

J Vet Diagn Invest 18:267–270 (2006)

Application of a dot enzyme-linked immunosorbent assay for evaluation of the immune status to canine parvovirus and distemper virus in adult dogs before revaccination

Trevor Waner,¹ Shlomit Mazar, Ephraim Keren-Kornblatt

Abstract. A growing body of literature has been published indicating that the current practice of annual vaccination of dogs may not be beneficial and in some cases may even be harmful. A number of publications have proposed assessing the immune status of dogs before annual revaccination. In this study the usefulness of a commercially available dot-ELISA kit was evaluated to determine the duration IgG antibody titers to canine parvovirus (CPV) and canine distemper virus (CDV) in 158 dogs vaccinated at least one year ago. Overall, the percentage of dogs with protective antibody titers to both CPV and CDV was 84%. The percentage of dogs with borderline antibody titers was 11% for CPV and 10% for CDV. Four percent of the dogs had no detectable antibody to CPV and 6% had no antibody to CDV. The results reported here are in good agreement with other studies measuring IgG antibody levels. It is concluded that the kit offers veterinarians the opportunity of determining antibody titers and revaccinating only those pets whose antibody titers to specific diseases have waned.

Key words: Annual revaccination; canine distemper; canine parvovirus; dot-ELISA.

From the Israel Institute for Biological Research, P.O. Box 19, Ness Ziona 70400, Israel (Waner), Biogal Galed Laboratories, Kibbutz Gal'ed, 19240, Israel (Mazar), and the Veterinary Clinic, P.O. Box 1708, Hashmonaim 73127, Israel (Keren-Kornblatt).

¹Corresponding Author: Dr. Trevor Waner, B.V.Sc., PhD, Dipl. ECLAM, Israel Institute for Biological Research, P.O. Box 19, Ness Ziona, 70400, Israel (e-mail: wanertnt@shani.net).

Historically, prophylaxis against important infectious diseases of dogs and cats has been based on annual vaccination. This approach has resulted in excellent disease control for infections that were once considered important causes of morbidity and mortality. Studies have shown that a high percentage of dogs and cats are protected for a number of years following a single vaccination. Among the conclusions made in a number of studies was the possibility of measuring antibody titers to assess the immune status of adult dogs and cats before revaccination.^{2,9} In this study, the use of an in-clinic dot-ELISA test for determining the duration immunoglobulin G (IgG) antibody titers to canine parvovirus (CPV) and canine distemper virus (CDV) in dogs vaccinated at least one ago was assessed.

One hundred and fifty-eight dogs that had not been vaccinated with a modified live vaccine (canine parvovirus, distemper virus, adenovirus, parainfluenza virus) for at least 12 months were sampled. The date of the last vaccination was obtained from the attending veterinarian. The dogs were client-owned and of a variety of breeds and both sexes, either sexually intact or neutered. Serum was collected and IgG antibody titers to CPV and CDV were determined on coded serum samples using a commercially available in-clinic dot-ELISA kit.^a The test was carried out as previously described.^{12,13} The concentration of CPV and CDV IgG antibodies for each sample was measured using a color-coded scale provided in the kit, and the results were expressed in "S units" on a scale of 0 to 6. For both CPV and CDV assays, an "S" value of 3 was standardized by the manufacturer to be the equivalence of a 1:80 serum antibody titer by the hemagglutination inhibition (HI) test and the serum neutralization (SN) assay for CPV and CDV, respectively. An HI and SN antibody titer of 1:80 ("S" = 3) was regarded as a protective titer for CPV and CDV, respectively. By extrapolation an "S" value of 1 represented a titer of about 1:20 and an "S" value of 2 was equivalent to a titer of about 1:40.^{12,13} For statistical analysis dogs were categorized into 6-monthly intervals according to their last vaccination and mean serum antibody titers for CPV and CDV calculated for each interval. A one-way ANOVA was used to compare the mean antibody titers for CPV and CDV for the different time intervals, and the level of significance was set at $P \leq 0.05$.

Of the 158 dogs examined, 42% had received their last vaccination between 12 and 18 months previously, 35% between 19 and 36 months, and 23% over 3 years

previously (Tables 1, 2). Overall, the percentage of dogs with protective antibody titers ("S" ≥ 3) for both CPV and CDV was 84% (Tables 1, 2). The percentage of dogs with borderline antibody titers ("S" = 1–2) was 11% for CPV and 10% for CDV. Four percent of the dogs had no detectable antibody to CPV, and 6% had no detectable antibody to CDV. There did not appear to be any correlation between the incidence of low or borderline titers to either CPV or CDV and time elapsed since the last vaccination.

A number of authors have suggested that instead of annual vaccination, pets may be tested for antibody titers on a regular basis, and only those pets showing negative, borderline, or low antibody titers should be revaccinated.^{4–6,8–10} The need for a reliable, affordable, and standardized serum antibody assay has been proposed.^{9,10} In response to this idea, this study evaluated the usefulness of a commercially available standardized in-clinic dot-ELISA test kit for the semiquantitative assay of CPV and CDV IgG antibodies in dogs that had not been vaccinated for at least one year. To the authors' knowledge, this is the first study to test the applicability of an in-clinic dot-ELISA assay for the assessment of immune status in adult dogs after vaccination.

High antibody titers have been shown to correlate with protective immunity; therefore, a critical issue in a study of this nature is to define the protective serum antibody titers to CPV and CDV. In a recent study, Böhm et al. chose CPV HI titers equal to or greater than 1:128 but pointed out that a cut-off titer of 1:80 has been used in other studies.² For CDV, Böhm et al. chose a cut-off SN titer of 1:64 or higher, although SN titers in the range of 1:16 to 1:32 have been shown to be protective.^{3,7} In a study to determine whether dogs either remained seropositive or responded serologically to revaccination to 5 viral agents after extended periods since their last vaccinations, dogs with SN CDV titers of $\geq 1:32$ and HI CPV titers of $\geq 1:80$ were regarded as being protective.⁵ In the present study a protective titer of $\geq 1:80$ was chosen for both CPV and CDV. The selected titer of $\geq 1:80$ is slightly lower than the titers chosen by some workers but higher by a factor of more than two when compared to the titers chosen by others.^{2,5}

An inherent difficulty in comparing serological studies relates to the manner in which the antibody titers were determined. The dot-ELISA method is a semiquantitative

Table 1. Canine parvovirus: statistical data of antibody titers for 158 dogs categorized by 6-monthly intervals following their last vaccination.*

Months	Overall	6-monthly interval after last vaccination						
		12–18	19–24	25–30	31–36	37–42	43–48	>48
No. of dogs	158	66	31	13	12	9	5	22
Mean "S" titer	NA	4.3	4.4	4.4	5.3	4.1	4.2	4.6
Standard deviation	NA	1.8	1.9	2.2	0.8	1.7	1.6	1.4
% Protected	84	80	84	85	100	78	80	91
% Borderline	8	12	6	0	0	11	20	5
% Low or undetectable	8	8	10	15	0	11	0	5

* NA = not applicable.

Table 2. Canine distemper virus: statistical data of antibody titers for 158 dogs categorized by 6-monthly intervals following their last vaccination.*

Months	6-monthly interval after last vaccination							
	Overall	12–18	19–24	25–30	31–36	37–42	43–48	>48
No. of dogs	158	66	31	13	12	9	5	22
Mean “S” titer	NA	4.0	4.6	4.5	4.2	5.0	5.0	3.9
Standard deviation	NA	2.0	1.4	1.6	1.5	1.7	1.2	2.2
% Protected	84	77	94	92	83	89	100	77
% Borderline	6	8	6	0	8	0	0	5
% Low or undetectable	10	15	0	8	8	11	0	18

* NA = not applicable.

method based on color comparisons between a standard and a test sample result. Previous studies have shown a good correlation between CPV HI titers and CDV SN titers, compared to the dot-ELISA “S” values.^{12,13} A limitation of the dot-ELISA method is the accurate determination of titers in the range of 1:10 to 1:40.¹³ This limitation is not considered to be a problem for the determination of protective antibody titers when a protective titer is defined as $\geq 1:80$, as used in the present study. On the other hand, setting the protective titer at this relatively high level may have resulted in the elimination of a percentage of dogs that may have been indeed protected, but had borderline CPV and/or CDV antibody titers. Dogs exhibiting borderline titers were estimated to have titers in the range of 1:20 to 1:40 (“S” = 1 and “S” = 2, respectively). The fact that these dogs possess specific antibodies to both of these infectious agents is evidence for their previous exposure to both CPV and CDV either through natural infection or vaccination. It is possible that these dogs are also protected by virtue of their immunologic memory despite their low antibody titers. Evidence for this inference is also based on challenge studies in which dogs were protected from virulent CPV and CDV challenge after only two vaccinations at 7 to 8 weeks of age.¹ Taking this into account, if the percentages of dogs with protective titers (“S” ≥ 3) and dogs with titers of “S” = 2 (equivalent to 1:40) for CPV and CDV are combined, the cumulative average percentage of dogs with protective titers reaches 92% and 89% for CPV and CDV, respectively.

Data collected in this study from a large group of dogs in Israel is in agreement with the general conclusions made by a large number of studies from different parts of the world that the vast majority of dogs retain their protective immunity to CPV and CDV after their initial vaccination. Use of the dot-ELISA kit in this study was able to distinguish a substantial number of dogs presenting to clinics for annual vaccination still harboring protective antibody titers to CPV and CDV, with only a small minority of dogs lacking IgG antibody to CPV and CDV or having low antibody titers to these agents.

A unique attribute of the dot-ELISA is the semiquantitative nature of the test.^{11–14} Quantification of the level of antibody is a necessary attribute of an IgG antibody test for the recognition of dogs with or without protective antibody to a specific disease. Quantification of IgG antibody levels to a variety of diseases offers the veterinarian the

opportunity of following antibody titers on a yearly basis and detecting trends over time which can be used for early detection of dogs that might have lost their immunity to specific diseases. Test kits that offer qualitative (positive or negative) results rely on the manufacturer to determine the cut-off titer of antibody that is protective. Under these circumstances, dogs with borderline titers would fall into the category of false negatives and would be regarded as lacking protection. From the data of the present study, this could account for between 5% and 13% of dogs presented for vaccination. It would be preferable to leave the decision for revaccination to the attending veterinarian, who should decide whether to vaccinate dogs with borderline antibody titers depending on the veterinarian’s knowledge of the risk conditions in his location and the lifestyle of the specific dog.

In conclusion, the data presented in this report demonstrate the value of a semiquantitative in-clinic dot-ELISA test for determining IgG antibody levels to CPV and CDV in dogs before annual revaccination. The results reported here are in agreement with other laboratory antibody studies indicating that a large percentage of healthy dogs have adequate serum antibody levels to CPV and CDV and do not, therefore, require annual vaccination. By requiring minimal use of laboratory equipment, the technique offers the practicing veterinarian the opportunity to easily monitor the vaccination status of dogs to revaccinate only those dogs whose immunity to specific diseases has waned. This practical approach allows the attending veterinarian to tailor a vaccination program for the individual patient as an alternative to annual vaccination, which may not be beneficial and in some cases may even be harmful.

Sources and manufacturers

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