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Detection of old and recently discovered parvoviruses in apparently healthy dogs in Campania region, Italy

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ABSTRACT

In addition to the more famous canine parvovirus 2 (CPV-2), the *Parvoviridae* family includes other viruses able to infect dogs [canine chaphamaparvovirus (CaChPV), canine bocavirus-1 (CBoV-1), and canine bufavirus (CBuV)], whose etiological role is still controversial (mostly identified in animals with diarrhea but also detected in asymptomatic animals). The aim of this work was to evaluate the shedding of these common and recently discovered viruses in the dog population from the Campania region (Italy). A total of 170 feces from apparently healthy dogs were sampled and tested with specific real-time PCR. The prevalences obtained are reported below: 6.5 % (11/117) for CPV-2, 4.1 % (7/170) for CaChPV, 11.8 % (20/170) for CBoV-1, and 7.6 % (13/170) for CBuV. The analysis of risk factors found a greater risk of parvoviruses detection for animals of stray origin, with an altered fecal score, and those living outdoors. Considering single parvoviruses, we found a significantly higher prevalence of CBoV in animals with impaired fecal scores and in dogs living outdoors. The detection of CaChPV in fecal samples was correlated to the origin (stray) of the dog.

We also evaluated the changes in the fecal microbiota in positive dogs, observing a reduction of *Bacteroides* and an increase of *Enterobacteriaceae* (up to 70 %) in CPV-2-positive dogs. Only minor changes, however, were observed in animals positive for other parvoviruses.

Moreover, we established that the shedding of these parvoviruses did not affect the result of rapid direct assays commonly used in clinical routine diagnostics for CPV-2.

1. Introduction

Parvoviruses have been known in dogs since 1967, when canine minute, later called canine parvovirus type 1 or minute virus of canines (MVC), was identified (Jager et al., 2021). These small single-stranded DNA viruses (4–5 kb) had and continue to have a substantial influence on pet health because of the severe forms that they may cause, as well as their resistance in the environment due to the lack of envelope (Decaro et al., 2020; Jager et al., 2021). The endemicity of these viruses is supported by the orofecal cycle and both direct and indirect transmission (Decaro et al., 2020). MCV was mainly responsible for reproductive disorders, whereas respiratory and gastrointestinal signs were occasionally described in young dogs (Jager et al., 2021). A second canine parvovirus (CPV-2) became endemic during 70s, in this case responsible for hemorrhagic gastroenteritis and fatal myocarditis. The latter has been considered the major cause of infective gastroenteritis in puppies in the world and has been classified into three variants (2a, 2b, 2c) and has been detected in other species, including cats (Amoroso et al., 2022; Tuteja et al., 2022). Furthermore, this parvovirus has also been widely described in wild animals (Conceição-Neto et al., 2017; Ferrara et al., 2023). In addition to these relatively "old" parvoviruses, further species have been described thanks to the introduction of metagenomic approaches: canine chaphamaparvovirus (CaChPV, most commonly known as cachavirus) and canine bufavirus (CBuV) (Capozza et al., 2023; Hu et al., 2020; Martella et al., 2018).

CaChPV was first described thanks to metagenomic analysis performed during an outbreak of diarrhea in dogs in the United States in 2017 (Capozza et al., 2023). Nowadays its association with diarrhea is controversial and not fully demonstrated because it has been commonly found also in healthy dogs (Capozza et al., 2023). In the last years, chaphamaparvovirus has been identified in several animal species, including mammals (cats, pigs, bats, and rodents) and birds (Łukaszuk

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et al., 2025; Tuteja et al., 2022). However, information on their epidemiology as well as their ability to cause disease and being associated with enteric signs was not fully defined.

CBuV was also identified using a metagenomic approach from puppies with respiratory signs in Italy (Martella et al., 2018). Like its human counterpart, human bufavirus (BuV), it can cause diarrhea, and recent studies have shown a positive correlation between detection of CBuV and enteric diseases (Yahiro et al., 2014). Moreover, further evidence has identified the CBuV DNA in the blood of dogs with enteritis (Li et al., 2019).

The canine bocavirus (CBoV) was discovered in the late 1800s and is now known to cause enteric and respiratory diseases in puppies and young dogs (Choi et al., 2015; Lau et al., 2012; Zhai et al., 2017). Furthermore, recently discovered parvoviruses include two further species of canine bocaviruses, CBoV-2 and CBoV-3, associated with respiratory and enteric disease in puppies (Bodewes et al., 2014; Kapoor et al., 2012; Li et al., 2013; Piewbang et al., 2018; Sobhy et al., 2022).

The discovery of these new viruses, mainly obtained through metagenomic approaches, has led to "complicated" changes in their taxonomy, and nowadays parvoviruses are included in three subfamilies: *Parvovirinae*, *Densovirinae*, and *Hamaparvovirinae*. The *Parvovirinae* subfamily is, in turn, divided into different genera, including *Protoparvovirus* (which includes the species *Protoparvovirus carnivoran1*, i.e. CPV-2, and the species *Protoparvovirus carnivoran3* which corresponds to the CBuVs) and *Bocaparvovirus* genus (which includes all CBoVs, including MVC) (Capozza et al., 2023). The subfamily *Hamaparvovirinae* includes the genus *Chaphamaparvovirus* which presents CaChPV (Capozza et al., 2023; Pénzes et al., 2020).

Given that most epidemiological studies regarding these viruses have been conducted on diseased animal populations (mainly affected by diarrhea or CPV-2-positive animals), the aim of this study was to evaluate the spread of parvoviruses in apparently healthy dogs in the Campania region. Further aims of the work were to evaluate the changes in the fecal microbiome associated with parvovirus detection and the possible interference of parvoviruses other than CPV-2 in rapid point-of-care diagnostic tests.

2. Materials and methods

2.1. Study design and dog sampling

In this study, fecal samples belonging to 170 dogs from the Southern Italy, Campania region were analyzed (Fig. 1, created with EpiInfo). This number was obtained by applying the sample size Thurshfield's formula for a theoretically infinite population and estimating a prevalence of 10 %, an absolute precision of 5 %, and 95 % confidence interval (CI). This estimation was based on the results of previous studies. The sampling was divided between the 5 provinces belonging to the Campania region in order to obtain a representative sample. A total of 54 districts were sampled.

A questionnaire was utilized to gather information about each sampled animal, including province, sex, age, breed, origin, and lifestyle (Ferrara et al., 2024a; Ferrara et al., 2024b). Age categories were determined as follows: $\leq$2 years = young, 2–6 years = adult, and $\geq$ 7 years = old. A fecal score (1–5) was assigned to each sample according to guidelines described in previous works (Freiche et al., 2025). Since the PCR methodology employed is unable to differentiate vaccine from wild-type strains, in order to minimise the risk of detecting vaccine-positive animals, only samples from vaccinated animals known to have been immunised at least one month prior to sampling were included in the study (Decaro et al., 2014). Each fecal sample was immediately transported to the Department of Veterinary Medicine and

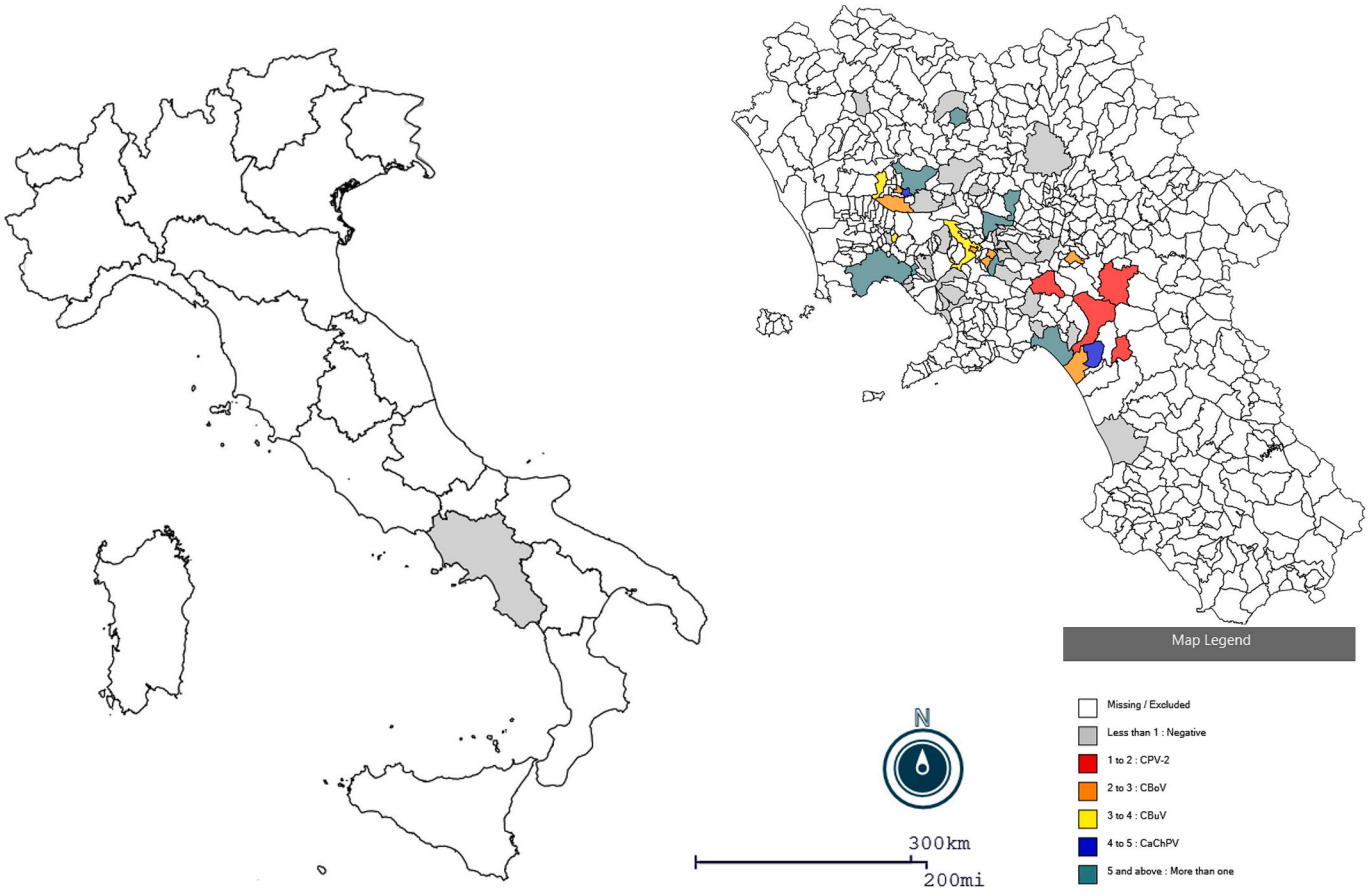

Fig. 1. Map of the study area and geographical distribution of positive samples. This figure was made using Epi Info software.

Animal Production Laboratory (University of Naples) under refrigeration conditions. Fecal DNA was extracted using QIAamp Fast DNA Stool Mini Kit (QIAGEN). The DNA samples were stored at −20 °C and quantified by NanoDrop before amplification protocols. The study protocol was approved by the Institutional Ethics Committee of the Department of Veterinary Medicine and Animal production (Centro Servizi Veterinari), University of Naples, Federico II (PG/2022/0093420, 21st July 2022) (Ferrara et al., 2024c; Ferrara et al., 2024d).

2.2. Molecular detection of parvoviruses and statistical analysis

Each DNA sample was used as templates in amplification reactions for the search for CaBuV, CaChPv, CaBoV, and CPV-2 nucleic acids using protocols already described in literature. Specifically, a real-time protocol was used for CaBuV consisting of 95 °C for 3 min, 42 cycles of denaturation at 95 °C for 10 s, and annealing extension at 60 °C for 30 s (Martella et al., 2018). CaChPV nucleic acid was detected using a real-time PCR described in a previous study and including 95 °C for 2 min, followed by 45 cycles of denaturation at 95 °C for 10 s and annealing/extension at 45 °C for 30 s (Palombieri et al., 2020). CBoV was analyzed using an end-point PCR amplifying a 530 bp fragment with the following thermal cycle: 95 °C for 15 min as initial denaturation step followed by 35 cycles of 94 °C for 30 s, 55 °C for 45 s, and 72 °C (Sobhy et al., 2022). Finally, CPV-2 DNA was detected using a real-time PCR including 95 °C for 10 min and 40 cycles consisting of denaturation at 95 °C for 15 s, primer annealing at 52 °C for 30 s and extension at 60 °C for 1 min (Decaro et al., 2005). All real-time PCR protocols used were based on TaqMan probes. All the PCR protocols were performed in 20 μL volume using iTaq Universal Probes Supermix (BioRad) and read using CFX96™ Real-Time PCR Detection System (BioRad). More information about the protocols and primers is available in Table 1. The number of positive dogs was divided by the total number to calculate each virus prevalence. Univariate analysis (chi-square test) was performed on the individual level for each parvovirus. PCR outcome was the dependent variable, and information regarding potential risk factors were independent variables (province, sex, age, breed, origin, and lifestyle). *P*-values lower than 0.05 were considered significant (MedCalc Statistical Software, Ostend, Belgium, version 16.4.3).

2.3. Evaluation of fecal microbiota in parvovirus-positive dogs

One μg of DNA extracted from positive and negative animals (two for each virus or negative sample) was subjected to NGS analysis to highlight any changes in the fecal microbiota (Langon, 2023). Briefly, DNA concentration and purity were monitored using a Qubit fluorometer (Thermo Scientific) and 1 % agarose gels, respectively. Shotgun metagenomic analyzes were conducted by an external company (BMR genomics, Padua, Italy) using Novaseq X Plus (format 150PE) and a previously described protocol (Rojas et al., 2024; Takahashi et al., 2014). Libraries were prepared with the Illumina DNAprep kit and sequenced on the Novaseq X Plus. Specifically, raw readings were initially verified for quality using DADA2, after which adapters and low-quality sequences were cut (Cutadapt) (Callahan et al., 2016). Finally, the assembly was assessed with the QIIME2 pipeline versus the Greengenes database with taxonomic assignment (Molano et al., 2024). A read limit of a minimum of 1 million was applied, and the abundance of bacterial phyla and families was assessed through a negative binomial generalized linear model using the differential expression analysis for sequence count data version 2 (DESeq2). Means obtained with two measurements (phyla and families) were compared using the one-sample-*t*-test ($p < 0.05$ was considered significant).

2.4. Influence of parvovirus on rapid assay for CPV-2 detection

Fecal samples resulting positive for at least one parvovirus were tested using Parvo IC (Agrolabo) a viral protein 2 (VP-2)-based qualitative immunochromatographic test for the detection of CPV-2 antigen in canine feces samples. Briefly, a fecal swab was diluted in a special diluent supplied by the company and, after shaking it, was loaded onto a lateral flow device. The results were read after 5 min, taking care not to exceed 10 min from loading as indicated by the manufacturer.

3. Results

In this study, a total of 170 apparently healthy dog stool samples were tested in real-time and end-point PCR, obtaining an overall prevalence of 25.3 % (Table 2). In particular, the highest prevalence was observed for CBoV (11.8 %), followed by CBuV (7.6 %), CPV-2 (6.5 %), and finally CaChPV (4.1 %) (Table 3). Members of the *Parvovirinae* and *Hamaparvovirinae* subfamilies were also widespread in the healthy population of dogs and were identified in 25/54 municipalities (Fig. 1). A very low co-infection rate (an overall 2.9 % and 11.6 % on positive animals) was obtained. A dog tested positive for CPV-2, CBoV, and CBuV, two for CBuV and CaChPV, one for CBoV and CBuV, and one for CPV-2, CBuV and CaChPV. Although there were differences in prevalence based on the variable, no risk factor was statistically associated with higher prevalences of CaBuV, CPV-2, and CaChPV (Table 3). Fecal score and outdoor lifestyle were correlated with higher CBoV prevalence (Table 3). Considering the overall prevalence, in addition to the fecal score and outdoor life, the origin of the animals was also correlated with more frequent positivity (Table 2). Animals that originated from stray animals were more likely to test positive for at least one virus (Table 2).

The analysis of the fecal microbiome of positive dogs for different parvoviruses has highlighted notable differences, especially in CPV-2 positive animals (Fig. 2A). At the phylum level, drastic reductions in *Fusobacteria*, *Firmicutes*, *Actinobacteria*, and Bacteroides were observed to the advantage of the phylum *Proteobacteria*, which was the predominant one in CPV-2 positive dogs (Fig. 2A). In CaBuV, CBoV, and CaChPV-positive animals, a reduction in *Fusobacteria* was observed. Furthermore, CaChPV-positive dogs had increased *Bacteroidetes*, and CaBuV-positive animals had increased *Firmicutes* (Fig. 2A).

Considering the bacterial families, an increase in *Enterobacteriaceae* (mainly *Escherichia coli*) was observed in CPV-2 shedding dogs (Fig. 2B).

Table 1
List of primers and probes used in this study.

	Forward	Reverse	Primer probe	Size (bp)	Reference
CaBuV	TGAACAAGAAATAGACAACATTGTCAT	AAAGAGCAGTTAGGTCATTGTTGT	Fam-CCAAACAAGGTACAGGACAGGAAGAAACAACACAA-BHQ1	208	(Martella et al., 2018)
CaChPV	AACAATCCTTACAATTGGCT	TTCCTGTTCTAGACAAGGAT	Fam-GACAATACAAGCTCAGTTTG-BHQ1	75	(Palombieri et al., 2020)
CaBoV	TCAGCTCCAGAAGCTCCGAC	CACGGATGCTGAGAGTACGG	None	530	(Sobhy et al., 2022)
CPV-2	AAACAGGAATTAACTATACTAATATATTTA	AAATTTGACCATTTGGATAAACT	Fam-TGGTCCTTTAACTGCATTAAATAATGTACC-BHQ1	125	(Decaro et al., 2005)

Table 2
Risk factor analysis of variables potentially associated with parvoviruses positivity.

Factor	Overall (at least one)	Positive	%	95 % CI	χ^2	p
	n					
Total	170	43	25.3			
Province						
Avellino	32	13	40.6			
Benevento	20	6	30			
Salerno	45	8	17.8		6.1	0.19
Caserta	39	8	20.5			
Napoli	34	8	23.5			
Sex						
Male	98	28	28.6		1.3	0.25
Female	72	15	20.8			
Age						
Young	34	11	32.3			
Adult	81	17	21		1.8	0.4
Old	55	15	27.2			
Bred						
Mix	84	25	29.8		1.75	0.18
Specific bred	86	18	20.9			
Origin						
Stray	91	29	31.9		4.48	0.03
Housed	79	14	17.7			
Fecal score						
≤2	89	15	16.8		7.04	0.008
>2	81	28	34.7			
Lifestyle						
In	46	6	13.04		5	0.025
Outside	124	37	29.8			

A slight increase in these bacteria was also observed in the microbiota of CaBuV and CaChPV-positive dogs. Similarly, a significant reduction in *Fusobacteria* characterized the microbiota of CPV-2-positive dogs, but only a slight reduction was evident in dogs positive for other parvoviruses (Fig. 2B). Bacteria from the *Lactobacillaceae* and *Prevotellaceae* families were practically absent in CPV-2 dogs. An important reduction of *Lactobacillaceae* was also observed in CaBuV-positive samples and a decrease in *Prevotellaceae* was identified in both CaBuV and CaChPV-positive samples (Fig. 2B). Data regarding both duplicates has been represented in Supplementary File 1.

All the positive samples were tested for VP-2 antigen detection using a commercial rapid immunochromatographic assay. We did not detect false positive results due to the presence of CBoV, CaBuV, and CaChPV. However, a very low sensitivity of these devices has been noted in animals not clinically affected by CPV-2. Only 2/11 of the PCR-positive animals also resulted positive to the rapid test (only those with the lowest threshold cycle).

4. Discussion

Since next-generation sequencing (NGS) allowed their discovery, new parvoviruses have been described worldwide among domestic and wild canid populations (Conceição-Neto et al., 2017; Canuti et al., 2022a; Canuti et al., 2022b). However, in most studies, populations of animals with gastrointestinal symptoms were examined, whereas in the present study, a population of apparently healthy dogs from the Campania region, southern Italy, was considered.

The prevalence of CaChPV identified in our study was in line with those described in other studies. For example, a case-control study performed in Italy detected CaChPV DNA in 1.9 % of stools belonging to symptomatic dogs and 1.6 % in that of healthy ones (Palombieri et al., 2020). Research carried out in China found a positive rate of 0 % and 1.55 % in healthy or diarrheic dogs, respectively. CaChPV-1 was detected in 6.56 % of samples (20/305, 14 diarrheic and 6 non-diarrheic dogs) and 1.61 % in two different samplings performed in Thailand (Hu et al., 2020). In a retrospective study conducted on 137 intestinal tissue samples and 168 fecal samples, CaChPV-1 was identified in intestinal tissue (mainly in the stromal and endothelial cells of intestinal villi) of some PCR-positive dogs by in situ hybridization (Piewbang et al., 2023). Considering these details, it is problematic to determine an association between CaChPV and diarrhea or to determine the virus's pathogenic role. In fact, if it were a non-pathogenic virus, its presence in diarrheal feces may simply be attributable to the increase in intestinal peristalsis typical of diarrhea (which would therefore improve the chances of

Table 3
Risk factor analysis of variables potentially associated with canine bocavirus (CBoV), canine bufavirus (CBuV), Canine chaphamaparvovirus (CaChPV), and canine parvovirus type 2 (CPV-2) positivity.

Factor	CBoV	Pos	%	χ^2	*p*	CBuV	%	χ^2	*p*	CaChPV	%	χ^2	*p*	CPV2	%	χ^2	*p*
	n					Pos				Pos				Pos			
Total	170	20	11.8			13	7.6			7	4.1			11	6.5		
Province																	
Avellino	32	6	18.7			5	15.6			3	9.4			5	15.6		
Benevento	20	4	20			2	10			0	0			0	0		
Salerno	45	2	4.4	6.7	0.14	2	4.4	5.18	0.27	2	4.4	3.46	0.48	3	6.7	6.8	0.14
Caserta	39	6	15.4			1	2.6			1	2.6			1	2.6		
Napoli	34	2	5.9			3	8.8			1	2.9			2	5.9		
Sex																	
Male	98	12	12.2	0.05	0.82	9	9.2	0.77	0.38	4	4.1	0.07	0.98	8	8.2	1.1	0.3
Female	72	8	11.1			4	5.5			3	4.2			3	4.2		
Age																	
Young	34	4	11.8			4	11.8			2	5.9			4	11.8		
Adult	81	9	11.1	1.8	0.4	4	4.9	1.8	0.4	3	3.7	0.33	0.84	3	3.7	2.65	0.26
Old	55	7	12.7			5	9.1			2	3.6			4	7.3		
Bred																	
Mix	84	12	14.3	1.01	0.31	7	8.3	0.1	0.74	5	5.9	1.41	0.23	7	8.3	0.95	0.32
Specific bred	86	8	9.3			6	7			2	2.3			4	4.6		
Origin																	
Stray	91	14	15.4	2.47	0.11	8	8.8	0.36	0.55	6	6.6	3.04	0.08	8	8.8	1.7	0.19
Housed	79	6	7.6			5	6.3			1	1.3			3	3.8		
Fecal score																	
≤2	89	6	6.7	4.5	0.03	5	5.6	1.1	0.3	2	2.2	1.65	0.2	4	4.5	1.2	0.27
>2	81	14	17.3			8	9.9			25	6.2			7	8.6		
Lifestyle																	
In	46	1	2.2	5.6	0.02	1	2.2	2.7	0.1	1	2.2	0.6	0.44	3	6.5	0.1	0.98
Outside	124	12	15.3			12	9.7			6	4.8			8	6.4		

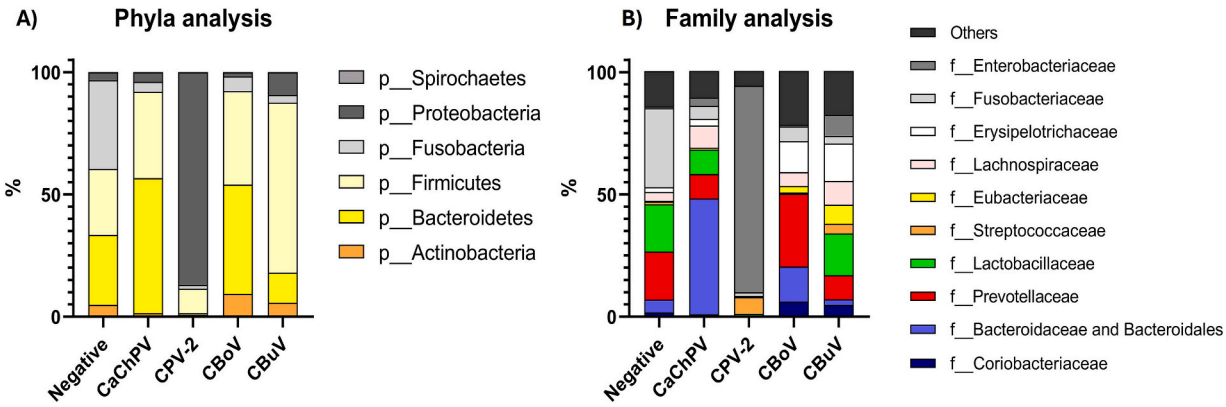

Fig. 2. Phylum and family-level molecular analysis of fecal microbiota in stool samples belonging to parvovirus-positive dogs.
The following are the supplementary data related to this article.

detecting the virus). Similar results have been observed in cats for fechavirus, also known as feline chaphamaparvovirus (FeChPV), highlighting the undefined pathogenic role of this genus also in other animal species (Ji et al., 2020).

The situation is different for CBuV, as the virus has been sporadically reported to cause intestinal or respiratory disease. One of the very first studies on this topic found a not statistically significant difference in the CBuV detection rate in animals with enteritis and control animals (Martella et al., 2018). However, descriptions of pathologic findings (bronchointerstitial pneumonia with segmental type II pneumocyte hyperplasia) associated with CBuV infection have been recently provided through postmortem investigations and in situ hybridization performed on 14 necropsied dogs (Piewbang et al., 2024). The same study also identified the presence of the virus in different matrices (lung, lymph node, and spinal cord) and massive colonization in puppies (Piewbang et al., 2024). However, the same evidence has not been reported regarding the colonization of the intestinal epithelium. Numerous studies have been conducted in China in different years, obtaining prevalence rates of 4.3 %, 2.2 %, and 1.7 % (Ji et al., 2024; Shao et al., 2020; Sun et al., 2019). Based on the population (diarrheal or non-diarrheal dogs) sampled, these prevalences did not differ much from that obtained in the present study (7.6 %). A study from Turkey detected CBuV DNA only in 3.22 % of fecal samples (Abayli et al., 2023). The only exception to these low prevalences described so far was that of a Chinese study in which a CBuV-88 potentially responsible for outbreaks of gastroenteritis was molecularly characterized. In fact, this strain was identified in 42.1 % of diarrheal dogs and, in some cases, also in serum (Li et al., 2019). Despite this, the virus was also identified in the feces and serum of healthy animals. In support of this, a large-scale Thai study described a prevalence of 9.4 % (50/531) regardless of the animal's health status (Charoenkul et al., 2024).

There have also been numerous reports on symptomatic animals, and a contributory role of enteric diseases has been attributed to CBoV. This virus, the most prevalent in the present study (11.8 %), was identified in 7.5 %, and 31 % in China and Austria, respectively (Doulidis et al., 2024).

Higher infection rates have been found in wild animals and, in particular, in the Canadian wolf, which revealed a prevalence of 42.6 %, 34 %, 5 %, and 2.6 % for CBuV, CPV-2, CBoV, CaChPV (Canuti et al., 2022a). A recent study also highlighted the presence of these viruses in three different populations of canids by evaluating the presence of the same viruses in companion dogs, stray dogs, and wild canids (Canuti et al., 2022b). Not only were these viruses identified with higher prevalences in wildlife, but once molecularly characterized, they also appeared divergent from the strains identified in pets, advancing the hypothesis of species-specific variants (Canuti et al., 2022b).

Although we identified a low rate of coinfection in our study (an overall 2.9 % and 11.6 % on positive animals), several statistically related coinfections have been described in the literature. Several studies have associated enteric viruses (such as CPV-2, canine adenovirus, or canine coronavirus) with higher prevalences of CaChPV, CBoV, and CBuV (Di Martino et al., 2021; Guo et al., 2016; Ji et al., 2024). For example, a previous study performed in Italy found an association between CBuV and CPV-2 (90.1 % of CBuV-positive samples were positive to CPV-2) (Di Martino et al., 2021). Usually, a significant rate of coinfections has been found in animals infected with symptomatic CPV-2 and/or dying from the virus. In this scenario, it is crucial to determine if these viruses play a copathogenic role or if their shedding is supported by the gastroenteritis and diarrhea caused by CPV-2 infection.

Although these parvoviruses have been described in numerous studies, few of them have evaluated the associated risk factors. In the present study, fecal score and lifestyle (dogs living outside) were correlated with higher CBoV prevalence. Considering the overall prevalence, fecal score, outdoor life, and origin (stray) were correlated with higher prevalence. A study performed in Thailand suggested a correlation between CBuV positivity and age (they found a higher prevalence of CBuV in dogs under five years of age), which was not confirmed by our results (Charoenkul et al., 2024).

CPV-2 was detected in 6.5 % of the animals tested. It seemed doubtful that the positive outcomes were related to vaccination strains, which are only shed for a few weeks (Decaro et al., 2014). Furthermore, substantial alterations in the fecal microbiota were discovered. The evaluation of the fecal microbiota in animals positive for different viruses has given indications about the changes caused by the colonization of the virus in the intestinal mucosa. The CPV-2 positive animals, although asymptomatic and therefore potentially animals with a history of CPV-2 infection, showed typical changes in the microbiota already described in previous studies. In particular, dogs experimentally infected with CPV-2 have shown a reduction in *Bacteroides*, elimination of *Prevotella* and *Lactobacillus*, and an important increase in *Enterobacteriaceae* (due to *E. coli* dissemination) (Park et al., 2019). These alterations were also highlighted by our results. A slight increase in *Enterobacteriaceae* was also observed in CBuV and CaChPV-positive dogs. Similarly, *Fusobacteria* were significantly reduced in CPV-2-positive dogs while slightly reduced in dogs positive for other parvoviruses. When evaluating the results of metagenomics, it is necessary to consider the limited number of animals analyzed (only two for each group) and the individual variations due to diet, habits, history, etc. (Dong et al., 2022.; Hand et al., 2013; Pilla and Suchodolski, 2021). Additional data is needed to confirm that CBoV, CBuV, and CaChPV infections change the fecal microbiota. Furthermore, it is uncertain how to interpret these findings and the temporal dynamics behind these changes.

However, it was obvious that the presence of additional parvoviruses had no effect on the results of a rapid direct test. The existence of cross-

responses in the lateral flow tests would have limited the quick identification of CPV-2, raising a number of problems. In terms of sensitivity, the rapid assay produced poorer results than real-time PCR (only 2/11), which was to be expected given the two methods' different detection limits. When performed on sick animals, these rapid tests detect huge amounts of virus, with results ranging from 80 to 90 %.

5. Conclusions

Although this study did not definitively establish the role of recently discovered canine parvoviruses, it did improve knowledge of these viruses by defining their prevalence in a clinically healthy population, risk factors associated with higher prevalences, changes in the fecal microbiota, and cross-reactions in CPV-2 diagnostic tests. More research is needed to properly identify the patterns and mechanisms that characterize infections caused by novel parvovirus strains.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.rvsc.2025.105887.

CRediT authorship contribution statement

Gianmarco Ferrara: Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Methodology, Investigation, Data curation, Conceptualization. **Michela Chianese:** Resources, Investigation. **Ugo Pagnini:** Writing – review & editing, Supervision, Formal analysis, Conceptualization. **Giuseppe Iovane:** Visualization, Supervision. **Serena Montagnaro:** Software, Resources, Data curation.

Ethics approval

The animal study protocol was approved by the Institutional Ethics Committee of Department of Veterinary Medicine and Animal production (Centro Servizi Veterinari), University of Naples, Federico II (PG/2022/0093420 20 July 2022).

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Not applicable (no data sets were generated or analyzed during the current study).

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