RESEARCH ARTICLE (Open Access)

Pathologic Findings in Dogs Died of CPV-2 in Kosovo

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Abstract

A necropsy was carried out in 14 puppies died from hemorrhagic enteritis, suspected of CPV-2. 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 clostridial infection and one resulted negative for CPV-2. The most lesion-pronounced organs affected by CPV-2 were the small intestine and duodenum. In most cases, the small intestine was congested containing hemorrhagic liquids. The serosal surface of the small intestine in four CPV-2 positive puppies had a granular appearance seen often during acute CPV-2 enteritis and the mucosal surface was congested, hemorrhagic and covered by exudate. At the histopathological examination of intestine sections the most prominent features observed were severe necrosis of epithelial cells, intestinal villi atrophy, dilated capillary vessels and desquamation of the epithelium. Anatomohistopathologic diagnosis is a tool that complements and supports best the diagnosis of CPV-2 enteritis.

Keywords: Gross pathology, histologic examination, CPV-2, dog necropsy

1. Introduction

Nearly four decades ago, almost simultaneously worldwide, as the causative of syndromes of an previously unknown disease in dogs, it was isolated canine parvovirus type 2 (CPV-2, canine parvovirus type 2) [12, 1, 5, 13, 18, 20]. This virus for many countries of the world and Europe today continues to be one of the most troubling health problems of dogs [21, 26]. 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 [24]. The disease first appeared with two syndromes; a non suppurative myocarditis associated with heart failure in dogs 4-8 weeks [17, 16, 9] and a severe enteritis accompanied by vomiting, diarrhea and death in puppies and adult dogs [1, 2, 23, 22]. With the introduction of regular mass vaccination at the beginning with live modified virus or inactivated feline panleukopenia virus (FPV) [4, 15] and later with modified live canine parvovirus type 2 produced in cell lines [8], the situation began to gradually improve. However, thanks to its high contagious properties and sustainability of the virus to environmental factors [14] even today, especially parvoviral enteritis, is a concern of the first hand to the importance of viral diseases in dogs. To date, in Europe and wider there are identified 3 serotypes of CPV-2 (CPV-2a, 2b and recently the 2c) [11]. What is the situation regarding parvoviral enteritis in Kosovo? Little can be said. There are reports of clinical symptoms similar to CPV-2 in dogs associated with high mortality. To the best of the authors´ knowledge up to currently in Kosovo still has no laboratory confirmation of CPV-2 in dogs.

2. Materials and Methods

A necropsy was carried out on 14 puppies dead from hemorrhagic enteritis, suspected of CPV-2. The 14 dogs belonged to the shelter of stray dogs from the communities of Gjakova, Rahovec, Peja and Kamenica. The dogs belonged to the age group 2-6 months, 8 of them were male and 6 female, all mixed breed. The necropsy was performed by routine techniques, focusing more on the study of the digestive organs.

During the necropsy, intestinal contents and/or feces were tested immediately for CPV-2 antigen and

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(Accepted for publication September 20, 20156)
***ISSN**: 2218-2020,*

based upon the quick result for CPV-2 antigen, positive intestinal samples (duodenum, ileum, jejunum) were taken and preserved in 10% formalin solution for histopathological studies. Samples for CPV-2 antigen were tested by means of a quick immunocromatographic test, Canine Parvovirus AG test - Quicking Biotech Co., Ltd., China. As a dog resulted negative for CPV-2 antigen and at the same time typical macroscopic signs of CPV-2 lacked, it was tested for the presence of bacterial infection at the Institute for Food Safety and Veterinary, Tirana.

Also, all histological samples were processed at the Institute for Food Safety and Veterinary, Tirana, Albania. Intestinal specimens after being fixed by immersion in formalin 10% were then processed with molten paraffin. Following processing, sections were cut on a microtome at a thickness of 4 µm ensuring that only a single layer of cells made up the section. Sections were then floated out on the surface of warm water in a flotation bath to flatten them and then picked up onto microscope slides. In the end, the specimens were stained with 10% hematoxylin and 0.5% eosin (H&E), covered with a glass coverslip and then examined under a light microscope.

3. Results and Discussion

Dogs submitted to this study had all clinical symptoms of diarrhea and/or vomiting lasting longer than 2 days. Depending on the onset of clinical signs, at the beginning, dogs were usually slightly hyperthermic with a rectal temperature varying from 39.3 to 39.9 ^{o}C being hypothermic as the symptoms progressed with a rectal temperature varying from 36.6 to 37.4 ^{o}C. 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 **(**Figure 8) and the remaining one resulted CPV-2 negative. 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 and duodenum. In most cases, the small intestine was congested containing aqueous hemorrhagic contents. In 4 puppies, ascarides in intestines were accompanying enteritis caused by CPV-2 (Figures 4 and 7). 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 [10, 22] and in one case (Figure 6), we noted serosal hemorrhages and hyperemic mesenteral vessels. While the intestinal mucosa was congested, haemorrhagic, covered by exudate (Figures 2, 3 and 4).

At the histopathological examination, in all CPV-2 positive puppies were seen lesions typical for hemorrhagic enteritis [10, 22, 19]. Microscopic examination of the ileum (Figure 9) and jejunum (Figure 10) 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. No such significant microscopic lesions were found in the duodenum (Figure 11).

Figure 1: A puppy confirmed with parvoviral enteritis. The duodenum is dilated and the distal two-thirds of the small intestine show patchy congestion. The serosal surface had a granular appearance often seen at post mortem in acute CPV-2 enteritis.

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 and mucosa appeared congested, haemorrhagic, covered by exudate.

Figure 4: 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 5: Content of hemorrhagic secretions in the stomach accompanied by a congested and hemorrhagic mucosa.

Figure 6: Serosal hemorrhages and hyperemic mesenteral vessels in a 11 - week old female, mixed breed puppy died of CPV-2.

Figure 7. Ascarides in the intestines were accompanying enteritis caused by the CPV-2 in this 2 months old, mixed breed puppy from Kamenica.

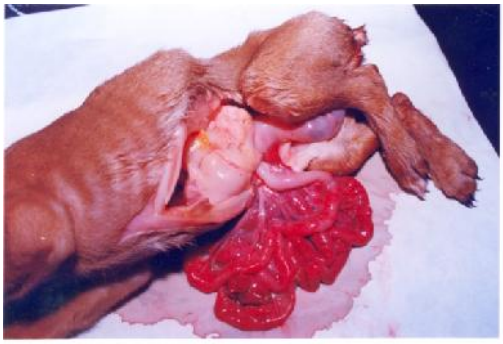

Figure 8: Pronounced hemorrhagic congestion of small intestinals due to *Clostridium perfinges* type A.

In a case of a puppy aged about 3 months old, which also presented with hemorrhagic enteritis, CPV-2 rapid test proved negative. Also macroscopic appearance of the small intestine had a pronounced hemorrhagic congestion different from that seen during CPV-2 (Figure 8, below). For that reason, it was taken intestinal content as well as a fecal specimen from colon and was sent in to the Institute for Food Safety and Veterinary, Tirana. From the bacterial culture it was isolated *Clostridium perfinges* type A **(**Figure 8). Such cases should be included in the differential diagnosis by being focused not only on the presence of hemorrhagic enteritis as an almost certain sign of CPV-2.

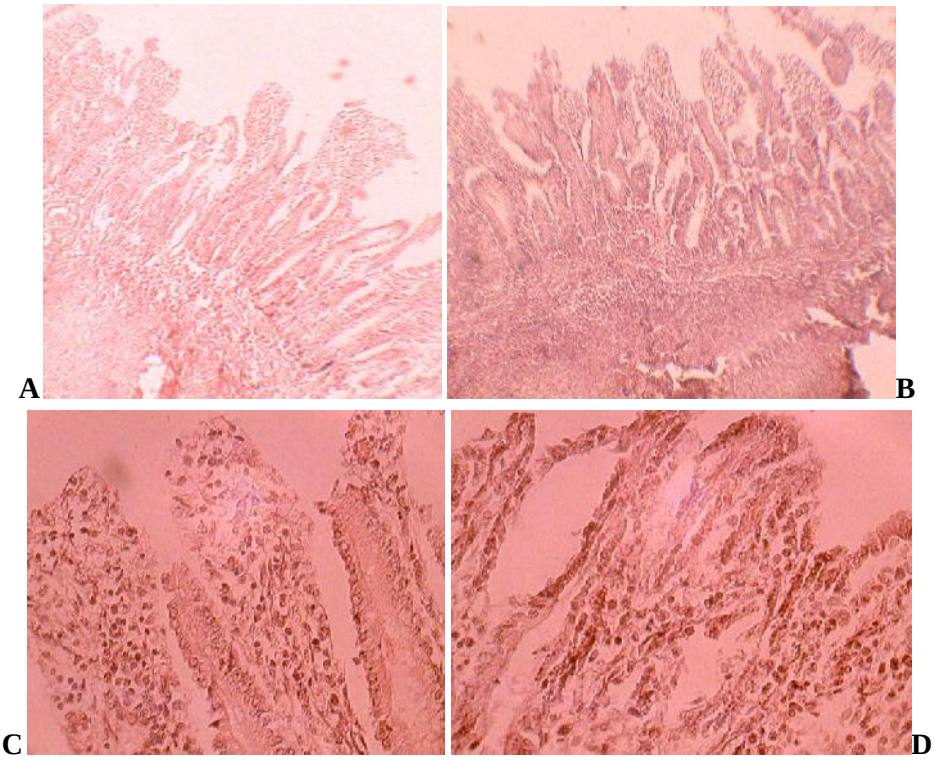

Figure 9: ***Ileum***, microphotography. Severe destruction of the villi and mucosal layers, necrosis and loss of surface epithelium, inflammatory cell infiltration into the lamina propria. **A**, **B** (X10 H&E), **C**, **D** (X40 H&E).

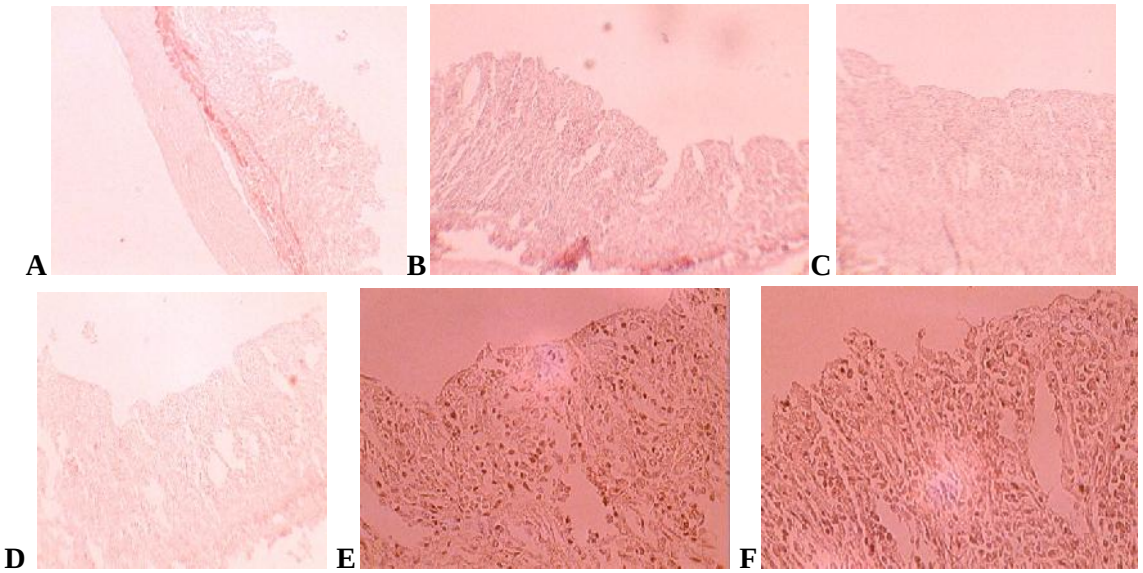

Figure 10: ***Jejunum***, microphotography. Loss of surface epithelium, shortened and blunting of villi, colapsed lamina propria followed by inflammatory and hemorrhagic cell infiltration and regeneration. **A, B, C, D** (X10 H&E), **E, F** (X40 H&E).

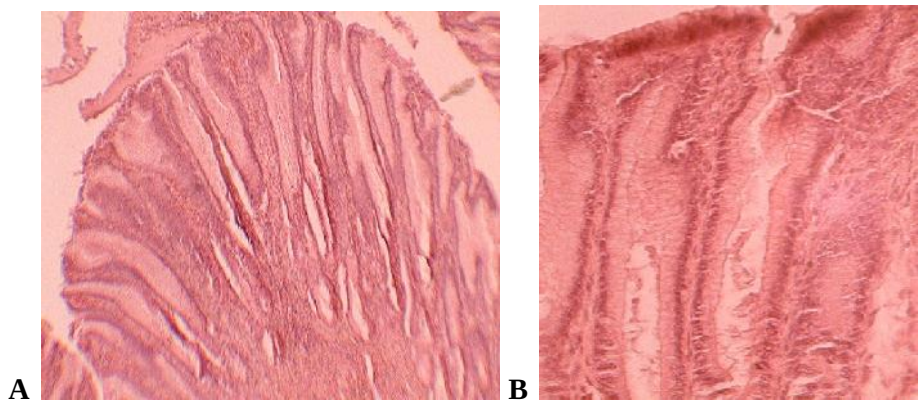

Figure 11: ***Duodenum,*** microphotography. Dilated crypts only. **A** (X10 H&E), **B** (X40 H&E).

4. Conclusions

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.

5. References

1. Appel, MJG., Cooper, BJ., Greisen, H., Carmichael, LE: **Status report: Canine viral enteritis**. *J. Am. Vet. Med. Assoc*, 1978, 173: 1516-1518.
2. Appel, MJG., Cooper, BJ., Greisen, H., Carmichael, LE: **Canine viral enteritis I: Status report on corona and parvo-like viral enteritides**. *Cornell. Vet*, 1979a, 69: 123-133.
3. Appel, MJG., Parrish, CR: **Canine parvovirus type 2**. In: *Virus infections of carnivores*. Elsevier Science Publishers B. V. Amsterdam. Appel, MJG ed; 1987: 69-92.
4. Appel, MJG., Scott, FW., Carmichael, LE: **Isolation and immunization studies of a canine parvo-like virus from dogs with heamorrhagic enteritis**. *Vet. Rec*, 1979b, 105: 156-159.
5. Burtonboy, G., Coignoul, F., Delferriere, N., Pastoret, P.-P: **Canine hemorrhagic enteritis: Detection of viral particles by electron microscopy**. *Arch. Virol*, 1979, 61: 1-11.
6. Carmichael, LE: **An annotated historical account of canine parvovirus**. *J Vet Med B Infectious Diseases and Veterinary Public Health*, 2005, 52: 303-311.
7. Carmichael, LE., Joubert, JC., Pollock, RVH: **Hemagglutination by canine parvovirus: serologic studies and diagnostic applications**. *Am. J. Vet. Res*, 1980, 41: 784-792.
8. Carmichael, LE., Joubert, JC., Pollock, RVH: **A modified live canine parvovirus strain with novel plaque characteristics. I.Viral attenuation and dog response**. *Cornell Vet*, 1981, 71: 408-427.
9. Carpenter, JL., Roberts, RM., Harpster, NK., King, NW: **Intestinal and cardiopulmonary forms of parvovirus infection in a litter of pups**. *J. Am. Vet. Med. Assoc*, 1980, 176: 1269-1273.
10. Cooper, BJ., Carmichael, LE, Appel, MJ., Greisen, H: **Canine viral enteritis. II. Morphologic lesions in naturally occurring parvovirus infection**. Cornell Vet, 1979, 69 (3): 134 – 144.
11. Decaro, N., Desario, C., Addie, DD et al: **Molecular epidemiology of canine parvovirus, Europe**. *Emerging Infectious Diseases*. www.cdc.gov/eid. August 2007: Vol. 13, No. 8.

12. Eugster, AK., Bendele, RA., Jones, LP: **Parvovirus infection in dogs**. *J. Am. Vet. Med. Assoc*, 1978, 173: 1340-1341.
13. Ganon, AN., Povey, RC: **A possible parvovirus associated with an epidemic gastroenteritis in Canada**. *Vet. Rec*, 1979, 104: 263-264.
14. Gordon, JC., Angrick, EJ: **Canine parvovirus: environmental effects on infectivity**. *Am. J. Vet. Res*, 1986, 47: 1464-1467.
15. Gordon, JC., Rogers, WA: **Field evaluation of a canine parvovirus vaccination program using feline modified live virus vaccine**. *J. Am. Vet. Med. Assoc*, 1982, 180: 1429-1431.
16. Hayes, MA., Russell, RG., Babiuk, LA: **Sudden death in young dogs with myocarditis caused by parvovirus**. *J. Am. Vet. Med. Assoc*, 1979, 174: 1197-1203.
17. Jezyk, PH., Haskins, ME., Jones, CL: **Myocarditis of probable viral origin in pups of weaning age**. *J. Am. Vet. Med. Assoc*, 1979, 174: 1204-1207.
18. Johnson, RH dhe Spradbrow, PB: **Isolation from dogs with severe enteritis of a parvovirus related to feline panleukopenia virus**. *Aust. Vet*. J, 1979, 55: 151.
19. Mason, MJ., Gillet, NA., Muggenburg, BA: **Clinical, pathological and epidemiological aspects of canine parvoviral enteritis in an unvaccinated closed beagle colony: 1978-1985**. *J. Am. Anim. Hosp. Assoc*, 1987, 23: 183-192.
20. McCandlish, IAP., Thompson,H., Cornwell, HJC., Laird, H., Wright, NC: **Isolation of a parvovirus from dogs in Britain**. *Vet. Rec*, 1979, 105: 167-168.
21. McCaw, DL., Thompson, M., Tate, D., Bonderer, A., Chen, YJ: **Serum distemper virus and parvovirus antibody titers among dogs brought to a veterinary hospital for vaccination**. *J. Am. Vet. Med. Assoc*, 1998, 213: 72-75.
22. Meunier, PC., Glickman, LT., Appel, MJG., Shin, S: **Canine parvovirus in a commercial kennel: Epidemiologic and pathologic findings**. *Cornell Vet*, 1981, 71: 96-110.
23. Osterhaus, ADME., van Steenis, G., de Kreek, P: **Isolation of a virus closely related to feline panleukopenia virus from dogs with diarrhea**. *Zbl. Vet. Med. B*, 1980, 27: 11-21.
24. Parrish, CR: **Emergence, natural history, and variation of canine, mink and feline parvoviruses**. *Adv. Virus Res*, 1990, 38: 403-450.
25. Parrish, CR., Aquadro, CF., Strassheim, ML., Evermann, JF., Sgro, J-Y., Mohammed, HO: **Rapid antigenic-type replacement and DNA sequence evolution of canine parvovirus**. *J. Virol*, 1991, 65: 6544-6552.
26. Pollok, RVH dhe Carmichael, LE: **Canine parvoviral enteritis**. In: *Infectious diseases of the dog and cat*. W.B Sounders Co., Philadelphia, Greene CE (Ed); 1990: 268-279.