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Antibody seroprevalence against canine distemper virus, parvovirus, and adenovirus in dogs from a brazilian animal shelter

[*Soroprevalência de anticorpos contra o vírus da cinomose canina, parvovírus e adenovírus em cães de um abrigo de animais brasileiro*]

L. *Galdioli*[1], Y.S.G. *Rocha*[1], S.T.O. *Stedile*[1], F. *Ferreira*[2], R.C.M. *Garcia*[1]

[1]Universidade Federal do Paraná, Curitiba, PR, Brasil
[2]Universidade de São Paulo, São Paulo, SP, Brasil

ABSTRACT

This study aimed to identify and compare the seroprevalence for canine distemper virus (CDV), canine parvovirus (CPV), and canine adenovirus (CAV) between newly admitted and already sheltered dogs. 122 dogs over six months of age and unvaccinated upon admission were sampled and divided into two groups: (A) newly admitted dogs and (B) dogs sheltered for at least two months. Sera were collected to determine IgG antibody titers against CDV, CPV, and CAV. We conducted a descriptive analysis of the samples and a chi-square test to investigate the relationship between potential elements associated with protective antibody titers. The results were: 56.5% of the dogs had protective levels of antibodies to all three pathogens. Dogs in group A had lower titers compared to those in group B for all three pathogens, with significant differences for CDV and CAV. We found no significant difference between the proportion of seropositive dogs and their age or reproductive status. The study suggests that the examined pathogens can circulate in the animal shelter and that dogs can be more exposed to these pathogens in the shelter than in the urban environment. Therefore, an effective immunization program should be carried out on all animals upon admission to shelters.

Keywords: protective antibodies, animal shelters, herd effect, immunization

RESUMO

Este estudo teve como objetivo identificar e comparar a seroprevalência para o vírus da cinomose canina (CDV), do parvovírus canino (CPV) e do adenovírus canino (CAV) entre cães recém-admitidos e já abrigados. Cento e vinte e dois cães com mais de seis meses de idade e não vacinados na admissão foram amostrados e divididos em dois grupos: (A) cães recém-admitidos e (B) cães abrigados por pelo menos dois meses. Os soros foram coletados para determinar os títulos de anticorpos IgG contra CDV, CPV, e CAV. Realizou-se uma análise descritiva das amostras e um teste de qui-quadrado para investigar a relação entre os elementos potenciais associados aos títulos de anticorpos protetores. Os resultados foram: 56,5% dos cães tinham níveis de anticorpos protetores para todos os três patógenos. Os cães do grupo A tinham títulos menores em comparação com os do grupo B para todos os três patógenos, com diferenças significativas para CDV e CAV. Não foi encontrada diferença significativa entre a proporção de cães soropositivos e a sua idade ou o seu estado reprodutivo. O estudo sugere que os patógenos examinados podem circular no abrigo de animais e que os cães podem estar mais expostos a esses patógenos no abrigo do que no ambiente urbano. Portanto, um programa de imunização eficaz deve ser realizado em todos os animais na admissão em abrigos.

Palavras-chave: anticorpos protetores, abrigos para animais, efeito rebanho, imunização

Corresponding author: lucasgaldioli@ufpr.br
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INTRODUCTION

Canine distemper, parvovirus, and infectious hepatitis are caused by highly contagious viruses associated with high mortality rates in dogs (Paul *et al*., 2006; Greene and Appel, 2006; Larson and Schultz, 2006; Taguchi *et al*., 2011; Greene and Levy, 2012; Miranda and Thompson, 2016; Mylonakis *et al*., 2016; Newbury, 2021). In Latin America, canine distemper virus and parvovirus are infectious diseases of clinical relevance and are perceived as a problem due to very low vaccination rates—and, consequently, low herd immunity—in addition to a large population of free-roaming urban dogs that have never been vaccinated (Hartmann *et al*., 2007). The situation can be even worse for unvaccinated dogs or those that have been improperly or insufficiently vaccinated in animal shelters, as they can become infected upon admission (Larson and Schultz, 2006; Horecka *et al*., 2020; Newbury, 2021). Controlling the outbreaks of these diseases in animal shelters is extremely challenging and difficult (Hurley and Miller, 2009; Day *et al*., 2016) due to the lack of effective treatment medications, which can lead to increased numbers of euthanasia, fewer animals being relocated to partner facilities, and loss of space and capacity to admit more animals into shelters until the outbreak is under control (Lechner *et al*., 2010).

Studies with shelter dogs in the United States have found insufficient antibodies against the canine distemper virus (CDV) and canine parvovirus (CPV), with more than half of the animals showing protective antibody titers below adequate at the time of their admission to the shelter (Lechner *et al*., 2010; Litster *et al.*, 2012). Regarding infectious canine hepatitis (ICH), caused by canine adenovirus (CAV), there are reports of outbreaks of this disease in animal shelters in Italy (Decaro *et al*., 2007) and some confirmed cases of CAV-1 infection in Brazil (Inkelmann *et al*. 2007; Headley *et al*., 2018, 2019). Despite this, there are few studies on the prevalence of antibody titers for this disease, and reports of confirmed diagnoses are rare.

One strategy to prevent these diseases in shelters is to vaccinate all animals either upon admission or one week in advance—even those that will remain in the shelter for a few days (Larson and Schultz, 2006; Larson *et al*., 2009; Hurley and Miller, 2009; Lechner *et al*., 2010; Newbury *et al.*, 2010; Spindel, 2013; Scherk *et al*., 2013; Day *et al*., 2016; Stone *et al*., 2020). With the development of vaccines for CDV, CPV, and CAV, widespread vaccination against these diseases has rendered outbreaks uncommon among dogs with guardians. However, outbreaks of CDV and CPV are still frequent in dog kennels and animal shelters worldwide (Pesavento and Murphy, 2014), posing a threat mainly to unvaccinated dogs (Lechner *et al*., 2010; Steneroden *et al*., 2011).

In Brazilian shelters, vaccinating dogs upon admission is not a standard practice, either due to unfamiliarity with a specific procedure established in the literature (Lima and Garcia, 2019) or to financial constraints. Furthermore, there is a gap regarding the immune status of admitted dogs against CDV, CPV, and CAV. Some circumstances observed in Brazilian animal shelters contribute to the propagation of diseases, such as the constant admission of new animals; insufficient physical space; inappropriate sanitary conditions (Cuglovici and Amaral; 2021); prolonged shelter stays; dense dog population; and possible mistakes in preventive care.

Serological tests can help diagnose infections, identify previous exposure to pathogens (particularly in unvaccinated animals), and assess immunity before and/or after vaccination (Böhm *et al*., 2004; Litster *et al*., 2012; Stone *et al*., 2020). Regarding animal shelters, antibody titers against species-specific diseases can be used to assess whether animals are protected against infections—especially CDV and CPV—since antibody levels are closely related to protection against these diseases, as well as to support a more effective approach to preventing and controlling outbreaks (Greene and Schultz, 2006; McCaw and Hoskins, 2006; Larson and Schultz, 2006; Larson *et al*., 2009; Day *et al*., 2016). Thus, this study aimed to identify and compare seroprevalence for canine distemper virus, parvovirus, and infectious hepatitis among newly admitted and long-term sheltered dogs at a Brazilian animal shelter.

MATERIALS AND METHODS

The study was conducted from June to December 2021 in a private animal shelter (non-

governmental organization), located in the municipality of Campo Magro (30,151 inhabitants) (Campo…, 2021), metropolitan region of Curitiba, state of Paraná, Brazil. In 2021, 190 animals were admitted in this shelter, of which 74.7% (142/190) were dogs. It was selected for being representative of most animal shelters in the area, with very dense population, collective stalls, inappropriate practices, and handling regarding the control of infectious diseases, precarious facilities, and no vaccination procedures, as well as history of outbreaks of canine distemper virus and parvovirus.

The dogs were divided into two groups (A and B). Group A consisted of all newly admitted dogs from June to December 2021, with the following criteria: (1) estimated minimum age of six months and (2) within seven days of admission. We chose the minimum age of six months because interpretation of antibody titers results would hardly be mistaken for the presence of maternal antibodies, given that protective titers can remain in some animals up to 20 weeks of age (Day *et al*., 2016). The period of seven days after the admission was due to three reasons: (1) feasibility to transport the researchers to the shelter for sample collection; (2) to match the quarantine period of the newly admitted dogs, when they have no contact with other animals that were already in the shelter; and (3) the test results would indicate the immune status prior to entering the shelter, when the animal was still exposed to its previous environment, since seroconversion requires at least seven days, longer than the quarantine period.

Group B was composed of already sheltered dogs and that were also sampled between June and December 2021, with the following criteria: (1) estimated minimum age of six months; (2) having been admitted to the shelter at least two months before sampling (this time was according to the determination of the shelter manager, who did not register the entries and exits, but knew the period in which the animal was sheltered); and (3) not having been vaccinated either upon admission or during their stay at the shelter. The age range was chosen for the same reason as group A; the accommodation period of at least two months in the shelter was decided because this is the average period dogs stay in the shelter until adoption and because the animals are highly susceptible to contamination in the shelter due to close interaction with other animals and circulation of the studied pathogens. Finally, the absence of vaccination either upon or after admission was chosen to avoid an immune response and seroconversion caused by the vaccine.

In total, 55 dogs were sampled in group A, and 67 in group B.

Information such as the admission date, animal identification (estimated age, sex, and breed), place of origin, and health status upon admission (healthy or unhealthy) was collected using a form. To assess the health status of the dogs, a veterinarian performed physical examinations, which included: assessment of body score (scaled from 1 to 9); evaluation of the oral cavity; cardiac auscultation; thoracic auscultation; secretion or injury assessment; abdominal palpation; and rectal temperature measurement. Blood samples were collected and submitted to serological tests to determine IgG antibody titers against CDV, CPV, and CAV.

The age of the dogs was estimated as young (less than 1 year old), young adult (between 1 and 2 years old), adult (between 2 and 8 years old), and old (more than 8 years old) by examining their dentition. The dogs' vaccination history before shelter admission was unknown. The dogs were considered unhealthy when any conditions associated with diseases or physical injuries were observed during the physical examination, or when a combination of these conditions was identified.

The study was approved by the Ethics and Animal Use Committee under No. 005/2020.

To collect the blood samples, the dogs were manually handled, and the blood was drawn by puncture of either the jugular or the cephalic vein into tubes without anticoagulant. The tubes were centrifuged, and the sera were frozen until analysis.

To determine IgG antibody titers against CDV, CPV, and CAV, the samples were analyzed using the VacciCheck® commercial kit (Biogal Laboratory), following the manufacturer's instructions. This kit is a useful, simple, rapid, and sensitive tool for routine assessment of

antibody titers against CDV, CPV, and CAV in dogs (Killey *et al*., 2018; Nayel *et al*., 2020), presenting a 92% specificity and 100% sensitivity for canine distemper virus; 100% specificity and 88% sensitivity for canine parvovirus; and 93% specificity and 94% sensitivity for canine infectious hepatitis. The kit is based on the Dot-ELISA principle and provides a semi-quantitative assessment of the concentration of antibodies against CDV, CPV, and CAV antigens.

The analysis followed the manufacturer's instructions to determine either positive or negative status of CPV, CDV, and CAV antibodies. A positive result for CAV indicates a titer of 16 in the serum neutralization test; a positive result for CPV indicates a titer of 80 in the hemagglutination inhibition test; and a positive result for CDV indicates a titer of 32 in the serum neutralization test. The results were graded in scores ranging from S0 (negative) to S6 (positive) (Table 1).

Table 1. Interpretation of the serological test in score, diagnosis, level of protection, and conversion of the analyzed titers using the immunocomb vaccicheck® parvovirus & hepatitis igg combscale (biogal laboratory)

Score	Titer conversion			Diagnosis	Level of protection
	Canine infectious hepatitis	Canine parvovirus	Canine distemper virus		
S0	-	-	-	Negative	Low
< S1	-	-	-	Negative	Low
S1–S2	1:4–1:8	< 1:40–1:40	< 1:8–1:16	Weak positive[a]	Low
S3–S4	1:16–1:32	1:80–1:160	1:32–1:64	Positive[b]	Strong
S5–S6	1:64–1:128	1:320– ≥ 1:640	1:128– ≥1:256	Strong positive[b]	Strong

[a] In this study, the "weak positive" diagnosis was considered negative, following the titer concentration and the manufacturer's instructions.
[b] Dogs diagnosed as "positive" and "strong positive" were considered to have sufficient/protective antibody titers.
Source: Adapted from the ImmunoComb VacciCheck® user manual.

We performed a descriptive analysis of the results, and the prevalence of seropositive animals for the studied pathogens was presented. We investigated the association between the potential risk variables and seropositivity for the different pathologies using Pearson's chi-square test. P-value < 0.05 was considered statistically significant.

RESULTS

We collected samples from 122 dogs, 55 from group A and 67 from group B. No dog in either group had been vaccinated for the studied pathogens either upon its admission or during its stay at the shelter. All dogs admitted during the study period were included in group A, except those that did not meet the inclusion criteria.

In group A, 12.7% (7/55) of the population was classified as young, 50.9% (28/55) as young adult, 29.1% (16/55) as adult, and 7.3% (4/55) as old; 98.2% (54/55) were of unknown breed; 70.9% (39/55) were females, of which 48.7% (19/39) were neutered, and 29.1% (16/55) were males, of which 12.5% (2/16) were neutered; and 23.6% (13/55) were considered unhealthy by physical examination. In group B, 3% (2/67) of the population was classified as young, 32.8% (22/67) as young adult, 53.7% (36/67) as adult, and 10.4% (7/67) as old; 100% were of unknown breed; 67.2% (45/67) were females, 32.8% (22/67) were males, and all dogs in this group were neutered, following the shelter policy; and 6% (4/67) were considered unhealthy by physical examination. All dogs in both groups were homeless and had unknown places of origin.

Regarding immune status, just over half of the dogs in group A (56.4%; 31/55) and most dogs in group B (83.6%; 56/67) were seropositive for CDV; most dogs in group A (90.5%; 50/55) and almost all in group B (98.5%; 66/67) were seropositive for CPV; and most dogs in both group A (60%; 33/55) and group B (83.6%; 56/67) were seropositive for CAV (Table 2). The chi-square test showed a statistical difference between the proportion of seropositive dogs for CDV ($p = 0.0019$) and CAV ($p = 0.0067$) in both groups.

Table 2. Immune status of 122 dogs for CDV, CPV and CAV (55 newly admitted dogs [Group A] and 67 dogs sheltered for at least two months [Group B]) in a private shelter in the state of Paraná, Brazil

Immune Status	CDV		CPV		CAV	
	Group A	Group B	Group A	Group B	Group A	Group B
Negative	24/55 (43.6%)	11/67 (16.4%)	5/55 (9.1%)	1/67 (1.5%)	22/55 (40%)	11/67 (16.4%)
Positive	31/55 (56.4%)	56/67 (83.6%)	50/55 (90.9%)	66/67 (98.5%)	33/55 (60%)	56/57 (83.6%)
p-value	0.0019[a]		0.1309		0.0067[a]	

[a] Note: p-value < 0.05 indicates significant difference.
CAV = canine adenovirus. CDV = canine distemper virus. CPV = canine parvovirus

The results showed that 56.5% (69/122) of the dogs had protective levels of antibodies against all three pathogens, with a higher percentage in group B (68.6%; 46/67) when compared to group A (41.8%; 23/55). When only the levels of antibodies against CDV and CPV were considered, 70.5% (86/122) of the study population was seropositive for both diseases, with 82.1% (55/67) seropositive dogs in group B and 56.4% (31/55) in group A. No dog was negative for all three viruses in group B, and in group A, only 5.4% (3/55).

In group A, CAV seropositivity had the same proportion in both younger (young and young adult) (60%; 21/35) and older dogs (adult and old) (60%; 12/20); positive CPV titers were slightly less prevalent in younger dogs (88.6%; 31/35) than in the older ones (95%; 19/20); and CDV seropositivity was more prevalent in younger dogs (51.4%; 18/35) than in the older ones (35%; 7/20).

In group B, CAV seropositivity was similar between younger (83.3%; 20/24) and older dogs (83.7%; 36/43); for CPV, 100% (24/24) of the younger dogs and 97.7% (42/43) of the older ones had positive titers; and for CDV, 87.5% (21/24) of the younger dogs and 81.4% (35/43) of the older ones were seropositive. In the statistical analysis, the chi-square test showed no significant difference between the three studied pathogens when the older dogs were compared with the younger ones, both within the groups and among the entire population (Table 3).

Table 3 - Association of CDV, CPV and CAV seropositivity and the age of the dogs per group (A and B).

Group	CDV		CPV		CAV	
	Younger	Older	Younger	Older	Younger	Older
A	18/35 (51.4%)	7/20 (35%)	31/35 (88.6%)	19/20 (95%)	21/35 (60%)	12/20 (60%)
p-value	0.4879		0.7564		1.0000	
B	21/24 (87.5%)	35/43 (81.4%)	24/24 (100%)	42/43 (97.7%)	20/24 (83.3%)	36/43 (83.7%)
p-value	0.7620		1.0000		1.0000	
Total	39/59 (66.1%)	42/63 (66.7%)	55/59 (93.2%)	61/63 (96.8%)	41/59 (69.5%)	48/63 (76.2%)
p-value	0.3026		0.6162		0.5297	

Note: p-value < 0.05 indicates significant difference.
CAV = canine adenovirus. CDV = canine distemper virus. CPV = canine parvovirus

In group B, all dogs were neutered, whereas, in group A, only 38.2% (21/55) were neutered. Overall, 57.1% (12/21) of the neutered dogs in group A were seropositive for CDV, 95.2% (20/21) for CPV, and 66.6% (14/21) for CAV. Still in group A, of the neutered females, most were seropositive for CDV (57.9%; 11/19), almost all for CPV (94.7%; 18/19), and most for CAV (68.4%; 13/19); as for the two neutered males, one was seropositive for CDV (50%), both for CPV (100%), and one for CAV (50%). Regarding the unneutered animals in group A (61.8%; 34/55), 55.9% (19/34) were seropositive for CDV, 88.2% (30/34) for CPV, and 55.9%

(19/34) for CAV. In the statistical analysis, the chi-square test showed no significant difference between the three studied pathogens when the neutered dogs were compared with the unneutered dogs in group A (p-value CDV = 1; p-value CPV = 0.6929; p-value = 0.6101).

DISCUSSION

This prospective study was conducted at a private animal shelter and revealed that most dogs had protective antibody titers against CDV, CPV, and CAV, with more than half of the dogs being seropositive for all three pathogens simultaneously. In the present study, the finding that the dogs had higher protective antibody titers against CPV than CDV and CAV may be related to the superior durability of CPV in the environment outside the host, providing more opportunities for natural exposure (McCaw and Hoskins, 2006).

Group A (newly admitted dogs) showed 56.4% (31/55), 90.5% (50/55), and 60% (33/55) seropositivity for CDV, CPV, and CAV, respectively. Studies conducted in the United States and Mexico found lower values for CDV and CPV compared to our study: they revealed 68.6% (35/51), 84.3% (43/51) (Litster *et al*., 2012), 31.5% (136/341) (Lechner *et al*., 2010) and 40% (422/1052) (Spindel *et al*., 2018) seroprevalence for CPV; and 37.3% (19/51), 41.2% (21/51) (Litster *et al*., 2012), 7.7% (33/431) (Lechner *et al*., 2010), and 4% (37/1052) (Spindel *et al*., 2018) for CDV. For CAV, a study carried out with unvaccinated shelter dogs in Turkey identified lower seropositivity (45.4%; 35/77) (Bulut *et al*., 2013) compared to our study.

Lechner *et al*. (2010) found that 64.5% (278/431) of the dogs in their study had insufficient antibody titers against CDV and CPV upon admission to a municipal animal shelter in the United States. In our study, the value of insufficient protective antibody titers against CDV and CPV in the group of newly admitted dogs was lower (43.6%; 24/55); however, when all three pathogens were considered, a higher percentage of dogs with insufficient protective antibody titers was observed in the shelter of our study (58.2%; 32/55).

We could not compare the seroprevalence of group B with other literature due to the lack of studies performing titration for dogs that stay longer in shelters and have unknown vaccination history, as most international shelters follow recommended guidelines and vaccinate the dogs upon admission. These shelters also have a higher turnover of animals and shorter stays. Nevertheless, we suggest the presence of the pathogens at the shelter of our study due to the high seropositivity presented by the dogs in group B, which had been at the shelter for longer and whose previous vaccination history was unknown upon admission, with no vaccines being administered during their stay. On the other hand, we cannot rule out the possibility of the seroprevalence of antibodies coming from natural infection or vaccination prior to admission among dogs in this group since a previous study has indicated that almost all dogs have antibodies against CPV and CDV after vaccination, even those with unknown vaccination histories (Litster *et al*., 2012). Other studies on serological challenges have also demonstrated that the immunity for CDV, CPV, and CAV is long-term, lasting up to nine years or more, depending on vaccine technology (Böhm *et al*., 2004; Ottiger *et al*., 2006; Larson and Schultz, 2006; Schultz *et al*., 2010; Taguchi *et al*., 2011; Mitchell *et al*., 2012; Day *et al*., 2016). Furthermore, the dogs' increased antibody titers may be explained due to natural boosters, which, in turn, would also explain the high seropositivity for all three pathogens in group B, and support the hypothesis of these pathogens circulating in the shelter. Böhm *et al*. (2004) suggest that the dogs in their study were likely to have had a natural boost against CPV, as they had significantly higher titers three years after primary vaccination compared to two weeks later.

In our study, newly admitted dogs (group A) showed lower titers for all three pathogens when compared to dogs that had been at the shelter for over two months (group B); the chi-square test also showed significant differences for CDV (p = 0.0019) and CAV (p = 0.0067). Protective antibody titers are usually not detected up to two weeks after exposure (Pollock and Carmichael, 1982; Larson and Schultz, 2006; Litster *et al*., 2012). Thus, all seropositive dogs in group A—which were quarantined at the shelter and had samples collected up to seven days after

admission—were likely exposed to the pathogens prior to their admission. This indicates that dogs may be more exposed to these pathogens in the shelter than in the urban environment (domiciled dogs, semi-domiciled dogs, and/or free-roaming dogs). To further investigate this hypothesis, the antibody titers of dogs in group A should have been analyzed throughout their stay at the shelter to verify whether their titers would be naturally boosted due to exposure to the studied viruses in the shelter; therefore, this is one of the limitations of our study. Based on the same hypothesis, a study in Philadelphia (USA) analyzed the results of the seroprevalence for canine influenza virus (CIV) in dogs at an animal shelter and suggested that dogs were more exposed to the pathogen at the shelter than in the urban environment (Holt *et al*., 2010).

Another limitation of the present study—and which is common in many seroprevalence surveys—concerns the fact that some results can be false-negative and false-positive even though the accuracy of the used antibody test has been validated. In our study, the VacciCheck™ test kit that was used in had a sensitivity of 88% to 100% and specificity of 92% to 100% for the three different antigenic components, as serum antibody titers are one way to assess immune protection in both vaccinated and unvaccinated animals (Mazar *et al*., 2009; Butler and Crawford, 2013; Meazi *et al*., 2022). It is worth mentioning that although fewer dogs were seronegative—i.e., presented low protective antibody levels at the time of testing—they may have responded adequately to prior vaccination or natural infection and, therefore, have immunological memory. Memory cells allow a rapid and efficient response, which may be sufficient to prevent the development of clinical disease even when antibody titers have decreased to low or undetectable concentrations (Tizard and Ni, 1998). Early protection probably occurs through stimulation of innate and cell-mediated immunity prior to the development of detectable seroconversion; thus, while protective antibodies are associated with resistance to infection, lower antibody titers do not necessarily indicate susceptibility to infection (Greene and Schultz, 2006).

Younger (young and young adult) and older (adult and old) dogs had similar seroprevalence in our study. The chi-squared test showed no significant differences when we evaluated the proportion of seropositivity and age in each group (A and B) separately, as well as in the entire study population. Previous studies conducted in the United States have reported that older dogs were more likely to have positive antibody titers for CPV (Litster *et al*., 2012; Lechner *et al*., 2010) and CDV (Lechner *et al*., 2012). In Japan, a study evaluating antibody titers against the same three viruses has also found significantly higher seropositivity for CDV in older dogs than in younger ones; however, antibodies against CPV were significantly higher in younger dogs compared to the older ones, and the titer for CAV was not associated with age (Taguchi *et al*., 2011).

There was no association between neutering and seroprevalence in the dogs in group B since all of them were neutered by shelter policy. In the neutered dogs in group A, we observed seropositivity of 57.1%, 95.2%, and 66.6% for CDV, CPV, and CAV, respectively. The chi-square test showed no significant difference in seropositivity between neutered and unneutered dogs. Lechner *et al.* (2010) found that the proportion of neutered dogs with protective antibody titers against CPV and CDV was significantly higher than the proportion of unneutered dogs; the authors reported that unneutered dogs were 8.3 times more likely to have protective antibodies against CDV, and 3.4 times more likely to have protective antibodies against CPV when compared to unneutered dogs. In our study, this finding may be explained by the fact that newly admitted dogs who were already neutered probably had guardians at some point in their lives and could also have been fully or partially vaccinated against the studied pathogens. As previously stated, we cannot rule out the possibility of exposure and infection by these pathogens in the urban environment, given their high morbidity rates.

Studies have already suggested a vaccination coverage of 70–75% as the minimum adequate to prevent disease outbreaks in populations of dogs with guardians (Horzinek, 2006; Riedl *et al*., 2015; Day *et al*., 2016). However, vaccination coverage should probably be higher to be effective in shelters. It is also of utmost importance that vaccination protocols be followed through, according to the specific

guidelines published for animal shelters (Decaro *et al*., 2020), to ensure a "herd effect"—i.e., to guarantee susceptible members of a certain population an indirect protection against a pathogen through immune members, as the risk of exposure to an infected individual decreases. This effect can be determined by the strength of previous infection transmission and recovery, as well as by herd immunity, that is, the proportion of immune individuals in each population (John and Samuel, 2000; Andrukonis *et al*., 2021). In our study, although a higher percentage of dogs with protective antibody levels against the studied pathogens were admitted to the shelter, the seroprevalence for CDV and CAV was insufficient to ensure a herd effect. A Brazilian study that evaluated 19 shelters reported that in 57.9% (11/19) of them, vaccination against rabies, parvovirus, canine distemper virus, leptospirosis, and infectious canine hepatitis was not performed (Cuglovici and Amaral, 2021).

We found seropositivity above 83% for all three pathogens in group B, suggesting that there was a herd effect in that environment. However, it was not possible to determine whether herd immunity was ensured by transmission of previous infection and recovery of the animals, or by possible vaccination prior to admission to the shelter.

Another limitation of our study concerns the ages, as they were only estimates based on the veterinarian's evaluation of the dogs' dentition. The age range (young, young adult, adult, and old) was adopted to reduce possible errors. Additionally, the number of days that dogs in group B stayed at the studied shelter was not assessed due to lack of entry and exit records; thus, it was not possible to identify whether the amount of time the dogs spent in the shelter was a risk factor associated with seropositive test results. According to Cuglovici and Amaral (2021), only 15.8% (3/19) of the managers of the Brazilian shelters they analyzed recorded the entry and exit of dogs over 12 months.

Finally, seroprevalence results for CDV, CPV, and CAV of the dog population in the studied shelter may not be representative of the entire dog population in Brazilian shelters, reinforcing the need for More studies in different shelters and regions.

CONCLUSION

Most dogs in the present study had protective antibody titers against CDV, CPV, and CAV, with more than half presenting seropositivity for all three pathogens simultaneously. Newly admitted dogs (group A) had insufficient levels of protective antibodies against CDV and CAV—but not against CPV—to ensure a herd effect. Dogs in group A also presented lower titers for all three pathogens compared to dogs in group B, which had been at the shelter for over two months, with the chi-squared test pointing to significant differences for CDV and CAV. Our study suggests a circulation of pathogens that cause parvovirus, canine infectious hepatitis, and canine distemper in the shelter, and that more dogs may be exposed to these pathogens in the shelter than in the urban environment.

Thus, due to the high risk of infectious disease outbreaks in animal shelters and the high morbidity and mortality rates of CDV, CPV, and CAV, an effective immunization program—capable of ensuring "herd effect"—aimed at the control, elimination, or eradication of vaccine-preventable infectious diseases should be rigorously carried out on all animals upon admission to shelters, especially considering that vaccination is the only method to protect individuals from these diseases and prevent the spread of these viruses in the population.

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REFERENCE

ANDRUKONIS, A.; BROWN, K.M.; HALL, N.J. *et al*. Intake vaccinations reduced signs of canine respiratory disease during an outbreak at an animal shelter. *Front. Vet. Sci.*, v.8, p.627580, 2021.

BÖHM, M.; THOMPSON, H.; WEIR, A. *et al.* Serum antibody Titres to canine parvovirus, adenovirus and distemper virus in dogs in the UK which had not been vaccinated for at least three years. *Vet. Rec.*, v.154, p.457-463, 2004.

BULUT, O.; YAPICI, O.; AVCI, O. *et al*. The serological and virological investigation of canine adenovirus infection on the dogs. *Sci. World J.*, v.2013, p.587024, 2013.

BUTLER, B.; CRAWFORD, P. Id-27 accuracy of a point-of-care immunoassay to determine protective antibody titers for canine parvovirus and canine distemper virus. *J. Vet. Intern. Med.*, v.27, n.3, 2013.

CAMPO MAGRO. Censo Brasileiro de 2010. Rio de Janeiro: IBGE, 2021. Available in: https://cidades.ibge.gov.br/brasil/pr/campo-magro/panorama. Accessed in: 16 Jan. 2021.

CUGLOVICI, D.A.; AMARAL, P.I.S. Dog welfare using the Shelter Quality Protocol in long-term shelters in Minas Gerais State, Brazil. *J. Vet. Behav.*, v.45, p.60-67, 2021.

DAY, M.J.; HORNIZEK, M.C.; SCHULTZ, R.D. *et al*. WSAVA guidelines for the vaccination of dogs and cats. *J. Small Anim. Pract.*, v.57, p.E1, 2016.

DECARO, N.; BUONAVOGLIA, C.B.V.R.; BARRS, V.R. Canine parvovirus vaccination and immunisation failures: Are we far from disease eradication? *Vet. Microbiol.*, v.247, p.108760, 2020.

DECARO, N.; CAMPOLO, M.; ELIA, G. *et al.* Infectious canine hepatitis: an "old" disease reemerging in Italy. *Res. Vet. Sci.*, v.83, p.269-273, 2007.

GREENE, C.E.; APPEL, M.J. Canine distemper. In: GREENE, C.E. (Ed.). *Infectious diseases of the dog and cat*. 3.ed. Philadelphia: Elsevier, 2006. p.25-41.

GREENE, C.E.; SCHULTZ, R.D. Immunoprophylaxis and immunotherapy. In: GREENE, C.E. (Ed.). *Infectious diseases of the dog and cat*. 3.ed. Philadelphia: WB Saunders, 2006. p.1069-1119.

GREENE, C.E.; LEVY, J.K. Imunoprofilaxia. In: GREENE, C. E, ed. *Infectious Diseases of the Dog and Cat*. 4 ed. Filadélfia: Saunders Elsevier Co, 2012. p. 1163–204.

HARTMANN, T.L.S.; BATISTA, H.B.D.C.R.; DEZEN, D. *et al*. Neutralizing antibodies to distemper and parainfluenza viruses in dogs in shelter kennels in the municipalities of Novo Hamburgo and Porto Alegre, RS, Brazil. *Ciênc. Rural*, v.37, p.1178-1181, 2007.

HEADLEY, S.A.; MELLO Z.M.; ELIAS B. *et al*. Disseminated melanized fungal infection due to Cladosporium halotolerans in a dog coinfected with canine adenovirus-1 and canine parvovirus-2. *Braz. J. Microbiol.*, v.50, p.859-870, 2019.

HEADLEY, S.A.; OLIVEIRA, T.E.; PEREIRA, A.H. *et al*. Canine morbillivirus (canine distemper virus) with concomitant canine adenovirus, canine parvovirus-2, and Neospora caninum in puppies: a retrospective immunohistochemical study. *Sci. Rep.*, v.8, p.1-16, 2018.

HOLT, D.E.; MOVER, M.R.; BROWN, D.C. Serologic prevalence of antibodies against canine influenza virus (H3N8) in dogs in a metropolitan animal shelter. *J. Am. Vet. Med. Assoc.*, v.237, p.71-73, 2010.

HORECKA, K.; PORTER, S.; AMIRIAN, E.S. *et al*. A decade of treatment of canine parvovirus in an animal shelter: a retrospective study. *Animals*, v.10, p.939, 2020.

HORZINEK, M.C. Vaccine use and disease prevalence in dogs and cats. *Vet. Microbiol.*, v.117, p.2-8, 2006.

HURLEY, K.F.; MILLER, L. Introduction to disease management in animal shelters. Infectious disease management in animal shelters. In: MILLER, L.; HURLEY, K. (Eds.). *Infectious disease management in animal shelters*. New York: John Wiley & Sons, 2009. p.2-16.

INKELMANN, M.A.; ROZZA, D.B.; FIGHERA, R.A. *et al.* Infectious canine hepatitis: 62 cases. *Pesqui. Vet. Bras.*, v.27, p.325-332, 2007.

JOHN, T.J.; SAMUEL, R. Herd immunity and herd effect: new insights and definitions. *Eur. J. Epidemiol.*, v.16, p.601-606, 2000.

KILLEY, R.; MYNORS, C.; PEARCE, R. *et al*. Long-lived immunity to canine core vaccine antigens in UK dogs as assessed by an in-practice test kit. *J. Small Anim. Pract.*, v. 59, p.27-31, 2018.

LARSON, L.; NEWBURY, S.; SCHULTZ, R.D. Canine and feline vaccinations and immunology. In: MILLER, L.; HURLEY, K. (Eds). *Infectious disease management in animal shelters*. Ames, IA: Wiley–Blackwell, 2009. p.61-82.

LARSON, L.J.; SCHULTZ, R.D. Effect of vaccination with recombinant canine distemper virus vaccine immediately before exposurem under shelter-like conditions. *Vet. Ther*, v.7, p.113-118, 2006.

LECHNER, E.S.; CRAWFORD, P.C.; LEVY, J.K. *et al*. Prevalence of protective antibody titers for canine distemper vírus and canine parvovirus in dogs entering a Florida animal shelter. *J. Am. Vet. Med. Assoc.*, v.236, p.1317-1321, 2010.

LIMA, L.; GARCIA, R.C.M. Experiência em medicina veterinária de abrigos. In: GARCIA, R.C.M.; CALDERÓN, N.; BRANDESPIM, D. *Medicina veterinária do coletivo:* fundamentos e práticas. [s.l.]: Integrativa Vet Brasil, 2019. p.326-327, 2019.

LITSTER, A.; NICHOLS, J.; VOLPE, A. Prevalence of positive antibody test results for canine parvovirus (CPV) and canine distemper virus (CDV) and response to modified live vaccination against CPV and CDV in dogs entering animal shelters. *Vet. Microbiol.*, v.157, p.86-90, 2012.

MAZAR, S.; LARSON, L.; LAVI, Y. Sensitivity-specificity accuracy and difference between positive and negative mean results of the ImmunoComb® Canine VacciCheck Antibody Test kit for canine distemper, parvo and adenovirus, 2009. Available in: https://vaccicheck.com/feline/wp-content/uploads/2014/02/sensitivity-specificty.pdf. Accessed in: 16 Jan. 2021

MCCAW, D.L.; HOSKINS, J.D. Canine viral enteritis. In: GREENE, C.E. (Ed.). *Infectious diseases of the dog and cat.* 3.ed. Philadelphia: Elsevier Inc., 2006. p.63-73.

MEAZZI, S.; FILIPE, J.; FIORE, A. *et al*. Agreement between in-clinics and virus neutralization tests in detecting antibodies against Canine Distemper Virus (CDV). *Viruses*, v.14, p.517, 2022.

MIRANDA, C.; THOMPSON G. Canine parvovirus: the worldwide occurrence of antigenic variants. *J. Gen. Virol.*, v.97, p.2043-2057, 2016.

MITCHELL, S.A.; ZWIJNENBERG, R.J.; HUANG, J. *et al*. Duration of serological response to canine parvovirus-type 2, canine distemper virus, canine adenovirus type 1 and canine parainfluenza virus in client-owned dogs in Australia. *Aust. Vet. J.*, v.90, p.468-473, 2012.

MYLONAKIS, M.; KALLI, I.; RALLIS, T. Canine parvoviral enteritis: an update on the clinical diagnosis, treatment, and prevention. *Vet. Med. Res. Rep.*, v.7, p.91-100, 2016.

NAYEL, M.; ZAGHAWA, A.; ZAYED, A. *et al.* Phylogenetic analysis and immunohematological response associated with two commercially available vaccines against canine distemper and canine parvovirus. *Vet. Med. Public Health J.*, v.1, p.5-14, 2020.

NEWBURY, S. Canine distemper virus. In: MILLER, L.; JANECZKO, S.; HURLEY, K. 2.ed. *Infectious disease management in animal shelters.* Ames, Iowa: Wiley-Blackwell, 2021. p.256-273.

NEWBURY, S.; BLINN, M.K.; BUSHBY, P.A. *et al*. Guidelines for standards of care in animal shelters. *Assoc. Shelter Vet*., p.1-64, 2010.

OTTIGER, H.P.; NEIMEIER-FÖRSTER, M.; BRUCKNER, L. *et al*. Serological responses of adult dogs to revaccination against distemper, parvovirus and rabies. *Vet. Rec.*, v.159, p.7-12, 2006.

PAUL, M. A. ; APPEL, M. ; BARRETT, R. *et al*. Report of the American Animal Hospital Association (AAHA) Canine Vaccine Task Force: executive summary and 2003 canine vaccine guidelines and recommendations. *J. Am. Anim. Hosp. Assoc.*, v. 39, n. 2, p. 119, 2003.

PESAVENTO, P.A.; MURPHY, B.G. Common and emerging infectious diseases in the animal shelter. *Vet. Pathol.*, v.51, p.478-491, 2014.

POLLOCK, R.V.; CARMICHAEL, L.E. Dog response to inactivated canine parvovirus and feline panleukopenia virus vaccines. *Cornell Vet*., v.72, p.16-35, 1982.

RIEDL, M.; TRUYEN, U.; REESE, S. *et al*. Prevalence of antibodies to canine parvovirus and reaction to vaccination in client-owned, healthy dogs. *Vet. Rec*., v.177, p.597-597, 2015.

SCHERK, M. A.; FORD, R. B.; GASKELL, R. M. *et al*. 2013 AAFP feline vaccination advisory panel report. *J. Feline. Med. Surg.*, v. 15, n. 9, p. 785-808, 2013.

SCHULTZ, R.D.; THIEL, B.; MUKHTAR E. *et al*. Age and long-term protective immunity in dogs and cats. *J. Comp. Psychol*., v.142, p.S102-S108, 2010.

SPINDEL, M. Strategies for management of infectious diseases in a shelter. In: ZAWISTOWSKI, S.; MILLER, L. *Shelter Medicine for Veterinarians and Staff* (Second). Ames, IA: Wiley-Blackwell, 2013. p. 279–86.

SPINDEL, M.E.; KRECIC, M.R.; SLATER, M.R. *et al*. Evaluation of a community's risk for canine parvovirus and distemper using antibody testing and GIS mapping of animal shelter intakes. *J. Appl. Anim. Welf. Sci.*, v.21, p.362-374, 2018.

STENERODEN, K.K.; HILL, A.E.; SALMAN, M.D. A needs-assessment and demographic survey of infection-control and disease awareness in western US animal shelters. *Prev. Vet. Med.*, v.98, p.52-57, 2011.

STONE, A.E.; BRUMMET, G.O.; CAROZZA, E.M. *et al*. AAHA/AAFP feline vaccination guidelines. *J. Feline Med. Surg.*, v.22, p.813-830, 2020.

TAGUCHI, M.; NAMIKAWA, K.; MARUO, T. *et al*. Antibody titers for canine parvovirus type-2, canine distemper virus, and canine adenovirus type-1 in adult household dogs. *Can. Vet. J.*, v.52, p.983, 2011.

TIZARD, I.; NI, Y. Use of serologic testing to assess immune status of companion animals. *J.* Am. Vet. Med. Assoc., v.213, p.54-60, 1998.