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Antibody Titers against Canine Parvovirus Type 2 in Vaccinated Dogs Attending Veterinary Clinics

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ABSTRACT

Canine parvovirus type 2 (CPV-2) represents one of the main challenges in veterinary clinical practice. Despite the availability of several commercial vaccines, morbidity and mortality continue to affect even vaccinated puppies. The aim of this study was to evaluate antibody titers against CPV-2 in vaccinated dogs attending veterinary clinics in Nicaragua. A total of 27 dogs aged 3 to 10 months were analyzed. Hemagglutination Inhibition (HI) assays were performed to determine antibody titers. Hematological parameters were measured, and vaccination histories were collected. Among the 27 dogs, 4 (14.8%) had antibody titers of 1:16, 6 (22.2%) had titers of 1:2, 3 (11.1%) had titers of 1:4, 7 (25.9%) had titers of 1:8, and 7 (25.9%) were seronegative. A significant difference was observed in mean corpuscular hemoglobin (MCH) ($p = 0.043$) between dogs with titers of 1:16 and seronegative dogs, with the latter showing higher MCH values compared to the other groups. Vaccine type and number of doses were associated with titer levels, with better responses observed in dogs that completed the full three-dose schedule. Overall, low levels of antibodies against CPV-2 were found in dogs that had received prior vaccination against this pathogen. These findings emphasize how important it is to monitor vaccination protocols in resource-limited regions.

Keywords: Antibody, Canine parvovirus, Immunity, Vaccine.

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INTRODUCTION

Canine parvovirus type 2 (CPV-2) is a virus with a single chain DNA genome, belonging to the Parvoviridae family. Represents one of the major pathogens at the veterinary clinic in dogs that are responsible for serious hemorrhagic gastroenteritis with high morbidity and mortality, especially in non-vaccinated animals, **(Wilkes, 2023).** In the environment, the virus can survive for months and remain infectious, complicating eradication and control efforts, **(Vargas Orozco and Bedoya Osorio 2021)**.

Variants of the CPV-2 sequence (CPV-2a, 2b, and 2c) are found globally, but there are regional differences in prevalence and pathogenicity. There is a risk of vaccine cross-protection and a chance of infection breakthroughs due to this antigenic diversity **(Wilson *et al.*, 2014).** The complex interplay between viral evolution, host factors, and vaccine practices has led to a number of field failures despite modern vaccines' broad-spectrum efficacy claims **(Schumann *et al.*, 2016).**

Vaccination remains the primary strategy for CPV-2 control. To overcome interference from maternally derived antibodies (MDA), It is recommended that vaccinations begin between the ages of 6 and 8 weeks and that additional doses be administered every 3 to 4 weeks until the puppies reaches the age of 16 weeks **(Squires *et al.*, 2024).** MDA interference is one of the leading causes of vaccine failure, as antibodies from the dam can neutralize vaccine virus before effective priming of the puppy's immune system occurs **(Escobar Pineda *et al.*, 2022).** Other critical factors include vaccine type (modified-live vs. inactivated), cold-chain integrity, manufacturer standards, and adherence to recommended protocols. In low-resource settings, inconsistent vaccine storage and administration practices further compromise outcomes **(Rubio *et al.*, 2018).**

Serological monitoring of vaccination responses can inform revaccination strategies and help identify at-risk populations. While virus neutralization assays remain the gold standard, they are costly and require specialized facilities. The Hemagglutination Inhibition (HI) assay offers a simpler, cost-effective alternative suitable for routine use in developing countries ***(De Cramer et al., 2011)***.

This study aimed to evaluate antibody titers against CPV-2 in vaccinated dogs attending veterinary clinics in Nicaragua, using the HI assay to measure antibody titers and analyze associations with vaccine type, dose number, and hematological parameters. Our goal is to inform vaccination strategies and support better disease control in resource-limited settings.

MATERIALS AND METHODS

Study area

This observational cross-sectional study was conducted from May to July 2024 in Rivas, a department in southwestern Nicaragua (11°26′20″N, 85°49′43″W), characterized by a tropical climate with marked wet and dry seasons. Two urban veterinary clinics participated, representing typical small animal practice settings in the region.

Sample

Twenty-seven dogs were included consecutively during routine veterinary clinics visits.

Demographics data

Dogs were between 3 and 10 months old, weighing between 3 and 15 kg. The sample consisted of 13 males and 14 females, with representation of purebred and mixed-breed animals.

Medical Status

Puppies without diarrhea and apparently healthy, with complete medical histories, were included.

Vaccination Data

Parvovirus vaccination history (verified in records) was reviewed. Unvaccinated puppies or puppies with unknown maternal colostrum intake were not included, and vaccination schedules were reviewed with the number of doses and brand/type of vaccine (Type 1 = lowest cost and Type 2 = medium cost).

Blood Sampling

Blood was collected aseptically from the cephalic vein using single-use needles and syringes. Samples were divided into Ethylenediaminetetraacetic Acid (EDTA) tubes for hematology and without anticoagulant tubes for serum separation. All samples were labeled, stored at 4 °C, and transported within 2 hours to the laboratory for processing.

Analysis of the results in the laboratory

Blood samples were processed for complete blood counts, with calculated red cell indices such as mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC), according to standard protocols **(Thrall *et al.,* 2012).**

Serum samples were tested for anti-CPV-2 antibodies using the Hemagglutination Inhibition (HI) technique adapted from De Cramer et al. (2011). Sera were inactivated at 56°C for 30min and were carried out in serial dilutions by duplicating 1:2 to 1:256 in phosphate-buffered saline (PBS). Twenty-five µl of Cornell vaccine strain (1000 TCID50) was added to wells, followed by incubation at room temperature for 90min. One percent porcine erythrocyte suspension in PBS was then added, and plates were incubated overnight at 4°C. Titers were read as the highest dilution showing ≥50% inhibition of hemagglutination. A threshold of ≥1:32 was considered indicative of effective immunity **(Cunha *et al.,* 2020).** Positive and negative control sera were included in each run to ensure assay validity.

Associations between HI titers and variables (vaccine type, number of doses) were analyzed using Fisher's exact test, while One-way analysis of variance (ANOVA One-way) was used to compare hematological values with antibody titers. A p value ≤ 0.05 was considered statistically significant.

Statistical analysis

Relative frequencies were used to describe the qualitative variables. Fisher's exact test was applied to compare antibody titers with vaccine types and the number of doses received. In addition, one-way analysis of variance (ANOVA) was used to compare hematological parameters with antibody titters.

RESULTS

Out of the 27 enrolled dogs, 48.1% (13/27) were male and 51.9% (14/27) female. The mean age was 6.4 ± 2.1 months, with animals distributed across the 3–10-month inclusion range. Twenty-four puppies were purebred, while three were mixed breed. Vaccination records indicated two vaccine brands used locally, designated here as Type 1 and Type 2.

HI testing revealed considerable variability in antibody responses. Seven dogs (25.9%) were seronegative despite reported vaccination. Among positive titers, 7 (25.9%) had 1:8, 4 (14.8%) had 1:16, 3 (11.1%) had 1:4, and 6 (22.2%) had 1:2. No animals reached the protective threshold of 1:32.

Analysis by vaccine brand showed trends suggesting differences in immunogenicity. Among 9 dogs vaccinated with Type 1, none achieved 1:16 titers; distributions included 2 at 1:8, 2 at 1:4, 2 at 1:2, and 3 seronegative. In contrast, among 18 dogs receiving

Type 2 vaccines, 4 achieved 1:16 titers, 5 had 1:8, 1 had 1:4, 4 had 1:2, and 4 were seronegative. Although differences were not statistically significant ($p \geq 0.05$), the pattern suggested better responses with Type 2 vaccines, **(Fig 1).**

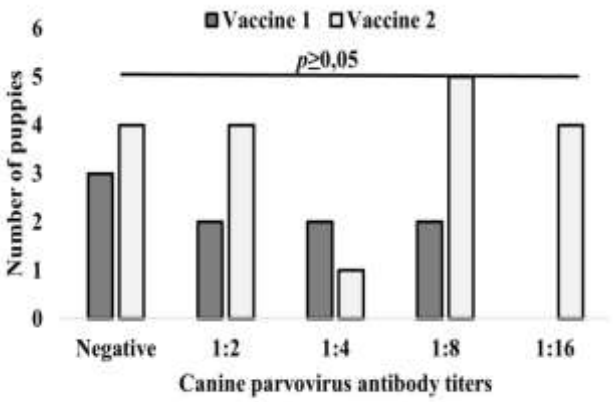

Fig. 1: Comparison of antibody titers against canine parvovirus type 2 by vaccine type

Dogs receiving three doses showed significantly higher titers ($p=0.007$). Among one-dose recipients, titers were limited to 1:2–1:8, with no 1:16 responses. Two-dose recipients included animals with 1:16 titers but also several seronegative cases, indicating partial protection. Dogs completing the three-dose series had the highest proportion of 1:16 titers, supporting the value of full adherence to recommended schedules, **(Fig. 2).**

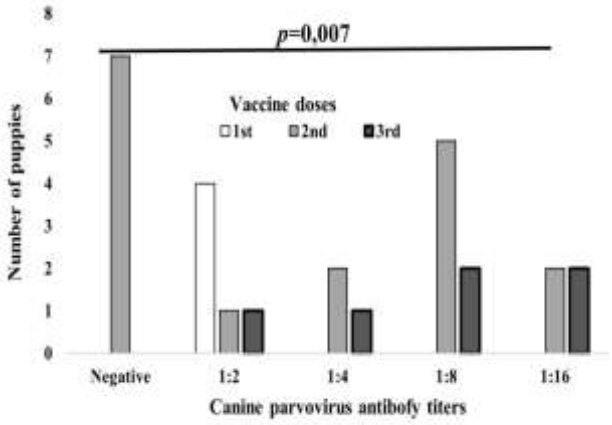

Fig.2: Antibody titers against canine parvovirus type 2 in relation to the number of vaccine doses administered.

Comparative analysis of hematological parameters across titer categories showed no significant differences for hematocrit, hemoglobin, MCV, MCHC, leukocyte counts, or differentials (all $p \geq 0.05$). However, mean corpuscular hemoglobin (MCH) was significantly higher in seronegative dogs versus those with 1:16 titers ($p=0.043$), suggesting potential age-related or subclinical variations influencing immunocompetence **(Fig.3).**

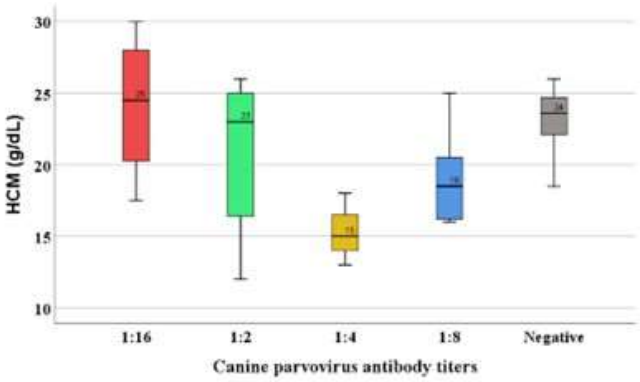

Fig.3: Mean corpuscular hemoglobin concentration in relation to antibody titters against canine parvovirus type 2.

DISCUSSION

This study revealed that a significant proportion (25.9%) of dogs in Nicaragua remained seronegative for CPV-2 despite reported vaccination, highlighting challenges in achieving adequate immunity in endemic settings. Such findings align with previous Nicaraguan studies documenting the widespread circulation of diverse CPV-2 variants, which may impact vaccine cross-protection **(Flores *et al.*, 2020)**.

Molecular studies in León and Matagalpa have identified both CPV-2b and CPV-2c variants circulating in the country, raising concerns about antigenic drift and the need for vaccines with broad-spectrum efficacy, genotyped field strains in Nicaragua, confirming the ongoing presence of multiple variants that could challenge immunity induced by older or poorly matched vaccines **(Flores *et al.*, 2020).**

There was a 14.8% HI titer of 1:16 in this study (still below many accepted protection thresholds, such as 1:32 or 1:64), suggesting suboptimal protection and possible infection susceptibility. This is consistent with field reports of Parvovirus outbreaks even in reportedly vaccinated puppies, underlining the multifactorial nature of vaccine failure, including MDA interference, incomplete protocols, and vaccine handling issues **(Rubio *et al.*, 2018; Escobar Pineda *et al.*, 2022).**

Vaccine type and dose number were both associated with observed titers. The results indicated higher antibody titers in puppies immunized with the type 2 vaccine. This difference may be explained by several factors, the most relevant being the distinct viral strains used in each formulation. The type 2 vaccine employs a strain that shares neutralizing epitopes with

circulating wild-type CPV isolates, potentially enhancing its ability to elicit effective cross-protective antibodies. Nevertheless, vaccine efficacy can also be influenced by additional factors, including viral dose, the degree of attenuation (or residual virulence), and possible interference from maternally derived antibodies **(Hoare *et al.*, 1997).**

Dogs completing the full three-dose series showed significantly better responses, reinforcing WSAVA recommendations for sequential vaccinations to overcome MDA interference. In endemic regions like Nicaragua, where environmental viral loads are high and vaccination practices vary, ensuring complete vaccination schedules is critical to achieving herd-level immunity **(Squires *et al.*, 2024).**

The limited association between most hematological parameters and antibody titers suggests that standard blood values cannot substitute for direct serological testing when evaluating vaccine efficacy (Ellis et al. 2022). However, the significant difference in MCH observed between seronegative dogs and those with higher titers may reflect underlying age-related or subclinical factors affecting immune competence.

Importantly, the HI assay demonstrated its practicality as a low-cost diagnostic tool for monitoring immunization status in resource-limited veterinary practices. Although less sensitive than virus neutralization assays, HI testing offers valuable, actionable information that can support targeted revaccination strategies and inform public health planning **(De Cramer *et al.* 2011).**

CONCLUSIONS

To improve CPV-2 control in Nicaragua and the broader Central American region, attention must also be given to vaccine cold chain logistics, standardization of administration protocols, and owner education about the importance of completing vaccination schedules. Enhanced surveillance, including molecular characterization of circulating strains, will be vital for ensuring that vaccines remain appropriately matched to local epidemiology and for reducing preventable morbidity and mortality in the canine population.

Conflict of interest

The authors declare that they have no competing interests

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