

Assessment of maternal antibody decay and response to canine parvovirus vaccination using a clinic-based enzyme-linked immunosorbent assay

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Abstract. Interference caused by maternal antibodies is considered a major cause of canine parvovirus (CPV) vaccination failure. In this study, an immunoblot clinic-based enzyme-linked immunosorbent assay (ELISA) method was used to detect CPV antibodies in sera of pregnant bitches and their offspring to study the response of pups to vaccination. With a easily accessible procedure for CPV antibody determination, the veterinarian should be able to gauge the response of pups after vaccination. The validity of the technique was tested in parallel against the standard hemagglutination inhibition (HI) test. Results of the ELISA were correlated with those of the standard HI method for quantification of CPV antibodies. With the ELISA, successfully immunized pups were identified, allowing for a more reliable and cost-effective program of vaccination. This simple clinic-based test could be used for the assessment of vaccination status of pups during the critical phase of 6 to about 16 weeks of age. This study is the first in which vaccination response to CPV in pups was followed, using a clinic-based ELISA for CPV antibody monitoring.

Maternal antibody transfer in the dog occurs primarily by intestinal absorption from the colostrum during the initial 2 days of the pup's life.¹ Interference by maternal antibodies is regarded as a major cause of canine parvovirus (CPV) vaccination failure in young dogs.^{1,7} Attempts to develop immunization strategies to overcome this problem have to date been based on administration of multiple vaccinations from about 6 weeks of age until about 16-18 weeks of age.²

In this study, we tested an immunoblot clinic-based enzyme-linked immunosorbent assay (ELISA) method to semiquantitatively assay CPV antibodies in sera of pregnant bitches and their offspring to study the kinetics of maternal antibodies in pups and the response of pups to vaccination. With an easily accessible in-clinic procedure for CPV antibody determination, it should be possible to monitor response of pups after vaccination with CPV. The validity of the technique was tested in parallel with the standard hemagglutination inhibition (HI) test.

Materials and methods

Animals. Ten pure-bred, pregnant beagle dogs^a were used in this study. The bitches were allowed to whelp normally, and 4 pups were selected at random from each litter for antibody studies.

Vaccination protocol. The pregnant bitches were vaccinated with modified attenuated CPV and canine distemper virus^b vaccines at 30-40 days of gestation. The pups were subsequently vaccinated with the same vaccine at 6 and 9 wk of age. In addition, all pups received *Bordetella bronchiseptica* vaccine^c at 4 wk of age, *Leptospira canicola* and *L. icterohaemorrhagiae* vaccine^d at 11 wk of age, leptospirosis and rabies vaccines at 14 wk of age, and hepatitis and parainfluenza^e vaccine at 16 wk of age.

Serum sample collection. Blood was collected from the pregnant bitches between 55 and 58 days of gestation. Blood samples were obtained from the pups at 1 day of age (after suckling) and then at 4, 6, 9, 12, 16, and 18 wk of age just prior to their respective vaccinations. Serum samples was stored at -20 C until tested.

Serologic assays. Each sample was assayed for CPV antibodies by both the HI test and ELISA. Antibody titers to CPV were determined by the HI test as previously described using CPV-2 virus antigen.³ Coded serum samples were assayed at the James A. Baker Institute, College of Veterinary Medicine, Cornell University. Titers were expressed as the reciprocal of the serum dilutions that inhibit 4-8 hemagglutination units.

Antibody titers also were determined on the coded serum samples using a commercially available ELISA kit.^{f,6} The test kit is based on solid phase immunoassay technology. Plastic combs were sensitized with CPV antigen (CPV-2 strain C-780916).^g The sera were diluted 1:20 in buffer and incubated with antigen spots for 5 min. After a wash period to displace unbound antibodies from the reaction spot, the combs were allowed to react with whole molecule goat anti-dog IgG alkaline phosphatase conjugate.^h After 2 successive washing steps, bound antibody was detected with a precipitating chromogen 5-bromo-4-chloro-3-indolyl phosphate and nitro-blue tetrazolium.ⁱ

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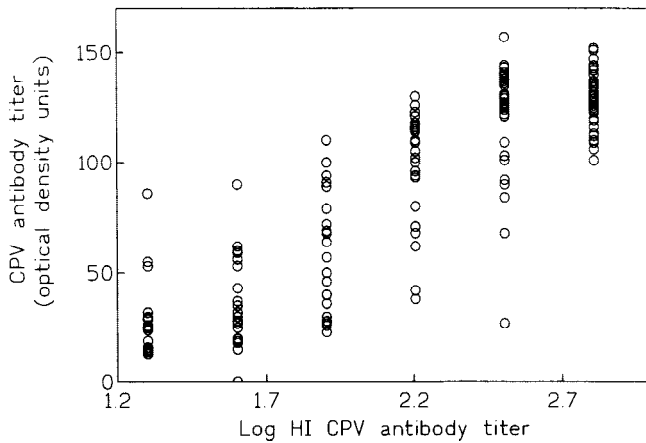


Figure 1. Correlation between serum canine parvovirus (CPV) hemagglutination inhibition (HI) titers and serum CPV antibody ELISA optical density results as measured by the immunoscan. ($r^2 = 0.728$).

The concentration of CPV antibodies for each sample was assessed in 2 ways. The first utilized the immunoscale method with the color scale provided in the kit. Translation of the color reaction to CPV antibody units was achieved by comparison of each spot with the color reaction of a spot from a known positive, pretitrated serum sample. The results were expressed in S units on a scale of 0 to 6. The positive control serum sample (HI titer = 1930) was adjusted to 3 S units. The second method, referred to as immunoscaning, employed a scanning device¹ designed for automatic reading of the color intensity of the reaction spots on the comb. The results were recorded as optical density.

Statistical analysis. Correlation between the HI and ELISA results was assessed using Pearson's correlation coefficient (r^2).

Results

Mortality. Two pups died at 4 weeks of age, and another died at 16 weeks of age. Those deaths were unrelated to CPV infection.

Antibody assays. A high correlation for the measurement of CPV serum antibody titers by the HI test and ELISA optical density immunoscan method was found ($r^2 = 0.728$), for the HI range of 1:20 to 1:640 (Fig. 1). Furthermore, the correlation between the objective immunoscale score and subjective optical density readings for assessment of antibody titer was also high ($r^2 = 0.860$) in the range of 1:20 to 1:640. For HI titers in the low range of 1:10 to 1:40, comparable color intensity results were obtained using the ELISA and, similarly, for HI titers in the high range of 1:1,280 to 1:10,240, respectively.

Maternally derived antibody titer. The mean serum CPV antibody titer for the 9 bitches before whelping was 1:320 (geometric mean) by the HI test, 127 ± 20 ($\bar{x} \pm SD$) for the immunoscan, and 5.8 ± 0.4 S scale units for the immunoscale. Serum titers of maternally

derived antibody in pups declined exponentially with time as detected by the HI technique. The mean litter half-life of maternal serum CPV antibody for the HI results was calculated as 11.6 ± 2.5 days.

Maternally derived antibody interference with vaccination. Antibody response to vaccination were clearly demonstrated by both the HI test and ELISA, with steep rises in antibody titers (Tables 1, 2; Fig. 2). A 4-fold or greater increase in HI titers or ELISA scores was considered indication of seroconversion.⁹ According to the HI results, vaccination was successful in 18 of the 38 pups (47%) at 6 weeks of age; 19 pups (50%) at 9 weeks of age, and 1 pup (S4) only at 16 weeks of age (Table 1).

Using the ELISA immunoscan results, successful immunization as judged by a 4-fold increase in serum antibody titer was also demonstrated in 18 of 38 pups at 9 weeks of age, 2 weeks after their first vaccination. In 6 pups (L2, L3, K3, I2, I3, I4), increases in CPV antibody titers detected at 9 weeks of age by the immunoscan ELISA method were not demonstrated by the HI test. According to the ELISA, vaccination carried out at 12 weeks of age successfully immunized the remaining 20 pups. Similar results were obtained for the immunoscale readings, which showed that all pups had seroconverted after the second vaccination at 9 weeks of age (Table 2).

The HI titer of maternal CPV antibody that interfered with vaccination was $\geq 1:80$, although some pups with lower titers failed to respond to the vaccine. Thirty-nine percent of the pups became responsive to vaccination at a HI titer of 1:10, 30% of the pups at 1:20, 26% at 1:40, and 5% at 1:80 (Table 1).

Immunization results for the ELISA immunoscan were calculated for all pups except those that appeared to have early unexpected rises in titer at 9 weeks of age after the first vaccination, i.e., L2, L3, K3, I2, I3, I4. For the other 32 pups, 3% were immunized at immunoscan serum titers of 1-10; 50% were immunized at 11-20, 41% at 21-30, and 6% at 31-40. ELISA immunoscale results revealed that 88% of pups were immunized with S scores of 0 and 1 and 12% with S scores of 2 (Table 2).

Discussion

To date, there is no commercially available test for the quantitative assay of CPV antibodies in serum of dogs. The HI technique is regarded as the "gold standard" and is generally available only at selected laboratories. The technique requires extensive equipment and is subject to error because of lack of standardization.⁴ The ELISA, although documented, has not gained popularity because it also requires elaborate laboratory equipment.⁸ The results of this study have shown that serum CPV antibodies in dogs can be quantitatively

Table 1. Hemagglutination inhibition (HI) titers of serum CPV antibody in pups evaluated over a period of 18 weeks.

Litter*/ Pup id.	Age (wk)						
	0	4	6	9	12	16	18
N (320)							
1	320	20	20	640	320	1,280	160
2	160	40	40	640	320	1,280	NR†
3	160	40	80	1,280	640	10,240	320
4	160	<10	320	640	640	2,560	320
S (1,280)							
1	5,120	160	80	<10	640	NR	640
2	640	80	≤80	<10	160	320	320
3	2,560	160	160	20	<10	1,280	1,280
4	1,280	160	160	<10	320	640	640
H (640)							
1	1,280	40	40	2,560	640	5,120	640
2	80	10	40	2,560	640	1,280	640
3	640	40	≥40	1,280	1,280‡
4	320	40	20	1,280	640	2,560	320
L (640)							
1	640	160	≤160	<10	NR	1,280	NR
2	1,280	160	80	<10	2,560	2,560	1,280
3	2,560	160	≤160	<10	1,280	5,120	1,280
4	1,280	160	80	<10	320	2,560	640
E (2,560)							
1	640	160	160	≤20	1,280	2,560	640
2	640	80	80	<10	1,280	1,280	640
3	1,280	160	80	<10	1,280	2,560	640
4	5,120	160	160	<10	640	1,280	320
K (320)							
1	320	40	20	640	320	2,560	320
2	1,280	40	10	<10	640	1,280	1,280
3	1,280	40	20	<10	1,280	1,280	320
4	1,280‡
M (NR)							
1	320	80	40	<10	640	2,560	640
2	2,560	80	80	≤20	640	NR	640
3	320	40	20	20	640	640	160
4	≥160	20	20	1,280	640	2,560	320
I (640)							
1	320	NR‡
2	1,280	160	80	20–40	640	NR	640
3	1,280	80	80	<10	640	2,560	640
4	160	80	80	<10	640	2,560	640
G (160)							
1	160	40	40	120	1,280	2,560	320
2	640	40	40	10,240	1,280	2,560	1,280
3	320	20	20	10,240	1,280	2,560	320
4	640	40	40	640	640	NR	320
J (1,280)							
1	640	80	40	1,280	640	2,560	640
2	1,280	320	80	640	320	640	640
3	320	80	20	320	640	≥2,560	320
4	1,280	80	40	320	1,280	2,560	1,280

* Value in parentheses is the bitch's prewhelping HI titer.

† NR = no result.

‡ Died.

Table 2. ELISA scores presented in S units of serum CPV antibody in pups evaluated over a period of 18 weeks.

Litter*/ Pup id.	Age (wk)						
	0	4	6	9	12	16	18
N (5)							
1	4	3	0	6	6	6	6
2	5	0	0	6	6	6	6
3	5	3	0	6	6	6	6
4	5	1	1	6	6	6	6
S (6)							
1	6	4	4	0	6	6	6
2	6	3	1	1	6	6	6
3	5	4	5	1	6	6	6
4	6	5	4	1	6	6	6
H (5)							
1	6	4	1	6	6	6	6
2	4	0	0	6	6	6	6
3	6	0	0	6	6†
4	5	4	1	6	6	6	6
L (6)							
1	6	4	4	1	6	6	6
2	NR‡	4	3	4	6	6	6
3	5	5	3	3	6	6	6
4	6	3	3	0	6	6	6
E (6)							
1	5	4	3	0	6	6	6
2	5	4	3	0	6	6	6
3	6	3	3	1	6	6	6
4	6	4	2	1	6	6	6
K (6)							
1	5	1	0	6	6	6	6
2	6	1	1	1	6	6	6
3	6	1	0	1	6	6	6
4	6†
M (NR)							
1	6	4	2	0	6	6	6
2	5	4	2	1	6	6	6
3	5	4	1	1	6	6	6
4	5	1	1	6	6	6	6
I (3)							
1	5	1†
2	6	2	0	0	6	6	6
3	6	2	0	2	6	6	6
4	6	2	1	4	6	6	6
G (6)							
1	5	0	1	6	6	6	6
2	5	1	1	6	6	6	6
3	5	0	1	6	6	6	6
4	5	2	1	6	6	6	6
J (6)							
1	5	3	2	6	6	6	6
2	5	2	3	6	6	6	6
3	6	4	3	6	6	6	6
4	6	3	1	6	6	6	6

* Value in parentheses is the bitch's prewhelping S unit score.

† Died.

‡ NR = no result.

measured with the minimum of laboratory equipment using a commercially available clinic-based ELISA on serum from both the bitch and the newborn pup.

The results of the ELISA optical density immunoscan and the HI titers were closely correlated. Taking into account the relative insensitivity of the HI test, where 2-4-fold titer variances are expected, the correlation coefficient should be regarded as evidence of a close correlation between the 2 techniques. The objective immunoscan titers and subjective immunoscale scores also were closely correlated. These findings indicate that the ELISA as scored by the immunoscale is a reliable method for the quantification of serum CPV antibodies in pups and adult dogs in the HI titer range of 1:40 to 1:640. However, it was not possible with the ELISA to accurately determine antibody titers in the HI range of 1:10 to 1:40 or 1:1,280 to 1:10,240. At those dilutions the color densities were similar. This limitation is not considered important in assessing immunity in pups because HI antibody titers from 1:10 to 1:40 are evidence of inadequate immunization, and most pups with these low titers can be successfully vaccinated with efficacious vaccines. However, titers in the high range indicate protective levels of immunity. This restriction therefore does not allow the method to be used for accurate calculation of maternal antibody half-lives or construction of a normogram for prediction of age of vaccination.

Interference with maternal antibodies is considered one of the most important factors in immunization failure.⁷ Current vaccination protocols are based on repeated vaccinations over a period of 6 to about 18 weeks of age, in an attempt to successfully vaccinate pups of unknown maternal antibody status.² The ELISA technique offers the opportunity of vaccinating pups and concurrently assessing immunization success or failure, therefore allowing a more reliable and cost efficient program of vaccination.

Ninety-five percent of pups could be successfully vaccinated with HI titers between 1:10 and 1:40. With the exception of 2 pups, a 1:80 maternal antibody titer was found to interfere with immunization. Similar results were demonstrated for the immunoscale and immunoscan results: 94% of pups were susceptible to vaccination at immunoscan titers of 0-30 and with immunoscale scores of 0 and 1.

Substantial increases in titers were clearly demonstrated in pups that responded to vaccination at 6 and 9 weeks. The increases in antibody levels were demonstrated by both the HI test and ELISA. This study revealed that after the second vaccination at 9 weeks of age, all pups were immunized, with serum antibody titers considered protective against the various strains of CPV. A rate of success for CPV strain 154 immu-

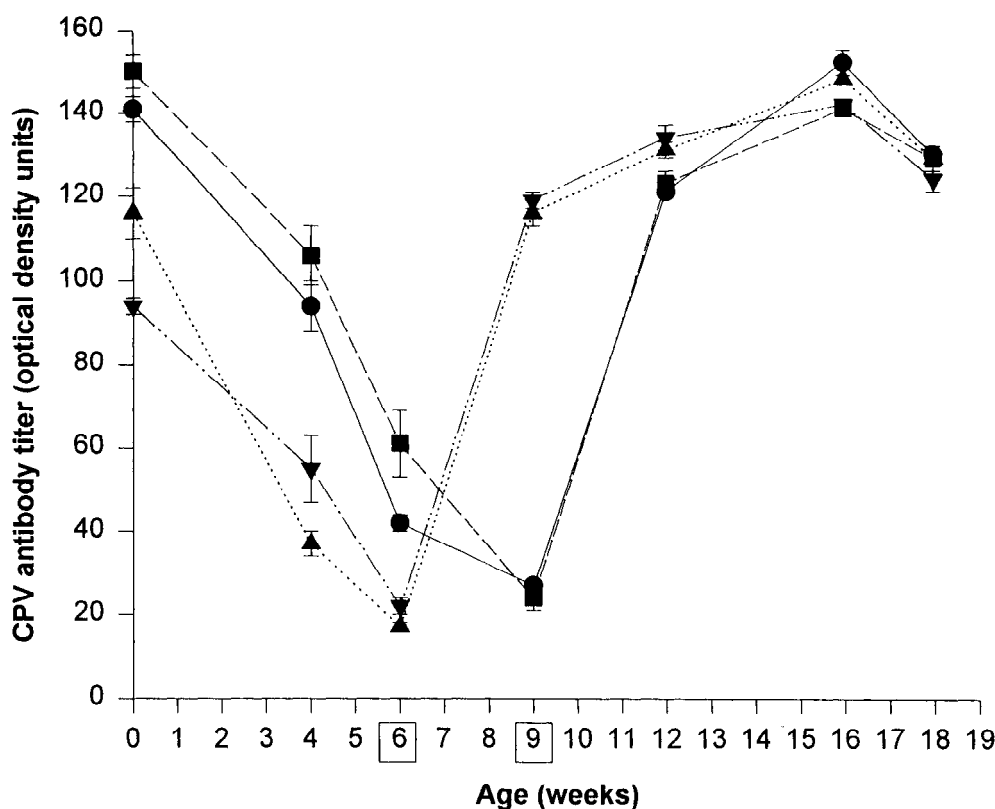


Figure 2. Response of 4 litters of pups to vaccination with Nobivac DP vaccine at 6 and 9 weeks of age. The mean CPV antibody titer expressed as the optical density ($\bar{x} \pm SE$) was measured by ELISA. Note the gradual decline in maternal antibody titer and the steep increase in CPV antibody titer following successful immunization. Two litters, G (.....) and N (- · - · -) were successfully immunized at 6 weeks, whereas litters E (—) and S (- - -) responded only after the vaccination at 9 weeks of age.

nization very similar to that found in this study has also been recently reported.⁵

The small increase in antibody titers in 6 pups after their first vaccination was unexpected and did not match the respective HI titers. The discrepancy may represent a low level of seroconversion by those pups that was not detectable by the HI technique.

There has been no commercially available method for the quantitative assay of CPV antibodies in dogs. The HI technique is available only in selected laboratories and the results can vary substantially among laboratories, although compared with other methods HI has been considered the most reliable.⁴ The new commercial ELISA is a simple and readily available method for the reliable measure of CPV antibodies in dogs.

Sources and manufacturers

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- Nobivac, Intervet, Boxmeer, The Netherlands.
- Intrac, Intervet, Boxmeer, The Netherlands.
- Novivac Lepto, Intervet, Boxmeer, The Netherlands.
- Novivac, Intervet, Boxmeer, The Netherlands.
- Immunocomb, Biogal, Kibbutz Gal'ed, Israel.

- American Type Culture Collection, Rockville, MD.
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- Biosynth International, Skokie, IL.
- Immunoscan, Orgenics, Yavneh, Israel.

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