VACCINE FAILURE AGAINST CANINE PARVOVIRUS INFECTIONS IN DOGS

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Canine parvovirus 2 (CPV-2) has been considered to be an important pathogen of domestic and wild canids and has spread worldwide since its emergence in 1978. It has been reported from Asia, Australia, New Zealand, the Americas and Europe. There are two distinct parvoviruses known to infect dogs – the pathogenic CPV-2 and CPV-1 or the minute virus of canine (MVC). The disease is characterized by two prominent clinical forms, enteritis with vomiting and diarrhea in dogs of all ages and myocarditis and subsequent heart failure in pups of less than 3 months of age with high morbidity (100%) and frequent mortality up to 10% in adult dogs and 91% in pups. The disease condition has been complicated further due to emergence of a number of variants namely CPV-2a, CPV-2b and CPV-2c over the years and involvement of domestic and wild canines. Vaccination is the most cost effective and ideal method to control the canine parvovirus infections in canines. Both live attenuated and inactivated vaccines are available to control the disease in animals. Vaccines used during the late 1970s and early 1980s were of feline panleukopenia virus (FPV) origin followed by canine-origin, inactivated and live attenuated vaccines of CPV-2, CPV-2a and CPV-2b. High-titer, low-passage CPV vaccines containing a canine-origin, attenuated virus are currently the vaccines of choice for use in pups of any breed. In spite of large scale vaccination to control the disease in dogs, the disease has been reported both in vaccinated and the unvaccinated dogs despite the progress in the field of diagnostics and immunoprophylactic agents. Considering the enormous importance of the disease, many reasons behind the vaccine failure in canine parvovirus infections have been discussed in this review for the benefit of the scientific fraternity, dog owners, veterinary practitioners, students, researchers and diagnosticians which in turn help in the better and effective management and ultimately control of the disease.

Keywords: Canine parvovirus 2 (CPV-2), CPV-2a, CPV-2b, CPV-2c, Hemorrhagic gastroenteritis, Myocarditis, Vaccination, Vaccination failure, Maternal antibodies, Inactivated vaccine, Live attenuated vaccine.

Introduction

Canine parvovirus 2, the causative agent of acute haemorrhagic enteritis and myocarditis in dogs, is one of the most important pathogenic viruses. It is a highly infectious 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% (Appel et al., 1978). The disease is characterized by two 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 (Appel and Parrish, 1987). 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). CPV is believed to have originated as a host range variant from feline panleukopenia virus (FPV), include a direct mutation from FPV, a mutation from a FPV vaccine virus and the adaptation to the new dog host via non-domestic carnivores, like mink and foxes. The original type (CPV-2) which emerged in the late 1970s was rapidly replaced by two antigenic variants, CPV-2a in 1979 and CPV-2b in 1983 (Parrish et al., 1985; Parrish et al., 1991). Further in 2000, a third type CPV-2c was first detected in Italy and found to be progressively replacing other variants in many countries of the European Union, South America, North America and Asia (Buonavoglia et al., 2001; Martella et al., 2004; Nakamura et al., 2004; Decaro et al., 2006, 07; Perez et al., 2007; Hong et al., 2007; Calderon et al., 2009; Nandi et al.,...
Canine parvovirus belongs to the genus Parvovirus and family Paroviridae. CPV has icosahedral symmetry, 25 nm in diameter and non-enveloped with a linear, single stranded DNA genome of 5.2 Kb. The infectious capsid contains ~55 copies of VP2 and ~5 copies of the VP1 protein which contains both the VP2 sequence and 143 additional N-terminal residues (Tsao et al., 1991; Xie and Chapman, 1996). VP2 (64 kDa) is an NH2-terminally truncated form of VP1 (84 kDa) and is the major component of the capsid. Elaborate loops forming most of the capsid surface make up most of the functional sites of the capsid, including those involved in receptor and antibody binding (Agbandje et al., 1993; Strassheim et al., 1994; Govindasamy et al., 2003; Hueffer et al., 2003). The cell receptor for CPV is the transferrin receptor (TfR), and appropriate TfR binding leads to cell infection followed by generation of large number of progeny virus particles (Hueffer et al., 2003b; Parker et al., 2001). There are at least 5 or 6 amino acid changes between the variants CPV-2a/2b and the original CPV-2 in the VP2 capsid proteins while the variant CPV-2a differs from the variant CPV-2b only in the 426 Asn-Asp within the major antigenic site of the capsid whereas in CPV-2c it is Glu-426 (Parrish et al., 1991; Buonavoglia et al., 2001). The few amino acid differences in CPV-2 and its variants have altered antigenic features of the virus and modified important biological properties such as the in vivo and in vitro host ranges, the interaction with the cellular receptor and the virulence (Cavalli et al., 2008).

**Epidemiology of CPV**

Canine parvovirus infection occurs worldwide in domestic dogs and other members of the dog family. Incidence is higher in animal shelters, pet shops, and breeding kennels. CPV can affect dogs at any age. Severe infection is most common in puppies between 6 weeks and 4 months old. All breeds of dogs are susceptible. The
crossbreds are less susceptible in comparison to pure breeds like Rottweilers, Doberman Pinschers, English Springer Spaniels and German Shepherd, the exception to this being Toy Poodles and Cocker Spaniels (Houston et al., 1996). The CPV infection is more severe in young puppies especially those younger than three months of age (Appel et al., 1979; Jacob et al., 1980). All infected dogs may not necessarily exhibit clinical manifestations but they may shed the virus in feces during the acute phase of enteric fever and show significant rise in the serum antibody titers (Stann et al., 1984).

The different antigenic variants of CPV-2 are prevalent in varying proportion in different countries. The prevalence of CPV-2b has been reported by various authors in several countries namely Brazil (Pereira et al., 2000), USA (Parrish et al., 1988), Japan (Hirasawa et al., 1996), Switzerland (Truyen et al., 2000) and South Africa (Steinel et al., 1998). Contrastingly, CPV-2a was found to be the prevalent antigenic type in France, Taiwan and Italy (Chang et al., 1992; Martella et al., 2004). However both CPV-2a and CPV-2b have been found to be distributed in equal proportion in Spain (de Ybanez et al., 1995) and U.K. (Greenwood et al., 1996). CPV-2c has also been found in Vietnam (Nakamura et al., 2004), Spain (Decaro et al., 2006b), United Kingdom (Decaro et al., 2007a), South America (Perez et al., 2007), North America (Kapil et al., 2007) and India (Nandi et al., 2010b).

CPV-2 for the first time isolated in India in 1982 (Ramadas and Khadher, 1982). After that, the incidence of CPV-2 variants in dogs were reported from different states viz. Kerala (Deepa and Saseendranath, 2000), Assam (Phukan et al., 2004), Tamil Nadu (Sanjukta et al., 2008), Orissa (Banja et al., 2002), West Bengal (Biswa et al., 2006), Pondicherry (Panneer et al., 2008), Haryana (Savi et al., 2009) and Uttar Pradesh (Panda et al., 2009; Nandi et al., 2010 a, b). The prevalence of CPV-2a has been documented in 2001 in Southern India (Narayanan et al., 2001; Chinchikar et al., 2006-7)). However, the incidence of CPV-2b is more common to other mutants in Northern India (Kumar and Nandi, 2010). Occurrence of CPV-2c was first reported in India by Nandi et al. (2010b) based on the sequence analysis of CPV-2b positive sample. Its presence in India supports the assumption that CPV-2c is going to reach a worldwide distribution and provides new information to understand the evolution of antigenic variants of CPV-2 (Nandi et al., 2010b).

Symptoms
There is a broad range in the severity of symptoms shown by dogs infected with parvovirus. Many adult dogs exposed to the virus remain apparently healthy but act as a carrier to transmit the virus to the susceptible animals. The disease in majority of the cases is seen in dogs less than 6 months of age with severe symptoms in puppies younger than 3 months of age. The most common form of the disease is enteritis. It is characterized by vomiting, diarrhea, dehydration, dark or bloody faeces and in severe cases fever and lowered WBC counts. Early symptoms are depression, loss of appetite, vomiting, high fever and severe diarrhea. There is slight rise of temperature in the initial stage of the disease but gradually turn to subnormal level with advancement of vomiting and diarrhoea (Kramer et al., 1980). There is no consistent character of the stool, it may be watery, yellow in color or tinged with frank blood in severe cases. Rapid dehydration is a danger, and dogs may continue to vomit and have diarrhoea until they die, usually three days after onset of symptoms. The course of illness is also highly variable depending on the infectious dose of the virus and clinical signs usually develop from 3 to 5 days following infection and typically persist for 5 to 7 days (Fletcher et
The acute parvovirus enteritis can be seen in dogs of any breed, sex or age. However, cross bred dogs are less susceptible than pure breed dogs such as Rottweilers, Doberman Pinchers, English Springer Spaniels, Labrador Retrievers and German Shepherd with the exception of Toy Poodles and Cocker Spaniels. The disease will progress rapidly and death occurs as early as 2 days after the onset of the disease. The presence of Gram-negative bacteria, parasites or other viruses can worsen the condition and slow down the process of recovery. The second form of CPV is cardiac syndrome, or myocarditis, which can affect puppies under three months old (Appel et al., 1979). 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 manifestation of CPV-2 myocarditis is the sudden death in young pups usually about 4 weeks of age (Mochizuki et al., 1996).

The tissue distribution of CPV was found to have similar patterns in dogs infected by types 2a, 2b and 2c, revealing that the variants have the same biological behaviour. Parvovirus replication in dogs and cats takes place mainly in highly mitotically active tissues, such as bone marrow, lymphoid organs and intestinal crypts (Appel and Parrish, 1987). The nervous tissue involvement has been described in cats (Csiza et al., 1972; Wilcox et al., 1984; Url et al., 2003), whereas in dogs CPV antigen has never been detected in neurons, despite the presence of neurodegeneration (Agungpriyono et al., 1999; Url and Schmidt, 2005) but these results were in contrast with the Decaro et al. (2007b), who demonstrated the presence of CPV nucleic acid in all tissues including brain, cerebellum and bulb.

Vaccines and Immunity

Puppies get protected during the first few weeks of their life through colostrums. The duration of immunity depends on how much colostrums a puppy received in its first 2-3 days of life with an average half life of 9-11 days (Decaro et al., 2005b). The decline of maternal antibody level starts from first week to 13 weeks in the pups. Immunity to CPV infection appears directly related to antibody titre. Vaccines used to date are unreliable when given in the presence of maternal antibodies. 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 titres 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 vaccines however, provide only a limited duration of immunity to infection and dogs are protected against disease for several months (Carmichael et al., 1993; Schultz, 2006). For paroviral prophylaxis, modified live virus (MLV) vaccines have proved to be much more effective than inactivated vaccines. MLV 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 (Carmichael et al., 1993).

There is a strong correlation between HI or serum neutralizing antibody titers and resistance to infection with CPV. The HI test has been useful to measure antibodies which correlated with immunity. Dogs vaccinated with killed vaccine developed a serum antibody titre of less than 1:80 in HI test and shed virulent CPV when challenged orally. It is indicated that dogs with low antibody titre support viral
replication in the intestine and are a source of infection for susceptible contacts. Dogs that recover from the infection have standard HI titres ranging from 1: 2560 to 1: 20480 which persist for at least one year and are solidly immune (Appel et al., 1980; Carmichael et al., 1993). Pups are fully susceptible to challenge CPV infection when the HI titre falls below 80 (Carmichael et al., 1993). This leaves a period of several weeks where the young pups are susceptible to infection but refractory to vaccination. This period has been termed as “immunity gap”. In 1985, a vaccine became available to use in dogs against CPV infection and when given at the age of 12 weeks 90% overcame the immunity gap. As the CPV evolves very rapidly the question arises about the efficacy of such vaccines against current field strains (Greenwood et al., 1995). Based on this observation, O’Brien (1994) suggested that all the susceptible pet population should be vaccinated at an interval of 3 weeks with a low passage MLV-CPV until they attain an age of 18-20 weeks.

The HI titre > 1:80 is considered protective. The highest rate of infection is reported in pups older than 6 weeks of age (Pratelli et al., 2000). Passively acquired antibody titers below 80 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 (Decaro et al., 2005b; Schultz, 2006).

Vaccination of dogs is generally performed using multivalent vaccines, which contain CDV, CPV, leptospira bacterin and inactivated rabies virus. Monovalent CPV-2 vaccines are also available, some of them containing very high titer (10⁷ TCID₅₀) virus and widely recommended for initial vaccination of pups. About 60% of all puppies seroconverted 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 seroconverted (Pratelli et al., 2000). 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 tittered or not (Decaro et al., 2005b). 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, CDV, CAV, 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.

**Antigenic variation and cross-protection**

There is a growing concern that the vaccines used currently to prevent CPV infection in dogs may fail to effectively protect pups against the new CPV antigenic variants (Martella et al., 2005; Truyen, 2006). Although the original CPV-2 was completely replaced by the antigenic variants a few years after its appearance,
the original CPV-2 is still used in most commercial vaccines (Nandi et al., 2010). Several studies have demonstrated that CPV-2 vaccines are still effective to induce protection against CPV variants (Greenwood et al., 1995). The antigenic relationships among the original CPV-2 and the variants CPV-2a, CPV-2b and CPV-2c were evaluated by HI and SN using the sera of immune dogs and rabbits (Cavalli et al., 2008). Cross-antigenic evaluation of the CPV-2 variants revealed clear differences, which were more appreciable by SN than by HI. These findings confirm preliminary observations and deserve particular attention, as HI is the gold standard test used in diagnostic laboratories for evaluation of humoral immunity to CPV-2 (Pratelli et al., 2001). Accordingly, the results obtained with HI may tend to overrate the real immune status of the animals. The greatest antigenic differences were found between the original CPV-2, which is still largely employed in vaccine formulations and the variants. The original CPV-2 differs in at least five or six amino acid changes from the recent CPV-2 variants (Parrish et al., 1991). However, it was also possible to observe antigenic differences among the CPV-2a, CPV-2b and CPV-2c variants, which may differ from each other even by a single amino acid change (Martella et al., 2005). In the animals immunized with CPV-2, the SN titers to the antigenic variants CPV-2a, CPV-2b and CPV-2c were significantly lower than the homologous titers (Cavalli et al., 2008). It is improbable that these differences may account for decreased protection against the variants in dogs that are protected by a strong active immune response, since after repeated immunizations the antibody titers in young dogs appear to be markedly higher than the minimum levels required for protection against disease and infection. However, it is possible that these differences may allow escape from the limited antibody repertoire of maternal origin in young, unvaccinated pups (Cavalli et al., 2008). Severe parvovirus outbreaks have been observed in pups with HI titers of maternally derived antibodies above the threshold (1:80) related to protection against disease and infection. Likewise, experimental infection by virulent CPV-2b strains of unvaccinated pups with high maternally derived antibody HI titers (>80) which are usually expected to prevent CPV infection and disease, resulted in clinical signs, virus shedding and an antibody response (Decaro et al., 2005b; Elia et al., 2005). Although animals immunized correctly with CPV-2 vaccines are fully protected clinically, there is evidence that the active immunity elicited by the vaccines may sometimes fail to protect adult dogs, and the reasons for this may rely on a physiological decline of the protective immunity or on the increased virulence/tropism inherent to some CPV strains (Greenwood et al., 1995). The sporadic cases of CPV-2c infection in adult dogs (>1 year) have been diagnosed. The disease outbreak caused by CPV-2c in adult dogs immunized 3 times with a vaccine containing the original CPV-2 has been reported. Marked antigenic differences were observed by SN in the sera of dogs and rabbits immunized with the CPV-2b vaccine, as the heterologous SN titers (versus CPV-2a and 2c) were significantly lower than the homologous SN titer (versus CPV-2b) (Cavalli et al., 2008).

The evaluation of the antigenic features of CPV-2c by cross-neutralization revealed a unique pattern for the variant CPV-2c. The CPV-2c variant was less effectively recognized by SN by the sera of dogs inoculated with the heterologous (CPV-2, CPV-2a and CPV-2b) viruses. Conversely, in dogs infected/inoculated with CPV-2c, the homologous (versus CPV-2c) titers tended to be lower than the heterologous titers, notably versus CPV-2b. To a lesser extent, a similar inconsistent pattern was observed in rabbits inoculated with the variant CPV-2a, as the
homologous (versus CPV-2a) titers tended to be lower than the heterologous titers to CPV-2b. The antigenic paradox exhibited by CPV-2c may generate a different selective pressure in the dog population and may have contributed to the spread of the variant CPV-2c. These findings warrant studies to evaluate the opportunity to develop ML vaccines based on the CPV-2c variant (Cavalli et al., 2008). It is indicated that the discrepancies between the HI and SN titers, suggesting that HI is not adequate to evaluate the real protective immunity of dogs, in particular against the antigenic variants. Also, by SN there are significant differences in the homologous and heterologous antibody titers, these differences were more marked between the original CPV-2 and the recent variants CPV-2a, CPV-2b and CPV-2c (Cavalli et al., 2008).

Vaccine failure

The primary cause of CPV vaccine is an interfering level of maternally derived antibodies against CPV. The genetic constitution of dogs also influences the susceptibility of particular breeds to CPV infections. Further, immunocompetence of the host at the time of vaccination also influences to elicit the effective immune response. Mismatching between vaccine strain and field strain of CPV may have variable protection level against various strains prevailing in the field. Maintenance of cold chain is an important parameter particularly in the tropical conditions to maintain the potency and efficacy of the vaccines. Improper administration of vaccine in the host may also play an important role in the vaccine failure. Other reasons behind vaccine failure in canine parvovirus infections may be improper zoosanitary measures, disinfection practices, follow up of improper vaccination schedule, presence of other intercurrent diseases of bacterial and viral origin, antigenic mass present in the vaccine, etc.

Therapy

The restoration of the electrolyte and fluid balance is the most important goal of therapy. The affected dogs 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 (Woods et al., 1980). During the early phase of the disease, the application of hyperimmune serum may help to reduce the virus load and render 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, Lopamide or bismuth subnitrate or other astringent preparations may be given (Kramer et al., 1980). A dog with persistent vomiting should not be given any food until the diarrhea and vomiting subsides.

Prevention and Control

As the canine parvovirus is not enveloped, it is especially hardy in the environment. It is able to withstand winter freezing temperatures in the ground outdoors and many household disinfectants are not capable of killing it indoors. Infected dogs shed virus in their stool in gigantic amounts during the 2 weeks following exposure. A typical/average infectious dose for an unvaccinated dog is 1000 viral particles. An infected dog sheds 35 million viral particles (35,000 times the typical infectious dose) per ounce of stool. Virus loses its infectivity within one month, therefore, it should be safe to introduce a new puppy indoors one month after the
active infection has ended. If the outdoors is contaminated and is frozen, one must wait for it to thaw out before safely introducing a new puppy. Shaded areas should be considered contaminated for seven months. Areas with good sunlight exposure should be considered contaminated for five months. Although most disinfectants cannot kill it, chlorine bleach (1 part bleach and 30 part water) is quite effective. There is no way to completely disinfect contaminated dirt and grass, although sunlight and drying has some effect. Mechanical decontamination through irrigation may also be helpful, but the area must be allowed to dry thoroughly between applications. Potassium peroxymonosulfate has relatively good activity in the face of organic matter, and can be sprayed on contaminated areas using a pesticide sprayer or other applicator.

Another strategy to reduce risk for parvoviral outbreaks is to segregate juvenile animals from adults. Puppies and kittens should not be house with adults. Puppies or kittens can be housed together using a planned all in-all out co-housing approach. In this approach, littermates can be housed together in small groups (3 per group), and unrelated puppies or kittens that were already living together before admission can also be housed together. Dogs and cats should be housed in separate areas because CPV-2b has the potential to infect cats and cause panleukopenia. Finally, all efforts to reduce stress should be pursued. The most effective way to reduce stress on animals is to prevent crowding by practicing population management principles. Limiting run and cage occupancy to 1–2 compatible animals each results in less stress and substantially reduces risk of contracting infectious disease.

References


parovirus type 2c in the dogs with haemorrhagic enteritis in India.

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