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Research Article

Circulating anti-Canine Parvovirus Antibody Titer Is Impacted by Colostrum Production in Breeding Bitches

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Abstract

This prospective study aimed to determine the impact of colostrogenesis on circulating serum titer against canine parvovirus (CPV-2) in the gravid bitch, and subsequent implications for timing of sample collection for nomograph analysis. CPV-2 is a deadly virus of global concern which mainly impacts susceptible puppies, inducing severe lymphopenia, gastroenteritis, and organ failure. Vaccinal blockade by maternally derived antibody is one of the main causes of modified-live CPV-2 vaccine "failure to immunize" in the puppy. Nomograph analysis intends to improve puppy immunization outcomes by providing a tailored vaccination schedule for a specific litter based on a conservative estimation of blockade length. To generate a nomograph, individual bitch antibody levels are determined and known half-life degradation is applied. The current study was undertaken to ensure optimal timing for serum sample collection to achieve the best diagnostic accuracy, and to prove our hypothesis that active transport and sequestration of immunoglobulin type G (IgG) specific for CPV-2 induces a temporary decline in circulating anti-CPV-2 antibody titer. Serum samples were collected from 56 pregnant beagle bitches at four timepoints: 4 weeks and 2 weeks pre-whelp, at whelp, and 2 weeks post-whelp. Sera were analyzed for specific antibody against CPV-2 by hemagglutination inhibition assay (HIA). Geometric mean titer values were statistically analyzed via repeated measures, one-way analysis of variance (ANOVA) test and Tukey's multiple comparisons *post hoc* correction, with p-value set at <0.05. Seven of the 56 bitches (12.5%) showed a significant decrease in circulating anti-parvovirus titer at whelp ($p<0.0001$). These results prove our hypothesis and indicate that serum for titer and nomograph analysis of breeding bitches should be collected outside of the colostrogenesis window for the greatest accuracy.

Keywords

Canine, Maternally Derived Antibody, Parvovirus, Nomograph, Colostrum

1. Introduction

Maternally derived antibody (MDA) is often considered a "double edged sword." For most mammalian species of veterinary concern, almost no antibody crosses the placental barrier between fetus and maternal circulation. Instead, immunoglobulin type G (IgG) is actively transferred from maternal circulation to the mammary gland where it is stored as colostrum. In the canine, this process is known to occur for approximately 2 weeks before whelping. For several hours after birth and suckling, neonatal mammals transfer antibody from colostrum across the gut lining mainly via active

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transcytosis mediated by receptors for the fragment crystallizable (Fc) portion of IgG [1-3]. Once these receptors are exhausted and loose junctions between enterocytes tighten, the neonatal gut is effectively closed [4, 5].

While passively transferred antibody is crucial to protect neonates from infectious diseases, MDA is also the main cause of vaccine failure through antibody blockade of modified live viral (MLV) vaccines, such as canine parvovirus (CPV-2). By applying the well-characterized MDA degradation rate to circulating bitch antibody titers, nomograph analysis has been shown to improve immunization outcomes for vaccinated puppies [6, 7]. While colostrogenesis in production animals has been thoroughly investigated, relatively fewer studies examine this process in the breeding bitch. This study aimed to determine if the process of colostrum production impacts circulating antibody specific for CPV-2, and consequently, breeding bitch nomograph analysis.

2. Methods

2.1. Animals

Ridglan Farms Institutional Animal Care and Use Committee (IACUC) approval was obtained before study was begun. Pregnant beagle bitches (n = 56) in a research breeding colony were bled for serology at timepoints 4 week and 2 weeks before expected whelp, day of whelp, and 2 weeks post whelp.

2.2. Serology

Serum samples were tested for specific antibody against CPV-2 by the hemagglutination inhibition assay (HIA) using the method developed by Carmichael [8]. Briefly, sera are doubly diluted in buffer containing bovine serum albumin (BSA) across a U-bottom 96 well plate. Sera dilutions are then incubated with 32 hemagglutinating (HA) units of infectious CPV-2 for 45-60 minutes at room temperature before addition of 1% porcine red blood cells (pRBC). A control plate containing dilutions of characterized positive and negative sera, viral back titration, and cell control is included. Plates are incubated at 4 ℃ overnight and then read for highest dilution of sera that inhibits CPV-2 induced agglutination of pRBC. Expected assay variability includes a single doubling dilution above or below reported value. Antibody against CPV-2 was chosen for examination because this virus has global impact, is highly stable in the environment, and is a common cause of fatal diarrhea and vomiting in susceptible pups.

2.3. Statistics

Average geometric mean titers at each time point were analyzed via a repeated measures, one-way ANOVA test with Tukey's multiple comparisons *post hoc* correction (GraphPad Prism). Significance at 95% confidence interval was set at $p<0.05$.

3. Results

Average geometric mean titer values were 8.1, 7.7, 7.1, and 8.1 over the 4 timepoints (Figure 1). Seven of the 56 bitches (12.5%) experienced a titer decrease for sera collected on day of whelp which was greater than inherent assay variability. One-way ANOVA with repeated measures revealed this decrease was highly significant ($p<0.0001$). All other timepoints did not differ significantly.

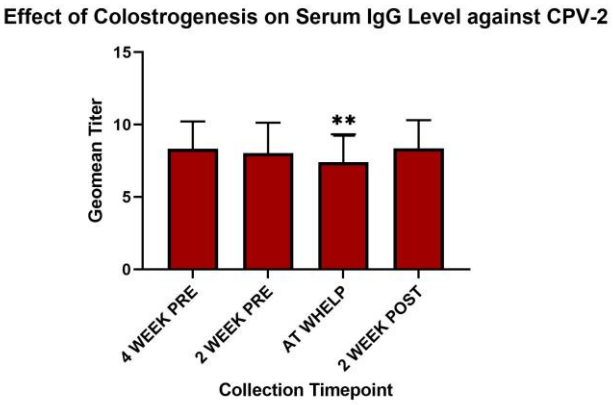

Figure 1. *Average geometric mean anti-CPV-2 titer for a group of 56 gravid bitches at timepoints before, after, and at whelping.*

4. Discussion

Maternally derived antibody (MDA) interference has long been known as the primary cause of immunization failure in the dog due to antibody neutralization of modified live viral (MLV) vaccines [9, 10]. Live CPV-2 vaccines will induce long lasting active antibody responses after a single effective dose with infection and replication of vaccinal virus in the dog. This process safely mimics an infection by virulent CPV-2. This benefit of MLV vaccines is offset by the "Achilles' heel" that these vaccines must infect to immunize. Any factor that decreases vaccine infectivity, such as improper handling or temperature control, will negatively impact efficacy. Specific antibody against CPV-2 is highly effective at neutralization of this virus, whether the antibody is passively derived (such as MDA) or due to active vaccinal response.

Depending on the maternal titer, some litters can be prevented from actively responding to CPV-2 vaccine until 22 weeks of age, which is beyond the standard age for final dose in the puppy series, which is generally recommended at 16 weeks of age [11]. Passive antibody blockade of immunization leaves the pup susceptible and immunologically naïve once passively transferred, maternally derived antibody has completely degraded. Veterinary clinicians will be well-aware of the juvenile dog that has been "well-vaccinated" but still not immunized against parvovirus,

and thus becomes seriously ill when exposed to virulent CPV-2 after maternal antibody has fully degraded.

Nomograph testing, which was originally developed in 1958 by Dr. James Baker [12], is a conservative analysis of the degradation of maternally derived antibody in a litter relative to the measured titer of the bitch and known half-life rate. This approach by-passes MDA interference by providing a suggested vaccine schedule that is tailored to a litter. In instances of high maternal titers against CPV-2 and/or canine distemper virus (CDV), the vaccination schedule will be extended beyond the standard recommendation. On the other hand, when maternal titers are low, the number of doses administered can be decreased, with an early finish and follow-up antibody testing applied earlier than 16 weeks of age.

In follow-up testing, pup titers are compared with those of the bitch in the context of time/degradation half-life, thus providing a method to easily differentiate active vaccinal antibody responses from residual declining maternally derived antibody. A study from our laboratory showed significant improvement in immunization outcomes for puppies when a nomograph had been completed as compared to a cohort of puppies that had not had a nomograph completed for their dam [7]. Puppies in the nomograph group were protected against CPV-2 at the same rate as adult vaccinates, whereas the group of pups vaccinated without a nomograph were highly significantly less likely to be protected (data not shown).

Follow-up titer testing of the litter is included in the nomograph-informed, tailored schedule, and is strongly recommended 2 weeks after the final dose of core MLV vaccine. In instances of low maternal titers, this testing may be as early as 12 weeks of age. When early immunization is proven through follow-up testing, pet owners can have peace of mind during an important puppy socialization and training "window" [13-15]. AAHA guidelines should be utilized when bitch titer is unknown, with a final dose of core vaccine administered at 16 weeks of age [11].

The authors strongly urge that all pups be tested for actively produced antibody against CPV-2 and CDV two weeks after the end of the initial vaccination series, whether a nomograph has been completed for the dam or not.

The current study indicates that the best timing for sample collection for nomograph testing of the breeding bitch is outside the timeframe of active antibody sequestration to the mammary gland which occurs during the 2 weeks pre-whelp and over the two weeks of recovery post-whelp. When sample must be drawn during this window, a small adjustment to the analysis needs to be considered.

5. Conclusions

Colostrogenesis significantly impacts maternal circulating anti-CPV-2 antibody titers at whelp. By two weeks post whelp, circulating titers return to pre-parturient levels. Sera for nomograph analysis in the breeding bitch should be collected outside of the 2-week period before and after expected whelp for best assessment accuracy. With the intention to ensure that nomograph application does not induce any long-term impacts on immune response, life-span studies are currently ongoing in this laboratory to compare immunity against CPV-2 between pet dogs which were vaccinated according to a nomograph informed initial puppy schedule and those that used a standardized schedule.

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Abbreviations

CPV-2	Canine Parvovirus Type 2
IgG	Immunoglobulin Type G
HIA	Hemagglutination Inhibition Assay
MDA	Maternally-Derived Antibody
Fc	Fragment Crystallizable
MLV	Modified Live Viral
IACUC	Institutional Animal Care and Use Committee
BSA	Bovine Serum Albumin
HA units	Hemagglutinating Units
pRBC	Porcine Red Blood Cells
ANOVA	Analysis of Variance

Author Contributions

Shay Lierman: Conceptualization, Data curation, Investigation, Methodology, Visualization

Azizeh Egerer: Investigation, Methodology

Laurie Larson: Conceptualization, Formal Analysis, Funding acquisition, Project administration, Writing – original draft, Writing – review & editing

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Data Availability Statement

The data supporting the outcome of this research work has been reported in this manuscript.

Conflicts of Interest

The authors declare no conflict of interest.

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Biography

Shay Lierman graduated from University of Wisconsin – Madison School of Veterinary Medicine in the class of 2023. During her time as a student, Shay was involved in many aspects of research, including investigations within the fields of infectious disease and immunology. She is currently working as a veterinary clinician in a mixed animal practice and has a special interest in equine medicine and surgery.

Azizeh Egerer is a fourth-year veterinary medical student at the University of Wisconsin – Madison School of Veterinary Medicine, and former employee of the Companion Animal Vaccines and ImmunoDiagnostic Service (CAVIDs) laboratory. Azizeh's aspirations involve working within the field of Public Health research with a focus on veterinary immunology, infectious diseases and zoonoses. Her interest in both clinical medicine and veterinary research is a driving factor in her public health goals.

Laurie Larson is a senior scientist, veterinary immunology instructor, and director of the Companion Animal Vaccines and ImmunoDiagnostic Service (CAVIDS) laboratory at the University of Wisconsin-Madison School of Veterinary Medicine. Dr. Larson received her DVM degree from Iowa State University in 1987. After several years in small animal clinical practice, she has worked in the field of veterinary vaccinology for 32 years. She owes much to her long-time mentor, Dr. Ronald Schultz.

Research Field

Shay Lierman: mixed animal clinical practice, veterinary immunology, veterinary vaccinology, infectious disease, primary care.

Azizeh Egerer: veterinary immunology, public health, infectious disease, One Health, wildlife/human interface, emerging zoonoses

Laurie Larson: veterinary immunology, veterinary vaccinology, infectious disease, canine and feline antibody responses, passively derived immunity. CPV