

## SEROPREVALENCE AGAINST CANINE DISTEMPER VIRUS (CDV) IN VACCINATED SHELTER DOGS FROM BULGARIA

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### ABSTRACT

Canine distemper virus (CDV) belongs to the genus *Morbillivirus* in the *Paramyxoviridae* and is the causative agent of a multi-systemic viral disease with high morbidity and mortality in susceptible animals. Disease control is based on the widespread use of vaccination of the most vulnerable age groups. The aim of the current study was to assess the serum antibody titers against canine distemper virus in vaccinated shelter dogs. Antibody prevalence was demonstrated in 88.44% (283/320) of the vaccinated animals through modified ELISA; 11.56% (37/320) were estimated as vaccination failure. The results confirmed that the approved shelter vaccination protocol could provide a successful post vaccination antibody titer. However, the relatively high rate of failed CDV vaccinations in this study may serve as an indication of possible breakthroughs in herd immunity and the risk of a potential disease outbreak.

**Key words:** canine distemper virus, CDV, antibodies, shelter dogs.

### Introduction

Canine distemper virus (CDV) belongs to the genus *Morbillivirus* in the *Paramyxoviridae* family and is closely related to the human measles virus and bovine rinderpest virus (Carter *et al.*, 2006). Disease caused by CDV is found primarily in young unvaccinated or vaccinated pups (Greene and Vandevelde, 2012). Vaccinated puppies sometimes fail to develop an immune response because of interference of active immunity by passively acquired maternal CDV antibody (Mila *et al.*, 2014). Disease can also affect unvaccinated adult animals. CDV infection in unprotected animals results in significant morbidity and mortality due to gastrointestinal, neurologic and respiratory abnormalities.

Two canine distemper virus (CDV) vaccine types are currently commercially available: modified-live virus (MLV) vaccines and a canarypox recombinant CDV (rCDV) vaccine (Pardo *et al.*, 1997; Larson *et al.*, 2007). The American Animal Hospital Association (AAHA) Canine Vaccine Guidelines and WSAVA Guidelines recommend that CDV vaccination should be a part of the core vaccination protocol for all puppies (AAHA, 2011; Day *et al.*, 2016). Notwithstanding, the incidence of canine distemper virus disease in canine populations throughout the world seems to have increased in the past decades, and several episodes in vaccinated animals have been reported (Martella *et al.*, 2008).

Increasing serological surveillance in different canine populations should be pivotal to understand the dynamics of CDV epidemiology. The purpose of the current study was to determine the serum antibody titers against canine distemper virus post vaccination in young dogs from a shelter.

## Materials and methods

### *Animals*

The study was carried out in a private dog shelter in Sofia, Bulgaria. The studied dog group consisted of 320 healthy mongrel dogs. The age of dogs was 6–8 weeks at the time of the first vaccination. No sex and weight features were taken into consideration.

### *Vaccine type and vaccination protocol*

The used vaccine was a commercially available polyvalent vaccine, containing attenuated Distemper strain BA5 > 10<sup>4.0</sup> CCID<sub>50</sub>. The applied vaccination protocol included initial subcutaneous inoculation of a single dose at D0, followed by a second injection at D14 and a booster dose at D28.

### *Sample collection*

Blood samples (2 ml) were collected by cephalic venipuncture into 5 ml blood collection tubes. Blood sampling was performed 14 days after the last vaccine application.

Serum was separated by centrifugation (3500 rpm/min for 10 min) and tested *ex tempore*.

### *ELISA immunoassay*

The serological assay used for estimation of the CDV antibody titers was Canine VacchiCheck (Biogal Laboratories, Israel). It is a rapid semiquantitative dot-ELISA-based system to determine the antibody titers in serum or whole blood. 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. CDV sensitivity and specificity were reported to be 100% and 92% respectively.

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 CDV antibody titer by the hemagglutination inhibition was assigned: S0– negative; S1 – 1:8; S2 – 1:16; S3 – 1:32; S4 – 1:64; S5 – 1:128; S6 – 1:256. Antibody titers equal to or higher than S3 values were considered indicative of a positive immunological response.

## Results

Total of 320 shelter dogs were serologically tested two weeks after the final vaccination. Protective antibody titers were demonstrated in 88.44% (283/320) of them and 11.56% (37/320) of the studied animals were without post vaccination protection based on their CDV antibody levels. All dogs with IgG titers of 3 “S” or more were accepted to have vaccination protection. Antibody titer of 3 “S” was detected in 15.63% (50/320) of all tested and 17.67% (50/283) of all positive animals; 4 “S” units were observed in 30.63% (98/320) and 34.63% (98/283) respectively; 5 “S” titer was reported in 33.92% (96/320) and 33.92% (96/283) respectively while 12.19% (39/320) and 13.78% (39/288) resulted in 6 “S” reading (Fig. 1).

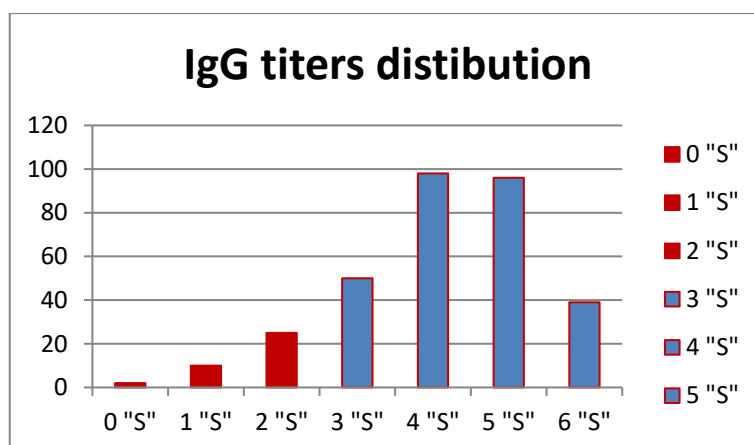


Figure 1: IgG titers distribution after CDV vaccine application in shelter dogs.

## Discussion

Assessment of immunization response to CDV vaccination in puppies using a clinic-based ELISA assay was successfully demonstrated to be a useful diagnostic tool (Tizard and Ni, 1998; Waner *et al.*, 1998; Waner *et al.*, 2003). Another application may be their use for evaluation before revaccination (Twark and Dodds, 2000; Waner *et al.*, 2006; Biogal, 2007).

The vaccination protocol which includes at least two consecutive applications of attenuated strains should induce virtually total seroconversion which was confirmed in the study of Eghafona *et al.* (2007). The results of Cunha *et al.* (2020) were comparable – 92.85% (26/28) of the vaccinated animals were positive. The mean antibody titer in vaccinated dogs ( $114 \pm 8.2$ ) indicated immunity and was significantly higher when compared to unvaccinated animals ( $29 \pm 8.8$ ,  $P < 0.05$ ) or dogs with unknown vaccination history ( $29 \pm 5.0$ ,  $P < 0.001$ ) (Jozwik *et al.*, 2004). The same conclusion has been made in the study of Oyedele *et al.* (2004). Another study postulated that 90.0% of the sampled dogs had protective immunity, with those given more than one dose having higher level of protective antibody (Ogbu, 2017). At the same time the antibody titer did not differ significantly in relation to sex, breed, age and location but significant difference was seen in relation to number of primary vaccination, i.e. dogs that received booster doses had more protective antibodies.

However the results in another study demonstrated a relatively low percent of CDV protective antibody titers in vaccinated dogs <1 year old – 54.7% (58/106) compared to 70.0% (666/951) for the whole tested canine population with different ages (Dall'Ara *et al.*, 2023).

The serological survey from shelters showed also that from 93.8% to 97.8% of vaccinated dogs were CDV antibody-positive after 13–15 days (Litster *et al.*, 2012). This data is inconsistent with the results of the present study which reported lower percent of animals with protective antibody titers and higher vaccination failure rates.

The data from another shelter indicated that overall, 64.5% (278/431) of dogs had insufficient titers for antibodies against CDV, CPV, or both. A total of 153 (35.5%) dogs had protective antibody titers for both CDV and CPV, 33 (7.7%) had titers for CDV but not CPV, 136 (31.5%) had antibodies for CPV but not CDV, and 109 (25.3%) did not have for either virus. Older dogs were more likely to have antibody titers for CDV and CPV and neutered were more likely to have for

CDV (Lechner *et al.*, 2010). The cited findings support the current guidelines recommending vaccination of all dogs immediately upon admission to shelters.

Among the factors which could cause distemper vaccination failure are the blocking effect of maternal antibodies up to the age of 12 weeks, the immunosuppression due to parvovirus and the use of corticosteroids in high doses (Povey, 1986). In case of vaccination failure an additional inoculation may lead to a booster effect resulting in increasing the antibody titers for CDV (Taguchi *et al.*, 2012).

Vaccine response to CDV is specific in each individual which makes it desirable to determine individual humoral immunity levels in order to assess vaccine efficacy (Nova *et al.*, 2018). Effective immune protection in primary vaccination depends mainly on the initial titer of maternal antibodies acquired by the neonate. Nevertheless, the use of a canarypox–vectored canine distemper vaccine in pups with maternal antibodies to CDV was able to induce protective immunity to the infective agent (Pardo *et al.*, 2007).

Results indicate that dogs vaccinated with modified live CDV can be protected from challenge for  $\leq 4.4$  years postvaccination and detection of specific antibodies is predictive of resistance to virulent strains (Abdelmagid *et al.*, 2004; Schultz, 2006; Jensen *et al.*, 2015). Even the administration of a single dose of a multivalent (DHPPiLR) vaccine prevents clinical signs and mortality following virulent challenge with canine distemper virus (Wilson *et al.*, 2014).

The relatively high rate of failed CDV vaccinations in this study may serve as an indicator of possible breakthroughs in herd immunity and the risk of potential disease outbreak.

## Conclusion

Based on the results of the current survey we can conclude that the widely accepted shelter vaccination protocol can provide a successful post vaccination cover in most of the treated dogs. Following a triple CDV vaccine application protocol a significant part of the animals developed high specific antibody titers which should insure a sustainable protection against pathogenic terrain strains. However, the relatively high vaccination failure rate in this study can indicate possible gaps in the development of sustainable herd immunity.

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