PAPERS

Prevalence of thermophilic *Campylobacter* species in household cats and dogs in Ireland

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Rectal swabs were collected from 147 household dogs and 35 household cats, including healthy animals, animals with gastrointestinal signs and animals with a variety of medical and surgical conditions. A combination of selective culture methods was used to optimise the recovery of *Campylobacter* species, and a PCR was used to confirm their isolation and to identify the species. The overall prevalence of *Campylobacter* species was 42·9 per cent in the cats and 41·5 per cent in the dogs. *Campylobacter upsaliensis* was the species most commonly isolated from the dogs and cats, and *Campylobacter jejuni* was the second most commonly isolated. Particularly high prevalences were detected in the few cats and dogs with diarrhoea, and in the cats and dogs that were six months old or younger.

Campylobacter species are the most prevalent cause of human bacterial gastroenteritis in the developed world. Risk factors for infection include ingesting or handling undercooked or contaminated meat products (especially poultry), consuming contaminated or unpasteurised milk and dairy products, drinking water from contaminated supplies, foreign travel and contact with pets (Altekruse and others 1994, Altekruse and Tollefson 2003, Foley and others 2003). Most human infections are caused by *Campylobacter jejuni*, with approximately 10 per cent of infections caused by *Campylobacter coli*, and a small number by less common species including *Campylobacter lari* and *Campylobacter upsaliensis* (Newell and others 2000). The clinical signs vary from abdominal cramping and mild diarrhoea to profuse, watery diarrhoea, haematochesia and vomiting. Severe complications are uncommon but extraintestinal infections with fatal bacteraemia may occur, especially in young children and immunosuppressed and elderly people (Skirrow and Blaser 2000). Guillain-Barré syndrome is a potentially fatal immune-mediated polyneuritis that has been linked with *Campylobacter* infection (Allos 1997).

Cats and dogs have been identified as asymptomatic carriers of *Campylobacter* species, but the organism may also act as a primary or secondary pathogen and cause gastrointestinal signs (McOrist and Browning 1982, Fleming 1983, Fox and others 1983a, Nair and others 1985, Burnens and others 1992). The main species of veterinary importance in companion animals include *C jejuni* and *C upsaliensis*. The rates of isolation reported from dogs and cats have varied widely and appear to be dependent on their age, clinical signs, environment, concomitant disease or infection with other enteropathogenic organisms, the species of *Campylobacter*, and the design of the study (Torre and Tello 1993, Engvall and others 2003, Bender and others 2005, Wieland and others 2005). The prevalence of *Campylobacter* species in Irish household cats and dogs has not been investigated. This study formed part of a study of the prevalence of *Campylobacter* species in pets in Ireland, including household cats and dogs, exotic pets and cats and dogs in shelters. Prevalences of 51·1 per cent and 75·0 per cent have been observed in healthy shelter dogs and cats, respectively, and a prevalence of 87·0 per cent was observed in the dogs at a shelter where 22 of 46 animals had signs of diarrhoea (Acke and others 2006).

Materials and methods

Rectal swabs were collected from 147 dogs and 35 cats during 2004 and 2005. Only animals that had not recently been treated with antimicrobial drugs or corticosteroids when they were sampled were included in the study. The majority of the animals were from County Dublin and were kept as indoor pets. Of the dogs, 43 were healthy (being presented for a health check, vaccination or neutering), 10 had diarrhoea and the other 94 had various medical or surgical conditions. Twenty-five of the cats were healthy, one had diarrhoea and nine had other medical or surgical conditions. The rectal swabs were transported in Amies transport medium (Copan), refrigerated at 4°C and cultured within 48 hours.

Selective solid media for the isolation of *Campylobacter* species was prepared with blood-free modified charcoal cefoperazone deoxycholate agar (mCCDA agar). For mCCDA agar, CCDA selective supplement (SR0155; Oxoid) was added to the basal agar in accordance with the manufacturer's instructions. For cefoperazone amphotericin teicoplanin (CAT) agar, CAT selective supplement (SR0174; Oxoid) was added to the mCCDA basal agar. Liquid selective enrichment media were prepared by using either the Preston broth or CAT broth formulations. Briefly, 25 g of the basal medium (nutrient broth number 2, CM0067; Oxoid) was dissolved in 950 ml of deionised water and autoclaved. The media were then cooled to 55°C, and Preston or CAT *Campylobacter* selective supplements (SR0204 or SR0174 respectively; Oxoid) and *Campylobacter* growth supplements (SR0232; Oxoid) were added together with 50 ml saponin-lysed defibrinated horse blood. The following five methods were used.

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Direct plating

Rectal swabs were streaked directly on CAT (method 1) and mCCDA (method 2) agar plates and incubated at 37°C in a microaerophilic environment (Genbox microaer sachets; bioMérieux) for 96 hours. The plates were examined after 48 hours of incubation and, if negative, were incubated for a further 48 hours. Any suspect colonies were subcultured on to Columbia blood agar plates and incubated for at least 48 hours at 37°C.

Selective enrichment

After direct plating, rectal swabs were also placed in tubes containing 5 ml of 0·1 per cent peptone water and vortexed for one minute; 1 ml aliquots of the vortexed diluent were then placed in 10 ml aliquots of CAT broth and 10 ml Preston broth, and the broths were incubated for 24 hours at 37°C.

After incubation, 200 μl CAT broth was filtered through a 47 mm diameter 0·65 μm pore cellulose acetate filter (Sartorius) in situ on Columbia blood agar (method 3) and CAT agar (method 4) plates. After one hour, the filter was removed and the filtrate was spread over the plate with a sterile glass hockey stick. The plates were incubated at 37°C and examined after 48 and 96 hours. Any suspect colonies on the CAT plates were transferred on to Columbia blood agar plates and incubated for at least 48 hours before being examined.

All the enriched samples in Preston broth were subcultured on to mCCDA agar (method 5) and incubated at 37°C, and the plates were examined after 48 hours incubation. Suspect colonies were subcultured on to Columbia blood agar and incubated for at least 48 hours at 37°C. All five culture methods were used for the first 29 samples, but on the basis of their results and the results of the study by Acke and others (2006) it was decided to use the three culture methods that yielded the highest recovery rates (methods 1, 2 and 3) for the remaining 153 samples.

Campylobacter speciation: DNA extraction and PCR

DNA was extracted from all the suspect *Campylobacter* cultures using the Wizard genomic DNA purification kit (Promega), and a multiplex PCR assay was used to identify the *Campylobacter* species (Wang and others 2002). Primer sequences specific for *C jejuni* subspecies *jejuni*, *C upsaliensis*, *C coli* and *C lari* were selected. Primers specific for 23S rRNA were used as an internal control to identify samples belonging to the closely related genera *Arcobacter* and *Helicobacter*. The PCR-amplified products were visualised by electrophoresis on 1·5 per cent agarose gels (Wang and others 2002). Because several samples revealed only the 23S rRNA band on electrophoresis, or could not be identified at all, analysis by restriction fragment length polymorphism (RFLP) was applied to each isolate that had not been identified fully (Marshall and others 1999). A restriction endonuclease *Dde*I (BioLabs) was used to identify *Campylobacter*, *Arcobacter* and *Helicobacter* isolates and species-specific digestion patterns were obtained for *Campylobacter* species that distinguished between *C jejuni*, *C coli*, *C lari*, *Campylobacter curvus*, *Campylobacter hyointestinalis*, two subspecies of *Campylobacter fetus*, three subspecies of *Campylobacter sputorum*, *C upsaliensis*, *Campylobacter helveticus* and *Campylobacter mucosalis*. The products were visualised by electrophoresis on 3 per cent agarose gel (Marshall and others 1999).

Statistical analysis

The prevalences of *Campylobacter* species recovered from the samples in each group were compared using chi-squared analysis with significance defined at the 95·0 per cent confidence level (P≤0·05). The analyses were carried out using STATVIEW version 5.0.1 (SAS Institute).

TABLE 1: Prevalence of *Campylobacter* species in healthy pets, pets with diarrhoea and pets with other conditions

| Species sampled | Number of animals (%) | | | |
	Healthy animals	With diarrhoea	With other conditions	Total prevalence
Dogs	20/43 (46·5)	6/10 (60·0)	35/94 (37·2)	61/147 (41·5)
Cats	11/25 (44·0)	1/1 (100·0)	3/9 (33·3)	15/35 (42·9)

Results

Association between animal species and clinical signs, and *Campylobacter* prevalence

Campylobacter species were isolated from 61 of the 147 dogs (41·5 per cent) and from 15 of the 35 cats (42·9 per cent). They were isolated from 20 of the 43 healthy dogs (46·5 per cent) and from 11 of the 25 healthy cats (44 per cent), and from six of the 10 dogs with diarrhoea. They were isolated from 35 of the 94 dogs with other conditions (37·2 per cent) and from three of the nine cats with other conditions (Table 1).

Association between *Campylobacter* species prevalence, animal species and clinical signs

Fifty-two of the 80 identified isolates (65·0 per cent) were *C upsaliensis*; this was significantly more than the 18 isolates (22·5 per cent) of *C jejuni* and other *Campylobacter* species (Table 2). There were three isolates of *C coli* and two of *C lari*. *C helveticus* was detected in only two cats. *C jejuni* was the most prevalent species in the animals with diarrhoea, and *C upsaliensis* was significantly more prevalent in the other two groups (P≤0·05). There were mixed infections of *Campylobacter* species in two dogs and two cats. In three of the 80 isolates, no distinction could be made between *C jejuni*, *C coli* and *C lari*.

Association between animal age and clinical signs

Campylobacter species were isolated from 10 of the 14 dogs that were aged six months old or younger (71·4 per cent) and from 51 of the 133 older dogs (38·3 per cent) (P≤0·05) (Table 3). A similar pattern was observed for the cats, with *Campylobacter* species being isolated from five of seven younger (71·4 per cent) and 10 of 28 (35·7 per cent) older cats. *Campylobacter* species were isolated from three of four younger healthy dogs and from four of six younger healthy cats; they were isolated from smaller proportions of older animals, 17 of the 39 dogs (43·6 per cent) and seven of the 19 cats (36·8 per cent). *Campylobacter* species were isolated from four of the six younger dogs with other conditions, and from 31 of the 38 older dogs (35·2 per cent). They were isolated from three of the nine older cats with other conditions.

Discussion

The results show that *Campylobacter* species are common in Irish household dogs and cats, with overall prevalences of 41·5 per cent in dogs and 42·9 per cent in cats (Table 1). Similar prevalences, ranging up to 41·2 per cent have been reported in adult household dogs (Fleming 1983,

TABLE 2: Numbers of healthy animals, animals with diarrhoea and other clinical signs, and numbers of cats and dogs from which different species of *Campylobacter* were isolated

| *Campylobacter* species | Clinical sign | | | Animal | | Total (%) |
	Healthy	Diarrhoea	Other	Dog	Cat	
Campylobacter jejuni	6	5	7	15	3	18/80 (22·5)
Campylobacter upsaliensis	22*	2	28*	43	9	52/80 (65·0)
Campylobacter coli	0	0	3	2	1	3/80 (3·8)
Campylobacter lari	1	0	1	1	1	2/80 (2·5)
Campylobacter helveticus	2	0	0	0	2	2/80 (2·5)
Campylobacter coli, jejuni or *lari*	1	0	2	2	1	3/80 (3·8)
Total	32	7	41	63	17	

* Significantly higher prevalence of *C upsaliensis* than in the diarrhoeic group and than *C jejuni* in these groups (P≤0·05)

TABLE 3: Prevalence of *Campylobacter* species in cats and dogs up to six months of age and more than six months showing different clinical signs

Species	Age	Number of animals (%)			
		Healthy animals	With diarrhoea	With other conditions	Total
Dogs	≤6 months	3/4 (75·0)	3/4 (75·0)	4/6 (66·7)	10/14 (71·4)*
	>6 months	17/39 (43·6)	3/6 (50·0)	31/88 (35·2)	51/133 (38·3)
Cats	≤6 months	4/6 (66·7)	1/1	0/0	5/7 (71·4)
	>6 months	7/19 (36·8)	0/0	3/9 (33·3)	10/28 (35·7)
Total (%)		31/68 (45·6)	7/11 (63·6)	38/103 (36.9)	76/182 (41·6)

* Significantly higher prevalence of *Campylobacter* species than in older dogs (P≤0·05)

Torre and Tello 1993, Sandberg and others 2002, Engvall and others 2003, Wieland and others 2005) and up to 40·9 per cent in household cats (Fox and others 1983b, Moser and others 2001, Spain and others 2001, Sandberg and others 2002, Bender and others 2005, Wieland and others 2005). All the suspect cultures in the present study were confirmed as *Campylobacter* species by PCR, and a combination of culture methods was used to optimise their recovery.

In previous studies, the prevalence of *Campylobacter* species in dogs has consistently been higher than in cats (Bruce and Ferguson 1980, Bruce and others 1980, McOrist and Browning 1982, Fox and others 1983b, Hald and Madsen 1997, Baker and others 1999, Lopez and others 2002, Sandberg and others 2002, Workman and others 2005). The rates of isolation of *Campylobacter* species depend on the environment the animals live in, and intensive housing is thought to be a significant risk factor. The prevalence in dogs and cats living in groups (for example pounds and kennels) is higher than in single household pets (Bruce and Ferguson 1980, Bruce and Fleming 1983, Fox and others 1985, Malik and Love 1989, Torre and Tello 1993, Baker and others 1999, Workman and others 2005), probably as a result of the stress, frequent dietary changes and increased incidence of gastrointestinal disease suffered by animals in pounds or kennels. Prevalences of up to 87·0 per cent in dogs and 75·0 per cent in cats have been reported in shelter dogs and cats in Ireland (Acke and others 2006).

The dogs with diarrhoea had the highest prevalence of *Campylobacter* species (Table 1). Of the eight dogs with diarrhoea and suspect cultures, three were diagnosed with concurrent gastrointestinal parasitism, three had histological changes consistent with inflammatory bowel disease (IBD) or lymphangiectasia/IBD in endoscopic biopsies, one had diabetes mellitus and self-resolving diarrhoea, and in one puppy no underlying cause was found. *C jejuni* was isolated from five of the dogs with diarrhoea and *C upsaliensis* from one. *C upsaliensis* was isolated from a kitten that had concurrent gastrointestinal parasitism (Table 1). Few of the animals had diarrhoea and a statistically significant association with the isolation of *Campylobacter* species could not be assessed. However, as the majority of these animals had concurrent gastrointestinal disease, *Campylobacter* infection was probably secondary to this underlying disease. *Campylobacter* species can be found in dogs and cats with gastrointestinal signs as an opportunistic infection (for example, secondary to exocrine pancreatic insufficiency, endoparasites, or coronavirus and parvovirus infections) and they may act as a primary or secondary pathogen. The link between the gastrointestinal signs and the presence of *Campylobacter* organisms has been investigated but is still uncertain. In addition, there have been large differences between the designs of the studies, and standardised studies are needed to determine whether *Campylobacter* species can cause significant disease as a primary pathogen (Bruce and Fleming 1983, Fleming 1983, Gondrosen and others 1985, Olson and Sandstedt 1987, Burnens and others 1992, Baker and others 1999, Lopez and others 2002, Sandberg and others 2002, Acke and others 2006). *Campylobacter*-confirmed cultures were detected in 46·5 per cent of the healthy dogs and 44·0 per cent of the healthy cats, compared with 37·2 per cent of the dogs with other conditions and 33·3 per cent of the cats with other conditions (Table 1), showing that asymptomatic animals can be carriers of *Campylobacter* species. These animals may be an important reservoir for *Campylobacter* species and a source of infection for other pets and

human beings (Olson and Sandstedt 1987, Newton and others 1988, Acke and others 2006).

In most studies, higher prevalences have been found in puppies and kittens than in adult dogs and cats, suggesting an age predisposition and the development of immunity with age (Blaser and others 1980, Fleming 1983, Fox and others 1983b, Nair and others 1985, Burnens and others 1992, Torre and Tello 1993, Sandberg and others 2002, Engvall and others 2003, Bender and others 2005, Workman and others 2005, Acke and others 2006). Similarly, in this study, the prevalence of *Campylobacter* species was significantly higher in the dogs that were six months of age or younger, and higher in the younger cats. There were too few animals with diarrhoea for any correlations with age to be analysed (Table 3).

Molecular techniques are considered to be the gold standard for the reliable identification of species of *Campylobacter* and are invaluable in epidemiological studies in human beings and animals (Newell and others 2000, Steinhauserova and others 2001, Gorkiewicz and others 2003), but there have been few studies based on molecular methods for *Campylobacter* species in pets. Wieland and others (2005), Hald and others (2004) and Engvall and others (2003) reported that the majority of isolates from dogs and/or cats were *C upsaliensis*. Workman and others (2005) detected a prevalence of 50 per cent for *C helveticus* in cats, and 51·5 per cent for *C jejuni* in dogs, suggesting that certain animals may be predisposed to carry specific *Campylobacter* species; further studies of the significance of animal and *Campylobacter* species in the epidemiology and pathogenicity of *Campylobacter* infections are needed (Wieland and others 2005, Workman and others 2005). In this study, *C upsaliensis* (65·0 per cent) was the *Campylobacter* species most commonly isolated (P≤0·05) from both the dogs and cats and *C jejuni* (22·5 per cent) was the second most common. *C jejuni* was the most prevalent species in the animals with diarrhoea, and *C upsaliensis* was significantly more prevalent in the other two groups (P≤0·05). *C coli* (3·8 per cent) and *C lari* (2·5 per cent) were rarely isolated and *C helveticus* was isolated from only two cats (Table 2). Confirmed mixed *Campylobacter* species infections were detected in two of the cats and two of the dogs. Similar mixtures of *Campylobacter* species have been isolated from the faeces of dogs and cats in previous studies (Hald and others 2004, Koene and others 2004, Wieland and others 2005).

C jejuni is the main *Campylobacter* species isolated from human beings (Newell and others 2000), but *C upsaliensis* was the species most commonly isolated from these dogs and cats. Most isolates of *C upsaliensis* in animals have been isolated from pets, and pets may be an important reservoir for *C upsaliensis* infections in people (Bourke and others 1998). Such infections have been associated with acute self-limiting diarrhoea, and with chronic recurrent gastrointestinal signs and weight loss (Goossens and others 1995), bacteraemia in paediatric patients (Lastovica and others 1989) and immunosuppressed patients (Jenkin and Tee 1998), haemolytic-uraemic syndrome (Carter and Cimolai 1996), and the Guillan-Barré syndrome (Bourke and others 1998).

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