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DYNAMICS OF POST-VACCINATION ANTIBODIES AGAINST CANINE PARVOVIRUS IN DOGS

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Abstract

Canine parvovirosis, or viral haemorrhagic gastroenteritis, is a contagious infectious disease of canids that is characterized by gastrointestinal syndrome and mortality, especially in young puppies. Canine parvovirus type 2 (CPV2) is the pathogen agent of disease, and it is considered antigenically distinct from CPV1, the etiological agent of a disease that causes neonatal mortality in puppies. The high rate of morbidity and mortality in young animals requires the use of specific prophylactic measures. Vaccination against canine parvovirosis is part of the common vaccination scheme; a live-attenuated vaccine is used. The purpose of our study is to evaluate the dynamics of anti-CPV antibodies as a result of vaccination, considering that CPV is extremely important in the sanitary management of dogs.

Key words*: postvaccination antibodies, CPV, dogs.*

INTRODUCTION

Canine parvovirosis, or viral haemorrhagic gastroenteritis, is a contagious infectious disease of canids that is characterized by gastrointestinal syndrome and mortality, especially in young puppies. The etiological agent is Canine parvovirus type 2 (CPV2), which is antigenically distinct from Canine parvovirus type 1 (CPV1), which is the etiological agent of a disease that causes neonatal mortality in puppies (Binn et al., 1970; Carmichel et al., 1994; Parrish, 1999). The origin of CPV2 remains unknown, possibly derived from feline panleukopenia virus (FPV) or wild carnivore FPV-like viruses (Truyen, 1999; Truyen et al., 1998). The first identification of canine parvovirus (CPV) was made by Binn in 1970 (Parrish, 1990), but the first description of illness as a clinical manifestation was made in 1977 in Texas (Burtonboy et al., 1979; Carmichel et al., 1994; Parrish, 1999). After 1977, CPV2 was reported as an emerging etiological agent for severe gastroenteritis in dogs, with epidemic evolution and characterized by decreased appetite, vomiting, mucous/haemorrhagic diarrhoea, and leukopenia (Apple et al., 1979; Burtonboy et al., 1979; Decaro et al., 2005; Kelly, 1978).

The canine parvovirus belongs to the family *Parvoviridae*, subfamily *Parvovirinae*, genus *Protoparvovirus*, and species *Protoparvovirus carnivoran1* (virus name and virus abbreviation are not official ICTV designations). The genome is enclosed in an icosahedral capsid constituted by the combination of two proteins, such as VP1 and VP2, translated from alternative mRNA chains (Martella et al., 2004). Regarding the antigenic structure, CPV presents five antigenic variants: CPV-2a, CPV-2b, the new CPV-2a, the new CPV-2b, and CPV-2c (Singh et al., 2023). Currently, the antigenic variants of the original CPV2 (CPV2a, CPV2b, and CPV2c) are widespread in canine populations around the world. The antigenic and genetic analysis of CPV2 variants isolated in Italy after 2002 highlighted that CPV2c is currently the replacement for CPV2b (Decaro et al., 2005; 2006).

The high resistance of CPV is characterized by its ability to survive at pH variations and, as well, at different temperatures, such as 6 months at 4-10°C, 2 weeks at 37°C, and during the months or years in faeces. It is not inactivated during the action of common disinfectants (e.g., formalin solutions, sodium hypochlorite, and oxidizing agents) and, as well, the action of UV radiation.

Currently, CPV2 affects dogs of all ages. The different variants of CPV2 represent pathogen agents of enteritis and myocarditis in dogs, being characterized by a severe clinical evolution, especially in puppies (Parrish, 1999). The high rate of morbidity (100%) and mortality (90%) is registered in puppies and young dogs between 6 weeks and 6 months of age. The high susceptibility at these ages is a result of decreasing the titre of anti-CPV2 maternal antibodies during the first month. The anti-CPV maternal antibodies start to decrease after 10 days of life (Singh et al., 2023). Due to the severe clinical evolution and the high rate of mortality, it requires the application of general and specific prevention measures. Regarding immunoprophylactic methods, the use of modified live vaccines (monovalent or polyvalent attenuated strains) represents a common practice. The first vaccination of puppies should start between the first 6-8 weeks of age (when the maternal antibody titre is declining), followed by several boosters every 2-4 weeks until the age of 16 weeks or older (Villa Nova et al., 2018). The effectiveness of vaccination depends on the results of both the humoral immune response and the cellular immune response. The duration of postvaccination immunity is related to the degree of memory cell stimulation (Schulz et al., 2010). The decrease in the incidence of CPV infection in the canine population is the result of anti-CPV vaccination.

MATERIALS AND METHODS

In order to assess the dynamics of anti-CPV antibodies as a result of vaccination, a trial was conducted for 12 months postvaccination at 18 adult dogs (different breeds and aged between 1 and 5 years) that have been split into two examination groups, such as **group I (GI)**, which includes 10 females (noted as F1-F10) and **group II (GII)** which includes 8 males (noted as M1-M8). The females in group I were unpregnant during the assessment, and two of them were neutered (F6 and F5).

For this purpose, two-time intervals of blood sampling were established for anti-CPV titre evaluation, during the 12 months after anti-CPV IgG vaccination, as follows:

T1: the first evaluation of anti-CPV IgG titre at 3 months after vaccination.

T2: the second evaluation of anti-CPV titre at 11 months after vaccination.

All dogs included in the trial were dewormed (as a preventive measure), had a good physiological status, and had a complete vaccination programme. All animals, at the time of vaccination as well as at the time of each testing (T1 and T2), were clinically examined, being clinically healthy, without clinic symptomatology of infectious diseases or other diseases. In addition, an evaluation of potential prior exposure to CPV infection has been done with Rapid Immuno-Migration (RIM) Assay for detection of Parvovirus antigen in the faeces of dogs (Witness®CPV, Zoetis, USA). The basic principle of the method is that one anti-CPV-Ab is conjugated with gold particles and another anti-CPV-Ab is fixed on a nitrocellulose membrane. If Ag is present: Ag couples with the conjugate, forming an Ag-Ab complex that will later be captured by Ab fixed on the nitrocellulose membrane with the development of a pink-purple band. All dogs included in the trial have had a negative response at T1 and T2. The last vaccination, before the trail, was carried out 3 months ago, and it has been done with a polyvalent vaccine DHPPI L (modified live strains of Canine Parvovirus 2, Canine Adenovirus type 2, Canine parainfluenza virus, Canine Distemper virus strains, and bacterin of *Leptospira icterohaemoragiae* and *Leptospira canicola*). The vaccination was carried out as part of the annual vaccination scheme for dogs.

In order to assess the anti-CPV postvaccination titre the ImmunoComb® Canine VacciCheck Test Kit (Biogal, Israel) has been used on blood samples. The ImmunoComb®, based on the *dot ELISA* method, is a modified immunoenzymatic test (ELISA), which is used to detect the antibodies and evaluate their titre on whole blood samples or blood serum. According to kit instructions, the measuring of IgG anti-CPV titre is done by a colorimetric interpretation scale (of the kit), and the results are expressed in S units on a scale from 0 to 6. The cut-off S3 represents a value that is equivalent to 1:80 hemagglutinin inhibition units for CPV, which is considered a protective anti-CPV infection. The uppermost spot is the positive control, and it has a distinct purple-grey colour. A colour tone identical to S1 or weaker is considered a negative result. The Comb Scale was used to

evaluate the antibody score. The scale of interpretation that has been done based on the instructions of the manufacturer, has four categories, such as:
S0 and S1: negative humoral response when it sees a poor tone of colour.
S2: inadequate humoral response
S3-S6: positive humoral response (the Ig anti-CPV titer is ≥1:80), when it sees a tone of colour that is equal to or darker than the reference (Biogal, Israel, 2010).

RESULTS AND DISCUSSIONS

The assessment of postvaccination anti-CPV IgG titre for group I (GI) for 12 months recorded the following results expressed both in S units and in hemagglutination inhibit units (HI), included in Table 1.

Table 1. Evolution of postvaccination Ig anti-CPV in group I for 12 months (10 female dogs) according to the Comb scale interpretation

Subjects of GI	Age	Titre of Ig anti-CPV at T1		Titre of Ig anti-CPV at T2	
		S	HI	S	HI
F1	3 years	5	1:320	3	1:80
F2	3 years	6	1:640	4	1:160
F3	2 years	5	1:320	3	1:80
F4	5 years	3	1:80	2	1:40
F5	5 years	3	1:80	2	1:40
F6	3 years	5	1:320	4	1:160
F7	1 year	6	1:640	4	1:160
F8	2 years	4	1:160	3	1:80
F9	4 years	5	1:320	3	1:80
F10	3 years	4	1:160	3	1:80
Titre average (as S)		**4.6**		**3.1**	

The evaluation of the IgG anti-CPV dynamics highlights a progressive reduction during the 11 months post-vaccination for group GI. The post-vaccination titre is maintained at ≥ 3 (1:80 HI units) in 80% of the female dogs tested at time T2. For two of them (F4, F5), an IgG titre was registered below the protection limit at T2 by comparing with the minimum protection value (1:80 SN units / S3). It can be noted that the highest postvaccination anti-CPV IgG titres were recorded at T1, where the average titre value has been 4.6, as S. At T2 time, the value of the average anti-CPV IgG titre was 3.1, as S.
The assessment of postvaccination antiCPV IgG titre for group II (GII) for 12 months recorded the following results expressed both in S units and in hemagglutination inhibit units (HI), included in Table 2.

Table 2. Evolution of postvaccination Ig anti-CPV of group II for 12 months (8 male dogs) according to the Comb scale interpretation

Subjects of GII	Age	Titre of Ig anti-CPV at T1		Titre of Ig anti-CPV at T2	
		S	HI	S	HI
M1	2 years	6	1:640	5	1:320
M2	3years	5	1:320	4	1:160
M3	2 years	6	1:640	5	1:320
M4	5 years	4	1:160	2	1:40
M5	2 years	5	1:320	4	1:160
M6	4 years	4	1:160	2	1:40
M7	1 year	6	1:320	5	1:160
M8	5 years	3	1:80	2	1:40
Titre average (as S)		**3.9**		**2.9**	

The evaluation of the IgG anti-CPV dynamics highlights a progressive reduction during the 11 months post-vaccination for group GII. The post-vaccination titre is maintained at ≥ 3 (1:80 HI units) in 70% of the male dogs tested at time T2. For 3 of them (M4, M6, and M8) an IgG titre was registered below the protection limit at T2 by comparing with the minimum protection value (1:80 SN units/S3). Based on these results, it can be observed that the highest postvaccination antiCPV IgG titres were recorded at T1, where the average titre value was 3.9 (as S), while at T2, the anti-CPV IgG titre was 2.9 (as S).
Based on the results obtained for group I, it can be observed that the postvaccination antiCPV IgG titre is protective for 80% of the subjects during the 11 months (Figure 1). The average titres at T1 and T2 are above the minimum protection level, which suggests that the vaccination was not effective.

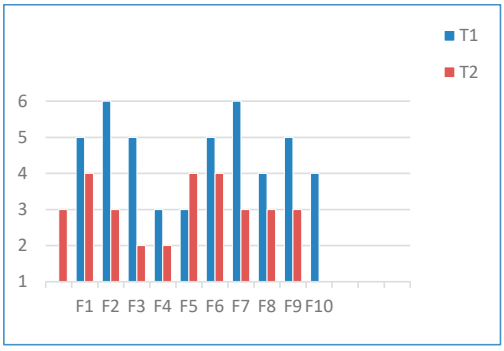

Figure 1. Graphic of S units for Group I

The assessment of the group II results pointed out that postvaccination anti-CPV IgG titre has been maintained at the minimum protective

level for 70% of the subjects during the 11 months. The average values of the anti-CPV IgG titre were at the minimum protection level at T1, respectively, below the minimum protection level at T2, which suggests that the level of protection was adequate against various CPV strains infection for 11 months for 70-80% of dogs (Figure 2.).

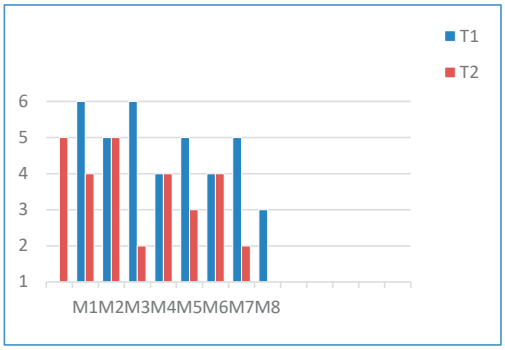

Figure 2. Graphic of S units for Group II

The values of anti-CPV IgG titres recorded at subjects F4 and F5 of GI, and subjects M4, M6, and M8 of GII (below the minimum level) at T2 suggests that the age of vaccinated animals or other exogen factors can influence the immunological reactivity during the 12 months postvaccination. The decrease in titres at the T2 has registered in the dogs aged 4-5 years.

The variations in immunological response are the results of various factors, such as the type of vaccine used, environmental factors, welfare conditions, and the immunocompetence of animal or individual factors (Vila Nova et al., 2018).

One of the important factors that can induce a failure of antiCPV is the high level of maternal antibodies (Nandi et al., 2013), but in our study only adult dogs were included. Other causes could be in relation to veterinary practice, such as storage of vaccine at an inadequate temperature, errors in vaccination, an inadequate programme of vaccination, or failure of the immunogenicity of the vaccine (Decaro et al., 2008; Altman et al., 2017).

CONCLUSIONS

The average values of the anti-CDV IgG titre are maintained above the minimum protection level for 70-80% of dogs, with no significant differences depending on the sex of the animals. The degree of protection against CPV infection is between 70 and 80% during the 11 months after the administration of a modified live vaccine (with CPV-2 strains), which allows us to conclude that during the 12-month period between two sessions of vaccination for canine parvovirus infection an appropriate level of protection is ensured for the majority of tested animals.

The post-vaccination immune response can be influenced by a series of factors, which can occur randomly at any moment of the period considered theoretically to be protective for CPV.

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