Canine nomograph evaluation improves puppy immunization

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Abstract

Immunization failure in puppies by modified live vaccines for canine distemper virus and canine parvovirus can occur due to interference from maternally derived antibody. Quantitative measurement of specific antibody (via half-life degradation analysis; nomograph) is available to determine passive transfer from dam. Nomograph enables vaccination timing and followup titer testing tailored for each litter. Objective was to evaluate effectiveness of this approach and use of a nomograph. Puppies (506 puppies < 1 year) that had nomograph completed for their dam were not different in protection rate compared to vaccinated adults and were proven immune at 15.9 weeks. A cohort of similar puppies at 21.6 weeks that did not have nomograph completed for their dam was more likely to have not responded to vaccination.

Keywords: Canine, nomograph, titer, vaccination, distemper, parvovirus

Introduction

Maternally derived antibody (MDA) interference with vaccination is considered as main cause for "failure to immunize" in puppies < 6 months old.[1-4] Determining breeding dam antibody titers and applying half life degradation analysis to litter (nomograph) improved timing for canine distemper virus (CDV) vaccination.[5] This concept was not widely adopted, since very few diagnostic laboratories offered canine vaccinal antibody testing. Beginning in late 1990's, an alarming increase in adverse reactions to vaccines, most notably feline vaccine associated sarcoma,[6] triggered veterinary profession to reevaluate annual vaccinations, standard practice at that time, for pet dogs and cats. American Association of Feline Practitioners and later American Animal Hospital Association developed vaccination guidelines for cats and dogs, respectively.[7,8] World Small Animal Veterinary Association (WSAVA) also published similar vaccination guidelines.[9] These guidelines described vaccines in 3 categories (core, noncore or not recommended). Core vaccines are able to provide complete protection against widespread diseases that induce high morbidity and mortality. For canine, these vaccines are CDV, canine parvovirus (CPV2), canine adenovirus (CAV1, CAV2), and rabies. Guidelines urged that all dogs receive CDV, CAV1, CAV2 and CPV2 vaccines to provide protection from infection for as long as 9 years.[10-15] As a result, early guidelines suggested administration of core vaccines to adult dogs at not more than 3 year intervals.

Sterile immunity is defined as stage of humoral immune response that "sterilizes" a specific virus, thus totally preventing infection. This applies both to vaccine and disease causing wildtype viruses. Modified live viral vaccines ability to infect and replicate are blocked in face of high antibody titers. This blockage happens whether antibody is produced actively by dog or passively acquired in puppy. Modified live viral vaccine neutralized by antibody provides no benefit to puppy or adult; actively immune dog usually has no increase in titer, whereas a puppy remains immunologically naïve.[4]

Currently, canine vaccinal antibody testing is fast becoming part of standard veterinary care. Multiple laboratories across North America offer quantitative antibody testing to determine immunity against CDV and CPV2, and qualitative, point of care antibody screening tests are also available to clinicians. Improved availability of testing, coupled with an increased interest in appropriate vaccine use, contributed to growth in this area.[16-18]

Although vaccine adverse reactions are rare, this risk is not offset by benefit in actively immune dog with titers above sterile immunity thresholds. Most recent version of AAHA canine vaccine guidelines suggest antibody testing helps clinicians balance risks and benefits of vaccination.[8]

Puppies present a unique challenge regarding antibody testing, due to potential for residual maternally derived immunoglobulin and likelihood of immunization failure. Most recent WSAVA

vaccine guidelines recommend antibody testing of puppies at 6 months of age[9] since all initial levels of MDA will have dissipated. This is also the earliest age that qualitative point of care tests may be applied, according to manufacturer. Testing pups at 24 weeks is a significant improvement over past practice of simply assuming vaccinated pups are immune. Unfortunately, however, testing at 6 months of age still leaves a population of puppies at potential risk of disease, especially during this critical period.

Because a clinician usually does not know potential MDA levels, multiple doses of vaccines are given to puppies over many weeks in an effort to both deliver an effective dose as early in puppy's life as possible and to continue until such time as maternal antibody is believed to have dissipated. In some situations, litters may be vaccinated as frequently as every week, beginning as early as 5 weeks of age, potentially increasing chances of adverse events (e.g. immunosuppression or allergic reaction).

Although a definite link between puppy core vaccination and hypertrophic osteodystrophy was not established,[19] producers of dog breeds at highest risk for hypertrophic osteodystrophy (Weimaraners, Irish Setters, etc.) were among first to request nomograph service through our laboratory. Although initial impetus was to avoid "shot gun" approach of administering multiple doses of vaccine over many weeks, it was soon realized that nomographs also allowed followup quantitative titer testing of puppies much younger than 24 weeks.

Based on our testing, average titers for breeding dams are approximately 1:640 for CPV2 and 1:64 for CDV. However, range of titers was quite large, from < 1:2 (negative) to 1:20,480. Depending on antibody amount transferred at birth, age that a litter had no maternal antibody ranged from day of birth to 22 weeks of age.

Because of possible failure of passive transfer, nomograph should not be used to predict protection against wildtype viruses. Breeders with known elevated risk of parvovirus in their kennels are urged to submit samples collected directly from selected pups at 3 - 4 weeks of age. This approach controls for failure of passive transfer and provides a direct measurement of maternal antibody for half life degradation analysis. Because it can be difficult and stressful to collect serum from small puppies, naïve puppy baseline titer testing is only suggested in the face of high disease risk.

Rather than an evaluation of protection for a litter, nomograph analysis of breeding dam antibody level is intended to be a conservative estimate of duration of maternal antibody interference with modified live viral vaccines. Reported percent transfer estimates of 60 - 70%[1] was confirmed by our laboratory (data not shown). However, because we had transfer rates up to 100% in some excellent colostrum producing dams, nomograph is calculated based on conservative assumption of 100% transfer from dam to litter.

Best timing to collect bitch sera for nomograph analysis is 2 weeks before expected whelping date or 2 weeks after whelping. Active colostrum production time is avoided, as circulating antibody titers are decreased due to sequestration of immunoglobulin G (IgG) in mammary gland. Ideally, nomograph should be completed for each litter in order to generate most accurate interpretation of puppy results followup testing. At minimum, a nomograph should be completed within 1 year before whelping.

Once dam's titer is known and set at 100% transfer to her litter at day 0, half life degradation analysis is applied. Rates of specific antibody decline for canine vary from 9 - 12 days.[1,5] Rate of 12 days was chosen to provide a more conservative estimate. Standard deviation inherent to assays is graphed by showing titers 1 dilution above and 1 below reported titer.

Variability of MDA titers between pups in a litter is within test variability parameters in majority of litters we tested for prevaccination baseline (complete data not shown). Litters tested include submissions from private breeders concerned about parvovirus in their kennels, as well as purpose bred Beagle litters for selection to vaccine research studies.

Vaccine administration is suggested when degradation analysis estimates indicate first successful immunization.[1,5] A second dose of vaccine is suggested when interfering MDA is very highly likely to be completely catabolized. These ages often vary between distemper and parvovirus; however, most often CPV2 titers are higher than those against CDV. In these litters, an additional dose of monovalent parvovirus vaccine is suggested at an older age. With high maternal titers against both CDV and CPV2, a dose of combination vaccine at 8 - 9 weeks is suggested as "optional". Although it is prudent to give a

dose of combination core vaccine to puppies before transfer to new homes, stakeholders will know that there is a good chance that this dose may not successfully immunize. Quantitative titer testing is suggested 2 weeks (completion of MDA degradation) after presumed final dose of vaccine.

Materials and methods

Serum samples submitted over a 3 year interval from across US and Canada to companion animal vaccine and immuno diagnostics Service (CAVIDS) laboratory were used. Samples were grouped based on whether nomograph had been completed for the dam or not. Protection rates for sera from these groups were compared against those of a group of sera from > 5,000 individual adult dogs with history of vaccination. For all groups, including adult dogs, only first sample submitted for an individual was included for analysis. Data (date of birth and vaccination history of commercial canine vaccinations) of dogs ≤ 1 year were used.

Nomograph followup group included 506 individual puppies, with an average age of 15.9 weeks (range 8.7 - 50.9). This group included 202 distinct litters from 188 dams, with 49 breeds represented. Golden Retriever made up 38.3% of this group, followed by the Labrador Retriever at 11.2% (Table 1).

Table 1. Top 5 breeds' percents in nomograph group

Number of pups	Top 5 breeds - Nomograph Group	Percent total
194	Golden Retriever	38.3
57	Labrador Retriever	11.2
24	Nova Scotia Duck Tolling Retriever	4.7
21	Rottweiler	4.1
21	Soft-Coated Wheaten Terrier	4.1

Group without nomograph included 235 individual puppies at average age of 21.6 weeks (range 7.7 - 49.4). This group was comprised of 90 breeds, including "mixed breed". Labrador Retriever was 18.3% of this group, followed by Golden Retriever at 13.2% (Table 2).

Table 2. Top 5 breeds' percents in without nomograph group

Number of pups	Top 5 breeds - Without nomograph Group	Percent Total
43	Labrador Retriever	18.3
31	Golden Retriever	13.2
24	Irish Setter	10.2
15	Mixed breed	6.4
12	Poodle, standard and toy	5.1

Serology methods included hemagglutination inhibition assay to determine antibody against CPV2 and serum virus neutralization assay to determine antibody against CDV. Both assays are considered "gold standard," directly test antibody function, and provide quantitative endpoint titers. Test sera are doubly diluted in duplicate across a 96 well plate and incubated with a standardized amount of infectious CDV or CPV2. After 1 hour, indicator cells are added to all wells. Plates are examined after further incubation. Endpoint titer is reported as the last dilution at which antibody is able to neutralize viral activity.[20,21] Threshold of protection determined by challenge of immunity.[10] Both HI and SVN assays detect IgG and IgM simultaneously and are well suited to test initial vaccine responses 2 - 3 weeks after presumed final dose of vaccine.

Data analyses

Chi square was used with significance of $p < 0.05$. Protection rates of both groups of pups were compared against protection rates of a general population of vaccinated adult dogs. Threshold of

protection for adult dogs was set at ≥1:8 (CDV) and ≥1:40 (CPV2). Using this threshold, expected rate of protection is 97% for CDV and 91% for CPV2. To provide a more stringent definition of protection for puppies, and to be able to compare groups, threshold of protection for puppies was set 2 fold higher for both viruses with ≥ 1:32 for (CDV) and ≥ 1:160 for CPV2.

Results

Protection rate for the nomograph group was 95.7% (484/506) against CDV (95% CI [95.5, 95.9]) and 90.5% (458/506) against CPV2 (95% CI [90.3, 90.7]). Average age of protected puppies was 15.9 weeks for both viruses. Compared to rates of protection in adult population, p values were 0.0755 (CDV) and 0.7024 (CPV2), indicating no significant differences.

Protection rates in the group without nomograph were lower at 85.5% (201/235) against CDV (95% CI [85.2, 85.8]) and 81.7% (192/235) against CPV2 (95% CI [81.4, 82]). Average age of protected puppies was 21.3 and 22.5 weeks of age for CDV and CPV2, respectively. Compared to expected values in adult dogs, p values were 0.0001 for both viruses, indicating a highly significant proportion of these pups were unprotected. (Figures 1, 2; Table 3, 4)
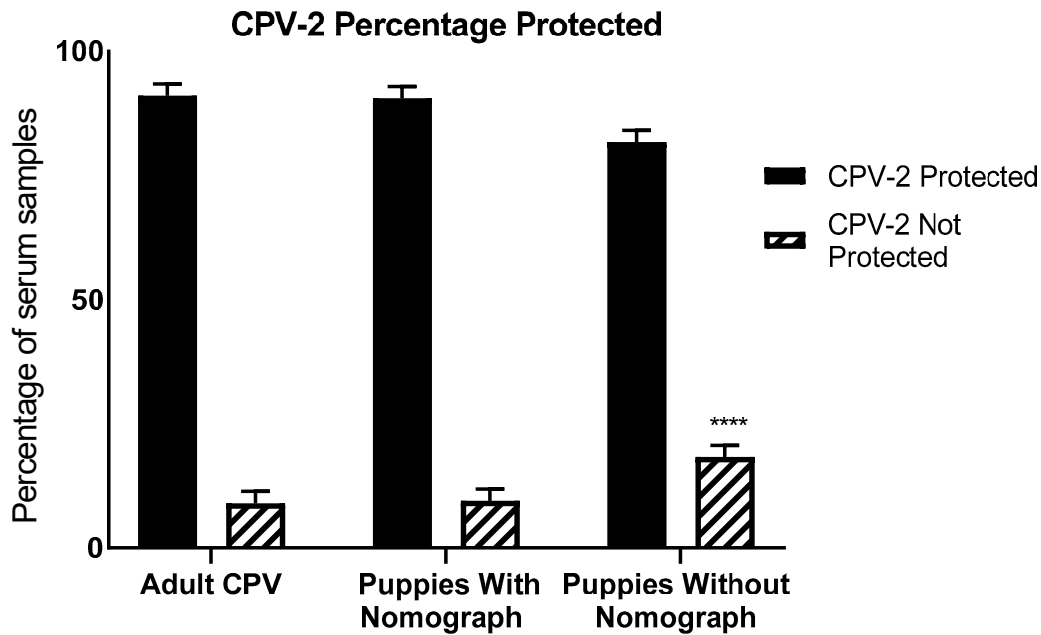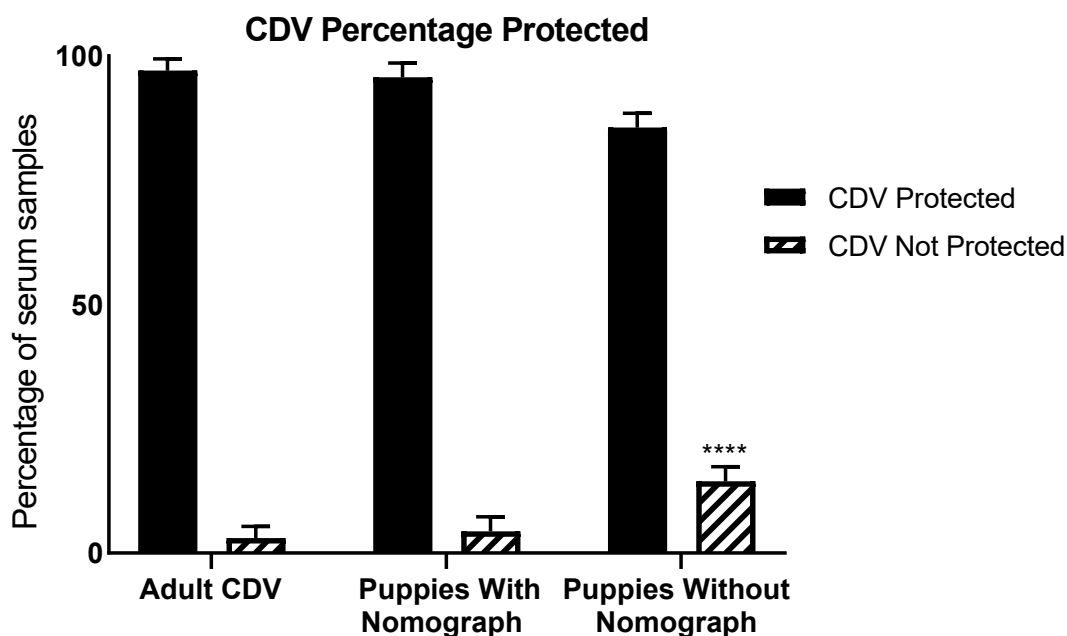

Figure 1

CDV Percentage Protected

Figure 2

Table 3. Percentage protected/not protected against CPV2 for groups of puppies

CPV-2 ≥160 Group	Percent protected (number)	Average age (weeks)	Percent not protected (number)	Average age (weeks)	P value compared to adults
Nomograph	90.5 (458/506)	15.9	9.5 (48/506)	15.7	0.7024
Without nomograph	81.7 (192/235)	22.5	18.3 (43/235)	17.8	0.0001**

Table 4. Percentage protected/not protected against CDV for groups of puppies

CDV ≥32 Group	Percent Protected (number)	Average age (weeks)	Percent not protected (number)	Average age (weeks)	P value compared to adults
Nomograph	95.6 (484/506)	15.9	4.3 (22/506)	14.3	0.0755
Without nomograph	85.5 (201/235)	21.3	14.5 (34/235)	23.5	0.0001**

Discussion

Maternally derived antibody is only 1 potential factor affecting vaccination failure. Other factors include poor vaccine handling and storage, incorrect administration and genetic issues. However, current study confirmed that MDA had a highly significant impact on vaccinal response in puppies.

Examining role of genetics as a risk factor for poor vaccine response was beyond the scope of this study; however, there were some noteworthy findings. Inclusion of 6.4% "mixed breed" puppies in group without nomograph did not seem to have a significant positive impact on protection rates for this group. Interestingly, in nomograph group, most puppies that failed to respond to vaccine were unique within their litters. In most cases, littermates of nomograph nonresponders responded well (data not shown). One litter of 10 Labrador Retrievers was the exception, with 7 pups that failed to respond to CPV2

vaccine. After a further dose of vaccine, 6 of 7 responded well. Seventh pup required 1 additional dose before eventually having an active response at 20 weeks. Mechanism of non-responsiveness in this litter is unknown, but the phenomenon emphasized importance of titer testing puppies to be certain of immunity. It is also interesting to note that this single litter (out of 202 litters tested) comprised 14.6% of 48 puppies that did not respond to parvovirus in the nomograph followup group.

Although nomograph report suggests tailored vaccination and follow-up testing schedules for each litter, veterinarians and puppy owners are free to interpret and implement nomograph data as they wish. In the current study, compliance was not determined precisely, but the nomograph group was generally in agreement with nomograph-generated schedules (data not shown). One notable exception was the single puppy in the nomograph group tested at 8.7 weeks of age. Although testing at this age was not in accordance with nomograph based suggestions for the litter, this sample was retained in the nomograph group data set. This puppy was negative for CPV2 antibody.

In another interesting finding, the youngest puppy in the group without a nomograph was protected against distemper, but not for parvovirus, at 7.7 weeks of age. This pup's distemper titer was very strong at 1:4,096, clearly an active response to vaccine. For this titer to be attributable to MDA, the dam would have had to have an extremely unlikely CDV antibody titer of 1:65,536 with a 100% transfer rate to her litter. In general, samples from very young pups are not submitted when dam's titers are not known. This is reflected in the smaller size and older average age of the group without nomographs. However, in instances where the puppy has had an adverse reaction to vaccination or other health issue that may complicate further vaccination, quantitative testing and "reverse" degradation analysis can be applied, along with a stringent threshold of protection.

Response to vaccination and protection rates for all groups, regardless of age or nomograph status, was much higher for CDV than for CPV2. Although mechanism responsible is not clearly understood, it possibly stems from the fact that parvovirus has more recently emerged into domestic canine population than distemper virus and has experienced relatively fewer generations of coevolution.

Current study confirmed that nomograph analysis of maternal antibody titers against CDV and CPV2 provides veterinarians and dog breeders with useful information to guide litter vaccination decisions and speed confirmation of immunity. In case of low maternal titers, much peace of mind can be gained by early proof of protection, especially for critical socialization experiences, e.g. puppy kindergarten. In the case of higher maternal titers, vaccination series will be extended, in some cases beyond standard final dose at 16 weeks of age. For all litters and stakeholders, overall outcomes are improved. Dog breeders can provide added value to their puppy buyers by providing information about dam's titers. Veterinarians can make better informed vaccination decisions. Most importantly, more families may be spared emotional and financial impacts of severe morbidity and mortality associated with CDV and CPV2 infections.

Future studies could examine factors that may influence nomograph, such as determining if active colostrum production induces change in circulating antibody titers for bitch; improving understanding of extent of influence of litter size on passive transfer rate; establishing if there are measurable differences in antibody degradation rate due to breed-specific body size, caloric restriction and/or metabolism; characterizing impacts of caesarian section on colostrum production and passive transfer; and determining effect of administration of supplemental antibody products (e.g. fresh frozen plasma) on passive antibody titers in recipient pups. Longer-term collection of titer data of puppies vaccinated according to nomograph as they enter young adulthood and beyond is currently underway.

Conclusion

Current study demonstrated that breeding dams nomograph testing provided important information to improve core immunization outcomes for puppies < 1 year and facilitated earlier followup titer testing for immunity. Regardless of whether nomograph was done for a dam, authors support WSAVA guidelines in urging that all puppies are tested for antibody titers by 6 months to detect failure of immunization during this highest risk period. Methods to detect unprotected puppies are readily available to veterinary practitioner, either through point of care screening tests at 24 weeks, or

quantitative testing earlier when maternal titers are known. Best medical practice should support applying this standard. Presumption that vaccination is equivalent to immunization may lead to unfortunate, but avoidable surprises.

Acknowledgement

Conflict of Interest

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