELISA.

Detailed history of each case was collected in the format (Appendix-I).

3.1.4 Agar gel immunodiffusion test

Reagents

a.	Noble agar (Difco)	- 1.2 g
	Sodium chloride	- 0.85 g
	Sodium azide	- 0.01 g
	Distilled water	- 100 ml

b. Agar coated slides

Clean microscopic slides were dipped in 1% melted agar and dried in air by keeping the slides horizontally over glass rods. The dried slides were stored at room temperature until use.

c. Staining solution

Amidoblack IOB	-	1 g
Sodium chloride	-	8.5 g
Distilled water	-	1000 ml

d. Destaining solution

Hypertonic saline (1.5%)

Glacial acetic acid (7%)

3.1.5 Counter immunoelectrophoresis (CIEP)

Reagents

a. Veronal acetate buffer (pH 8.6)

Barbitone sodium	-	10.31 g
Barbituric acid	-	1.84 g
Sodium acetate	-	6.8 g
Sodium azide	-	0.5 g
Distilled water to make	-	1000 ml

b. Agarose - 1.2 g

Veronal acetate buffer - 100 ml

c. Agar coated slides

3.1.6 Haemagglutination and Haemagglutination inhibition test

Reagents

a. Alsever's solution

Dextrose	- 10.25 g
Trisodium citrate	- 4.00 g
Sodium chloride	- 2.10 g
Citric acid	- 0.275 g
Distilled water to make	- 500 ml

The pH of the solution was adjusted to 7.2 and autoclaved at 115°C for 30 min and stored at 4°C till use.

b. Phosphate buffered saline (PBS)

PBS stock (10x)

Sodium chloride	- 80 g
Potassium chloride	- 2 g
Disodium hydrogen phosphate	- 11.33 g
Potassium dihydrogen phosphate	- 2 g
Distilled water to make	- 1000 ml

The pH of the solution was adjusted to 7.2 and autoclaved at 121°C for 15 min.

PBS working solution (pH 7.2)

PBS (10x)	- 100 ml
Distilled water to make	- 1000 ml

c. Diluent for haemagglutination test (PBS-BSA)

Bovine serum albumin, fraction V (Sigma)	- 0.1 g
Phosphate buffered saline	- 100 ml

Diluent was maintained at 4°C till use.

3.1.7 Globulin separation

a. Saturated ammonium sulphate solution (SAS)

This was prepared by adding 760 g of ammonium sulphate to one litre of double distilled water. This was heated at 56°C for 30 min in a water bath with continuous stirring. The solution was filtered by using whatman No.1 filter paper to remove insoluble impurities and then cooled at room temperature. The pH of the solution was adjusted to 7.0 with ammonia solution just prior to use.

b. Working ammonium sulphate solution

Solution of 66 per cent strength was prepared (v/v) freshly from the stock solution of saturated ammonium sulphate.

c. Ammonium hydroxide solution

d. Normal saline

e. 10% barium chloride solution

f. Borate buffered saline, pH 8.5

Borate buffer

Boric acid	-	6.184 g
Borax	-	9.53 g
Sodium chloride	-	4.384 g
Distilled water	-	1000 ml

The above reagents were transferred to a one litre volumetric flask containing 600-800 ml of distilled water and shaken until the contents were completely dissolved. To this, distilled water was added to make the volume to one litre and the pH was adjusted to 8.5 using 1N NaOH.

Borate buffered saline

Borate buffer	-	5 ml
Normal saline	-	95 ml

3.1.8 Conjugation

Reagents

a. 0.1 M potassium phosphate solution

Potassium phosphate (KH_2PO_4)	- 1.36 g
Distilled water	- 100 ml

b. 1 per cent gluteraldehyde solution

Gluteraldehyde (25 per cent solution)	- 0.2 ml
Distilled water	- 9.8 ml

c. Total protein and albumin estimation

Reagent 1	Biuret reagent
Reagent 2	Buffered dye reagent
Reagent 3	Protein standard

3.1.9 Immunoelectrophoresis

Reagents

a. Tris-barbital buffer

Barbitone sodium	- 9.9 g
Tris (hydroxy methyl amino methane)	- 17.7 g
Sodium azide	- 0.3 g
Distilled water	- 1000 ml

pH adjusted to 8.6 with 1N Hcl

b. Agar coated slides

c. Melted agarose

Agarose	-	0.8 g
Tris barbital buffer	-	100 ml

3.1.10 Dot ELISA

Reagents

a. Canine parvovirus antigen

Canine parvovirus antigen supplied by M/s Indian Immunologicals, Hyderabad was used for the present study.

b. PBS-Tween 20 (PBST)

PBS (10x) solution	-	100 ml
Tween 20	-	500 µl
Distilled water to make	-	1000 ml

pH was adjusted to 7.2

c. 5 per cent skim milk powder solution

Milk powder	-	5 g
PBST to make	-	100 ml

d. Peroxidase conjugated Rabbit Anti-dog Immunoglobulin (1:10) solution

Peroxidase-conjugated rabbit anti-dog Immunologbulin	-	10 µl
PBST	-	0.09 ml

Peroxidase conjugated Rabbit Anti-dog Immunoglobulin (1:1000) solution (Sigma, VSA)

Peroxidase conjugated rabbit anti-dog Immunologbulin	- 10 µl
PBST	- 9.99 ml

e. Substrate solution

3-3' Diamino benzidine tetrahydrochloride	- 5 mg
Hydrogen peroxide (30 per cent)	- 30 µl
PBS (pH 7.4)	- 10 ml

3.2 Methods

3.2.1 Preparation of anti-canine parvovirus (Anti-CPV) hyperimmune serum

The inactivated canine parvovirus vaccine (PARVOCINE, Tech America) was used to produce anti-canine parvovirus serum in rabbits as per Ramadass and Khader (1982).

Two healthy male rabbits aged six months procured from Rabbit Research Station of Kerala Agricultural University were used for this purpose.

Rabbits were injected intramuscularly with one ml of vaccine antigen emulsified in Freund's adjuvant (DIFCO). Totally four injections were given at an interval of 10 days. Freund's complete adjuvant (FCA) was used for the first

injection and Freund's incomplete adjuvant (FICA) was used for subsequent injections. Ten days after the last injection, the rabbits were test bled from ear vein and serum samples tested by AGID for the antibody. When the results were found satisfactory, the rabbits were bled and the serum was separated, inactivated at 56°C for 30 minutes and stored in small aliquotes of 2 ml each at -20°C.

Positive control sera

Anti-CPV hyperimmune sera prepared in rabbits were employed in the tests, as known positive sera, for screening the faecal samples for the detection of antigen.

Negative sera

Sera from healthy dogs which were not immunized and were not infected with CPV were taken as negative controls.

Positive canine parvovirus antigen

Attenuated parvoviral vaccine supplied by M/s Indian Immunologicals and inactivated parvovirus vaccine (PARVOCINE, Tech America) were used as positive control antigens.

3.2.2 Agar gel immunodiffusion test (AGID)

The test was carried out as per the method described by Williams and Chase (1971) with some modifications.

Preparation of agar gel slides

Noble agar (DIFCO) 1.2 g was melted with 100 ml of normal saline solution, cooled to 60°C and 0.01 per cent sodium azide was added. Four ml of melted agar was poured on to a clean microscope slide and allowed to solidify and then kept at 4°C till use.

Test proper

Wells of 5 mm diameter were punched out on the solidified agar over the slide at a distance of 3-4 mm. The wells were charged with about two to three drops of the positive control serum, suspected sample, and positive control antigen in separate wells. The slides were incubated in a moist chamber at room temperature for about 24 to 48 hours.

Washing and drying of slides

The slides were kept in 1.5 per cent hypertonic saline solution with three changes at 8 hours interval, to remove the soluble non-reacting constituents.

Drying was done by placing a strip of good quality filter paper over the surface of the gel and kept at 37°C for 24 hrs. The filter paper was removed and the slide was cleaned for a few seconds in running water to remove the adhering particles of filter paper.

Staining procedure

The method followed by Williams and Chase (1971) was used for staining the dried gels.

The dried slides were immersed in 0.1 per cent Amido Black stain solution for about 10 minutes.

Destaining was carried out using 7 per cent aqueous glacial acetic acid till the background was decolourised.

When complete decolouration was obtained, the slides were dried in air and the results obtained were recorded.

A white precipitate between the antigen and the suspected serum sample was taken as a positive result.

3.2.3 Counter immunoelectrophoresis

This test was carried out as per the method described by Ramadas *et al*. (1983b).

Staining and preservation

Staining was carried out as described in AGID. A white precipitate between anodal and cathodal wells was taken as positive.

3.2.4 Immunoelectrophoresis

The test was carried out as per the method of Williams and Chase (1971) with some modifications.

The 0.8 g agar in Tris-barbital buffer was melted and three ml of melted agar at 50°C was poured on to each slide. The agar was allowed to solidify initially at room temperature and subsequently at 4°C. Wells and central trough were cut on each slide.

After removing the agar, the wells were filled with antigens. A drop of bromophenol blue dye was added to the side of the well as an indicator. The slides were then placed in the electrophoresis chamber in such a way that the antigen wells were nearer to the cathode than to anode. Contact between the slides and the buffer was effected by filter paper wicks on each end of the slide. Power supply at the rate of 5 mA per slide was given and the electrophoresis was continued till the indicator dye reached 1 cm away from the anode end of the slide.

The power supply was disconnected, slides were taken and the agar in the troughs were removed carefully. The troughs were then filled with respective antisera (antidog whole serum) and left at room temperature in the electrophoretic chamber for 20-24 hrs.

The slides were examined against a light for the development of precipitin arcs and then the slides were washed and stained as for AGID.

3.2.5 Haemagglutination test

The haemagglutination (HA) test was carried out as per the method of Carmichael *et al.* (1980) with few modifications in a 96 well U bottom microtitre plates (Laxbro).

Preparation of pig red blood cells suspension

Swine blood was collected in Alsever's solution to prepare pig red blood cell (PRBC) suspension. It was centrifuged at 3000 rpm for 10 minutes to remove the plasma portions and the buffy coat layers. They were then washed three times in ice cold PBS-BSA and packed at 3000 rpm in a refrigerated centrifuge for ten minutes. After the final wash, a one per cent PRBC suspension was made in PBS-BSA.

Test proper

A two fold dilution (0.05 ml) of the test faecal samples starting from an initial 1:2 dilution was made in ice cold PBS-BSA and 0.05 ml of cold one per cent PRBC were added to all wells. The plate was incubated at 4°C for 2-4 hours. Controls were included with positive and negative faecal samples and all controls in separate rows of the microtitre plates. The results were read when buttons were formed in the control wells. The highest dilution forming a uniform mat was considered as the end point.

3.2.6 Haemagglutination inhibition test

This test was carried out as per the method of Carmichael *et al.* (1980) with few modifications using 96 well U-microtitre plates.

The sera samples were inactivated at 56°C for 30 minutes and 1:10 dilution in PBS was made. This was treated with 0.1 ml of 50 per cent PRBC to remove non-specific inhibitors of HA and allowed to stand overnight at 4°C. The sera were then centrifuged at 1500 rpm for 15 minutes to remove PRBC. The supernatent which was inactivated and PRBC treated was used for serology.

Test proper

A two fold dilution (0.025 ml) of the test serum was made starting with a 1 in 20 through 1 in 40,960 in ice cold PBS-BSA. After adding 0.025 ml of antigen from Indian immunologicals, titrated to (4 to 8 HA units) to the serum, and then kept at room temperature for one hour. Then 0.05 ml of one per cent PRBC was added to all wells and plates were incubated at 4°C, HI end points were determined after an overnight incubation.

The controls consisted of PRBC, positive control serum sample and HA virus antigen. The haemagglutination inhibition (HI) end points were determined after the incubation period and read as reciprocals of serum dilutions that completely inhibited 4 to 8 units of HA antigens and were expressed as HI titres.

3.2.7 Preparation of antidog whole serum

The animal was immunized by the following schedule.

One ml of whole dog serum was homogenized with one ml of Freund's complete adjuvant and was given intramuscularly to a healthy rabbit aged 6 months. Three booster doses of 1 ml each of whole dog serum without adjuvant was given at weekly intervals by the same route.

Ten days following the last injection, test bleeding was done to assess the antibody response by AGID, and immunoelectrophoresis. When the results were found satisfactory, the animal was bled, the serum separated, inactivated at 56°C for 30 minutes and stored at -20°C in small aliquotes of 1 ml each to be used as rabbit antidog whole serum.

3.2.8 Globulin separation

a. **Globulin from the normal dog serum was separated using the procedure described by Garvey (1977)**

Fifty ml of 66 per cent ammonium sulphate solution (ASS) was added dropwise to a 50 ml of serum sample while stirring. The stirring of serum ASS mixture was continued for 30 minutes after the addition of last drop of ASS and the precipitate was allowed to stand overnight at 4°C. Next day the suspension was centrifuged in a refrigerated centrifuge at 3000 rpm for 30 minutes. The precipitate so obtained was dissolved in enough saline to restore the original volume of serum and reprecipitated twice following the above procedure, omitting overnight keeping of suspension at 4°C. The precipitate from the third precipitation was dissolved in borate buffered saline to a final volume of 20 ml. The ammonium sulphate was removed from the precipitate by dialysing against borate buffered saline at 4°C.

b. Estimation of globulins

Globulin was estimated using the Qualigens kit. Serum globulin was calculated by substracting the serum albumin value from the total serum protein (Benjamin, 1985). The results were recorded in gm/dl.

c. Preparation of antidog globulin

Antidog globulin was raised in two rabbits by dissolving the serum globulins in borate buffered saline having a concentration of 15 mg/ml. A total of three injections at interval of 10 days was given. Ten days following the last injection rabbits were bled, serum separated inactivated at 56°C for 30 min and tested by AGID and immunoelectrophoresis. When the results were satisfactory, globulins were used for HRPO conjugation after checking its purity and concentration.

d. Conjugation

The labelling of antidog globulin with horse radish peroxidase was done as per the procedure described by Avrameas (1969) with slight modifications.

The antidog globulin was reconstituted with borate buffered saline to obtain 7 mg of globulin/ml and the pH was adjusted to 6.9 by the addition of 0.1 M solution of potassium phosphate. For each ml of the above solution, 12 mg of HRPO

enzyme was added and after its complete dissolution, 0.05 ml of gluteraldehyde was added. The mixture was shaken for 2 hours at room temperature by end-over-end rotation. The product thus obtained was then dialysed overnight at 4°C against physiological saline, pH 7.4. Next day the solution was centrifuged for 15 minutes at 1500-2000 g. The supernatent was collected and stored in small aliquates at -20°C..

3.2.9 Dot-ELISA

This test was carried out as per the method described by Hawkes (1986) with minor modifications.

One microlitre of an optimal dilution (1:10) of canine parvovirus antigen in PBS (pH 7.2) were dotted on to a nitrocellulose membrane. After air drying the antigen was fixed to the membrane by baking at 80°C for 30 minutes. The unreacted sites were blocked by incubating the membrane at 37°C in five per cent solution of skim milk powder for 30 minutes. The membrane was then washed thrice in PBST for 10 minutes and incubated in 1:100 dilution of known positive, known negative and test serum at 37°C for 45 minutes and then washed thrice in PBST. After washing, the membrane was treated with 1:1000 dilution of peroxidase conjugated rabbit antidog globulin and incubated at 37°C for 30 minutes. the HRPO conjugate prepared in our laboratory did not give results even at 1:10 dilution. The reason might be the addition of

horse radish peroxidase enzyme in the conjugation procedure. The NC membrane was then washed thrice for 10 minutes each time in PBST solution and treated with freshly prepared DAB substrate solution for 10 minutes. The reaction was stopped by rinsing the membrane in tap water.

Positive reaction was evidenced by the appearance of brown dots.

3.2.10 Electronmicroscopy (EM)

Electronmicroscopy was carried out as per the method described by Cooper *et al.* (1979) with slight modifications.

Faecal samples were clarified twice by low speed centrifugation at 5000 rpm for 30 min at 4°C. The supernatents were subjected to ultracentrifugation at 100000 g in a Beckman type 28 rotar for 2 hours at 4°C. The pellet was resuspended in 0.2 ml of distilled water and was used for electronmicroscopic examination. One drop of this virus suspension was placed on a formavar coated grid. After 30 seconds the excess fluid was absorbed in a whatman No.1 filter paper. The grid was then stained with phosphotungstic acid (PTA 2 per cent aqueous solution, pH 6.5) for 30 sec. After blotting the excess PTA, the grid was dried at 37°C overnight and examined in a Hitachi 500 G electronmicroscope at 75 KV, at a magnification of 1,20,000.

3.2.11 Immunoelectronmicroscopy (IEM)

Immunoelectronmicroscopy was carried out as per the method described by Hammond and Timoney (1983) with slight modifications.

Faecal samples were centrifuged at 1000 g for 15 minutes and the supernatant was mixed with anti CPV hyper immune serum in a ratio at 1:4. The mixture was kept at 4°C overnight and centrifuged at 1,00,000 g for two hours. The resultant pellet was resuspended in 2-8 drops of distilled water. Staining and examination of the grid was done as per the method described in EM procedure.

Results

Chapter-IV

RESULTS

The canine parvovirus (CPV) antigen in the faecal samples of 57 clinically suspected dogs was studied using agar gel immunodiffusion (AGID) and counter immunoelectrophoresis (CIEP). The virus titre was assessed by haemagglutination (HA) test.

The sera samples collected from 57 clinically suspected dogs and 292 apparently healthy dogs including vaccinated ones were examined for the presence of CPV antibodies using AGID, CIEP and Dot-ELISA. Antibody titre was assessed by haemagglutination inhibition (HI) test.

4.1 Detection of antigen

4.1.1 Agar gel immunodiffusion test (AGID)

The results of AGID are presented in Table 1 and Fig.1.

Out of the 57 tested faecal samples collected from clinically suspected dogs tested, 9 (15.7 per cent) were found to be positive for CPV antigen. All positive samples were also positive by HA test with titres between 512 and 2048. Of the negative samples tested by AGID, 26 were positive by HA with titres between 64 and 512, and the remaining negative

samples showed HA titres ranging from 0 to 32. All positive cases showed pyrexia, inappetance, vomition, dysentry and dehydration.

4.1.2 Counter immunoelectrophoresis (CIEP)

The results are presented in Table 1, Fig.1.

Faecal samples from eleven cases (19.3 per cent) out of the 57 collected from clinically suspected dogs were positive by CIEP. The HA titres of these positive cases were between 256 and 2048. The two samples that were negative by AGID, but positive by CIEP, had HA titres of 256 and 512. All positive cases showed clinical signs like pyrexia, inappetance, vomition, dysentry and dehydration.

4.1.3 Haemagglutination (HA) test

The results are presented in Table 1 and 2, Fig.1 and 2 and Plate 1.

Among the 57 clinically suspected dogs, 35 (61.4 per cent) were having the canine parvoviral antigen in the faecal samples. The HA titres of the faecal samples ranged between 0 and 2048. Titres of 64 and above were taken as positive as suggested by Carmichael *et al*. (1980). The frequency of the samples with their corresponding HA titre are shown in Table 2 and Fig.2.

Among these positives, five (8.8 per cent), seven (12.2 per cent), nine (15.7 per cent), six (10.5 per cent), five (8.8 per cent) and three (5.3 per cent) were showing HA titres of 64, 128, 256, 512, 1024 and 2048 respectively.

Positive cases were showing clinical signs like pyrexia in 31 cases (88.6 per cent), inappetance in 35 cases (100 per cent), vomition in 32 cases (91.4 per cent), dysentry in 29 cases (82.8 per cent), and respiratory distress in 5 cases (14.3 per cent).

One 10 month old male Dalmation dog (O.P. No.922) showing inappetance, pyrexia, blood tinched vomition, and dysentry for about three days had a HA titre of 64. Another a six month old male Dachshund dog (O.P. No.13574) with HA titre of 2048 had shown pyrexia, inappetance, vomition and was passing fresh blood along with faeces on the first day and died immediately.

A one year old male Alsation dog (O.P. No.12311) vaccinated 6 months back with the killed vaccine showed pyrexia, inappetance, vomition and dysentry for about three days and had a HA titre of 32. While, a three month old male Alsation dog (O.P. No.22459) vaccinated with killed vaccine 1 month back, a five month old male Alsation dog (O.P. No.5619) vaccinated with killed vaccine 2 months back and a one and half year old female Doberman dog (O.P. No.14119) vaccinated with killed vaccine at 3 months of age, showed

Table 1. Diagnostic tests to detect CPV antigen in faecal samples

Diagnostic tests	Number of samples tested	Samples positive	Percentage
Agar gel immunodiffusion test (AGID)	57	9	15.7
Counter immuno-electrophoresis (CIEP)	57	11	19.3
Haemagglutination test (HA)	57	35	61.4**
Electronmicroscopy (EM)	8	1	13
Immunoelectron-microscopy (IEM)	6	1	17

** Highly significant $P \leq 0.01$

Table 2. Frequency of canine parvovirus haemagglutination assay titres in faecal samples

HA titre	Sample frequency	Per cent of total samples
Nil	3	5.3
2	4	7.0
4	3	5.3
8	5	8.8
16	3	5.3
32	4	7.0
64	5	8.8
128	7	12.2
256	9	15.7
512	6	10.5
1024	5	8.8
2048	3	5.3

HA titre equal to or above 64 was taken as positive

Fig. 1 COMPARISON OF AGID, CIEP AND HA TO DETECT CPV ANTIGEN IN FAECAL SAMPLES

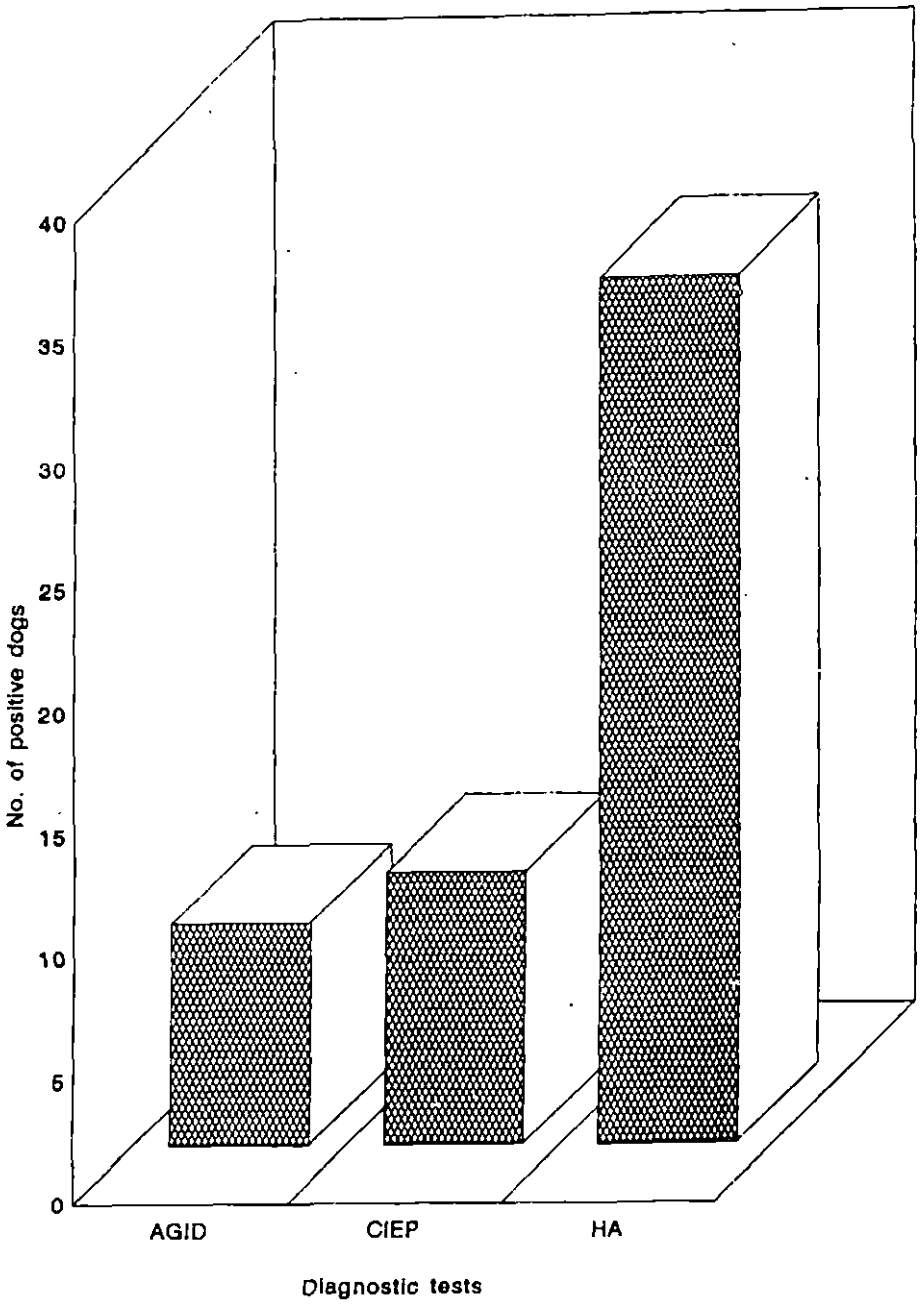


Fig.2 FREQUENCY OF CANINE PARVOVIRUS HA TITRES IN FEACAL SAMPLES

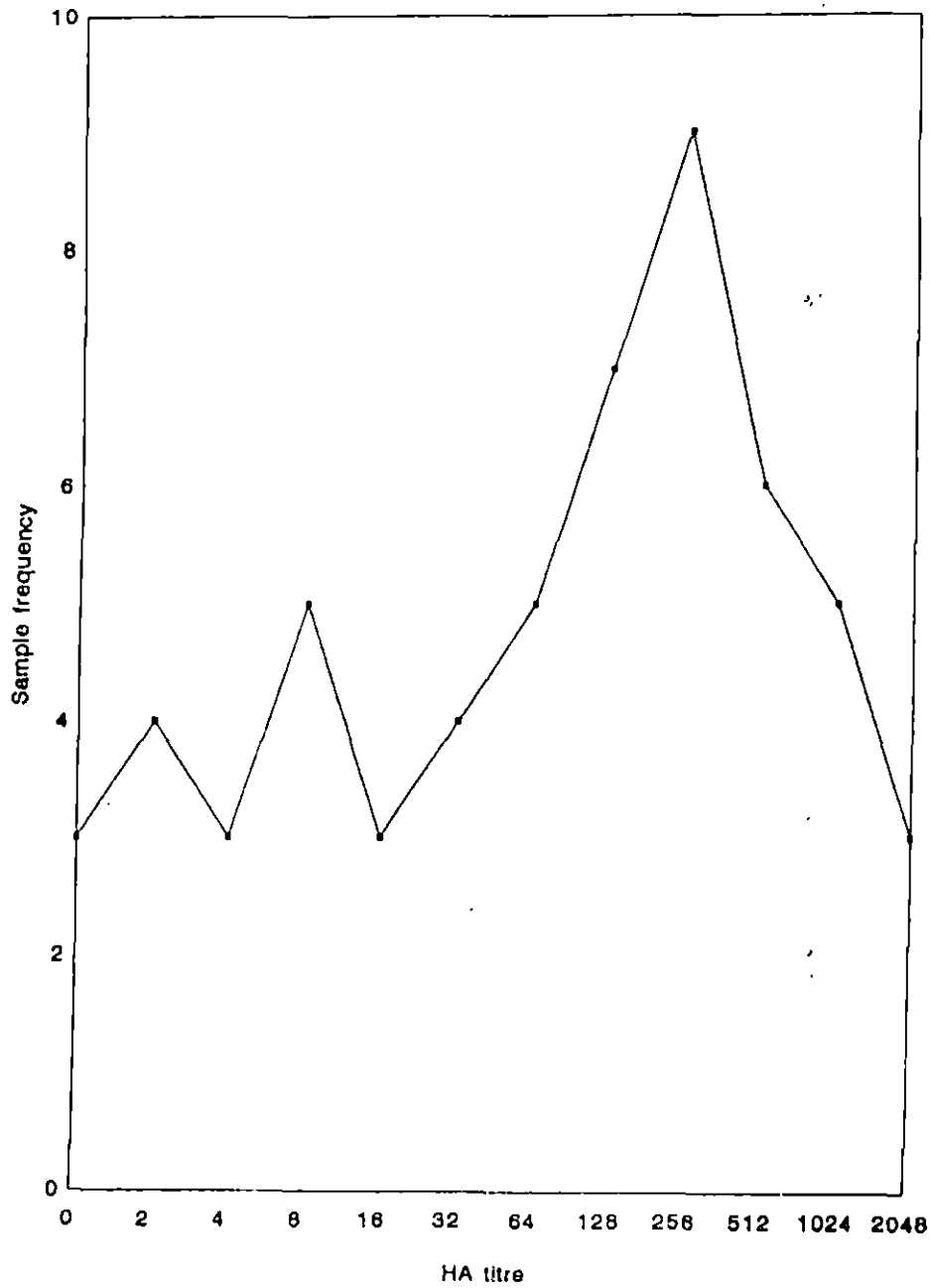
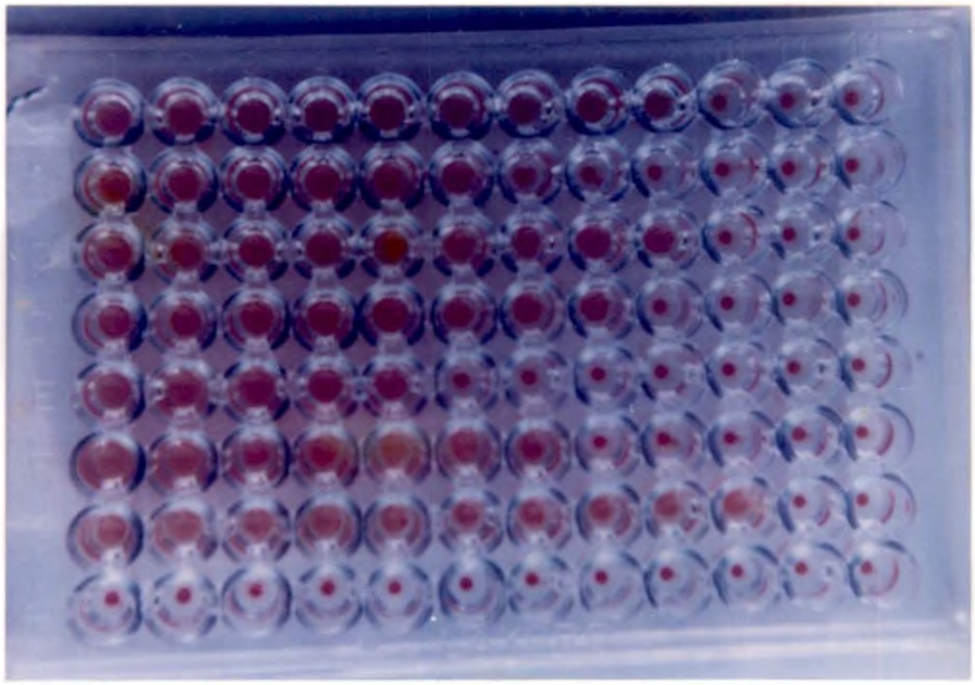


Plate 1. Haemagglutination assay for CPV in faecal samples

Rows A to F	:	Test faecal samples
Row G	:	Positive control sample
Row H	:	Cell control



pyrexia, inappetance, vomition and dysentry for two days and had HA titre of 128, 256 and 64 respectively.

A three months old Doberman pup vaccinated 10 days back with modified live virus vaccine and a two years old Doberman dog vaccinated 4 months back with killed vaccine, showed pyrexia, inappetance, vomition, and dysentry for about two days had HA titres of 256 and 64.

4.1.4 Electronmicroscopy

The results are presented in Table 1. Out of eight faecal samples from clinically suspected dogs examined, one (13 per cent) were found to be positive for CPV by EM. Virus was the size of 22 nm.

4.1.5 Immunoelectronmicroscopy

The results are presented in Table 1. Faecal samples from one case (17 per cent) out of six clinically suspected dogs were positive for CPV by IEM.

4.2 Detection of antibody

4.2.1 Agar gel immunodiffusion test

Among the 349 animals, including healthy vaccinated dogs, 38 (10.9 per cent) were positive for CPV antibody. The results are presented in Table 3, Fig.3 and Plate 2.

Out of the 57 clinically suspected dogs screened, 7 (12.3 per cent) were positive for CPV antibody (Table 4). Sera from 14 dogs (36.8 per cent) out of the 38 healthy vaccinated dogs tested, were positive for CPV antibodies (Table 5). Out of the 254 healthy nonvaccinated dogs studied, 17 (6.7 per cent) were found to be positive for CPV antibody (Table 6).

4.2.2 Counter immunoelectrophoresis

The sera collected from 349 dogs examined, 39 (11.2 per cent) were found to be positive for CPV anibody and the results are presented in Table 3, Fig.3 and Plate 3.

A total of 57 sera samples from clinically suspected dogs were screened, and 8 (14 per cent) were found to be positive for CPV antibody (Table 4). Among 38 vaccinated dogs, 14 (36.8 per cent) were found to be positive for CPV antibody (Table 5) and 17 (6.7 per cent) were positive out of 254 healthy nonvaccinated dogs examined (Table 6).

4.2.3 Haemagglutination inhibition test

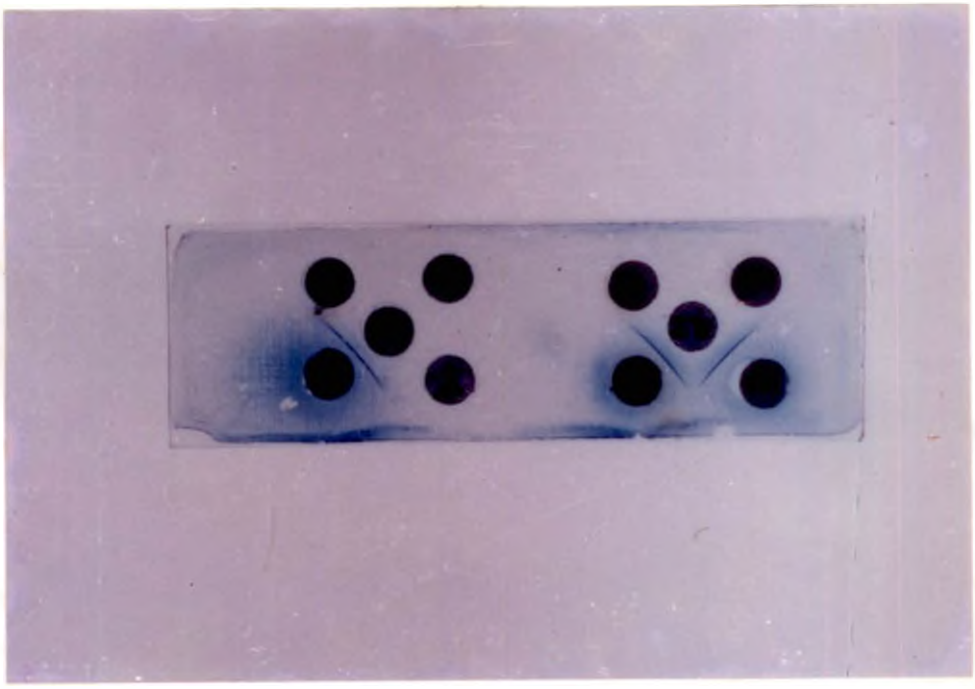
The sera collected from 57 clinically suspected dogs and 292 apparently healthy dogs including vaccinated ones were screened for antibody to CPV and the results are presented in Table 3, Fig.3 and Plate 4.

Plate 2. **Agar gel immunodiffusion test - precipitation pattern of CPV antigen with sera samples**

A	:	**Known positive antigen**
KP	:	**Known positive serum**
KN	:	**Known negative serum**
T	:	**Test serum samples**

Plate 3. **Counter immunoelectrophoresis - precipitation pattern of CPV antigen with sera samples**

A to F anode wells	:	**Known positive antigen**
A,B,E and F cathode wells	:	**Test sera samples**
C cathode well	:	**Positive control serum**
D cathode well	:	**Negative control serum**



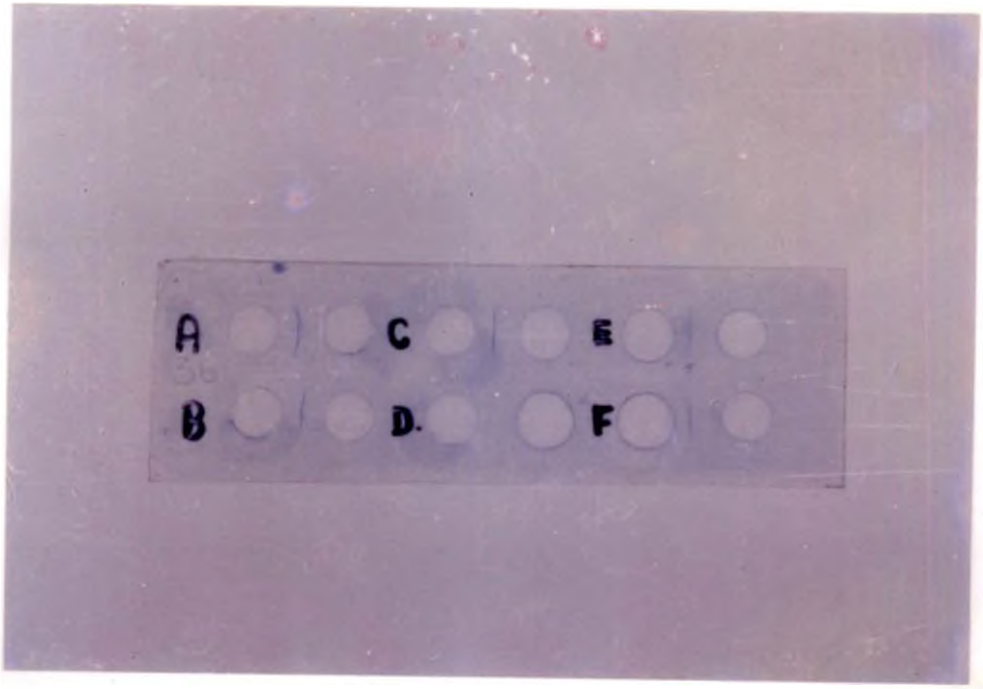

A
C
E
B
D
F

Table 3. Diagnostic tests to detect CPV antibody in serum samples

Diagnostic test	Samples tested	Samples positive	Percentage positive
Agar gel immunodiffusion test (AGID)	349	38	10.9
Counter immuno-electrophoresis (CIEP)	349	39	11.2
Haemagglutination inhibition test (HI)	349	246	70.5
Dot-ELISA	349	249	71.3

Table 4. Diagnostic tests to detect CPV antibodies in suspected dogs

Diagnostic tests	Samples tested	Samples positive	Percentage
Agar gel immunodiffusion test (AGID)	57	7	12.3
Counter immuno-electrophoresis (CIEP)	57	8	14.0
Haemagglutination inhibition test (HI)	57	36	63.2
Dot-ELISA	57	38	66.7

Table 5. Diagnostic tests to detect CPV antibodies in healthy vaccinated dogs

Diagnostic tests	Samples tested	Samples positive	Percentage
Agar gel immunodiffusion test (AGID)	38	14	36.8
Counter immuno-electrophoresis (CIEP)	38	14	36.8
Haemagglutination inhibition test (HI)	38	37	97.3
Dot-ELISA	38	37	97.3

Table 6. Diagnostic tests to detect CPV antibodies in healthy nonvaccinated dogs

Diagnostic tests	Samples tested	Samples positive	Percentage
Agar gel immunodiffusion test (AGID)	254	17	6.7
Counter immuno-electrophoresis (CIEP)	254	17	6.7
Haemagglutination inhibition test (HI)	254	173	68.1
Dot-ELISA	254	174	68.5

Plate 4. Haemagglutination inhibition assay

Rows A to E	**:**	**Test sera samples**
Row F	**:**	**Positive control serum**
Row G	**:**	**Virus control**
Row H	**:**	**Cell control**

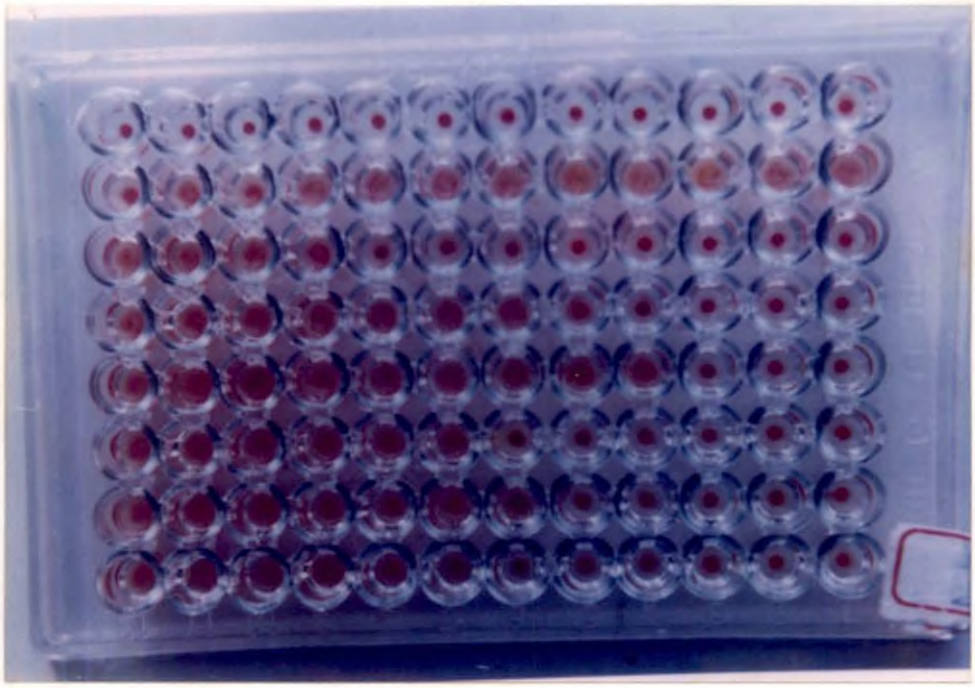
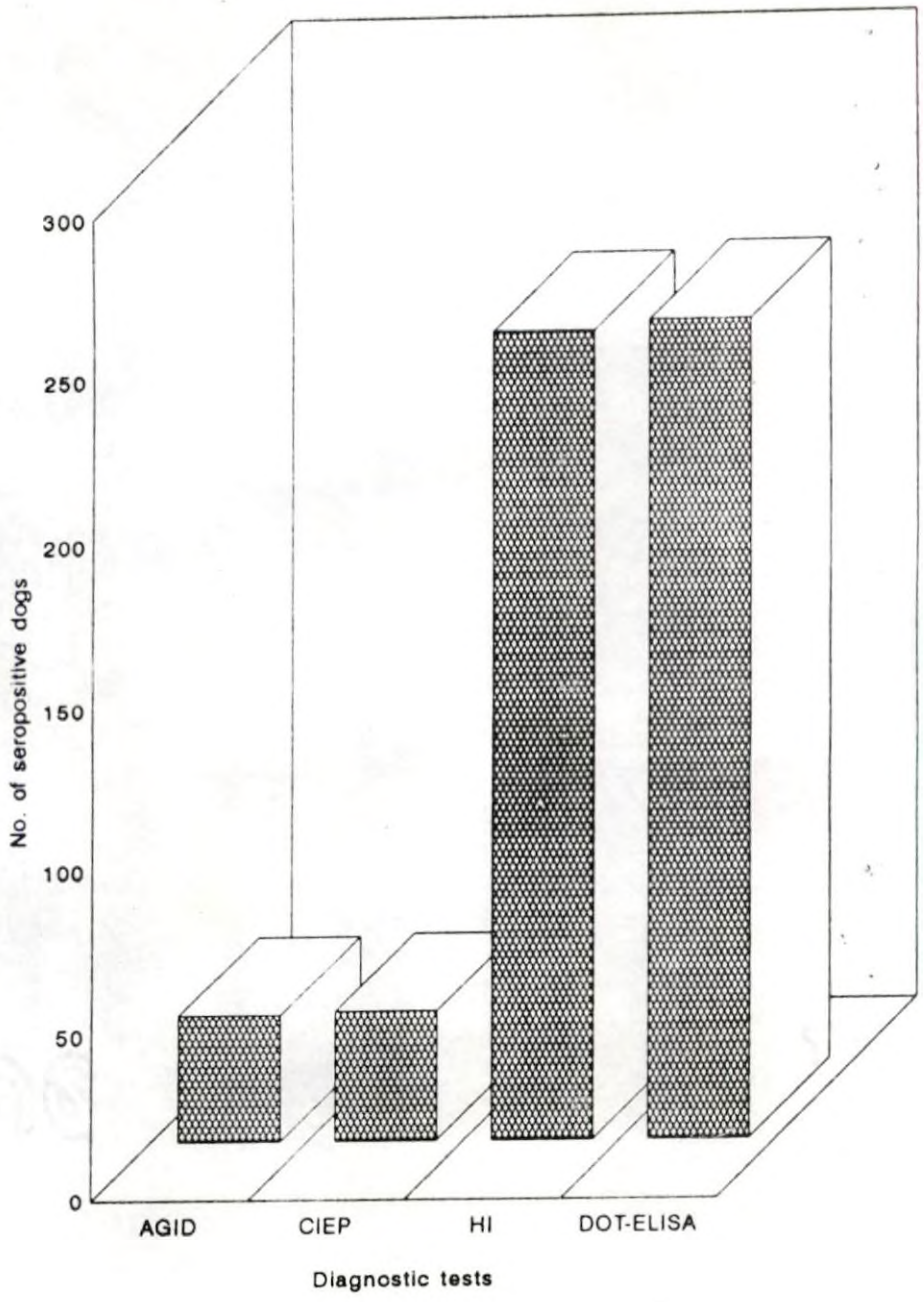


Fig. 3 DIAGNOSTIC TESTS TO DETECT CPV ANTIBODY BY DIFFERENT SEROLOGICAL TESTS



The Haemagglutination Inhibition (HI) titres were ranged between 0 and 20480 (Table 7). The HI titres of ≥80 were considered as positive as this titre was found to protect animals against virulent CPV infection (Pollock and Carmichael, 1982).

4.2.3.1 Suspected dogs

Out of the 57 clinically suspected cases, 36 (63.2 per cent) dogs were positive for CPV antibody and the results are presented in Table 4. The HI titres were ranging between 80 and 5120.

Among the 36 seropositive dogs, three (5.2 per cent) five (8.8 per cent), five (8.8 per cent), seven (12.3 per cent), eleven (19.3 per cent), three (5.3 per cent) and two (3.5 per cent) dogs were showing the antibody titres of 80, 160, 320, 640, 1280, 2560 and 5120 respectively (Table 7, Fig.4). Out of these positive cases, one, four month old Dachshund dog (O.P. No.22501) and a seven month old Alsation dog (O.P. No.12312) showed pyrexia, inappetance, vomition and diarrhoea for five days and had HI titres of 2560 and 5120 respectively. A one year old male Alsation dog (O.P. No.12311) vaccinated 6 months back and a one and half year old female Doberman dog (O.P. No.14119) vaccinated at 3 months of age showed pyrexia, inappetance, vomition and dysentry for about three and two days had HI titres of 160 and 80 respectively. Two animals,

a three month old male Alsation dog (O.P. No.22459) vaccinated 1 month back, and a five month old male Alsation dog (O.P. No.5619) vaccinated 2 months back with antibody titres of 20 and zero showed pyrexia, inappetance, vomition and dysentry for about two days.

A three months old Doberman pup vaccinated 10 days back and a two years old Doberman dog vaccinated 4 months back with antibody titres of zero and 40 showed pyrexia, inappetance, vomition and dysentry for about two days.

4.2.3.2 Vaccinated dogs

Out of the sera samples collected from 38 healthy vaccinates, 37 (97.3 per cent) were found to be seropositive (Table 5). The HI titres were ranged between 80 and 10240. Among these seropositive dogs, three (7.9 per cent), five (13.2 per cent), seven (18.4 per cent), eight (21.1 per cent), 10 (26.3 per cent), two (5.2 per cent) and one (2.6 per cent) cases were showing the antibody titres of 80, 160, 320, 640, 1280, 2560, 5120 and 10240 respectively (Table 7, Fig.4).

One Alsation pup (O.P. No.12316) 5 months of age, vaccinated 2 months back with killed vaccine showed HI titre of 40.

Table 7. Frequency of samples with their corresponding HI titres in suspected and healthy dogs

HI titre	Suspected vaccinates	Percentage	Suspected non-vaccinates	Percentage	Healthy vaccinates	Percentage	Healthy non-vaccinates	Percentage
0	2	33.3	7	13.7	0		38	14.9
20	1	16.7	4	7.8	0		23	9.1
40	1	16.7	6	11.8	1	2.6	20	7.9
80	1	16.7	2	3.9	3	7.9	42	16.5
160	1	16.7	4	7.8	5	13.2	34	13.4
320			5	9.8	7	18.4	38	14.9
640			7	13.7	8	21.1	43	16.9
1280			11	19.3	10	26.3	11	4.3
2560			3	5.9	2	5.2	2	0.78
5120			2	3.9	1	2.6	1	0.39
10240					1	2.6	1	0.39
20480							1	0.39
40960								

HI titres = 80 or above were taken as positive

Fig.4 FREQUENCY OF SAMPLES WITH THEIR CORRESPONDING HI TITRES IN SUSPECTED AND VACCINATED DOGS

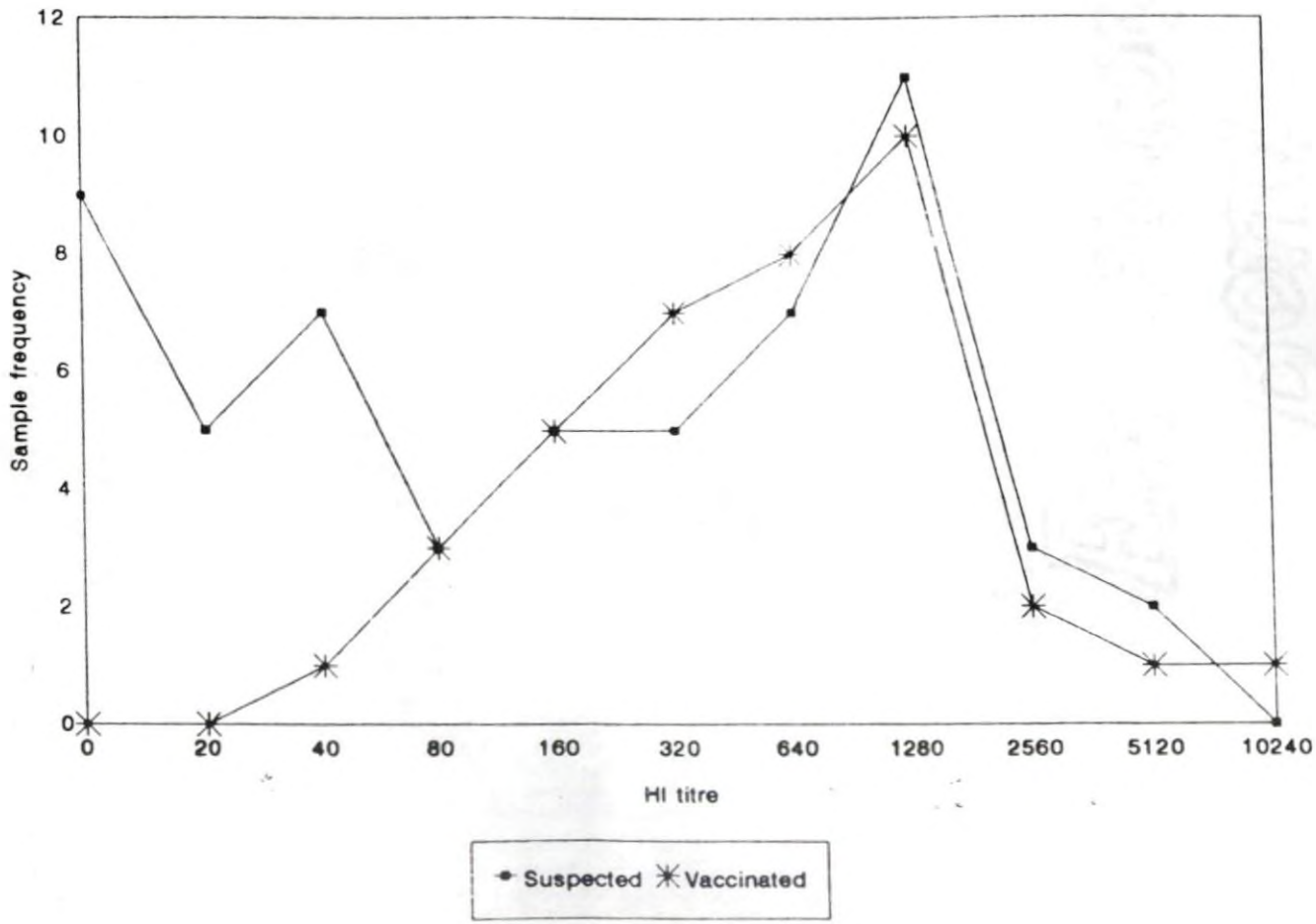
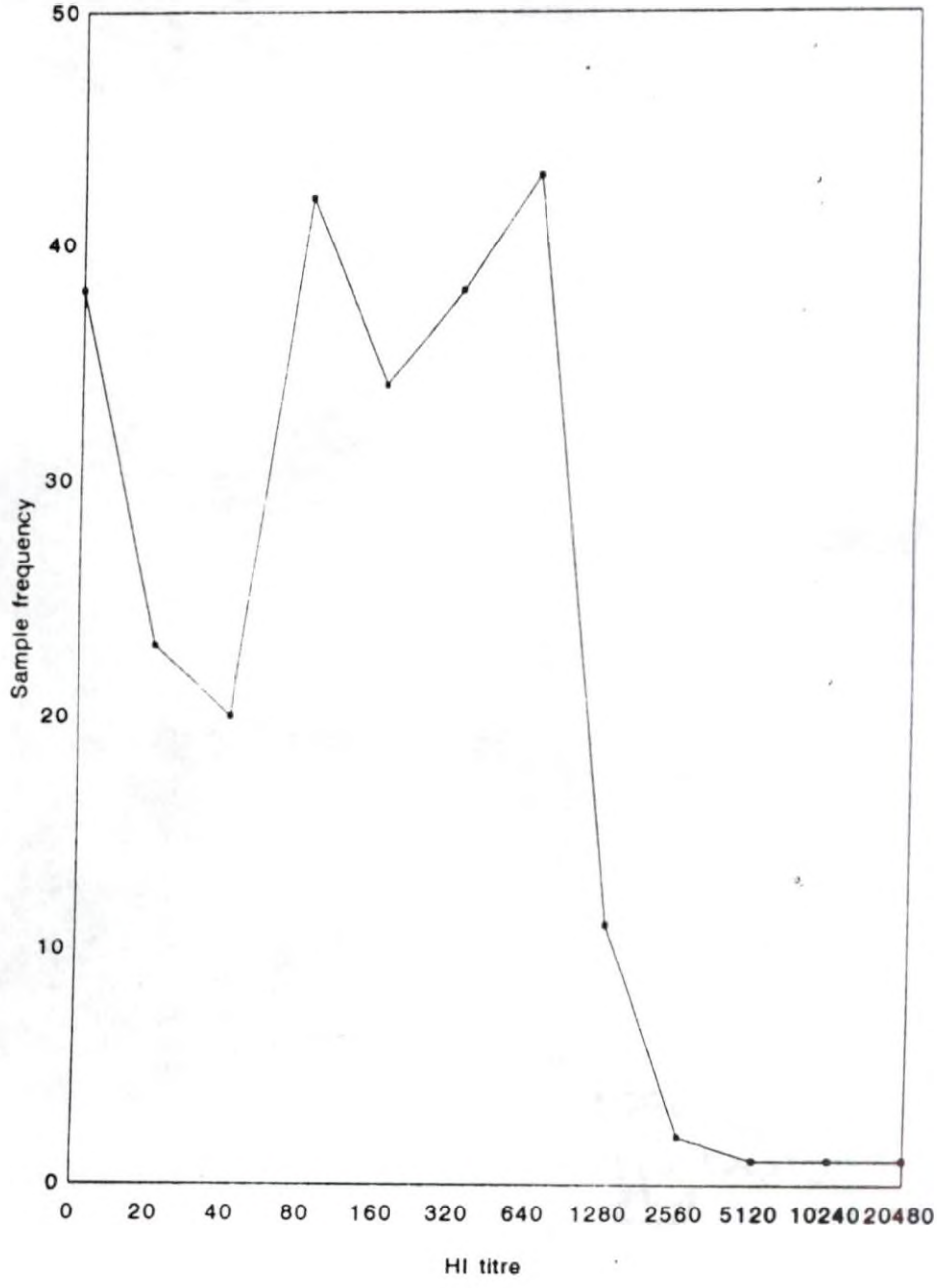


Fig.5 FREQUENCY OF SAMPLES WITH THEIR CORRESPONDING HI TITRES IN HEALTHY NON-VACCINATED DOGS



4.2.3.3 Healthy dogs

Out of the 292 healthy dogs surveyed, 210 (71.9 per cent) were found to be seropositive.

Out of the 254 healthy nonvaccinates screened, 173 (68.1 per cent) were found to be positive for CPV antibody (Table 6). Among the positives, 16.9 per cent of the non-vaccinates, showed a HI titre of 640 (Table 7, Fig.5).

The sera samples collected from 52 non-descript dogs were examined for CPV antibody and 38 (73.1 per cent) were found to be seropositive. Among these positives 10 (26.3 per cent) showed HI titre of 320. One, two year old male non-descript dogs which suffered from canine parvoviral infection 2 months back showed a HI titre of 20480.

4.2.4 Dot ELISA

The screening of 349 serum samples by Dot-ELISA showed 249 (71.3 per cent) positive for CPV antibody. The results are presented in Table 3, Fig.3 and Plate 5.

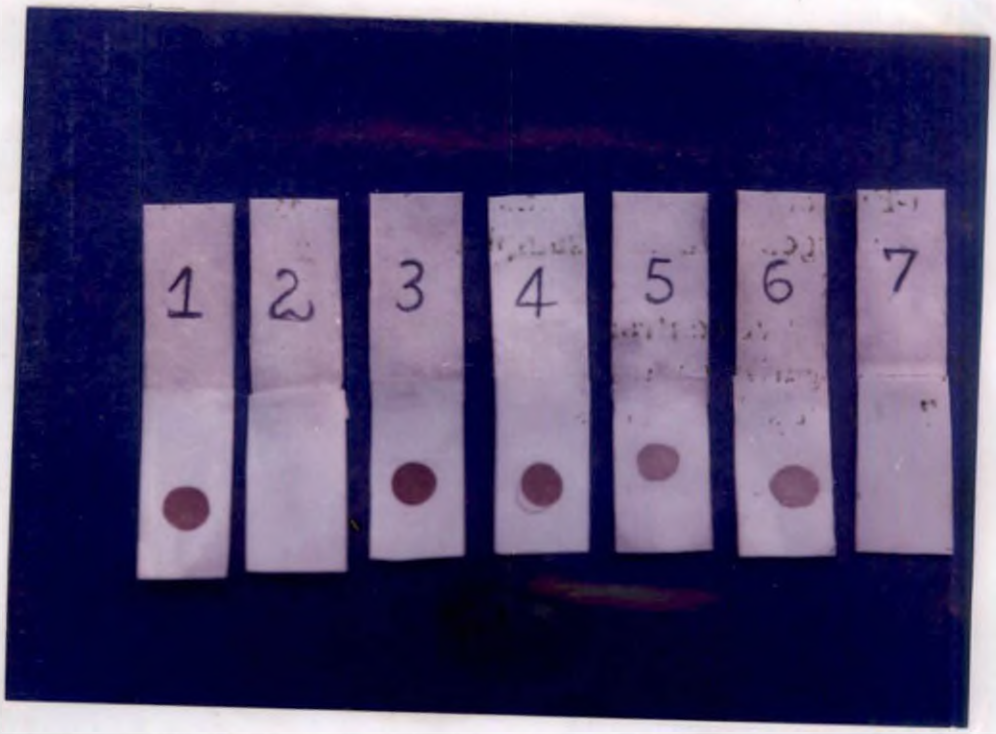
4.2.4.1 Suspected dogs

Out of 57 clinically suspected dogs screened 38 (66.7 per cent) were found to be positive for CPV antibody (Table 4). It was observed that 36 samples positive by HA with titres between 80 and 5120 were positive by Dot-ELISA,

Plate 5. Dot-Enzyme linked immunosorbent assay - Reaction of CPV antigen with sera samples

1. Positive control
2. Negative control

3-7 Test sera samples


1
2
3
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while 2 samples positive by Dot-ELISA were negative by HI with titres of 20 and 40.

4.2.4.2 Vaccinated dogs

Out of 38 healthy vaccinated dogs screened, 37 (97.3 per cent) were found to be positive for CPV antibody (Table 5). All positive samples were positive by HI with titres between 80 and 10240.

4.2.4.3 Healthy dogs

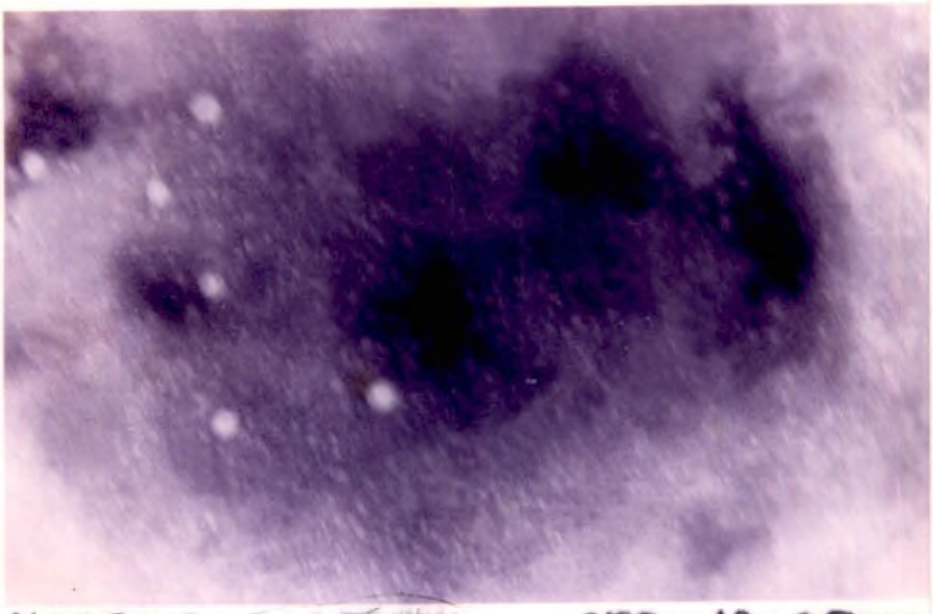
Out of 292 healthy dogs screened, 211 (72.3 per cent) were found to be seropositive.

Out of 254 healthy nonvaccinated dogs screened 174 (68.5 per cent) were found to be positive for CPV antibody (Table 6). It was further observed that 167 samples positive by Dot-ELISA were also positive by HI with titres ranging between 80 and 20480, while 7 samples positive by Dot-ELISA were negative by HI with titres between 0 and 40.

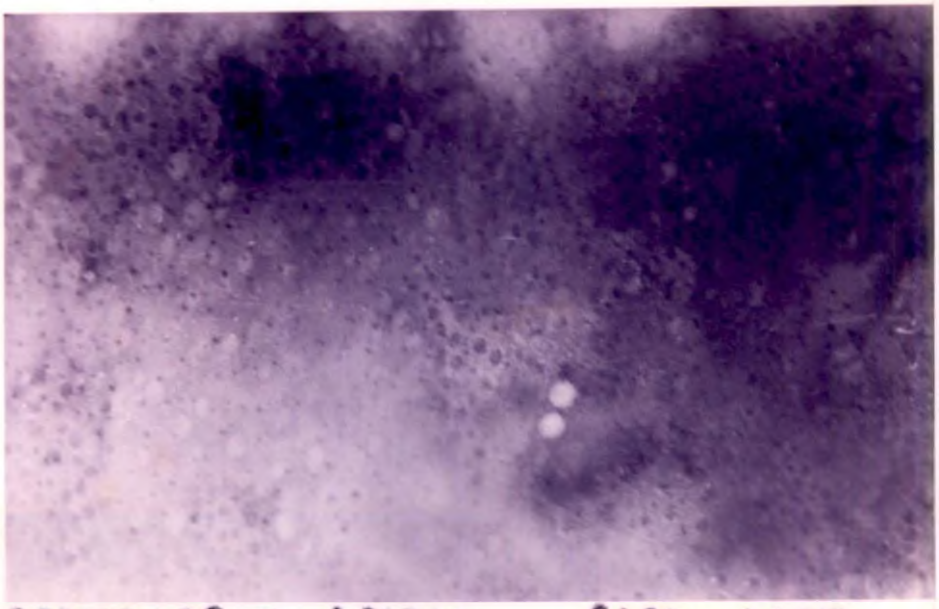
Again out of the 80 samples negative by Dot-ELISA, 74 were also negative by HI with titres between 0 and 40, while 6 samples were positive by HI with titres between 80 to 640.

Sera samples collected from different districts of Kerala was screened for CPV antibody. It was observed that 12/15 (80 per cent) in Trivandrum, 43/54 (79.6 per cent) in Ernakulam,

Plate 6&7. Canine parvovirus under electronmicroscope



MAGNIFICATION - 150 000 SIZE - 18-25nm

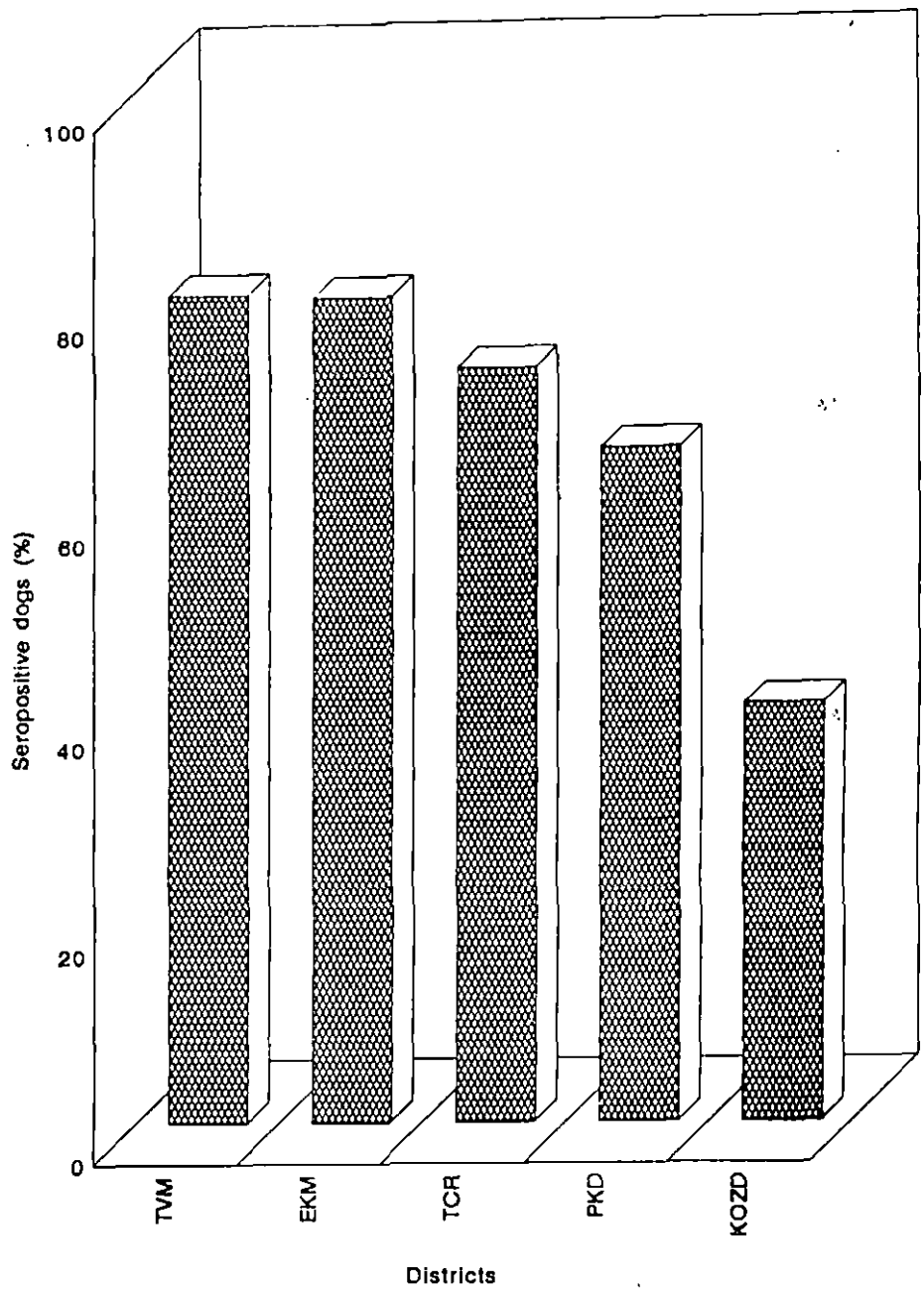


MAGNIFICATION - 120000 SIZE - 22 nm

Table 8. Distribution of seropositive dogs in different districts of Kerala

Districts	Number of samples tested	Samples positive	Percentage
1. Trivandrum	15	12	80
2. Ernakulam	54	43	79.6
3. Trichur	223	162	72.6
4. Palakkad	37	24	64.9
5. Calicut	20	8	40.0

Fig. 6 DISTRIBUTION OF CPV SEROPOSITIVE DOGS IN DIFFERENT DISTRICTS OF KERALA



162/223 (72.6 per cent) in Trichur, 24/37 (64.9 per cent) in Palakkad and 8/20 (40 per cent) in Calicut, of cases were seropositive (Table 8, Fig.6).

4.3 Prevalence

4.3.1 Age-wise prevalence

The age-wise distribution of seropositive dogs are shown in Table 9 and Fig.7. The prevalence is highest (85.3 per cent) amongst 0 to 6 months age group and the highest number of samples were also from this age group numbering 163 (46.7 per cent) and the number of positive cases when compared to the total positive ones is 55.8 per cent in this age group.

Next higher prevalence of CPV is in the >12 months age group with 69.2 per cent. The prevalence of CPV in 6-12 months age group is 46.3 per cent. The percentage of positives is 28.9 per cent when compared to the total positives as against only 15.3 per cent in 6-12 months age group.

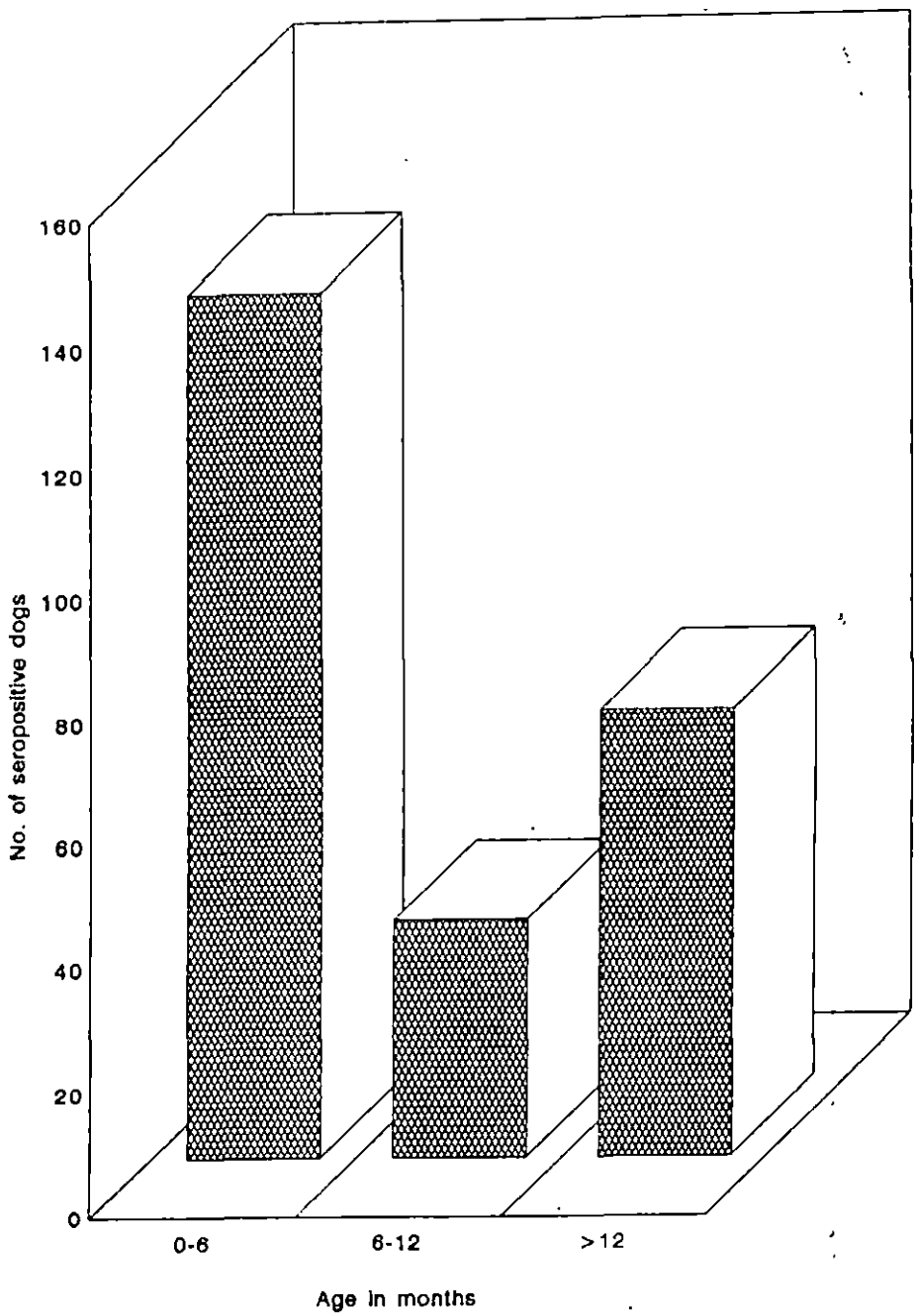
Statistical analysis revealed a highly significant ($P \leq 0.01$) prevalence rate amongst the 0 to 6 months age group when compared to the 6-12 months age group.

Table 9. Age wise distribution of CPV seropositive dogs

Age in months	Number of samples	Number of positive	Percentage	Per cent of total samples	Per cent of total positive
0-6	163	139	85.3**	46.7	55.8
6-12	82	38	46.3	23.5	15.3
>12	104	72	69.2	29.8	28.9
Total	349	249	71.3	100	100

** Highly significant P≤0.01

Fig. 7 AGE-WISE DISTRIBUTION OF CPV SEROPOSITIVE DOGS



4.3.2 Breed-wise prevalence

More seropositive cases were noted among the German shepherd dogs with 100 numbering (40.2 per cent) followed by Doberman Pinsher 39 (15.7 per cent), Dachshund 19 (7.6 per cent), pomeranian 23 (9.2 per cent), other breeds and crossbreds 30 (12 per cent) and non-descript 38 (15.3 per cent) (Table 10, Fig.8).

Statistical analysis revealed a significnt ($P \leq 0.05$) prevalence rate amongst the German shepherd dogs.

4.3.3 Sex

Male dogs were more seropositive 137/187 (73.2 per cent) compared to the females 112/162 (69.1 per cent) (Table 11, Fig.9). But on statistical analysis no significant difference was observed between positive males and females.

4.3.4 Seasonal variation

More number of seropositive cases were recorded during July with (46 cases, 18.5 per cent); followed by April (39 cases, 15.7 per cent); June (32 cases, 12.9 per cent); May (25 cases, 10 per cent); August (22 cases, 8.8 per cent); September (18 cases, 7.2 per cent); March (16 cases, 6.4 per cent); February and October (12 cases, 4.8 per cent); January

Table 10. Breed-wise distribution of CPV seropositive dogs

Breed	Number of seropositive	Per cent
German shepherd	100	40.2*
Doberman pinscher	39	15.7
Dachshund	19	7.6
Pomeranian	23	9.2
Other breds and crossbreds	30	12.0
Non-descript	38	15.3
Total	249	

* Significant $P \leq 0.05$

Table 11. Sex distribution of CPV seropositive dogs

Sex	Samples tested	Samples positive	Percentage
Male	187	137	73.2 NS
Female	162	112	69.1 NS
Total	349	249	71.3

NS : Non-significant (P>0.05)

Fig. 8 BREEDWISE DISTRIBUTION OF CPV SEROPOSITIVE DOGS

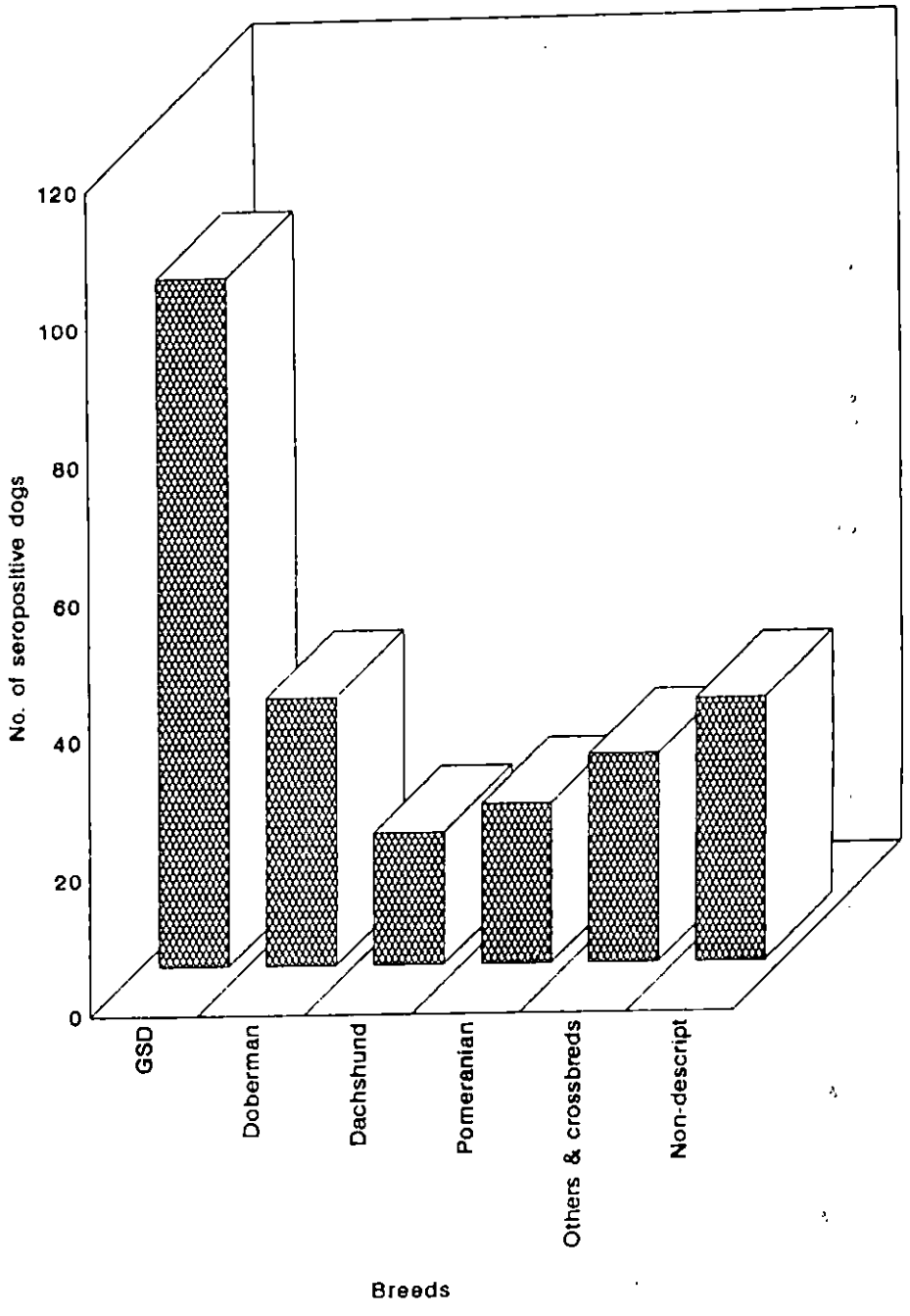
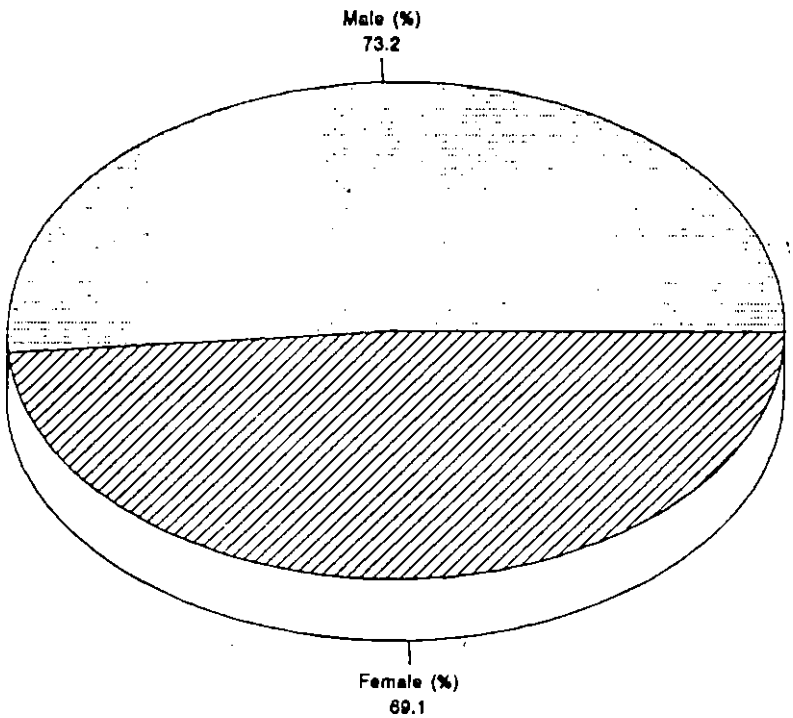


Fig. 9 SEX DISTRIBUTION OF CPV SEROPOSITIVE DOGS



(10 cases, 4.0 per cent); November (9 cases, 3.6 per cent) and December (8 cases 3.2 per cent) (Table 12, Fig.10).

On statistical analysis, no significant difference was observed. However, the percentage of positive cases were comparatively higher during April, June and July.

Table 12. Month-wise distribution of CPV seropositive dogs

Month	Number of seropositive	Per cent
January	10	4.0
February	12	4.8
March	16	6.4
April	39	15.7
May	25	10.0
June	32	12.9
July	46	18.5
August	22	8.8
September	18	7.2
October	12	4.8
November	9	3.6
December	8	3.2

Non-significant

Fig. 10 MONTHWISE DISTRIBUTION OF CPV SEROPOSITIVE DOGS

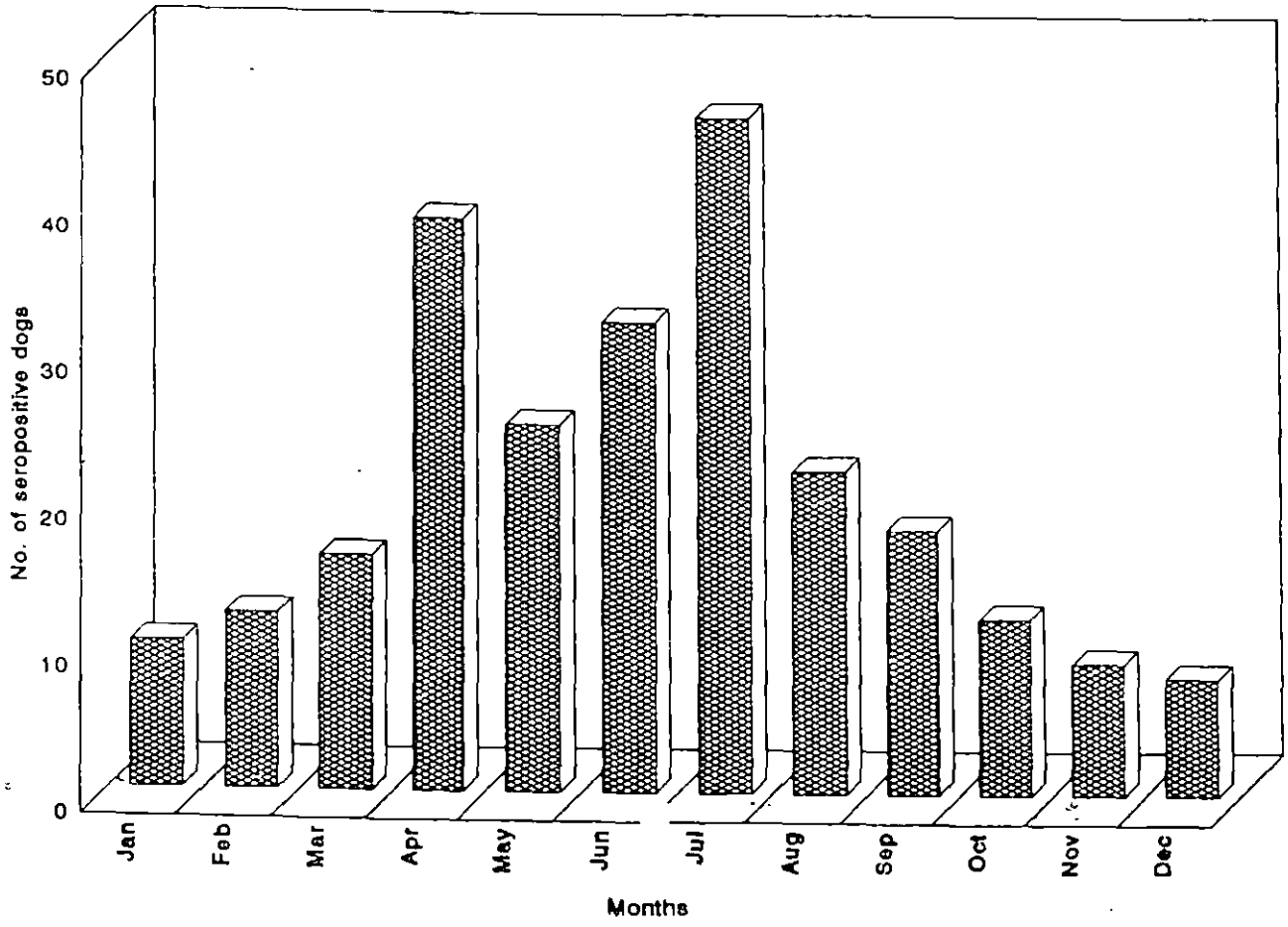


Table 13. Comparison of Dot-ELISA with HI, AGID and CIEP for detection of CPV antibodies in dogs

Dot-ELISA		HI Test		AGID		CIEP	
		Positive (No.)	Negative (No.)	Positive (No.)	Negative (No.)	Positive (No.)	Negative (No.)
Positive	249	240	9	38	211	39	210
Negative	100	6	94	-	100	-	100
Total	349	246	103	38	311	39	310

Table 14. Relationship of clinical signs to CPV in infected dogs

Clinical symptom	Number positive	Percentage
Pyrexia	31	88.6
Inappetance	35	100.0
Vomition	32	91.4
Haemorrhagic diarrhoea	29	82.8
Respiratory distress	5	14.3
Total	35	

Discussion

Chapter-V

DISCUSSION

In the present study, the prevalence of canine parvoviral infection all over Kerala was assessed. The faecal samples from fifty seven clinically suspected dogs were subjected to Agar gel immunodiffusion (AGID) test and counter immunoelectrophoresis (CIEP) test. The virus titre in the faecal sample were assessed by Haemagglutination (HA) test.

A total of three hundred and forty nine dogs were tested for CPV antibody by AGID, CIEP and Dot-ELISA. The CPV antibody titre was also assessed by Haemagglutination Inhibition (HI) test.

5.1 Antigen detection

5.1.1 Agar gel immunodiffusion test

Agar gel immunodiffusion test detected canine parvoviral antigen in the faeces of 15.7 per cent of clinically suspected dogs (Table 1, Fig.1).

The positive animals were showing pyrexia, inappetance, vomition, dysentry and dehydration. All positive samples showed high HA titre between 512 and 2048.

Ramadass and Khader (1982) had detected canine parvoviral antigen in 51.7 per cent of clinically suspected dogs with symptoms of haemorrhagic enteritis by using AGID and also observed that AGID was as good as FAT in the detection of CPV. The results obtained in the present study was not agreeing with these findings. The low percentage of antigen detected by AGID could be due to low sensitivity of the test.

However, Gunaseelan (1993) observed CPV antigen in the faecal samples of only 11.2 per cent of clinically suspected dogs which confirms with the present observation.

5.1.2 Counter immunoelectrophoresis

CIEP detected CPV antigen in faecal samples of 19.3 per cent of clinically suspected dogs (Table 1, Fig.1).

The HA titres of these positive samples were between 256 and 2048. On analysis of positive cases, the symptoms exhibited were pyrexia, inappetance, vomition, dysentry and dehydration.

The observations of the present study are not in agreement with the work of Schwers *et al.* (1980) who detected canine parvoviral antigen in 52 per cent of clinically suspected dogs using CIEP.

However, Ganesan *et al.* (1990) observed that only five per cent of clinically suspected dogs showed presence of CPV antigen in faecal samples by CIEP test.

In the present study, no significant difference observed between CIEP and AGID, which is in agreement with the observation of Saseendranath *et al.* (1992) who demonstrated CPV antigen in faecal samples of 28 per cent of clinically suspected dogs by AGID and CIEP.

5.1.3 Haemagglutination (HA) test

The haemagglutination (HA) activity of CPV was utilized diagnostically in estimating amounts of viral haemagglutinins in faecal samples (Carmichael *et al.*, 1980).

In the present study, haemagglutination test on faecal materials of 57 clinically suspected dogs revealed that 61.4 per cent of them were shedding CPV in their faeces (Table 1, Fig.1).

HA titre of 64 and above was considered as positive as suggested by Carmichael *et al.* (1980). The CPV titres in this study ranged from 0 to 2048/0.05 ml. While, Carmichael *et al.* (1980) observed HA titres ranging between 320 to 10,240 between 4 and 7 days after experimental infection, Janthur and Kokkles (1984) found HA titres ranging from 40-40960 and

Sherikar and Paranjape (1985) observed HA titres ranging between 8 and 2048.

Many workers (Mohan *et al.*, 1982, Sherikar and Paranjape, 1985; Sulochana *et al.*, 1986; Szaniszlo and Horvath, 1989 and Rai *et al.*, 1994) demonstrated that 71 per cent, 75.5 per cent, 79.5 per cent, 91.7 per cent and 85 per cent respectively of clinically suspected dogs were actively excreting CPV antigen in the faeces. The results of the present study are in agreement with the results of the above studies.

However, Sabine *et al.* (1982) and Studdert *et al.* (1983) observed that only about 30 per cent of the faecal samples from dogs with characteristic clinical signs were positive by antigen detection and Gunaseelan (1993) detected CPV antigen in only 18.5 per cent of dogs with characteristic signs of CPV, while 81.5 per cent were negative.

The high percentage of positive cases in the present study may be due to collection of faecal samples between 1 to 4 days after the onset of clinical illness. Rai *et al.* (1994) found that majority of the HA activity of 320 to 20480 was obtained from faecal samples collected 3 to 6 days after the onset of clinical illness.

Studdert *et al.* (1983) stated that the presence of the virus in the faeces is only for a short period (2-3 days) after the onset of clinical signs, consequent to the presence of coproantibodies. However, Komalafe (1988) suggested the existence of carrier status in dogs recovered from CPV infection, which can act as a potential source of infection to other dogs.

Treatment of faecal samples with chloroform as suggested by Carmichael *et al.* (1980) to remove non-specific haemagglutinins was adopted in this study. In the present study, it was observed that the procedure did not significantly alter the HA titres of samples, signifying the absence of non-specific haemagglutinins. However, Mathys *et al.* (1983) and Gunaseelan (1993) had contended that the utility value of chloroform in removing non-specific haemagglutinins is only limited.

In the present study, it was observed that dogs vaccinated with killed vaccine showed characteristic clinical signs of canine parvoviral infection and positive HA titres for CPV in faecal sample. The reason for the failure of immune response to vaccination could be either due to vaccine factors or host factors (Greene, 1984).

Janssen *et al.* (1982) suggested that maternal antibody may interfered with immunization and may cause vaccine

failures. Ramadass *et al.* (1983) observed the occurrence of CPV infection in dogs immunized with live distemper virus vaccines indicating that vaccination might have reduced the immune response to parvoviral infection.

In the present study, also, vaccine failure was observed in a dog vaccinated with modified live virus vaccine and this may be due to the fact that live modified parvovirus vaccine might have immunosuppressive properties as stated by Brenner *et al.* (1989).

Statistical analysis revealed that HA was significantly superior than AGID and CIEP in the detection of CPV antigen in the faeces. Gunaseelan (1993) observed that the highest positive correlation was between FAT and HA (84.4 per cent), while the correlation between AGID and FAT, and HA and AGID was of decreasing order.

In the study, HA was found to be simple, and convenient for screening and its specificity could also be ascertained by inhibition as demonstrated by many workers (Carmichael *et al.*, 1980; Mochizuki *et al.*, 1989).

HA can be considered as a rapid and convenient test for the detection of CPV in faecal samples at the field level as suggested by Sherikar *et al.* (1989).

5.1.4 Electromicroscopy

Electronmicroscopy detected CPV in the faeces of 13 per cent of clinically suspected dogs (Table 1). This was found in agreement with Vieler and Herbst (1995) who demonstrated CPV in 17.2 per cent of faecal samples from clinically suspected dogs.

5.1.5 Immunoelectronmicroscopy

IEM detected CPV in faecal samples of 17 per cent of clinically suspected dogs (Table 1). In the present study, IEM was found to be more sensitive than EM. This is in agreement with observation of Hammond and Timoney (1983).

5.2 Detection of antibody

5.2.1 Agar gel immunodiffusion test

In the present study, canine parvoviral antibodies were detected in 12.3 per cent of clinically suspected dogs by AGID (Table 4). The sera collected from 38 apparently healthy vaccinates and 254 apparently healthy non vaccinates showed 36.8 per cent (Table 5) and 6.7 per cent (Table 6) antibodies respectively to CPV by this method.

All positive cases by AGID were also positive by HI with titres between 640 and 5120 in clinically suspected dogs, 640

and 10240 in healthy vaccinated dogs and 640 to 20480 in healthy non vaccinated dogs.

5.2.2 Counter immunoelectrophoresis

Counter immunoelectrophoresis detected canine parvoviral antibodies in 14 per cent of dogs suffering from canine parvoviral infection (Table 4). The sera samples from 38 healthy vaccinated dogs and 254 healthy non vaccinated dogs showed 36.8 per cent (Table 5) and 6.7 per cent (Table 6) antibodies respectively to CPV by CIEP.

Among the clinically suspected dogs, all positive samples by CIEP were also positive by HI with titres between 640 and 5120. One positive case (O.P. No. 12612) recorded negative by AGID, was positive by HI with a titre of 640. In vaccinated dogs, all positive samples were also positive by AGID and HI with titres between 640 and 10240 and in apparently healthy non vaccinated dogs, all positive samples showed positive reaction by AGID and HI with titres between 640 and 20480.

The results of the present study were not found to be in agreement with the reports of Schwers *et al.* (1980) who observed a clear precipitating band for all serum samples in which HI titres were above 40.

Gaitamonova (1990) suggested that CIEP was sensitive in 81.3 per cent for the detection of parvoviral antibodies, whereas HI test was sensitive in 75.5 per cent only.

Zuffa and Rejhalcova (1988) observed 75.9 per ent of mink to be positive for antibodies to Aleutian disease.

However, Schwers *et al.* (1983) demonstrated antibodies to CPV infection in 2.8 per cent of fox serum samples by using counter immunoelectrophoresis.

5.2.3 Haemagglutination inhibition test

The haemagglutination inhibition (HI) test has been employed in this study for serosurvey as it is widely used by many workers as an evidence of occurrence of CPV infection (Pollack and Carmichael, 1979; Appel *et al.*, 1980; Carmichael *et al.*, 1980; Mohri *et al.*, 1982).

Out of the 349 dogs screened, 70.5 per cent of the dogs including suspected and healthy vaccinated ones showed antibodies to CPV by HI test (Table 3, Fig.3).

The seropositivity of HI test has not been clearly established since absolute values vary among laboratories depending on the method and source of antigen used.

A HI titre of >80 considered as positive in this study is claimed to protect against virulent CPV infection (Pollack and Carmichael, 1982; Olson *et al.* 1988; Udupa and Sastry, 1996).

However, Carmichael *et al.*(1980), Walker *et al.* (1980) and Azetaka *et al.* (1981) have suggested HI titres of >320, >256 and >128 to be positive, respectively.

5.2.3.1 Suspected dogs

Out of 57 clinically suspected dogs, 63.2 per cent had protective level of antibody against CPV (Table 4) and all positive cases showed pyrexia, inappetance, vomition, dysentry and dehydration.

Many workers (Binn *et al.*, 1981, Mohan *et al.*, 1992; Rai *et al.*, 1994; and Udupa and Sastry, 1997) demonstrated CPV antibodies in 75.3 per cent, 91.6 per cent, 87.6 per cent and 78 per cent of dogs with characteristic clinical signs of CPV infection.

The antibody titres of 80, 160, 320, 640, 1280, 2560 and 5120 in three (5.2 per cent), five (8 per cent), five (8.8 per cent), seven (12.3 per cent), eleven (19.3 per cent), three (5.3 per cent) and two (3.5 per cent), cases respectively of clinically suspected dogs were due to the different stages of infection at the time of blood collection. The animals in the

early stages of infection showing symptom of canine parvoviral infection for about two days had the antibody titre of 80 and 320 and the animals in mid stage of infection showing the symptom of canine parvoviral infection for about 3-5 days had antibody titre of 320 and 5120. High antibody titres of 2560 and 5120 were recorded in 5.9 and 3.9 per cent cases respectively showing pyrexia, inappetance, vomition, and dysentry were in animals with CPV infection about for five days. These results were found in agreement with the observations of Rai *et al.* (1994) who detected CPV antibodies in serum samples from clinically suspected dogs between 4 to 10 days after the onset of illness with titres varying from 40 to 1280.

Similar finding was recorded by MaCartney (1984) who detected CPV antibody at day 5 after inoculation in an experimental infection and the antibody level increases rapidly with titres at 1024 or greater on or after day 7.

In the present study, four vaccinated dogs with clinical signs like inappetance, pyrexia, vomition and dysentry for about two days were negative for CPV antibody and two vaccinated dogs with clinical signs of canine parvoviral infection for three days showed protective level of antibody to CPV infection.

5.2.3.2 Vaccinated dogs

Of the 38 healthy vaccinates studied, 97.3 per cent had good seroconversion (>80) (Table 5) and among the positives, 25 per cent of the total vaccinates had HI titres of 1280. In direct contrast to these findings Olson *et al.* (1988) had observed that only 11.5 per cent of the vaccinates had HI titres of >80, while 67.2 per cent of vaccinates did not have any detectable titre.

In the present study, one five month old dog vaccinated 2 months back showed negative results with a HI titre of 40, probably due to the fact that maternal antibodies interfered with vaccination response as stated by Jenssen *et al.* (1982) or due to vaccine factors.

5.2.3.3 Healthy dogs

Out of the sera samples collected from two hundred and fifty four healthy non vaccinated dogs, 68.1 per cent were found to be seropositive against CPV infection (Table 6) and among these positive samples, 16.9 per cent of the non vaccinates showed HI titres of 640.

In the present study, it was observed that apparently healthy dogs. were having the protective level of antibody

against canine parvoviral infection. This could be due to subclinical CPV infection and the animal became immune to the disease.

Lenghaus (1980) observed that the majority of urban dogs showed serological evidence of CPV infection without any apparent illness. Similarly, Mohri *et al.* (1982) in Japan found more of seropositive dogs not to have had clinical CPV. Sabine (1982) observed that 60 per cent of urban dogs and 30 per cent of rural dogs in Sydney showed high titres of CPV antibody without clinical illness.

Carmichael *et al.* (1980) suggested that CPV antibodies in sera persist at high levels for at least one year.

The observation of HI titre upto 20, 480 amongst the non-descript can be explained due to the constant natural challenge as suggested by Lenghaus (1980) which can be expected to occur more amongst the non-descript.

In the present study, serum HI test was found to be more sensitive and specific in detecting antibodies against canine parvoviral infection.

5.2.4 Dot ELISA

Dot ELISA which is an alternative of plate ELISA for detection of antibodies to soluble antigens has been known to

possess high specificity and field application in terms of its convenience and easiness to perform.

Out of 349 sera samples screened by Dot ELISA 71.3 per cent were found to be positive for CPV antibody (Table 3, Fig.3).

5.2.4.1 Suspected dogs

Dot ELISA detected CPV antibody in 66.7 per cent of clinically suspected dogs (Table 4). All samples that were positive by HI with titres between 80 and 5120 were also positive by Dot ELISA, while 2 samples positive by Dot ELISA were negative by HI with titres of 20 and 40.

5.2.4.2 Vaccinated dogs

While screening healthy vaccinated dogs for CPV antibody by Dot ELISA, showed 97.3 per cent positive reactors for CPV (Table 5). All positive samples were also positive by HI with titres between 80 and 10240.

5.2.4.3 Healthy dogs

Detection of CPV antibody in healthy non vaccinated dogs showed 68.5 per cent seropositive (Table 6). It was observed that 167 samples positive by Dot ELISA were also positive by HI with titres ranging between 80 and 20480, while 7 samples

positive by Dot ELISA were negative by HI with titres between 0 and 40.

Again out of 80 samples shown negative by Dot ELISA, 74 were also negative by HI with titres between 0 and 40, while 6 samples were positive by HI with titres between 80 to 640.

The results of the present study are found to be in agreement with the observation of EI-Sanousi-A (1990) who suggested that Dot ELISA was more sensitive compared with the standard solid phase ELISA, simple to perform and was recommended as a rapid technique for the diagnosis of CPV infection.

The observation of the present study revealed no statistically significant difference between Dot ELISA and HI for the detection of CPV antibody. However, Dot ELISA was found to be slightly more sensitive than HI for the detection of CPV antibody. Similar finding was observed by Hara *et al.* (1994) who demonstrated the higher sensitivity of the ELISA with its titres showing good correlation with HI test are in agreement with the present study.

Compared to other tests, a positive reaction in Dot ELISA can be read by the naked eye without difficulty. Dot ELISA also facilitates easy method of transport of specimens from remote corners by just dotting the suspected materials on to

the nitrocellulose membrane. Moreover, the antigen dotted strips can be stored in the dark for years without any significant loss of colour, thus maintaining a permanent record of the results (Walton *et al.*, 1986). Hence Dot ELISA can be considered as an excellent test for the detection of CPV antibodies in geographically remote areas.

Sera samples collected from different districts of Kerala showed a prevalence rate of 80 per cent in Trivandrum, 79.6 per cent in Ernakulam, 72.6 per cent in Thrissur, 64.9 per cent in Palakkad and 40 per cent in Calicut. This may be probably due to the fact that there are more number of susceptible populations in the Southern part of Kerala compared to the Northern part of Kerala. Wiesrup (1983) suggested that prevalence of CPV infection was highest when the density of susceptible population exceeds 12/km^2 and ceases when the density was 6/km^2.

5.3 Prevalence

5.3.1 Age wise prevalence

Many workers suggested that dogs <6 months of age were more susceptible compared to others, though all age groups seemed to be affected (Smith *et al.*, 1980; Ramadass and Khader, 1982; Hammond and Timoney, 1983; Studdert *et al.*, 1983; Mason *et al.*, 1987 and Ernst *et al.*, 1988).

In the present study too, the dogs in the age group below 6 months of age were more affected compared to other age groups and a significant difference ($P \leq 0.01$) in the number of seropositive dogs was noticed between the age groups (Table 9, Fig.7).

Next higher prevalence of CPV is in the above 12 months age group which is in agreement with the study of Gunaseelan (1993). In this study, more number of samples and positives were from dogs less than 6 months of age, followed by dogs above 12 months of age.

In the observation of Mason *et al.* (1987) aged dogs died intermittently due to CPV enteritis and this was attributed to stressful events in such cases, while Jaroslow *et al.* (1974) and Schultz (1984) suggested that as age advanced, protective titres declined leading to susceptibility. These could be attributed for the incidence in dogs above 12 months of age.

5.3.2 Breedwise prevalence

The breed-wise prevalence of canine parvoviral infection in the present study is found to be higher in the German shepherd dogs compared to other breeds and the results indicated a significant difference in seroprevalence between the breeds ($P \leq 0.05$) (Table 10, Fig.8).

Similar findings have been described by Mizak and Mizak (1994) who observed that 85.9 per cent of German Shepherd dogs had CPV antibodies and 33.3 per cent had higher titres ≥1280 suggesting a natural infection. Rogers (1987) and Houston *et al.* (1996) observed that Rottweilers, Doberman Pinschers and German Shepherd dogs were more at risk, while toy poodles and cocker spaniels were at lesser risk for CPV infection.

However, Gunaseelan (1993) suggested that breeds other than Doberman and German shepherd are significantly at higher risk for CPV enteritis.

In the observation of some other workers all breeds are equally susceptible (Eugster *et al.*, 1978; Ramadass and Khader, 1982; Udupa and Sastry, 1996).

5.3.3 Sex

In the present study more number of male dogs are seropositive than females (Table 11, Fig.9). However, no statistically significant difference was obtained between the sexes. Similar findings were observed by Fluckinger (1980). Prange *et al.* (1982) observed that males account for 72 per cent of total deaths due to CPV infection and Houston *et al.* (1996) suggested that males below 6 months were at twice greater risk than females for CPV enteritis.

However, many workers suggested that the influence of sex on the incidence of CPV infection was not significant (Ramadass and Khader, 1982; Rogers, 1987; Ernst *et al.*, 1988; Gunaseelan, 1993; Udupa and Sastry, 1997).

5.3.4 Season

On analysis of the results, in the present study more number of seropositive dogs are recorded during April, June and July. Otherwise the incidence is evenly spread out throughout the year. The incidence among months is not significant as shown in Table 12, Fig.10.

This finding is in accordance with Gunaseelan (1993). Mason *et al.* (1987) who observed higher prevalence during March and April. The reasons may be as attributed by other workers, viz., increase in susceptible population through whelpings and weaned puppies. This could be applied in the present study to coincide with the observed seasonal prevalence. Ernst *et al.* (1987) stated that the peak seasonality of CPV infection occurred during periods of high temperature and humidity.

However, Ernst *et al.* (1988) observed a significantly lower risk for CPV infection during May, June, July and September.

The peaks observed in June and July in the present study are perhaps due to the onset of incliment weather or continued whelping and weanings throughout the year besides the two principal breeding seasons.

Summary

Chapter-VI

SUMMARY

The prevalence of CPV infection in three hundred and forty nine dogs from all over Kerala was studied. The canine parvoviral antigen and antibody were detected for the diagnosis of the case. CPV antigen was detected in faecal samples by using AGID, CIEP and EM and the virus titre was assessed by HA test.

Canine parvoviral antibody was detected by using AGID, CIEP and Dot-ELISA and the antibody titre was assessed by HI test.

Agar gel immunodiffusion test detected CPV antigen in faecal samples of 15.7 per cent of 57 clinically suspected dogs.

The clinically suspected dogs were also tested for the presence of CPV antigen by CIEP and 19.3 per cent were found to be seropositive. All positive cases were also positive by HA with titres between 256 and 2048.

The virus titres in faecal samples were assessed by HA test and found that 61.4 per cent of clinically suspected dogs were having the canine parvoviral antigen in the faecal sample. The HA titres in the faecal samples ranged between

0 and 2048 and cases having titres of 64 and above were taken as positive. Among these positives, five (8.8 per cent), seven (12.2 per cent), nine (15.7 per cent), six (10.5 per cent), five (8.8 per cent) and three (5.3 per cent) were showing HA titres of 64, 128, 256, 512, 1024 and 2048 respectively. The animals in the early stage of infection showing the symptoms of canine parvoviral infection for about 1-2 days had the virus titre of 256 to 2048 and the animals showing symptoms of CPV for about 3-4 day had virus titre of 64 to 256. The virus titre was found to decrease with progression of clinical illness. So faecal sample should be collected during early stage of illness (1-2 days) for demonstration of viral antigen. Haemagglutination test was found to be simple, convenient and rapid test for detection of CPV antigen in faecal samples at the field level.

Vaccination failure had been observed in dogs vaccinated with killed or live vaccine.

EM detected CPV in faecal samples of 13 per cent of eight clinically suspected dogs, whereas IEM detected CPV in faecal samples of 17 per cent of six clinically suspected dogs.

The agar gel immunodiffusion test was also used to detect the CPV antibody in sera. Out of 57 clinically suspected dogs screened, 12.3 per cent showed antibodies to CPV infection. Among 38 healthy vaccinated dogs and 254 healthy

non-vaccinated dogs tested, 14 (36.8 per cent) and 17 (6.7 per cent) of cases were seropositive by AGID. All positive samples by AGID were also found to be positive by HI with titres between 640 and 20480.

Counterimmunoelectrophoresis detected CPV antibodies in 14 per cent of clinically suspected dogs. Out of 38 healthy vaccinated dogs and 254 healthy non-vaccinated dogs, 36.8 per cent and 6.7 per cent respectively showed antibodies to CPV by CIEP. All positive cases were positive by HI with titres between 640 and 20480.

The canine parvoviral antibody was also assessed by HI test. A total of 349 dogs including 57 clinically suspected and 292 healthy and vaccinated dogs, tested 246 (70.5 per cent) cases were having the protective level of antibody. Among 36 seropositive dogs of 57 suspected, the antibody titre of 80, 160, 320, 640, 1280, 2560 and 5120 were present in three (52. per cent), five (8.8 per cent), five (8.8 per cent), seven (12.3 per cent), eleven (19.3 per cent), three (5.3 per cent) and two (3.5 per cent) cases, respectively.

In 38 healthy vaccinated dogs, 37 (97.3 per cent) were seropositive with antibody titre of 80, 160, 320, 640, 1280, 2560, 5120 and 10240 in three (7.9 per cent), five (13.2 per cent), seven (18.4 per cent), eight (21.1 per cent), 10 (26.3

per cent), two (5.2 per cent), one (2.6 per cent) and one (2.6 per cent) cases, respectively.

The healthy non-vaccinated dogs screened for the presence of CPV antibody, 173 (68.1 per cent) were positive and the antibody titre of 80, 160, 320, 640, 1280, 2560, 5120, 10240 and 20480 were present in 42 (16.5 per cent), 34 (13.4 per cent), 38 (14.9 per cent), 43 (16.9 per cent), 11 (4.3 per cent), 2 (0.75 per cent), 1 (0.39 per cent), 1 (0.39 per cent) and 1 (0.39 per cent) cases, respectively. This indicates subclinical infection is more common in CPV infection and play an important role in the epidemiology of CPV infection.

The animals in the early stage of infection showing the symptoms of CPV infection for about two days had antibody titre of 80 and 320, and the animals in mid stage of infection showing the symptom of CPV infection for about 3-5 days had antibody titre of 320 and 5120. It was also found a rapid establishment of antibody titre on 2nd day of illness, reaching the peak on 5-7 days and persisted for long period of time. So, HI test was found to be rapid, sensitive and specific test for screening of sera samples for CPV antibodies.

On screening 57 clinically suspected dogs, 38 (66.7 per cent) were found to be seropositive by Dot-ELISA. Two samples that were positive by Dot-ELISA, were negative by HI with

titres of 20 and 40. Out of 38 healthy vaccinated dogs and 254 healthy non-vaccinated dogs, 37 (97.3 per cent) and 174 (68.5 per cent) showed CPV antibodies by Dot-ELISA. Dot-ELISA was found to be slightly more sensitive than HI in detection of CPV antibodies. It was found to be simple, rapid and sensitive test and hence can be considered as excellent test for detection of CPV antibodies in geographically remote areas.

Screening of sera samples from different districts of Kerala, showed 12 (80 per cent) in Trivandrum, 43 (79.6 per cent) in Ernakulam, 162 (72.6 per cent) in Trichur, 24 (64.9 per cent) in Palakkad and 8 (40 per cent) in Calicut, were seropositive.

The dogs in the age group of six months and below were more seropositive in this study and had the higher prevalence rate (85.3 per cent) followed by 69.2 per cent in more than 12 months age group and 46.3 per cent in age group of 6 to 12 months. Eventhough no statistical significant difference was observed between sexes, the prevalence of CPV was found more in male dogs. Among the breeds, prevalence was significantly higher ($P<0.05$) in the German shepherd dogs. Seasonal prevalence was higher during April, June and July. However, the attack rate was found to be constant throughout the year.

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* Originals not seen

SEROPREVALENCE AND DIAGNOSIS OF CANINE PARVOVIRAL INFECTION

By
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ABSTRACT OF A THESIS

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ABSTRACT

The prevalence of canine parvoviral infection in three hundred and forty nine dogs from all over Kerala including 57 clinically suspected dogs and 292 healthy and vaccinated dogs was studied.

Among 57 clinically suspected dogs, 15.7 per cent and 19.3 per cent respectively, had CPV antigen in the faecal samples by AGID and CIEP.

Canine parvovirus (CPV) antigen were detected in faecal samples of 61.4 per cent of 57 clinically suspected dogs by HA test.

EM detected CPV in one (13 per cent) of eight clinically suspected dogs and 17 per cent of six clinically suspected dogs had CPV in the faecal samples by IEM.

Agar gel immunodiffusion test detected canine parvoviral antibody in seven (12.3 per cent) of clinically suspected dogs, 14 (36.8 per cent) of 38 healthy vaccinated dogs and 17 (6.7 per cent) of 254 healthy non-vaccinated dogs.

Counterimmunoelectrophoresis (CIE) test detected CPV antibody in 8 (14 per cent) out of 57 clinically suspected

dogs 14 (36.8 per cent) of 38 healthy vaccinated dogs and 17 (6.7 per cent) of 254 healthy non-vaccinated dogs.

Out of 349 dogs screened by HI test, 70.5 per cent dogs had the protective level of antibody against canine parvoviral infection. Haemagglutination inhibition test detected CPV antibodies in 36 (63.2 per cent) of 57 clinically suspected dogs, 37 (97.3 per cent) of 38 healthy vaccinated dogs and 173 (68.1 per cent) of 254 healthy non-vaccinated dogs.

Dot-ELISA detected CPV antibodies in 38 (66.7 per cent) of 57 clinically suspected dogs, 37 (97.3 per cent) of 38 healthy vaccinated dogs and 174 (68.5 per cent) of 254 healthy non-vaccinated dogs.

More seropositive dogs were recorded in dogs below six months of age. Prevalence rate observed was more in male dogs, eventhough no significant difference was observed between sexes. Among the breeds, German shepherd were more affected, followed by Doberman Pinscher and non-descript. Seasonal prevalence in the present study was higher during April, June and July. However, no significant difference was observed between different months.

Appendix

APPENDIX

Proforma for collection of details from the dog owner

1.	Serial No.	:	
2.	Case No.	:	
3.	Name & address of the owner	:	
4.	Species	:	
5.	Breed	:	
6.	Age & date of birth	:	
7.	Sex	:	
8.	Colour	:	
9.	In case of pup, whether the dam was immunized against canine parvoviral enteritis	:	
10.	History	:	
11.	Whether vaccinated against canine parvoviral enteritis	:	Yes/No
	a. If yes		
	(i) Type of vaccine used	:	
	(ii) Age at vaccination	:	
	(iii) Whether dewormed before vaccination	:	
12.	Whether the animal suffered from canine parvoviral enteritis	:	Yes/No
	a. If yes		
	(i) When did it suffer	:	
	(ii) Name of doctor treated	:	
	(iii) Treatment given	:	
	(iv) Course of the disease	:	