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Research Article

Diagnostic approach in canine parvovirus infection in vaccinated and non-vaccinated dogs

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Abstract. The significance of Canine parvovirus 2 (CPV-2) infection for the domesticated canides is well understood. The current study compares the morbidity rate and clinical outcome in a group of vaccinated and non-vaccinated dogs (11 and 27 individuals, respectively) presented for treatment between October 2009 and March 2011. Fecal samples from the dogs with clinical signs of Canine Parvovirosis (CPV) were tested using a rapid test based on detection of CPV antigens, and a polymerase chain reaction (PCR) was applied for detection of viral DNA. For further confirmation two of the positive samples were sequenced, and after alignment with the Basic Local Alignment Search Tool (BLAST) they showed over 99% of overlapping with the data in GenBank. There wasn't significant difference in morbidity between vaccinated and non-vaccinated dogs, but the recovery period was shorter for the first group (mean value 7 days+/-1d, versus 12 days+/-2d). Some of the dogs were vaccinated twice or even thrice, but fail to resist, which might be due to an inadequate vaccination scheme or vaccine, or an early exposure to the wild virus. The latter suggests the increasing need of a modern diagnostic approach, for discrimination between the wild virus and the strains used for vaccination.

Key Words: Canine Parvovirus, Vaccination, Therapy

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Introduction

Canine parvovirus type 2 (CPV-2) emerged in 1978 (Appel et al 1979; Kelly 1978) in two clinical forms - gastroenteritis and myocarditis, causing high morbidity and high mortality among the canine population worldwide. The new antigenic variants (biotypes) of the virus, designated as CPV-2a and CPV-2b, totally replaced the original type 2 between 1979 and 1984 (Parrish, 1994). In 2000, a new variant, CPV-2c, was firstly detected in Italy (Buonavoglia et al 2001) and now is spread in many countries (Decaro & Buonavoglia 2012).

The spreading of this new variant according to some recent studies (Decaro et al 2007; Wang et al 2016) may compromise the routine vaccination plan. This rises the necessity of further investigation both on diversity of virus variants and on the real percentage of vaccinated and non-vaccinated dogs among the city population.

Development of well-established treatment algorithm for the two aforementioned groups of virus as well as for the susceptible breeds (e.g. Rottweiler, Labrador Retrievers etc.) is of paramount significance (Goddard & Leisewitz, 2010). According to Ikeda et al (2002) and Mech and Goyal (1993) parvovirus affects the population of certain feline and wild-life species thus the propagation and impact on different species of the virus should be a subject of thorough investigation. The phylogenetic history and the quick evolution of the Parvoviridae family as well as involvement of some strains in human pathophysiology (i.e. cardiomyopathy) may be adopted as plausible model for epizootology of an actual infection spreading in rising populations of susceptible species which lives in close proximity (Cotmore et al 2013).

Different studies in Bulgaria showed prevalence of CPV type 2a, less CPV type 2b and only a few CPV type 2c (Filipov et al 2011, Filipov et al 2016). The aim of the current study is to present cases of canine parvovirosis, with methods for in-clinic and molecular diagnostics, and to compare the morbidity rate and clinical outcome of treated dogs, patients of veterinary clinics.

Material and method

Two groups of dogs with clinical signs of gastroenteritis with known immune status have been discussed: group A (vaccinated, n=11) and group B (non-vaccinated, n=27). The dogs were presented for treatment in veterinary clinics in Sofia between October 2009 and March 2011. Fecal samples from the dogs with clinical signs of the disease have been tested using in-clinic tests based on detection of CPV antigens, and a polymerase chain reaction (PCR) was applied for detection of viral DNA. We used TC-3000 PCR Thermal Cycler (Thermo, Cole-Parmer, Staffordshire, UK) and a set of two primers: CPV-For (5'-AAACAGGAATTAACTATACTAATATATTTA-3') and CPV-Rev (5'AAATTTGACCATTTGGATAAACT-3'). A thermal cycling protocol described in previous publication (Filipov et al 2011) was applyed: activation of DNA polymerase at 95ºC for 10 min, and 40 cycles consisting of denaturation at 95ºC

Table 1 Samples Results

Sample №	Date obtained	Patient data			Vaccination status (Yes/No/ Number of inoculations)	PCR results
		Breed	Age	Gender		
1	2/15/2009	Mixed breed	2 M	M	No	positive
2	2/22/2009	Mops	1 M	M	1	positive
3	2/28/2009	Mops	1 M	F	1	positive
4	3/11/2009	German Shepherd	2 M	F	2	positive
5	4/24/2009	Dachshund	1 M	M	1	positive
6	5/23/2009	Mixed breed	N/A	M	No	positive
7	7/15/2009	Mixed breed	N/A	F	No	positive
8	9/17/2009	N/A	N/A	F	No	negative
9	10/16/2009	Mixed breed	5 M	M	No	negative
10	10/16/2009	Cocker Spaniel	2 M	F	No	positive
11	2/16/2010	Spitz	N/A	F	No	positive
12	2/27/2010	Mixed breed	5 years	F	No	positive
13	3/6/2010	N/A	2 M	M	No	positive
14	3/16/2010	N/A	N/A	M	No	positive
15	3/17/2010	Golden Retriever	6 M	M	2	positive
16	3/17/2010	Golden Retriever	2 M	F	2	positive
17	3/22/2010	Dachshund	2 M	F	1	positive
18	3/29/2010	N/A	N/A	M	No	positive
19	4/5/2010	Jack russell terrier	2 months	M	2	positive
20	5/3/2010	N/A	N/A	F	No	positive
21	5/3/2010	Epagneul breton	4 months	M	No	positive
22	5/9/2010	Mixed breed	2 months	F	No	positive
23	7/1/2010	Pomeranian spitz	3 M	F	No	positive
24	7/18/2010	Mixed breed	N/A	M	No	positive
25	8/23/2010	Beagle	2 M	M	No	positive
26	9/14/2010	Bolognese	5 M	F	No	positive
27	9/24/2010	Mixed breed	2 M	F	No	positive
28	1/16/2011	Mixed breed	5 M	F	2	positive
29	2/3/2011	Rottweiler	5 M	M	2	positive
30	2/5/2011	Rottweiler	2 Y	F	3	positive
31	3/13/2011	Miniature Pinscher	2 M	F	No	positive
32	3/22/2011	Yorkshire terrier	1 M	M	No	positive
33	6/12/2011	Mixed breed	N/A	M	No	positive
34	6/21/2011	Mixed breed	N/A	F	No	positive
35	9/4/2011	Mixed breed	2 M	F	No	positive
36	12/12/2011	Mixed breed	2,5 M	M	No	positive
37	12/21/2011	N/A	N/A	M	No	positive
38	12/27/2011	Rottweiler	N/A	F	No	positive

Table 2 DNA sequence of randomly selected samples. The definite matching regions are highlighted.

Sample	DNA sequence
№10	GAAAANTAACTTTACCTCTGTACAGNTGATAATG **TATTGCTACCACCAGATCCAATTGGAGGTAAAACAGGAATTAACTAT ACTAATATATTTAATACTTATGGTCCTTTAACTGCATTAAATAATGTA CCACCAGTTTATCCAAATGGA**
№28	NGCGTAAGGCTAGTTTAATATATTAGTATAGTTa **atTCCTGTTTTACCTCCATTGGATCTGTTGGTAGCATACATTATCATT TGTTACAGGAAGGTTAAAGTTAATATTTTGAATCCATCTCCTTCTGG ATATC**

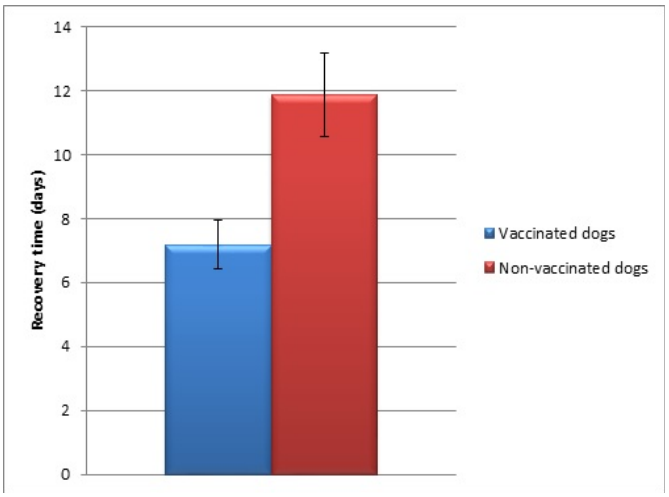

Fig. 1 Average recovery time

for 15 sec, primer annealing at 52ºC for 30 sec, and extension at 60ºC for 1 min.

For further confirmation two of the positive PCR samples were sequenced. Sequencing was performed using the Sanger's method on ABI Prism®310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Treatment was focused on fighting opportunistic infections, maintaining of hydration status and control hypoalbuminemia.

Results

All samples tested by in-clinic tests were positive for CPV antigen and only two of them were negative for viral DNA in following PCR testing (Table 1). Sequenced samples - one of vaccinated and one of nonvaccinated dog - showed over 99% of overlapping with the data in GenBank after the alignment with the Basic Local Alignment Search Tool (BLAST) (Table 2). Although there wasn't significant difference in morbidity between vaccinated and non-vaccinated dogs, the recovery period was shorter for group A - mean value 7 days+/-1d, versus 12 days +/- 2days for group B (Fig.1). Three of the patients (one vaccinated and two non-vaccinated dogs) were presented in severe condition and died 24 hours after hospitalization.

Discussion.

There were a good correlation between the used in-clinic tests in practice and the polymerase chain reaction (PCR). Some of the dogs were vaccinated twice or even thrice, but failed to resist the natural infection. Cases of canine parvovirosis (sometimes with fatal end) in young and old vaccinated dogs have been described worldwide and confirmed by molecular methods, despite the strictly applied vaccine schedules according the companies' recommendations. This problem arises again the contradictions among the veterinary practitioners and the dog's owners. It is difficult to be concluded always why that happens: the long-term presence of high titer maternally derived antibodies (MDA) in puppies, which interferes the vaccine strains; immunocompromised patients or incomplete cross-protection of commercial used vaccines against the circulating field strains. An effort has to be made for complete investigation of the possible reasons for vaccine failures. This could help us to understand better the CPV evolutional potential and to respond to the need of new vaccine (Truyen, 2006), in order to minimize the natural infection and the morbidity rate of dogs.

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