

Canine parvoviral enteritis: a review of diagnosis, management, and prevention

Jennifer Prittie, DVM, DACVIM (Internal Medicine), DACVECC

Abstract

Objective: To review and summarize current information regarding epidemiology, risk factors, and pathophysiology associated with canine parvoviral infection, and to outline diagnostic and treatment modalities for this disease. Preventative and vaccination strategies will also be discussed, as serologic documentation of immunocompetence and adoption of safe and effective vaccination protocols are crucial in limiting infection and spread of canine parvoviral enteritis.

Etiology: Parvoviruses (Parvoviridae) are small, nonenveloped, single-stranded DNA viruses that replicate in rapidly dividing cells. Canine parvovirus 2 (CPV-2) remains a significant worldwide canine pathogen and the most common cause of viral enteritis in this species.

Diagnosis: Classic presentation of CPV infection includes acute-onset enteritis, fever, and leukopenia. Definitive diagnostic tests include detection of CPV in the feces of affected dogs, serology, and necropsy with histopathology.

Therapy: Standard therapeutic practices for both mildly and severely affected puppies will be discussed. The ability of this virus to incite not only local gastrointestinal injury, but also a significant systemic inflammatory response has recently been reviewed in the literature, and novel innovative experimental and clinical therapeutic strategies, such as antagonism of proinflammatory cytokines and immunostimulation, are introduced in this article.

Prognosis: CPV remains a significant worldwide canine pathogen. In experimentally affected dogs, mortality without treatment has been reported as high as 91%. However, with prompt recognition of dogs infected with CPV-2, and aggressive in-hospital supportive therapy of severely affected puppies, survival rates may approach 80–95%.

(*J Vet Emerg Crit Care* 2004; 14(3): 167–176)

Keywords: immunomodulation, leukopenia, parvovirus, SIRS, vaccination

Introduction

Canine parvovirus (CPV) remains a significant worldwide canine pathogen. In experimentally affected dogs, mortality without treatment has been reported as high as 91%.¹ No definitive treatment has been established, with mortality rates of 4–48% reported despite aggressive supportive care.^{2–5} Survival in affected dogs has recently been shown to vary depending on place of treatment, with higher survival rates reported in ter-

tiary care hospitals *versus* private practices.⁵ The following discussion will review the pathophysiology associated with canine parvoviral infection, standard treatment interventions, innovative experimental and clinical therapeutic strategies, and current vaccination protocols.

Parvoviridae

The most important cause of canine viral enteritis is CPV, which emerged as a clinical problem in 1978.⁶ Parvoviruses (Parvoviridae) are small, nonenveloped, single-stranded DNA viruses that replicate in actively dividing cells.⁷ These viruses are hardy, persisting for long periods of time in the environment (5–7 months), and are ubiquitous.

From the Department of Critical Care, the Bobst Hospital of the Animal Medical Center, New York, NY.

Address correspondence and reprint requests to:

Dr. Jennifer Prittie, The Animal Medical Center, Bobst Hospital, 510 East 62nd Street, New York, NY 10021. Fax: (212) 752-2992. E-mail: jennifer.prittie@amcn.org

Canine parvovirus 2 (CPV-2) believed to have emerged from feline panleukopenia virus or from a parvovirus of another wildlife species was the first variant of this virus associated with the development of hemorrhagic diarrhea. Between 1979 and 1985, CPV-2 was largely replaced by 2 more virulent strains of parvovirus, CPV-2a and CPV-2b. More than 80% of the isolated cases of CPV in the United States today are CPV-2b.^{6,7}

Epidemiology

Initial outbreaks of parvovirus were characterized by groups of adult and/or young dogs becoming clinically ill simultaneously, and were associated with high morbidity and mortality rates. Today, this virus almost exclusively affects puppies between the ages of approximately 6 weeks to 6 months. Most adult dogs are immune to the disease, either via natural infection or immunization. Subclinical infections, characterized by seroconversion without evidence of clinical disease, are common in the unvaccinated adult.⁸ Immunity is long-lived, perhaps lifelong, which leaves only a pool of susceptible puppies born into the population.

If the bitch has antibodies to CPV, either by previous infection or vaccination, her puppies are protected against parvovirus infection for the first several weeks of life by maternal antibody. The CPV-2 antibody titer transferred to the neonate by absorbed colostrum antibody is 50–60% of the mother's titer.⁹ Therefore, maternal antibody titer is determined by the serum titer of the mother at whelping, the amount of colostrum ingested, and litter size.

Maternal antibody to parvovirus has a half-life of approximately 10 days.⁹ As this antibody titer declines, puppies become susceptible to infection, and active immunization is required for protection against disease. A window of increased susceptibility to viral infection occurs when maternal antibody interferes with the immune response to CPV vaccine but cannot protect against CPV infection.

Risk Factors

Predisposing factors for parvoviral infection in puppies include lack of protective immunity, unsanitary/overcrowded environments, and endoparasitism. Certain breeds are at increased risk for severe CPV enteritis, including the Rottweiler, Doberman Pinscher, American Pit Bull terrier, Labrador retriever, and German Shepherd dog.^{7,10,11} Inherited immunodeficiency in Rottweilers, and common ancestry and von Willebrand's disease in both Rottweilers and Doberman Pinschers are postulated as reasons for increased sus-

ceptibility to severe disease manifestations in these breeds.^{3,11} While genetics appear to play a role in breed-associated risk, other factors that may account for increased disease occurrence in certain breeds, such as breed popularity and lack of appropriate vaccination protocols, cannot be excluded. A distinct seasonality has been reported, with a higher incidence of clinical disease occurring between July and September than during the other months.¹⁰ All studies report increased susceptibility in puppies less than 6 months of age. Among dogs older than 6 months, sexually intact males are twice as likely as sexually intact females to develop CPV enteritis.¹⁰

Disease Transmission and Pathogenesis

Infection is acquired by the fecal–oral route of transmission.⁷ During the first 2 days after ingestion, viral replication occurs in the oropharynx and local lymphoid tissue. Viremia ensues and is marked by the third to fifth days post-infection, with CPV preferentially targeting tissues with rapid cell turnover.^{6,7} The cells most affected in puppies are the lymphoid tissues, intestinal epithelium, bone marrow, and heart. Myocarditis occurs only if neonates are affected during the period of rapid myocardial cell proliferation (beginning *in utero* and completed within the first 2 weeks of life). This latter form of CPV infection is rare today because of population immunity and maternal antibody protection.^{6,7}

In a puppy greater than 2 weeks of age, the tissues most affected by CPV are lymphoid tissue, intestinal epithelium, and bone marrow. The virus reaches the intestinal mucosa by way of the blood stream, replicating in the germinal epithelium of the intestinal crypts. Subsequent villous loss results in collapse of the intestinal epithelium, loss of absorptive capacity, and the development of hemorrhagic diarrhea 4–5 days after oral exposure.^{6,7} Characteristic microscopic intestinal lesions consist of necrosis of the epithelium of the intestinal crypts, villous atrophy or collapse, and/or disruption of the lamina propria. Dilated intestinal glands filled with necrotic debris, and evidence of epithelial regeneration are also commonly observed.¹² Recently weaned puppies are at increased risk for CPV enteritis, as enterocytes in these puppies have a higher mitotic index due to diet change and changes in bacterial flora.¹⁰ Disease may be more rapid or severe in dogs with an already compromised gastrointestinal barrier, as seen with concurrent endoparasitism or canine coronavirus infection.^{6,7}

Lymphopenia, and in severe cases, panleukopenia, occur secondary to lymphoid necrosis and destruction of myeloproliferative cells in the bone marrow.^{6,7} A

neurologic form of CPV infection, characterized by diffuse leukoencephalomalacia, has also been reported.¹³ Liquefactive necrosis of the cerebral white matter secondary to severe myocardial lesions producing cerebral hypoxia and ischemia is the postulated mechanism for this manifestation of CPV infection.

While local antibody is detectable early in the course of CPV infection, it is the systemic humoral immune response that confers protection, as the virus enters the intestinal tract by way of the bloodstream as opposed to via the intestinal lumen. Circulating antibodies to CPV are usually detectable at the commencement of clinical signs, and peak during the course of clinical illness.^{6,7} Disease severity and duration is largely determined by the rapidity of this systemic immune response.

Because cell turnover in the gastrointestinal tract is rapid (1–3 days), intestinal malabsorption is short-lived, and recovery from the enteric form of the disease is rapid. Even in severe and/or fatal cases, there is evidence of intestinal regeneration. Fecal shedding of virus is variable and short-lived (typically for less than 2 weeks following infection). Immunity is long lasting and complete.^{6,7}

Clinical Findings Associated with CPV Infection

Initial clinical signs associated with CPV enteritis are nonspecific, and include anorexia, depression, and fever. Most affected puppies begin vomiting and develop small bowel diarrhea within 24–48 hours of initial clinical signs.¹⁴ Large fluid and protein losses through the gastrointestinal tract may result in severe dehydration and hypovolemic shock. Classic signs associated with impairment in tissue perfusion, including changes in mentation, prolonged capillary refill time, tachycardia, poor pulse quality/hypotension, cool extremities, and low rectal temperature, may be evident. Clinical disease is more severe in puppies with underlying compromise of humoral immunity, secondary to concomitant infection, low maternal antibody titers, or environmental stresses.

Abdominal pain secondary to acute gastroenteritis or intussusception may be evident on physical examination. Intestinal intussusception warrants immediate surgical intervention, and may be diagnosed via abdominal palpation, radiography, and ultrasonography. Nonspecific radiographic findings consistent with gastroenteritis are fluid- and gas-filled small intestinal loops.

The most consistent hematologic finding associated with CPV infection is lymphopenia, but panleukopenia may be observed in severe cases.^{6,11} Abnormalities on serum chemistry analysis are nonspecific, and may include prerenal azotemia and elevations of hepatocellu-

lar enzymes secondary to severe dehydration and tissue hypoperfusion; hypoalbuminemia secondary to gastrointestinal losses; hypokalemia secondary to gastrointestinal losses and inadequate intake; and hypoglycemia associated with severe malnutrition and/or underlying sepsis.

Systemic Manifestations of CPV Infection

While clinical signs of CPV infection are typically limited to severe gastrointestinal upset and immunosuppression, a less clinically apparent, more global systemic inflammatory response occurs in many affected puppies. *Escherichia coli* has been recovered from the lungs or liver of the majority of puppies that have died secondary to severe CPV infection, and pathologic findings compatible with acute respiratory distress syndrome (ARDS) have been reported.¹⁵ These findings imply that intestinal tract damage secondary to viral infection increases the risk of bacterial translocation and subsequent coliform septicemia, development of a systemic inflammatory response, and death.⁴ Furthermore, secondary bacterial infections with salmonellae, clostridia, and campylobacter species have been reported to occur with CPV infection, and may directly result in septicemia and/or endotoxemia.^{16,17}

Bacteremia is not necessary for the development of ARDS and sepsis. Endotoxin, through its elaboration of pro-inflammatory cytokines such as tumor necrosis factor (TNF), is a potent mediator of the systemic inflammatory response. The release of TNF and interleukin-1 (among others) into circulation results in peripheral vasodilation, increased capillary permeability, depressed cardiac function, and activation of the coagulation cascade.^{18,19} In a recent study by Otto et al., 82% of dogs with CPV enteritis had measurable endotoxin in circulation. Approximately 33% of the dogs in this study had evidence of TNF activity during hospitalization. There was a significant association between increasing TNF activity and mortality in this population.⁴

Evidence of hypercoagulability without disseminated intravascular coagulopathy has been documented in dogs with CPV enteritis.²⁰ An endotoxin- or cytokine-mediated procoagulant effect on endothelial cells, loss of the natural anticoagulant, antithrombin (AT), through the gastrointestinal tract along with albumin, consumption of AT as a result on endotoxin-mediated activation of coagulation, and hyperfibrinogenemia are postulated as contributing to the hypercoagulable state seen with CPV infection.²⁰

Diagnosis of CPV Infection

Classic presentation of CPV infection includes acute-onset enteritis, fever, and leukopenia.⁶ However, while

often considered a hallmark of parvoviral enteritis, leukopenia is present in less than half of infected dogs at the time of hospital admission.²¹ Definitive diagnostic tests include detection of CPV in the feces of affected dogs, serology, and necropsy with histopathology.¹²

Practitioners can utilize a readily available in-office enzyme-linked immunosorbent assay (ELISA) test^a to demonstrate CPV in the stool of infected puppies. Viral particles are shed in the feces from approximately days 3 through 12 post-infection, and are readily detectable at the peak of shedding (4–7 days after infection).¹² False-positive results may occur 3–10 days after vaccination with a modified live CPV vaccine secondary to fecal shedding of vaccine virus.¹² False-negative results may occur secondary to binding of serum-neutralizing antibodies with antigen in diarrhea or cessation of fecal viral shed (10–12 days post-infection).^{6,14} Other methods of detecting parvoviral antigen in feces include electron microscopy, viral isolation, stool hemagglutination, latex agglutination, and counterimmunoelectrophoresis.^{12,14} Assays that utilize a polymerase chain reaction (PCR) have been developed to detect parvovirus in feces. PCR tests have higher sensitivities and specificities than conventional methods of viral antigen determination in feces.^{14,22}

Serologic tests are widely available for documentation of CPV infection. Typically, antibodies to CPV become detectable at the commencement of clinical signs, and titers increase rapidly and remain elevated for several years. However, because 25–90% of healthy unvaccinated dogs can be seropositive secondary to previous, often subclinical infection,^{21,23} positive serology is not by itself diagnostic for active CPV infection. Serodiagnosis of active CPV infection requires detection of anti-CPV antibody that is of recent origin (i.e. IgM class antibodies) in the face of typical clinical signs. Lack of anti-CPV antibodies in a puppy presenting with acute gastroenteritis is usually sufficient to rule out CPV as the causative agent. Fecal antigen testing is a more widely utilized diagnostic tool, as serologic tests are more expensive and are associated with longer turnaround times.

Treatment of CPV Infection

Outpatient management

The cornerstone of management of CPV enteritis remains supportive care. Mildly affected puppies may be treated as outpatients. Standard treatments include subcutaneous fluid administration and dietary restriction. All food should be withheld for a minimum of 12–24 hours to allow the inflamed gastrointestinal tract to rest. Water should also be withheld if there is a history of vomiting. Gradual reintroduction of water

and then small amounts of a bland diet should then be attempted.

There are limited veterinary studies evaluating the efficacy of gastrointestinal medications in the symptomatic treatment of CPV enteritis. Motility modifiers should be used with caution. Anticholinergic antidiarrheal medications may result in gastric atony and small intestinal ileus, and increase the risk of intussusception. Synthetic opioids and narcotic analgesics inhibit the flow of intestinal contents and may thereby limit diarrheal losses, but delaying gastrointestinal motility may facilitate microbial proliferation and mucosal invasion resulting in increased toxin absorption from the gastrointestinal tract. Clinical human trials have documented a reduction in the severity and duration of diarrhea following treatment with preparations that contain bismuth subsalicylate, secondary to a local antiprostaglandin effect, and such preparations may be beneficial in dogs that can tolerate oral medications.⁶ Patients should be reevaluated by phone or recheck appointment 2–3 days after discharge from the hospital.

In-hospital management

Fluid therapy: Puppies that fail outpatient management due to persistent or recurrent diarrhea and/or vomiting, and puppies that initially present to the veterinarian with more severe clinical signs, including severe dehydration/hypoperfusion, fever, abdominal pain, and protracted diarrhea and/or vomiting should be hospitalized immediately and managed aggressively. Outpatient management of severely affected puppies is rarely successful. Subcutaneous fluids are ineffective in restoring adequate intravascular volume in dogs that present with significant dehydration, hypoperfusion, and ongoing fluid losses secondary to vomiting/diarrhea. Fluids administered subcutaneously to dehydrated and/or leukopenic puppies may actually worsen the clinical picture by resulting in subcutaneous infections and skin sloughing at the administration site.

Re-establishment of effective circulating blood volume in puppies that present in hypovolemic shock and fluid replacement for losses secondary to ongoing diarrhea and vomiting is mandatory in severely affected puppies. The initial fluid of choice is a balanced electrolyte solution (i.e., lactated Ringer's solution). The preferred rate and route of initial fluid therapy varies with the condition of the patient. Fluid deficits should be replaced as soon as possible (within 1–2 hours of presentation) in dogs that present in hypovolemic shock. A short, large-bore catheter (18 or 20 gauge) can be placed in the cephalic or lateral saphenous vein, and fluids for treatment of shock should be administered at a rate and volume dictated by physiologic endpoints (initial rate 90 mL/kg/hr). If venous access is

prevented secondary to circulatory collapse, intraosseous fluid administration is an effective means of achieving intravascular volume expansion. Frequent reassessment of perfusion parameters during initial resuscitative efforts is imperative for optimal treatment of shock. Animals that are dehydrated but not in shock should be rehydrated over a period of 6–24 hours (deficit is calculated by multiplying percent dehydration \times body weight in kilograms). Subcutaneous and intraperitoneal fluids are not recommended in the face of circulatory compromise because of inadequate distribution secondary to peripheral vasoconstriction.

Once perfusion has been restored, the intravenous fluid rate is typically decreased to 4–10 mL/kg/hr (this rate may need to be adjusted to account for ongoing fluid losses through the gastrointestinal tract). Ideally, the dog's maintenance fluid plan should be based on analysis of systemic acid–base status and serum electrolyte concentrations. Once again, a balanced electrolyte solution, isotonic to blood, is the initial fluid of choice. Puppies with ongoing anorexia, vomiting, and diarrhea are prone to the development of hypokalemia, which can result in profound muscle weakness/paralysis, gastrointestinal ileus, cardiac arrhythmias, and polyuria. Potassium chloride is added to the fluids as needed to prevent the development or worsening of hypokalemia. The clinician should be cognizant of the fact that many of these patients are being treated aggressively with high fluid rates to replace fluid deficits and accommodate for ongoing losses. The amount of potassium chloride the dog is receiving hourly in these fluids should be calculated, as the administration of greater than 0.5 mEq/kg/hr of this electrolyte may adversely affect normal cardiac function.

Hypoglycemia, secondary to profound malnutrition, hypermetabolism, underlying liver dysfunction, or sepsis, is commonly observed with CPV enteritis. Enteral feedings are frequently withheld from affected puppies for several days because of ongoing vomiting, which may perpetuate clinical hypoglycemia. After rehydration, intravenous supplementation of 2.5–5% dextrose added to the balanced electrolyte solution, may be necessary to combat this metabolic disturbance.

A severe protein-losing enteropathy may accompany CPV enteritis. The clinician should consider the addition of a nonprotein colloid (i.e. hetastarch or dextran 70) to the replacement and/or maintenance fluid plan if the albumin decreases below 2 g/dL, the total protein decreases below 4 g/dL, or if the patient develops evidence of third spacing of fluids (i.e., conjunctival or peripheral edema, ascites, pleural effusion) on physical examination. During initial resuscitative efforts, colloidal fluids can be administered in 5 mL/kg slow intra-

venous boluses to effect. Colloids are dosed at a maintenance rate of 20 mL/kg/day. The crystalloid administration rate is typically decreased by 40–60% if a colloidal solution is added to the fluid plan. Daily measurement of the dog's colloid osmotic pressure (COP) is warranted during colloidal therapy, as over-supplementation can blunt endogenous hepatic albumin production.

The role of blood products in the treatment of CPV enteritis is not clear. Packed red blood cells (RBCs) should be administered for anemia resulting from blood loss secondary to hemorrhagic diarrhea or concurrent endoparasitism. A dose of 10 mL/kg administered intravenously over 4–6 hours will raise the dog's packed cell count (PCV) by approximately 10%. The decision to transfuse RBC products should be based on clinical signs referable to anemia (i.e., dull mentation; tachycardia; tachypnea; or bounding femoral pulses) and not on the absolute hematocrit.

Plasma transfusion has been recommended as an adjunctive treatment for CPV enteritis, for its ability to provide albumin, immunoglobulins, and serum protease inhibitors that may help to neutralize circulating virus and diminish the accompanying systemic inflammatory response.¹⁴ In human medicine, albumin supplementation has been recommended for the treatment of severe hypoalbuminemia (<2 g/dL) in the face of the protracted diarrhea when enteral feedings have failed. But in general, nonprotein colloids are preferred over 25% albumin solution for human patients with severe hypoalbuminemia.²⁴ Furthermore, 2 recent meta-analyses reviewing the use of albumin solutions in critically ill human patients have found no significant beneficial effect of albumin supplementation on survival, with one study documenting increased mortality associated with the administration of this product.^{25,26}

A recent veterinary review article recommended plasma infusion to raise the plasma albumin to 2.0–2.5 g/dL, and the preferential administration of nonprotein, synthetic colloids to maintain the plasma COP between 13 and 20 mmHg.²⁷ However, a large volume of plasma is required to achieve a small increase in plasma albumin (22.5 mL/kg plasma will raise plasma albumin by 0.5 g/dL).

Use of convalescent serum from dogs that have recovered for CPV infection (1.1–2.2 mL/kg intravenously or subcutaneously) as a means of providing passive immunization has been reported only anecdotally.^{11,14} To the author's knowledge, there are no randomized, controlled studies evaluating the efficacy or survival benefit of plasma transfusion for treatment of CPV enteritis. Plasma administration is certainly indicated in CPV puppies with documented hypocoagulability associated with disseminated intravascular coagulation.

The role of plasma for albumin/immunoglobulin delivery and serum protease inhibitor replenishment is less defined.

Antibiotic therapy: In dogs with CPV enteritis, disruption of the mucosal barrier (which can lead to bacterial translocation, endotoxemia, and/or sepsis) and severe neutropenia, warrants treatment with broad-spectrum, bacteriocidal antibiotics. Although reports are conflicting, the clinician must be cognizant of the fact that antibiotic therapy may increase the release of endotoxin and exacerbate any ongoing systemic inflammatory response.^{4,28} Furthermore, antibiotic therapy may lead to bacterial overgrowth of *Clostridium perfringens*, resulting in bloody diarrhea.^{16,29} Mildly affected dogs with normal white blood cell counts do not require aggressive combination antibiotic therapy.

When antibiotic therapy is deemed necessary, the parenteral route is preferred over enteral delivery, as gastroenteritis is commonly accompanied by vomiting, delayed gastric emptying, and changes in gastrointestinal microflora that may result in malabsorption of oral medications. A combination of a β lactam antibiotic (ampicillin,^b 22 mg/kg IV TID) with an aminoglycoside (gentamicin,^c 6 mg/kg IV SID) or baytil^d (5 mg/kg IV SID) will provide excellent coverage against gram-negative and anaerobic bacteria that may translocate from the gut. Aminoglycosides may cause acute renal failure, and should be administered only in well-hydrated patients; enrofloxacin has been associated with the development of cartilage abnormalities in young, growing dogs.

Antiemetic therapy: Antiemetics may be necessary in cases of severe vomiting. The 2 drugs most commonly used in the face of CPV enteritis are chlorpromazine and metoclopramide. Chlorpromazine^e (0.5 mg/kg intramuscularly or subcutaneously every 6 hours) is a phenothiazine derivative that blocks both the chemoreceptor trigger zone and the vomiting center in the brain. This antiemetic should not be used in dehydrated patients because arteriolar vasodilation occurs secondary to alpha-adrenergic blockade and may result in hypotension. Metoclopramide^f is a dopaminergic antagonist antiemetic that blocks the chemoreceptor trigger zone, stimulates and coordinates motility of the upper intestinal tract, and increases pressure in the lower esophageal sphincter. The recommended dose is 0.2–0.4 mg/kg intramuscularly or subcutaneously every 8 hours, or as an intravenous continuous rate infusion at 1–2 mg/kg/day. Ondansetron HCL (Zofran^{®g}), a 5-HT₃ receptor antagonist that acts peripherally and centrally to inhibit vomiting, at a dose of 0.1–0.15 mg/kg intravenously every 6–12 hours, may be used in cases of intractable vomiting. Vomiting that does not

respond to the above antiemetics should alert the clinician to evaluate the patient for foreign body obstruction, intussusception, reflux esophagitis, or pancreatitis.

Immunotherapy: Dogs suffering from CPV enteritis often experience leukopenia secondary to destruction of hematopoietic progenitor cells in the bone marrow, resulting in inadequate delivery of neutrophils to the inflamed gastrointestinal tract. Leukopenia thus increases susceptibility to bacterial translocation and subsequent septicemia, thereby influencing disease course and affecting mortality.^{6,30}

Granulocyte colony-stimulating factor (G-CSF) is a cytokine produced by bone marrow stromal cells, endothelial cells, macrophages/monocytes, and fibroblasts, whose actions include release of granulocytes from the storage pool of the bone marrow, shortened neutrophil maturation time, and enhanced granulopoiesis.^{30,31} In healthy veterinary patients, a single dose of recombinant human G-CSF (Neupogen^{®h}) results in a significant increase in both neutrophil and total leukocyte counts in the peripheral blood within 12–24 hours.³²

In sick dogs, recombinant human G-CSF (rhG-CSF) has been utilized effectively in the treatment of chemotherapy- or radiation-induced neutropenia and cyclic neutropenia in gray collies.^{31–33} The use of rhG-CSF to combat severe leukopenia associated with CPV infection has also been investigated. Kraft et al. demonstrated an increase in the neutrophil count in puppies treated with rhG-CSF during hospitalization.³⁴ Other investigators have found neither an increase in white blood cell count nor a survival advantage following the administration of this product to puppies with CPV. Therefore, use of this recombinant cytokine for immune stimulation in the face of CPV infection remains controversial.^{30,35–37} Postulated reasons for lack of a treatment effect include depletion of the storage pool and of more mature progenitor cells in the bone marrow, lack of C-GSF receptors secondary to granulopoietic progenitor cell depletion, and a lag time of 2–3 days before C-GSFs effect of accelerated maturation of progenitor cells is measurable in the blood.³⁰

In addition to lack of proven efficacy, one complication associated with the administration of rhG-CSF to canine patients is the development of neutralizing antibodies within 3 weeks of initiation of therapy, resulting in a decline in leukocyte counts.³¹ Human G-CSF has 80% amino acid sequence homology with canine G-CSF, which is thought to be responsible for the finding.³¹ A recombinant canine G-CSF has recently been developed, but it is not commercially available.

As previously discussed, endotoxemia and/or bacterial septicemia are documented sequelae to severe

CPV infection, and may result in terminal acute shock secondary to activation of a systemic inflammatory response. Neutralization of lipopolysaccharide (LPS; also called endotoxin) with a polyvalent equine-origin anti-endotoxin antiserum has been attempted, and results of studies are conflicting.^{2,38} In one study, addition of anti-endotoxin to conventional therapeutic techniques resulted in a decrease in mortality by 31%.³⁸ Other investigators have reported an increase in mortality rate when puppies 16 weeks old or less were treated with antiendotoxin antibody.²

More recently, use of human recombinant bactericidal/permeability-increasing protein (rBPI₂₁) to curb the systemic inflammatory response associated with CPV infection has been investigated.⁵ BPI is produced and stored in PMN azurophilic granules, and, when released, is cytotoxic to gram-negative bacteria and inhibitory to free LPS.^{39,40} Recombinant rBPI₂₁ has been shown to offer a survival advantage when used in humans with septicemia and in experimental animal models.^{40,41} However, in a recent veterinary hospital-based study, administration of recombinant rBPI₂₁ to dogs affected with CPV enteritis had no significant effect on plasma endotoxin concentration, and conferred no survival advantage over standard treatment.⁵ Postulated reasons for lack of efficacy included insufficient dose and/or infusion period, socioeconomic study biases, and the use of diluted canine plasma as a placebo.⁵ Furthermore, neutralizing endotoxin prior to its participation in the initiation of the inflammatory cascade, and targeting several mediators of systemic inflammation at once may prove to be more effective treatment strategies.

Interferons modulate immune function through their potent antiviral and antitumor activities. In 2 experimental models of parvovirus infection, administration of recombinant feline interferon- ω (rFeIFN- ω), at a dose of 1–2.5 MU/kg/day intravenously for 3 successive days after inoculation with CPV-2 virus, resulted in a significant reduction in the severity of enteritis in affected dogs and decreased morbidity and mortality.^{42,43} More recently, administration of rFeIFN- ω (2.5 MU/kg intravenously for 3 consecutive days) to a group of dogs with naturally acquired CPV enteritis resulted in a 4.4-fold overall reduction in mortality.⁴⁴ No side effects were observed following treatment with this IFN product. These preliminary results are encouraging and warrant further investigation.

Nutrition: Dogs suffering from CPV enteritis often have prolonged anorexia, decreased voluntary caloric intake, and protein-losing enteropathies. Protein loss through the gastrointestinal tract may result in hypoalbuminemia, which has been associated with feeding

intolerance, multiple organ dysfunction, and increased mortality.²⁷ Clinicians should be cognizant of the fact the administration of nonprotein colloids for support of intravascular volume and/or dextrose supplementation in intravenous fluids does not constitute nutritional support. Enteral feeding has been shown to help maintain mucosal integrity and decrease the risk of bacterial translocation. Enteral feeding can be achieved through syringe/forced feedings or via placement of a nasoesophageal/nasogastric, esophagostomy, gastrostomy, or jejunal feeding tube. Nasoesophageal/nasogastric tubes can be placed with minimal sedation and are a reasonable choice in patients who are unable to tolerate general anesthesia. Nasogastric feeding tubes allow the clinician to intermittently measure gastric residual volume and thereby assess gastrointestinal motility. During intermittent bolus feeding, it is generally recommended that if greater than 50% of the last fed volume remains in the stomach, gastric emptying is delayed and promotility agents may be indicated.⁴⁵ During continuous infusion feeding gastroparesis exists if gastric suction results in volumes greater than twice that fed in 1 hour.⁴⁵ In dogs that cannot handle full maintenance enteral feedings due to ongoing diarrhea and/or vomiting, the addition of partial parenteral nutrition (PPN) has been advocated.¹⁴ PPN solutions are administered through an aseptically placed catheter in a designated peripheral vein, at a maintenance rate of 40–60 mL/kg/day. These formulations are hypertonic, and can cause phlebitis near the catheter site. Total parenteral nutrition (TPN) should be reserved for dogs that fail all attempts at enteral feeding.

Prevention and Vaccination

Historically, outbreaks of CPV enteritis have been difficult to control. The virus is ubiquitous, can survive for more than 6 months at room temperature, and is readily transported among dogs via cages, soiled bedding, or humans. In kennel situations, good hygienic practices, including vigilant disinfection of all exposed surfaces and personnel, are paramount for prevention of nosocomial transmission of viral infection.

More important than good hygiene for prevention of CPV infection is assurance of strong individual-dog immunity via adoption of effective immunization protocols. Serum antibody titer is correlated with immunity. Seronegative dogs are susceptible; dogs with low titers typically do not become systemically ill but will shed virus in their feces. Dogs with high antibody titers do not develop active infection or contribute to spread of virus.^{6,12}

The interference of maternal antibody is considered one of the most important causes of immunization

failure.^{46,47} Effective vaccination therefore depends on both the maternal antibody titer and the type of vaccine used. Several laboratory methods exist for determination of CPV antibody titers in pregnant bitches and their offspring, including ELISAs, indirect immunofluorescent antibody (IFA) tests, and hemagglutination inhibition (HI) tests. These tests can be utilized to establish when and if active immunization is necessary, and how effective vaccination will be in conferring protection against infection with CPV.⁴⁷ Measurement of HI antibody titers is considered the 'gold standard' for quantification of CPV antibodies. Puppies with a HI titer $\leq 1:80$ are susceptible to infection and are in need of immunization.¹² A HI titer from maternally derived CPV antibody of $\geq 1:80$, and in some cases 1:10 to 1:40, will interfere with vaccination.^{47,48}

The type of vaccine utilized also affects immunization success. The current vaccines of choice are canine-origin attenuated vaccines with high-titer, low-passage CPV.⁷ The term *high-titer* refers to the amount of virus in the vaccine dose; the term *low passage* refers to the time spent in various tissue cultures in an effort to decrease virulence of the virus. High-titer, low-passage vaccines are better able to effectively vaccinate dogs during the period of maternal antibody interference.⁷ According to vaccine manufacturers, the reported incidence of post-vaccination reactions is less than 1%.^{7,12} Reactions are typically limited to mild hypersensitivity reactions, characterized by facial swelling, local inflammation, and pruritus. Immunization with certain modified-live vaccines may result in fecal viral shedding several days post-vaccination, interfering with interpretation of fecal antigen results.

Inactivated (killed) feline panleukopenia and CPV vaccines may be utilized to immunize against CPV. These vaccines protect against infection for at least 6 months; 2 injections 3–4 weeks apart are required for an adequate immune response.¹² Inactivated CPV vaccines are considered inferior to attenuated vaccines, in that they do not prevent viral shedding and may allow spread of the virus, and they are less likely to effectively immunize a puppy during maternal antibody interference.^{7,12} Inactivated vaccines may still be advantageous in certain situations (i.e., vaccination of a pregnant bitch or an immunosuppressed animal).⁷

The currently accepted vaccination protocol for protection against CPV infection recommends immunization every 2–3 weeks starting at the ages of 6–8 weeks until 16–18 weeks of age, and then annually. Additional recommendations for ensuring protection in high-risk breeds have included extending the vaccination period to 20 weeks of age, evaluation of CPV titers several weeks following the last vaccination, biannual boosting, and boosting 2–3 weeks prior to potential CPV

exposure (i.e., dog shows, kennels).^{7,11} The CPV vaccines utilized most commonly today (attenuated, high-titer, low-passage vaccines) have been proven in several studies to provide adequate protection following 2 or 3 vaccinations administered at 6, 9, and/or 12 weeks of age.^{7,47,48} This suggests a reconsideration of standard vaccination protocols in dogs.

Furthermore, the requirement of annual boosters has recently come into question. In a recent study, 94.8% of the sample dog population had an adequate response to CPV (as determined by the IFA test) for more than 1 year following vaccination, and 93.7% of the dogs had an adequate response after more than 2 years following vaccination.⁴⁹ Dogs that have recovered from active CPV infection have HI titers ranging from 1:640 to 1:2560 2 years post-infection, suggesting that immunity following infection is long-lived and perhaps lifelong.⁴⁸ Titers only confirm immunologic response and do not ensure protection against infection. Because of the increased risk of immune-mediated disease associated with over-vaccination, practitioners should consider basing their decision to booster annually on serologic evaluation of antibody titers.

Conclusions

CPV remains a significant canine pathogen. Despite early and aggressive supportive care, morbidity and mortality remain high. The ability of this virus to incite not only local gastrointestinal injury but also a significant systemic inflammatory response has recently been documented. This more global tissue injury is due in part to the ability of endotoxin released from the gastrointestinal tract to activate, among other systems, the inflammatory and coagulation systems. Early enteral nutrition and novel therapeutic interventions, such as treatment with specific antagonists of proinflammatory cytokines and general immunostimulation with interferon, may prove to help curb the systemic inflammatory response and improve outcome in dogs suffering from CPV infection. Isolation of immunonaive puppies, serologic documentation of immunocompetence in vaccinated dogs, and adoption of safe and effective vaccination protocols are also paramount in protecting dogs against infection and spread of CPV.

Footnotes

- ^a Cite test, IDEXX, Portland, ME.
- ^b Apothecon[®], Bristol-Myers Squibb Co., Princeton, NJ.
- ^c Gentozen[™], Schering-Plough Animal Health, Union, NJ.
- ^d Baytril, Bayer Corporation, Shawnee Mission, KS.
- ^e Compazine[®], GlaxoSmithKline, Research Triangle Park, NC.

- ^f Metoclopramide, Gensia Sicor Pharmaceuticals, Inc., Irvine, CA.
^g Zofran[®], GlaxoSmithKline, Research Triangle Park, NC.
^h Neupogen[®], Amgen Inc., Thousand Oaks, CA.

References

- Kariuki NM, Nyaga PN, Buoro IBJ, et al. Effectiveness of fluids and antibiotics as supportive therapy of canine parvovirus-2 enteritis in puppies. *Bull Anim Health Prod Afr* 1990; 38:379–389.
- Mann FA, Boon GD, Wagner-Mann CC, et al. Ionized and total magnesium concentrations in blood from dogs with naturally acquired parvoviral enteritis. *J Am Vet Med Assoc* 1998; 212:1398–1401.
- Glickman LT, Domanski LM, Patronek GJ, et al. Breed-related risk factors for canine parvovirus enteritis. *J Am Vet Med Assoc* 1985; 187:589–584.
- Otto CM, Drobatz K, Soter C. Endotoxemia and tumor necrosis factor in clinical canine parvoviral enteritis. *J Vet Intern Med* 1997; 11:65–70.
- Otto CM, Jackson CB, Rogell EJ, et al. Recombinant bactericidal/permeability-increasing protein (rBPI₂₁) for treatment of parvovirus enteritis: a randomized, double-blinded, placebo-controlled trial. *J Vet Intern Med* 2001; 15:355–360.
- Pollock RV, Coyne MJ. Canine parvovirus. *Vet Clin North Am Small Anim Pract* 1993; 23(3):555–568.
- Carr-Smith S, Macintire DK, Swango LJ. Canine parvovirus: Part 1. Pathogenesis and Vaccination. *Comp Cont Ed Pract Vet* 1997; 19(2):125–133.
- Mason MJ, Gillet NA, Muggenburh BA. Clinical, pathological, and epidemiological aspects of canine parvovirus in an unvaccinated beagle colony: 1978–1985. *J Amer Anim Hosp Assoc* 1987; 23:183.
- O'Brien SE. Serologic response of pups to the low-passage modified-live canine parvovirus-2 component in a compound vaccine. *J Am Vet Med Assoc* 1994; 204:1207–1209.
- Houston DM, Ribble CS, Head LL. Risk factors associated with parvovirus enteritis in dogs: 283 cases (1982–1991). *J Am Vet Med Assoc* 1996; 208:542–546.
- Brunner CJ, Swango LJ. Canine parvovirus infection: effects on the immune system and factors that predispose to severe disease. *Comp Cont Ed Pract Vet* 1985; 7(12): 979–989.
- Pollock RH, Carmichael LE. Canine viral enteritis. *Vet Clin North Am Small Anim Pract* 1983; 13(3):551–566.
- Agungpriyono DR, Uchida K, Tabaru H, et al. Subacute massive necrotizing myocarditis by canine parvovirus type-2 infection with diffuse leukoencephalomalacia in a puppy. *Vet Path* 1999; 36:77–80.
- Macintire DK, Smith-Carr S. Canine parvovirus. Part II. Clinical signs, diagnosis, and treatment. *Comp Cont Ed Pract Vet* 1997; 19(3):291–300.
- Turk J, Miller M, Brown T, et al. Coliform septicemia and pulmonary disease associated with canine parvoviral enteritis: 88 cases (1987–1988). *J Am Vet Med Assoc* 1990; 200:991–994.
- Turk J, Fales W, Miller M, et al. Enteric *Clostridium perfringens* infection associated with parvoviral enteritis in dogs: 74 cases (1987–1990). *J Am Vet Med Assoc* 1992; 200:991–994.
- Sandstedt K, Wienup M. Concomitant occurrence of *Campylobacter* and parvoviruses in dogs with gastroenteritis. *Vet Res Comm* 1981; 4:271–273.
- Weiss DJ, Rashid J. The sepsis-coagulant axis: a review. *J Vet Intern Med* 1998; 12:317–324.
- Gando S, Nanzaki S, Sasaki S, et al. Activation of the extrinsic coagulation pathway in patients with severe sepsis and septic shock. *Crit Care Med* 1998; 26:2005–2009.
- Otto CM, Rieser TM, Brooks MB, et al. Evidence of hypercoagulability in dogs with parvoviral enteritis. *J Am Vet Med Assoc* 2000; 217(10):1500–1504.
- Kramer JM, Meunier PC, Pollock RVH. Canine parvovirus: update. *Vet Med Small Anim Clin* 1980; 75:1541.
- Mochizuki M, San Gabriel MC, Nakatani H, et al. Comparison of polymerase chain reaction with virus isolation and hemoagglutination assays for the detection of canine parvoviruses in faecal specimens. *Res Vet Sci* 1993; 55: 60–63.
- Helper-Baker C, Evermann JF, McKeirnan AJ, et al. Serologic studies on the incidence of canine viral enteritis viruses. *Canine Pract* 1980; 7:37.
- Vermeulen LC, Ratko TA, Erstad BL, et al. A paradigm for consensus: the university hospital consortium guidelines for the use of albumin, nonprotein colloid, and crystalloid solutions. *Arch Intern Med* 1995; 155:373–379.
- Cochrane Injuries Group Albumin Reviewers. Human albumin administration in critically ill patients: systematic review of randomized controlled trial. *Br Med J* 1998; 317:235–240.
- Wilkes MM, Navickis RJ. Patient survival after human albumin administration: a meta-analysis of randomized, controlled trials. *Ann Intern Med* 2001; 135:149–164.
- Mazzaferro EM, Rudloff E, Kirby R. The role of albumin replacement in the critically ill veterinary patient. *J Vet Emer Crit Care* 2002; 12(2):113–124.
- Stockwell JA, Huang YC, Su YF, et al. Bactericidal antibiotics increase tumor necrosis factor alpha and cardiac output in rats after cecal ligation and puncture. *Circ Shock* 1994; 42:68–75.
- Tsukada K, Katoh H, Shiojima M, et al. Mortality rate and bacteremia, endotoxin, and endothelin-1 levels in antibiotic therapy for *E. coli* septic peritonitis. *APMIS (Acta Pathologica, Microbiologica et Immunologica Scandinavica)* 1993; 101:97–100.
- Mischke R, Barth T, Wohlsein P, et al. Effect of recombinant human granulocyte colony-stimulating factor (rhG-CSF) on leukocyte count and survival rate of dogs with parvoviral enteritis. *Res Vet Sci* 2001; 70:221–225.
- Henry CJ, Buss MS, Lothrop CD. Veterinary uses of recombinant human granulocyte colony-stimulating factor. Part 1. Oncology. *Comp Cont Ed Pract Vet* 1998; 20(6): 728–735.
- Lothrop CD, Warren DJ, Souza LM, et al. Correction of canine cyclic hematopoiesis with recombinant human

- granulocyte colony-stimulating factor. *Blood* 1988; 72:1324–1328.
33. Rewarts JM, Heny CJ. Veterinary uses of recombinant human granulocyte colony-stimulating factor. Part II. Infectious diseases. *Comp Cont Ed Pract Vet* 1998; 20(7): 823–829.
 34. Kraft W, Kuffer M. Treatment of severe neutropenias in the dog and cat with Filgrastim. *Tierarztl Prax* 1995; 23:609–613.
 35. Hammond WP, Csiba E, Canin A, et al. Chronic neutropenia: a new canine model induced by human granulocyte colony-stimulating factor. *J Clin Invest* 1991; 87:704–710.
 36. Brunner CJ, Swango LJ. Canine parvovirus infection: effects on the immune system and factors that predispose to severe disease. *Comp Cont Ed Pract Vet* 1985; 7(12): 979–989.
 37. Rewerts JM, McCaw DL, Cohn LA, et al. Recombinant human granulocyte colony-stimulating factor for treatment of puppies with neutropenia secondary to canine parvovirus infection. *J Am Vet Med Assoc* 1998; 213: 991–992.
 38. Dimmit R. Clinical experience with cross-protective antitendotoxin antiserum in dogs with parvoviral enteritis. *Canine Pract* 1991; 16:23–26.
 39. Elsbach P. The bactericidal/permeability-increasing protein (BPI) in antibacterial host defense. *J Leukoc Biol* 1998; 64:14–18.
 40. Giroir BP, Quint PA, Baron P, et al. Preliminary evaluation of recombinant amino-terminal fragment of bactericidal/permeability increasing protein in children with severe meningococcal sepsis. *Lancet* 1997; 350:1439–1443.
 41. Lin Y, Leach WJ, Ammons WS. Synergistic effect of a recombinant N-terminal fragment of bactericidal/permeability increasing protein and cefamandole in treatment of rabbit gram-negative sepsis. *Antimicrob Agents Chemother* 1996; 40:65–69.
 42. Ishiwata K, Minagawa T, Kajimoto T. Clinical effects of the recombinant feline interferon- ω on experimental parvovirus infection in beagle dogs. *J Vet Med Sci* 1998; 60(8): 911–917.
 43. Martin V, Najbar W, Gueguen S, et al. Treatment of canine parvoviral enteritis with interferon-omega in a placebo-controlled challenge trial. *Vet Microbiol* 2002; 89: 115–127.
 44. de Mari K, Maynard L, Eun HM, et al. Treatment of canine parvoviral enteritis with interferon-omega in a placebo-controlled field trial. *Vet Rec* 2003; 152:105–108.
 45. Marks S. Enteral feeding devices: what's old, what's new: 2001 Scientific Proceedings, WSAVA World Congress, Vancouver, Canada. WSAVA 2001, pp. 342–348.
 46. Pollock RVH, Carmichael LE. Maternally derived immunity to canine parvovirus infection: transfer, decline, and interference with vaccination. *J Am Vet Med Assoc* 1982; 180:37–42.
 47. Waner T, Naveh A, Wudovsky I, et al. Assessment of maternal antibody decay and response to canine parvovirus vaccination using a clinic-based enzyme-linked immunosorbent assay. *J Vet Diag Invest* 1996; 8:427–432.
 48. Buonavoglia C, Tollis M, Buonavoglia D, et al. Response of pups with maternal derived antibody to modified-live canine parvovirus vaccine. *Comp Immunol Microbiol Infect Dis* 1992; 15:281–283.
 49. Twark L, Dodds WJ. Clinical use of serum parvovirus and distemper virus antibody titers for determining revaccination strategies in healthy dogs. *J Am Vet Med Assoc* 2000; 217(7):1021–1025.