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SEROLOGICAL STUDY OF CANINE PARVOVIRUS-2 ANTIBODY TITERS FROM A DOG SHELTER IN BULGARIA

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ABSTRACT

Canine parvovirosis represents one of the most communicable and fatal gastrointestinal infections. The rapid spread and the extreme contagiousness of the canine parvovirus-2 (CPV-2) makes this disease a serious challenge especially in dog shelters and kennels. Disease control is based on vaccination of the most susceptible age groups. The aim of the current study was to determine the CPV-2 post vaccination antibody titers in a shelter population of young dogs, vaccinated according to a three-dose regimen. Anti CPV humoral immunity was demonstrated in 90% (288/320) of vaccinated animals while 10% (32/320) showed low to no antibody titer. The results confirmed that the three-regimen CPV vaccination protocol can provide a successful post vaccination cover in the majority of shelter dogs. However, the relatively high vaccination failure rate indicates possible gaps in the development of sustainable herd immunity.

Key words: canine parvovirus, antibodies, shelter dogs.

Introduction

Canine parvovirus 2 (CPV-2) belongs to the species *Carnivore protoparvovirus 1*, genus *Parvovirus* of the family *Parvoviridae* (Xu et al., 2020). It is the causative agent of one of the most contagious and fatal gastrointestinal infections in domestic and wild canids (Lamm and Rezabek, 2008; Mazzaferro, 2020). The infection spread is extremely rapid especially in shelters and kennels.

CPV-2 emerged as a new virus in 1978 and became distributed in the global dog population within 2 years (Carmichael, 2005). A few years later, the virus's original type was replaced by a new genetic and antigenic variant, called CPV-2a. Around 1984 and 2000, virus variants termed CPV-2b and CPV-2c were detected. However the different CPV variants are more than ~99% identical in nucleotide sequence (Voorhees et al., 2020). The circulation of all strains was already confirmed in Bulgaria (Filipov et al., 2016).

Disease control is accomplished by the application of modified live virus vaccines to obtain long-term immunity (Truyen, 2006; AAHA, 2011). The vaccination with canine parvovirus type 2b (CPV-2b) induces neutralizing antibody responses to CPV-2a and CPV-2c (Larson and Schultz, 2008; Wilson et al., 2014) while injection of CPV-2 strain stimulates immunity against virulent CPV-2b and CPV-2c (Siedek et al., 2011). Nevertheless some researchers made inconclusive inferences regarding the cross-protection of licensed CPV-2 and CPV-2b vaccines against the subtype 2c in up to 4 months old puppies (Hernández-Blanco and Catala-López, 2015). However postvaccinal efficacy and immunity can significantly vary depending on the vaccination protocol, health status of the animals and the presence of maternal antibodies and with regard of the virulence of the circulating antigenic variants (Lida et al., 1990; Miranda and Thompson, 2016). Attention is paid on early life disease protection as well as on vaccination failure due to maternally derived antibodies particularly in crowded kennel and shelter populations (Mila et al., 2014; Mila et al., 2018). Establishment of the factors which can induce a vaccination failure can contribute to better management and control of the parvovirus infection.

The CPV strain used in vaccine (CPV-2 or CPV-2b) was not associated with vaccination failure. On the other hand the age of administration of the final CPV puppy dose was a significant risk factor for vaccination failure, regardless if it was inoculated on the age of 10 or 12 weeks (Altman et al., 2017). The current canine guidelines for pet dogs recommend that the last dose of core vaccines be given to puppies 12 weeks of age or older, with revaccination after year, then not more often than every 3 years (Schultz, 2006).

Vaccination failure is a logical explanation for parvovirus outbreaks and CPV circulation in dog shelters (Kanwar, 2014). The loading factors for immunization failure include i) the presence of interfering titers of maternally-derived antibodies; ii) the presence of non-responders and iii) possible reversion to virulence (Chastant and Mila, 2019; Decaro et al., 2020). Nova et al. (2018) speculated on the fact that antibody response to vaccination is specific to each animal.

A clinic-based enzyme-linked immunosorbent assays for assessment of canine parvovirus vaccination response has been successfully implemented at the end of XX century (Waner et al., 1996). Rapid antibody tests for the evaluation of immune status before or after vaccination can be used as a valuable marker for disease protection level and a guideline for revaccination strategies in healthy dogs (Tizard and Ni, 1998; Twark and Dodds, 2000; Schultz et al., 2002; Waner et al., 2006).

The aim of the current study was to determine the CPV-2 post vaccination antibody titers in a community of young shelter dogs, vaccinated according to a three-dose regimen.

Materials and methods

Animals: The study was carried out in a private dog shelter in Sofia, Bulgaria, in 2019. The studied canine population consisted of 320 mongrel dogs. Their age was 6-8 weeks at the time of the first vaccination. No sex and weight features were taken into consideration. All dogs were clinically healthy based on a clinical examination at the day of the first vaccination and on a complete blood count. The animals were dewormed and fecal samples were parasitologically tested. Constant veterinary control was carried out.

Vaccine type and vaccination protocol: Products used were mono- and polyvalent CPV vaccines containing attenuated Canine Parvovirus strain CAG2: $10^{4.9}$ $CCID_{50}$-$10^{7.1}$ $CCID_{50}$.

The vaccination protocol was based on initial inoculation of monovalent CPV vaccine (D0), followed by second injection of polyvalent vaccine (D14) and booster with the same vaccine (D28). All vaccines were injected subcutaneously.

Sample collection: Blood samples (1 ml) were collected by cephalic venipuncture into 5 ml blood collection tubes. Blood sampling was performed 14 days after the last vaccine booster. Serum was separated by centrifugation (3500 rpm for 10 min) and tested *ex tempore*.

ELISA immunoassay: The serological assay used for estimation of the parvovirus antibody titers was ImmunoComb® Canine VacciCheck (Biogal Laboratories, Israel). It is a modified ELISA, an enzyme labeled "dot blot assay" that detects antibodies in serum or whole blood and provides a semiquantitative score for serum antibody titers. Although results are not read as end-point titers, the test kit utilizes a graduated (gray-purple) color scale to determine the relative amount of antibody present compared to a “positive” reference (control) color. It can be performed with 5 µl serum or 10 µl of whole blood. CPV sensitivity and specificity were reported to be 88% and 100% respectively. Based on the manufacturer’s recommendation the test should be implemented at least 14 days after the last vaccination.

Analysis of results: The results were expressed in "S" units on a scale of 0 to 6, where 3 "S" units were assigned as the positive serum titer which can provide protective immunity. Samples showing 0-2 "S" units, were interpreted as non-immunized and unprotective. The following correlation between "S" units and antibody titer by the hemoglutination inhibition was assigned:

- 0 "S" – no titer
- 1 "S" – 1:20
- 2 "S" – 1:40
- 3 "S" – 1:80
- 4 "S" – 1:160
- 5 "S" – 1:320
- 6 "S" – 1:640

Animals that did not respond to vaccination for CPV components were revaccinated with a booster dose of the same vaccine 4 weeks after the last inoculation.

Results and Discussion

The results of the tested animals are presented in Table 1 and Fig.1.

Table 1: Count and percent of the vaccination success and failure in the tested dog population

	Number	Percent (%)
Studied dogs	320	100
Vaccination success to CPV	288	90
Vaccination failure to CPV	32	10

Total 320 shelter dogs were serologically tested two weeks after the final (third) vaccination. Protective antibody titers were demonstrated in 90% (288/320) of the studied animals while 10% (32/320) lacked post vaccination protection based on their CPV antibody levels. All dogs with IgG titers of 3 "S" or more were estimated as having vaccination protection, based on the manufacturer's recommendation. Antibody titer of 0 "S" was estimated in 4.69 % (15/320), 1 "S" in 4.06 % (13/320) and 2 "S" in 1.25 % (4/320) of the tested animals. The distribution of the positively responding animals was as follow: 3 "S" was detected in 1.56 % (5/320) of all tested and 1.74 % (5/288) of all positive animals; 4 "S" units were documented in 7.19 % (23/320) and 7.99% (23/288) respectively; individuals with 5 "S" titer were 36.25 % (116/320) and 40.28% (116/288) respectively and with 6 "S" – 45 % (144/320) and 50% (144/2880 respectively (fig. 1).

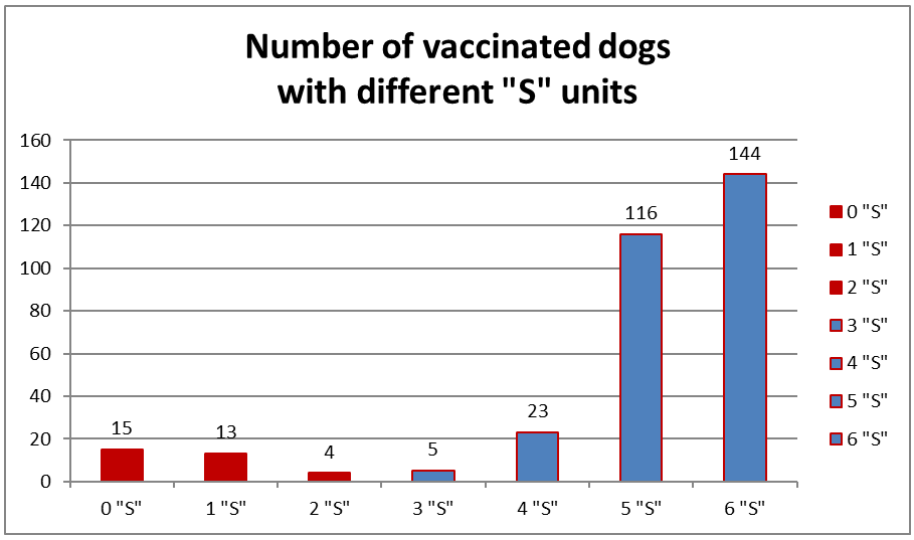

Figure 1: Number of shelter animals with different "S" units after CPV vaccine applications.

The current guidelines of WSAVA recommend initial core vaccination at 6–8 weeks of age, then every 2–4 weeks until 16 weeks of age or older (Day et al., 2016). However animals in shelters are characterized by unknown vaccination history, high population turnover and high infectious disease risk which necessitate protocol to be modified. The core vaccination may be started as early as 4–6 weeks of age, and revaccination can be every 2 weeks until the animal reaches 20 weeks of age (Day et al., 2016).

A puppy that is seronegative at 18 weeks of age should be revaccinated not less than 2 weeks following the last injection. Antibody testing can then be performed as early as 2 weeks following administration of this last vaccine dose. Nevertheless additional inoculation of CPV booster to adult dogs with suboptimal antibody titers was not able to stimulate immune response 2 months later (Taguchi et al., 2012a).

Satisfactory solid herd immunity was present in the majority of breeding kennels and only 4.6 % of the tested adult dogs were without protective antibody titers (Rota et al., 2019). It should be noted that the studied animals belong to the group of adult animals.

Studies with similar design (n=102) (same vaccination protocol, same ELISA assay) showed that 76 % of the vaccinated pups were with positive titers, i.e. can be interpreted as protected and 13 % represented vaccination failure (Waner et al., 2003). Eghafona et al. (2007) reported that all of the tested animals (n=120) were seropositive to CPV.

Available data concerning shelters reported that 97.9% of the 4 months old pups (n=51) were CPV antibody-positive (Litster et al., 2012). At the same time a significant part of the older dogs entering a shelter (31.5 %; n=431) may demonstrate a protective antibody titer (Lechner et al., 2010).

Post-vaccination immunity can vary according to the body weight in adult dogs and CPV-2 antibody titers were significantly higher in dog group < 5 kg (Taguchi et al., 2012b). To the best our knowledge no such correlation regarding pups is available.

Immunity duration is significantly influenced by the booster rate. The double booster dogs maintained a higher immune response throughout a 6 months period after vaccination compared to single booster although the difference in titers was not statistically significant (Vasu et al., 2019).

The almost total vaccination success (76–100%) against CPV can be interpreted with care since the fact that terrain CPV-2c strain can overcome the postvaccinal immunity in adult kennel dogs (Decaro et al., 2008).

Conclusion

Based on the results of the current survey we can conclude that the three-regimen CPV vaccination protocol can provide a successful post vaccination cover in the majority of shelter dogs. The used CPV regime resulted in high specific antibody titers which should guarantee an effective protection against pathogenic terrain strains. However, the relatively high vaccination failure rate in the population can indicate possible gaps in the development of sustainable herd immunity.

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