

CANINE PARVOVIRUS INFECTIONS IN DOGS - AN EMERGING DISEASE OF PARAMOUNT IMPORTANCE

S. Nandi, S. D. Audarya and R.S. Chauhan

Centre for Animal Disease Research and Diagnosis Indian Veterinary Research Institute,
Izatnagar, U.P. (243122)

Canine parvovirus 2 (CPV-2), the causative agent of acute haemorrhagic enteritis and myocarditis in dogs, is one of the most important pathogenic viruses. It is a highly contagious and often fatal disease. CPV-2 was first recognized in 1977 and since then it has been well established as an enteric pathogen of dogs throughout the world with high morbidity (100%) and frequent mortality up to 10%. The disease is characterized by 2 prominent clinical forms (i) enteritis with vomiting and diarrhea in dogs of all ages (ii) myocarditis and subsequent heart failure in pups of less than 3 months of age. The virus was named CPV-2 in order to differentiate it from a closely related parvovirus of canine known as CPV -1 or minute virus of canine (MVC). MVC, a completely different parvovirus, had not been associated with natural disease until 1992. MVC may cause pneumonia, myocarditis and enteritis in young pups or transplacental infections in pregnant dams, with embryo resorptions and fetal death. About 30 confirmed cases of CPV-1 have been reported in USA, Sweden, Germany and more recently in Italy. The canine parvovirus infections have been confined to be a problem in dogs in spite of a number of potent and efficacious live as well as inactivated vaccines available to be used in the dogs. So, it is the prime time to create awareness among the dog owners, pet lovers, Kennel club owners, pet shop owners and defense personnel about the disease in order to prevent and control the disease in a more effective and efficient manner.

Etiology: CPV is extremely small, only 22 nm in diameter with an icosahedral capsid enclosing a genome composed of single stranded negative sense DNA of about 5.2 Kb in length coding two structural proteins (VP1 and VP2) and two non-structural proteins (NS 1 and NS2). The CPV -2 is closely related antigenically to feline panleukopenia virus (FPL V) and appears to have arisen as a result of mutation of feline virus. The virus lacks envelope and essential lipid. Simply, one

million virus particles laid end to end would measure slightly under one inch. There are a total of 60 copies of the VP 1 and VP2 with about 5-6 copies of VP1 and 54-55 copies of VP2. Like all other parvoviruses, it can replicate only in actively dividing cells.

The virus can be grown in both primary dog kidney and cat kidney and CRFK, MDCK and An cell lines. But the growth in cell culture is not generally accompanied by a gross cytopathic effect although I/N inclusions may be found on microscopic examination. The presence of virus is normally detected by haemagglutination, immunofluorescence, IPT or ELISA. Viral haemagglutination is also easily detected in the faeces of dogs with parvovirus enteritis.

As the Virus is naked, it is resistant to ether and chloroform and stable at pH 3 and 9. The virus can withstand high temperature and can remain viable in fomites for many years. The virus is destroyed by 2% formalin, 2% aOH and 2% sodium hypochloride. A typical average infectious dose for an unvaccinated dog is 1000 virus particles and chances of getting infections depend on the breed, age, health status of the dogs and virulence of the virus. An infected dog sheds 35 million viral particles (35000 times the typical infectious dose) per ounce of stool.

Antigenic variants of CPV -2: Since the emergence of CPV-2 in 1978, an antigenic variant was identified in 1979 in many different countries of the world using monoclonal antibody testing and that strain was termed as CPV-2a. Although those viruses differed only in 5-6 amino acids from the CPV-2, they differed in two different neutralizing antigenic sites on the surface of the capsid. In 1984, a further antigenically variant virus strain was detected which differed in only a single epitope compared to CPV -2a and designated as CPV -2b. The only significant difference between CPV -2a and CPV-2b variants is the substitution of one amino acid (Asn to Asp) at position 426 of VP2 protein. Despite the small

number of differences between those viruses in each case the viruses became globally distributed indicating that they must have been under strong selection. These viruses also differ in host range as well as antigenicity. The different antigenic variants of CPV are prevalent in varying proportion in different countries with >80% of isolates being CPV-2b and <20% CPV-2a in USA, while 60% are CPV-2a and 40% CPV-2b in Germany. In India, there are also large number of positive cases of CPV infections are detected in samples of dogs collected from the IVRI, Polyclinics or samples of dogs received from BSF, Tekanpur, Gwalior, Veterinary Polyclinics, Durg, Chhattisgarh on the basis of the results revealed in PCR. Besides, the disease has been reported by various workers viz., G.B. Pant University of Agriculture and Technology, Pantnagar, Uttranchal., GADVASU, Ludhiana, Veterinary College, Parbhani and Veterinary College, Madras etc on the basis of HA test, ELISA, LAT or PCR. From this study, the gravity of problem can be easily realized and suitable measures must be undertaken immediately to check the spread of the disease in the form of effective and efficient preventive and control programmes against the disease.

Host range: All the members of the group comprising CPV /FPL V replicate in feline cells in tissue culture, but only isolates from dogs replicate in cultured canine cells. Their in vivo host ranges also differ since FPLV isolates replicate efficiently only in cats whereas CPV isolate show variable replication in cats depending on the strain of CPV. The original CPV -2 isolates do not replicate in cats but the variants of CPV -2 designated CPV2a and CPV -2b replicate efficiently in cats.

Pathogenesis: CPV -2 replicates in several lymphoid tissues and the intestinal epithelium of dogs and FPL V replicates in the corresponding tissues in the cat. However, there are differences in the extent of viral growth in tissues of the two species. The pathogenesis of CPV-2 infections in dogs and FPLV infections in cats are very similar. The route of entry and initial site of virus replication are cells of nasal and oropharynx including tonsils and other lymphoid tissues. Animals can be experimentally infected by most of the parenteral routes but oral route is the most common natural route of infection. During viraemia, virus spread systematically

and is found in tonsils, retropharyngeal lymph nodes, thymus and mesenteric lymph node after 1-3 days. The virus can be recovered by 3 days in the Peyer's patches. Cytokines may play an important role in the pathogenesis of CPV /FPL V infections.

In general FPLV replicate in feline tissues, lymph node, thymus, spleen, intestinal epithelial cells and virus is present in large numbers in stools. It also replicates in thymus and bone marrow of dog but not in gut or mesenteric lymph node and there is no shedding of virus in the stool. On the other hand, CPV-2, 2a and 2b have the ability to infect the gut tissues of dogs and virus is present in huge quantity in the stool. However, CPV-2 does not replicate in cats but CPV-2a and CPV-2b replicate in cats and high titre virus is present in lymphoid and intestinal tissue.

Epidemiology: In 1978, the entire canine populations were susceptible to CPV -2. Consequently, the infection and disease occurred in all ages of dogs from neonate pups to aged animals. The disease was first noticed predominantly in Kennels and both myocardial and enteric forms were seen.

Epidemiology is concerned with the frequency and distribution of diseases in populations. The pattern of disease experienced in a population is largely influenced by the susceptibility of the host, environmental and management conditions such as housing, hygiene and population density and pathogenicity of the infectious agent. The virus is present in high titers in the faeces of dogs during the acute phase of the disease and the faeces may remain infectious to other dogs for several weeks following clinical recovery. CPV-2 is transmitted from dog to dog primarily by faecal oral route. The constant introduction of susceptible pups (e.g. kennels) or import (pet shops) and improper environmental sanitation makes the dogs exposed to CPV-2 constantly.

Since CPV -2 viruses excreted in the faeces is extremely resistant to inactivation and persists in the environment for prolonged periods of time, CPV -2 infections typically occurs in pups from weaning to about 20 weeks of age at a low but constant rate. However, due to interruptions in the vaccination programme or lapses in hygiene, outbreaks of CPV-2 enteritis associated with high rates of morbidity and mortality are common. All the vaccinated dogs in a Kennel will be naturally infected with

CPV-2 when their maternal antibody titer declines below a critical level, although all infected dogs will not necessarily develop clinical disease. Adult dogs in kennel and pet shops are usually immune to CPV -2 infection either as a result of previous vaccination or natural exposures.

The differences in exposure patterns to CPV-2 between kennel dogs and household pups partially explain why CPV-2 vaccination has been very effective in reducing the incidence of CPV -2 enteritis in the pet dog populations. The annual number of enteritis cases and death due to CPV -2 in pet dogs has been drastically reduced. Routine use of modified live CPV -2 vaccines for pups has resulted in a dramatic decrease in the incidence of CPV -2 enteritis in pet dogs. In contrast, outbreaks of CPV -2 enteritis continue to be a problem in kennel, pet shops and street dog populations. CPV-2 associated myocarditis resulting from infection of neonatal pups born to CPV-2 naive dams has virtually disappeared as a clinical entity, because virtually all dams are now immune to the disease and passively transfer protective maternal antibodies through colostrums to the offspring.

Clinical signs: Two forms of clinical manifestations are observed in parvovirus infections (a) Parvovirus enteritis (b) Parvovirus myocarditis.

(a) Parvovirus enteritis: This occurs in dogs of any age but appears in serious proportions in pups. The most commonly encountered clinical signs include in order of decreasing frequency depression, vomiting, diarrhea, anorexia and fever. There is slight rise of temperature in the initial stage of the disease but gradually turn to subnormal level with advancement of vomiting and diarrhea. There is no consistent character of the stool, it may be watery, yellow in color or tinged with frank blood. The course of illness is also highly variable depending on the infectious dose of the virus and a clinical sign usually develop from 3 to 5 days following infection and typically persist for 5 to 7 days. The morbidity and mortality vary according to the age of the animals, the severity of challenge and the presence of intercurrent disease problems. Faeces can vary from simply soft or pasty in mild cases to grossly haemorrhagic in severe cases. Death ensues due to dehydration leading to the peripheral circulatory failure.

It is important to realize that within a group of dogs or litter of dogs, there may be one death, two pups seriously ill, two pups slightly ill and 3 apparently unaffected. All however will excrete the virus in the faeces and develop a high antibody titer to the virus.

(b) Parvovirus myocarditis: CPV-2 myocarditis is a condition of young dogs (less than 10 weeks) and outstandingly a litter problem. Within an infected litter, 70% pups will die in heart failure by 8 weeks of age and the remaining 30% will have pathological changes which may result in death many months or even years later. The most dramatic manifestations of CPV-2 myocarditis is the sudden death in young pups usually about 4 weeks of age. The collapsed dying pup may have cold extremities, pale mucosae and show gasping respiration or terminal convulsions. Acute heart failure with respiratory distress occurs in pups between 4 to 8 weeks of age. Subacute heart failure occurs in older pups usually 8 weeks or more. They are tachypnoeic or dyspnoeic especially on exercise. The abdomen is swollen with hepatomegaly and ascitic fluid is blood tinged. There is tachycardia, sometimes with arrhythmias and a weak pulse. Most animals die due to cardiogenic shock. However, if the animal survives it will suffer from chronic myocardial and circulatory complications.

Pathology: The mesenteric and peripheral lymph nodes are enlarged and oedematous soon after infection and later may decrease in size. The thymus undergoes atrophy and can be reduced to 1/3rd of the original size. There is extensive lysis of lymphocytes in all lymphoid tissues resulting in lymphocyte depletion. Lymphocytolysis is more severe in the cortex than in the medulla of the thymus. In other tissues, germinal centres are severely affected. There may be erythrocytic, myeloid and megakaryocytic cells in the bone marrow. Intranuclear (I/N) inclusion are rarely seen in the lymphoid tissue. Varying degree of immunosuppression is observed in CPV-2 infected dogs due to pathogenic changes in the lymphoid tissues.

The serosa of the small intestine is often haemorrhagic and has a fine granular appearance due to fibrinous effusion. The intestinal contents may be mucoid or haemorrhagic. The microscopic lesions vary from villus atrophy and crypt cell necrosis to

severe loss of enterocytes. Later, there is epithelial regeneration characterized by presence of cuboidal or large irregularly shaped enterocytes and crypt cell hyperplasia. There is infiltration of neutrophils and eosinophils in the mucosa. Eosinophilic IIN inclusions are occasionally observed in intact crypt cells.

Post mortem examination of pups with myocarditis which have died suddenly or in acute heart failure may reveal severe pulmonary oedema with frothy fluid in the tracheobronchial tree. Microscopically, IIN basophilic inclusion bodies are commonly present in cardiac myocytes in the early stages and EM shows parvovirus particles in these nuclei.

In older animals with subacute heart failure usually 8 weeks or older, there is pulmonary oedema, but also hydrothorax, hydropericardium and ascites with hepatic enlargement and congestion. The heart is enlarged and dilated with pale streaks in the heart wall. Histologically, lymphoid tissues in myocarditis cases show reactive hyperplasia rather than lymphocytolysis.

Diagnosis: A presumptive diagnosis of CPV-2 enteritis can be made based on the age of the dog (<24 weeks of age), a history of inadequate vaccination for CPV-2 and clinical signs. Several laboratory tests have been developed and are available for specific viral diagnosis. Rapid diagnosis can be made by electron microscopy of faecal material from cases with typical signs of diseases. The virus also can be isolated in several feline and canine cell lines such as MDCK, CRFK and A-72 cell lines. But isolation is seldom practiced since cell cultures require at least 1 week to provide diagnosis. Faecal HA and HI test have provided a simple and rapid method for detecting virus in faecal and tissue samples and are adopted by several diagnostic laboratories. However, the HA test is less sensitive than EM or ELISA. Several species of RBC e.g. pig, rhesus monkey or cat can be used to perform the test. For specificity, a HI test is needed to be carried out using specific antiserum or MAbs. Although there is no ELISA kit available in India to diagnose the CPV disease, it is available in other countries. This test is based on the antigen-antibody reactions with specific MAbs fixed on plastic, nitrocellulose membranes, latex or gold particles. The tests are rapid, relatively cheap and can be performed in any veterinary clinic.

Recently, polymerase chain reaction has been adopted to detect the presence of CPV in the stool samples at CADRAD, IVRI, Izatnagar. It has been widely applied to provide rapid, sensitive and accurate diagnosis of the disease. The PCR has been found to detect fewer particles of CPV-2 than other tests like HA and ELISA. The PCR can now be used to differentiate the different mutants (CPV-2, 2a and 2b) of CPV-2 using the primers specific for particular mutants. Either the nested PCR using internal primers or the RE analysis of PCR product of conventional PCR can also be used to further confirm the result of the PCR. The confirmatory diagnosis of the mutants of the CPV responsible for the disease can be ascertained by sequencing the genes coding the structural proteins. The confirmatory diagnosis of the mutants of the CPV -2 responsible for the disease can be ascertained by sequencing the gene coding the structural proteins.

Therapy: The restoration of the electrolyte and fluid balance is the most important goal of therapy. The affected dog should be put under broad spectrum antibiotic umbrella (ampicillin, chloramphenicol, erythromycin, gentamycin etc.). Norfloxacin and nalidixic acid have been proved to be effective against canine haemorrhagic gastroenteritis. The symptomatic treatment with steroid, broad spectrum antibiotic, fluid and electrolyte may save the life of the animal. During the early phase of the disease, the application of hyperimmune serum may help to reduce the virus load and render the infection less dramatic. Such treatment has been shown to reduce the mortality and shorten the length of the disease however hyperimmune serum is difficult to obtain. In case of vomiting, Reglan @ 0.5 mg /kg body weight (Metaclopramide) may be given at 8 hours interval. To correct the gastric problem cimetidine, ranitidine, famotidine and to check diarrhea, loperamide or bismuth subnitrate or other astringent preparations may be given. A dog with persistent vomiting should not be given any food until the diarrhea and vomiting subsides.

Immuno-prophylactic agents: Effective vaccines are available for the prevention of CPV -2 infections. Both modified live and inactivated parvovirus vaccines have been used to fully susceptible sero-negative pups. Attenuated strains of CPV have been derived by repeated passage of the viruses in cell

culture. The vaccine viruses are shed at much lower titers in the faeces suggesting that the absence of enteritis results from decreased viral replication in the intestine. Experimentally live virus vaccines have been shown to protect dogs for at least 3 years or longer. Inactivated vaccine however, provides only a limited duration of immunity to infection and dogs are protected against disease for several months. For parvoviral prophylaxis, modified live virus (ML V) vaccines have proved to be much more effective than inactivated vaccines. ML V vaccines have been shown to be safer and neither vaccine induced diseases, reversion of virulence or the involvement of vaccine viruses in the generation of new viruses have been confirmed.

There is a strong correlation between HI or serum neutralizing antibody titres and resistance to infection with CPV. The HI test has been useful to measure antibodies which correlated with immunity. The HI titre > 1:40 or 1:80 is considered protective. The highest rate of infection is reported in pups older than 6 weeks of age. As with other infectious diseases of dogs, puppies from immune bitches are protected for the first week of life by maternal antibodies which are acquired via the colostrums. Successful immunization with most vaccines can be accomplished with a high degree of confidence only in sero-negative pups, or in pups with very low antibody titers. Maternal antibodies are acquired during the initial 2-3 days of life and then decline, with an average half life of about 9-10 days. Passively acquired antibody titers below 40 are not considered protective against infection but they commonly interfere with immunization. There is a critical period where maternal antibodies are no longer present in sufficient quantity to confer protection. But 90% of the pups from vaccinated populations respond to vaccines at 12 weeks of age.

Vaccination of dogs is generally performed using multivalent vaccines, which contain CD V, CPV, leptospira bacterin and inactivated rabies virus. Monovalent CPV-2 vaccines are also available, some of them containing very high titer (10^7 TCID₅₀) and widely recommended for initial vaccination of pups. About 60% of all puppies sero-converted after a single vaccination either at 6 weeks of age with a CPV monovalent vaccine or at 8 weeks of age with a multivalent vaccine. At 12 weeks of age another shot is given when all pups had received 2-3 inoculation at this age

but nearly 10% pups still had not sero-converted. The principal reason for the non-responders was the persistence of interfering levels of maternal antibodies. None of the vaccines tested were capable of breaking through a maternal antibody titer of 1: 160 or higher, regardless whether the vaccines were high titered or not.

The following general vaccination schedule is recommended.

1. Vaccination at 6 weeks of age with a CPV-2 monovalent vaccine.
2. Vaccination at 8 weeks of age with a multivalent vaccine CPV, CDV, canine adenovirus (CAV), leptospira and rabies antigen.
3. Vaccination at 15 of 16 weeks of age with a multivalent vaccine CPV, CD V, CAY, leptospira and rabies antigen.

If it is necessary to develop an individual vaccination schedule, determination of the antibody titer of one or two pups in the litter could be determined at 5 or 6 weeks of age, then vaccination of the litter may be calculated on the basis of titer, using an estimated antibody half life of 9.5 days. Vaccination is likely to be successful when the maternal antibody titer has declined to less than 1: 10. Titer below 1:40 is variably protective, but they may interfere with vaccination.

An attenuated live FPLV vaccine for CPV-2b infection has shown that vaccinated specific pathogen free (SPF) cats are protected from challenge with CPV -2b at 2 weeks after vaccination. However, antibody titers induced by a FPL V vaccine are significantly lower against CPVs than FPL V. It is better to use homologous inactivated vaccines that use CPV-2a or CPV-2b for cats. CPV-2a/CPV-2b based vaccines are expected to protect cats more efficiently from CPV-2 infection than conventional FPL V vaccines. Again, like FPL V vaccine for CPV - 2 infection, CPV2a12b based vaccines may be less efficient for FPL V infection which would be a major concern. Interestingly, CPV -2c infected cats showed similar neutralization antibody titers against FPLV, CPV-2a, CPV- 2b as well as CPV2c. An inactivated CPV-2c based vaccine for cats could be a promising vaccine candidate against both CPV and FPL V infection.

Recombinant Vaccine: Recombinant vaccine containing the baculovirus expressed VP2 protein was found to be structurally and immunologically indistinguishable from

authentic VP2. The recombinant VP2 also shows the capability to self assemble, forming virus like particles similar in size and appearance to CPV virions. The VP2 protein at the rate of 10 µg was able to elicit good protective response as measured by ELISA and shown to be better than commercially available inactivated CPV vaccine in terms of immune response. The expressed VP2 was administered along with the Quil A (50 µg/ animal), alumina or both adjuvants on 0 day and 28 days to improve the immunogenicity of the vaccine at different doses (10µg, 25µg, 50µg and 100µg). All the vaccinated dogs maintained the protective antibody response up to 6 days observation period and withstood challenge virus infection 42 days after the booster doses.

DNA vaccines: Both the prokaryotic and eukaryotic vectors harbouring the genes coding for the structural proteins of the canine parvovirus has shown the encouraging results. The dogs immunized with the DNA vaccines withstood the challenge with virulent canine parvovirus. However, the DNA vaccines still is in the experimental stage and not yet licensed to be used in the field condition.

Conclusion: Canine parvovirus infections in dogs have been regarded as one of the most important diseases of dogs. Since it was first reported in 1977, the disease has been reported from many countries of the world including India. Although potent and efficacious live attenuated and inactivated vaccines are available in India, large number of cases are diagnosed by HA, HI, ELISA or PCR mostly from the unvaccinated dogs. As the CPV-2 is very resistant to environmental conditions, they remain viable for long period of time in nature. The stray dogs are not vaccinated against the disease and they remain carrier of the virus and source of infection to other susceptible dogs. Extensive studies must be undertaken to know the molecular epidemiology of the canine parvovirus infections in different species canines the variants of the CPV involved in the

outbreak of the disease. The necessary preventive measures must be undertaken to immunize the susceptible dogs including the stray dogs with the potent and efficacious vaccines against the disease to check the spread of the disease.

Reference:

Afshar, A (1981). Canine parvovirus infections - a review. *Vet Bull.* 51 : 605-612..

Battilani, M., Scagliarini, A, Tisato, E., Turilli, C., Jacoboni, I., Casadio, R. and Prospero, S. (2001). Analysis of canine parvovirus sequences from wolves and dogs isolated in Italy. *J. Gen. Virol.* 82 : 1555-1560.

Hirasawa, T, Kaneshige, T. and Mikazuki, K. (1994). Sensitive detection of canine Parvovirus DNA by the nested PCR. *Vet. Microbiol.* 41 : 135-145.

Ikeda, Y, Mochizuki, M, Naito, R., Nakamura, K., Miyazawa, T., Mikami, T. and Takahashi, E. (2000). Predominance of canine parvovirus (CPV) in unvaccinated cat populations and emergence of new antigenic types of CPV s in cats. *J. Virol.* 278 : 13-19

Parrish, C. R. (1999). Host range relationship and the evolution of canine parvovirus. *Vet. Microbiol.* 69 : 29-40.

Pereira, C.A, Monezi, T.A, Mehnert, D.D., D' Angelo, M. and Durigon, E.L. (2000). Molecular characterization of canine parvovirus in Brazil by PCR. *Vet. Microbiol.* 75: 127-133.

Sagazio, P., Tempesta, M., Buonavoglia, D., Cirone, F. and Buonavoglia, C. (1998). Antigenic characterization of canine parvovirus strains isolated in Italy. *J. Virol. Meth.* 73 : 197- 200.

Uwatoko, K., Sunairi, M., Yamamoto, A, Nakajima, M. and Yamaura, K. (1996). Rapid and efficient method to eliminate substances inhibitory to the PCR from animal faecal samples. *Vet. Microbiol.* 52 : 73-79.

&&&&&&&&