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Salmonellae and Campylobacters in Household and Stray Dogs in Northern Taiwan

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ABSTRACT

Rectal swabs were collected from 437 household and 491 stray dogs in northern Taiwan from May 2003 to June 2005 to investigate the prevalence and antimicrobial susceptibilities of salmonellae and campylobacters. The results revealed that 2.1% of household dogs and 6.3% of stray dogs were positive for salmonellae, with *Salmonella* Duesseldorf being the most dominant serotype in both. Additionally, 2.7% of the household dogs and 23.8% of the stray dogs were positive for campylobacters. *Campylobacter jejuni* was the most prevalent species (86.8%), followed by *C. upsaliensis* (9.3%) and *C. coli* (3.9%). Both salmonella and campylobacter isolation rates from the stray dogs were significantly higher than those from the household dogs ($p < 0.01$). The susceptibility of 33 *C. jejuni* isolates to eight antimicrobials was studied by the *E*-test. A high rate of resistance was observed to azithromycin (93.9%), clindamycin (87.9%), erythromycin (81.8%), tetracycline (78.8%), chloramphenicol (69.7%), nalidixic acid (51.5%), gentamicin (33.3%), and ciprofloxacin (18.2%). The susceptibility of 40 *Salmonella* isolates to 15 antimicrobials was also studied by the disc-diffusion method. All the *Salmonella* isolates were susceptible to ciprofloxacin and ceftriaxone. Resistance was observed most frequently to tetracycline (77.5%), chloramphenicol (52.5%), and ampicillin (50%).

Keywords: antimicrobial agents, campylobacters, household dogs, salmonellae, stray dogs

Abbreviations: CAT, cefoperazone amphotericin teicoplanin; MIC, minimum inhibitory concentrations; PCR, polymerase chain reaction

INTRODUCTION

Infectious enteric pathogens have long been recognized as a significant problem owing to their pathogenicity potential to animals and their zoonotic risk to humans. Among them, two gastrointestinal bacterial pathogens, salmonellae and campylobacters have been considered to be important food-borne pathogens causing human enteritis worldwide and leading to serious public health concern (Ethelberg *et al*., 2004). In addition to causing enteritis, these organisms have also been reported in association with bacteraemia, reactive arthritis, and meningitis (Goldberg and Rubin, 1988; Peterson, 1994).

Salmonellae and campylobacters are ubiquitous and can be isolated from many kinds of farm and pet animals. The majority of human salmonellosis and campylobacteriosis cases in developed countries are most likely caused through consumption of undercooked poultry, raw milk, or untreated surface water (Goldberg and Rubin, 1988; Kapperud *et al*., 1992; Altekruse *et al*., 1994). Furthermore, living with a household dog has previously been identified as a risk factor for these diseases (Kapperud *et al*., 1992; Robinson and Pugh, 2002). Most dogs are asymptomatic when they act as reservoirs shedding salmonellae or campylobacters in their faeces. Pathogens in their faeces may ultimately infect other animals by contaminating the environment (Morse and Duncan, 1975; Fox, 1990; Hald and Madsen, 1997). Recently, among immunocompromised populations, i.e. those using immunosuppressive drugs having acquired immunodeficiency syndrome, and the elderly these bacteria have become a great pathogenic risk (Robinson and Pugh, 2002).

The prevalence of salmonellae and campylobacters in dogs in Taiwan is still unknown; this study was done to determine the prevalence of these bacteria in household and stray dogs and to assess bacterial antibiotic susceptibilities.

MATERIALS AND METHODS

Sampling and experimental design

Rectal swabs were collected from household dogs at the National Taiwan University Veterinary Hospital, and four other private veterinary clinics located in Taipei city. For the stray dogs, swabs were collected from six municipal animal shelters located in northern Taiwan. Each shelter was visited twice in a 3-month period and all the dogs were sampled upon each visit. The usual holding period for the stray dogs in the public shelter was 10 days, so the dogs were sampled within 1–10 days after their arrival. The swabs were transported in Cary and Blair transport medium (Oxoid, Basingstoke, Hampshire, UK) to the laboratory on the day of collection.

Isolation and identification of salmonellae

Each sample was enriched using selenite brilliant-green enrichment broth (Difco, Detroit, MI, USA) for 18 h at 37°C. Samples were then plated onto brilliant-green phenol-red lactose sucrose agar (BPLS agar, Merck, Whitehouse Station, NJ, USA) and incubated for 24 h at 37°C. Isolated pink colonies surrounded by a red zone were then subcultured on tryptic soy broth (Merck) for 18 h at 37°C and then subjected to identification by the following biochemical tests: triple sugar iron agar, sulphide-indole-motility medium, lysine decarboxylase test, and urease test (Merck). Once salmonellae were identified, serotyping was performed according to the Kauffmann–White scheme using a commercial antiserum kit (Difco).

Isolation and identification of campylobacters

Rectal swabs were streaked on two cefoperazone amphotericin teicoplanin (CAT) agar plates (Oxoid). Under microaerophilic conditions (10% CO_2 and 5% O_2), one plate was incubated at 42°C and the other one at 37°C in order that *C. upsaliensis* growth would be uninhibited (Corry *et al*., 1995). The plates were checked after 2–3 days and again after 5 days for growth of campylobacters. Preliminary identifications were based on phenotypic characteristics: colony morphology, microscopic morphology, motility and oxidase and catalase reactions.

Polymerase chain reaction (PCR) was used to detect and differentiate three major species of campylobacters (*C. jejuni*, *C. coli* and *C. upsaliensis*). The genomic DNA of isolates was extracted using a commercial kit (Genomic DNA Purification Kit, MBI Fermentas GMBH, St. Leon-Rot, Germany). For the detection of *C. jejuni and C. coli*, the procedures described by Harmon and colleagues (1997) were used with a primer set of pg-3 (5′-GAACTTGAACCGATTTG-3′) and pg-50 (5′-ATGGGATTTCGTATTAAC-3′). To further differentiate *C. jejuni* and *C. coli*, the primers 5′-TACTACAGGAGTTCAAGCTT-3′ and 5′-GTTGATGTAACTTGATTTTG-3′ described by Nishimura and colleagues (1996) were used. The PCR described by Linton and colleagues (1996) was used to specifically detect *C. upsaliensis*. The primer set used was CHCU146F (5′-GGGACAACACTTAGAAATGAG-3′) and CU1024R (5′-CACTTCCGTATCTCTACAGA-3′).

Antimicrobial susceptibility test

All the 40 *Salmonella* isolates were tested for antimicrobial susceptibility testing by the disc-diffusion method following the NCCLS (2002) guidelines. The following antimicrobial agents were used at the indicated concentrations (μg/disc except where specified): amoxicillin/clavulanic acid (20/10), ampicillin (10), apramycin (15), cefoxitin (30), ceftriaxone (30), cephalothin (30), chloramphenicol (30), ciprofloxacin (5), gentamicin (10), kanamycin (30), nalidixic acid (30), nitrofurantoin (300), streptomycin (10), sulfamethoxazole/trimethoprim (23.5/1.5) and tetracycline (30).

A total of 33 *C. jejuni* isolates were randomly chosen and tested with the E-test system (AB BIODISC, Solna, Sweden) to determine minimum inhibitory concentrations (MICs) for eight antimicrobial agents (azithromycin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin, gentamicin, nalidixic acid, and tetracycline). These eight antimicrobial agents were those included in the National Antimicrobial Resistance Monitoring System in the USA for the monitoring the antimicrobial resistance of *Campylobacter* spp by the E-test system (Gupta *et al*., 2004). The *E*-test was performed on Mueller–Hinton agar supplemented with 5% sheep blood according to the manufacturer's instructions. Inocula were prepared by incubating the strains for 24 h at 42°C under microaerobic conditions in trypticase soy broth. After application of the *E*-test strips, plates were incubated at 42°C for 48 h. MIC values were read directly from the test strip according to the instructions of the manufacturer, where the elliptical zone of inhibition intersected with the MIC scale on the strip.

Statistical analysis

Chi-squared and Fisher's exact tests were used to evaluate the differences in prevalence with Microsoft Excel (Microsoft, WA, USA).

RESULTS

Isolation of salmonellae

In the total of 437 household dogs aged 3 months to 19 years, 9 dogs (2.1%) were positive for salmonellae (Table I). Thirteen household dogs showed symptom of diarrhoea during sampling. Of these dogs, only one *Salmonella* isolate was recovered from one dog. There was no statistical difference in isolation rates between male (1.8%, 4/222) and female (2.3%, 5/215) groups ($p < 0.05$), and no statistical difference between young (3–12 months old) (1.4%, 1/70) and adult (>1 year old) (2.2%, 8/367) groups ($p < 0.05$). Six serotypes of *S. enterica* were identified: *Salmonella* Duesseldorf ($n = 3$), *Salmonella* Derby ($n = 2$), *Salmonella* Bardo ($n = 1$), *Salmonella* Bellevue ($n = 1$), *Salmonella* Panama ($n = 1$) and *Salmonella* Stanley ($n = 1$). Serogroup B based on O-antigen grouping was the most prevalent serogroup (42.5%), followed by serogroups C_2 (35.0%), D_1 (17.5%), and E_1 (5%) (Table II).

Of the 491 stray dogs, 2/6 animal shelters (16.7%) and 31 stray dogs (6.3%) were salmonellae positive (Table I). The isolation rates between household dogs (2.1%) and stray dogs (6.3%) were significantly different ($p < 0.01$). There was no statistical difference in isolation rates between male (6.1%, 13/212) and female (5.7%, 16/279) stray dogs ($p < 0.05$). The difference between the two age groups was unavailable because their ages were unknown. At least 17 *Salmonella* serotypes were recognized, among which *Salmonella* Dusseldorf ($n = 6$) and *Salmonella* Enteritidis ($n = 5$) were predominant (Table II).

Isolation of campylobacters

Campylobacter spp. were isolated from 2.7% (12/437) of the household dogs, contrasting with 23.8% (117/491) of the stray dogs in all the six animal shelters. Campylobacter isolation

TABLE I
The numbers and percentages of salmonellae and campylobacters isolated from household dogs and stray dogs in Taiwan

	Household dogs ($n = 437$)		Stray dogs ($n = 491$)		Total ($n = 928$)	
Agent	*n*	(%)	*n*	(%)	*n*	(%)
Salmonella spp.	9	(2.1)	31	(6.3)	40	(4.3)
Campylobacter spp.	12	(2.7)	117	(23.8)	129	(13.9)
C. jejuni	11	(91.7)[a]	101	(86.3)	112	(86.8)
C. coli	1	(8.3)	4	(3.4)	5	(3.9)
C. upsaliensis	0	(0)	12	(10.3)	12	(9.3)

[a]Percentage values for each campylobacter species relate to the proportion relative to the total campylobacter numbers isolated.

TABLE II
Salmonella serotypes and numbers isolated from dogs in Taiwan

O antigen serogroup	Serotype	Household dogs	Stray dogs	Total
B	*Salmonella* Branderburg	0	1	1
B	*Salmonella* Derby	2	2	4 (10%)
B	*Salmonella* Eppendorf	0	1	1
B	*Salmonella* Essen	0	2	2
B	*Salmonella* Fyris	0	1	1
B	*Salmonella* Lagos	0	1	1
B	*Salmonella* Schwarzengrund	0	1	1
B	*Salmonella* Stanley	1	0	1
B	*Salmonella* Typhimurium	0	1	1
B	*Salmonella* spp.	0	3	3
C_2	*Salmonella* Bardo	1	2	3
C_2	*Salmonella* Bellevue	1	0	1
C_2	*Salmonella* Dusseldorf	3	6 (19.4%)	9 (22.5%)
C_2	*Salmonella* Newport	0	1	1
C_2	*Salmonella* spp.	0	1	1
D_1	*Salmonella* Enteritidis	0	5 (16.1%)	5 (12.5%)
D_1	*Salmonella* Itami	0	1	1
D_1	*Salmonella* Panama	1	0	1
E_1	*Salmonella* Goelzau	0	1	1
E_1	*Salmonella* Weltevreden	0	1	1
Total		9	31	40

rates between household and stray dogs were significantly different ($p < 0.01$). However, there was no significant difference in the isolation rates between male (2.3%, 5/222) and female (3.3%, 7/215) household dogs, nor between male (22.2%, 47/212) and female (25.1%, 70/279) stray dogs ($p < 0.05$). There was also no significant difference in isolation rate between young (4.3%, 3/70) and adult household dogs (2.5%, 9/367) ($p < 0.05$). *C. jejuni* was the most prevalent species in both household (91.7%) and stray dogs (86.3%). *C. coli* was isolated from 8.3% of the household dogs and 3.4% of the stray dogs. *C. upsaliensis* was isolated from 10.3% of the stray dogs but was not found in the household dogs (Table I).

Antimicrobial susceptibility test

Using the disc diffusion method, 8 out of 40 strains of *Salmonella* (22.2%) were resistant to amoxicillin, 20 (50%) to ampicillin/clavulanic acid, 9 (22.5%) to apramycin, 1 (2.8%) to cefoxitin, none to ceftriaxone, 2 (5%) to cephalothin, 21 (52.5%) to chloramphenicol, none to ciprofloxacin, 2 (5%) to gentamicin, 2 (5%) to kanamycin, 17 (42.5%) to nalidixic acid, 4 (10%) to nitrofurantoin, 14 (38.9%) to streptomycin, 15 (37.5%) to sulfamethoxazole/trimethoprim and 31 (77.5%) to tetracycline.

TABLE III
Antimicrobial resistance of 33 strains of *C. jejuni* as determined by *E* test

Antibiotic	Break point	% Resistant	Minimum inhibitory concentrations (µg/ml)																						
			0.125	0.19	0.25	0.38	0.50	0.75	1.0	1.5	2	3	4	6	8	12	16	24	32	48	64	96	128	192	256
Azithromycin	≥2	93.9			1	1							2	1	3			1	1	1	2		1		19
Chloramphenicol	≥32	69.7				1						3	2	1	2			1				1	2		20
Ciprofloxacin	≥4	18.2	8		1	4	1	1	2	5	3	2							6						
Clindamycin	≥4	87.9	1	2			1								1	1	2	3					1	1	20
Erythromycin	≥8	81.8					2	1	2	1								1	1	2			2		21
Gentamicin	≥16	33.3				1			1	3	1	2	3	1	5		1		1	1					8
Nalidixic acid	≥32	51.5									4	4	4		1	3						3	1	1	12
Tetracycline	≥16	78.8							1			3	1		2		1	1		2	3				19

The MICs of the eight antimicrobial agents for *Campylobacter* strains are shown in Table III. High rates of resistance were observed to azithromycin (93.9%), clindamycin (87.9%), erythromycin (81.8%), tetracycline (78.8%), chloramphenicol (69.7%), nalidixic acid (51.5%), gentamicin (33.3%), and ciprofloxacin (18.2%).

DISCUSSION

Multiple serotypes of salmonellae commonly exist in dog populations. In the southern USA, 53 serotypes have been isolated, with *Salmonella* Anatum and *Salmonella* Typhimurium being predominant (Morse and Duncan, 1975); in Trinidad, 28 serotypes were isolated, with *Salmonella* Javiana, *Salmonella* Newport, *Salmonella* Arechavaleta and *Salmonella* Heidelberg being predominant (Seepersadsingh *et al*., 2004). Ten serotypes were previously isolated from dogs in Taiwan in 1963–1967, with the most prevalent serotypes being *Salmonella* Weltevreden, *Salmonella* Meleagridis, *Salmonella* Derby and *Salmonella* Tananarive (Cheng *et al*., 1968). In this study, at least 20 serotypes were detected and *Salmonella* Dusseldorf was the most prevalent followed by *Salmonella* Enteritidis and *Salmonella* Derby. It was concluded that the prevalent serotypes of salmonellae in dog populations are quite variable among different countries and at different times within the same country.

In Taiwan, *Salmonella* Typhimurium, *Salmonella* Weltevreden, *Salmonella* Derby, *Salmonella* Muenchen, *Salmonella* Agona, and *Salmonella* Panama were the six most frequent serotypes from humans in 1983–1993 (Wang *et al*., 1994). Except for few host-adapted serotypes such as *Salmonella* Gallinarum and *Salmonella* Pullorum, other serotypes are considered pathogenic to humans and have various kinds of hosts. Serotypes *Salmonella* Derby, *Salmonella* Panama, and *Salmonella* Enteritidis, the causative agents of human disease and found in dogs, have also been found commonly in chickens and ducks in Taiwan (Chou and Tsai, 2001; Tsai and Hsiang, 2005). Thus the human and canine infections might both be due to the consumption of contaminated poultry. The antimicrobial resistant patterns of the Taiwanese canine and human *Salmonella* isolates are quite different. Increasing resistance to ceftriaxazone and ciprofloxacin in human Taiwanese *Salmonella* isolates has been reported recently (Su *et al*., 2005); however, we demonstrated that all the canine isolates were susceptible to ceftriaxazone and ciprofloxacin.

The species distribution of *Campylobacter* isolates from dogs differs considerably between publications and years. *C. upsaliensis* (Sandberg *et al*., 2002; Hald *et al*., 2004; Wieland *et al*., 2005) and *C. jejuni* (Hald and Madsen, 1997; Lopez *et al*., 2002; Workman *et al*., 2005) have been demonstrated to be the predominant species in dogs in different studies. Additionally, younger dogs have been reported to carry higher rates of campylobacters (Lopez *et al*., 2002; Engvall *et al*., 2003; Hald *et al*., 2004), and have higher odds of carrying *C. upsaliensis* than older dogs (Wieland *et al*., 2005). In this study, no significantly higher isolation rate was found in young household dogs, and this might have been the result of most of our isolates being *C. jejuni,* in which age is not associated with carriage (Wieland *et al*., 2005).

In Taiwan, it has been shown that human *Campylobacter* isolates were significantly more susceptible than chicken isolates to erythromycin, clindamycin and ciprofloxacin (Li *et al*., 1998). From our study, it appears that the canine isolates were also significantly more resistant to clindamycin and erythromycin than were human isolates. Whereas nearly all human and chicken *Campylobacter* isolates were susceptible to gentamicin (Li *et al*., 1998), our results showed a higher level of resistance in canine isolates (33.3%). However, the resistance rates to ciprofloxacin were much lower in canine isolates (18.2%) in this study than human isolates (79%) (Li *et al*., 1998). Different antimicrobial susceptibility of human and canine isolates may reflect the different use of antimicrobials in pet animal veterinary medical practice and human medical practice, and may also indicate that exchange of *Campylobacter* spp. between the human and canine population is rare.

The fact that household dogs generally show lower isolation rates of salmonellae and campylobacters than stray dogs (Shimi *et al*., 1976; Simpson *et al*., 1981; Fox, 1990; Workman *et al*., 2005) was shown by our findings as well. These highly prevalent gastrointestinal pathogens in shelters may increase the risks of (1) nosocomial transmission between dogs, (2) zoonotic transmission to workers in shelters and people adopting dogs from shelters, and (3) environment contamination potential (Sokolow *et al*., 2005; Wright *et al*., 2005). Because of financial constraints and crowded dog housing with frequent turnover, surveillance for specific pathogens may not be feasible and therapeutic measures to reduce the contamination level may also not be easily achieved. The public health importance of salmonellae and campylobacters in dogs in Taiwan has not been established, especially in the case of stray dogs. However, from the findings reported here, the contamination in the dog shelters appeared to be a potential risk to public health, although this assumption would still need to be verified by molecular methods. Concerning potential zoonotic risks, the implementation of nonspecific prevention methods such as prophylactic disinfection in the animal holding areas, self-sanitation procedures for the employees, quarantine before dog adoption, and clear emphasis on warnings of the zoonotic risk of transmission to the adopters is recommended (Sokolow *et al*., 2005; Wright *et al*., 2005).

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