Serum Antibody Titers against Distemper, Parvovirus and In-fectious Hepatitis in Dogs from Central Spain

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Article

Serum Antibody Titers against Distemper, Parvovirus and Infectious Hepatitis in Dogs from Central Spain

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Simple Summary: Vaccination is considered a safe and cost-effective practice with minimal risks. However, in recent years, the excessive vaccination of companion animals against diseases such as distemper, infectious hepatitis, and parvovirus has attracted concern. The main topic of debate is whether excessive immunization could undermine vaccines' efficacy rather than benefit animals' immune resistance. In our study, no significant differences were observed between vaccinated and non-vaccinated animals in terms of immune defense against the three viruses tested. These results suggest a high rate of circulation of canine distemper virus (CDV), canine parvovirus (CPV), and infectious hepatitis virus (CAV-1) in the studied population and highlight the need to re-evaluate the benefits of using commercialized vaccines to treat these viruses. We encourage clinical veterinarians to use the specific antibody determination methods outlined in this study prior to vaccinating dogs, allowing the establishment of individualized vaccination protocols.

Abstract: A canine population's immune resistance to canine distemper virus (CDV), canine parvovirus (CPV), and infectious hepatitis virus (CAV-1) was evaluated. In this study, a total of 112 sera were analyzed. Animals were considered as vaccinated if, in the last two years, they had received at least one dose of a vaccine that provides joint protection against CDV, CPV, and CAV-1. Animals that had never received any dose of these vaccines were designated as non-vaccinated. CDV, CPV, and CAV-1 antibodies were detected via a modified solid-phase enzyme-linked immunosorbent assay (ELISA), which detects IgG antibody levels in sera and provides semi-quantitative results in less than 30 min. In total, 41.1% of the dogs had been vaccinated, and 58.9% of dogs were designated as non-vaccinated. Overall, 90.2%, 92.0%, and 78.6% of the tested dogs had positive results for the presence of IgG antibodies against CPV, CDV, and CAV-1, respectively. CPV antibodies were present in 93.5% (43/46) of the vaccinated and 87.9% (58/66) of the non-vaccinated dogs, while CDV antibodies were present in 95.5% (63/66) of the vaccinated and 87.0% (40/46) of the non-vaccinated dogs. Finally, CAV-1 antibodies were present in 84.8% (56/66) of the vaccinated and of 69.6% (32/46) the non-vaccinated dogs.

Keywords: canine distemper virus; canine parvovirus; infectious hepatitis virus; IgG level; Vaccicheck

1. Introduction

Canine distemper virus (CDV), canine parvovirus (CPV), and canine infectious hepatitis virus (CAV-1) are the etiological agents of three major infectious diseases that affect dogs. The World Small Animal Veterinary Association (WSAVA) regards vaccines that bolster immunity against these diseases as essential, stating that they should be administered to all individuals, regardless of their geographic location or lifestyle, since they provide protection against globally distributed, life-threatening diseases [1].

Herd immunity refers to the collective immune resistance of a host population to a specific microorganism [2], i.e., the higher the proportion of vaccinated individuals within a population, the lower the proportion of individuals susceptible to infection. In general, for an entire canine

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population to be protected against a disease, at least 70% of the population must be immunized [3]. Herd immunity, as it is based on the use of vaccines that provide long-lasting immunity, is more dependent on the total proportion of vaccinated animals in the population than the number of individuals receiving annual revaccinations [1].

Though serum antibodies only represent a part of the immune response, multiple studies have shown that, in the cases of dog diseases caused by CDV, CPV, and CAV-1 or by the rabies virus, there is a good correlation between the presence of serum antibodies and increased protective immunity. Different techniques are available for determining these antibodies, with diverse enzyme-linked immunosorbent assay (ELISA)-based methods being the most widely used techniques, as they are fast, versatile, reliable, and cost-effective methods [4–6].

Nevertheless, though numerous studies have investigated the immune responses to CDV, CPV, and CAV-1 in vaccinated dogs in various countries [7–9], research on the immune statuses of non-vaccinated dogs remains notably limited [10,11]. Moreover, a comprehensive exploration of these pathogens' presence in dogs in Spain is currently lacking within the established scientific literature. This study aims to determine the immune status of a canine population in central Spain, using an in-practice test kit to determine the presence of antibodies against CDV, CPV, and CAV-1.

2. Material and Methods

2.1. Animals

In this study, a total of 112 sera samples were analyzed. Each sample was collected from a different dog residing in central Spain in 2021 and 2022. Data regarding each dog's gender, age, and vaccination status against canine distemper virus (CDV), canine parvovirus (CPV), and canine adenovirus-1 (CAV-1) were collected. The dogs were classified as vaccinated if they had received at least one dose of the combined CDV, CPV, and CAV-1 vaccine within the previous two years. Dogs that had never received this vaccine were categorized as non-vaccinated. We used a convenience-based sampling approach due to the challenges involved in obtaining samples from non-vaccinated dogs. The non-vaccinated dogs primarily corresponded to hunting dogs living in rural areas, often found in hunting estates. Conversely, the group of vaccinated dogs predominantly consisted of companion animals that lived with their owners in urban environments. All dogs included in this study were healthy, which was defined based on the absence of clinical signs, a normal clinical examination, and no clinical issues related to immune function, such as Leishmaniosis, Cushing's syndrome, Addison's syndrome, hypothyroidism, lymphoma, mass cell tumors, and other neoplastic disorders.

2.2. Samples

Blood collection was carried out by licensed veterinarians after receiving informed consent from the dogs' owners, with this process following animal welfare protocols. Upon their arrival at our laboratory, all serum samples were assigned with a sample code and preserved at −80 °C until blind analysis (i.e., the staff involved in their analysis did not know any details about their origin) of the samples via the enzyme-linked immunosorbent assay (ELISA) protocol, as detailed below.

2.3. Commercial Solid-Phase ELISA

Antibodies against CDV, CPV, and CAV-1 were determined via the modified solid-phase ELISA ImmunoComb® Canine VacciCheck (Biogal Galed Labs, Kibbutz Galed, Israel), which detects IgG antibody levels in serum, plasma, or whole blood and provides semi-quantitative results in less than 30 min. This process was performed according to the manufacturer's instructions. At the end of the protocol, we predicted that a purple-grey color would appear on both the positive control and positive samples, but the color intensity was expected to vary depending on the antibody level. Color intensity was read using a CombCam automatic reader (Biogal Galed Labs) and interpreted following the manufacturer's instructions. Color intensity results were categorized using the following scale: level 0: negative result; level 1: inconclusive; level 2: weak positive; levels 3 and 4: positive results;

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and levels 5 and 6: highly positive results. Samples for which any of the dots corresponding to different antibodies showed a color intensity of level 2 or higher were considered positive results, with those dogs having protection against the respective antigens [12].

2.4. Statistical Analysis

Categorical variables are presented as percentages. For continuous variables, the normality of data distribution was evaluated via the Kolmogorov–Smirnov test. Continuous data are presented as either the mean with standard deviation (SD) or the median with interquartile range (IQR).

A univariate logistic regression model was used to study the influence of the vaccination statuses of dogs in which IgG antibodies (negative/positive) against CDV, CPV, and CAV-1 were present. These models were adjusted based on gender and age. Odds ratios (ORs) were calculated at confidence intervals (CIs) of 95%. During statistical analyses, we tested the null hypothesis, which was rejected if the alpha error was less than 0.05. Values with $p < 0.100$ were considered as having a tendency toward reliability. Statistical analyses were performed using Stata software version 13.0 (Stata Corp., College Station, TX, USA).

3. Results

Of the 112 dogs that we studied, 41.1% were non-vaccinated, while the remaining 58.9% had received at least one vaccination. Of the non-vaccinated dogs, 56.5% were male, and 43.5% were female. Conversely, within the vaccinated group, 48.5% of the dogs were female, and 51.5% of the dogs were male. Regarding the age distribution, the median age of the non-vaccinated dogs was 5.8 years old (IQR: 4.9–7.9), whereas for the vaccinated dogs, the median age was 3.6 years old (IQR: 1.5–8.4). The most prevalent breeds among the non-vaccinated group were Greyhounds (67.4%) and Podencos (13.0%). In contrast, mixed-breed dogs were highly represented in the vaccinated group, comprising 15.2% of the total number, followed by Belgian Shepherds (15.2%) and German Shepherds (13.6%).

Of the dogs that had IgG antibodies against CPV, 93.5% (43/46) had been vaccinated, while 87.9% (58/66) of these dogs had not been vaccinated. Of the dogs with IgG antibodies against CDV, 95.5% (63/66) had been vaccinated, while 87.0% (40/46) of these dogs had not been vaccinated. Finally, of the dogs with IgG antibodies against CAV-1, 69.6% (32/46) had not been vaccinated, while 84.8% (56/66) of these dogs had been vaccinated (Table 1).

In Table S1, IgG antibody presence against CPV, CDV, and CAV-1 is presented for both vaccinated and non-vaccinated dogs, with results segregated by a colorimetric scale for clarity in visualising antibody levels within each dog group.

Table 1. Presence of IgG antibodies against CPV, CDV, and CAV-1 in non-vaccinated and vaccinated dogs.

	IgG against CPV		IgG against CDV		IgG against CAV-1	
Vaccination Status	**Negative n (%)**	**Positive n (%)**	**Negative n (%)**	**Positive n (%)**	**Negative n (%)**	**Positive n (%)**
Non-vaccinated	3 (6.5)	43 (93.5)	6 (13.0)	40 (87.0)	14 (30.4)	32 (69.6)
Vaccinated	8 (12.1)	58 (87.9)	3 (4.5)	63 (95.5)	10 (15.2)	56 (84.8)

We used a logistic regression model to evaluate the influence of vaccination status on the presence of IgG antibodies against CPV, CDV, and CAV-1, and, when adjusted for age and gender, the logistic regression model did not show a significant difference between OR and adjusted OR; therefore, age and gender did not have a significant influence on the presence of antibodies. No statistically significant association was found between vaccination status and the presence of IgG antibodies against CPV (Adjusted OR = 0.52; 95%CI, 0.13–2.12; $p = 0.360$) and CDV (Adjusted OR = 3.43; 95%CI, 0.74–16.00; $p = 0.116$). However, a tendency was observed in the case of antibodies against CAV-1 (Adjusted OR = 2.38; 95%CI, 0.90–6.25; $p = 0.077$) (Table 2).

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Table 2. OR and adjusted OR of presence of IgG antibodies against CPV, CDV, and CAV-1 in vaccinated dogs compared to non-vaccinated dogs.

	OR			Adjusted OR Based on Age and Gender		
Positive IgG Antibodies	**OR**	**95%CI**	***p*-Value**	**OR**	**95%CI**	***p*-Value**
CPV	0.51	0.13–2.02	0.335	0.52	0.13–2.12	0.360
CDV	3.15	0.75–13.3	0.119	3.43	0.74–16.0	0.116
CAV-1	2.45	0.98–6.15	0.056	2.38	0.91–6.25	0.077

4. Discussion

This study is one of the first investigations conducted in Spain to assess the use of serum antibody titers against core vaccine antigens (CPV, CDV, CAV-1) in dogs, notably featuring a substantial number of non-vaccinated subjects. Our findings reveal that many dogs exhibited IgG antibodies against CPV and CDV, with no significant differences being observed between vaccinated and non-vaccinated individuals; these observations were also independent age and gender. Moreover, many dogs exhibited IgG antibodies against CAV-1, with vaccinated dogs tending to have a higher proportion of antibodies than non-vaccinated dogs.

When assessing protective antibody levels, it is essential to note that the gold standard for CDV and CAV is the viral neutralization (VN) assay, while for CPV, the established gold standard is the hemagglutination inhibition (HI) assay. However, these methods are limited by their need to be administered in specialized laboratory settings by skilled personnel, rendering them impractical for routine clinical use. Practical methods, such as VacciCheck, use an enzyme-linked immunosorbent assay (ELISA) to generate semi-quantitative results regarding the presence of IgG antibodies against CPV, CDV, and CAV-1. VacciCheck uses a dot-ELISA method, where the intensity of the colors of the dots corresponding to different viral antigens correlates with antibody titers, ranging from no color (0) to shades of color (S1 to S6). These cost-effective, user-friendly tests can easily be conducted in a clinical setting. Multiple past studies have explored the diagnostic accuracy of this point-of-care dot blot ELISA method (the index test) in comparison to reference standard laboratory-based assays [12,13]. In these studies, when a cutoff point of two or higher was applied, the results demonstrated impressive diagnostic performance. For all three viral antigens, the index test exhibited sensitivity ranging from 96.03% to 96.75%, while specificity ranged from 87.50% to 94.33%. Moreover, the overall accuracy of these tests ranged from 93.43% to 95.91%.

Studies conducted across multiple nations, including the UK, the USA, Canada, Japan, India, South Korea, Netherlands, Brazil, Australia, Ecuador, and India, consistently indicate that both vaccinated and non-vaccinated dog populations display a high degree of immunity to canine parvovirus (CPV) [7,8,11,14]. This resilience is attributed to CPV-2's pervasiveness in both urban and rural ecosystems, its exceptional environmental robustness, and its potent immunogenicity [5,9,15]. CPV-2 serves as a natural immunological booster, sustaining elevated seroprotection rates, even among elderly canines. Variations in CPV strains, like CPV-2a, CPV-2b, and CPV-2c, can contribute to differences in immunity patterns [16]. Our study aligns with similar observations made in Spain, with the prevalence of CPV in Iberian wolf and fox populations, primarily due to virus circulation among domestic canids, underscoring the significant role played by non-vaccinated dogs in bolstering CPV immunity [17]. Comparable findings in Italy and France also emphasize that CPV antibody titers in vaccinated dogs tend to stabilize after one year of age, possibly due to contact with the field virus, resulting in a notable percentage of 'protected' dogs [18]. Additionally, the prevalence of free-roaming cat colonies, as in Italy, raises concerns about the potential for CPV transmission from feline hosts to dogs in Spain. Cats infected with feline parvovirus (FPV) risk infecting dogs, emphasizing the importance of considering this factor in central Spain, as was noted in the observations made in other studies, such as those conducted in Italy [19].

Overall, protection against canine distemper virus (CDV) is notably high, though slightly lower than for canine parvovirus (CPV), which is consistent with various studies' findings [5,9,20,21]. Notably, a significant number of non-vaccinated dogs also exhibit CDV protection, in contrast to the

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findings of other studies [11,22–24]. Some studies report lower protection in non-vaccinated dogs, often attributing this trend to CDV's susceptibility to environmental inactivation and its lower immunogenicity [1]. However, another hypothesis suggests that high natural protection in non-vaccinated dogs may be linked to the presence of wildlife reservoirs. Studies have found that the presence of CDV in wildlife species, such as the Iberian wolf (*Canis lupus*) and fox (*Vulpes vulpes*) in Spain, can lead to virus transmission via interactions with domestic canids, and these connections have led to documented cases of CDV transmission from domestic dogs to various wild species [17,25], including severe outbreaks, as observed in the case of Masai lions (*Panthera leo masaica*) in the Serengeti [18]. Moreover, prior serological surveys conducted in central Italy and south-eastern France provided evidence of CDV exposure in free-ranging brown bears and wolves, with CDV exposure also being identified in foxes and badgers within the same geographical areas [18]. These findings suggest a broad ecological influence on CDV transmission dynamics beyond domestic canine populations.

In our study, we observed that the proportion of dogs vaccinated against canine adenovirus 1 (CAV-1) exhibited protection levels similar to those reported in previous studies [26,27]. Remarkably, even among non-vaccinated dogs, the levels of protection were relatively high, being comparable to those of some past studies and notably higher than those of other studies [22,24], although they were lower than in vaccinated animals. Regarding CAV-1, there have been no reported outbreaks of infectious hepatitis in dogs in central Europe between 1987 and 2001. However, more recently, outbreaks occurred in Italy in 2001, 2004, and 2006 [28], as well as in Germany in 2008 [29,30]. In the outbreaks in Italy, which mainly affected puppies, mixed infections of CAV-1 and CDV were detected in 2001, while CPV alone was detected in 2004. Notably, when the disease affected adult dogs, the mortality rate remained low, even in non-vaccinated animals [28]. In Spain, a study of the European brown bear population revealed that CAV-1 posed a significant threat to the species, leading to the deaths of an adult bear in 2014 and two cubs in 2015 and 2017 [31]. The presence of the virus in the environment and its circulation among sympatric species, such as dogs living in rural areas, contributed to CAV-1-related bear deaths. These observations highlight the virus's resilience in the environment, which may explain the persistence of CAV-1 [32]. Furthermore, the potential for cross-immunity between CAV-1 and CAV-2 should be considered, as these closely related viruses can provide natural protection. The results suggest that distinct interplay between environmental resistance and cross-immunity could be a contributing factor to the elevated protection levels observed in our study, despite the limited number of recent hepatitis infections in central Europe.

This study had several limitations. The research area was confined to central Spain, limiting its broad applicability to the entire Spanish dog population, with this regional focus being used for convenience. A more comprehensive study spanning across Spain is required to address the variation in vaccination practices and disease prevalence across the country. Moreover, the predominant focus on young adult dogs constrained the generalization of our findings to other age groups, although it allowed for valuable comparisons between vaccinated and non-vaccinated dogs. Furthermore, the absence of the gold standard VN or HI tests could influence the accuracy of the determination of protective antibody levels, although VacciCheck's recommendation by WSAVA and AAHA and its official approval in several countries provide some support to the study findings. The lack of data regarding antibody titers for these viruses in Spain is also a significant limitation. However, this study has several strengths, including confirming the health statuses of all participating dogs prior to the testing stage, thus eliminating potential confounding factors related to immune function, and offering a valuable comparison between vaccinated and non-vaccinated animals.

This study provides valuable insights into the immune statuses of dogs in Spain with regard to CDV, CPV, and CAV-1. Notably, many of the dogs exhibited some IgG antibodies against CPV, CDV, and CAV-1, regardless of their vaccination status, age, or gender. These findings align with observations from other countries, highlighting the presence of robust herd immunity against these viruses, even in non-vaccinated dogs. This study underscores the potential influence of wildlife reservoirs to enhance non-vaccinated dogs' immunity. Moreover, practical diagnostic tools such as VacciCheck enable an efficient assessment of a dog's immune status. These findings support the

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recommendation that clinical veterinarians incorporate rapid diagnostic tests, like VacciCheck, to enable individualized vaccination protocols. Further comprehensive research is needed to more comprehensively establish disease prevalence and immunity, which could serve as a baseline for future epidemiological studies and vaccination campaigns.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

Author Contributions: Conceptualization, J.L.B. and M.E.G.; formal analysis, G.O.-D.; investigation, E.P. and S.R.; methodology, E.P. and S.R.; supervision, J.L.B. and M.E.G.; validation, G.O.-D.; writing—original draft, J.L.B.; writing—review and editing, G.O.-D. and M.E.G. All authors have read and agreed to the published version of the manuscript.

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Informed Consent Statement: Samples were provided by veterinary surgeons in the course of their professional duties inside veterinary clinics. Blood samples were taken for metabolic or biochemical profiles during routine clinical examinations in veterinary practices. A small amount of serum was given to us by veterinary surgeons, and these samples were included in this study. The dogs' owners were informed of this study's purpose and signed a consent form allowing us to use the samples for research purposes. According to European Directive 2010/63/EU (article 1, point 5), the clinical veterinary procedures followed were non-experimental, and ethical approval was not necessary. This information can be found in the following working document: https://ec.europa.eu/environment/chemicals/lab_animals/pdf/Consensus_document.pdf (accessed on June 15th, 2023). We hereby confirm the following statements: All methods used in this project were carried out in accordance with European guidelines and regulations, as defined by European Directive 2010/63/EU, regarding the protection of animals used for scientific purposes. All methods are reported to be in accordance with the ARRIVE guidelines for the reporting of animal experiments regarding sample size, blinding assays, statistical methods, etc.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Conflicts of Interest: The ImmunoComb® Canine VacciCheck was provided free of charge by Biogal Galed Labs. This product was sent to Dr. Marta E. Garcia. The remaining authors have no competing interests.

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