

RESEARCH ARTICLE

Antimicrobial Susceptibility Test and Prevalence of *Campylobacter* spp. Isolated from Client-Owned Dogs Visiting Veterinary Clinics in Khon Kaen Province

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Abstract

Objective—The present study aimed to determine the prevalence and antimicrobial susceptibility pattern of *Campylobacter* spp. in dogs brought to the veterinary clinics in Khon Kaen province during January, 2010 – January, 2011.

Materials and Methods—A total of 301 fecal swabs from dogs were sampled and used for the isolation of *Campylobacter* species. Colony Multiplex PCR for species identification was performed. Antibiotic sensitivity testing was done using disk diffusion technique.

Results—Forty-two dogs (14.0%) from 301 fecal swab samples were positive for *Campylobacter* species. Colony multiplex PCR for species identification showed that *C. jejuni*, *C. coli*, co-infection and other *Campylobacter* species were identified at 1.0% (3/301), 7.0% (21/301), 4.7% (14/301), and 1.0% (4/301), respectively. Of the 42 positive isolates, *C. jejuni*, *C. coli*, co-infection, and other *Campylobacter* species were 40.5 (17/42), 83.3 (35/42), 33.3 (14/42) and 9.5% (4/42), respectively. The antimicrobial sensitivity study showed that 95.0% of isolates were sensitive to amoxicillin-clavulanic acid while enrofloxacin, norfloxacin, nalidixic acid, oxytetracycline and Trimethoprim – Sulfamethoxazole were 60.0-69.0% susceptible.

Conclusion—Prevalence of *Campylobacter* spp. in dogs was 14.0%. In total 42 isolates were identified. Co-infection was 4.7%. Antimicrobial susceptibility tests noted that amoxicillin-clavulanic acid works best for the treatment of *Campylobacter* species infection.

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Keywords: Antimicrobial susceptibility test; *Campylobacter*; Dogs; Prevalence

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การทดสอบความไวต่อยาปฏิชีวนะและความชุกของเชื้อแคมไพโลแบคเตอร์ที่เพาะแยกจากสุนัขที่ถูกนำเข้ามารักษาในคลินิกสัตวแพทย์ในจังหวัดขอนแก่น

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บทคัดย่อ

วัตถุประสงค์ เพื่อหาความชุกของเชื้อแคมไพโลแบคเตอร์และทดสอบความไวต่อยาปฏิชีวนะจากเชื้อที่เพาะแยกได้จากสุนัขที่เข้ามาทำการรักษาที่คลินิกสัตวแพทย์ในจังหวัดขอนแก่นในระหว่างเดือนมกราคม 2553 ถึงเดือน มกราคม 2554

วัสดุ อุปกรณ์ และวิธีการ ทำการเก็บตัวอย่างอุจจาระของสุนัขจำนวน 301 ตัว เพื่อเพาะหาเชื้อแคมไพโลแบคเตอร์ โดยตรวจหาสปีชีส์ด้วยเทคนิค Colony Multiplex PCR และทดสอบความไวต่อยาปฏิชีวนะโดยใช้วิธี Disc Diffusion

ผลการศึกษา พบเชื้อแคมไพโลแบคเตอร์จากสุนัขจำนวน 42 ตัว จาก 301 ตัว (14.0%) โดยเมื่อทำ Colony Multiplex PCR พบความชุกของเชื้อ *C. jejuni*, *C. coli*, co-infection และเชื้อแคมไพโลแบคเตอร์สปีชีส์อื่น เท่ากับ 1.0 (3/301), 7.0 (21/301), 4.7 (14/301) และ 1.0% (4/301) ตามลำดับ โดยจาก 42 ไอโซเลทที่แยกได้ พบความชุกของเชื้อ *C. jejuni*, *C. coli*, co-infection และเชื้อแคมไพโลแบคเตอร์สปีชีส์อื่น เท่ากับ 40.5 (17/42), 83.3 (35/42), 33.3 (14/42) และ 9.5% (4/42) ตามลำดับ การทดสอบความไวต่อยาปฏิชีวนะพบว่าเชื้อมีความไวต่อยา amoxicillin-clavulanic acid คิดเป็น 95.0% ในขณะที่มีความไวต่อยา enrofloxacin, norfloxacin, nalidixic acid, oxytetracycline และ trimethoprim-sulfamethoxazole คิดเป็น 60.0-69.0%

ข้อสรุป ความชุกของเชื้อแคมไพโลแบคเตอร์ในสุนัข 301 ตัว เท่ากับ 14.0% โดยจำนวนเชื้อทั้งหมดที่แยกได้มี 42 ไอโซเลท ความชุกของการพบเชื้อร่วมกันระหว่าง *C. jejuni* และ *C. coli* คิดเป็น 4.7% จากการทดสอบความไวต่อยาปฏิชีวนะพบว่ายา amoxicillin-clavulanic acid ให้ผลในการยับยั้งการติดเชื้อแคมไพโลแบคเตอร์ได้มากที่สุด

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คำสำคัญ: การทดสอบความไวต่อยาปฏิชีวนะ แคมไพโลแบคเตอร์ สุนัข ความชุก

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Introduction

Campylobacter is a major cause of gastroenteritis in human. It is also commonly found in the intestines of many wild and domestic animals, wide spread in mammals, foods, water and environment [1-5]. The bacteria normally live in the intestinal tract and are discharged through their feces. *Campylobacter* could be transmitted from person to person by contacting infected feces [6]. It was reported that household pets carry and transmit the bacteria to their owners with all ages affected [2, 7-10]. In 1994, Gurgan and Diker [9] isolated *C. upsaliensis* from blood sample and fetoplacental material of 18-week-pregnant woman who had contact with a household cat. The pathogen was isolated from the cat without any clinical sign. The report suggested that *Campylobacter* infection during pregnancy may have serious consequences and that this organism should be considered as a potential pathogen for pregnant women, especially when there is a history of contact with animals. In addition, diarrheal puppies were frequently infected with *Campylobacter* and may be an important reservoir for human infection [9, 11]. Previous studies indicated that the prevalence of *Campylobacter* in UK, Norway and Denmark were 38.0, 18.0 and 11.0% respectively [8, 12, 13]. In Thailand, data concerning *Campylobacter* epidemiology in dogs was insufficient. Diarrhea caused by *Campylobacter* spp. in human is often a mild and self limited disease but, in serious case such as septicemia and meningitis, may require antibiotic treatment. The information on antimicrobial sensitivity of *Campylobacter* spp. differs somewhat between different countries [14-17].

The present study aimed at determining the prevalence and antimicrobial susceptibility pattern for *Campylobacter* spp. in dogs from veterinary clinics in Khon Kaen, Thailand. The Colony Multiplex PCR was employed for investigating the distribution of *C. jejuni*, *C. coli*, *C. lari*, *C. upsaliensis*, *C. fetus* subsp. *fetus* and *C. helveticus* from canine feces attending the veterinary clinics.

Materials and Methods

Sampling and culturing

The study area was in Khon Kaen province, northeastern Thailand. The total area was 10,886 km² which ranked 15th among the 76 provinces of Thailand (excluding Bangkok). Faecal swabs from 301 dogs visiting veterinary clinics and hospital were collected. Then, the sample was put into Bolton Broth (CM-983, Oxoid, UK) supplemented with 10 µg/ml cefoperazone, 10 µg/ml vancomycin, 10 µg/ml trimethoprim and 5 µg/ml amphotericin B (*Campylobacter* selective supplement, SR 0208, Oxoid). The samples were kept in a refrigerator and transferred to the laboratory within 2 hours. The membrane filtration method was used [18-20] by adding 5 drops of fecal suspension onto a 0.45-µm-pore-size, 47 mm in diameter cellulose nitrate filter (WhatmanTM, United Kingdom) on modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA). After incubation at 37°C under aerobic condition for 1 h, the filter was removed. All plates were incubated in jars (Oxoid anaerobic jar HP0011, United Kingdom) under microaerophilic condition (GasPakTM EZ, CampyPouchTM System) at 37°C for 48 h. The plates showing suspected colonies of *Campylobacter* were confirmed based on their characteristic cell morphology. Then

they were subcultured into Mueller-Hinton Broth (CM0405, Oxoid) containing 20.0% (v/v) glycerol and stored at -80°C until use.

Antimicrobial susceptibility test

The disc diffusion method of Bauer et al. (1996) [21] was used to determine the antibiotic resistance of *Campylobacter* spp. on Mueller-Hinton Agar plates containing 5.0% defibrinated sheep blood (PB5007A, Oxoid, UK). The incubation was done under microaerophilic condition (GasPak™ EZ, CampyPouch™ System) at 37°C, 48 h. The suspension was adjusted to 0.5% McFarland standard turbidity as recommend by The Clinical and Laboratory Standard Institute [22].

Antimicrobial susceptibility test was performed using standard disc (Oxoid, UK) containing norfloxacin (10 µg), enrofloxacin (5 µg), nalidixic acid (30 µg), oxytetracycline (30 µg), sulphamethoxazole/trimethoprim (23.75/1.25 µg) and amoxy-clavulanic acid (20/10 µg). The results were interpreted as sensitive, intermediate or resistant according to The Clinical and Laboratory Standard Institute [22].

Molecular Study

DNA template preparation: *Campylobacter* isolates were grown on mCCDA supplemented with SR0155 (Oxoid) and incubation at 37°C under microaerophilic condition. DNA was prepared by the whole-cell boiling procedure. Each DNA template was prepared using a loopful of culture transferred to sterile deionized water, and heated at 100°C for 10 min. The suspension was subsequently centrifuged at 4,000 x g (Heraeus Labofuge 400R Centrifuges, Germany) for 10 min. The supernatant containing the DNA was transferred to a sterile microcentrifuge tube for PCR amplification [13].

PCR primers: The oligonucleotide primers used for amplification of the gene *hipO* from *C. jejuni*, *glyA* from *C. coli*, *C. lari* and *C. upsaliensis*, *sapB2* from *C. fetus* subsp. *fetus*, 16S rDNA from *C. helveticus* and 23s rRNA from *C. jejuni* (Table 1). The 23s rRNA from *C. jejuni* was included to serve as an internal validation control to monitor PCR conditions and reagents as a conserve gene.

Multiplex PCR conditions: The reactions were performed in the GeneAmp PCR system 2400 (Perkin Elmer, USA). A total volume of 50 µl PCR mixture containing 5 µl of DNA template, 8 µl of 2 mM MgCl₂²⁺, 2 µl of each primers, 5 µl of 10 x PCR buffer, 1 µl of 10 mM dNTP, 1 µl of 5u/µl Fermentas *Taq* DNA Polymerase (Fermentas, Canada). The volume was adjusted with sterile deionized water to 50 µl. PCR condition were 95°C, 6 min for initial denaturing of DNA templates to single strand DNA, then starting at 95°C, 30s; 59°C, 30s; 72°C, 30s for 30 cycles for denaturing-annealing-extension. The final extension was conducted at 72°C for 7 min. The amplicons were stored at 4°C until needed. The amplified DNAs were subjected to gel-electrophoresis using 1.5% (w/v) agarose in TBE buffer for 30 minutes at 100 V (Mupid®-2 plus Submarine Electrophoresis System Advance, Japan). Agarose gel was stained with ethidium bromide and photographed under UV light transilluminator (Quantum ST4, Germany). The extracted DNA of *C. coli* ATCC 33559 was used as a positive control.

Statistical analysis

Preliminary descriptive and univariable questionnaire analyses were employed for statistical analysis with SPSS version 17.0.

Table 1. Oligonucleotide Primers Used in the Multiplex PCR Assay

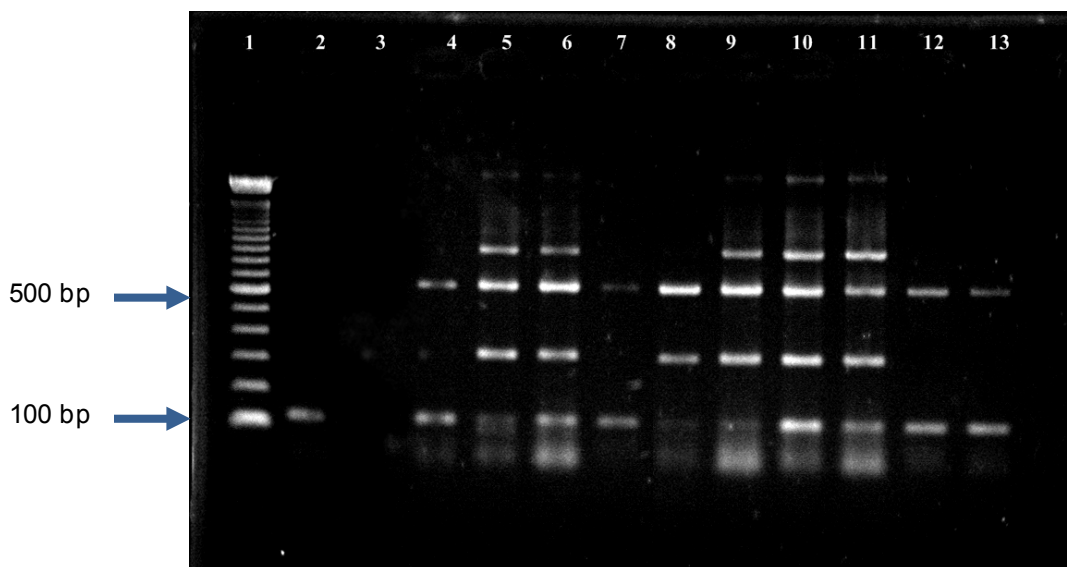
Primers	Size (in bp)	Sequence(5'-3')	Target gene	Gene location (bp)
CJF	323	ACTTCTTTATTGCTTGCTGC	<i>C. jejuni</i> <i>hipO</i>	1662-1681
CJR		GCCACAACAAGTAAAGAAGC		1984-1965
CCF	126	GTAAAACCAAAGCTTATCGTG	<i>C. coli</i> <i>glyA</i>	337-357
CCR		TCCAGCAATGTGTGCAATG		462-444
CLF	251	TAGAGAGATAGCAAAAGAGA	<i>C. lari</i> <i>glyA</i>	318-337
CLR		TACACATAATAATCCCACCC		568-549
CUF	204	AATTGAAACTCTTGCTATCC	<i>C. upsaliensis</i> <i>glyA</i>	63-82
CUR		TCATACATTTTACCCGAGCT		266-247
23SF	650	TATACCGGTAAGGAGTGCTGGAG	<i>C. jejuni</i> <i>23s</i> <i>rRNA</i>	3807-3829
23SR		ATCAATTAACCTTCGAGCACCG		4456-4435
Chcu146F	1225-	GGGACAACACTTAGAAATGAG	<i>C. helveticus</i>	146-166
Ch1371R	1375	CCTGTACATGGCTGATTAC		1351-1371
	435		<i>C. fetus</i> <i>sapB2</i>	
CFF		GCAAATATAAATGTAAGCGGAGAG		2509-2532
CFR		TGCAGCGGCCCCACCTAT		2943-2926

Source: Inglis and Kalischuk (2003) [23], Shen et al. (2001) [24], Wang et al. (2002) [25]

Results

Forty-two dogs (14.0%) were positive for *Campylobacter*. They were confirmed to species level by multiplex PCR technique (**Figure 1**). The colony multiplex PCR indicated that *C. jejuni*, *C. coli*, co-infection and other *Campylobacter* species were determined at 1.0% (3/301), 7.0% (21/301), 4.7% (14/301), and 1.0% (4/301), respectively. Of the 42 positive samples, *C. jejuni* were 40.5% (17/42), *C. coli* were 83.3% (35/42), co-infection were 33.3% (14/42) and other *Campylobacter* species were 9.5% (4/42) (**Figure 2**). **Figure 3** shows that 54.0% (23/42) of diarrheal dogs were *C. coli* positive while 15.0% (6/42) were *C. jejuni* and co-infection.

The antimicrobial susceptibility of 42 *Campylobacter* isolates was determined against 6 antimicrobial agents, and results are presented in **Table 2** and **3**. Ninety five percent of the isolates were susceptible to Amoxicillin-clavulanic acid. One of *C. coli*, and one of co-infection were intermediate

Figure 1. Agarose Gel electrophoresis of Multiplex PCR Detection, and Identification

Lane assignments: Lane 1, Molecular weight marker, 100 bp DNA ladder (Takara BIO INC, Japan); Lane 2, positive control: 126 bp, *C. coli* ATCC 33559; Lane 3, negative control; Lane 4 to 13, *Campylobacter* isolates from dog feces.

resistant to amoxicillin-clavulanic acid. Furthermore, 26.0, 14.0%, 21.0%, 36.0% and 21.0% of the isolates were resistance to enrofloxacin, nalidixic acid, oxytetracycline, norfloxacin and trimethoprim-sulfamethoxazole (**Table 3**).

Discussion

The prevalence of *Campylobacter* in pets was noted between 11.0 to 92.0% in previous studies in other country [8, 10, 12, 13, 24, 26- 28]. There was a co-infection *Campylobacter* species. It was noted from previous studies that many individual samples contained multiple species of *Campylobacter* [29, 30]. Of the 301 dogs, 42 dogs were positive for *Campylobacter*. The 14.0% prevalence of *Campylobacter* in canine fecal samples reported in the present study is in harmony with previous studies. However, the species distribution is differed. The present study has considerably higher prevalence of *C. coli* than previously reported between 0.7-5.0% [10, 26], and no other *Campylobacter* spp. was identified. Previous studies suggested that *Campylobacter* populations were genetically diversified when multiple isolation methods were used. In multiple genotype variant samples, there were different species distributed over different media rather than a single isolation method [13, 24, 26, 27, 31, 32]. The selective media contained cefoperazone which probably inhibit the growth of *C. upsaliensis* [33]. Hald et al. (2004) [26]

Figure 2. Distribution of *Campylobacter* spp. Isolates from Positive Dog Fecal Specimens for *Campylobacter* spp. (42 Samples)

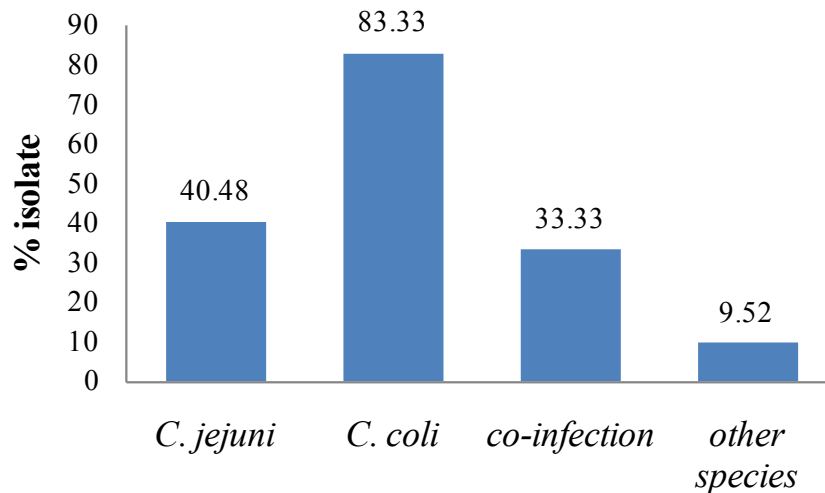
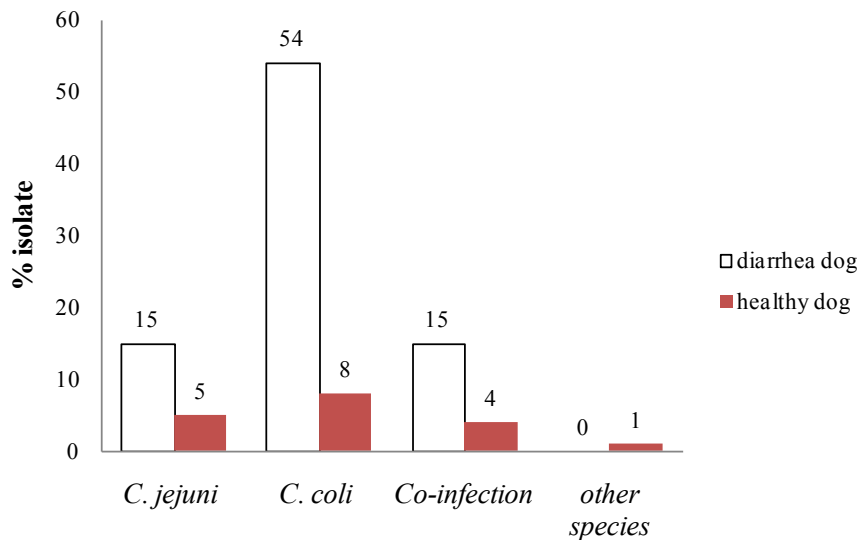


Figure 3. Comparison of the Prevalence of *Campylobacter* spp. from Dog Feces



suggested that *C. upsaliensis* were slowly growing and required at least 3 to 4 days to develop visible growth. This study was similar to Moreno et al. (1993) [34] who found that no *C. upsaliensis* was isolated within the first 48 h of incubation. There was no difference between the present study and previous study

Table 2. Antimicrobial Susceptibility Patterns of 42 *Campylobacter* Isolates from Dogs Visiting Veterinary Clinics during January, 2010 - January, 2011

Drugs	Sensitive strains	
	No.	(%)
Amoxicillin-clavulanic acid	40	(95.0)
Enrofloxacin	25	(60.0)
Nalidixic acid	27	(64.0)
Norfloxacin	26	(62.0)
Oxytetracycline	29	(69.0)
Trimethoprim - Sulfamethoxazole	29	(69.0)

Table 3. Resistance of *Campylobacter* Isolates from Dogs Against 6 Antibiotics

Drugs	<i>Campylobacter</i> spp. (n=42)		<i>C. coli</i> (n=21)		<i>C. jejuni</i> (n=3)		Co-infection (n=14)		Other spp. (n=4)	
	No. R (%)	No. I	No. R (%)	No. I	No. R (%)	No. I	No. R (%)	No. I	No. R (%)	No. I
Amoxicillin-clavulanic acid	0 (0.0)	2	0 (0.0)	1	0 (0.0)	0	0 (0.0)	1	0 (0.0)	0
Enrofloxacin	11 (26.0)	6	6 (29.0)	2	1 (33.0)	1	4 (29.0)	2	0 (0.0)	1
Nalidixic acid	6 (14.0)	9	4 (19.0)	4	0 (0.0)	1	1 (7.0)	2	1 (25.0)	2
Norfloxacin	15 (36.0)	1	6 (29.0)	1	2 (67.0)	0	5 (36.0)	0	2 (50.0)	0
Oxytetracycline	9 (21.0)	4	4 (19.0)	3	1 (33.0)	0	4 (29.0)	1	0 (0.0)	0
Trimethoprim - Sulfamethoxazole	9 (21.0)	4	3 (14.0)	2	2 (67.0)	0	4 (29.0)	1	0 (0.0)	2

Abbreviations: R = resistance; I = intermediate resistance

in the prevalence of co-infection between *C. jejuni* and *C. coli* (i.e. 33.3%) [35].

The present study noted that 35 of 42 (83.3%) positive samples were detected in dogs at less than 1 year of age which was similar to other studies. Other studies revealed the increase in number of positive samples in young dog [10, 12, 13, 26, 36].

Antimicrobial resistant patterns of nalidixic acid, norfloxacin and trimethoprim-sulfamethoxazole in previous studies in poultry and human were noted at lower percentage than *Campylobacter* spp. from canine feces in this study [17, 37, 38]. The high fluoroquinolone resistant rates were the most probably caused by the broad use of this antibiotic in veterinary medicine. Amoxicillin-clavulanic acid still has high potency towards *Campylobacter* spp. since the susceptibility was more than 95.0% which was similar to the work by Wardak et al. in 2007 [38]. This may be attributable to the fact that this drug was less frequently administered to diarrheal cases in dogs. Nalidixic acid resistance was higher in *C. coli* than in *C. jejuni*, which was similar to previous study [39].

Prevalence of *Campylobacter* spp. in dogs was 14.0%. In total 42 isolates were identified. Co-infection was 4.7%. Antimicrobial susceptibility tests noted that amoxicillin-clavulanic acid works best for the treatment of *Campylobacter* species infection. Dogs carried *Campylobacter* may be a reservoir for infection among dog owner in Thailand. High number of *Campylobacter coli* has been found in this study.

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