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Vaccination of racing greyhounds: effects on humoral and cellular immunity

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Abstract

Greyhound kennel owners frequently employ multiple vaccination schedules in an attempt to reduce financial losses incurred as a result of infectious diseases. In order to determine the effects of multiple vaccination schedules on the immune system of racing greyhounds, three litters of greyhound pups raised in laboratory conditions were divided into two groups and subjected to either a maximum or a minimum vaccination schedule. Blood samples were collected biweekly for 6 months beginning at 2 weeks of age and analyzed to establish 'baseline' values for the lymphatic system of greyhounds. Lymphocyte transformation, total and differential leukocyte counts, and flow cytometry were used to evaluate cellular immunity. Humoral immunity was evaluated using serum neutralization and hemagglutination inhibition tests.

Proliferation of peripheral blood lymphocytes in response to the mitogen concanavalin A (Con A) was higher for the maximum vaccination groups. The frequency distribution of circulating CD4 and IgG labeled lymphocytes was higher in the minimum vaccination groups. A significant treatment by time interaction in CD4, IgG, and IgM labeled cells was observed. This interaction, however, was not significant at any point in time for CD4 and IgG labeled cells. The percentage of lymphocytes expressing surface IgM was significantly higher in the minimum vaccination groups at 10 and 14 weeks of age. No significant differences were detected in humoral immunity between the maximum and minimum groups of each litter. Results of this study indicate that maximum vaccination schedules do not appear to be more effective or more immunosuppressive than minimum vaccination schedules.

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1. Introduction

Viral pathogens, in particular canine parvovirus (CPV) and canine distemper virus (CDV), cause a significant financial loss to the greyhound industry. In order to circumvent these losses, kennel owners attempt to elicit an early and continuous immune response by administering multiple doses of vaccines. Simultaneous administration of CPV and CDV vaccines has been reported to decrease the number of circulating lymphocytes which may in fact produce a temporary state of immunosuppression (Kesel and Neil, 1983; Mastro et al., 1986; Phillips et al., 1989).

The host defense system is traditionally divided into two functionally overlapping categories: the humoral immune system and the cellular immune system (Schultz, 1982a). The humoral immune system consists of B-lymphocytes which produce immunoglobulins, helper T-lymphocytes, macrophages, killer cells, and effector molecules. This aspect of the immune system is evaluated through the use of antigen–antibody interactions, effector cell assays, and through the evaluation of complement-mediated activity. The cellular immune system consists of T-lymphocytes, macrophages, and cytokines. Lymphocyte proliferation, phenotyping, and delayed type hypersensitivity are commonly used to evaluate cellular immunity.

The correlation between antibody titer and protective effect after immunization with CPV and CDV vaccines indicates that humoral immunity is important in the host defense to these pathogens. The role of cellular immunity in particular to CPV vaccination has received less attention. Current vaccination protocols in the greyhound industry administer viral antigens more frequently and at a younger age. The response of pups less than 3 weeks of age to viral antigens is reported to be lower than that of adult dogs and their lymphocyte proliferation in response to mitogen stimulation reaches adult values between 6 weeks and 6 months of age (Banks, 1982). The following study was designed to determine the effect of extensive vaccination protocols on the developing immune system of racing greyhounds.

2. Materials and methods

2.1. Animals, vaccination, and blood collection

At weaning, 18 greyhound pups from three different dams and the same sire were randomly divided into two groups. The vaccination history of the dams was unknown except that one dam was vaccinated with a multivalent vaccine ($DA_2LP + Pv$) prior to breeding. Each group was housed in a separate pen and vaccinated with either a maximum or a minimum vaccination schedule (Table 1). The maximum vaccination schedule was modeled after current vaccination protocols being used in the racing greyhound industry. The minimum vaccination schedule more closely resembled recom-

Table 1
Vaccination schedules

Maximum vaccination schedule	
2 weeks	killed parvo
3 weeks	killed parvo, parainfluenza-bordetella
4 weeks	killed parvo, DA_2PL
5 weeks	modified live parvo, killed corona
6 weeks	killed parvo, DA_2PL
7 weeks	modified live parvo, parainfluenza-bordetella
8 weeks	DA_2PL
9 weeks	killed corona
10 weeks	modified live parvo
12 weeks	$DA_2LP + Pv$
16 weeks	$DA_2LP + Pv$
18 weeks	Rabies
24 weeks	$DA_2PP + CvK/LCI$, parainfluenza-bordetella
Minimum vaccination schedule	
5 weeks	killed parvo
9 weeks	modified live parvo, $DA_2PP + CvK/LCI$
13 weeks	$DA_2PP + CvK/LCI$
17 weeks	$DA_2PP + CvK/LCI$, rabies
22 weeks	$DA_2PP + CvK/LCI$, parainfluenza-bordetella

DA_2PL, distemper–adenovirus–parainfluenza vaccine–leptospira bacterin.
$DA_2LP + Pv$, distemper–adenovirus–parainfluenza–parvovirus vaccine–leptospira bactrin.
$DA_2PP + CvK/LCI$, distemper–adenovirus–parainfluenza–parvovirus–coronavirus vaccine–leptospira bactrin.

mendations of the American Veterinary Medical Association (AVMA Council on Biologic and Therapeutic Agents, 1989). All vaccines were administered subcutaneously with the exception of rabies (intra-muscular) and parainfluenza–bordetella (intra-nasal). Blood samples were collected from adults 2 weeks prior to whelping and from pups every 2 weeks beginning at 2 weeks of age and ending at 6 months of age. Blood samples were collected in untreated, preservative-free heparinized, and EDTA treated tubes.

2.2. Humoral immunity

CPV serum antibody titers were assayed biweekly using the hemagglutination inhibition (HAI) test as previously described (Carmichael et al., 1980). HAI titers were recorded as the reciprocal of the highest serum dilution showing complete inhibition of hemagglutination.

CDV serum antibody titers were assayed biweekly using the serum-neutralization (SN) test as previously described (Appel and Robson, 1973). The SN titer was recorded as the reciprocal of the highest serum dilution that neutralized viral cytopathic effect on Vero cells (ATCC CCL 81). Canine herpesvirus (CHV), canine coronavirus (CCV), and infectious canine hepatitis (ICH) virus serum antibody levels were assayed monthly

using a SN test on primary dog kidney, swine testicle (ST) (ATCC CCL 1746), and Mandin–Darby canine kidney (MDCK) (ATCC CCL 34) cells respectively.

2.3. Lymphocyte blastogenesis and hematology

Peripheral blood mononuclear cells (PBMC) were isolated using density gradient centrifugation (Fletcher et al., 1992). Heparinized blood was diluted 1:1 in 0.01 M phosphate-buffered saline (PBS) pH 7.0, layered onto Histopaque-1077 (Sigma), and centrifuged at $400 \times g$ for 30 min. The leukocyte layer was collected, washed twice in RPMI 1640 media (GIBCO Laboratories), and resuspended to a concentration of 1×10^5 cells ml^{-1} in RPMI 1640 containing 10% heat inactivated fetal bovine serum (FBS) (Hyclone Laboratories), L-glutamine (2 mM), penicillin (100 units ml^{-1}), streptomycin (0.1 mg ml^{-1}), kanamycin (5 μg ml^{-1}), and gentamicin (12.8 μg ml^{-1}).

Lymphocyte responsiveness to mitogen stimulation was assayed as previously described (Schultz, 1982b) with minor modifications. Briefly, PBMCs were dispensed in 0.2 ml amounts (20000 cells per well) into 96-well flat bottom tissue culture plates (Corning Glass Works) and subjected to the following experimental conditions: cells only; cells + 1.0 μg per well phytohemagglutinin-P (PHA) (Sigma L-9132); cells + 0.5 μg per well concanavalin A (Con A) (Sigma C-5275); cells + CPV (ATCC VR-953) at a multiplicity of infection (moi) of 64; cells + CDV (Onderstepoort strain, kindly donated by the James A. Baker Institute for Animal Health) at a moi of 0.004. All tests were conducted in replicates of four. Tritiated thymidine (^{3}HTdr) at a concentration of 0.2 μCi per well was added after 48 h incubation at 37°C in a 5% CO_2 incubator. Cells were harvested 18–24 h later using a PHD™ cell harvester (Cambridge Technology, Inc.) and counts per minute (cpm) were determined on a Beckman LS 1701 Scintillation Counter. The blastogenic response of lymphocytes was expressed as a stimulation index (*SI*) which was defined as the ratio of experimental to control cpm.

Leukocyte differential counts were performed on Wright-stained smears made from EDTA blood samples. Total white blood cell (WBC) and red blood cell (RBC) counts were determined on a Coulter Model S-Plus IV (Coulter Electronics, Inc., Hialeah, FL).

2.4. Flow cytometry

PBMCs were dispensed into 12 ml tubes at a concentration of 2×10^6 cells per tube and washed once with 0.01 M PBS pH 7.0. Cell suspensions were analyzed by one-color flow microfluorometry using monoclonal antibodies. Staining was conducted in either a direct or an indirect method with the following fluorescein isothiocyanate (FITC) conjugated antibodies: 0.5 μg anti-dog IgM FITC conjugated (Bethyl Laboratories, Inc.); 0.5 μg anti-dog IgG FITC conjugated (Bethyl Laboratories, Inc.); 2.5 μg mouse anti-feline CD4 FITC conjugated (Fisher Biotech); 1.25 μg biotinylated mouse anti-feline CD8 (Fisher Biotech) followed by 2.5 μg avidin–FITC (Boehring Mannheim Biochemicals); 0.02 μg H2OA (Class I major histocompatibility complex (MHC) antigen, VMRD) followed by 1.0 μg goat anti-mouse IgM_{mu}-FITC (Bethyl Laboratories, Inc.); 0.02 μg TH81A5 (Class II MHC, VMRD) followed by 0.5 μg goat anti-mouse $IgG_{(H+L)}$-FITC (Bethyl Laboratories, Inc). Our laboratory has demonstrated that anti-

feline CD4 and CD8 antibodies (Fisher Biotech) selectively bind mutually exclusive populations of canine peripheral blood lymphocytes (D.S. McVey, unpublished data, 1984) in proportions similar to canine specific CD4 (8.53/12.125) and CD8 (1.140/4.78) antibodies reported by Gebhard and Carter (1992). Antibodies were incubated at 4°C for 30 min. After staining, cells were washed twice with 2 ml of 0.01 M PBS pH 7.0, fixed with 50 μl of 1% paraformaldehyde, resuspended in 250 μl of sheath fluid and read at a wavelength of 488 nm on a Becton Dickinson FACScan™ flow cytometer equipped with a 5 W argon laser. Unstained, avidin–FITC stained, anti-mouse IgM-FITC stained, and anti-mouse IgG-FITC stained cells served as controls. Forward light scatter gates were set on the lymphocyte fraction to exclude dead cells and erythrocytes and 5000 lymphocytes were analyzed for each test. Filter systems were used to measure light emitted at 522 nm for detection of FITC. Analysis was performed using the LYSIS II Version 1.0 program. The percentage of positive cells was expressed as the percent positive staining for each cell marker minus the control.

2.5. Statistics

Analysis was conducted using a mixed model analysis of variance and the PROCGLM program of the Statistical Analysis System with significance at $P < 0.05$. Comparisons were made between treatments within litters and within treatments over time.

3. Results

3.1. Humoral immunity

The two dams that were not vaccinated pre-breeding had average antibody titers of 1:40 for CPV and 1:32 for CDV. The dam that was vaccinated pre-breeding had CPV and CDV antibody titers of 1:160 and 1:16 respectively. Maternally derived antibody levels to CPV were non-protective (HAI < 1:80) (Pollock and Carmichael, 1982) in pups as early as 2 weeks of age and in all pups by 4 weeks of age (Table 2). Average maternally derived antibody levels to CDV were less than 1:4 by 6–8 weeks of age (Table 3).

Although antibody titers varied over time, no significant difference was detected between the maximum and minimum vaccination groups in the antibody response to CPV and CDV vaccination. Average CPV antibody titers were near protective values (HAI 1:40–1:80) by 8–10 weeks of age. Antibody titers subsequently declined in two of the litters at 12–16 weeks of age then increased to protective levels in all litters by 6 months of age (Fig. 1). Average CDV antibody titers were over 1:16 by 14–16 weeks of age (Table 3). Maximum CDV antibody titers were observed at 18–22 weeks of age (Fig. 2).

3.2. Cellular immunity

Lymphocyte proliferation in response to Con A stimulation was generally higher ($SI = 15.09–20.39$) than response to PHA ($SI = 7.50–10.07$) stimulation (Table 4).

Table 2
CPV antibody titers (weekly means)

Age (weeks)	Maximum pups			Minimum pups		
	Litter 1	Litter 2	Litter 3 [a]	Litter 1	Litter 2	Litter 3 [a]
2	< 10(1)	< 10(1)	ND	< 10(1)	< 10(1)	ND
4	< 10(1)	30(1)	67(3)	< 10(1)	< 10(1)	40(3)
6	26(4)	23(3)	37(3)	15(3)	20(2)	33(3)
8	43(4)	53(3)	60(3)	32(3)	28(2)	37(3)
10	65(4)	15(3)	67(3)	40(3)	30(2)	40(3)
12	75(4)	10(3)	80(3)	50(3)	< 10(2)	53(3)
14	23(4)	12(3)	67(3)	45(3)	15(2)	67(3)
16	10(4)	12(3)	93(3)	15(3)	10(2)	93(3)
18	23(4)	25(3)	133(3)	27(3)	30(2)	133(3)
20	30(4)	38(3)	107(3)	32(3)	38(2)	133(3)
22	40(4)	20(3)	53(3)	20(3)	13(2)	80(3)
24	63(4)	73(3)	100(3)	90(3)	80(2)	120(3)
26	125(4)	73(3)	160(3)	ND	ND	ND

[a] Litter from dam vaccinated immediately prior to breeding.
Numbers in parentheses are the numbers of pups tested. Litter 1, $n = 7$; Litter 2, $n = 5$; Litter 3, $n = 6$. ND, test not done.

Table 3
CDV antibody titers (weekly means)

Age (weeks)	Maximum pups			Minimum pups		
	Litter 1	Litter 2	Litter 3 [a]	Litter 1	Litter 2	Litter 3 [a]
2	16(1)	4(1)	ND	16(1)	4(1)	ND
4	4(1)	2(1)	2(2)	16(1)	2(1)	2(2)
6	5(4)	2(3)	3(3)	6(3)	3(2)	2(3)
8	3(4)	5(3)	< 2(2)	3(3)	< 2(2)	2(3)
10	< 2(4)	64(3)	< 2(3)	< 2(3)	< 2(2)	2(3)
12	< 2(4)	48(3)	75(3)	11(3)	18(2)	64(3)
14	22(4)	64(3)	85(3)	11(3)	48(2)	85(3)
16	65(4)	37(3)	107(3)	53(3)	24(2)	107(3)
18	136(4)	37(3)	64(3)	107(3)	96(2)	85(3)
20	98(4)	48(3)	75(3)	139(3)	128(2)	75(3)
22	106(4)	85(3)	32(3)	213(3)	256(2)	75(3)
24	105(4)	53(3)	48(3)	149(3)	128(2)	85(3)
26	58(4)	75(3)	35(3)	ND	ND	ND

[a] Litter from dam vaccinated immediately prior to breeding.
Numbers in parentheses are number of pups tested. Litter 1, $n = 7$; Litter 2, $n = 5$; Litter 3, $n = 6$. ND, test not done.

Table 4
Average weekly means of maximum vs. minimum vaccination groups

Treatment	PHA	ConA	CPV	CDV	CD4	CD8	IgG	IgM	Class I	Class II
Max.	10.1	20.4 *	4.0	4.2	16.8	33.9	18.3	19.1	9.0	7.9
Min.	7.5	15.1 *	3.4	3.6	19.7	38.4	22.2	23.1	10.0	7.3

* Significant difference ($P = 0.04$) existed between the maximum and minimum vaccination groups.

Table 5
Significant differences between maximum and minimum vaccination groups

Variable	Effect	*P*-value
ConA	Treatment	0.04
IgM	Week × Tretment	0.01
	10 weeks	0.02
	14 weeks	0.01
IgG	Treatment	0.04
	Week × Treatment	0.02
CD4	Week × Treatment	0.03
	Treatment	0.02

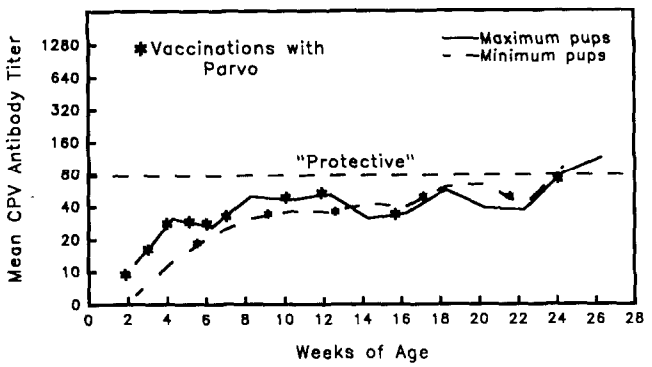

Fig. 1. Average CPV antibody titers assayed using the HAI test. No significant difference existed between the maximum and minimum vaccination groups.

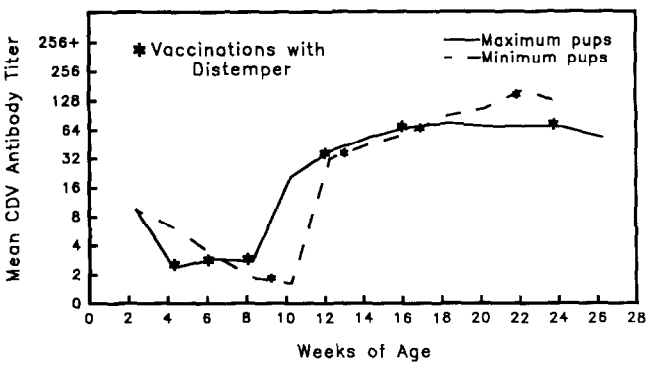

Fig. 2. Average CDV antibody titers assayed using the SN test. No significant difference existed between the maximum and minimum vaccination groups.

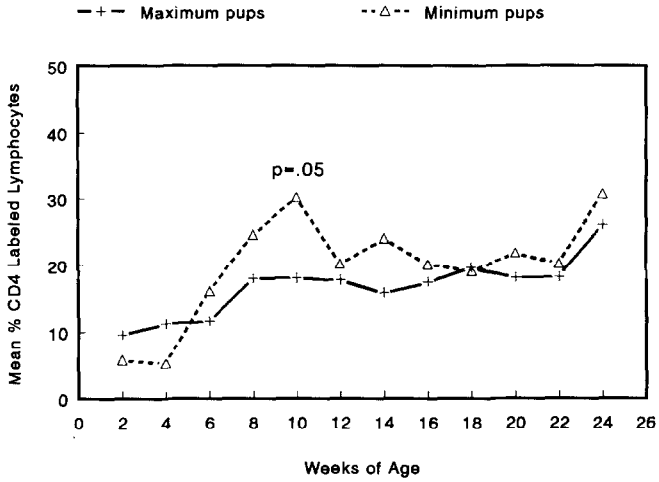

Fig. 3. Average frequency distribution of CD4 labeled peripheral blood lymphocytes as determined by flow cytometry. A significant treatment ($P = 0.02$) and treatment by time interaction ($P = 0.03$) existed. This interaction, however, was not significant at any specific time interval.

Proliferation in response to antigens CPV ($SI = 3.4–4.0$) and CDV ($SI = 3.6–4.2$) was lower than response to mitogen stimulation (Table 4). There was no significant difference between the two vaccination groups in response to PHA, CPV, and CDV. A significant difference was detected ($P = 0.04$) in response to Con A stimulation with the

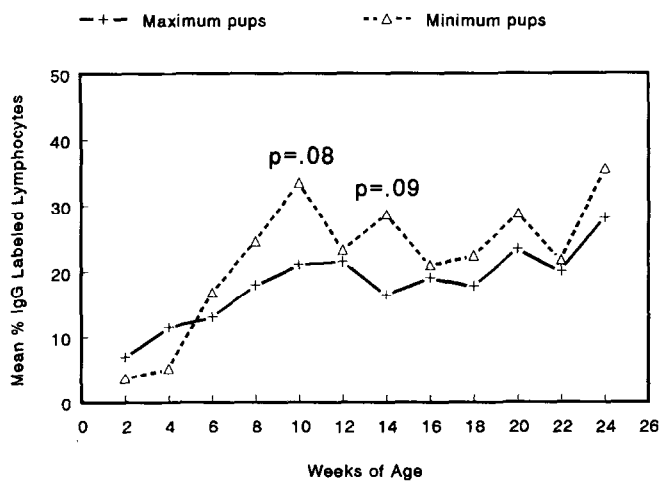

Fig. 4. Average frequency distribution of peripheral blood lymphocytes expressing surface IgG as determined by flow cytometry. A significant treatment ($P = 0.04$) and treatment by time interaction (0.03) existed. This interaction, however, was not significant at any specific time interval.

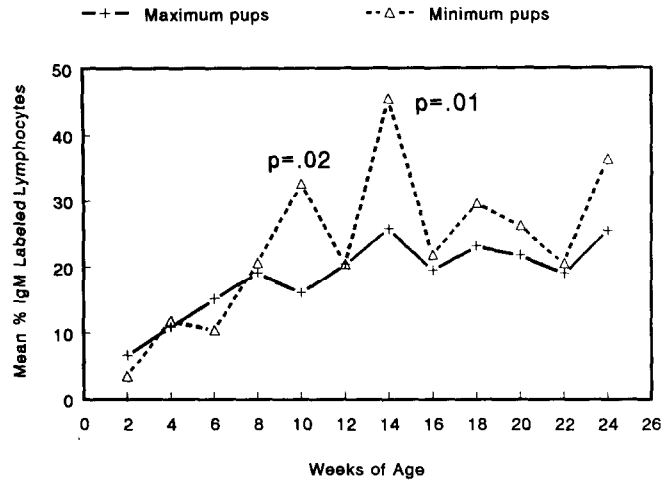

Fig. 5. Average frequency distribution of peripheral blood lymphocytes expressing surface IgM as determined by flow cytometry. A significant treatment by time interaction ($P = 0.01$) existed and at 10 and 14 weeks of age the minimum vaccination groups had a significantly higher percentage of IgM expressing lymphocytes than the maximum vaccination groups ($P = 0.02$ and $P = 0.01$).

average response over time being higher for the maximum vaccination groups ($SI = 20.39$) than the minimum vaccination groups ($SI = 15.09$) (Tables 4 and 5).

No significant differences were detected in the expression of class I and class II antigens of the major histocompatibility complex or in the percentage of CD8 labeled cells between the two vaccination groups (Table 4). A significant treatment effect was detected for CD4 ($P = 0.02$) and IgG ($P = 0.04$) labeled cells (Table 5). In addition, a treatment by time interaction was observed for CD4 ($P = 0.03$) and IgG ($P = 0.03$) labeled cells with the maximum vaccination groups being higher at 2 and 4 weeks of age followed by the minimum vaccination groups being equal or higher from 5 weeks of age on (Figs. 3 and 4, Table 5). At no specific point in time, however, was this interaction significant. A treatment by time interaction was observed for IgM labeled cells ($P = 0.01$) with the maximum vaccination groups having a higher percentage of positive cells at 2 weeks of age followed by the minimum vaccination groups being equal or higher from 8 weeks of age on. At 10 and 14 weeks of age the minimum vaccination groups had a significantly higher percentage of lymphocytes expressing surface IgM than the maximum vaccination groups (P-values 0.02 and 0.01 respectively) (Fig. 5 and Table 5).

4. Discussion

Data presented here indicate that no significant difference occurred in the humoral immune response between racing greyhounds subjected to multiple vs. minimal vaccination schedules. Antibody levels to CPV and CDV did not increase at an earlier time period in greyhounds vaccinated at 2 weeks of age compared with those vaccinated at 5

weeks of age. In addition, antibody levels did not reach higher levels in greyhounds vaccinated with multiple vaccination schedules (Figs. 1 and 2). This suggests that there was no apparent increased protection against disease in greyhounds that were vaccinated both at an earlier age and more frequently.

Maternal antibody levels to canine parvovirus were non-protective at the time of whelping in two of our adult greyhounds even though they were obtained from track or kennel environments where exposure to natural CPV was likely. Our third female was vaccinated pre-breeding but only achieved a CPV antibody titer of 1:160 at the time of whelping. The reason for these poor antibody levels in our adult greyhounds is unknown but could be attributed to environmental stresses.

Average antibody titers to CPV vaccination were poor in both experimental groups with all puppies not achieving protective antibody titers until 22 weeks of age. Although the reason for this poor antibody response to CPV is unknown, it should be noted that both schedules initially used killed parvo vaccines. A recent report (Blythe et al., 1994) advocating the use of killed parvo vaccines until 12–13 weeks of age in kennel situations states that modified live parvo vaccines can lower the immune status of pups for up to 3 days. Recent work in this laboratory investigating the use of modified live parvo vaccines in greyhound pups beginning at 6 weeks of age and then vaccinating every 3 weeks have recorded higher CPV antibody titers than when killed vaccines are used (unpublished data). Modified live vaccines typically give a better immune response than killed vaccines by virtue of their ability to undergo limited replication in the host which stimulates both cell-mediated and antibody-mediated immunity (Ellis, 1988). Since killed vaccines do not replicate in the host they are inherently less efficient inducers of the cell-mediated immune system. It is possible that the killed parvo vaccine administration to these young greyhounds resulted in enough seroconversion to inhibit the ability of the modified live vaccine to replicate. Consequently, lower antibody titers to CPV resulted. A decline in CPV antibody titers was observed in two litters at 12–16 weeks of age. A similar decline in CDV antibody titers post-vaccination has been reported in greyhounds (Webster, 1977).

CPV and CDV vaccinations have been reported to be immunosuppressive in that they cause a decrease in the number of circulating lymphocytes and their ability to respond to mitogens (Povey, 1986; Mastro et al., 1986; Krakowka et al., 1987; Phillips et al., 1989). Another study, however, found no evidence of immunosuppression as a result of CPV vaccination (Phillips and Schultz, 1987). A recent study (Miyamoto et al., 1992) reported a decrease in leukocyte and lymphocyte counts on day 7 post vaccination in both puppies and adult dogs. This study also demonstrated an increase in the blastogenic response of lymphocytes after vaccination in puppies and no change in adult dogs. Consequently, suppression as indicated by lymphopenia or decreased response to mitogen stimulation may be dependent upon sampling time post-vaccination. Our investigations were conducted in order to examine the difference in the immune response to traditional vs. aggressive vaccination protocols and to determine if aggressive vaccination is beneficial in terms of immune function.

Throughout the study, leukocyte and lymphocyte counts remained within normal reference ranges (data not shown) and the ability of peripheral blood lymphocytes to respond normally to mitogens was not diminished as a result of multiple vaccine

administration. The maximum vaccination group exhibited a greater response to stimulation by Con A than did the minimum vaccination group. This effect could be due to the more frequent stimulation of the immune system by the maximum vaccination schedule resulting in the proliferation of immature T-lymphocyte subpopulations. Or, as Miyamoto et al. (1992) suggested, vaccination may act in an immunomodulatory fashion in puppies resulting in an increased blastogenic response. The maximum vaccination group did not exhibit a greater response than the minimum vaccination group to stimulation by PHA. Con A has been shown to be a strict T-cell mitogen for the dog. PHA is capable of causing both T-lymphocyte and B-lymphocyte differentiation (Krakowka and Ringler, 1986). If immature T-lymphocytes comprise a greater percentage of the peripheral blood population in the maximum vaccination group, these immature T-lymphocytes would be more responsive to Con A than to PHA.

While lymphocyte phenotyping has been performed for many years in humans and mice, relatively few reagents exist for phenotyping in other animal species, in particular the dog. Owing to a lack of commercially available specific canine reagents, cross-reactive anti-feline CD4 and CD8 markers were used. These antibodies bind to mutually exclusive populations of canine peripheral blood lymphocytes in proportions similar to monoclonals described by Gebhard and Carter (1992) and are co-expressed on approximately 50% of thymocytes from Beagle pups. This is in agreement with the proportion of double positive lymphocyte expression in the thymus of all species where monoclonals have been defined (Tompkins et al., 1990). Anti-feline CD4 antibody additionally binds to canine peripheral blood granulocytes and immunoprecipitates reduced peptides in the range of 32–37 kDa. This is comparable to that reported for canine specific CD4 antibody (Gebhard and Carter, 1992). Reported normal values of these lymphocytes subpopulations for adult dogs and cats are similar with 39% CD4 + and 18% CD8 + lymphocytes in the dog and 34% CD4 + and 19% CD8 + lymphocytes in the cat (Dean, et al., 1991; Gebhard and Carter, 1992). Age, antigenic load, and environmental conditions, however, may greatly influence lymphocyte subpopulations (Joling et al., 1994).

Multiple vaccine administration did not significantly alter the frequency distribution of CD4 and IgG labeled lymphocytes. A significant treatment effect and a treatment by time interaction existed for CD4 labeled lymphocytes and for lymphocytes expressing surface IgG. The average weekly means for the minimum vaccination groups were higher than the maximum vaccination groups from 5 weeks of age until the completion of the study (Figs. 3 and 4). This difference, however, was not significant at any one point in time when the least squares means were compared. Consequently, the difference in treatment appears to depend on the week or on the sampling time post vaccination and not on the specific treatment.

The significant treatment by time interaction observed in IgM expressing cells indicated that the minimum vaccination groups had a significantly higher percentage of peripheral blood lymphocytes expressing surface IgM at 10 and 14 weeks of age compared to the maximum vaccination groups (Fig 5). This could be the result of different sampling times post vaccination in the two treatment groups. The minimum vaccination groups received a modified live vaccine 1 week prior to both of these sampling times. The maximum vaccination groups received a killed vaccine at 9 weeks

of age and a modified live vaccine at 12 weeks of age. The higher percentage of IgM expressing lymphocytes may be a reflection of seroconversion in the minimum vaccination groups. A similar proliferation could have occurred in the maximum vaccination groups after the 12 week modified live vaccination but due to a difference in sampling time post vaccination this response was not detected. In addition, no overall significant treatment effect was detected for IgM expressing lymphocytes. Consequently, this does not likely represent a deficiency in the immune response of the maximum vaccination groups.

The administration of extensive vaccination schedules as described here is a common practice in the racing greyhound industry. These extensive vaccination protocols are used in an attempt to provide early and complete protection against the common viral pathogens and to prevent extensive financial losses due to disease outbreaks. The cost of supporting the extensive vaccination protocols in the greyhound industry in one county alone in Kansas is estimated to be \$50 000–75 000 $year^{-1}$. Experimental evidence presented in this report indicates that the use of multiple vaccination protocols in racing greyhounds raised in laboratory conditions does not increase the ability of their humoral immune system to respond to viral antigens. Although this practice does not appear to be detrimental to the immune system, the cost vs. benefit of implementing such extensive vaccination schedules is questionable. It is important to note, however, that most racing greyhounds in kennel situations are exposed to environmental conditions that were not present in the laboratory experiments described here. Therefore, further evaluation of the influence of multiple vaccination schedules on the immune system of racing greyhounds raised in kennel situations and exposed to environmental stress is currently being conducted in this laboratory.

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References

Appel, M.J.G. and Robson, D.S., 1973. A microneutralization test for canine distemper virus. Am. J. Vet. Res., 34: 1459–1463.

AVMA Council on Biologic and Therapeutic Agents, 1989. Canine and feline immunization guidelines. J. Am. Vet. Med. Assoc., 195(3): 314–317.

Banks, K.L., 1982. Host defense in the newborn animal. J. Am. Vet. Med. Assoc., 181(10): 1053–1056.

Blythe, L.L., Gannon, J.R. and Craig, A.M., 1994. The immune system, infectious diseases, and vaccination programs. In: L.L. Blythe, J.R. Gannon and A.M. Craig (Eds.), Care of the Racing Greyhound: A Guide for Trainers, Breeders and Veterinarians. Veterinary Practice Publ. Co., Santa Barbara, CA, Chap. 6, pp. 85–86.

Carmichael, L.E., Joubert, J.C. and Pollock, R.V.H., 1980. Hemagglutination by canine parvovirus: serologic studies and diagnostic applications. Am. J. Vet. Res., 41(5): 784–791.

Dean, G.A., Quackenbush, S.L., Ackley, C.D., Cooper, M.D. and Hoover, E.A., 1991. Flow cytometric analysis of T-lymphocyte subsets in cats. Vet. Immunol. Immunopathol., 28: 327–335.

Ellis, R.W., 1988. New technologies for making vaccines. In: S.A. Plotkin and E.A. Mortimer, Jr. (Editors), Vaccines. W.B. Saunders, Philadelphia, PA, pp. 568–570.

Fletcher, M.A., Klimas, N., Morgan, R. and Gjerset, G., 1992. Lymphocyte proliferation. In: N.R. Rose, E.C. de Macario, J.L. Fahey, H. Friedman and G.M. Penn (Editors), Manual of Clinical Laboratory Immunology, 4th edn. American Society for Microbiology, Washington DC, p. 218.

Gebhard, D.H. and Carter, P.B., 1992. Identification of canine T-lymphocyte subsets with monoclonal antibodies. Vet. Immunol. Immunopathol., 33: 187–199.

Joling, P., Bianchi, A.T.J., Kappe, A.L. and Zwart, R.J., 1994. Distribution of lymphocyte subpopulations in thymus, spleen, and peripheral blood of specific pathogen free pigs from 1 to 40 weeks of age. Vet. Immunol. Immunopathol., 40: 105–117.

Kesel, M.L. and Neil, D.H., 1983. Combined MLV canine parvovirus vaccine: immunosuppression with infective shedding. Vet. Med. Small Anim. Clin., 78: 687–691.

Krakowka, S. and Ringler, S.S., 1986. Activation specificity of commonly employed mitogens for canine B- and T- lymphocytes. Vet. Immunol. Immunopathol., 11: 281–289.

Krakowka, S., Ringler, S.S., Lewis, M., Olsen, R.G. and Axthelm, M.K., 1987. Immunosuppression by canine distemper virus: Modulation of in vitro immunoglobulin synthesis, interleukin release and prostaglandin E_2 production. Vet. Immunol. Immunopathol., 15: 181–201.

Mastro, J.M., Axthelm, M., Mathes, L.E., Krakowka, S., Ladiges, W. and Olsen, R.G., 1986. Repeated suppression of lymphocyte blastogenesis following vaccinations of CPV-immune dogs with modified-live CPV vaccines. Vet. Microbiol., 12: 201–211.

Miyamoto, T., Taura, Y., Une, S., Yoshitake, M., Nakama, S. and Watanabe, S., 1992. Changes in blastogenic responses of lymphocytes and delayed type hypersensitivity responses after vaccination in dogs. J. Vet. Med. Sci., 54(5): 945–950.

Phillips, T.R. and Schultz, R.D., 1987. Failure of vaccine or virulent strains of canine parvovirus to induce immunosuppressive effects on the immune system of the dog. Viral Immunol., 1(2): 135–144.

Phillips, T.R., Jensen, J.L., Rubino, M.J., Yang, W.C. and Schultz, R.D., 1989. Effects of vaccines on the canine immune system. Can. J. Vet. Res., 53: 154–160.

Pollock, R.V.H. and Carmichael, L.E., 1982. Maternally derived immunity to canine parvovirus infection: transfer, decline, and interference with vaccination. J. Am. Vet. Med. Assoc., 180(1): 37–42.

Povey, R.C., 1986. Distemper vaccination of dogs: factors which could cause vaccine failure. Can. Vet. J., 27: 321–323.

Schultz, R.D., 1982a. Theoretical and practical aspects of an immunization program for dogs and cats. J. Am. Vet. Med. Assoc., 181(10): 1142–1149.

Schultz, R.D., 1982b. Assays of cellular immunity. J. Am. Vet. Med. Assoc., 181(10): 1169–1176.

Tompkins, M.B., Gebhard, D.H., Bingham, H.R., Hamilton, M.J., Davis, W.C. and Tompkins, W.A.F., 1990. Characterization of monoclonal antibodies to feline T lymphocytes and their use in the analysis of lymphocyte tissue distribution in the cat. Vet. Immunol. Immunopathol., 26: 305–317.

Webster, A.C., 1977. Distemper vaccination response in greyhounds: breed variation. Aust. Vet. Pract., 7(3–4): 196–200.