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Occurrence of Arcobacter in dogs and cats in Selangor, Malaysia, and associated risk factors

Dauda Mohammed GONI[1], Saleha ABDULAZIZ[1,*], Gurmeet Kaur DHALIWA[2], Zunita ZAKARIA[1], Ibrahim Jalo MUHAMMAD[1], Mohamed Abdelrahman MOHAMED[1], Abdulrasheed Aliyu BELLO[1], Asinamai Athliamai BITRUS[1]

[1]Department of Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

[2]Department of Clinical Studies, Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia

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Abstract: *Arcobacter* is widely regarded as an emerging foodborne pathogen because of its increasing occurrence in food production causing gastroenteritis in humans. In addition, it is also reported as a potential zoonosis. The objectives of this study were to determine the occurrence of *Arcobacter* in dogs and cats and the associated risk factors. Rectal and buccal cavity swab samples were taken from dogs (n = 40) and cats (n = 40) owned by individuals who brought the animals to a university teaching hospital and stray dogs (n = 61) and cats (n = 46). Suspected colonies of *Arcobacter* were subjected to biochemical tests (catalase, oxidase, and hippurate hydrolysis and indoxyl acetate hydrolysis tests). Multiplex polymerase chain reaction (mPCR) was employed for the confirmation and differentiation of the isolates. Results showed that the rates of *Arcobacter* carriage were 34.8% and 45.0% in stray and pet cats, respectively, while in stray and pet dogs the occurrences were detected at 50.8% and 60.0%, respectively. *Arcobacter butzleri* was the only species identified. The risk factors for *Arcobacter* infections in dogs and cats were determined through a questionnaire and analyzed statistically. The factors that were found to significantly increase the risk of *Arcobacter* infection were households with multiple pets and the source of drinking water.

Key words: *Arcobacter*, dogs, cats, Malaysia, risk factors

1. Introduction

The ability of *Arcobacter* to grow at lower temperatures (15–25 °C) and being aerotolerant makes it different from *Campylobacter* and other related taxa although they are phenotypically and morphologically similar (1). The prevalence of *Arcobacter* in humans and animals can be determined more accurately with more sensitive techniques for their isolation and identification. *Arcobacter* species. particularly *A. butzleri*, *A. skirrowii*, and *A. cryaerophilus*, have been known to be of veterinary importance and can be isolated from farm animals, wild animals, and animal products (2–4). Today they are gaining attention as emerging foodborne organisms (5). In addition, *Arcobacter* can be transmitted to humans through close contact with pets (6). *Arcobacter butzleri* is reported as one of the species isolated and found in 'traveler's disease', a common disease that can affect those visiting developing countries and usually caused by consumption of contaminated food. It was reported that *Arcobacter* was isolated from major restaurants in Bangkok, Thailand, in 13% of samples and it was detected in 8% of diarrhea cases in Mexico, Guatemala, and India (7,8). The prevalence of *Arcobacter* infections in domestic animals varies in different parts of the world and various studies have shown that the highest prevalence is found in chicken meat, followed by pork and beef (9). Regarding geographical distribution, the prevalence ranged from 77.8% in Italy to 22.1% in Nigeria, 2.4% in Thailand, 12.9% in South Africa, and 1.2% in France (9–14). In Malaysia, *Arcobacter* was isolated from beef and milk at rates of 26.3% and 7.6%, respectively (2). Water has also been reported as a good medium for *Arcobacter* transmission and *Arcobacter* may be considered as a potential waterborne pathogen (15,16).

Apart from food animals, dogs and cats have also been shown to be carriers of this emerging pathogen across the globe (9,17–20). In Malaysia, there is a lack of published studies on the presence of *Arcobacter* in pet animals. *Arcobacter* infection in animals may result commonly in mastitis, abortion, and diarrhea that is more persistent and watery than in *Campylobacter jejuni* infections; other clinical signs include nausea, fever, and abdominal pain (21,22).

* Correspondence: saleha@upm.edu.my

The main objectives of this study were to determine the occurrence of *Arcobacter* in dogs and cats in Selangor, Malaysia, and to identify the risk factors associated with their occurrence.

2. Materials and methods

2.1. Collection of samples

Samples were collected after receiving due approval from the Institutional Animal Care and Use Committee of Universiti Putra Malaysia (AUP No.: R001 / 2013). The samples collected were from client-owned dogs and cats at a university veterinary hospital after seeking the consent of the owners, and from stray cats at an animal shelter and stray dogs at an animal pound. Each pet owner was requested to complete a questionnaire. A total of 101 rectal and buccal swabs each were aseptically collected from pet (n = 40) and stray (n = 61) dogs. Similarly, 86 rectal and buccal cavity swabs each were collected from stray (n = 46) and pet (n = 40) cats. Each swab was placed in a universal bottle containing 0.9% NaCl and appropriately labeled. All the samples were kept in a cool box containing ice and transported to the laboratory for culturing within 2–4 h of collection.

2.2. Risk factors

The factors investigated to assess their association with occurrence of *Arcobacter* in dogs and cats included age, breed, sex, single or multipet household, recent treatment with antibiotics, housing of the dogs and cats sampled, source of drinking water, and place of residence of the owner. The results were considered statistically significant at $P \leq 0.05$ at a 95% confidence interval. The Pearson chi-square test and logistic regression statistics using SPSS 20.0 (IBM Corp, Armonk, NY, USA) were used to determine the association between risk factors and occurrence of *Arcobacter* based on the answers provided by the pet owners in the questionnaires.

2.3. Isolation and identification of *Arcobacter* species

Swab samples were vortexed and 1 mL was transferred into an *Arcobacter* broth (CM0965, Oxoid, Hampshire, UK) supplemented with cefoperazone, amphotericin, and teicoplanin (SR0174, Oxoid) and incubated under microaerobic conditions (BD Campy Pak, Becton, Dickinson & Company, Plymouth, UK) at 30 °C for 4 h. Plating of broth cultures was done according to the protocol described by Atabay and Corry (20) and Ridsdale et al. (23) with slight modification such that 5% defibrinated horse blood was used instead of 5% sheep blood. A cellulose acetate membrane filter with pore size of 0.65 µm and diameter of 47 mm was placed earlier on the surface of the blood agar plates (Blood Agar Base No. 2; CM0271, Oxoid), and 5–6 drops of each enriched culture were dispensed onto the membrane filter. Passive filtration was carried out by incubating the plates aerobically at 37 °C for 1 h and then each membrane filter was gently removed from the surface of the agar and the plate was incubated aerobically at 30 °C for 48 h. Initial identification of *Arcobacter* isolates was carried out based on colony morphology. Small, convex, smooth, white, whitish-gray, or transparent colonies were picked from each blood agar plate and examined for motility by hanging drop method for characteristic cork-screw motility and Gram staining to show gram-negative spiral-shaped organisms. Presumptive *Arcobacter* isolates were then subcultured on blood agar plates and incubated at 30 °C for 48 h. All presumptive *Arcobacter* isolates were further examined for species identification using biochemical tests, namely oxidase, catalase production, hippurate hydrolysis, and indoxyl acetate hydrolysis tests. Positive isolates were preserved in cryobeads at –20 °C.

2.4. Confirmation of isolates by multiplex polymerase chain reaction (mPCR) assay

Stock cultures of *Arcobacter* isolates were revived on blood agar. The extraction of DNA was conducted using a genomic DNA extraction kit (Promega, Madison, WI, USA). Amplification of 16S RNA species-specific genes for *A. butzleri* (CCUG 17812), *A. cryaerophilus* (CCUG 17801), and *A. skirrowii* (CCU 30483) was carried out using primers in appropriate cycling conditions as described by Houf et al. (24). The primers used are shown in Table 1. The reaction was performed in a reaction

Table 1. Primers used for the amplification of *Arcobacter* genes.

Arcobacter spp.	Target genes	Primer sequences (5' to 3')	Expected band length (bp)
A. butzleri	16S rRNA	BUTZ: CCTGGACTTGACATAGTAAGAATGA ARCO: CGTATTCACCGTAGCATAGC	401
A. skirrowii	16S rRNA	SKIR: GGCGATTTACTGGAACACA ARCO: CGTATTCACCGTAGCATAGC	641
A. cryaerophilus	23S rRNA	CRY 1A: TGCTGGAGCGGATAGAAGTA CRY 1B: AACAACCTACGTCCTTCGAC	257

Source: Houf et al. (24).

volume of 50 µL containing 25 µL of TopTaq multiplex master mix (QIAGEN, Hilden, Germany), 5 µL of primer mix at 0.2 mM final concentration (ARCO, BUTZ, SKIR, CRY1, and CRY2), 2 µL of DNA, and 18 µL of RNase-free water. The mPCR reaction was performed in a thermocycler (Eppendorf) with the following conditions: initial temperature 95 °C for 15 min followed by 32 cycles of denaturation at 94 °C for 45 s, annealing at 61 °C for 45 s, and extension at 72 °C for 30 s, with final extension at 72 °C for 10 min. Amplified products were electrophoresed on 1% agarose gel prepared in 1X Tris-borate-EDTA buffer (40 mM Tris-borate, 2 mM EDTA, pH 7.5) at 80 V for 60 min. The gels were stained with ethidium bromide and viewed under ultraviolet transilluminator light with a gel documentation system (Bio-Rad, Hercules, CA, USA).

3. Results

Morphologically, all *Arcobacter* colonies were small, white to whitish gray, convex, smooth, and translucent on blood agar. Presumptive *Arcobacter* isolates were gram-negative and showed a characteristic "S" shape. In addition, the isolates exhibited corkscrew motility when viewed under phase contrast microscopy by hanging drop method prepared from the fresh cultures. Furthermore, all suspected *Arcobacter* isolates were positive for catalase, oxidase, and indoxyl acetate, and negative for hippurate hydrolysis tests. The isolates that were subjected to biochemical tests were confirmed as *Arcobacter* using mPCR assay (Figure 1). All the isolates were identified as *A. butzleri*. Overall, *Arcobacter* was isolated in 54.4% (55/101) of dogs and 39.5% (34/86) of cats. In cats, 34.8% (16/46) of the stray cats and 45.0% (18/40) of pet cats were found positive for *Arcobacter*. In stray and pet dogs, *Arcobacter* was isolated at the rate of 50.8% (31/61) and 60.0% (24/40), respectively. These rates were not statistically significant (χ^2 = 0.8209, P = 0.364). The isolation of *Arcobacter* was 17.4% (8/46) each in the buccal cavity and rectum of stray cats, and in pet cats, it was isolated at 22.5% (9/40) from each. In stray dogs, *Arcobacter* was isolated from the rectum and buccal cavity at 22.9% (14/61) and 27.9% (17/61), respectively, while 25.0% (10/40) of *Arcobacter* in pet dogs was isolated from the rectum and 35.0% (14/40) from the buccal cavity (Figure 2). The differences in the rates of isolation of *Arcobacter* from the buccal cavity and the rectum were not statistically significant, although a higher carriage rate was observed in the buccal cavity than in the rectum. There was also no significant difference in the occurrence of *Arcobacter* in dogs among the various age groups, although the occurrence rate was higher in puppies (18.2%) than adults (16.7%) and (11.1%) juvenile dogs. Dogs that consumed raw meat and fish showed a significant difference (P = 0.053) in the occurrence of *Arcobacter*. The presence of other pets at home (P = 0.873) and predatory habits (P = 0.894) did not show significant difference. Antibiotic usage, sex, and housing of the pets also showed no significant difference in the occurrence of *Arcobacter* in dogs and cats; however, animals from town areas and those kept outdoors had higher occurrence rates. The type of household of the pets (P = 0.873) and water source (P = 0.873) also showed no significant difference in the occurrence of *Arcobacter* in dogs. As shown in Table 3, *Arcobacter* occurrence in cats showed no significant difference among the different age categories; however, it was higher in juveniles (52.2%), followed by kittens

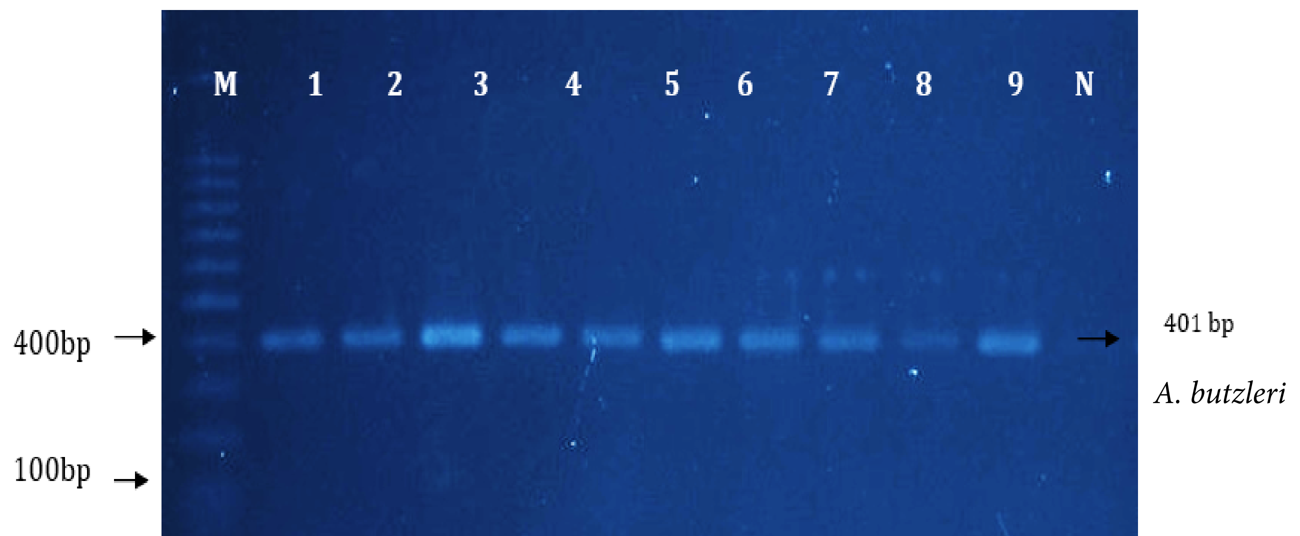

Figure 1. Confirmation of *Arcobacter* spp. using mPCR. Lane M: Ladder (100-bp DNA ladder), Lanes 1–9: *A. butzleri* isolates; Lane 10: positive control (CCUG 17812); Lane N: negative control.

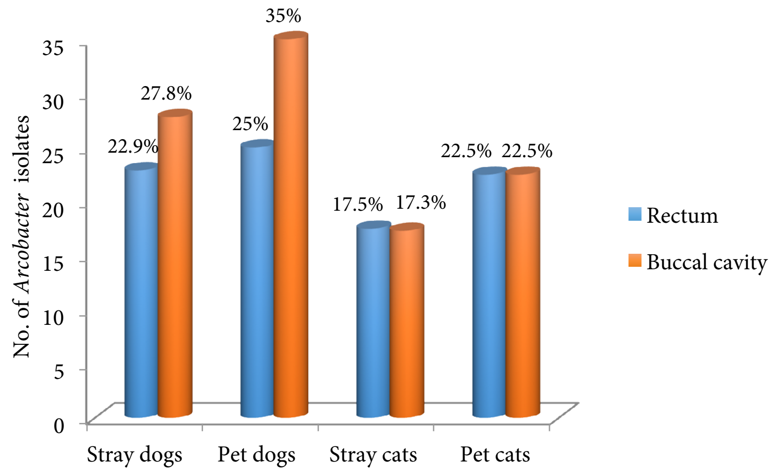

Figure 2. Occurrence of *Arcobacter butzleri* at two sampling sites in the animals.

Table 2. Univariate analysis of risk factors and occurrence of *Arcobacter* in dogs.

Variable	Category	Prevalence (%)	P-value	Odds ratio	95% confidence interval	
					Lower	Upper
Age	Puppy	63.6	0.728	0.764	0.167	3.487
	Juvenile	45.5	0.395	2.100	0.381	11.589
	Adult	38.9	NA	Ref	Ref	Ref
Sex	Female	47.6	0.987	0.990	0.286	3.430
	Male	47.4	NA	Ref	Ref	Ref
Breed category	Local	52.9	0.554	0.684	0.194	2.410
	Pedigree	43.5	NA	Ref	Ref	Ref
Owner's residence	Town	51.7	0.385	0.533	0.128	2.225
	Urban	36.4	NA	Ref	Ref	Ref
Housing	Outdoor	50	0.796	0.846	0.238	3.004
	Indoor	45.8	NA	Ref	Ref	Ref
Household type	Multipet	48.3	0.873	1.120	0.278	4.508
	Single	45.5	NA	Ref	Ref	Ref
Antibiotic history	No	57.1	0.199	2.286	0.641	8.149
	Yes	36.8	NA	Ref	Ref	Ref
Antibiotic duration	>1 month	57.1	0.199	2.286	0.641	8.149
	<1 month	36.8	NA	Ref	Ref	Ref
Predatory habits	Yes	50.0	0.894	1.125	0.198	6.385
	No	47.1	NA	Ref	Ref	Ref
Water source	Unfiltered	48.3	0.873	1.120	0.278	4.508
	Filtered	45.5	NA	Ref	Ref	Ref
Raw meat or fish consumption	Yes	54.5	0.053*	0.139	0.015	1.285
	No	14.3	NA	Ref	Ref	Ref
Contact with other animals	Yes	100	0.287	1.056	0.949	1.174
	No	46.2	NA	Ref	Ref	Ref

*: Statistically significant.

Table 3. Univariate analysis of risk factors and occurrence of *Arcobacter* in cats.

Variable	Category	Prevalence	P-value	Odds ratio	95% confidence interval	
					Lower	Upper
Age	Juvenile	52.2	0.762	1.500	0.109	20.675
	Kittens	42.9	0.547	2.182	0.173	27.556
	Adult	33.3	NA	Ref	Ref	Ref
Sex	Female	50	0.752	0.818	0.236	2.835
	Male	45	NA	Ref	Ref	Ref
Breed category	Local	50	0.364	3.000	0.279	32.209
	Mixed	50	0.437	3.000	0.188	47.963
	Pedigree	25	NA	Ref	Ref	Ref
Owner's residence	Town	55.5	0.141	0.356	0.088	1.444
	Urban	30.8	NA	Ref	Ref	Ref
Housing	Outdoor	45.5	0.775	1.200	0.344	4.181
	Indoor	50	NA	Ref	Ref	Ref
Household type	Multipet	60	0.006*	13.500	1.509	120.783
	Single	10	NA	Ref	Ref	Ref
Antibiotic history	No	55	0.342	0.545	0.155	1.914
	Yes	40	NA	Ref	Ref	Ref
Antibiotic duration	>1 month	52.4	0.516	0.661	0.189	
	<1 month	42.1	NA	Ref	Ref	Ref
Predatory habits	Yes	50	0.583	0.667	0.156	2.852
	No	40	NA	Ref	Ref	Ref
Water source	Unfiltered	65	0.027*	0.231	0.061	0.869
	Filtered	30	NA	Ref	Ref	Ref
Raw meat or fish consumption	Yes	50	0.629	0.714	0.182	2.800
	No	41.70	NA	Ref	Ref	Ref
Contact with other animals	Yes	48.6	0.720	0.706	0.105	4.758
	No	40	NA	Ref	Ref	Ref

*: Statistically significant.

(42.9%) and then adults (33.3%). Similarly, the presence of other pets at home, antibiotic usage, sex, housing, and contact with other animals showed no significant difference in occurrence of *Arcobacter* in cats, although cats kept outdoors and those in urban areas had higher occurrence rates. On the other hand, household type (P = 0.006) and water source (P = 0.027) showed significant difference in the occurrence of *Arcobacter* in cats (Table 3).

4. Discussion

Several studies have been conducted to determine the presence of *Arcobacter* in dogs, cats, and food animals globally. This study is the first to be conducted in Malaysia with regards to dogs and cats. The overall carriage of *Arcobacter butzleri* in dogs and cats was 54.4% and 39.5%, respectively. The only species identified in the study was *A. butzleri*. These findings are similar to the results of studies conducted worldwide. In southern Italy, it was reported that the prevalence of *Arcobacter* in cats was 78.8% (67/85); among the *Arcobacter* specimens isolated, 66 (77.6%) and 29 (34.1%) were *A. butzleri* and *A. cryaerophilus*, respectively, and of the 29 samples positive for *A. cryaerophilus*, 28 were also found to be positive for *A. butzleri* (9). In Chile, the prevalence of *Arcobacter* species in the feces of dogs reported by Fernandez et al. (17) was 3.3%. However, Aydin et al. (18) did not find *Arcobacter* species in the feces of dogs in Turkey. In Belgium, Houf et al. (19) isolated *Arcobacter* from dogs at 2.6% but not from the oral cavity or feces of cats. In a study by Petersen et al. (20), they found *A. cryaerophilus* in feces (1.5%) and

the oral cavity (0.7%) of dogs; however, *A. butzleri* was recovered only from feces (0.75%). *Arcobacter butzleri* was described as a novel isolate from the oral cavity of dogs and cats and reported to be associated with enteric pathogens in causing diarrhea in humans and animals (25). In this study, it was observed that *Arcobacter* species can be isolated from the oral or buccal cavities of pet and stray dogs and cats. Fera et al. (10) isolated A. *butzleri* and *A. cryaerophilus* from dogs and cats. These animals may play a role in the dissemination of *Arcobacter* in domestic animals' habitat. They can also be isolated from clinically healthy people and other animals, regardless of age and system of management of the animals (3). According to Houf et al. (19), *A. skirrowii* and *A. cryaerophilus* were seldom detected in both dogs and cats, probably due to their slow-growing nature and the likelihood of being susceptible to the antibiotic supplements used in isolation media. The absence of a standard protocol for the isolation made it difficult to compare the results of the isolation rates from other studies (18). Moreover, *Arcobacter* can progress to a viable but nonculturable (VBNC) state due to environmental stresses and thrive for an extended period within that environment (9). The growth and propagation of *Arcobacter* species may be hindered due to starvation and physical stress. However, VBNC cells are significant in public health because the cells could remain in virulent form although the initiation of infection in human is not yet understood. Prior to this study, the presence of *Arcobacter* in Malaysia was examined by Amare et al. (25), who reported the occurrence of *Arcobacter* in chicken meat in markets in parts of Selangor at 39% with *A. butzleri* being the most common species. The study also showed that *Arcobacter* was not isolated from broiler chickens on farms. Shah et al. (2) reported the prevalence of *Arcobacter* in adult and young cattle at 7.27% and 4.81%, respectively, and none in goats; the study also detected *Arcobacter* spp. from various sources, which included 26.7% on cattle house floor, 26.3% in beef, 11.1% in water, and 7.6% in milk.

Molecular techniques for the confirmation of *Arcobacter* species are imperative due to differences in the isolation techniques that can affect the isolation of *Arcobacter* species. The simultaneous detection of different species may be suggestive of different sources of infection. The colonization of the oral cavity of dogs and cats by *Arcobacter* is of concern although its role in causing infection is not well recognized. *Arcobacter* is not routinely tested for in clinical samples compared to *Salmonella* and *Campylobacter*; hence, it may be underreported, particularly in foodborne disease outbreaks. The occurrence of *Arcobacter* in pets has potential public health implications and pets may transmit the organisms to humans through biting and licking and in the dissemination of the organisms in the environment.

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