Epidemiol. Infect. (2011), **139**, 1531–1541. © Cambridge University Press 2011
doi:10.1017/S0950268810002906

Factors related to *Campylobacter* spp. carriage in client-owned dogs visiting veterinary clinics in a region of Ontario, Canada

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(*Accepted 16 November 2010; first published online 6 January 2011*)

SUMMARY

From July 2008 until May 2009, 240 client-owned pet dogs from seven veterinary clinics in the Region of Waterloo, Ontario, Canada participated in a study to determine pet-related management factors that may be associated with the presence of *Campylobacter* spp. in dogs. The prevalence of *Campylobacter* spp. carriage in our study population of pet dogs was 22%, with 19% of the dogs positive for *C. upsaliensis*, and 3% positive for *C. jejuni*. A significant risk factor from multivariable logistic regression models for both *Campylobacter* spp. and *C. upsaliensis* carriage was having homemade cooked food as the dog's diet or added to its diet, and a significant sparing factor for both models was treatment with antibiotics in the previous month. Increasing age of the dog decreased the odds of *Campylobacter* spp. and *C. upsaliensis* carriage. Based on the high prevalence of *Campylobacter*, and specifically *C. upsaliensis*, further research concerning pet dogs as a risk factor for campylobacteriosis in humans is warranted.

Key words: *Campylobacter*, *C. upsaliensis*, dogs, public health, zoonoses.

INTRODUCTION

Campylobacteriosis is the most common cause of bacterial enteritis in people in Canada, with about 9500 laboratory-confirmed cases each year [1]. The most commonly recovered species is *Campylobacter jejuni*, followed distantly by *C. coli* and *C. lari* [1, 2]. *Campylobacter* usually causes mild to severe gastrointestinal infection in humans, including nausea, vomiting and watery diarrhoea, but potentially life-threatening sequelae can occur (e.g. Guillain–Barré syndrome) [3, 4]. The majority of human cases are sporadic and believed to be foodborne; however, since the early 1980s, several studies have investigated the role of companion animals as potential sources of human infections (e.g. [5, 6]). Many studies have identified having a household pet, especially a puppy, or a dog with diarrhoea, as a risk factor for *Campylobacter* infection in people [7–12]. Dogs have also been suspected as the source of transmission in several cases of campylobacteriosis [13–15].

Domestic dogs have long been recognized as potential sources of zoonotic enteric pathogens like *Salmonella*, *Campylobacter* and *Giardia* [16, 17]. The prevalence of *Campylobacter* carriage in clinically healthy pet dogs has been estimated to be between

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15% and 58% [18–24], but can be as high as 87% in stray animals [25]. *Campylobacter* can cause both clinical and non-clinical infections in dogs, with the most severe sequelae occurring in young and immunocompromised dogs; often associated with *C. jejuni* infection [6, 26]. The most commonly isolated species of *Campylobacter* in dogs has varied in studies due to microbiological methods, but in recent work, *C. upsaliensis* has been the most frequently recovered species in dogs [21, 22, 24, 27].

It is estimated that there are about six million dogs in Canada, with 32% of households owning at least one dog [28]. In North America, a strong human–animal bond means that dogs are often considered family members rather than simply 'pets'. It is this close relationship that causes concern with respect to the potential transmission of zoonotic infections from dogs to humans. Several studies have identified young age, the presence of diarrhoea, season, and high-density housing, like kennels and shelters, as significant risk factors for the carriage of *Campylobacter* in dogs [20–24, 29]. Due to a lack of detailed investigations examining pet-related management factors and their association with *Campylobacter* carriage in dogs in North America, investigations are required to identify these factors, and determine which management practices may potentially put pet-owners at increased risk of infection from their pets. The purpose of this study was to determine which pet-related management factors, including type of diet fed to the dog, the dog's exposure/access to other pets and livestock, the dog's involvement in group activities (e.g. obedience), and veterinary treatments, are associated with the carriage of *Campylobacter*. In addition, human-related factors, including the presence of children in the home, household members' exposure/access to other animals and livestock, whether household members have visited or worked in a hospital, and any household members experiencing vomiting or diarrhoea in the previous week, have also been examined. This study will be used to explore the epidemiology of carriage of *Campylobacter* and specific *Campylobacter* spp. in a population of client-owned pet dogs from the Region of Waterloo, Ontario, Canada.

METHODS

Recruitment

Between July 2008 and May 2009, dogs visiting seven veterinary clinics in the Region of Waterloo, Ontario were recruited to participate in a study to investigate the occurrence of *Campylobacter*, *Salmonella*, *Giardia*, and antimicrobial resistance in generic *Escherichia coli* in client-owned pet dogs. This paper contains only the *Campylobacter* results. Veterinarians from all 44 veterinary clinics in the Region of Waterloo, Ontario were sent letters inviting them to participate in the study, with nine clinics responding and seven clinics agreeing to participate. Once a clinic agreed to take part, the primary author (E.L.) visited each of the clinics every 7–14 days for 10 months to recruit client-owned pet dogs for the study. Any dog visiting the clinic was eligible to participate, including those with signs of gastrointestinal disease and those being treated with antimicrobials; however, only one dog per household was included in the study and dogs were only eligible to participate in the study once. Dog-owners visiting the veterinary clinics were asked by their veterinarian or the primary author to participate in the study. Those who agreed to participate then spoke with the primary author, the owner questionnaire was administered, and the owner was provided with a faecal collection kit to collect and return a single faecal swab per day for two consecutive days. The study was approved by the University of Guelph Research Ethics Board.

Samples

The faecal kit provided to the dog-owners contained an instruction guide, tongue depressors, disposable gloves, biohazard bags, sterile specimen containers, two sterile Cary–Blair agar swabs (CultureSwab™ Cary–Blair agar; Becton Dickson and Company; USA), and pre-addressed, postage-paid cushioned envelopes for mailing the samples. Faecal swabs were used for *Campylobacter* isolation because the samples had to travel in the mail, and due to the fastidiousness of *Campylobacter*, it was felt the agar in the Cary–Blair swabs would provide better recovery. In a small trial completed by our laboratory using faecal samples spiked with *Campylobacter*, the faecal swabs remained positive after being mailed, whereas the full faecal samples did not (unpublished results). The fecal swab was plunged into the freshly passed faeces collected in the sterile specimen containers, and then placed in the Cary–Blair agar tube. Proper use of the Cary–Blair swabs was demonstrated for each owner at the time of recruitment. The two Cary–Blair swabs were tested for *Campylobacter* spp. only.

Microbiological analysis

All samples were received via express post at the University of Guelph. Upon arrival, information pertaining to the faecal swabs was documented and swabs were immediately sent to the Laboratory Services Division (LSD), University of Guelph for *Campylobacter* isolation. The faecal swab was streaked directly on to modified cefoperazone charcoal deoxycholate agar (mCCDA) plates [*Campylobacter* selective blood-free agar (CM0739) and CCDA selective supplement (SR0155), Oxoid, Canada] and the swab was then inserted into 5 ml Bolton broth (Oxoid). A 1-ml aliquot of the inoculated Bolton broth was then added to 9 ml new Bolton broth for further enrichment. The plates and broth were incubated for 48 h at 42 °C in a microaerophilic atmosphere, based on standard *Campylobacter* spp. isolation methods at LSD. The mCCDA plate with the direct streak was then read and both Bolton broth dilutions were plated onto mCCDA and incubated for another 72 h. Controls were used at every stage of the procedure. All mCCDA plates were observed for *Campylobacter* based on the presence of grey colonies. If present, colonies were re-streaked for purity, and tested for oxygen tolerance and growth at 25 °C. Additionally, dark-field microscopy, catalase and oxidase tests, and antibiotic sensitivity tests for cephalothin and nalidixic acid, were conducted on all suspect colonies to confirm the presence of *Campylobacter*. All *Campylobacter* isolates were frozen in glycerol at −70 °C to allow for future molecular typing. A dog was considered positive for *Campylobacter* if at least one swab tested positive.

Polymerase chain reaction (PCR) species identification

A series of PCR assays were performed targeting the 16S rRNA encoding genes to determine the species of *Campylobacter*. A loopful of the glycerol frozen broth containing the isolate was inoculated onto Columbia agar and incubated with CampyGen™ (Oxoid), an atmosphere generation system, at 37 °C for 2 days. This culture was then subcultured on another Columbia agar plate and incubated with CampyGen™ at 37 °C for 1 day. Once the isolate had been purified, the DNA was extracted using InstaGene™ (Bio-Rad Laboratories, USA) and the remaining culture was stored on Cryostor™ beads (Oxoid) at −70 °C. If the culture was catalase positive from previous biochemical testing performed at LSD, PCR methods previously described were used to identify the isolate [30]. If the catalase-positive culture was negative for both *C. jejuni* and *C. coli* based on the above PCR methods, a second PCR method was used to identify *C. lari* [31]. Finally, if the culture was catalase negative, a previously described PCR method for *C. upsaliensis* and *C. helveticus* was used to identify the isolate [31]. The primers and targets used for *Campylobacter* spp. identification can be found in previously published work [30, 31].

Questionnaire

Each questionnaire was administered by the primary author to the primary caregiver of the recruited dog during their visit at the veterinary clinic. The questionnaire included questions concerning the following: the dog's main diet and whether additional animal products were added to the diet; the presence of other pets in the home; the dog's activities; the occurrence of vomiting and diarrhoea in the previous month; veterinary care, including de-worming; any contact with livestock; and the use of antibiotics in the previous month. Breed, age, sex and neuter status were also collected for all dogs. The variables investigated in this study can be found in Table 1 and the questionnaire is available upon request.

Statistical analysis

Data from the questionnaires were entered into EpiData© version 3.1 (EpiData Association, Denmark) and analysed in Intercooled Stata/MP® 11.0 for Windows (USA). All tests were two-tailed with a statistical significance level of 5%. Univariable logistic regression models were used to screen all variables from the questionnaire for an association with *Campylobacter* spp. carriage and for each species of *Campylobacter* isolated if sufficient data were available (e.g. *C. upsaliensis*, *C. jejuni*). Significant continuous variables were evaluated for linearity with the log odds of the outcome using lowess curves and categorical linear trends (lintrend plots) [32]. Pair-wise correlations between significant variables from the univariable analysis ($P \leqslant 0{\cdot}20$) were examined using Spearman's correlation test. Variables with correlation values $>0{\cdot}7$ were investigated and the variable that was more biologically plausible, or had the least number of missing values, was included in the model [32].

Multivariable models were constructed for the dogs *Campylobacter* spp. status (positive/negative) and for individual *Campylobacter* species where data were

https://doi.org/10.1017/S0950268810002906 Published online by Cambridge University Press

Table 1. *List of pet-related management variables evaluated for an association with* Campylobacter *spp. carriage in client-owned pet dogs in the Region of Waterloo, Ontario 2008–2009* ($n=240$)

Demographic and dog information
- Age (years)
- Breed size[a]
- Sex (M/F)
- Intact (Y/N)
- Source of dog
  - Pet store
  - Breeder
  - Humane society
  - Other
- Reason for vet visit (open)
- Type of home
  - Urban
  - Suburban
  - Small town rural
  - Non-farm rural
  - Farm
- Season (determined from date of enrolment)
- Veterinary Clinic (A-G)

Activites & pet information (Y/N)
- Involved in hunting
- Has contact with other dogs
- Visits dog park
- Goes to dog day care
- Group activity[b]
- Involved in therapy programme
- Vet clinic stay in last 6 months
- Kennel stay in last 6 months

Other pet information (Y/N)
- More than one dog
- Cats in home
- Other pets in home
- Access to livestock[c]
- Catch and eat prey
- Access to garbage in past 2 weeks
- Licked plates/bowls in past 2 weeks
- Access to compost in past 2 weeks
- Access to cat litter in past 2 weeks
- Access to dead animals in past 2 weeks
- Access to animal faeces in past 2 weeks

Water exposure (Y/N)
- Given tap water
- Given well water
- Given bottled water
- Drinks out of toilet
- Access to lakes/rivers/creeks in past 6 months
- Access to ditches or puddles in last 6 months

Dog health information (Y/N)
- De-wormed in past 6 months
- Diagnosed with enteric illness in past 6 months[d]
- Given a probiotic in past month
- Given antibiotics in past month
- Given any other medications or supplements in past month
- Diarrhoea in past month
- Vomiting in past month

Exposure to humans (Y/N)
- Children living in home
- Household member with vomiting in past month
- Household member with diarrhea in past month
- Household member worked/visited/been in hospital in past month
- Household member treated with antibiotics in past month
- Household member had contact with other cats in past week
- Household member had contact with other birds in past week
- Household member visited a petting zoo in past week
- Household member had contact with livestock in past week

Diet information (Y/N)
- Store-bought/commercial processed food (dry or can)
- Homemade cooked
- Homemade raw
- Commercial cooked
- Commercial raw
- Combination of diets
- Food added to diet (daily or weekly) (Y/N)
  - Table scraps
  - Raw beef
  - Cooked beef
  - Raw chicken
  - Cooked chicken
  - Raw turkey
  - Cooked turkey
  - Raw pork
  - Cooked pork
  - Raw eggs
  - Cooked eggs
  - Fish
- Treats given more than once a month (Y/N)
  - Dried pig's ears
  - Raw bones
  - Cooked bones
  - Store-bought bones
  - Rawhide chews
- Homemade cooked food fed or added to diet (collapsed variable)

[a] Small, medium, large/giant breed or mixed breed.
[b] Participation in activities like obedience, flyball, agility.
[c] Livestock includes cattle, sheep, goats, pigs, or horses.
[d] Diagnosed with *Salmonella*, *Campylobacter*, *Giardia* or *C. difficile*.

sufficient. The main-effects models were created with the significant variables from the univariable analysis ($P \leqslant 0{\cdot}20$). A manual backwards step-wise procedure was used to construct the multivariable model. Likelihood ratio (LR) tests were used to assess the significance of each model as variables were removed. Confounding was evaluated by examining the effect of the removed variables on the coefficients of the remaining variables. A variable was determined to be a confounder if the log odds of the other independent variables changed by $\geqslant 20\,\%$, and it was not an intervening variable [32, 33]. The potential confounding effects and interactions of breed (mixed, pure small, pure medium, pure large); age (years); sex (male, female); and neuter status (intact, neutered) were examined regardless of statistical significance due to the suspected impact of these demographic characteristics on management-related risk factors. Interaction terms were examined for all remaining variables in the final model. To assess clustering, clinic was modelled using two approaches. In the first, clinic was modelled as a random effect. Clinic was also modelled as a fixed effect because of concerns that due to the limited number of clinics, the random effects would not be properly

estimated. The significance of clinic both as a random effect and fixed effect was assessed based on a LR test. If the clinic variables were not significant and did not confound any measures of association, the simpler multivariable models were reported. For the multilevel models, the normality of best linear unbiased predictors (BLUPs) were assessed with normal quantile plots to determine model fit [32]. For standard logistic regression, residuals and Hosmer–Lemeshow goodness-of-fit tests for the final models were assessed. A P value $\leqslant 0{\cdot}05$ from the goodness-of-fit test indicated that the model did not fit the data [32].

RESULTS

In total, 240 client-owned pet dogs were recruited for the study. From the seven participating veterinary clinics, 492 pet-owners were approached to participate in the study, 279 dogs were recruited for the study, and complete samples were received for 240 dogs. Both faecal swabs were received from 97·5% [234/240, 95% confidence interval (CI) 94·64–99·08] of the dogs, and only one swab was received from 2·5% (6/240, 95% CI 0·92–5·36) of the dogs. The median number of dogs recruited from the clinics was 35, with a minimum of three and maximum of 80. Questionnaires were completed for all dogs recruited for the study. Of the participating dogs, 52·9% (127/240, 95% CI 46·39–59·37) were female and 16·7% (40/240, 95% CI 12·18–21·99) were aged <1 year, with the average age of all participating dogs being 4·9 years (95% CI 4·39–5·39). Demographic information of the participating dogs can be found in Table 2.

About 21·7% (52/240, 95% CI 16·63–27·42) of the dogs enrolled in this study had at least one faecal swab positive for *Campylobacter*. The predominant species of *Campylobacter* recovered was *C. upsaliensis*, which was shed by 19·2% (46/240, 95% CI 14·39–24·73) of the dogs in the study. Of the *Campylobacter*-positive dogs, 88·5% (46/52, 95% CI 76·56–95·65) carried *C. upsaliensis*, and 13·5% (7/52, 95% CI 5·59–25·79) carried *C. jejuni*. One dog had both *C. upsaliensis* and *C. jejuni* and no other species of *Campylobacter* were recovered.

In total, 81 variables relating to the dogs' health, diet, and common exposures were examined in univariable models (Table 1). The variables found to be significant at the 20% level in the *Campylobacter* spp. and *C. upsaliensis* univariable logistic regression models are given in Table 3. There were no statistically significant associations found in any of the models between *Campylobacter* carriage and vomiting or diarrhoea, or *Campylobacter* carriage and season. Moreover, clinic was not found to be significant as a random effect or fixed effect in any multilevel logistic regression models, with P values $>0{\cdot}99$ and variance $<0{\cdot}001$, and insignificant LR tests. Age (years) was statistically significant and was found to be linearly associated with carrier status on the lowess and lintrend plots, and was kept as a continuous variable in all of the models. Univariable and multivariable logistic regression models were not examined for *C. jejuni* carriage because only seven dogs were found to be carrying *C. jejuni* resulting in a very small effective sample size.

From the multivariable model for *Campylobacter* spp. carriage, not being treated with antibiotics in the previous month, not having children in the home, and having homemade cooked food as the dog's diet or added to the dog's diet increased the odds of carriage. The odds of *Campylobacter* spp. carriage decreased as the age of the dog increased (Table 4).

In the multivariable model for *C. upsaliensis* carriage, not being treated with antibiotics in the previous month, a household member having contact with a cat that was not their own in the previous week, and having homemade cooked food as the dog's diet or added to the dog's diet increased the odds of carriage. The odds of *C. upsaliensis* carriage decreased as the age of the dog increased (Table 4).

In the multivariable models for *Campylobacter* spp. and *C. upsaliensis* carriage, interactions between the significant variables were not found to be statistically significant ($P>0{\cdot}05$). Residuals from both final multivariable models were examined for outliers and influential covariate observations. There were several observations with large residuals; however, the data were examined and found to be correct, and therefore all observations were kept in the final models. The final models for *Campylobacter* spp. and *C. upsaliensis* were not significant at the 5% level with the Hosmer–Lemeshow goodness-of-fit tests ($P=0{\cdot}64$ for both models), indicating that the models fit the data.

DISCUSSION

This study offers a detailed investigation of pet-related risk factors for *Campylobacter* carriage in client-owned pet dogs in North America. Previous risk-factor research has been completed mostly in Europe and Australia. The occurrences of

https://doi.org/10.1017/S0950268810002906 Published online by Cambridge University Press

Table 2. *Demographic information of the client-owned pet dogs sampled in this study from the Region of Waterloo, Ontario 2008–2009* ($n=240$)

	Number of dogs $n=240$ (%)	*Campylobacter* spp. (positive/negative)	*C. upsaliensis* (positive/negative)	*C. jejuni* (positive/negative)
Clinic (clinic type)				
A (small animal, urban)	60 (25·0 %)	10/50	8/52	2/58
B (small animal, suburban)	80 (33·3 %)	17/63	16/64	1/79
C (mixed practice, rural)	8 (3·3 %)	5/3	5/3[a]	1/7[a]
D (small animal, urban)	3 (1·3 %)	0/3	0/3	0/3
E (small animal, suburban)	47 (19·6 %)	13/34	11/36	2/45
F (small animal, suburban)	35 (14·6 %)	6/29	5/30	1/34
G (small animal & exotics, urban)	7 (2·9 %)	1/6	1/6	0/7
Dog demographics				
Sex				
Male	113 (47·1 %)	25/88	22/91[a]	4/99[a]
Female	127 (52·9 %)	27/100	24/103	3/124
Intact				
Yes	50 (20·8 %)	16/34	15/35[a]	2/48[a]
No	190 (79·2 %)	36/154	31/159	5/185
Breed size				
Pure small (<25 lb)	68 (28·3 %)	15/53	14/54	1/67
Pure medium (25–60 lb)	27 (11·3 %)	7/20	6/21[a]	2/25[a]
Pure large/giant (>60 lb)	69 (28·8 %)	17/52	15/54	2/67
Mixed (any size)	76 (31·7 %)	13/63	11/65	2/74
Source of dog				
Pet store	16 (6·7 %)	1/15	1/15	0/15
Breeder	122 (50·8 %)	24/98	21/101	3/119
Humane society	20 (8·3 %)	2/18	2/18	0/20
Other[b]	82 (34·2 %)	25/57	22/60[a]	4/78[a]
Type of home				
Urban	55 (22·9 %)	11/44	8/47	3/52
Suburban	165 (68·8 %)	36/129	33/132	3/162
Rural	20 (8·3 %)	5/15	5/15[a]	1/19[a]
Season when recruited				
Spring	43 (17·9 %)	6/37	6/37	0/43
Summer	76 (31·7 %)	18/58	16/60[a]	3/73[a]
Autumn	71 (29·6 %)	21/50	18/53	3/68
Winter	50 (20·8 %)	7/43	6/44	1/49
Age (years)				
Mean (minimum, maximum)	4·9 (0·14, 17)	2·6 (0·14, 9·6)/ 5·5 (0·14, 17)	2·8 (0·14, 9·6)/ 5·4 (0·14, 17)	2·0 (0·19, 8)/ 5·0 (0·14, 17)

[a] Total is greater than total number positive because one dog had both *C. upsaliensis* and *C. jejuni*.
[b] Included farms, friends, rescue groups, etc.

Campylobacter, *C. upsaliensis*, and *C. jejuni* found in this study were consistent with the estimated prevalences previously reported for household dogs [21–24]. A number of the investigated pet-related management factors (i.e. age and antibiotic use) associated with the carriage of *Campylobacter*, were consistent with those found in previous studies [20–22, 24]. The potential role of adding cooked human food to a pet dog's diet as a risk factor for *C. upsaliensis* carriage needs to be investigated further, as a similar association has also been demonstrated in a recent study [22]. Our study also demonstrated that *C. upsaliensis* was much more common in this population of pet dogs than *C. jejuni*, which is in agreement with several earlier studies [21, 22, 24, 27]. This study highlights the fact that pet dogs may be an important source of *Campylobacter*, especially *C. upsaliensis*, and exposure to dogs must be considered in human cases of campylobacteriosis. Moreover, *Campylobacter* from positive dogs should be

https://doi.org/10.1017/S0950268810002906 Published online by Cambridge University Press

Table 3. *Descriptive statistics and significant associations* ($P \leqslant 0{\cdot}20$) *from univariable logistic regression analysis of pet-related management factors and* Campylobacter *spp. and* C. upsaliensis *carriage in client-owned pet dogs, recruited through veterinary clinics in the Region of Waterloo, Ontario, 2008–2009* ($n = 240$)

			Campylobacter spp.			*C. upsaliensis*		
Variable	Exp.[a]	Unexp.[b]	OR[c]	*P* value	95% CI[d]	OR[c]	*P* value	95% CI[d]
Age (years)	n.a.	n.a.	0·8	0·000	0·7–0·9	0·8	0·000	0·7–0·9
Intact	50	190	2·0	0·049	1·0–4·0	2·2	0·031	1·1–4·5
Participates in a group activity[e]	16	224	4·1	0·008	1·5–11·5	2·8	0·063	1·0–8·0
No antibiotics in previous month	180	60	5·1	0·003	1·8–14·8	6·0	0·004	1·8–20·0
Homemade cooked food fed or added to diet	11	229	4·8	0·013	1·4–16·3	5·7	0·006	1·7–19·5
Fed table scraps	71	169	0·4	0·014	0·2–0·8	0·4	0·021	0·2–0·9
Catches or hunts prey animals[f]	26	214	5·1	0·095	0·9–5·1	2·5	0·039	1·1–6·1
Drinks out of ditches and puddles	103	137	3·3	0·074	1·0–3·3	2·0	0·040	1·0–3·8
Contact with other dogs	190	50	4·5	0·144	0·8–4·5	2·0	0·154	0·8–4·9
Kennel stay in past 6 months	42	198	1·8	0·111	0·9–3·8	1·9	0·092	0·9–4·1
More than one dog in the house	61	179	0·6	0·133	0·3–1·2	0·6	0·133	0·3–1·3
No children in the household[g]	161	79	1·9	0·091	0·9–3·8	1·7	0·152	0·8–3·6
Household member works in/has visited a hospital in the past week	51	189	0·5	0·126	0·2–1·2	0·4	0·063	0·2–1·1
Household member had contact with other cats in past week[h]	57	183	1·8	0·089	0·9–3·5	2·0	0·053	1·0–4·0
Household member had contact with livestock in past week[i]	13	227	2·4	0·141	0·8–7·7	2·8	0·080	0·9–9·1
Dog has had access to dead animals in past 2 weeks	11	229	—	—	—	2·6	0·151	0·7–9·1
Dog drinks out of toilet	28	212	—	—	—	1·8	0·184	0·8–4·5
Household member has had vomiting or diarrhoea in past week	35	205	—	—	—	0·4	0·143	0·1–1·4

OR, Odds ratio; CI, confidence interval.
Dashes (–) signify that the variable was not significant at the 20% level in that univariable model.
[a] Exposed dogs (i.e. those that were positive for the risk factor).
[b] Unexposed dogs (i.e. those that were negative for the risk factor).
[c] Odds ratio calculated in Stata/MP 11.0.
[d] 95% confidence interval of the odds ratio calculated in Stata/MP 11.0.
[e] Included obedience, flyball and agility classes.
[f] Included birds, small rodents and other small prey.
[g] Children aged <18 years that live in the home on a regular basis.
[h] Someone who lives in the home had contact with a cat that was not their own in the previous 7 days.
[i] Someone who lives in the home had contact with livestock (cattle, sheep, goats, pigs, or horses) in the previous 7 days.

speciated in order to determine the risk for human infection and any species-specific control methods that may be necessary.

This study has the following limitations that need to be considered to avoid over-interpreting our results: the subjects were not recruited randomly; the response rates by clinic and client were poor; and the exploratory nature of the study resulted in many variables being examined. Without a random sample, the reader should be cautious about extrapolating the prevalence in our study population to the Region of Waterloo or Ontario. However, similar prevalences of *Campylobacter* carriage in pet dogs have been found in several recent studies [21–24]. In terms of poor response level, non-response is a form of selection bias that could have altered the size and direction of the odds ratios estimated from our models. However, for this selection bias to occur, non-participation (non-response) by dog-owners or veterinary clinics needs to relate to both the examined pet-related risk factors and *Campylobacter* carriage [32]. Considering that few animals were showing clinical signs, and no association was found between diarrhoea or vomiting and *Campylobacter* carriage, it is unlikely that owners'

https://doi.org/10.1017/S0950268810002906 Published online by Cambridge University Press

Table 4. *Significant risk factors* ($P \leqslant 0{\cdot}05$) *from multivariable logistic regression analysis of pet-related management factors and* Campylobacter *spp. and* C. upsaliensis *carriage for client-owned pet dogs recruited through veterinary clinics in the Region of Waterloo, Ontario, 2008–2009* ($n = 240$)

		Campylobacter spp. model			*Campylobacter upsaliensis* model		
Variable		OR	*P* value	95% CI[a]	OR	*P* value	95% CI[a]
Age (years)		0·8	0·000	0·7–0·9	0·8	0·000	0·7–0·9
Participates in a group activity[b]	No (referent)						
	Yes	4·0	0·027	1·2–13·8	—	—	—
Children in the home[c]	Yes (referent)						
	No	2·5	0·029	1·1–5·6	—	—	—
Antibiotics in previous month	Yes (referent)						
	No	8·7	0·001	2·4–31·7	10·7	0·003	2·3–50·0
Homemade cooked food fed or added to diet	No (referent)						
	Yes	17·8	0·001	3·4–93·2	25·3	0·000	4·3–148·0
Household member had contact with other cats in the previous week[d]	No (referent)						
	Yes	—	—	—	2·3	0·040	1·0–5·2

OR, Odds ratio; CI, confidence interval.
Dashes (—) signify that the variable was not significant at the 5% level in that multivariable model.
[a] 95% confidence interval of the odds ratio.
[b] Participation in activities like obedience, flyball, agility.
[c] Children aged <18 years that live in the home on a regular basis.
[d] Someone who lived in the home had contact with a cat that was not their own in the previous 7 days.

willingness to participate was related to both the outcome and the exposures of interest. Further, no association was found between clinic and *Campylobacter* carriage, therefore it is unlikely that clinics' willingness to participate was related to both the outcome and the exposures of interest. Finally, like many exploratory studies, a large number of variables were examined, so the possibility of type I errors should be noted. Where we have identified novel risk factors for *Campylobacter* carriage, we suggest these variables be examined in future studies. Also, in view of the fact that this study was cross-sectional in nature, we cannot determine which factors cause *Campylobacter* carriage and which factors prolong carriage since prevalence is a function of incidence and duration [32, 34]. However, controlling management factors related to prevalence itself would be useful for protecting public health.

Feeding homemade cooked food was found to increase the odds of *Campylobacter* spp. and *C. upsaliensis* carriage in dogs in our study. Previously, *C. jejuni*-contaminated food has been associated with infection with *Campylobacter* in humans and animals [35, 36]. To date, dogs and cats have been assumed to be the only reservoir for *C. upsaliensis* [37]. However, a recent study by Westgarth *et al.* [22] also found an association between feeding leftover human food and *C. upsaliensis* carriage in community dogs. In our study, only one participating dog was fed a raw food diet; therefore, the association with feeding human food may be due to poor food-handling practices rather than direct exposure from raw food. Nonetheless, these findings of an association between feeding homemade cooked food and leftovers, and the presence of *C. upsaliensis* in canine faeces, may warrant the inclusion of *C. upsaliensis* in food safety surveillance programmes in the future. Microbiological testing of the foods fed to the dogs in this study was not done, so a direct connection cannot be made. However, sample size needs to be taken into account with this association in our study, as only 11/240 dogs were fed homemade cooked food, either as their main diet or added to their diet.

Similar to previous studies, a significant difference in *Campylobacter* and *C. upsaliensis* carriage was observed based on age [20–22, 24, 38], and with every year increase in age, the odds of *Campylobacter* and *C. upsaliensis* carriage decreased by 0·8 (Table 4). This is probably due to the inexperienced immune systems of the younger dogs; as dogs mature the occurrence of *Campylobacter* carriage decreases [6, 29].

Lack of exposure to antibiotics in the month prior to sample testing was found to increase the odds

https://doi.org/10.1017/S0950268810002906 Published online by Cambridge University Press

of *Campylobacter* carriage in our study. A similar finding has been discussed in previous studies, but, unlike in our study, the association was not found to be statistically significant [22, 24]. The association between lack of antibiotic use and an increase in the risk of *Campylobacter* carriage is logical given the antibacterial function of most antibiotics; however, treatment with antibiotics is controversial and only recommended in severely ill animals [26]. Consequently, the use of antibiotics to prevent the carriage of *Campylobacter* in clinically healthy dogs is not normally recommended.

Interestingly, two previously unreported findings, not having children living in the home, and a household member having contact with a cat that was not their own, were associated with an increase in the odds of carriage of *Campylobacter* spp. and *C. upsaliensis* in pet dogs, respectively. In previous studies, having a dog or puppy was associated with an increase in the risk of *Campylobacter* carriage in children [8, 10]; however, current research has not studied the association in the opposite direction. For *C. upsaliensis* carriage and cat contact, it is possible that these owners were experiencing a greater deal of contact with other animals and may have been acting as a vector of *Campylobacter* for their pets. Cats have been found to carry *Campylobacter*, including *C. upsaliensis* [39] and have been identified as a significant risk factor for *C. jejuni* infection [40]. It is also possible that these variables are acting as proxies for other statistical associations, or could simply be due to chance because of the large number of variables investigated. Nonetheless, these associations should be investigated in future studies.

Finally, given that only 52 dogs were found to be shedding *Campylobacter* spp., caution should be taken when interpreting the non-significant results in this study. Potential risk factors for carriage of *Campylobacter* spp. may have been missed due to the large effect and/or small amount of variation that is often needed to observe statistical significance in small studies [32]. Weaker associations could have been disguised by the small sample size.

This study identified several novel risk factors for *Campylobacter* spp. carriage in pet dogs, including lack of antibiotic exposure, not having children in the home, exposure to cats and other pets, and including homemade cooked food in the dog's diet, that require further investigation. These results may warrant a change in the current surveillance of *Campylobacter* spp. in food sources, specifically in the case of *C. upsaliensis*. Recent changes in laboratory methods for processing canine faecal samples have given rise to an increased prevalence of *C. upsaliensis* in dogs [41, 42]. It is possible that *C. upsaliensis* is more common in food sources and in human cases of campylobacteriosis than is currently appreciated, since it may be missed as a result of using laboratory methods designed to detect *C. jejuni* and *C. coli* (i.e. catalase-positive *Campylobacter* spp.). Current laboratory methods used for isolation of *Campylobacter* spp. from human faecal samples and food samples often involve the use of agar plates and broth suspensions that contain cefaperazone, nalidixic acid, and cephalothin at levels that prevent the growth of *C. upsaliensis* [37]. Using a previously described filtration method, Lastovica & Le Roux [43] found that almost 25% of campylobacteriosis cases in humans in South Africa were due to *C. upsaliensis*. A study from the USA has suggested that *C. upsaliensis* is the second most commonly isolated *Campylobacter* spp. in humans, after *C. jejuni* [44]. A Belgian study also found that *C. upsaliensis* was recovered more often than *C. coli* in humans, indicating that *C. upsaliensis* may be of greater importance than previously thought [45]. *C. upsaliensis* is certainly capable of causing disease in humans and may be more common than believed in Canadian human infections [37, 46]. Further research into the prevalence of *C. upsaliensis* in human gastrointestinal disease and the potential sources of *C. upsaliensis* is warranted. The information collected from this study and similar future studies, is crucial for the development of evidence-based guidelines for safe dog-ownership and to protect the public through responsible pet management.

ACKNOWLEDGEMENTS

Sample collection and testing for this study were supported by the Public Health Agency of Canada and the Ontario Veterinary College Pet Trust Fund. The infrastructure for statistical analyses was supported through a grant to D. L. Pearl from the Canada Foundation for Innovation and the Ontario Research Fund. The primary author was supported through the Blake Graham Fellowship from the Ontario Veterinary College. Isolation of *Campylobacter* was completed by Laboratory Services Division, University of Guelph (Dimitrinka Oke and Susan Lee). PCR was completed by Joyce Rousseau of Dr J. S. Weese's laboratory in the Department of Pathobiology, University of Guelph.

DECLARATION OF INTEREST

None.

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