Papers

Epidemiology of canine parvovirus and coronavirus in dogs presented with severe diarrhoea to PDSA PetAid hospitals

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Canine parvovirus (CPV) and canine enteric coronavirus (CECoV) are often cited as causes of diarrhoea in dogs. This study aimed to determine the prevalence of CPV and CECoV in dogs presenting with severe diarrhoea to PDSA PetAid hospitals throughout the UK. A total of 355 samples were collected from the PDSA between 2006 and 2008. All samples were tested for CPV using a long range PCR and for CECoV using RT-PCR. The prevalence of CPV was 58 per cent (95 per cent confidence interval [CI] 52 to 63 per cent), with some evidence for regional variation. The prevalence of CECoV was 7.9 per cent (95 per cent CI 5.1 to 10.7 per cent). Analysis showed that animals with no history of vaccination were more likely to be CPV positive, with greatest effect in younger animals. CPV-positive animals were more likely to present with depression/lethargy than CPV-negative cases. The volume of diarrhoea and the presence of haemorrhage did not appear to be associated with the likelihood of detecting CPV. This study shows that CPV is a common finding in dogs presenting to PDSA hospitals with severe diarrhoea, and that CECoV is a less common but still potentially important pathogen. It also confirms that young and unvaccinated animals appear to be more at risk of presenting with CPV.

CANINE diarrhoea has many known viral causes, including canine enteric coronavirus (CECoV), canine parvovirus (CPV), rotavirus, adenovirus and astrovirus, as well as many bacterial and protozoal causes (Tennant 2001). A large proportion of canine diarrhoea cases goes undiagnosed, however, either because the disease is self-limiting or due to financial constraints on diagnostic procedures.

Although CPV is frequently cited as a cause of severe haemorrhagic diarrhoea in dogs (Pratelli and others 2001, Schulz and others 2006), the prevalence of the disease in the UK dog population as a whole, and in dogs in the UK presenting with diarrhoea, has not been determined. A German study reviewing clinical records of 936 dogs with acute haemorrhagic diarrhoea over an 11-year period (Schulz and others 2006) reported a prevalence of CPV of 16.6 per cent. This study also examined 100 healthy dogs and found only one to be shedding CPV asymptomatically. However, Schulz and others (2006) used electron microscopy, which for enteric pathogens is considered relatively insensitive compared with modern molecular assays such as PCR (Logan and others 2006). Therefore, these figures are likely to underestimate the true prevalence of CPV in the tested population.

CECoV is also associated with diarrhoea in dogs. Clinical infection is typified by mild diarrhoea that resolves with no, or with only symptomatic, treatment (Tennant and others 1993, Pratelli 2005). However, more severe signs can occur, including vomiting, lethargy, haemorrhagic diarrhoea and death (Evermann and others 2005, Buonavoglia and others 2006). Previous reports have linked outbreaks of haemorrhagic diarrhoea to CPV and CECoV co-infection (Pratelli and others 1999b). In diarrhoeic dogs the prevalence of CECoV by RT-PCR has been reported to range from 15 to 42 per cent in pet dogs (Bandai and others 1999, Pratelli and others 2000, Yesilbag and others 2004) and up to 73 per cent in kennelled dogs (Sokolow and others 2005). The prevalence in the general dog population in the UK has been estimated at approximately 3 per cent (Stavisky and others 2010).

The PDSA is a small animal veterinary charity that provides first opinion care to eligible, low-income pet-owning households in receipt of local authority means tested benefit, through its network of PetAid services in the UK. The PDSA's flagship service is delivered through its 47 PetAid hospitals, which are geographically located in most major cities in the UK. It also provides care for eligible patients though private practices offering its PetAid scheme and national special requests. In 2008, from its hospitals alone, the PDSA treated over 310,000 pets, over 60 per cent of which were dogs. Analysis of PDSA's clinical database indicated that in the first six months of 2006, staff at the hospitals administered intravenous fluids to over 2000 animals whose clinical records indicated that they presented with signs of gastroenteritis. However, as laboratory analysis was rarely conducted, there was no confirmation as to the causative agents in most of these cases.

This study aimed to determine the prevalence of faecal shedding of CPV and CECoV in the population of dogs presented at PDSA hospitals with severe gastroenteritis, and to evaluate whether history and presenting clinical signs were associated with the likelihood of detection of CPV and CECoV, as determined by PCR.

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TABLE 1: Prevalence of canine parvovirus determined by PCR in 355 diarrhoeic dogs presented to PDSA hospitals in different regions of the UK

	London	Midlands	North	Scotland	South	Wales	Total
Negative	44	34	23	21	25	3	150
Positive	37	63	81	3	14	7	205
Total	81	97	104	24	39	10	355
Regional prevalence (% [95% CI])	46 (35-57)	65 (55-74)	78 (69-85)	13 (4-30)	36 (22-52)	70 (39-91)	58 (53-63)

CI Confidence interval

Materials and methods

Study population and sample collection

The study was conducted at PDSA PetAid hospitals between September 2006 and March 2008. All of the PDSA's 47 hospitals were eligible to take part.

Sample size estimates showed that a sample of 938 would give 95 per cent confidence to detect an expected CPV prevalence of 15 per cent (Schulz and others 2006) with 2 per cent precision. The initial aim for a sample size of 1000 cases was set. Based on an estimated population size of 4000 (the number of acutely diarrhoeic dogs the PDSA was estimated to have seen in the previous year), it was envisaged that this should be achievable within a 12-month period.

The instructions to participate in the study were electronically distributed to clinical staff along with requests that samples of faeces for testing, from all eligible cases, should be sent immediately by post to the University of Liverpool, in sterile containers. Initially, samples needed to be addressed and posted manually by the individual veterinary surgeon. However, this was associated with poor compliance. To address this, suitable sample pots in pre-addressed, prepaid packs were subsequently provided to all hospitals. Patients for which a sample was submitted were identified in the PDSA's clinical database (PremVet; Vet Solutions) by an individual code.

Clinicians were advised that all dogs presenting with diarrhoea that, in the opinion of the attending veterinary surgeon, required anything more than conservative treatment (starvation and oral fluids) were eligible for inclusion in the study. The case definition was therefore either: dogs initially presenting with diarrhoea with or without vomiting that in the opinion of the attending veterinary surgeon required more than conservative treatment (starvation and oral fluids); or dogs that, following an initial consultation and conservative treatment, were presented again for diarrhoea with or without vomiting that now required additional treatment.

Monthly updates of numbers of samples submitted, as well as results to date, were fed back to the hospitals throughout the study.

At the laboratory, samples were either stored at −80°C until use (CPV), or diluted 1:10 w/v in a 10 per cent dilution of minimum essential medium with 10 per cent fetal calf serum, which was homogenised and the supernatant removed and stored at −80°C until use (CECoV).

Diagnosis of CPV

DNA was extracted as described by Desario and others (2005) with a slight modification. Samples were homogenised (10 per cent w/v) in phosphate buffered saline (PBS) and centrifuged for 15 minutes at 8100 *g*. The supernatant was boiled for 15 minutes, before being chilled on ice. Samples were then centrifuged again as before for five minutes. The supernatant was removed, and refrigerated until use.

Primers were designed to amplify the full VP2 region, using an alignment of publicly available sequences from Genbank and aligned using Mega 4 (Tamura and others 2007). Two primers were developed to amplify a ~2000 bp fragment from the viral VP2 gene: EF (2748–2765) GCCGGTGCAGGACAAGTA, and JS2R (Meers and others 2007) (4818–4799) CAACCCACACCATAACAACA. Primers were manufactured at MWG Eurofins (Germany).

In order to minimise PCR inhibition, the DNA extraction supernatant was diluted one in 10 before being exposed to PCR (Schunck and others 1995). An initial diagnostic screen was carried out in a 25 µl reaction, consisting of 12.5 µl extensor PCR master mix (Abgene), 8.5 µl of molecular water (Sigma), 1 µl primer EF (12.5 pm/µl), 1 µl primer JS2R (12.5 pm/µl) (Meers and others 2007) and 2 µl DNA. Any negatives were re-extracted and re-run. Negative controls were included at both the DNA extraction and PCR stages to control for contamination.

Diagnosis of CECoV

Genetic material was extracted using the Qiamp Viral RNA mini kit (Qiagen) as recommended by the manufacturer. Reverse transcription was carried out using 6 µl RNA, random primers and Superscript III MuLV Reverse Transcriptase (Invitrogen), according to the manufacturer's instructions.

A 409 bp fragment of the M gene was amplified by RT-PCR, using Thermoprime Plus DNA polymerase with 10X ReddyMix PCR buffer (Abgene), and the primer pair CECOV1/ CECOV2 (Pratelli and others 1999a). These primers have previously been used successfully to detect both types I and II CECoV (Benetka and others 2006). Negative controls were included at every stage, and at least one positive control was included with every PCR run.

Data collection

The PremVet (VetSolutions) practice management system is used at all PDSA hospitals. Each hospital has its own clinical database; however, the clinical data from each hospital is synchronised onto a central server, allowing easy access to the information across all hospitals from one single point of access via proprietary SQL tools (Navicat [MySQL GUI] 2006).

Every animal in the PDSA's clinical database has a unique identification number. This number was submitted with each sample to the laboratory and linked to the results data. This allowed animal breed, colour, age, sex and neuter status data to be extracted alongside the result information.

The clinical records for each animal were reviewed to extract data on vaccination history, presenting signs and treatment for each case. Few animals during or before the study had been vaccinated by the PDSA, so the investigators were reliant on the history provided by the client rather than a documented history of vaccination or presentation of vaccination records.

Statistical analysis

Descriptive statistics were used to depict the prevalence of CPV and CECoV. Screening of variables was performed using chi-squared analysis and a univariable logistic regression model, with CPV status as the dependent variable followed by CECoV as the dependent variable. Separate models were used to evaluate possible risk factors for disease and clinical signs associated with disease. The continuous variable 'age' was examined in continuous form and the functional form of the relationship was explored using generalised additive models (GAM) (Hastie and Tibshirani 1990). The relationship between age and CPV status was not linear and so polynomial relationships were explored to see if they significantly improved the fit of the model.

All variables with a univariable value of $P<0.3$ were considered for subsequent inclusion in a multivariable model. The model was built using a backwards stepwise approach where variables were retained in the model if their exclusion resulted in a likelihood ratio test statistic (LRTS) of $P<0.05$. The effects of biologically plausible interaction terms were tested in the model. Model fit was addressed by the Hosmer-Lemeshow goodness-of-fit test. Statistical analyses were performed with SPSS 15.0 and S-plus (MathSoft 2005).

Results

A total of 355 suitable samples were collected during the study period. Two of these samples were subsequently excluded from multivariable analysis as insufficient clinical information was held about these cases within the database.

Samples were received from 36 of the 47 hospitals, with considerable variation in the total numbers obtained from each site. The two hospitals in Liverpool (Everton and Huyton) accounted for 22 per cent of the total submissions received nationally. The

TABLE 2: Results of univariable analyses of dog characteristics/variables associated with canine parvovirus status in 353 diarrhoeic dogs presented to PDSA hospitals in the UK

Variable	Parvovirus status Negative (n)	Parvovirus status Positive (n)	Odds ratio	95% confidence interval	LRS P value
Breed					0.07
Pedigree	114	140	Ref		
Crossbreed	34	65	1.6	1.0-2.5	
Breed size					0.05
Large (>25 kg adult weight)	43	62	Ref		
Medium (11 to 24 kg adult weight)	38	51	0.9	0.5-1.7	
Small (<10 kg adult weight)	36	29	0.6	0.3-1.0	
Unknown	31	63	1.4	0.8-2.5	
Vaccination					<0.001
Vaccination status up to date	20	19	Ref		
History of previous vaccination	12	10	0.9	0.3-2.5	
Not vaccinated	50	123	2.6	1.3-5.2	
Unknown	66	53	0.8	0.4-1.7	
Black or tan					0.2
No	134	177	Ref		
Yes	14	28	1.5	0.8-3.0	
Sex					0.4
Female	66	101	Ref		
Male	82	104	0.8	0.5-1.3	
Neutered status					<0.001
Entire	113	194	Ref		
Neutered	35	11	0.2	0.1-0.4	
Region					<0.001
London	44	37	Ref		
Midlands	34	63	2.2	1.2-4.0	
North	23	81	4.2	2.2-7.9	
Scotland	21	3	0.2	0.05-0.6	
South	25	14	0.7	0.3-1.5	
Wales	3	7	2.8	0.7-11.5	
Age (continuous variable piecewise to 55 months)			0.95	0.94-0.97	<0.001

LRS Likelihood ratio test statistic, Ref Reference category

TABLE 3: Results of univariable analyses of presenting clinical signs in 353 diarrhoeic dogs presented to PDSA hospitals in the UK and association with canine parvovirus status

Variable	Parvovirus status Negative	Parvovirus status Positive	Odds ratio	95% confidence interval	LRS P value
Depression/lethargy					<0.001
No	50	31	Ref		
Yes	98	174	2.9	1.7-4.8	
Outcome					0.3
Survived	111	143	Ref		
Died	37	62	1.3	0.8-2.13	
Vomiting					<0.001
No	31	14	Ref		
Yes	117	191	3.6	1.8-7.1	
Volume of diarrhoea					0.8
Copious	43	66	Ref		
Mild	37	50	0.9	0.5-1.6	
Moderate	68	89	0.9	0.5-1.4	
Diarrhoea type					0.3
Haemorrhagic	100	148	Ref		
Non-haemorrhagic	48	57	0.8	0.5-1.3	
Body temperature					0.07
Normal	90	113	Ref	Ref	
Elevated	34	69	1.6	1.0-2.6	
Sub-normal	24	23	0.8	0.4-1.4	
Age (continuous variable piecewise to 55 months)			0.95	0.94-0.97	<0.001

LRS Likelihood ratio test statistic, Ref Reference category

Birmingham region hospitals submitted 13 per cent of total samples and one London hospital submitted 11 per cent of the total samples. The remaining samples were more evenly distributed among the remaining hospitals.

A relatively consistent total number (mean 25, range 17 to 32) of samples was received each month through the core period of the study (2006); however, the monthly total was reduced in both the initial and final sample collection months.

The age of animals from which samples were submitted ranged from one to 16 years and one month with both the mean and median figure being seven months.

Overall, the case fatality rate was 28 per cent (99 of 353). Antimicrobials were prescribed to 98 per cent (346 of 353) of cases.

Prevalence of CPV and risk factors for infection

The overall prevalence of CPV in these samples was 58 per cent (95 per cent confidence interval (CI) 53 to 63 per cent). There was an apparent variation by region (Table 1). The northern part of England had the highest prevalence of 78 per cent (95 per cent CI 69 to 85 per cent) and also submitted the greatest number of samples (104 of 355). Wales showed a similarly high prevalence of 70 per cent (95 per cent CI 39 to 91 per cent). However, in light of the smaller sample numbers and subsequent wide CI, the significance of this figure is less certain. The lowest prevalence was seen in Scotland, at 13 per cent (95 per cent CI 4 to 30 per cent).

Univariable analysis (Table 2) also showed that unvaccinated animals and entire animals were more likely to be CPV positive compared with vaccinated and neutered animals, respectively. Older animals were less likely to be CPV positive.

Analysis of the presenting clinical signs (Table 3) showed that CPV-positive animals were more likely to be depressed or lethargic on presentation, and were also more likely to be vomiting. Neither estimated diarrhoea volume, nor the presence of haemorrhage, was associated with CPV infection.

CPV-positive dogs were more likely to receive intravenous fluids than dogs negative for CPV (96 v 75 per cent) and were more likely to receive antiemetic treatment (91 v 66 per cent). The mortality outcome showed no significant difference between the CPV positive and CPV negative groups (30 v 25 per cent).

Whether a dog was pedigree or crossbreed did not appear to influence its CPV status, and particular breeds, including rottweilers, dobermanns and German shepherd dogs, were no more likely to be CPV positive; however, the numbers of these breeds in the study were not high.

The final multivariable model showed that age, vaccination status and region were the only factors associated with CPV status (Table 4). However, this relationship was complicated. The age of the animal did not have a linear relationship with the outcome (Fig 1), so this was modelled as a piecewise fit with a decreasing risk up to the age 55 months followed by no change in risk. Furthermore, there was a notable interaction between age and vaccination status (Fig 2). At younger ages, unvaccinated animals were more likely to be CPV positive than those animals that had been vaccinated. As age increased, however, the vaccination status appeared to have less effect. The Hosmer-Lemeshow goodness-of-fit statistic was P=0.8 (ie, not significant), indicating good model fit.

Multivariable analysis of presenting clinical signs and isolation of CPV is shown in Table 5. These complement the findings of the univariable analysis and show that the presence of lethargy and depression were more likely to be presenting clinical signs in CPV-positive dogs; CPV-positive dogs also tended (P=0.1) to be more likely to present with vomiting. These estimates were not affected by including the age of the animal. The Hosmer-Lemeshow goodness-of-fit statistic was P=0.5, indicating good model fit.

None of the other presenting signs were associated with CPV status.

Prevalence of CECoV and risk factors for infection

The prevalence of CECoV infection was 7.9 per cent (95 per cent CI 5.3 to 11.2 per cent) (28 of 355). Co-infection with CPV and CECoV was not common (2 per cent; seven of 353), and by chi-squared test was less likely than would occur by chance – that is, dogs that were CECoV positive were less likely to also be CPV positive (P<0.001).

Dogs infected with CECoV were no more likely to be male than female (odds ratio [OR] 0.6, 95 per cent CI 0.3 to 1.4), crossbreed than pedigree (OR 1.2, 95 per cent CI 0.5 to 2.7) or neutered than entire (OR 1.7, 95 per cent CI 0.5 to 5). Clinical outcomes such as vomiting (OR 0.5, 95 per cent CI 0.2 to 1.3), non-haemorrhagic diarrhoea (as

TABLE 4: Multivariable analyses of dog variables associated with canine parvovirus status in 353 diarrhoeic dogs presented to PDSA hospitals in the UK

Variable	Odds ratio	95% confidence interval	LRS P value
Age in months (piecewise to 55 months)	0.99	0.9-1.0	0.3
Vaccination status			
History of previous vaccination	Ref		0.09
Not vaccinated	2.8	1.1-6.9	
Unknown	2.2	0.8-6.1	
Age: vaccination interaction			
Age x history of previous vaccination	Ref		0.04
Age x not vaccinated	0.9	0.9-1.0	
Age x unknown vaccination status	0.9	0.9-1.0	
Region			
London	Ref		<0.001
Midlands	2.0	1.0-3.9	
North	2.8	1.3-5.6	
Scotland	0.2	0.1-1.0	
South	0.8	0.3-1.9	
Wales	2.7	0.5-13.9	

LRS Likelihood ratio test statistic, Ref Reference category
Hosmer-Lemeshow P=0.8

TABLE 5: Multivariable analyses of presenting clinical signs associated with canine parvovirus in 353 diarrhoeic dogs presented to PDSA hospitals in the UK

Variable	Yes/no	Odds ratio	95% confidence interval	LRS P value
Depression/lethargy	No	Ref		
	Yes	2.2	1.2-3.9	0.009
Vomiting	No	Ref		
	Yes	1.9	0.9-4.3	0.1
Age in months (piecewise to 55 months)		0.9	0.9-1.0	<0.001

LRS Likelihood ratio test statistic, Ref Reference category
Hosmer-Lemeshow P value=0.5

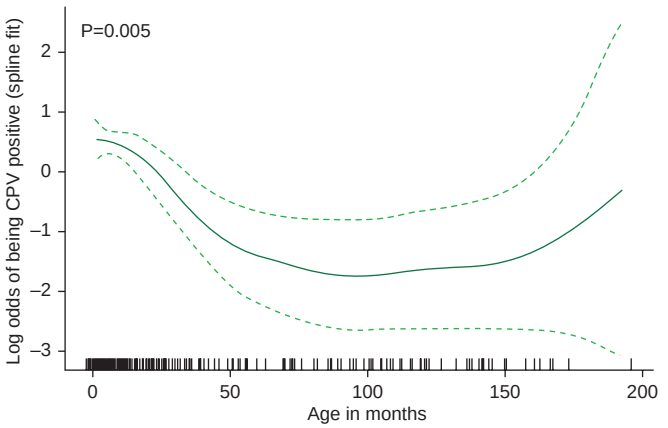

FIG 1: Plot representing the functional form of the continuous variable 'age' modelled in a multivariable generalised additive model (where the continuous fixed effects are fitted using smoothers) to determine the shape of the relationship between the predictor variable and the outcome (log odds of canine parvovirus [CPV] positive). The plots show the fitted curves with 95 per cent confidence intervals (dashed lines). The rug plots along the x axis represent the number of data points. The P value (P=0.005) indicates that the relationship was significantly different linearly

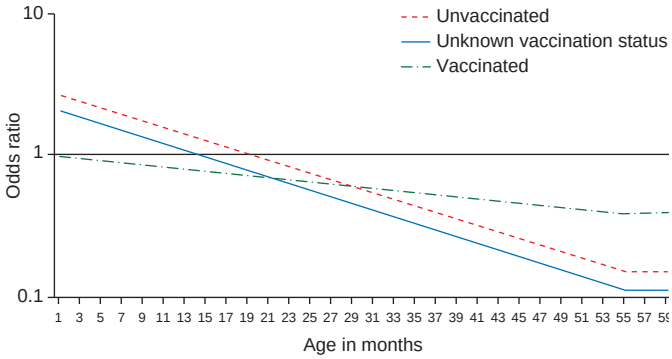

FIG 2: Plot to demonstrate the relationship between age and vaccination status on the odds of being canine parvovirus positive in 353 diarrhoeic dogs presented to PDSA hospitals in the UK

opposed to haemorrhagic diarrhoea) (OR 1.5, 95 per cent CI 0.7 to 3.5), depression (OR 0.8, 95 per cent CI 0.3 to 2.1) and death (OR 0.8, 95 per cent CI 0.3 to 2.3) were not significantly different in CECoV-positive dogs when compared with the rest of the study population. Unvaccinated dogs were likewise at no significantly different risk of CECoV infection (OR 0.4, 95 per cent CI 0.1 to 1.2).

Treatments given to CECoV-positive dogs had some differences when compared with the remainder of the study population. Although there was no significant difference in the likelihood that a CECoV-positive dog would receive antibacterials (OR 0.2, 95 per cent CI 0.04 to 1.7) or have intravenous fluids administered (OR 0.5, 95 per cent CI 0.2 to 1.4), CECoV-positive dogs were significantly less likely to be given antiemetics (OR 0.4, 95 per cent CI 0.1 to 0.9) than the other dogs in the study population.

Discussion

Severe diarrhoea remains an important cause of morbidity and mortality in dogs. Using PCR, this study showed the prevalence of CPV and CECoV was 58 per cent and 7.9 per cent, respectively, in a population of 355 dogs presenting to PDSA hospitals with severe gastroenteritis.

The prevalence of CPV was much higher than previous estimates by Schulz and others (2006) of 16.6 per cent. However, this latter study used electron microscopy to diagnose infection, which is known to be less sensitive than PCR for similar pathogens (Logan and others 2006). Within the present study, there was evidence of geographical variation in the CPV prevalence, even after allowing for age and reported vaccination status, with estimates ranging from 78 per cent in the north of England to as low as 13 per cent in Scotland. The precise reason for these observations is uncertain but may reflect differences in vaccine uptake in the general population, or population density, or may be due to sampling bias. Whether the higher prevalence of CPV seen in certain areas reflects long-term differences in different populations, or is a reflection of local epidemics of disease and/or sampling bias, is not clear. For example, most of the cases (75 per cent) from the northern regions were submitted from one city, Liverpool, so it is possible that these results were due to an outbreak of CPV infection in that area during the study. Clearly, the geographical variation in CPV disease warrants further investigation.

The reported prevalence of CECoV in the study population (7.9 per cent) was lower than that previously found in dogs with diarrhoea using similar molecular assays (–15 to 42 per cent in pet dogs and up to 73 per cent in kennelled dogs (Bandai and others 1999, Pratelli and others 2000, Yesilbag and others 2004, Sokolow and others 2005). However, it was higher than the 3 per cent prevalence reported for the general dog population (Stavisky and others 2010), suggesting an association between CECoV shedding and diarrhoea in the dogs in the present study. This may be a reflection of the fact that infection with CECoV is generally thought to cause mild diarrhoea that resolves with little or no treatment (Tennant and others 1993, Pratelli 2005). However, more severe signs can occur more rarely, including vomiting, lethargy, haemorrhagic diarrhoea and death (Evermann and others 2005, Buonavoglia and others 2006), and it is perhaps these cases that are being seen in the present study.

Co-infection with CPV and CECoV was not a common feature in the present study (2 per cent) and was notably less common than would be expected by chance. This contrasts with previous suggestions that CPV and CECoV may be important co-infecting agents in animals with severe diarrhoea (Evermann and others 1980, Pratelli and others 1999b), and this discrepancy may be due to differences in the dog populations from which the samples were obtained in different studies. It seems unlikely that infection with one virus is in

itself protective against infection with the other. Perhaps more likely, this suggests that different risk factors exist for infection for CPV and CECoV within this population, but that the present study lacked the power to characterise these variations fully. The CPV-positive dogs tended to be younger than the CECoV-positive dogs, suggesting that age may be one of the differing risk factors.

Perhaps not surprisingly, vaccination status was identified as a risk factor for CPV infection, with unvaccinated animals more likely to be CPV positive than their contemporaries with a history of vaccination. It was difficult to ascertain the precise vaccination history of many of the dogs as most had not had a vaccine administered by the PDSA. The vaccine history was therefore dependent on the owner's recall and may have been subject to recall bias. Those animals with an uncertain vaccination status were shown to have an intermediate risk of being CPV positive, between the vaccinated and unvaccinated groups, suggesting that this group included both vaccinated and unvaccinated animals. The lack of association of vaccine status with CECoV status is perhaps predictable, considering that CECoV is not included in many vaccination protocols.

Increasing age had an inverse relationship with the likelihood of CPV infection; the odds of CPV decreased as the animal's age increased up until approximately 55 months of age. This relationship was much more pronounced in unvaccinated dogs and in those with an unknown vaccination history than it was in vaccinated dogs. However, after approximately 30 months there was little effect of vaccination history, suggesting that low-level environmental exposure to field or vaccine virus may have a protective effect over time. No significant relationship was detected between age and the likelihood of CECoV infection. Although CECoV can affect dogs of any age, it is considered more common in younger dogs; in particular, virulent CECoV is generally reported in young puppies (Evermann and others 2005, Buonavoglia and others 2006). A larger sample size than that used in the current study would be required to characterise risk factors for CECoV.

In this study, the animals infected with CPV were more likely to be depressed and vomiting than those animals that were CPV negative. In contrast, neither the severity of the diarrhoea nor the presence of haemorrhagic diarrhoea was associated with CPV in this study. This has previously been suggested (Houston and others 1996). Therapeutically, CPV-positive cases were more likely to receive antiemetics, consistent with them being more likely to present with vomiting. In contrast, although CECoV-positive dogs were no less likely to vomit, they were significantly less likely to receive antiemetics, suggesting that the vomiting seen with CECoV may be less severe or prolonged than that seen with CPV infections.

Regardless of what agents were eventually identified, 307 (87 per cent) of the cases received intravenous fluid therapy, although CPV-positive cases were more likely to receive this treatment. Antibiotics were prescribed to 348 (98 per cent) of the cases. The dogs that did not receive antibiotics had generally died before antibiotics could be administered. It seems likely that the high levels of antibiotic usage and intravenous fluid therapy in this population represents a sampling bias towards those dogs that were most severely ill and required hospitalisation, making obtaining a faecal sample logistically straightforward.

The observed overall mortality in this study (28 per cent) was high relative to some previously published studies (8 per cent; six of 77) (Mantione and Otto 2005). This is likely because all cases in this study were severe cases of gastroenteritis and therefore, regardless of the aetiological agent, might be expected to have a high mortality compared with milder cases of diarrhoea. Mortality rates were similar within the CPV (30 per cent), CECoV (21.4 per cent) and cause-not-identified (25 per cent) groups.

The results showed that the pedigree/non-pedigree status of the animal had no influence on the likelihood of being CPV or CECoV positive. In addition, no evidence was found to suggest that rottweilers, dobermanns or German shepherd dogs were more likely to be CPV positive; however, the numbers of these breeds were not high. This is in contrast to a study by Houston and others (1996), which showed a higher risk of CPV infection in rottweilers and German shepherd dogs. Houston and others (1996) also showed that neutered animals were at a lower risk of contracting CPV than entire animals, and also that sexually intact males were at a greater risk than intact females. No such association with sex was shown in the present study, and although univariable analysis showed that neutered animals were at lower risk, this did not remain in the final multivariable model. This is likely due to confounding between the neuter status, vaccination status and age of the animal (ie, entire animals are likely to be younger and may be more likely to be unvaccinated). The reason for these discrepancies between the two studies is likely to be due to differences in analytical methods and the inclusion of the potential confounders' age and vaccination status in our model.

Of the cases presented here, 38 per cent (136 of 355) of the dogs were negative for both CPV and CECoV, raising the question of what the aetiological agent might have been in these cases. Other known causes of severe diarrhoea that were not looked for in this study include other pathogens, such as *Salmonella* species (Schotte and others 2007) and *Giardia* species (Paoletti and others 2008), and non-infectious causes such as scavenging (Stavisky and others 2010).

Compliance by PDSA staff was poorer than initially anticipated; it is estimated that approximately 6000 eligible cases would have been presented at a hospital during the eventual 18-month study period, but only 355 samples were received, representing a compliance of 5.9 per cent. This may explain why the prevalence estimate for CPV in this study is so high, and reflects a selection bias at the level of the PDSA staff collecting and submitting samples. This underlines the difficulty of obtaining clinical samples of this type from a busy practice environment; however, despite the challenges in obtaining these samples, they represent the most direct measure of field clinical cases actually presenting at veterinary surgeries.

In conclusion, CPV is a common agent isolated in cases of severe gastroenteritis presenting to PDSA hospitals. Those patients presenting with signs of depression, lethargy and vomiting are more likely to be infected with CPV. Vaccination appears to be beneficial in the prevention of CPV infection, especially in younger animals. CECoV should also be considered as a differential diagnosis in cases of severe enteritis, particularly when CPV is not present.

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