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A case–control study of pathogen and lifestyle risk factors for diarrhoea in dogs

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ABSTRACT

Diarrhoea is a common and multi-factorial condition in dogs, the aetiology of which is often incompletely understood. A case–control study was carried out to compare the carriage of some common canine enteric pathogens (enteric coronavirus, parvovirus, distemper, endoparasites, *Campylobacter* and *Salmonella* spp.), as well as lifestyle factors such as vaccination history, diet and contact with other species, in dogs presenting at first opinion veterinary practices with and without diarrhoea.

Multivariable conditional logistic regression showed that dogs in the study which scavenged or had had a recent change of diet (OR 3.5, $p = 0.002$), had recently stayed in kennels (OR 9.5, $p = 0.01$), or were fed a home-cooked diet (OR 4, $p = 0.002$) were at a significantly greater risk of diarrhoea, whilst being female (OR 0.4, $p = 0.01$), currently up to date with routine vaccinations (OR 0.4, $p = 0.05$) and having contact with horse faeces (OR 0.4, $p = 0.06$) were associated with a reduced risk. None of the pathogens tested for was a significant factor in the final multivariable model suggesting that in this predominantly vaccinated population, diarrhoea may be more associated with lifestyle risk factors than specific pathogens.

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1. Introduction

Diarrhoea is a common condition in dogs. In a questionnaire study of dog owners in the UK, 14.9% (266/1784) of dogs had had diarrhoea within the previous two week period (Hubbard et al., 2007). Similarly, in a cross-sectional study of vet-visiting dogs, 28.6% (68/249) of dogs either had a presenting complaint of diarrhoea, or had had diarrhoea within the previous month (Stavisky et al., 2010). However, risk factors and aetiological agents remain poorly understood, partly because many cases resolve with no, or

only symptomatic treatment. Whilst many dogs do recover, their wellbeing must be considered impaired by this syndrome, and additionally, outbreaks of even self-limiting diarrhoea can have extensive implications both in terms of welfare and economics, especially when large numbers of dogs are involved. These negative consequences of diarrhoea become magnified in outbreaks of severe disease, when affected dogs suffer systemic illness, and the cost of their care is increased. Consequently, an improved understanding of potential causes of this condition is desirable.

Many cases of acute diarrhoea in dogs may be multi-factorial, resulting from a combination of pathogens and lifestyle risk factors. A small number of studies have been performed to clarify the role of infectious agents in canine diarrhoea in veterinary practice. In faecal samples from 71 vet-visiting dogs and 59 age-matched non-diarrhoeic

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controls, 30% of cases and 22% of controls were found to contain potential pathogens, including canine enteric coronavirus (CECoV), canine parvovirus 2 (CPV-2), *Campylobacter* spp., *Salmonella* spp, *Giardia* spp, cryptosporidium and helminths (Hackett and Lappin, 2003). These prevalence figures may have been an underestimate, as some of the methods used in the study, for example electron microscopy for viruses, are relatively insensitive. Another study compared the prevalence of pathogens, again using electron microscopy, in the faeces of 936 dogs presenting at a veterinary hospital with haemorrhagic diarrhoea with samples from 200 healthy controls (Schulz et al., 2008). Potential pathogens were detected in 44% of cases and 18% of controls, and included CECOV, CPV-2 and a non-specified paramyxovirus.

Despite this work, there appears to be no published report of a case-control study where participants are recruited contemporaneously, and such a large number of potential risk factors, including lifestyle and infectious agents, have been evaluated in cases of diarrhoea presenting to first opinion veterinary practices. A case-control study was therefore performed to identify such risk factors in dogs presented to first opinion veterinary practices for diarrhoea.

2. Materials and methods

2.1. Study design

A matched case-control study design was used. An estimate of the sample size required in a 1:2 case-control study was made, using an algorithm for an unmatched study. To detect an odds ratio of 3 or greater, for exposures of 10% or greater in the control group with a confidence level of 95% and a power of 80%, the required sample size was 71 cases. We therefore aimed to collect 80 cases and 160 matched controls to allow for losses to follow-up. Matching would also increase the power. For rarer exposures, such as that of 3% expected for CECOV (Stavisky et al., 2010), the study would be able to detect an odds ratio of 5 or greater.

2.2. Case definition

A case was defined as any dog presented to a first opinion veterinary clinic with diarrhoea. Diarrhoea was defined as an owner having observed an increase in the fluidity, volume and/or frequency of the faeces passed by the dog (Battersby and Harvey, 2006). Data regarding the severity of the diarrhoea were recorded, using the Walthams faecal scale as a descriptive guide (Waltham Centre for Pet Nutrition, Melton Mowbray, Leicestershire, UK). Dogs were only included in the study if the owner's description corresponded to a score of 3.5 or more out of a possible score of 5 (1/5 being very dry, hard faeces, and 5/5 being very watery diarrhoea).

Controls were the next two dogs to visit the same clinic, giving a 1:2 case-control format with each set matched on practice and time. Cases could be visiting the practice for any reason, including preventive care and illness of any kind. A control was excluded if it had had diarrhoea in the two week period before presentation. Exclusion criteria for controls were: having an owner-reported history of

diarrhoea in the two week period before presentation at the veterinary practice; the occurrence of any diarrhoea between the veterinary visit and faecal sample submission (if a sample was not collected at the time of presentation), and previous participation in the study. Control dogs could be presented for any reason, provided none of the exclusion criteria were violated.

2.3. Sample collection

The staff of 13 veterinary practices were recruited on the basis of their willingness to participate in the study. Selection of practices was convenience based; all were known to the investigators either from their participation in previous studies or through other professional association. Two practices were located in south-west Scotland (Lanarkshire), seven in north-west England (Merseyside, Cheshire, Manchester, West Yorkshire) and four in southern England (Buckinghamshire, Berkshire, Dorset, Wiltshire).

The study ran from May to October 2008. The period of participation of the practices ranged from three to five months. Veterinary staff were asked to recruit all eligible cases and matched controls as described above. Target numbers were suggested for each practice, based on their caseload as estimated by their staff. Each practice was contacted on an approximately two-weekly basis during the study, to address any problems promptly and encourage compliance. As incentives, each practice received a £5 shopping voucher for each complete case-control set submitted, and these were returned to each practice at two-weekly intervals through the study. Participating owners were also entered into a draw for shopping vouchers, which were awarded at the end of the study.

A faecal sample was obtained from each recruited dog, collected either by the veterinary practice if the dog was admitted (or defecated during its visit), or by the dog owner as soon as possible after the visit, which was generally within 2–3 days. On recruitment, owners were given a pack including information about the study, a faecal sample pot, a prepaid addressed padded envelope and a prepaid addressed postcard to fill in with their contact details. All participating owners were contacted on receipt of their postcard to complete a questionnaire, which was administered by the author (JS) as soon as possible following recruitment. All questionnaires were administered over the telephone, with the exception of a single questionnaire conducted face to face as the respondent was deaf. Respondents were asked to respond to questions regarding risk factors in the period immediately preceding the onset of diarrhoea (cases) or when the faecal sample was obtained (controls). A copy of the questionnaire is available on request.

The questionnaires used for the cases and controls were identical, apart from some additional details collected for the case dogs to characterise their diarrhoea. Data collected are shown in Table 1, and include signalment, vaccination and worming status, any history of kennelling and contact with other species. Questions about diet and contact with other species were asked regarding the dog's usual habits; questions about change in diet referred to the previous week, and questions about any stay in kennels referred

Table 1

Univariable analysis of risk factors for diarrhoea in 86 case and 167 control dogs presenting at veterinary practices in the United Kingdom, 2008.

Variable	Controls (%)	Cases (%)	Total	Parameter estimates		
				p-Value	Odds ratio	95% CI
<i>Questionnaire data</i>						
<i>Population data</i>						
Total age (months)				0.8	1	1–1.004
Age ≤ 1 year	39 (67.2)	19 (32.8)	58			
Age > 1 year	127 (65.5)	67 (34.5)	194	0.8	1.07	0.5–2.1
Male	79 (61.2)	50 (38.8)	129			
Female	88 (71)	36 (29)	124	0.1	0.6	0.4–1.09
Sexually intact	55 (60.4)	36 (39.6)	91			
Neutered	111 (68.9)	50 (31.1)	161	0.2	0.7	0.4–1.2
Crossbreed	38 (76)	12 (24)	50			
Pedigree	128 (63.4)	74 (36.6)	202	0.08	1.9	0.9–3.9
One dog household	110 (67.9)	52 (32.1)	162			
Two dog household	36 (57.1)	27 (42.9)	63	0.04	2	1.02–3.8
3 or more dogs in household	20 (76.9)	6 (23.1)	26	0.5	0.7	0.3–2
<i>Vaccination history</i>						
Up to date with routine vacs (i.e. having had a booster or primary course within the previous year)	132 (73.3)	48 (26.7)	180			
All other vaccinal histories	32 (48.5)	34 (51.5)	66	<0.001	0.3	0.2–0.6
<i>Diet</i>						
Wet dog food (0)	91 (69.5)	40 (30.5)	131			
Wet dog food (1)	76 (62.3)	46 (37.7)	122	0.2	1.4	0.8–2.6
Raw meat/offal (0)	149 (64.5)	82 (35.5)	231			
Raw meat/offal (1)	18 (81.8)	4 (18.2)	22	0.1	0.4	0.1–1.3
Home cooked diet (0)	126 (70.8)	52 (29.2)	178			
Home cooked diet (1)	41 (54.7)	34 (45.3)	75	0.009	2.2	1.2–4.03
Table scraps (0)	81 (66.9)	40 (33.1)	121			
Table scraps (1)	86 (65.2)	46 (34.8)	132	0.9	1.02	0.6–1.7
Dry dog food (0)	15 (57.7)	11 (42.3)	26			
Dry dog food (1)	152 (67)	75 (33)	227	0.3	0.6	0.3–1.6
Meaty titbits (pigs ears, rawhide etc.) (0)	95 (62.1)	58 (37.9)	153			
Meaty titbits (pigs ears, rawhide etc.) (1)	72 (72)	28 (28)	100	0.07	0.6	0.3–1.05
No scavenging or diet change in week before sampling	126 (72.8)	47 (27.2)	173			
Scavenged	16 (57.1)	12 (42.9)	28	0.07	2.3	0.9–5.4
Changed diet	5 (26.3)	14 (73.7)	19	<0.001	10	2.8–35.8
Other unusual dietary occurrence	14 (51.9)	13 (48.2)	27	0.08	2.2	0.9–5.5
No scavenging/diet change in previous week	126 (72.8)	47 (27.2)	173			
Scavenging/diet change in previous week (all categories combined)	35 (47.3)	39 (52.7)	74	<0.001	3.1	1.7–5.8
<i>Worming</i>						
Never wormed	3 (42.9)	4 (57.1)	7			
Wormed in the last 3 months	108 (67.1)	53 (32.9)	161	0.2	0.3	0.05–2.07
Wormed in the last year	38 (63.3)	22 (36.7)	60	0.3	0.3	0.05–2.4
Last wormed over a year ago/never wormed	8 (61.5)	5 (38.6)	13	0.5	0.5	0.07–3.7
Wormed over 3 months ago (all categories)	49 (61.3)	31 (38.7)	80			
Wormed within last 3 months	108 (67.1)	53 (32.9)	161	0.5	0.8	0.4–1.5
<i>Contact with other animals (physical)</i>						
Regular physical contact with cats (0)	126 (66.7)	63 (33.3)	189			
Regular physical contact with cats (1)	41 (64.1)	23 (35.9)	64	0.7	1.1	0.6–2.05
Regular physical contact with pigs (0)	167 (66)	86 (34)	253			
Regular physical contact with cattle/sheep (0)	156 (65.5)	82 (34.5)	238			
Regular physical contact with cattle/sheep (1)	11 (73.3)	4 (26.7)	15	0.6	0.7	0.2–2.5
Regular physical contact with horses (0)	151 (65.4)	80 (34.6)	231			
Regular physical contact with horses (1)	16 (72.7)	6 (27.3)	22	0.6	0.8	0.3–2.2
Regular physical contact with other animals (0)	136 (66.7)	68 (33.3)	204			
Regular physical contact with other animals (1)	31 (63.3)	18 (36.7)	49	0.7	1.1	0.6–2.3
<i>Contact with other animals (faeces)</i>						
Regular contact with cats' faeces (0)	143 (66.2)	73 (33.8)	216			
Regular contact with cats' faeces (1)	24 (64.9)	13 (35.1)	37	0.8	1.1	0.5–2.4
Regular contact with pigs' faeces (0)	163 (65.5)	86 (34.5)	249			
Regular contact with pigs' faeces (1)	4 (100)	0 (0)	4			
Regular contact with cattle/sheep faeces (0)	119 (62.3)	72 (37.7)	191			

Table 1 (Continued)

Variable	Controls (%)	Cases (%)	Total	Parameter estimates		
				p-Value	Odds ratio	95% CI
Regular contact with cattle/sheep faeces (1)	48 (77.4)	14 (22.6)	62	0.02	0.4	0.2–0.9
Regular contact with horse faeces (0)	113 (61.8)	70 (38.3)	183			
Regular contact with horse faeces (1)	54 (77.1)	16 (22.9)	70	0.03	0.4	0.2–0.9
Regular contact with other animals' faeces (0)	123 (63.7)	70 (36.3)	193			
Regular contact with other animals' faeces (1)	44 (73.3)	16 (26.7)	60	0.1	0.6	0.3–1.2
Visit to kennel/shelter/veterinary clinic						
No overnight stay at boarding, rescue or vet kennels	153 (67.7)	73 (32.3)	226			
Any overnight stay at boarding, rescue or vet kennels (all categories combined)	10 (43.5)	13 (56.5)	23	0.02	3	1.2–7.6
Pathogen data						
CECoV (0)	145 (66.5)	73 (33.5)	218			
CECoV (1)	2 (22.2)	7 (77.8)	9	0.02	6.1	1.3–29.8
CPV (0)	147 (65.9)	76 (34.1)	223			
CPV (1)	0 (0)	4 (100)	4	0.015*		
Campylobacter (0)	84 (60)	56 (40)	140			
Campylobacter (1)	63 (72.4)	24 (27.6)	87	0.2	0.7	0.4–1.2
Helminths (0)	136 (64.5)	75 (35.6)	211			
Helminths (1)	11 (68.8)	5 (31.3)	16	0.8	0.9	0.3–2.8

* p-Value is from fishers exact test, as there were zero controls that were parvovirus positive. p-values in bold were submitted for inclusion in multivariable model.

to the previous two-week period. In order to ameliorate the effect of selective recall bias, the study was simply described as being about canine health, rather than about diarrhoea. A range of questions was asked in order to avoid focussing the owner's attention on diarrhoea.

Samples were assigned a sequential number on arrival at the laboratory to allow testing to be performed blind to the case status of each sample.

2.4. Nucleic acid extraction, RT-PCR and PCR

Nucleic acid extraction and reverse transcription were carried out as previously described (Godsall et al., 2010; Stavisky et al., 2010). For CECoV, real-time RT-PCR was carried out using primer/probe combinations targeting a fragment of the M gene as previously described (Decaro et al., 2005), with the addition of a modified IPC consisting of a real-time BVDV assay (Willoughby et al., 2006). Assays were carried out separately for type I and type II CECoV, and performed in triplicate.

For CDV, RT-PCR targeting the conserved large polymerase gene, and for CPIV, nested RT-PCR targeting the nucleocapsid gene were carried out as previously described (Demeter et al., 2007). The positive control used was a multivalent vaccine with fractions of both CDV (strain Onderstepoort) and CPIV (strain FDL) (Duramune® DAPPi + Lc, Fort Dodge).

For the CPV-2 PCR, the DNA extraction and PCR, targeting a 2034 bp fragment containing the viral protein-2 gene, was carried out as described previously described (Meers et al., 2007; Godsall et al., 2010).

2.5. Bacteriology

Samples were screened for *Campylobacter* and *Salmonella* spp. *Campylobacter* spp. were identified by

both culture with prior filtration, and direct PCR, as previously described (Parsons et al., 2010).

Salmonella culture was performed using enrichment with Rappaport-Vassiliadis Medium broth (RVB) and isolates were serotyped using the Kauffman-White scheme, as previously described (Hughes et al., 2008).

2.6. Microscopy

For endoparasitic screening, a modified McMaster method was used, as previously described (Ward et al., 1997). Electron microscopy was carried out on a Philips EM 301 Transmission Electron Microscope (Philips Electron Optics UK Division, Cambridge) at a screen magnification of 45,000 \times . Grids were scanned horizontally, along the rows of grid squares, from end to end, at three different locations.

2.7. Statistical techniques

Descriptive statistics were calculated using SPSS (SPSS for Windows, Rel. 16.0 2007. Chicago SPSS Inc.), and some graphical summaries were generated using Excel (Microsoft Excel 2007). Screening of all variables was performed by univariable conditional logistic regression using matched case-control sets. All factors with a $p \leq 0.3$ were considered for inclusion in the multivariable conditional logistic regression model which was built using backwards elimination. Factors with a likelihood ratio test statistic p-value of >0.05 were excluded step-wise from the model. All eliminated factors were then rechecked against the final model. All terms which had a univariable $p \leq 0.05$ or were included in the final model were checked for interactions, and those with a $p \leq 0.05$ were included in the multivariable model. Model fit was checked using unstandardised delta-betas. All case-control sets with a member with a delta-beta of >0.4 or <-0.4 were excluded, and the model

re-run and checked for consistency. Analyses were performed using EGRET (Egret for Windows 2.0, Cytel Software Corporation 1999). For continuous variables such as age, a generalised additive model (Hastie et al., 2009) (ignoring matching) was used to check for linearity (S-Plus). Collinearity was assessed using Pearson's test for continuous data, and crosstabulation for categorical variables using SPSS.

3. Results

3.1. Response

Nine of the practices returned samples, whilst the remaining four practices withdrew from the study, citing time pressures or inadequate case numbers to warrant participation. In total, 272 dogs were recruited; for 26 of these dogs, faecal samples were not submitted, but questionnaires were completed. Twelve dogs (four cases and eight controls) were completely withdrawn from the study. Of these twelve dogs, two died (neither of diarrhoea) before the questionnaire could be administered, and the others either declined to take part or could not be contacted after numerous attempts. The total of 260 dogs was further reduced to 253 when dogs which did not fit the case or control criteria were excluded before analysis was performed (usually these were dogs which were enrolled as controls but were found to have had diarrhoea within the previous two-week period and were therefore ineligible). Therefore data from 86 cases and 167 controls (86 matched sets, 5 of which had only one control) were included in the final analyses for lifestyle risk factors. For pathogen risk factors, data from the 80 cases and 147 controls which had a faecal sample submitted were used for analysis.

The number of samples submitted by each practice was variable, ranging from two to twenty-two case-control groups (median 10). This was partly due to practice size; the caseload varied from approximately 200 to 580 consultations per week during the study period. The four practices which withdrew cited a similar variation in caseload, having from 150 to 500 consultations per week, suggesting that the size of practice was not related to non-response.

The reason for the visit was specified for all 167 of the control dogs. When more than one reason was mentioned, the first one given was used to categorise the consultation. The most common single reason was vaccination (61/150, 36.5%), followed by skin complaints (16/167, 9.6%), 'social/accompanying other dog' (12/167, 7.2%), ear complaints (8/167, 4.8%), eye complaints (6/167, 3.6%) and post-operative checks (including suture removal) (6/167, 3.6%). The remaining 34.7% of cases were presented for a variety of complaints, including routine neutering, lameness, wound suturing and pregnancy diagnosis.

Several of the dogs in the study were from the same household, and when this occurred, they were placed in the same analysis set. In the final analysis there were five case-control sets in which both control dogs lived in the same household, and a single case-control set where one control and the case were from the same household.

3.2. Characterisation of clinical disease

Using the questionnaire, a more detailed description of the cases was obtained. For a small number of cases, some information was missing, because the respondent had not observed or remembered the behaviour (e.g. defaecation). As described by 86 case owners, the nature of diarrhoea was mild (slightly soft; Waltham score 3.5–4) in 8 (9%), moderate (cow-pat like; Waltham score 4.5) in 27 (31%), and severe (very watery; Waltham score 5) in 51 (59%). Of 85 cases for which the information was known, 50 (58%) had no blood in their diarrhoea, 18 (21%) were passing a little blood or blood spots in their faeces, 9 (10%) had a moderate amount of blood, and 8 (9%) were producing faeces described as very bloody by the owner. Of the 80 dogs for which the information was known, for 13 (16%), the diarrhoea had begun within the previous 48 h, for 45 (56%) the diarrhoea had been ongoing for 3–7 days, and for 14 (18%) 7–14 days.

Only 8 (11%) of the cases in the study had had diarrhoea for >14 days. Of the 86 cases, 46 (53%) had no vomiting, 26 (30%) had vomited once or twice in the previous 48 h, 6 (7%) had vomited 3–4 times, and 8 (9%) had had >4 episodes of vomiting in the previous 48 h. Owners reported a normal demeanour in 29 (34%) dogs, 24 (30%) were 'a little quiet', 30 dogs (35%) were lethargic, and 3 (4%) were collapsed or comatose on presentation at the veterinary practice.

3.3. Univariable analysis (Table 1)

3.3.1. Pathogens

Pathogen analysis was conducted on the 227 dogs for which a faecal sample was submitted. CECoV was more common in cases (7/80, 8.8%,) than in controls (2/147, 1.4%,), OR 6.1 (95% CI 1.3–29.8). Of the positive samples, five were type I CECoV (3 cases, 2 controls), three were type II (all cases) and one (case) dog had a mixed type I and type II infection. Only four dogs within the study tested positive for CPV-2, and all had presented with acute diarrhoea of varying severity. Of these cases, two survived, one died, and for the fourth case the outcome was unknown. No samples tested positive for CDV or CPIV.

Shedding of *Campylobacter* spp. was higher in controls (63/147, 43%,) than the cases (24/80, 30%,), but this was not statistically significant on univariable or multivariable analysis. A single case was found to be shedding *Salmonella* sp.

Overall, evidence of helminth infestation was detected in 7% of the samples, with no significant association between the presence of helminth eggs and diarrhoea. The helminths detected were *Toxocara canis* (two cases, six controls), *Toxascaris leonina* (one case, co-infected with *T. canis*), *Taenia* spp. (three controls), *Uncinaria stenocephala* (two cases) and *Dipylidium caninum* (one case, two controls).

Electron microscopy (EM) was conducted on all cases and on the first 60 controls received. CECoV was observed in two of the case samples, which were both also positive by RT-PCR. CPV was found by EM in two case samples,

which were both also positive by PCR. In two cases and one control, myxovirus-like particles were detected.

3.3.2. Lifestyle risk factors

Lifestyle risk factors were determined using data from the owner questionnaires ($n=253$). Overall, univariable analysis of risk factors for diarrhoea identified that belonging to a multi-dog household, being fed a home cooked diet, having had a recent overnight stay at a boarding kennel, rescue shelter or veterinary practice, having had a change in diet, and shedding CECoV or CPV-2 were all significantly positively associated with a risk of diarrhoea. Having regular contact with the faeces of horses, sheep or cattle, being female and being up to date with routine vaccines were all associated with a reduced risk of diarrhoea.

3.4. Multivariable analysis

The final multivariable model is shown in Table 2. A number of factors significantly affected the likelihood of diarrhoea. A change in diet (whether via the owner, or through scavenging) and being fed a home-cooked diet were each associated with an increased risk of diarrhoea. Being female and being up to date with routine vaccines were each associated with a reduced risk of diarrhoea. Having regular contact with horse faeces was also associated with a reduced risk of diarrhoea. In the final model, the confidence intervals for the odds ratio of this term included one, but the term was retained as it improved model fit (likelihood ratio statistic [LRS] $p < 0.05$).

All terms were checked for collinearity, and no significant associations were found. All terms with $p < 0.05$ on univariable analysis, and all of the terms from the multivariable model were checked for interactions. An interaction was found between having stayed in kennels in the previous two-week period (including boarding kennels, rescue shelter or overnight at a veterinary practice) and being fed a home-cooked diet. This interaction term was therefore included in the final model and showed that, although staying in kennels and eating a home-cooked diet increased the risk of diarrhoea, the combined effect of these two covariates was lower than expected suggesting that the effect of staying in kennels is less in those dogs normally fed a home cooked diet.

3.5. Model fitting

Data points with delta-betas of more than 0.4 or less than -0.4 were removed from the data set, along with associated cases or controls. There were a number of points with high residuals in the data set 'stay in kennels'; these were equally distributed between the cases and controls and, when removed from the data set, they did not affect the predictions of the model except to increase further the odds ratio of the effect of a stay in kennels (OR 21.2, 95% CI 1.9, 233.4). Since removal of residuals would have only reinforced the findings of the model, these cases were retained in the analysis.

4. Discussion

This study has identified a number of factors which contribute significantly to the occurrence of diarrhoea in dogs. Several lifestyle and dietary practices were shown to be potentially associated with canine gastrointestinal disease on univariable and multivariable analysis, and in a small number of cases, CECoV and CPV-2 appeared to be involved.

The finding that a history of scavenging or change of diet in the week prior to the diarrhoea gave increased odds of having diarrhoea is supported by a previous study of dog-owning veterinary clients (Hubbard et al., 2007). However, it is possible that owners of dogs with diarrhoea might be more likely to recall occasions of dietary indiscretion than owners of healthy dogs, and this potential bias could affect the results, making some risk factors appear more probable in case versus control dogs.

A home-cooked diet was also positively associated with diarrhoea. This was detailed separately from the feeding of scraps and left-overs, so would perhaps be unlikely to be due to the feeding of food thought to be unfit for human consumption. A possible explanation is that owners might be more likely to feed a dog known to be fussy or prone to digestive upsets on a home-cooked diet, or may be more likely to seek veterinary advice if their dog has diarrhoea. However it may be that home cooked diets are poorly prepared or composed, in comparison to commercially prepared foods. Feeding of raw meat, including bones, rawhide and tripe was detailed separately, and had no significant association with the risk of diarrhoea.

Female dogs were at a slightly decreased risk of diarrhoea compared to male dogs. This agrees with a previous study in which male dogs were found to be at increased risk of diarrhoea (Hubbard et al., 2007). Although the reason for such a finding is unclear, it may be that males are more likely to encounter pathogens whilst engaged in investigatory and roaming behaviour. No statistical association was observed between gender and scavenging behaviour; however this was not a primary objective of the study. Others have suggested a relationship between male hormones and roaming behaviour (Maarschalkwerd et al., 1997) and sniffing contacts with other dogs (Westgarth et al., 2008), all of which may give increased opportunity for the acquisition of gastrointestinal (and other) pathogens.

Having an up-to-date vaccination history was also associated with reduced risk of diarrhoea. Routine vaccination in the UK, when administered, typically includes vaccines protective against diseases caused by CDV, CPV-2, CPIV and canine adenovirus, and *Leptospira* serovars *canicola* and *icterohaemorrhagiae* (Gaskell et al., 2002). Of these, CPV-2 and CDV are the most likely to be associated with diarrhoea, although CDV infection is typically also accompanied by respiratory and other signs. Despite using sensitive molecular techniques, no CDV was detected, and only four case dogs were found to be shedding CPV-2. None of these four positive dogs was known to have a complete vaccination history; two dogs had not yet been vaccinated, and for the other two dogs the vaccine history was unknown.

The reason why there was a relatively low prevalence of the recognised enteric viral pathogens such as CPV-2 and CECoV in this study is not clear. One possible expla-

Table 2

Final multivariable conditional logistic regression model showing risk factors for diarrhoea in vet-visiting dogs in the United Kingdom, 2008, using 86 matched 1:2 case–control sets.

	Parameter estimates		p-Value	Odds ratio	95% CI	
	Coefficient	Standard error			Lower	Upper
<i>Terms</i>						
Stay in kennels	2.2	0.9	0.01	9.5	1.6	54.7
Recent scavenging/diet change	1.2	0.4	0.002	3.5	1.6	7.5
Home-cooked diet	1.4	0.4	0.002	4	1.6	9.6
Female	−0.9	0.4	0.01	0.4	0.2	0.8
Up to date with vaccinations	−0.9	0.4	0.05	0.4	0.2	1
Contact with horse faeces	−0.9	0.5	0.06	0.4	0.1	1.02
Interaction term home-cooked diet × recent stay in kennel ^a	−2.4	1.2	0.04	0.09	0.009	0.9
<i>Interaction terms</i>						
Dog did not stay in kennels and was not fed home cooked diet				1		
Dog stayed in kennels and was not fed home cooked diet	2.3			9.5		
Dog did not stay in kennels and was fed home cooked diet	1.4			4		
Dog stayed in kennels and was fed home cooked diet	1.2			3.4		

^a Stay in kennels included any overnight stay in a boarding kennel, rescue shelter or veterinary clinic.

nation is that the cases were obtained from first opinion practice and the case definition was relatively broad, thus potentially leading to the inclusion of a variety of other aetiologies, including lifestyle factors, as well as other potential pathogens for which we did not screen. Another possibility is that the sensitivity and specificity of our tests was too low. In general, sensitivity and specificity of individual tests have not been reported for the majority of canine pathogens, partly because of insufficient data, and partly because of a lack of agreement for what constitutes a 'gold standard'. However in a recent study where we focused on more severe, haemorrhagic cases of diarrhoea, we detected CPV-2 in 58% of 355 diarrhoeic dogs attending a charitable hospital practice, using an identical laboratory protocol (Godsall et al., 2010). Similarly in previous work we have been able to detect a prevalence of CECoV of up to 20% using the same real-time RT-PCR assay in kennelled dogs (Stavisky et al., 2010).

Overall, it is perhaps more likely that the apparent association between up to date vaccinations and a reduced risk of diarrhoea may be a proxy for better owner care including vaccination, or due to sampling bias within the study. For example control dogs visiting the practice may be more likely to be up to date with vaccinations, or veterinarians may have preferentially recruited dogs presented for booster vaccination rather than more complex consultations. This may have resulted in dogs visiting for boosters being over-represented in the control population.

In general the control population had similar reasons for presentation when compared to a previous study of vet-visiting dogs (Hill et al., 2006) in that preventive care was the most common reason given for presentation, followed by dermatological complaints. An un-anticipated reason given was 'social', and this encompassed both dogs visiting for habituation to the practice, and dogs accompanying another dog. This reason was unexpected, and may suggest either that a relatively large number of dogs visit the vet for social reasons, or that a small number of control participants were recruited on a convenience basis, rather than strictly according to the study protocol. It is difficult to predict what effect, if any, this may have had on the composition of the control group of dogs; it is how-

ever possible that some bias may have been introduced in this way.

Regular contact with cattle/sheep or horse faeces appeared to be associated with a reduced risk of diarrhoea, having a strong association in the univariable analysis and a marginal association in the multivariable model. Horse faeces have been suggested to contain probiotic bacteria which discourage gut colonisation of several pathogenic bacteria, including *Escherichia coli*, *Clostridium difficile* and *Salmonella* spp. (Weese et al., 2004), whilst probiotic supplementation in dogs with food-responsive diarrhoea has been shown to improve clinical signs of disease (Sauter et al., 2006). In addition, it has been suggested that the consumption of cellulose may reduce the water content of faeces (Wichert et al., 2002). The question asked was limited to whether the dog had had contact with the faeces, rather than whether it had consumed them; therefore whilst it is possible that there is some genuine protective effect evoked by the contact with or consumption of some herbivore faeces in the dog, this would require further study.

The cases and controls in this study were defined by the dogs' owners. Owners were asked if their dog had had diarrhoea, defined as an increase in the fluidity, volume and/or frequency of faeces (Battersby and Harvey, 2006). This is obviously a subjective assessment and could have given rise to misclassification bias in either the cases or the controls. In order to control for this, an objective scale was also used; however alternative methods, such as the calculation of faecal dry matter, could also be considered.

Although the case definition in this study did not specify how long the dog had had diarrhoea, the large majority (90%) of the dogs in the study had experienced diarrhoea for less than 14 days, which is used in practice as the threshold for defining chronic diarrhoea (Chandler, 2002). This suggests that acute diarrhoea is a much more common presentation in first opinion veterinary practice than chronic diarrhoea. The study is therefore by default largely focussed on acute diarrhoea in dogs, and it is possible that chronic diarrhoea may have a different aetiology; however this was not further explored in the present study.

The use of vet-visiting dogs is a commonly used technique when investigating diseases in companion animals

(Hill et al., 2006; Summers et al., 2009); however questions must be raised as to how representative these animals are of the population as a whole. It seems likely that there may be characteristics of dogs whose owners take them to the vets that differ from those dogs whose owners rarely or never present them to a veterinary surgeon; however, accessing this community of dogs for research purposes presents considerable challenges.

A limitation of the study is that it was not possible to include a number of potential pathogens in the faecal screening. Numerous organisms have been suggested to have a role in canine diarrhoea, including *Giardia* spp. (Hackett and Lappin, 2003), calicivirus (Mochizuki et al., 1993), norovirus (Martella et al., 2008), *C. difficile* (Berry and Levett, 1986), *E. coli* (Sancak et al., 2004), *Brachyspira* spp. (Hidalgo et al., 2010) and *Isospora* and *Cryptosporidium* spp. (Batchelor et al., 2008). For many of these organisms, as for *Campylobacter* spp. and *Salmonella* spp., a formal causal relationship has not been established, and would benefit from further investigation.

In conclusion, this represents the first case–control study of diarrhoea in dogs presented to first opinion veterinary practices in which samples were obtained contemporaneously, and where a wide range of pathogens were screened for using sensitive molecular techniques. The identification of the possible role of various lifestyle risk factors, and infectious agents such as CPV-2 and CECoV in some cases, should aid in the prevention and control of this common syndrome. However, the role of other pathogens should also be investigated in future work, as many cases of diarrhoea in dogs currently remain undiagnosed.

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