Volume 5, issue 9, 2016 • e-ISSN: 1857-8187 • p-ISSN: 1857-8179

Research Article

Studies on Canine Parvovirus 2 in Kosovo

Healthcare

Keywords: Serology, gross pathology, histological examination, CPV-2.

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Abstract

A serologic survey was carried out and blood samples were taken from 124 unvaccinated, clinically healthy dogs of different ages (from 6 to 156 months). Serum samples were tested by using hemagglutination inhibition (HI) test having titers of HI antibodies to CPV-2 varying from 0 to 1:2048.Out of 124 dogs tested, 96 (77.42%) had HI titers of 1:16 or greater (a criteria that established the positivity) and 28 of them had HI titers less than 1:16 that meant a negative result. The seroprevalence in communities of Kamenica, Rahovec, Peja, Prishtina and Prizren was 78.0%, 76.5%, 64.2%, 75.0% and 100%, respectively. It showed a high prevalence of CPV-2 antibodies in each community involved in the study as well as that at the nation level 96 (77.42%). Such a finding indicates that the prevalence of anti-CPV-2 antibodies is high, and as a consequence, the CPV-2 itself is widespread all-over the country. In addition to the serological studies, clinical and pathological findings were taken into account as well. The more frequent clinical symptoms noticed were depression, anorexia, watery and/or bloody diarrhea, vomiting, tachycardia and severe dehydration. In spite of the supportive therapy, a diet consisting of 50% yoghurt of homemade cow milk mixed with 50% water was introduced. Of 6 puppies that received the yoghurt, 5 survived (83.3%) and only one died (16.6%). A necropsy was carried out in 14 puppies died from hemorrhagic enteritis. All 12 puppies belonged to the shelter of stray dogs of Rahovec from the communities of Gjakova, Rahovec, Kamenica and Peja. Based on a rapid test for CPV-2 antigen detection, 12 puppies tested positive for CPV-2, one was identified with clostridia infection and one resulted negative for CPV-2. The most lesion-pronounced organs affected by CPV-2 were the small intestine.

Introduction

Canine parvovirus 2, the causative agent of acute hemorrhagic enteritis and myocarditis in dogs, is one of the most important pathogenic viruses. It is a highly contagious and often fatal disease. CPV-2 was first recognized in 1977 and since then it has been well established as an enteric pathogen of dogs throughout the world with high morbidity (100%) and frequent mortality up to 10% (Appel et al., 1979; Black et al., 1979). This virus for many countries of the world and Europe today continues to be one of the most troubling health problems of dogs (McCow et al., 1998; Pollock and Charmichael, 1990). In the first two years of its appearance, CPV-2 as a new virus spread to every corner of the globe in a panzootic form infecting almost every populations of domestic and wild dogs examined (Parrish, 1990). The disease first appeared with two syndromes; a non suppurative myocarditis associated with heart failure in dogs 4-8 weeks (Jezyk et al., 1979; Mason et al., 1987; Carpenter et al., 1980) and a severe enteritis accompanied by vomiting, diarrhea and death in puppies and adult dogs (Appel et al., 1979; Osterhaus et al., 1980; Meunier et al., 1981). Thanks to its high contagious properties and sustainability of the virus to environmental factors (Gordon and Angrick, 1986)] even today, especially parvoviral enteritis, is a concern of the first hand to the importance of viral diseases in dogs (Carmichael et al., 1980). To date, in Europe and wider there are identified 3 serotypes of CPV-2 (CPV-2a, 2b and recently the 2c) (Decaro et al., 2007). To the best of the authors´ knowledge, up to currently in Kosovo, except for clinical record, there is no any laboratory confirmation of CPV-2 in domestic dogs.

Materials and Methods

Serologic Study

A serologic survey was performed on 124 never-vaccinated, clinically healthy dogs of various age, breed and sex. The age ranged from 6 to 156 months. Only seven of dogs were confined as puppies belonging to the age group equal or greater than 6 to 12 months old and 116 others were from greater than 12 to 156 months old. Geographical coverage of dogs that were serologically tested included Kamenica (41), Rahovec (47), Peja (14), Prishtina (12) and Prizeren (10). As the test to detect anti-CPV-2 antibodies was the hemagglutination-

inhibition (HI) test performed according to an already well established method (Carmichael et al., 1980; Kumar et al., 2004).

The serum samples for HI test were heat-inactivated at 56 ° C for 30 minutes and serial 2-fold dilutions were made of each serum in PBS, in 96-well U-bottom microtiter plates using 25 µl droppers and diluters. A total of 10 units of hemagglutination of canine parvovirus antigen with a hemaglutination (HA) titer of 1:1024/0.025 ml was added to each serum dilution and plates were mixed. The CPV-2 antigen used was prepared from a fecal sample of a 9-week old female puppy from Kamenica diagnosed of CPV-2 enteritis by HA test. After standing for 1 hour at room temperature, 50 µl of a 0.5% pig RBC suspension was added to each serum-virus mixture and incubated at 4°C until erythrocyte controls had formed clear buttons, usually after 3 hours. Each test series included erythrocyte, virus and serum controls. Intestinal specimens after being fixed by immersion in formalin 10% were then processed for microscopical examination and, in the end,the specimens were stained with 10% hematoxylin and 0.5% eosin (H&E), and examined under a light microscope.

Results and Discussion

Out of 124 sera tested with HI test, 96 (77.42%) resulted positive (titers ≥1:16 were considered positive), what meant that those dogs had already contracted the virus. HI titers varied from 1:16 to 1:2048. Out of 96 HI positive dogs, 48 (38.7%) were males and also 48 (38.7%) were females, being in a perfect equality of positivity. Thus no difference was shown between positive male dogs and positive female dogs.

The overall seroprevalence of 77.42% in this study was similar high to another serologic survey carried out in dogs in Albania 2004, which was 83.9% (Kusi, 2004).

The seroprevalence in communities of Kamenica, Rahovec, Peja, Prishtina and Prizeren was 78.0%, 76.5%, 64.2%, 75.0% and 100% , respectively. It showed a high prevalence of CPV-2 antibodies in each community involved in the study as well as that at the nation level (Table 1). Nonetheless, no significant difference was found between the communities involved in the study. Such a finding indicates that the prevalence of anti-CPV-2 antibodies is high, and as a consequence, the CPV-2 itself is widespread all-over the country.

Table 1. *Seroprevalence in each district involved in the study and overall*

Communities	**No. of dogs tested**	**No. of dogs having a HI titer ≥ 1:16**	**Seroprevalence in %**
Kamenica	41	32	78.0
Rahovec	47	36	76.5
Peja	14	9	64.2
Prishtina	12	9	75.0
Prizeren	10	10	100.0
T O T A L	124	96	77.42

In conclusion, based on the serologic, the situation allows little space for discussion about the necessity of strict and regular vaccination of puppies, considering every dog population nationwide.

As per sick puppies submitted to clinical and pathologic studies all of them had clinical symptoms of diarrhea and/or vomiting lasting longer than 2 days. The more frequent clinical symptoms noticed were depression, anorexia, watery and/or bloody diarrhea, vomiting, tachycardia and severe dehydration. In addition to the supportive therapy, it was introduced a diet consisting of 50% joghurt of homemade cow milk mixed with 50% water, given *per os* 4-5 times daily. Of 6 puppies that received the joghurt, 5 survived (83.3%) and only one

died (16.6%). Right after taking the joghurt the puppies increased the appetite and in 2 to 4 days after the onset of the symptoms the clinical signs almost vanished and the puppies survived. Although the number of puppies receiving the joghurt was a few, making the data at the anecdotal level, in our experience, we could note a surprising rate of survival (83.3%) of puppies having CPV-2 enteritis.

Among 14 dogs having hemorrhagic enteritis suspected of CPV-2, based on a rapid test for capturing antigen of CPV-2, 12 tested positive for CPV-2, one was identified with clostridial infection and the remaining one resulted CPV-2 negative. Most pronounced gross pathologic and microscopic lesions of 12 dogs died from parvoviral enteritis are shown below in a summarized form. All puppies at necropsy were cachectic and dehydrated. The abdominal organs having more pronounced lesions were small intestine. In most cases, the small intestine was congested containing aqueous hemorrhagic contents.

The serosal surface of the small intestine in 12 positive dogs for CPV-2 had a typical granular appearance seen often during acute enteritis due to CPV-2 (Cooper et al., 1979; Meunier et al., 1981), and in one case (Figure 4), we noted serosal hemorrhages and hyperemic mesenteral vessels. While the intestinal mucosa was congested, haemorrhagic, covered by exudate (Figures 1, 2 and 3).

At the histopathological examination, in all CPV-2 positive puppies were seen lesions typical for hemorrhagic enteritis (Cooper et al., 1979; Munier et al., 1981; Mason et al., 1987). Microscopic examination of the ileum (Figure 5) and jejunum (Figure 6) showed severe destruction of the villi and mucosal layers including severe necrosis and loss of surface epithelium and also distention of crypts lumen. Shortened and blunting of villi as well as inflammatory and hemorrhagic infiltration into the lamina propia, were seen especially in the jejunum.

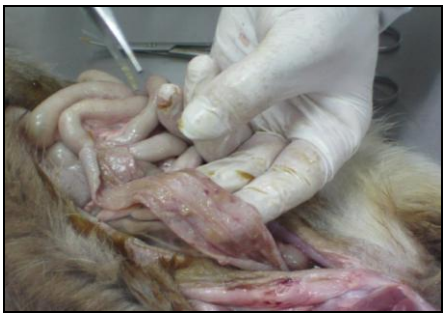

Figure 1: Serosal surface of the intestine had a granular appearance and mucosa appeared congested, haemorrhagic, covered by exudate.

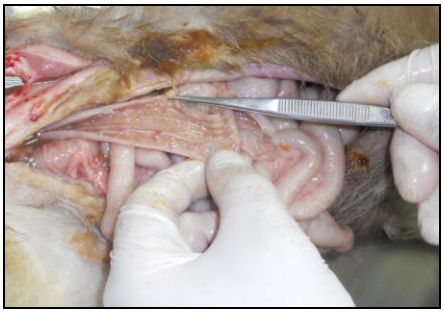

Figure 2: Serosal surface of the intestine had a granular appearance and mucosa appeared congested, haemorrhagic, covered by exudate.

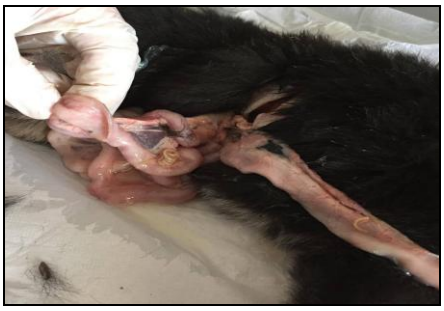

Figure 3: Serosal surface of the intestine had a granular appearance, petecial hemorrhagic on the mucosa and ascarides in a 8-week old female puppy died of CPV-2.

Figure 4: Serosal hemorrhages and hyperemic mesenteral vessels in a 11 - week old female, mixed breed puppy died of CPV-2.

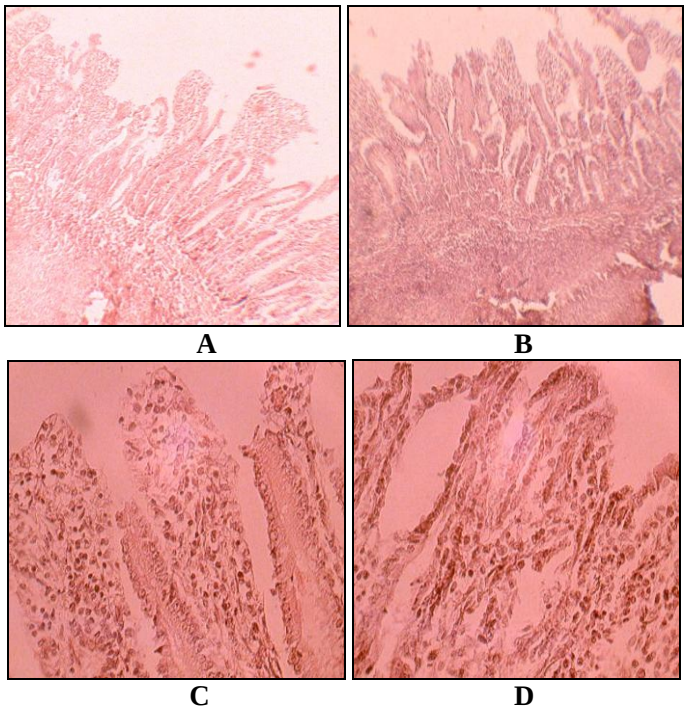

Figure 5: ***Ileum***, microphotography. Severe destruction of the villi and mucosal layers, necrosis and loss of surface epithelium, inflammatory cell infiltration into the lamina propria. **A**, **B** (X10 H&E), **C**, **D** (X40 H&E).

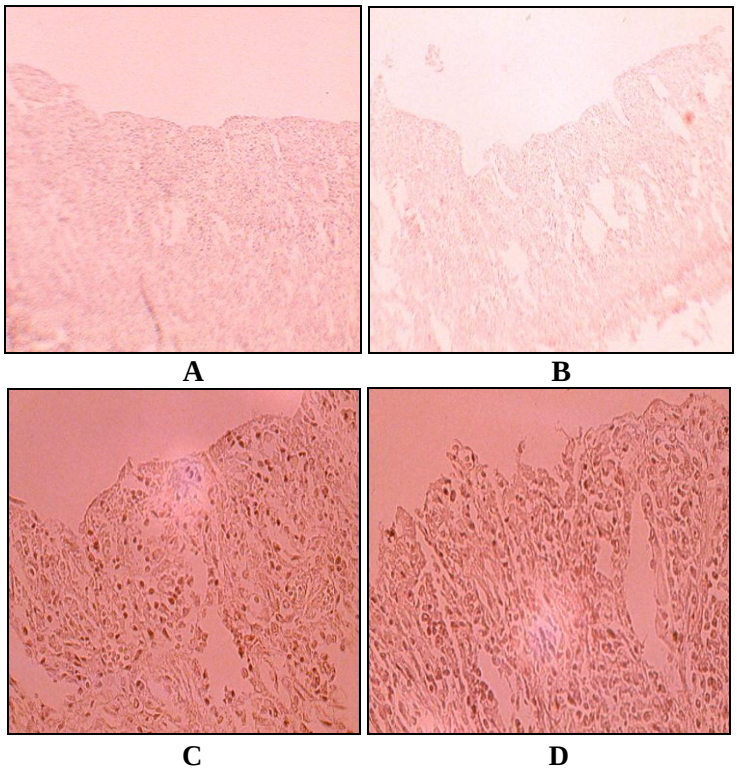

Figure 6:***Jejunum***, microphotography. Loss of surface epithelium, shortened and blunting of villi, colapsed lamina propria followed by inflammatory and hemorrhagic cell infiltration and regeneration. **A, B,** (X10 H&E), **C, D** (X40 H&E)

Diagnosis based on gross pathologic and microscopic changes is a tool that complements and supports best the diagnosis of CPV-2. Concerning 14 dogs submitted to necropsy, suspected of CPV-2, besides the macroscopic and microscopic changes, there were feces or intestinal content of those who underwent a laboratory diagnosis and according to the case they either helped to make a diagnosis or ruled out the disease caused by CPV-2.

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