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**Original Scientific Paper****CANINE VIRAL ENTERITIS – OLD AND NEW PATHOGENS****Dragan BACIĆ\*, Sonja OBRENOVIĆ**

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\* Corresponding author: Dragan Bacić, [bacid@vet.bg.ac.rs](mailto:bacid@vet.bg.ac.rs)**Summary**

Canine viral enteritis is a frequent problem in clinical veterinary practice. From the discovery of canine parvovirus in the late 1970s to the present day, viruses have been one of the most common causes of diarrhea, especially in younger categories of dogs. The following primary causes of enteritis in dogs are most often isolated: parvovirus types 1 and 2, corona and rota virus. Modern molecular methods of diagnosis in the feces of dogs have proven the presence of new viruses, which lead to milder forms of enteritis (circoviruses, picornaviruses, astroviruses, calici and paramyxoviruses) in dogs. The presence of the virus in the feces of dogs with symptoms of diarrhea was proven in 40–60% of the examined samples.

**Keywords:** dogs, viral enteritis, clinical signs, diagnosis, prophylaxis

**INTRODUCTION**

The widespread use of modern, innovative molecular techniques, as well as the mass sequencing of the virus genome, have led to revolutionary discoveries and new knowledge about the causes of viral enteritis in dogs. Some of these newly discovered viruses have probably been present and circulating within the population of dogs and other animal species for decades, but their presence has not been proven so far. The question arises, are these viruses capable of causing enteritis or are they part of the normal intestinal microbiome. A large number of different viruses have been isolated from the feces of dogs with or without diarrhea. In most cases, the pathogenic potential of new viruses is not completely understood. Therefore, research related to this topic continues.

**Canine parvovirus infection in dogs**

Canine parvovirus infection is a highly contagious infectious disease to which all members of the Canidae family are susceptible. The causative agent is widespread in nature and is very resistant. In the first weeks of life of puppies infection is characterized by myocarditis, while in older puppies intestinal disorders are present. Puppies of unvaccinated bitches are particularly susceptible (Buonavoglia et al., 2006).

Canine parvovirus (CPV-1) was first discovered in 1967 in the feces of clinically healthy dogs, and was called the Minute virus of canines. The much more pathogenic Canine parvovirus (CPV-2) was discovered in 1978 in the United States and was most likely

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caused by a mutation in the feline panleukopenia virus. The virus can be transmitted through objects, clothing, footwear and as such can be introduced and present in kennels and quarantines. The source of infection in most cases is the feces of infected dogs, which contain large amounts of the virus. So the primary route of infection is the feco-oral route. One gram of feces of dog with acute infection can infect a million other dogs (Green and Decaro, 2012).

As it is a highly resistant virus in the external environment, infectious material can remain on hair, insects and people who can transmit the infection over long distances. Virus excretion could be detected up to 14 days after the infection. However, infected dogs can carry the virus on their paws and hair for a longer period of time. In the uninfected population of dogs, the disease is usually introduced with contaminated material, as well as animals that have survived the infection, and which carry the virus. Subclinically infected animals play the most important role in the epidemiological chain, because they excrete the virus through feces and do not show signs of disease. Predisposing factors also play a significant role in the occurrence of the disease, such as: poor breeding, stress, poor diet, other viral, bacterial and parasitic diseases, breed and individual predisposition (Rottweiler, Doberman Pinscher, German Shepherd, Labrador Retriever). Mortality in adults dogs is less than 1%, but in young categories, aged 6 to 14 weeks, it can reach 100%. In closed populations of susceptible dogs where immunoprophylaxis is not performed and where there is no treatment option, mortality is up to 90% (Bányai et al., 2018).

The clinical symptoms of the disease are getting worst if there is a secondary infection with *Salmonella spp*, *Clostridium perfringens*, *Escherichia coli*, *Campylobacter spp*, Canine distemper and intestinal parasites. The incubation period for the enteral form of the disease is 4-7 days. The disease is characterized by fever (40-41°C), depression, anorexia, vomiting and diarrhea. The clinical picture varies from asymptomatic cases, through apathy and anorexia to acute hemorrhagic enteritis (Green and Decaro, 2012). The vomit is usually light, and bile or blood may appear. Diarrhea is grayish or yellow-gray in color, with an unpleasant odor, and traces of blood and mucus originating from the intestines are noticed later. In some puppies, the diarrhea is brown, watery, and can be completely hemorrhagic. Diarrhea can last for a week, although if the puppies are not treated, they may die in the first few days. Animals that survive the first 3-4 days of the disease usually recover with minimal complications, unless secondary bacterial infections occur, when recovery takes longer and the outcome is uncertain. Morbidity and mortality are 20% to 100% in suckling puppies and 10% to 50% in older puppies and adult dogs. The mortality rate depends on the accurate diagnosis and the time when adequate and quality therapy begins.

The myocardial form of the disease is characterized by a longer incubation. Clinical symptoms of myocarditis appear 3-7 weeks after the enteral phase. Usually more than 50% of puppies from the same litter die at the age of 8 weeks, and some puppies may die later, due to heart failure (Carmichael, 2005).

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An accurate diagnosis of parvovirus infection is made by determining the presence of viral Ag in the feces of dogs or by determining the titer of antibodies in the blood serum of the diseased animal.

To determine the presence of antigen in the examined fecal samples, the Antigen Rapid CPV test is used, whose sensitivity is 98.50% and specificity 98%.

The indirect ELISA test method is used to determine the antibody titer. Precise determination of the antibody titer against CPV in puppies can be used in clinical practice to determine the titer of maternal antibodies, the optimal time of vaccination, as well as the level of antibodies in infected puppies (Decaro and Buonavoglia, 2012).

There is still no adequate therapy for canine parvovirus infection in dogs. Therapy is nonspecific and symptomatic. The main goal of therapy is to compensate lost fluid and electrolytes and to prevent their continuous loss. Treatment of enteritis is based on diet and parenteral replacement of lost fluid and electrolytes. It is necessary to give medications that will relax the digestive tract (stomach and intestines), i.e. that will stop vomiting and diarrhea. Saline, Ringer's solution, 5% glucose, Hartmann's solution and oral rehydration agents are recommended. Antiemetics (Metoclopramide), then antispasmodics (Buscopan), as well as broad-spectrum antibiotics should be given. Antihemorrhages and vitamins K<sub>2</sub> and K<sub>3</sub> should be given to stop bleeding from the digestive tract. In practice, a combination of penicillin and streptomycin, then ampicillin, gentamicin, sulfa medicines, etc. were proved to be the best, in order to prevent secondary bacterial infection. Medications for coating the intestinal mucosa may also be given orally. Probiotics can be given to regenerate the disturbed intestinal flora. At the beginning of the disease itself, the administration of medical charcoal is indicated (Green and Decaro, 2012).

Hyperimmune sera may be given in the viremia stage. If given in the first four days after infection, milder symptoms can be expected, and they can also be given after the appearance of the clinical signs (as therapeutics). In case of large blood loss, blood transfusion can be performed, especially with the blood of animals immune to parvovirus infection. Treatment of myocarditis does not give any special results, but some of the medicines for heart can be used, as well as drugs against arrhythmia. As for the use of corticosteroids, they can be given only at the beginning of the disease, and their longer use is contraindicated because they have an immunosuppressive effect. The sick animal should be provided with peaceful environment, and if necessary, some of the sedatives should be given - diazepam, bromazepam, etc.

Specific prophylaxis is the best way to protect dogs from parvovirus infection. There are inactivated and attenuated vaccines on the market today (Day et al., 2016).

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### **Coronavirus infection in dogs**

Canine coronavirus enteritis is a disease of dogs that is widespread throughout the world, characterized by lethargy, anorexia, muscular diarrhea and dehydration. Coronavirus infection in dogs is a fairly mild disease, but if it is mixed with parvovirus infection, the disease is fatal.

Coronaviruses were discovered in 1971 in the feces of military dogs in Germany. Canine coronavirus belongs to a group of single-stranded RNA viruses that includes transmissible porcine gastroenteritis and feline enteric coronaviruses. The virus leads to the appearance of CPE on cell cultures of kidney, thymus, synovium, feline kidney and embryonic fibroblasts after only 2 days (Decaro et al., 2010). The disease is transmitted by contact of patients with healthy animals, mainly by feco-oral route. Dogs of all ages are affected. Feces is the most important source of infection. The appearance of the disease can be influenced by age, breed, stress, overcrowding and breeding in unhygienic conditions. The virus is sensitive to high temperatures, and it is resistant to cold, so the disease occurs more often in the winter. The virus is resistant to acids, so it can pass through the stomach undamaged. Dogs excrete the virus in their feces 2 weeks after infection. Serological tests in some dog populations have proven up to 80% of seropositive individuals (Green and Decaro, 2012).

The infection spreads distally for 24h to 48h, until the small intestine is completely affected. The virus attacks mature epithelial cells in the lumen of the small intestine villus. Infection of the intestinal epithelium is limited to mature epitheliocytes of the intestinal villi. Viremia and systemic spread are not as pronounced as in parvovirus enteritis. Viral replication causes the death and desquamation of mature epithelial cells, resulting in the detachment of the villus. Loss and damage of the absorptive and digestive epithelium leads to diarrhea. The germinal epithelium is preserved, so that the recovery is relatively fast and occurs in a week.

The incubation period lasts from one to several days, usually no longer than 5 days. The disease begins with sudden vomiting, and immediately after that there is a moderate to severe diarrhea, which is initially darkened to yellow, soft, mucous, very rarely with blood impurities. The disease lasts 4-8 days and mostly ends with a complete spontaneous recovery, although cases of sudden death have been reported. The disease is mild, but if it is complicated by a parvovirus infection, it has a much more severe course.

The diagnosis is made by proving the presence of viruses or viral antigens in the intestines or feces. The virus can be detected in the intestinal contents by electron microscopy or immunofluorescence. In dogs that have survived the infection, neutralizing antibodies can be detected on the 21<sup>st</sup> day after the infection. There are commercial tests for rapid diagnosis that prove the presence of antigen in the feces. The definitive diagnosis is made by isolating the virus on cat cell cultures.

Differential diagnosis should pay attention to diseases that are characterized by diarrhea and vomiting: canine parvovirus infection, rotavirus infection, Canine distemper, endoparasites, eating disorders, motility disorders, bacterial infections, poisoning. The therapy is supportive and should be performed following the instructions for the treatment of parvovirus infection. Adequate diet regimen, fluid replacement, electrolytes (5%

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glucose, saline, Ringer's and Hartmann's solution), astringents and absorbents are very useful. In severe general conditions, broad-spectrum antibiotics should be used. Contact of healthy dogs with sick ones should be prevented, non-specific factors, including stress, should be eliminated. The best way to prevent the disease is vaccination. An inactivated vaccine is used for vaccination, but there is also an attenuated vaccine, although it has been withdrawn from the market due to numerous side effects.

### **Rotavirus infections in dogs**

Rotavirus was first detected in dog feces by electron microscopy in 1979, and serologically a few years before that. Canine rotavirus is antigenically related to rotaviruses of other animal species, so cross-infections of different animal species are not excluded. Rotaviruses are widespread throughout the world (Eugster and Sidwa, 1979).

Rotaviruses belong to the family *Reoviridae*. They often appear as causes of diarrhea in many animal species and humans. In humans, they are main causes of sporadic acute enteritis in infants and young children. Rotaviruses are transmitted by the feco-oral route. Viruses are well adapted to survive in the external environment and in the upper parts of the digestive tract. Serological studies have shown that most adult dogs are infected with canine rotavirus. The disease has low mortality (Mihalov-Kovács et al., 2015). Rotaviruses infect most mature epithelial cells on the luminal or absorptive surfaces of the intestine. Infected cells are swollen, degenerate and desquamate into the intestinal lumen, from where they release a large number of virions that become a source of infection for the lower segments of the intestine and for other animals. Damaged villus contract and villous atrophy occurs. Necrosis of rotavirus-infected cells is most pronounced 18 to 24 hours after oral infection.

Rotavirus diarrhea occurs in puppies younger than 12 weeks, but most often in puppies younger than two weeks of age. Experimentally, it is not possible to cause the disease in animals older than 6 months. After oral inoculation of the virus in a two-week-old gnotobiotic puppy, severe diarrhea and dehydration occurred, but no hematological changes were observed. Most natural infections have been shown to be subclinical or limited to mild, moderate diarrhea, anorexia, and lethargy. Most pathogenic rotaviruses have a common group antigen that can be detected by many methods, including a commercial ELISA test. Rotaviruses can also be detected in fecal samples by electron microscopy, but it is necessary to distinguish rotaviruses from non-pathogenic reoviruses that may occasionally be found in dog feces. Immunoelectron microscopy increases the specificity of the test. The virus can be detected by the fluorescent antibody technique. The therapy is symptomatic, the same as with parvo and corona viral enteritis. The duration and nature of immunity to rotaviruses have not been sufficiently studied. The main source of protection for newborn puppies are antibodies present in the milk of immune bitches. Since the virus has a pathogenic effect on the surface of the epithelium of the digestive tract, secretory antibodies are of greater importance in protection than humoral immunity. There is no vaccine, and the frequency and severity of the disease do not justify the cost of developing the vaccine.

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### **Canine caliciviruses in dogs**

Caliciviruses (fam. *Caliciviridae*) are a large family of RNA viruses, which cause various clinical manifestations in different species of animals. Caliciviruses are currently classified into 11 genera, and only three genera have been isolated in dogs (*Norovirus*, *Sapovirus*, *Vesivirus*).

Caliciviruses have been detected in dogs of all ages, although the seroprevalence rate increases with age, and clinical symptoms have only been reported in young animals. Noroviruses are the leading cause of acute gastroenteritis in humans worldwide. Although different strains of calicivirus are associated with the occurrence of canine gastroenteritis, the role of these viruses in the pathogenesis of the disease has not been fully discovered (Banyai, 2018).

### **Canine astroviruses in dogs**

Astroviruses of dogs belonging to the genus *Mamastrovirus*, family *Astroviridae*, and were first isolated from the feces of puppies with diarrhea in the United States in 1980, but in 2009 the first detailed description and molecular characteristics of the virus were published. The genus *Mamastrovirus* includes 19 species (1-19). Astroviruses are RNA viruses, spherical, uncoated. The presence of the virus has been proven in dogs, wild carnivores and humans. Puppies from 2-3 months of age are especially susceptible to the appearance of the disease. Astroviruses are the second leading cause of acute diarrhea in humans after rotavirus (Choi et al., 2014; Martella et al., 2011).

### **Canine circoviruses in dogs**

Canine circovirus belongs to fam. *Circoviridae*, genus *Cirkovirus*, which includes 43 species of virus. Circoviruses are small, spherical DNA viruses, without envelopes. Dogs are natural hosts of circovirus, but the presence of the virus has also been proven in wild carnivores. Virus was first isolated from the feces of dogs with diarrhea, vasculitis and hemorrhages in 2012.

In routine serological tests performed in the United States, circovirus was detected in 2.9% of dog sera, and the prevalence rate in dogs with or without clinical symptoms is estimated to be between 2.9-11.3%. (Zaccaria et al., 2016; Todd et al., 2001).

### **Canine kobuviruses of dogs**

Kobuviruses are small RNA viruses without envelopes, belonging to the genus *Kobuvirus* and the family Picornaviridae. In addition to domestic dogs, virus has been isolated from wild carnivores in Africa and Europe. It was discovered in 2011 in dogs with diarrhea (Melegari et al., 2018; Choi et al., 2014).

### **Canine bocavirus in dogs**

Canine bocaviruses belong to the genus *Bocaparvovirus* (supfam. *Parvovirinae*, fam. *Parvoviridae*), which currently includes 25 different types of viruses, of which six (*Bocaparvovirus* carnivores 1-6) are pathogenic to carnivores. They belong to DNA viruses and are structurally similar to other parvoviruses. Two canine bocaviruses have

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been isolated in dogs: the canine minute virus known since the 1960s, which was renamed canine bocavirus 1 (CaBoV-1), and canine bocavirus 2 (CaBoV-2). CaBoV-2 was first proven in 2012 in dogs with respiratory symptoms, and then in puppies with severe and fatal gastroenteritis. The presence of canine bocavirus has also been demonstrated in cat feces (Miranda and Thompson, 2016).

### CONCLUSION

Studies on other animal species have shown that some viruses (as astrovirus) may be enteral pathogens, while others are most likely present by chance. Particles similar to astroviruses have been found in the feces of both clinically healthy dogs and dogs with diarrhea. The herpes virus is antigenically very similar to the feline rhinotracheitis virus and has been isolated in dogs with diarrhea. In some dogs, human echo and coxsackie viruses have been isolated. Canine calicivirus is isolated from the feces of dogs with enteritis, sometimes alone and sometimes with other viruses. Parainfluenza virus has been isolated in dogs with bloody diarrhea. The significance of coronavirus infection for dogs' health has not yet been fully established. Coronaviruses are not strictly host-specific, so the possibility of transmission to humans has not yet been ruled out. Serological tests performed on over 1000 veterinary medicine students did not prove seroconversion and the presence of infection. When it comes to canine parvovirus, there is no danger to public health, however, it is necessary to pay attention when there is a contact with sick animals and fecal material because other enteropathogens may be present in the feces. Rotaviruses are not species specific as human rotavirus can reproduce in dogs. Puppies with rotavirus enteritis can be a potential danger to humans, especially children. It is necessary to be careful when handling the feces of dogs with diarrhea, although the risk of human infection with *Campylobacter* is higher than that of rotavirus enteritis.

Conflict of interest statement: The authors declare that there is no conflict of interest.

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