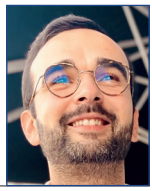
Health Sentinels: Canine Parvovirus and Coronavirus in Portuguese Shelter Dogs



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Abstract

This study investigates the prevalence of Canine Parvovirus (CPV) and Canine Coronavirus (CCoV) among shelter dogs in Portugal. Despite advancements in veterinary medicine and widespread vaccination efforts, CPV and CCoV continue to pose significant health threats to the canine population, particularly in high-density environments such as shelters. Through a cross-sectional study involving 240 shelter dogs in five municipalities in Portugal, this study utilised an immunochromatographic technique for the simultaneous detection of CPV and CCoV antigens. The findings reveal a 6.2% and 9.2% prevalence of CPV and CCoV, respectively, with a co-infection prevalence of 4.6%, highlighting the persistent challenge these viruses represent. The study further explores the lack of significant association between infection prevalence and variables such as age, sex, breed, and municipality, suggesting that susceptibility to these infections may be broadly distributed among shelter dogs. By providing new insights into the epidemiology of CPV and CCoV within Portuguese shelters, this study contributes to the body of knowledge necessary for developing targeted strategies to manage and prevent these infectious diseases in high-risk canine populations.

Key words: *CCoV; CPV; dogs; Portugal; shelter*

Introduction

Canine parvovirus (CPV) and canine coronavirus (CCoV) are two highly contagious pathogens that pose important health threats to the canine population, particularly in densely populated environments such as animal shelters (Decaro et al., 2011; Horecka et al., 2020). These viruses are responsible for severe and potentially life-threatening diseases in dogs, making them a considerable concern for animal welfare organisations and veterinary professionals. The effective manage-

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ment and containment of CPV and CCoV outbreaks are vital for safeguarding the health of shelter dogs and preventing the spread of these pathogens to the broader dog population (Mazzaferro, 2020; DeTar et al., 2022).

CPV, a member of the *Parvoviridae* family, was first recognised in 1978 (Zhao et al., 2011) and is characterised by its high stability in the environment, allowing it to persist in contaminated areas for extended periods (Doyle, 2021). This virus primarily targets the rapidly dividing cells of the gastrointestinal tract and bone marrow, leading to clinical signs such as severe gastroenteritis, dehydration, lethargy, and often a high mortality rate if left untreated (Tuteja et al., 2022). Less reported is the possibility of puppies infected during the perinatal period developing parvoviral myocarditis (Dines et al., 2023). However, parvoviral enteritis is a considerable cause of morbidity and mortality despite vaccination protocols (Doyle, 2021). CCoV, on the other hand, is a member of the *Coronaviridae* family and, although it typically causes mild gastrointestinal clinical signs in infected dogs, it can exacerbate signs when co-infecting with CPV or other pathogens (Buonavoglia et al., 2023). Despite enteric CCoV infection being characterised by high morbidity and low mortality, serology and virology studies have shown that CCoV is extensively present across the dog population, with a notably high occurrence in kennels and animal shelters (Decaro and Buonavoglia, 2008, 2011).

Animal shelters play a crucial role in providing temporary housing and care for stray, abandoned, and relinquished dogs, making them vulnerable to the introduction and rapid spread of these viral diseases (Turner et al., 2012; Horecka and Neal, 2022). CPV and CCoV outbreaks in shelter environments can result in substantial economic losses due to the cost of medical treatment, euthanasia, and facility decontamination. Moreover, the welfare of sheltered dogs is at risk, as these diseases can cause suffering and increase the likelihood of euthanasia for infected animals (Horecka et al., 2020).

A recent survey involving Portuguese municipal and non-profit private animal shelters revealed that CPV (62%) was one of the most frequently reported infections, highlighting its impact and presence despite vaccination efforts (Marques et al., 2023). Canine coronavirus, although less common (12%), was also indicated. Despite the considerable impact highlighted by the study of Marques et al. (2023), a lack of comprehensive research on the prevalence of CPV and CCoV remains in Portugal, particularly in shelter environments.

Traditional diagnostic methods for CPV and CCoV, such as polymerase chain reaction (PCR) and enzyme-linked immunosorbent assay (ELISA), are accurate but often require sophisticated laboratory equipment and time-consuming procedures. In shelter settings, rapid and cost-effective screening methods are essential for early detection, isolation, and treatment of infected animals to minimise disease spread (Marques et al., 2023). Point-of-care faecal ELISA antigen testing remains the diagnostic test of choice for CPV in the shelter setting (Doyle, 2021).

Despite vaccination against CPV-2 strains, the disease continues to be of significant concern in both veterinary medicine and economically (Mazzaferro, 2020).

While CPV and CCoV have seen declines in occurrence among domesticated dog populations following successful vaccination campaigns, the situation in animal shelters presents a more complex challenge. Notwithstanding overall pro-

gress, geographical and socioeconomic factors leading to pockets of unvaccinated populations and sporadic disease outbreaks, these diseases continue to threaten the welfare of stray and shelter dogs (Day et al., 2016).

Despite some studies in wildlife and one study on domestic dog population, there is a lack of studies specifically addressing the prevalence of CPV and CCoV within Portuguese shelters (Santos et al., 2009; Duarte et al., 2013; Miranda et al., 2016; Rosa et al., 2020).

The prevalence of CPV and CCoV in such settings remains a significant concern, necessitating a deeper understanding to improve management and containment strategies. This study aims to explore the prevalence of these viruses in Portuguese shelters, offering new insights into the epidemiology of CPV and CCoV within these high-risk communities.

Material and Methods

Population and samples

Faeces were collected individually from each dog during routine medical check-ups upon admission. A swab was used to collect the sample from the rectum.

To detect CPV and CCoV, a kit based on an immunochromatographic technique (PARVO-CORONA Diagnostic Kit Uranotest®, Urano®vet, S.L, Barcelona, Spain) was used following the manufacturer's instructions. This test allows simultaneous detection of the CPV and CCoV antigens.

The PARVO-CORONA Diagnostic Kit Uranotest® reported a sensitivity of 100% versus hemagglutination, a specificity of 99% versus hemagglutination, both for CPV, and a sensitivity of 94% versus real-time PCR and a specificity of 97% also versus real-time PCR, both for CCoV.

This study was approved by the Ethics Committee of the University of Trás-os-Montes e Alto Douro (UTAD) (process reference: Doc6-CE-UTAD-2022).

Data analysis

Statistical analysis was conducted using JMP® 17.2.0 (SAS Institute Inc., SAS Campus Drive, Cary, NC, USA). Chi-square tests were initially utilised to assess the relationship between categorical independent variables (sex, breed, hair type, and municipality) and the proportions of positivity for CPV and CCoV, as well as co-infections of both viruses.

Age was treated as a continuous variable in a logistic regression analysis to determine its effect on the likelihood of testing positive for CPV, CCoV, and their co-infections. The model's goodness-of-fit was evaluated using the likelihood ratio test (-2 Log likelihood), and the significance of the age predictor was determined using the Wald chi-square test.

Results

During the study period, 240 dogs entering an official shelter were tested. The mean age was 67 days, and there were 133 females (55.4%) and 107 males (44.6%). The sample population was sex-balanced (Tables 2, 3 and 4). All dogs were sexually intact.

Dogs came from the five municipalities of Terra Quente Transmontana: Mirandela (31.2%, n = 75), Macedo de Cavaleiros (22.5%, n = 54), Vila Flor (18.8%, n = 45), Alfândega da Fé (16.6%, n = 40), and Carrazeda de Ansiães (10.8%, n = 26). The 240 samples were collected from 2020 until 2023: 2020 (34.6%, n = 83), 2021 (38.3%, n = 92), 2022 (19.6%, n = 47) and 2023 (7.5%, n = 18) (Figure 1).

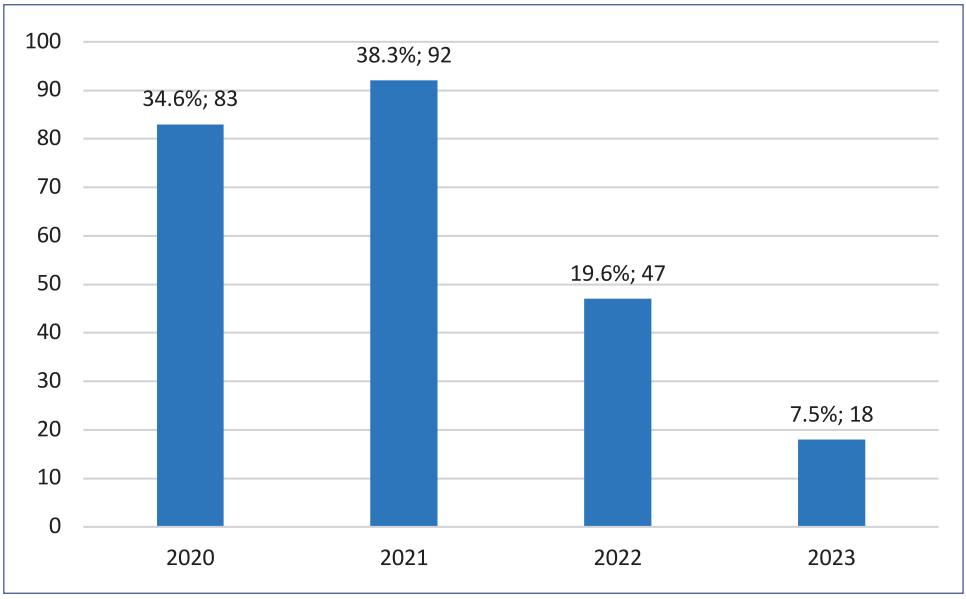

Figure 1. Number of dogs tested yearly between 2020 and 2023.

The overall seroprevalence of CPV and CCoV was 20.0% (48/240, 95% confidence interval [CI]: 15.4–25.5) of the dogs tested (Table 1). The proportion of dogs positive to CPV only, CCOV only, and both CPV and CCOV was 6.2% (15/240, 95% CI: 3.8–10.1%), 9.2% (22/240, 95% CI: 6.1–13.5%) and 4.6% (11/240, 95% CI: 2.6–8.0%), respectively.

Table 2 presents the seroprevalence of CPV based on different variables, such as sex, breed, hair length, and municipality.

No statistically significant differences were found between the categories of the independent variables sex, breed, hair length, and municipality (Table 2). The overall prevalence of CPV was 6.2% (15/240, 95% CI: 3.8–10.1).

Concerning CCoV prevalence, no statistical association was found between CCoV and the independent variables of sex, breed, and municipality (Table 3). A significant difference (p = 0.045) was found in the prevalence between medium (50.0%) and short-haired dogs (8.8%). However, the confidence interval for medium-haired dogs was extremely wide (9.5–90.5%), since only two dogs being tested, indicating a high degree of uncertainty. The narrower CI for short-haired dogs (5.8–13.1%) provides a more reliable estimate of prevalence within this group. The overall prevalence of CCoV was 9.2% (22/240, 95% CI: 0.9–4.4).

Table 4 provides insights into the seroprevalence of both CPV and CCoV based on independent variables of sex, breed, hair length, and municipality. No significant differences were observed in CPV and CCoV prevalence based on sex, breed, or municipality (Table 4). However, a statistically significant association between hair length and CCoV presence was identified ($P = 0.045$). This should be interpreted with caution due to the limited number of observations for medium hair length, which consists of only two cases, thereby diminishing the value of this statistical significance. The overall seroprevalence of CPV and CCoV was 4.6% (11/240, 95% CI: 2.6–8.0).

Table 1. Seroprevalence of Canine Parvovirus (CPV) and Canine Coronavirus (CCoV) in stray dogs (n = 240) between 2020 and 2023.

Pathogen	Seropositive dogs	Prevalence (%)	95% CI
CPV (only)	15	6.2	3.8–10.1
CCoV (only)	22	9.2	6.1–13.5
CPV and CCoV	11	4.6	2.6–8.0
Overall seroprevalence	48	15.3	15.4–25.5

Table 2. Prevalence of Canine Parvovirus by sex, breed, hair length, and municipality.

Variable	Title	Dogs tested (n)	Relative distribution (%)	Positive (n)	Positive (%)	95% CI
Sex (P = 0.365)	Female	133	55.4	10	7.5	4.1–13.3
	Male	107	44.6	5	4.7	2.0–10.5
Breed (P = 0.560)	Defined	5	2.1	0	0.0	0.0–43.4
	Moggy	235	97.9	15	6.4	3.9–10.3
Hair (P = 0.714)	Medium	2	0.8	0	0	0.0–65.8
	Short	238	99.2	15	6.3	3.9–10.1
Municipality (P = 0.122)	Medium	75	31.2	2	2.7	0.7–9.2
	Small	165	68.8	13	7.9	4.7–13.0
Total	All	240	100	15	6.2	3.8–10.1

Table 3. Prevalence of Canine Coronavirus by sex, breed, hair, and municipality.

Variable	Title	Dogs tested (n)	Relative distribution (%)	Positive (n)	Positive (%)	95% CI
Sex (P = 0.416)	Female	133	55.4	14	10.5	6.4–16.9
	Male	107	44.6	8	7.5	3.8–14.1
Breed (P = 0.473)	Defined	5	2.1	0	0.0	0.0–43.4
	Moggy	235	97.9	22	9.4	6.3–13.8
Hair (P = 0.045)	Medium	2	0.8	1	50.0	9.5–90.5
	Short	238	99.2	21	8.8	5.8–13.1
Municipality (P = 0.305)	Medium	75	31.2	9	12.0	6.4–21.3
	Small	165	68.8	13	7.9	4.7–13.0
Total	All	240	100	22	9.2	6.1–13.5

Table 4. Seroprevalence of Canine Parvovirus and Canine Coronavirus by sex, breed, hair length, and municipality.

Variable	Title	Dogs tested (*n*)	Relative distribution (%)	Positive (*n*)	Positive (%)	95% CI
Sex (*P* = 0.237)	Female	133	55.4	8	6.0	3.1–11.4
	Male	107	44.6	3	2.8	1.0–7.9
Breed (*P* = 0.620)	Defined	5	2.1	0	0	0.0–43.4
	Moggy	235	97.9	11	4.7	2.6–8.2
Hair length (*P* = 0.756)	Medium	2	0.8	0	0	0.0–65.8
	Short	238	99.2	11	4.6	2.6–8.1
Municipality (*P* = 0.305)	Medium	75	31.2	4	5.3	2.1–12.9
	Small	165	68.8	7	4.2	2.1–8.5
Total	All	240	100	11	4.6	2.6–8.0

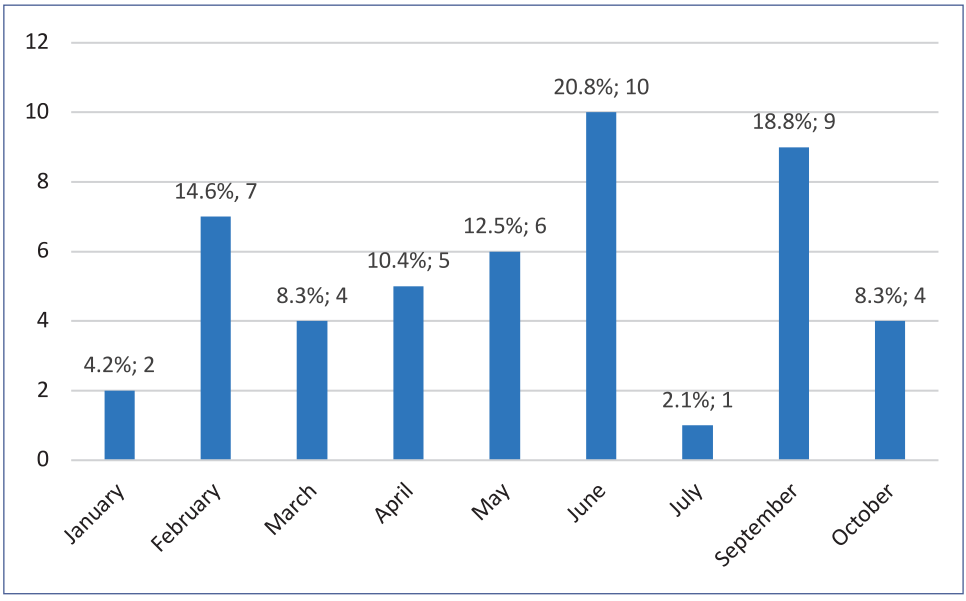

Figure 2. Monthly distribution of dogs that tested positive for Canine Parvovirus and Canine Coronavirus.

Figure 2 presents the monthly distribution of dogs testing positive for CPV, CCoV and co-infection: it was highest in June (20.8%) and lowest in July (2.1%). These results indicate variability in CPV, CCoV and co-infection prevalence throughout the year, with the highest rates observed in the summer months of July and September.

Logistic Regression Analysis based on age Canine Parvovirus (CPV)

The logistic regression model was not statistically significant ($p = 0.85$), suggesting that age, as a sole predictor, does not significantly contribute to the CPV status in dogs.

Table 5 presents the parameter estimates for the logistic regression model, implying that the probability of CPV positivity does not increase with age in the studied population.

The model showed a low entropy R-square value of 0.0003, indicating minimal explanatory power for CPV status variance. While the intercept was significant, suggesting the baseline log odds of a dog being CPV negative is -2.76039, age was not a significant predictor (B = 0.0008682, $P = 0.8530$). Despite a high classification rate of 93.75% for negatives, the model's overall predictive accuracy was compromised, with a 6.25% misclassification rate, highlighting its limitations in accurately predicting positive cases

Canine Coronavirus (CCoV)

The model did not reveal age as a significant predictor of CCoV positivity ($P = 0.55$). This finding indicates that the age of the dogs, when treated as a continuous variable, does not significantly affect the likelihood of testing positive for CCoV within the sampled population.

Table 6 summarises the logistic regression model parameters, illustrating that the probability of a dog being positive for CCoV does not significantly change with age. These findings highlight the necessi-

Table 5. Logistic regression analysis predicting Canine Parvovirus (CPV) status based on age.

Variable	B (SE)	Wald χ^2	df	*P*
Intercept	-2.76039 (0.41924)	43.56	1	< 0.0001*
Age (days)	0.0008682 (0.00466)	0.03	1	0.8530

B = regression coefficient; SE = standard error; df = degrees of freedom; $\chi^2(1, N = 240) = 0.03125$; *$P < 0.05$.

Table 6. Logistic regression analysis predicting Canine Coronavirus (CCoV) status based on age.

Variable	B (SE)	Wald χ^2	df	*P*
Intercept	-1.97615 (0.56197)	12.37	1	< 0.0004*
Age (days)	-0.049615 (0.083595)	0.35	1	0.5528

B = regression coefficient; SE = standard error; df = degrees of freedom; $\chi^2(1, N = 240) = 0.03125$; *$P < 0.05$.

Table 7. Logistic regression analysis predicting co-infection of Canine Parvovirus (CPV) and Canine Coronavirus (CCoV) based on age.

Variable	B (SE)	Wald χ^2	df	*P*
Intercept	-2.692046 (0.80352)	11.22	1	< 0.0008*
Age (days)	-0.050409 (0.01214)	0.20	1	0.6558

CCoV+ CPV+ indicates co-infection with Canine Coronavirus and Canine Parvovirus; B = regression coefficient; SE = standard error; df = degrees of freedom; $\chi^2(1, N = 240) = 0.293319$; *$P < 0.05$.

ty to explore other factors that might influence CCoV positivity in dogs.

Logistic regression analysis revealed a significant intercept (B = -1.97615, p < 0.0004), indicating a baseline tendency for CCoV negativity. The age factor, however, did not significantly influence CCoV presence (B = -0.049615, $P = 0.5528$). The model's low explanatory power (entropy R-square = 0.0035) and high misclassification rate suggest limited predictive capability for CCoV status.

Canine Parvovirus (CPV) and Canine Coronavirus (CCoV)

The analysis did not demonstrate a significant association between age and the likelihood of co-infection ($p = 0.66$, suggesting that age, considered as a continuous variable, does not have a significant impact on the probability of dogs testing positive for both CPV and CCoV.

Table 7 encapsulates the regression analysis findings, providing evidence that age does not significantly influence the log odds of co-infection with CPV and CCoV in the canine population under study. These insights direct further research towards other potential determinants of co-infection.

Parameter estimates showed that the intercept was statistically significant (B = -2.692046, $P < 0.0008$), indicating the log odds of being negative for both CPV and CCoV when age is zero. Nevertheless, age itself was not a significant predictor of co-infection status (B = -0.050409, P = 0.6558). The model explained a minimal amount of the variance in co-infection status ($R^2 = 0.0033$), and with the Akaike Information Criterion (AICc) and Bayesian Information Criterion (BIC) values being relatively high, the model's overall predictive strength is limited.

Discussion

To the best of our knowledge, this study is the most comprehensive research conducted in Portugal to determine the prevalence of CPV and CCoV among shelter dogs. The study provides valuable insights into the epidemiology of viral infections within this specific population.

The findings underscore the ongoing prevalence of CPV and CCoV within dog shelters in Portugal, despite vaccination efforts and biosecurity measures (Marques et al., 2023). A prevalence of 6.2% for CPV and 9.2% for CCoV, along with a co-infection prevalence of 4.6%, underscores the persistence of these pathogens in shelter environments. These figures are particularly concerning given the high risk of morbidity and mortality

associated with these diseases, especially in unvaccinated populations and in high-density settings such as shelters.

Scarce literature is available about CPV and CCoV prevalences in shelters. Our findings contrast with a report identifying enteropathogens in 100 dogs shortly after admission to a Florida animal shelter identified in dogs with and without diarrhoea due to CPV (2% and 2%, respectively; though the possibility of vaccine virus detection in some of these cases could not be ruled out) and CCoV (2% and 18%, respectively) (Tupler et al., 2012). Another study in the USA investigating gastrointestinal diseases in 280 adult dogs and puppies, as reported by adopters after relocation (shelter to shelter), revealed that only one of these dogs was confirmed to have a diagnosis of CPV (Doyle et al., 2020). In another study in the USA based on 4088 puppies relocated for adoption, the incidence of post-transport CPV diagnoses was 2.3% (DiGangi et al., 2021).

In one study in California, CPV and CCoV were tested in a 1:1 matched case-control study design in diarrheic dogs in an animal shelter by PCR assay and ELISA, respectively. Canine coronavirus was detected in 73.3% (44/60) case dogs and 59.3% (35/59) control dogs. Parvovirus was detected in only 1.7% (1/59) (1.7%) case dogs and no control dogs (Sokolow et al., 2005).

A study tested 1172 samples faecal samples from dogs with gastroenteritis in Europe to CCoV, 493 (42.1%) were positive, ranging from 100% (1/1) in Slovenia, (1/1) Poland, (3/3) Sweden and (3/3) Germany, 78.0% (32/41) in Hungary, 66.7% (4/6) in Romania, 57.1% (4/7) in Belgium, 55.6% (45/81) in Greece, 50% (1/2) in Slovakia, 43.4% (330/760) in Italy, 36.4% (12/33) in Portugal, 33.3% (1/3) in Bulgaria, 27.1% (54/199) in the UK, and 6.3% (2/32) in Spain (Decaro et al., 2010).

In a Western European study by PCR in faecal samples 48.7% (75) tested positive for CPV, including 87.5% (7/8) in UK, 71.4% (15/21) in Germany, 61.5% (16/26) in France, 53.8% (21/39) in Italy, 40% (4/10) in Belgium, 27.7% (13/47) in Spain, and 0% (0/5) in the Netherlands. Canine Coronavirus was detected in 38.5% (60/156) tested samples with higher detection in Belgium (80%; 8/10), the Netherlands (60%; 3/5), Italy (51.3%; 20/39), and the UK (12.5%; 1/8). Mixed infections in 17.9% (28/156) samples, including 40% (4/10) in Belgium, 30.8% (12/39) in Italy, 28.6% (6/21) in Germany, 12.5% (1/8) in the UK, 8.5% (4/47) in Spain, and 3.8% (1/26) in France (Decaro et al., 2011).

A study in 355 animals presenting with severe diarrhoea in the UK revealed a prevalence of 58% CPV and 7.9% CCoV determined by faecal PCR. Analysis showed that animals with no history of vaccination were more likely to be CPV positive, with the greatest effect in younger animals (Godsall et al., 2010).

In Japan, the prevalence of CCoV and CPV in owned dogs with diarrhoea was significantly different between age groups. For dogs aged less than 1 year, the CCoV prevalence was 66.3% and the CPV prevalence 43.8%, while for dogs aged 1 year or older they were only 6.9% and 10.3%, respectively (Soma et al., 2011).

In Turkey, 179 dogs tested for antibodies to CCoV, 112 (62.5%) were found to be positive by serum neutralisation test, while 133 (74.3%) were positive by ELISA. The highest prevalence (94.2%) was detected in kennel dogs. Detection of the CCoV genome in faeces was performed in samples from 90 diarrhoeic puppies by reverse transcription PCR. Fourteen (15.5%) faeces were positive for CCoV (Yeşilbağ et al., 2004).

In one study in the UK, 2.8% of pet dogs (7/248) were positive for CCoV (Stavisky et al., 2010). A study conducted in Ireland on 250 dogs found seven (2.8%) CPV-positive, two (0.8%) CCoV-positive, and one (0.4%) both CPV- and CCoV-positive. Curiously, all the dogs that tested positive belonged to a group of clinically suspected dogs that had been previously vaccinated (McElligott et al., 2011).

More recently, in a study in Italy, CPV-2 was the most prevalent viral species and CPV-2b was the most prevalent variant (80%), while CPV-2a (10%) and CPV-2c (10%) were both present but with a much lower frequency, and CCoV was constantly detected in samples together with CPV-2 strains, confirming that these viruses are often present as coinfections (Zobba et al., 2021). In Greece, 116 dogs presenting diarrhoea were tested by PCR for the presence of CPV and 58.6% (68/116) were found to be positive (Kantere et al., 2021).

The prevalence of CCoV antibodies in different countries seems to be highly variable. Seroprevalence of infection has been reported to be 15.8% in pet dogs and 40.8% in shelter dogs in Australia (Naylor et al., 2001), 73.4% in Italy (Pratelli et al., 2002), 44.1% in Japan (Bandai et al., 1999) and 76% seroprevalence and 45% faecal isolation from a rescue kennel in England (Tennant et al., 1993). However, the extent of variations may be influenced by social interactions among dogs and the sensitivity of the employed methods.

Despite vaccination has resulting in a decline in major infectious diseases within pet populations in developed nations, the reality is more complex. Geographical clusters of infection persist, and sporadic outbreaks are not uncommon, even due to the virus's capacity to mutate and different variants to become dominant and spill over (Lamm and Rezabek, 2008; Hao et al., 2022). This is particularly acute within stray and shelter environments, contrasting starkly with household pets due to the absence of vaccination in the firsts or lack of or incomplete vaccination in the seconds due to financial constraints. Furthermore, in developing regions, these diseases remain prevalent, exacerbated by lower vaccination rates, estimated at merely 30–50% even in affluent countries, a figure declining in the wake of economic downturns (Day et al., 2016). Such a backdrop underscores the urgency of examining vaccination practices, especially for diseases like CPV and CCoV in shelters, where the prevalence of these viruses remains an important concern. CPV and CCoV are highly contagious and can lead to severe outbreaks within these high-density environments, where animals with unknown medical histories come into close contact. Understanding their prevalence is crucial for implementing effective disease prevention and control measures, which can mitigate the impact on sheltered dogs and ensure their health and well-being prior to adoption.

Although there is a lack of studies specifically addressing the prevalence of CPV and CCoV within Portuguese shelters and the broader pet population, some research has been conducted on these viruses within the country's wildlife and a study on domestic dog population was realised (Santos et al., 2009; Duarte et al., 2013; Miranda et al., 2016; Rosa et al., 2020;).

Santos et al. (2009) discovered antibodies to CPV in free-ranging Iberian carnivores between 1995 and 2006, indicating a widespread presence of CPV antibodies among these wild animal populations. Duarte et al. (2013) further highlighted that exposure to parvovirus is a common and geographically widespread phenom-

enon among wild carnivores, posing potential risks to susceptible groups at the interface between wildlife and domestic animals, as well as to endangered species. A notable study in northern Portugal found a high prevalence of CPV across all species of wild carnivores, suggesting its endemic nature, while CCoV occurrences were more sporadic, with instances of co-infection by both viruses (Rosa et al., 2020).

Miranda et al. (2016) collected 260 faecal samples in domestic dogs in Portugal; 76.2% (n = 198) were CPV-positive by PCR, and the CPV antigen was detected in 60.0% (61/109) samples by an immunochromatographic (IC) test. Sequence analysis of the 198 strains confirmed that CPV-2c were the dominant variant (51.5%), followed by CPV-2b (47.5%) and CPV-2a (1%).

These findings emphasise the importance of these viruses not only to wild carnivores but also to stray dogs and cats, especially in rural areas where the multi-host nature of these viruses represents a considerable risk. In 1978, canine parvovirus type 2 originated from a spillover of a feline panleukopenia-like virus, causing a worldwide pandemic of enteritis and myocarditis among canids. In 2020, the virus was identified in pigs in South Dakota, USA, by PCR, sequencing, *in situ* hybridisation, and serology. Genetic analysis suggests spillover from wildlife (Temeeyasen et al., 2022). CPV-2 has been associated with severe enteritis in insectivorous Taiwanese pangolin (*Manis pentadactyla pentadactyla*), further demonstrating the propensity of CPV-2 to overcome host barriers (Chang et al., 2021). A further challenge is likely to be due to viruses jumping species and the emergence of more virulent variants of established viruses resulting from mutations, as has been the case for the canine parvovirus and coronaviruses (Patel and Heldens, 2009).

The mutation of these viruses complicates pathogen detection and management in wild carnivore populations, underscoring the importance of ongoing vigilance and adaptive conservation strategies. The implementation of comprehensive vaccination and control measures for domestic animals is crucial to prevent pathogen spillover. Additionally, local shelters serve as sentinel sites for monitoring and managing the spread of these pathogens, playing a vital role in safeguarding the health of domestic and wild animals, as well as public health.

Of reference is a survey covering municipal and non-profit animal shelters in Portugal, where CPV was the most commonly reported infection in dogs at 62%. Canine coronavirus was also among the most frequently reported infections in dogs at 12%. This highlights CPV prevalence despite being preventable by vaccines. The persistence of CPV in shelters was attributed to the virus's environmental resistance and issues related to maternal immunity affecting primovaccination (Marques et al., 2023). The findings of this study confirm that CPV and CCoV remain significant concerns for the health of dogs in Portuguese shelters, with notable prevalence even among vaccinated populations (Marques et al., 2023).

The lack of a significant association between age and infection positivity suggests that effective prevention and control require more than age-based vaccination strategies.

While our statistical analysis did not reveal a significant correlation between age and positivity for CPV, CCoV, or co-infection, suggesting that susceptibility to infection may be influenced by factors other than age, findings by Ellis et al. (2022) indicate that puppies are at

an increased risk of disease compared to adult dogs. This heightened risk in puppies may be attributed to the interference of maternal antibodies with the vaccination response. Moreover, the absence of a reliable association of enteropathogens with discernible risk factors complicates the efforts of shelter staff in pinpointing and isolating dogs that may transmit disease or require targeted treatment. This uncertainty also challenges the perceived direct relationship between the presence of an organism and its effect on animal health. Identifying enteropathogens in the faeces of dogs with diarrhoea does not necessarily implicate these pathogens as the causative agents of the condition, thereby necessitating further diagnostic evaluations or therapeutic trials to establish a definitive link (Tupler et al., 2012).

Our study found no significant differences in the prevalence of canine parvovirus CPV, CCoV, and both CPV and CCoV infections when considering factors such as sex, breed, hair length, or municipality, suggesting that these factors may not play a decisive role in the susceptibility to these infections. It's important to note that while a statistical significance was observed for hair length in CCoV cases, this finding is not deemed reliable due to the extremely low number of observations in the medium hair length category—only two cases, one of which is positive for CCoV. Such a small sample size can greatly amplify the effect of random variation and may not be representative of the population. Therefore, the statistical significance reported here does not necessarily imply a meaningful or generalizable relationship between hair length and CCoV infection. Further research with a more balanced and larger sample size across hair length categories would be necessary to draw any robust conclusions. Therefore, our conclusion that these factors may not play a decisive role in the susceptibility to these infections stands, with the caveat that further research with a more representative sample size for hair length is needed to confirm this observation.

Contrarily, the literature indicates that all dogs, regardless of age or breed, are susceptible to parvovirus, with puppies aged 6–16 weeks being particularly vulnerable (Mylonakis et al., 2016). This heightened risk is attributed to the decline in maternally derived passive immunity, which puppies receive from vaccinated bitches through colostrum. While this maternal immunity offers initial protection, its gradual decrease exposes neonates to a greater risk of infection as they reach 8–12 weeks of age, underscoring the importance of timely vaccination (Pollock and Carmichael, 1982; Lamm and Rezabek, 2008; Mila et al., 2014;).

Furthermore, CPV is a major cause of illness and death in puppies under 6 months, emphasising its critical impact on young dogs (Kalli et al., 2010). Although the odds of CPV enteritis were found to be higher in purebreds, suggesting a possible breed-specific susceptibility, no links were established between CPV enteritis and the gender or body weight of the puppies (Kalli et al., 2010). This observation aligns with our findings and suggests that while certain factors may influence the risk of CPV and CCoV, others, like sex and body weight, do not significantly affect the likelihood of enteritis due to CPV. Together, these findings highlight the need for vigilant vaccination and health management practices to protect all puppies, especially during the critical window when maternal immunity wanes and the inherent risk of infection escalates.

Our findings reveal a distinct variability in the prevalence of CPV, CCoV, and

co-infections throughout the year. The most significant prevalence rates were observed in June with 20.8% (n = 10), followed by September at 18.8% (n = 9), and February at 14.6% (n = 7). Conversely, the lowest prevalence was recorded in July at 2.1% (n = 1). These results suggest that the summer months, particularly June and September, are associated with higher rates of infection. In contrast, other researchers have identified a different seasonal pattern, where the incidence of parvovirus peaked during May and June and then declined to its lowest points in August, September, December, and January (Horecka et al., 2020). The discrepancy between these findings could indicate regional variations or differences in data collection periods. The fluctuations noted by Horecka et al. (2020) suggest a considerable variance in the monthly population, potentially by as many as 41 animals, with a secondary smaller peak possibly occurring in October. Together, these observations underscore the complex dynamics of CPV and CCoV prevalence and the influence of seasonal factors on disease transmission within canine populations.

Several factors have been consistently identified as risk factors for the development of parvoviral enteritis. These include a lack of prior vaccination against canine parvovirus (CPV) and an age less than 6 months (Godsall et al., 2010; Kalli et al., 2010; Zourkas et al., 2015). While previous literature suggested breed as a risk factor, recent research has not supported this association (Godsall et al., 2010; Kalli et al., 2010). Ultimately, CPV can cause clinical disease in dogs of any age, breed, or gender.

Shelter-related risk factors for the development of CPV outbreaks include overcrowding, insufficient isolation of infected dogs, poor biosecurity/sanitation, and an inadequate vaccination protocol (Doyle, 2021). Additionally, factors such as vaccination status, the overall health condition of dogs upon arrival, and the effectiveness of infection control and prevention measures implemented by the shelter can influence the risk of CPV outbreaks.

In one study in the region of Thessaly, Greece, age and utility were identified as significant risk factors associated with parvoviral enteritis, while environmental variables such as livestock density, land uses, and human population density were also described as important factors related to virus infection (Kantere et al., 2021). This study recognised favourable areas for the potential occurrence of CPV-2 infection.

Factors such as lack of protective immunity, intestinal parasitism, overcrowding, and stress are commonly presumed to be present in shelter populations and thought to predispose puppies to parvoviral infection (Mazzaferro, 2020). For most enteropathogens, the risk of infection was not correlated with diarrhoea, signs of disease, or other risk factors in one study (Tupler et al., 2012).

Furthermore, this study highlights the need for further research into factors influencing the prevalence of CPV and CCoV in shelters, including vaccination practices, shelter environment management, and the socioeconomic characteristics of the regions under study. Understanding these factors could inform more effective strategies for the prevention and control of these infectious diseases.

A study suggests that the risks of prolonged shelter stays, even with additional vaccinations, may outweigh the benefits against CPV, considering the low risk of disease exposure during transport. The study emphasises the importance of strong biosafety measures and modi-

fied-live virus vaccinations as more effective in minimising disease exposure and transmission (DiGangi et al., 2021).

The management of CPV and CCoV in shelter environments presents significant challenges, necessitating stringent biosecurity and vaccination protocols. The World Small Animal Veterinary Association (WSAVA) guidelines for the vaccination of dogs and cats advocate for core vaccinations against these pathogens before or immediately upon a dog's entry into a shelter (Day et al., 2016). This approach aims to provide all dogs with essential protection as promptly as possible. Considering the financial limitations many shelters face, adopting this protocol as a minimum standard is imperative since a single vaccination could reduce the risk of developing CPV enteritis by 2.3 times, underlining the efficacy of preventive measures (Kalli et al., 2010).

The guidelines further recommend initiating vaccinations at an early age − between 4 to 6 weeks − and continuing revaccination every 2 weeks until the dog reaches 20 weeks of age, assuming they remain within the shelter. This regimen is particularly vital due to the high risk and severe outcomes associated with CPV and CCoV infections in such settings. Moreover, Day et al. (2016) endorsed serological testing for CPV to assess protective immunity and guide the management of outbreaks.

The necessity of adopting the WSAVA's recommended vaccination and biosecurity measures cannot be overstated. Early and consistent vaccination schedules, combined with serological tests for CPV, form the cornerstone of efforts to curb CPV and CCoV outbreaks in shelters. However, the sporadic nature of these outbreaks and the persistence of geographical infection clusters call for a broader strategy in shelter animal health management.

Treatment outcomes for CPV-infected dogs vary widely, with survival values ranging from as low as 9% in untreated cases to 80−90% in animals receiving care in tertiary facilities. The treatment costs in private practice can range from $1,000 to $2,000 USD, a factor that often leads to euthanasia decisions in financially constrained shelters rather than providing care. This is especially true in nonprofit rescues and shelters, where euthanasia is sometimes chosen over treatment due to the high costs and the highly contagious nature of CPV (Horecka et al., 2020). The same authors found an average treatment duration of 9.03 hours by staff and volunteers, with an 86.6% survival rate in a shelter that treated all CPV-infected dogs. This highlights the potential for successful outcomes with adequate resources. However, overcrowding is a critical issue that hampers the ability to monitor and care for shelter populations effectively, increasing the risk of disease exposure (Doyle, 2021).

Environmental sanitation practices, such as the use of a 0.75% sodium hypochlorite solution have been shown to significantly reduce the spread of CPV in crowded settings like shelters and veterinary hospitals (Cavalli et al., 2018). While CCoV is a notable concern in shelter dogs, particularly among juveniles with diarrhoea, specific measures against CCoV may not be necessary beyond standard disinfection and disease monitoring protocols (Doyle, 2021).

Comprehensive vaccination, biosecurity, and management strategies are crucial in mitigating the impact of CPV and CCoV in shelter environments. These approaches not only help in preventing outbreaks but also ensure that shelters are better equipped to handle cases that do arise, ultimately reducing the overall burden of these diseases.

To prevent CPV infection, isolating at-risk puppies is essential. It is vital to educate people to keep puppies away from other dogs until their vaccinations have been completed, as vaccinated adults can still spread CPV. In shelters and veterinary settings, strict hygiene practices, such as thorough handwashing and using fresh gloves for each patient, are critical. Surfaces and equipment need regular disinfection with appropriate solutions. Even if a diarrhoeic patient tests negative for CPV, barrier precautions are recommended to prevent infection spread (Mazzaferro, 2020).

Canine parvovirus remains a critical viral pathogen for dogs, challenging despite widespread vaccination. Continuous monitoring is essential to identify emerging CPV variants. Dogs infected with CPV and CCoV may shed the virus once clinical signs have ceased. In addition, recovered dogs may serve as carriers and shed the virus periodically. This is an important mechanism for the continued circulation and persistence of CPV and CCoV in the environment.

A study in southern Ireland provided evidence that both CPV and CCoV are causative agents of gastroenteritis in symptomatic dogs, with no evidence of viral shedding in asymptomatic dogs (McElligott et al., 2011). Dual infection of CCoV along with CPV is of significant concern regarding animal health and well-being. Continued surveillance would be beneficial to evaluate better vaccine efficacy, to understand the underlying mechanisms of vaccine breakthroughs and to implement successful prophylactic measures.

The adoption of recommended vaccination and biosecurity practices, as per WSAVA guidelines (Day et al., 2016), together with the implementation of customised infection control measures based on the specific characteristics of each shelter, are essential to reduce the prevalence of these diseases. Moreover, continuous research to explore other variables that may influence susceptibility to infection and the effectiveness of interventions, including socioeconomic and geographical factors, is crucial.

Finally, this study emphasises the importance of a collaborative approach involving veterinarians, researchers, shelter managers, and policymakers to develop integrated, evidence-based strategies for managing and preventing CPV and CCoV in shelter environments. Through concerted, data-informed efforts, we can better protect the health and well-being of dogs in shelters and, by extension, wildlife and owned pets and public health.

Conclusions

The study provides significant perspectives into the prevalence and impact of CPV and CCoV in Portuguese shelters. Despite advancements in vaccination and disease management practices, CPV and CCoV continue to pose substantial health threats to sheltered dogs, underscoring the persistent challenge these viral pathogens represent to animal welfare and shelter routines. The findings reveal CPV and CCoV prevalences of 6.2% and 9.2%, respectively, with a 4.6% rate of co-infection, a circumstance that highlights the resilience of these viruses in shelter environments and their potential to cause significant morbidity and mortality among the canine population.

Moreover, this study reinforces the idea that shelters can act as sentinels for public health, highlighting their role not only in safeguarding animal health but also in providing early warnings for potential zoonotic diseases and public health threats. This sentinel function

underscores the necessity of integrating animal health surveillance with broader public health efforts, facilitating timely responses to emerging infectious diseases that may affect both animal and human populations.

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Opasnosti za zdravlje – pseći parvovirus i koronavirus u pasa u portugalskim skloništima

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Ova studija istražuje prevalenciju psećeg parvovirusa (CPV) i psećeg koronavirusa (CCoV) u pasa u portugalskim skloništima. Unatoč napretcima u veterinarskoj medicini i raširenim nastojanjima da psi budu cijepljeni, CPV i CCoV i dalje predstavljaju važne opasnosti za zdravlje pseće populacije, naročito u okruženjima s visokom gustoćom nastanjenosti, kao što su skloništa. Pomoću unakrsne studije koja je uključivala 240 pasa iz skloništa u pet općina u Portugalu, ovo istraživanje koristilo je imunokromatografsku tehniku za istovremeno otkrivanje CPV i CCoV antigena. Rezultati su otkrili prevalenciju od 6,2 % i 9,2 % za CPV, odnosno CCoV, uz prevalenciju koinfekcije od 4,6 %, naglašavajući stalnu opasnost kojeg ovi virusi predstavljaju. Studija istražuje i izostanak značajne veze između prevalencija infekcije i varijabli poput dobi, spola, pasmine i općine, što ukazuje na to da osjetljivost na ove infekcije može biti široko rasprostranjena među psima iz skloništa. Osiguravajući nove uvide u epidemiologiju CPV i CCoV u portugalskim skloništima, ovo istraživanje doprinosi saznanjima potrebnim za razvijanje ciljanih strategija za upravljanje i sprječavanje ovih zaraznih bolesti u visokorizičnim psećim populacijama.

Ključne riječi: *CcoV, CPV, psi, Portugal, sklonište*