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Prevalence, diversity and public health implications of *Helicobacter* species in pet and stray dogs

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ABSTRACT

Background: It is probable that humans can acquire *H. pylori* and non—*H. pylori Helicobacter* infections via domestic animals. The prevalence and risk factors of infections of *Helicobacter* species in canines of Taipei city were therefore analysed in this study.
Materials and methods: A total of 95 canine faecal samples were collected from different animal shelters and hospitals in Taipei city. Total DNA was extracted for semi-nested PCR detection of *Helicobacter* species. The PCR products were sequenced for further comparative database and phylogenetic analyses.
Results: The overall prevalence of *Helicobacter* species in canines of Taipei city was 75.79% (72/95). Two gastric, seven enterohepatic and two unclassified *Helicobacter* species were identified, all of which have been implicated in the aetiology of human diseases. The predominant species detected included *H. canis* (27.78%), *H. pylori* (26.39%), *H. canicola* (18.06%), and *H. bilis* (13.89%) in decreasing order, while *H. canadensis* and *H. typhlonius* were identified for the first time in canines. The genotypes in *H. pylori* and *H. canicola* clusters grouped together, with their respective reference strains, showed a close evolutionary distance in the phylogenetic tree, indicating a common ancestry may have existed in these clusters respectively. The residential region of canines, dog living status (pet or stray) and breed (purebred or mixed-breed) are the risk factors associated with *Helicobacter* infections in the canines examined.
Conclusion: The high prevalence of *Helicobacter* infections in canines highlights a potential public health risk of zoonotic transmission among dogs, humans and other animals, and therefore, the need for proper methods in controlling the transmission routes. In addition, the 16S rRNA gene amplification method was found to be useful for bacterial identification and phylogenetic analysis.

1. Introduction

Helicobacter species are spiral, microaerophilic, gram-negative bacteria colonizing the biliary tract and gut of various animals, causing several gastrointestinal disorders such as peptic ulcer, chronic gastritis, gastric adenocarcinoma, and lymphoid tissue lymphoma [1,2]. To date, the genus *Helicobacter* contains about 47 species, with each of the species having a different preference for colonizing different anatomical regions of the host where they incite their pathogenicity [3]. Although *H. pylori* has been indicated as the most important species in the genus, infecting an estimated 50% of the global human population, recently, about 23 other species are also reported to be significantly associated with human infections [4,5].

Helicobacter spp. have been categorized into two groups depending on their preferred place of colonization on the gastrointestinal tract. The first group is the gastric *Helicobacter* spp. (GH), which colonizes the upper gastrointestinal region (stomach and duodenum), while the second group, the enterohepatic *Helicobacter* spp. (EHH), occupies the lower gastrointestinal region (ileum, colon, liver, and rectum) [6]. Examples of GH include *H. pylori, H. felis,* and *H. heilmannii* sensu stricto (s. s.), while EHH include *H. canicola, H. canis,* and *H. bilis* [7,8] respectively.

The route of transmission of these *Helicobacter* spp. has not been clearly proven, although several theories are postulated. For instance,

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individual-to-individual transmission of the bacteria including gastro-oral and faecal-oral, are the major routes that have been suggested, yet, there is no conclusive data supporting the predominance of any of these routes [9,10]. Subsequently, the vehicles for transmission within individual-to-individual have been highlighted to include vomitus, saliva, gastric juice, contaminated food (e.g. milk, meat, vegetables, etc.), and water [11].

Indirect transmission routes of *Helicobacter* spp. through animals such as dogs, cats, birds, monkeys, etc. have been suggested, because of the high incidence of *Helicobacter* spp. in the gut of these animals. Domesticated animals, especially pets, are considered a potential risk factor for the transmission of infection to human hosts as a result of their close interactions [9,12]. For example, studies in Iran, Poland, and Brazil showed that 87.5%, 93.9%, and 94.7% of dogs were infected with *Helicobacter* spp., respectively [6,13]. In Taiwan however, data obtained from veterinary clinics and animal quarantine centres disclosed the prevalence of *Helicobacter* infections in pet dogs and cats as 60% and 64% respectively [14]. Moreover, a direct correlation of transmission between human host and pet has been recently reported [15], due to the similarity in the sequences of *ure*AB gene of *H. pylori* from both humans and dogs.

In a systematic review with meta-analysis, the prevalence of *H. pylori* infection in the Taiwanese population using urea breath test was 53.9% [16]. This data covers the entire Taiwanese population strata. For instance, *H. pylori* virulence factors induced mixed infections such as chronic gastritis, gastric ulcer, duodenal ulcer, and gastric carcinoma in patients with gastrointestinal disease [17]. Moreover, the clinical manifestations of *Helicobacter* infections in children [18] and the high rate of standard first-line antibiotic resistance in patients [19] had been reported. Therefore, the quest for further understanding of the infection and transmission routes of *Helicobacter* bacteria is critical for public health awareness.

Understanding the prevalence of *Helicobacter* infections in animals is important as these may underlie *Helicobacter* infections in humans as well, and therefore contributes to the identification of appropriate public health mitigation strategies, especially with regard to pet owners [20]. Breaking this highly suspected transmission route of infection may lead to the reduction of infection rate in the Taiwanese and animal population, hence achieving the One Health goal. The aim of this study therefore, was to determine the prevalence, distribution, and risk factors for *Helicobacter* spp. infections in canines of Taipei city, and to establish the phylogenetic relationship between the *Helicobacter* spp. detected.

2. Materials and methods

2.1. Animal data collection and animal samples

This study was carried out on stool samples of 95 dogs conveniently collected from 6 animal hospitals and an animal shelter in various districts of Taipei city, Taiwan (Fig. 1). Data on the subject animals were collected by the development of a sample questionnaire (Supplement 1). Information such as age, sex, breed type, living status, region of residence and symptoms of each animal was collected using the questionnaires filled in by the veterinary doctors and health workers from the respective veterinary hospitals and animal shelter. Demographic characteristics were summarized in Table 1. Taipei city was divided into 4 regions: Northern region consisting of two districts (Districts A and B); Eastern region consisting of five districts (Districts C, D, E, F, and G); Western region consisting of four districts (Districts H, I, J and K); and Southern region of only one district (District L), for further residential analysis of the canines (Supplementary figure). Bacterial DNA was extracted from 220 mg each of stool sample according to the QIAamp DNA Stool Mini Kit Manufacturer's instructions.

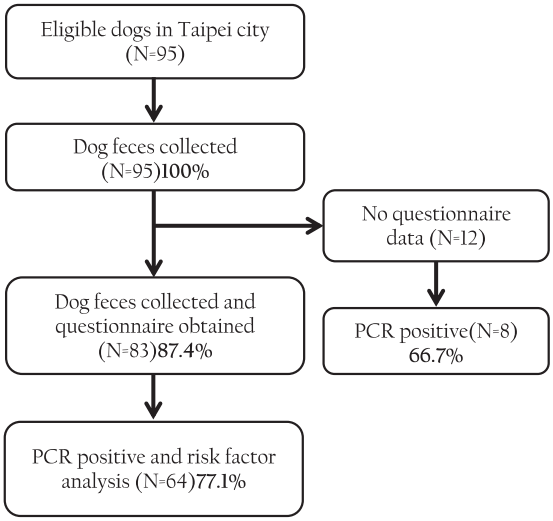

Fig. 1. Flow diagram of recruitment of dogs in Taipei city.

Table 1
Demographic characteristics of Taipei city canines sampled for this study.

Canines characteristics collected (n = 95) (%)	Categories	Total samples	Percentage
Sex	Male	41	43.16
	Female	42	44.21
	Missing	**12**	**12.63**
Age(Months)	Puppies	38	40.00
	Young	30	31.58
	Adults	15	15.79
	Missing	**12**	**12.63**
Living Status	Pet	61	64.21
	Stray	22	23.16
	Missing	**12**	**12.63**
Breed	Mixed breed	41	43.16
	Single breed	42	44.21
	Missing	**12**	**12.63**
Region	North	22	23.16
	South	12	12.63
	East	17	17.90
	West	32	33.68
	Missing	**12**	**12.63**
Symptomatic	**Yes**	**36**	**37.90**
	No	**47**	**49.47**
	Missing	**12**	**12.63**

2.2. Semi-nested-PCR

Primer sets designed by Fox et al., 1997 [21] were modified in order to detect the 16S rRNA gene of *Helicobacter* spp. in the stool samples used for the PCR screening. The forward primer C97b: GCTATGACGGGTATCCGGC and reverse primer, C05: ACTTCACCCCAGTCGCTG sets were modified in order to increase the melting temperature of the first PCR step. The amplified first PCR products were used for the second step of the semi-nested PCR. PCR was carried out using the modified forward primer C97b: GCTATGACGGGTATCCGGC and the existing reverse primer C98: GATTTTACCCCTACACCA. The amplification condition included denaturation at 94 °C for 3 min, followed by 35 cycles at 94 °C for 30s denaturation, 58 °C for 30s annealing, and 72 °C for 90s extension, and a final extension at 72 °C for 10 min. This gives a PCR amplicon product of 1200 bp. The nested PCR condition was similar to the first step except that the annealing temperature was changed from 58 °C to 55 °C and the extension time to 30s with expected amplicon product of size ≤ 398 bp).

2.3. Sequencing and phylogenetic analysis

The semi-nested PCR products of the 16S rRNA gene were sequenced at the National Yang Ming Chiao Tung University genomic centre. Sequences obtained were submitted to NCBI GenBank (Accession nos. ON000364 – ON000391) and used for BLAST identification of *Helicobacter* species and subsequent phylogenetic analysis using MEGA7 software [22]. Specific related sequences were retrieved from the GenBank and aligned to our sequences using the Muscle Alignment method [23]. The cut-off point used for percentage identity with respect to determining similarity to the group reference strain was >95%. A phylogenetic tree was reconstructed using the Neighbour-Joining algorithm with 1000 bootstrap replicates [24].

2.4. Data statistical analysis

Descriptive statistics and chi-square assessment of the possible risk factors for canine infection were carried out using SPSS at a 95% confident interval to determine possible significant differences. The statistical significance level was determined at $P < 0.05$. Subsequently, binomial logistic regression was used to determine the relationship between the suspected risk factors and the PCR test outcome.

3. Results

3.1. Prevalence and species of Helicobacter

A total of 95 canine faecal samples were collected and subjected to nested PCR based on the 16S rRNA gene. Of these 95, 72 canine samples were positive and subsequently sequenced, giving a prevalence of 75.79% *Helicobacter* infections (Fig. 2). After blasting against the NCBI database, these 72 PCR sequences were designated into 11 *Helicobacter* spp. as shown in Table 2. The 11 *Helicobacter* spp. were categorized into the gastric group (20/72, 27.78%) which included *H. pylori and H. heilmannii* s.s., and the enterohepatic group (50/72, 69.44%) which included *H. bilis, H. canis, H. cinaedi, H. canadensis, H. canicola, H winghamensis,* and *H. typhlonius*, and 2 unclassified species. Incidentally, *H. pylori* infections occurred in 26.39% (19/72) of the cases and non—*H. pylori Helicobacter* spp. (NHPH) in 73.61% (53/72). *H. canis* is the most prevalent species detected in 20 dogs (27.78%), followed by *H. pylori* in 19 dogs (26.39%), *H. canicola* in 13 dogs (18.06%), and *H. bilis* in 10 dogs (13.89%). Except for the 2 unclassified species, all the species identified in this study have been documented as aetiological agents in humans. Another gastric *Helicobacter* spp. detected in this study was *H. heilmannii* s.s., which was detected in one dog. Moreover, *H. canadensis* and *H. typhlonius* were detected for the first time in dogs of Taiwan.

3.2. Phylogenetic analysis using 16S rRNA sequence

The 72 partial sequences of the 16S rRNA gene were used to reconstruct a phylogenetic tree in order to determine the molecular and evolutionary relationship between the *Helicobacter* spp. identified. A total of 28 genotypes were identified and classified into 9 *Helicobacter* spp. clusters designated 1 to 9, and two unclassified genotypes as shown on the phylogenetic tree (Fig. 3). The 9 *Helicobacter* clusters were categorized into two major clades, gastric and enterohepatic *Helicobacter* groups. Clusters 1 and 2 had an average of 99.1% similarities to their reference strains and they belonged to the GH group, while Clusters 3, 4, 5, 6, 7, 8, and 9 with an average of 98.5% similarities to their reference strains belonged to the EHH group. *H. pylori, H. canicola, H. typhlonius, H. canis,* and *H. bilis* contain 6, 4, 3, 5, and 3 genotypes respectively, while others contain one in each of their clusters. The most similar NCBI genotypes to the detected genotypes identified in this study are shown in Table 3. Each of these genotypes within their respective clusters have an average of 98.7% similarity to the reference strain in that cluster. Moreover, whereas the evolutionary distances between genotypes of *H. pylori* and *H. canicola* were closer within their clusters, these evolutionary distances were longer in genotypes of *H. bilis* and *H. canis* clusters respectively.

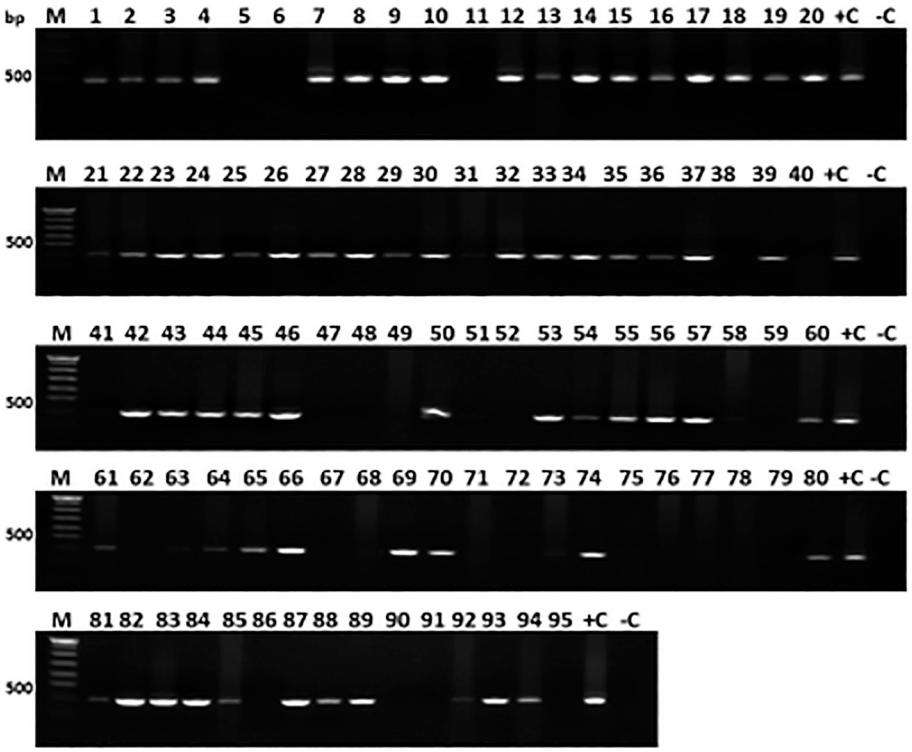

Fig. 2. Gel electrophoresis of the nested 16S rRNA PCR products of canine stool samples using modified Fox et al. primers. M: 100 bp DNA ladder, 1-95: stool samples, +C: positive control, -C: negative control. Positive band is~ 398 bp.

Table 2
List of most closely related *Helicobacter* species detected in this study and their reported human pathogenicity.

Species	Number (n)	Percentage (%)	Natural hosts	Human diseases	References***
Gastric					
H. pylori	19	26.39	Human	Gastritis, PU, GAC, and MALToma	Marshall et al. 1984
H. heilmannii s.s.	1	1.39	Dogs, cats	Gastritis, PU, GC and MALToma	Smet et al. 2013 Kubota-Aizawa et al. 2017
Enterohepatic					
H. canis	20	27.78	Dogs, cats	CD, Bacteraemia	Kaakoush et al. 2010 Gerrard et al. 2001
H. canicola	13	18.06	Dogs	GE	Fresia et al. 2017
H. bilis	10	13.89	Mice, rats, gerbils, dogs, cats, sheep	CC AIH	Fox et al. 1998 Casswall et al. 2010
H. typhlonius	3	4.17	Rats	IBD, IBS	Chichlowski et al. 2008
H. winghamensis	2	2.77	Human, Wild, rodents	GE, AA	Melito et al. 2001
H. canadensis	1	1.39	Birds, Pigs	CD, UC	Laharie et al. 2009 Thomson et al. 2011
H. cinaedi	1	1.39	Hamsters, rats, cats, rhesus monkeys, dogs, baboons	AAAI, Arthritis	Kakuta et al. 2014
Unclassified *Helicobacter* spp.*	2	2.77	**	**	**
Total	72	100.00			

AA: Abdominal abscess; AAAI: Abdominal Aortic Aneurysm infection; AIH: Autoimmune hepatitis; CC: Chronic cholecystitis; CD: Crohn's disease; GAC: gastric adenocarcinoma; GC: gastric carcinoma; GE: Gastroenteritis; IBD: Inflammatory Bowel Disease; IBS: Irritable bowel syndrome; MALToma: MALT lymphoma; PU: peptic ulcer; UC: Ulcerative colitis; *: The species belongs to a category of *Helicobacter* yet to be classified. **: Not available. ***: References listed in the Supplement 2.

3.3. Risk factors of Helicobacter infection

Data from 83 completed questionnaires were used to analyse the risk factors using a binomial logistic regression model. The prevalence of *Helicobacter* infections in dogs was 95.45% (21/22), 82.35% (14/17), 74.19% (23/31), and 46.15% (6/13) in the Northern, Eastern, Western and Southern regions of Taipei city, respectively (Table 4). The prevalence in the Northern region was significantly higher than in the Eastern, Western, and Southern ($P = 0.020$, $P = 0.006$, and $P = 0.080$) regions respectively. Moreover, stray dogs showed a higher risk of *Helicobacter* infection than pet dogs ($P = 0.050$), whereas infections in mixed breed dogs were more frequent than in purebred ($P = 0.040$). Other variables such as age, sex, and symptomatic status of the canines were not significantly associated with being infected. Interestingly, among the 16 samples in the *H. pylori* cluster, 11 (68.75%) were from Districts B and I respectively. Only 15/48 (31.25%) of enterohepatic *Helicobacter* spp. were identified from Districts B and I as shown in Table 3.

4. Discussion

The zoonotic potential of *Helicobacter* spp. and its possible interference with achieving the One Health goal at the human-animal-ecosystem interface have been documented [25]. Domesticated animals such as dogs and cats have been considered to be important sources of *Helicobacter* spp. transmission because of their intimate interaction with humans, although other mammals such as monkeys, sheep, rats, etc. were also implicated as natural hosts of this genus. In this study, the prevalence of *Helicobacter* infections in dogs is 75.79%, which is relatively similar to studies from Korea (76%), Italy (85.0%), Brazil (94.7%), and Sweden (66-100%) [13,26,27]. Considerably, these data are higher than a previous study (60.0%) detected by duplex PCR based on *Helicobacter* 16S rRNA and dog/cat β-actin genes in pet dogs of Taipei [14]. The difference in prevalence rates in these two studies might be due to the years of surveillance and/or semi-nested PCR used, having a higher sensitivity and accuracy than the traditional PCR [6].

The GH detected in this study were *H. pylori* and *H. heilmannii* s.s. Incidentally, *H. pylori* was the second most prevalent (26.39%) species detected, unlike *H. felis, H. bizzozeronii,* and *H. heilmannii* s.s. often highlighted as the predominant GH in canines from some other studies [28]. In contrast, however, a high prevalence of *H. pylori* infection (62.5% and 41.43%) in canine stool samples from Egypt has been reported [29,30]. Interestingly, *H. pylori* genotypes identified in this study were most similar to *H. pylori* LVRN-53 (MT477178) from pregnant Chilean women, LPB13-03 (EU020083), LPB-28 V (AY304557), and LPB151-02 (EU033946) from hepatic disease patients in Brazil, SR2-GB (HM596601) from gallbladder disease patients in Pakistan and Hpfe0001 (CP094173) from human gastric biopsies in China (Table 3). Similarly, in Japan, two dogs and their owner were reported to be infected with an identical *H. pylori* strain [31], and in Taiwan, mixed infections of *H. pylori* in patients with gastrointestinal diseases had been reported [17]. In addition, only one *H. heilmannii* s.s. strain, another gastric *Helicobacter*, was detected in this study. However, since gastric NHPH are rarely detected in stool samples, the detection of *H. heilmannii* s.s. strain in dog faeces will subsequently be evaluated by performing DNA PCR on samples from dog gastric biopsies. Notably, a case of *H. heilmannii* s.s. infection in Taiwan had been reported from a patient who complained of epigastralgia and heartburn sensation and has had a long history of domestic dog contact [32]. Therefore, the high prevalence of *H. pylori* infection detected in this study with their close similarity to genotypes found in humans highlight the potential zoonotic transmission of *H. pylori* and gastric NHPH, raising the One Health issue between canines and humans.

EHH have increasingly gained attention, not only because they are associated with human enterohepatic diseases but also because of their potential zoonosis in humans, canines, and other animals [33]. *H. canis* was detected as the most prevalent species (27.78%) in the EHH group, and this high prevalence has been substantiated previously [8,33]. *H. canis* has been demonstrated to be associated with bacteraemia, Crohn's disease, and gastroenteritis in humans [34–36]. In fact, Sabry et al. 2016, [37] isolated identical *H. canis* strains from sheep and their animal caretakers, and several immunocompromised patients with *H. canis* bacteraemia have reported close contact with canines [35]. However, how *H. canis* uses canines and farm animals as reservoirs and then finds its way to colonizing the human enterohepatic tract requires further studies. Furthermore, *H. canicola* (18.06%) and *H. bilis* (13.89%) are two other dominant EHH detected in our study. *H. bilis* has been proven to colonize the large intestine of dogs and is commonly detected in canine faeces [2,33]. It can also cause a wide range of diseases in humans such as gastroenteritis, inflammatory bowel disease, bacteraemia, hepatobiliary disease, and even several cancers [33,38]. *H. canicola,* spectacularly, has been re-classified into the *H. cinaedi/ canicola /'magdeburgenesis'* complex, as a new zoophilic species especially in canines, based on phenotypic and genotypic analyses, and genomic comparison [39,40]. *H. cinaedi*, a human-adapted species close to *H. canicola* and associated with gastroenteritis, proctitis, bacteremia, cellutitis, and neonatal meningitis in humans, was also detected in a

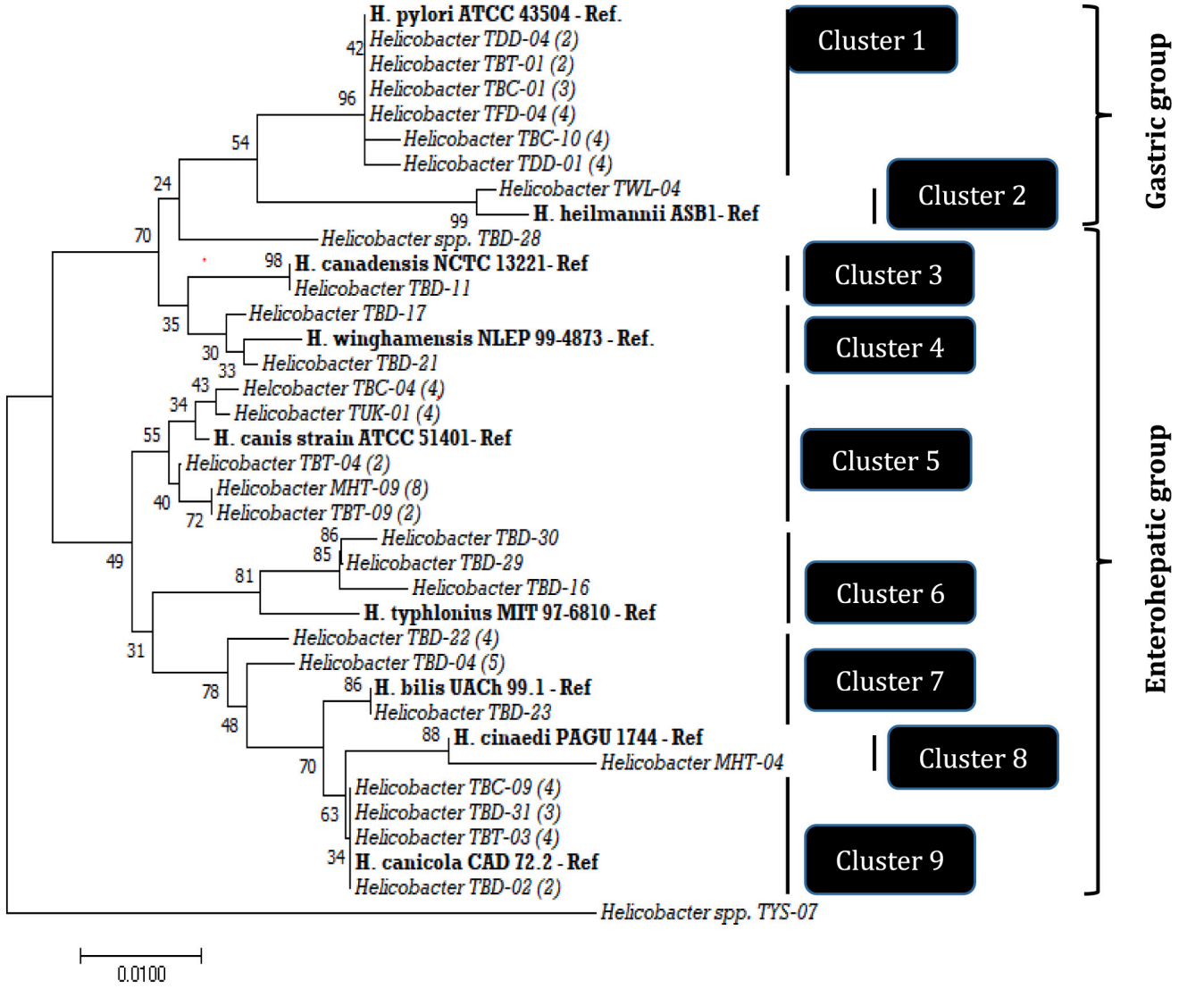

Fig. 3. Distribution and evolutionary relationships of *Helicobacter* strains in Taipei city using Neighbour-Joining Phylogenetic tree. The percentage of replicate trees (1000 replicates) is shown next to the branches. The evolutionary distance was computed using the JC method and is in the units of the number of base substitution per site. Evolutionary analyses were conducted using MEGA7.

canine faecal sample in this study. Thus, the public health impact and the One Health concept of *H. bilis/H. canicola/H. cinaedi* can be determined through diagnosing patients with any of these infections and further evaluating them for their animal contact history, and vice versa.

Newly identified *H. canicola* genotypes are phylogenetically identical to *H. canicola* CAD 72.2 (MK279513) and PAGU 1410 (NR_146694) with >98% identity (Table 3, Fig. 3). One possible explanation is that the 4 genotypes might have come from a common ancestor, and then very slowly evolved and migrated to different regions of the city. A similar observation was seen in the *H. pylori* cluster where six new genotypes were phylogenetically identical but were divided into two lineages, implying that the 6*H. pylori* genotypes might have also come from the same ancestor. However, a better BLAST match in the NCBI is obtained for *H. pylori* because there are more *H. pylori* 16S rRNA sequences in the NCBI database compared to *H. canicola*. *H. bilis* genotypes nevertheless, are more diverse as shown in the phylogenetic tree (Fig. 3), which may indicate that different genotypes came from different ancestors.

Three variables, which include region of dog residence, dog living status, and dog breed, have been identified as significant risk factors associated with *Helicobacter* infections. Overall, the infection rate of canines in the northern region (95.45%) was significantly higher ($p <$ 0.05) than in the eastern (82.35%), western (74.19%), and southern (46.15%) regions of the city. Previous studies also supported our observations. For instance, Dore et al. [41] reported that the seroprevalence of *H. pylori* infection was significantly higher among children in rural areas (37%) than in urban areas (13%) in Italy, while Jankowski et al. [6] submitted that the prevalence of *H. heilmannii* s.s., *H. felis, H. salomonis, H. bizzozeronii and H. pylori* vary with geographical location. Surprisingly, *H. pylori* (12/16) was predominantly found in Districts I and B of Taipei city, although there is no significant statistical association probably because of the small number of cases. There is an indication, however, of a unique focal distribution of *H. pylori* in Taipei city. The possible reasons may be that District I is one of the lower socioeconomic areas in Taipei with a higher aging index, lower education population, and more underprivileged groups, whereas District B located on the margin of northern Taipei features the mountains, platforms, and plain topographical areas as a ruralized area of Taipei with lowest population density [42]. Therefore, the geographic distribution of *Helicobacter* spp. in canines associated with human infections needs to be further investigated, especially in District B, which does not only have a higher prevalence of *H. pylori,* but also other EHH infections.

The higher prevalence of certain infections in stray dogs compared to

Table 3
Helicobacter species detected in this study with their location and distribution compared to the standard strains.

Cluster	Genotype/ Sample No.	Most similar genotype in NCBI	District
Cluster 1	*Helicobacter*	*H. pylori* LVRN-53	District I
H. pylori	TDD-01	(MT477178)	
	Helicobacter		District
	TBT-01		B
	Helicobacter		District
	TBT-02		B
	Helicobacter		District
	TBT-10		B
	Helicobacter	*H. pylori* LPB13-03	District I
	TBC-10	(EU020083)	
	Helicobacter		District
	TBC-03		A
	Helicobacter		NA
	TDD-05		
	Helicobacter		District
	TBD-27		F
	Helicobacter	*H. pylori* LPB-28 V	NA
	TFD-04	(AY304557)	
	Helicobacter		District I
	TDD-01		
	Helicobacter		District I
	TDD-04		
	Helicobacter		NA
	TBT-08		
	Helicobacter	*H. pylori* LPB151-02	District I
	TBC-01	(EU033946)	
	Helicobacter		District
	TWL-03		L
	Helicobacter		District
	TBD-06		B
	Helicobacter	*H. pylori* SR2-GB	District
	TBT-01	(HM596601)	B
	Helicobacter		District
	MHT-03		E
	Helicobacter	Or Hpfe0001 (CP094173)	District I
	TDD-04		
	Helicobacter		District
	TBC-02		L
Cluster 2	*Helicobacter*	*H. heilmannii* ASB1	District
H. heilmannii	TWL-04	(HE984298)	L
Cluster 3	*Helicobacter*	*H. canadensis* NCTC	District I
H. canadensis	TBD-11	13221 (KJ534296)	
Cluster 4	*Helicobacter*	*H. winghamensis* NLEP 98-	NA
H. winghamensis	TBD-17	0305 (AF363063)	
	Helicobacter	*H. winghamensis* NLEP 98-	District
	TBD-21	0305 (AF363063)	F
Cluster 5	*Helicobacter*	*H. canis* MIT 12-7728	District
H. canis	TBT-09	(KC878294)	B
	Helicobacter		District
	TBT-08		B
	Helicobacter	*H. canis* MIT 12-7728	District
	MHT-09	(KC878294)	E
	Helicobacter		NA
	TFD-05		
	Helicobacter		NA
	TBT-06		
	Helicobacter		District
	TWL-10		L
	Helicobacter		District
	MHT-03		E
	Helicobacter		District
	TYS-04		J
	Helicobacter		District
	TYS-08		J
	Helicobacter		District
	TYS-09		J
	Helicobacter	*H. canis* ClinIsoA01	District
	TBT-04	(KC293823)	B
	Helicobacter		District I
	TBC-07		
	Helicobacter	*H. canis* ARUP1	NA
	TUK-01	(MF542253)	
	Helicobacter		NA
	TFD-06		
	Helicobacter		District
	TBT-05		B
	Helicobacter		District
	TBD-06		B
	Helicobacter	*H. canis* ARUP1	District I
	TBC-04		
	Helicobacter	(MF542253)	District
	TWL-10		L
	Helicobacter		District
	TBD-10		B
	Helicobacter		District
	TYS-03		C
Cluster 6	*Helicobacter*	*H. typhlonius* MIT 97-	District
H. typhlonius	TBD-16	6810 (NG_042883)	F
	Helicobacter		District
	TBD-29		D
	Helicobacter		District
	TBD-30		H
Cluster 7	*Helicobacter*	*H. bilis* Hb1 (NG_041960)	District
H. bilis	TBD-23		B
	Helicobacter	*H. bilis* KO220	District
	TBD-04	(AY578100)	H
	Helicobacter		District
	TBD-09		H
	Helicobacter		District
	TBD-10		B
	Helicobacter		District I
	TDD-03		
	Helicobacter		District
	MHT-06		E
	Helicobacter	*H. bilis* UACh 99.1	NA
	TBD-22	(MK849616)	
	Helicobacter		NA
	TBD-08		
	Helicobacter		District
	MHT-10		E
	Helicobacter		District
	MHT-05		E
Cluster 8	*Helicobacter*	*H. cinaedi* PAGU 1744	District
H. cinaedi	MHT-04	(LC102852)	E
Cluster 9	*Helicobacter*	*H. canicola* PAGU 1410	NA
H. canicola	TBD-02	(NR_146694)	
	Helicobacter		District
	MHT-01		E
	Helicobacter	*H. canicola* CAD 72.2	District
	TBT-03	(MK279513)	B
	Helicobacter		NA
	TBD-25		
	Helicobacter		District I
	TDD-06		
	Helicobacter		District
	TBD-05		L
	Helicobacter	*H. canicola* CAD 72.2	District
	TBD-31	(MK279513)	F
	Helicobacter		District
	TBD-03		A
	Helicobacter		NA
	TDD-02		
	Helicobacter	*H. canicola* CAD 72.2	District I
	TBC-09	(MK279513)	
	Helicobacter		District
	TBD-13		H
	Helicobacter		District
	TBD-18		A
	Helicobacter		District
	TYS-02		J
Unclassified	*Helicobacter*	*Helicobacter* sp. strain 91-	District
Helicobacter spp. **	TBD-28	266-11	B
		(M88152)	
Unclassified	*Helicobacter*	*Helicobacter* sp. in dog	District
Helicobacter spp. **	TYS-07		J

* Highlighted strains are group representative used for building phylogenetic tree. **: The species belongs to a category of *Helicobacter* yet to be classified. NA: Not available.

Table 4
Overall prevalence of *Helicobacter* spp. in Taipei city dogs based on risk factors.

Variables	Dogs (n)	Infected dogs (n%)	95% CI	OR	*P*-value
Age (ref = puppies)	28	22 (78.57)			
Young	28	19 (67.85)	(0.39–6.32)	1.57	0.527
Adult	27	23 (85.19)	(0.60–8.80)	2.30	0.224
Sex (ref = male)	42	34 (80.95)			
Female	41	30 (73.17)	(0.19–1.58)	0.55	0.265
Region (ref = North)	22	21 (95.45)			
South	13	6 (46.15)	(0. 08–1.16)	0.30	0.080
East	17	14 (82.35)	(0.02–0.72)	0.14	*0.020
West	31	23 (74.19)	(0.00–0.40)	0.04	*0.006
Living Status (ref = pet)	57	40 (70.17)			
Stray	26	24 (92.13)	(0.99–22.15)	4.63	*0.050
Dog breed (ref = Mixed)	41	35 (85.37)			
Pure	42	29 (60.05)	(0.10–0.97)	0.31	*0.044
Symptomatic status (ref = No)	47	35(74.46)			
Yes	36	29 (80.56)	(0.51–3.89)	1.41	0.504

Total of 83 samples were used for risk factors analysis using SPSS analytical software. Samples with missing data were excluded from the final analysis. * indicates existing significant association between the variable(s) and being positive to PCR test.

pet dogs has been documented in a *Helicobacter* study [43] and other studies related to *Campylobacter* [44]. Stray dogs in Taiwan usually free-roam in their living territories. They can easily transmit infectious disease by playing, biting, licking each other and passing stool into the environment thereby directly contaminating soil and water and getting infected from each other – a phenomenon that may also happen between canines and humans. Furthermore, there are a few reports comparing infectious diseases between purebred and mixed-breed dogs. *Bartonella* and *Giardia* infections were reported to be higher in mixed-breed than in purebred dogs, respectively [45,46]. It is unknown what underlies either of these risk factors for *Helicobacter* infection, however, speculation is that mixed-breed dogs often come from stray dogs and acquire *Helicobacter* infection during early life.

Due to the relatively small sample size and the convenient sampling method used, the results obtained in this study may not be completely generalizable in Taiwan. Nevertheless, this study provides useful, enormous information for further holistic surveillance of gastric and enterohepatic *Helicobacter* infections in both humans and canines and for implementation of the One Health approach approach to address the zoonotic infection by *Helicobacter*.

In conclusion, our study reveals that strains of gastric and enterohepatic *Helicobacter* species found in canines are highly similar to those found in humans. This portends possible cross-transmission between humans and canines and is an indication that special attention should be given to eradicating these bacteria in the guts of pets, especially pets in order to forestall possible pet-to-human infections.

Conflict of interests

The authors declare that there are no any conflicts of interests associated with this work.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.onehlt.2022.100430.

References

[1] B. Marshall, J.R. Warren, Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration, Lancet 323 (8390) (1984) 1311–1315.
[2] C. Recordati, V. Gualdi, M. Craven, L. Sala, M. Luini, A. Lanzoni, M. Rishniw, K. W. Simpson, E. Scanziani, Spatial distribution of *Helicobacter* spp. in the gastrointestinal tract of dogs, Helicobacter. 14 (3) (2009 Jun) 180–191, https://doi.org/10.1111/j.1523-5378.2009.00674.x. PMID: 19702848.
[3] Sofia Ochoa, Luis Collado, Enterohepatic *Helicobacter* species –clinical importance, host range, and zoonotic potential, Crit. Rev. Microbiol. 47 (6) (2021) 728–761, https://doi.org/10.1080/1040841X.2021.1924117.
[4] M.V. Powers-Fletcher, M.R. Couturier, Non-*Helicobacter* pylori *Helicobacter* species associated with human disease: a primer for the clinical microbiology laboratory, Clin. Microbiol. Newsl. 37 (12) (2015) 93–101.
[5] A.C. Parte, Sard a Carbasse J, Meier-Kolthoff JP, Reimer LC, Goker M., List of Prokaryotic names withstanding in Nomenclature (LPSN) moves to the DSMZ, Int. J. Syst. Evol. Microbiol. 70 (11) (2020) 5607–5612.
[6] M. Jankowski, et al., Detection of gastric *Helicobacter* spp. in stool samples of dogs with gastritis, Pol. J. Vet. Sci. 19 (2) (2016) 237–243.
[7] F. Haesebrouck, et al., Gastric *Helicobacters* in domestic animals and nonhuman primates and their significance for human health, Clin. Microbiol. Rev. 22 (2) (2009) 202–223.
[8] E. Ekman, M. Fredriksson, G. Trowald-Wigh, *Helicobacter* spp. in the saliva, stomach, duodenum and faeces of colony dogs, Vet. J. 195 (1) (2013) 127–129.
[9] F.S. Abdi, et al., Detection of *Helicobacter* spp. DNA in the colonic biopsies of stray dogs: molecular and histopathological investigations, Diagn. Pathol. 9 (2014) 50.
[10] J.G. Kusters, A.H. Van Vliet, E.J. Kuipers, Pathogenesis of *Helicobacter* pylori infection, Clin. Microbiol. Rev. 19 (3) (2006) 449–490.
[11] N.F. Azevedo, J. Huntington, K.J. Goodman, The epidemiology of *Helicobacter* pylori and public health implications, Helicobacter 14 (2009) 1–7.
[12] F.F. Vale, J.M. Vítor, Transmission pathway of *Helicobacter* pylori: does food play a role in rural and urban areas? Int. J. Food Microbiol. 138 (1-2) (2010) 1–12.
[13] Brunna Mary Okubo, Rafael Ricci-Azevedo, Nathalia Novak Zobiole, Danieli Fernanda Buccini, Susana Elisa Moreno, Prevalência de *Helicobacter* spp. Em Cães De Campo Grande-MS. Ciência Animal Brasileira 18, 2017, e17286. Epub April 10, 2017, https://doi.org/10.1590/1089-6891v18e-17286.
[14] Tsung-Yu Chen, Hui-Pi Huang, Cho-Hua Wan, Prevalence of the enterohepatic *Helicobacter* infection in pet dogs and cats from the national Taiwan university veterinary hospital in-patients and quarantine center, Taiwan Vet. J. 38 (3) (2012) 199–207.
[15] S. Kubota-Aizawa, et al., Epidemiological study of gastric *Helicobacter* spp. in dogs with gastrointestinal disease in Japan and diversity of *Helicobacter* heilmannii sensu stricto, Vet. J. 225 (2017) 56–62.
[16] J.K.Y. Hooi, et al., Global prevalence of *Helicobacter* pylori infection: systematic review and meta-analysis, Gastroenterology 153 (2) (2017) 420–429.
[17] C.H. Lai, et al., Mixed infections of *Helicobacter* pylori isolated from patients with gastrointestinal diseases in Taiwan, Gastroenterol. Res. Pract. 2016 (2016) 7521913.
[18] C.-Y. Yeung, H.-C. Lee, Paediatric *Helicobacter* pylori infection in Taiwan: current status and perspectives, Gastroenterology 6 (1) (2017) 90–97.
[19] I.T. Wu, et al., Five-year sequential changes in secondary antibiotic resistance of *Helicobacter* pylori in Taiwan, World J. Gastroenterol. 21 (37) (2015) 10669–10674.
[20] I. Mladenova-Hristova, O. Grekova, A. Patel, Zoonotic potential of *Helicobacter* spp, J. Microbiol. Immunol. Infect. 50 (3) (2017) 265–269.
[21] J.G. Fox, A. Lee, The role of *Helicobacter* species in newly recognized gastrointestinal tract diseases of animals, Lab. Anim. Sci. 47 (3) (1997) 222–255.
[22] S. Kumar, G. Stecher, K. Tamura, MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets, Mol. Biol. Evol. 33 (7) (2016) 1870–1874.
[23] R.C. Edgar, MUSCLE: a multiple sequence alignment method with reduced time and space complexity, BMC Bioinformatics 5 (2004 Aug 19) 113.
[24] N. Saitou, M., Neighbour-joining method: a new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (4) (1987) 406–425.
[25] V. Castiglioni, et al., Enterohepatic *Helicobacter* spp. in colonic biopsies of dogs: molecular, histopathological and immunohistochemical investigations, Vet. Microbiol. 159 (1–2) (2012) 107–114.
[26] I. Amorim, A. Smet, O. Alves, S. Teixeira, A.L. Saraiva, M. Taulescu, C. Reis, F. Haesebrouck, F. Gärtner, Presence and significance of *Helicobacter* spp. in the gastric mucosa of Portuguese dogs, Gut Pathog. 7 (2015 Apr 16) 12.
[27] Enterohepatic Helicobacter species, in: D.B. Schauer, H.L.T. Mobley, G.L. Mendz, S. L. Hazell (Eds.), *Helicobacter* pylori: Physiology and Genetics, ASM Press Copyright © 2001, ASM Press, Washington (DC), 2001.

[28] M. Fatemi Khader, M. Pourmahdi Borujeni, N. Moori Bakhtiari, R. Avizeh, An exploratory study on the presence of *Helicobacter* heilmannii and *Helicobacter* billis in the feces of companion dogs, Vet. Med. Sci. 8 (2) (2022 Mar) 537–545.
[29] Rehab Elhelw, Mahmoud Elhariri, Eman Ragab, Sara M. Nader, Dalia Hamza, Dog as potential source of *Helicobacter* pylori in Egypt, Public Health Significance (2020), https://doi.org/10.21203/rs.3.rs-26490/v1.
[30] M. Abdel-Raouf, Y. Abdel-Gleel, A. Enab, Study on the role of pet animals for *Helicobacter* pylori transmission, J. Am. Sci. 10 (2014) 20–28.
[31] S. Kubota-Aizawa, Y. Matsubara, H. Kanemoto, H. Mimuro, K. Uchida, J. Chambers, M. Tsuboi, K. Ohno, K. Fukushima, N. Kato, H. Yotsuyanagi, H. Tsujimoto, Transmission of *Helicobacter* pylori between a human and two dogs: a case report, Helicobacter. 26 (3) (2021 Jun) e12798.
[32] J.E. Tzeng, Y.L. Lin, Y.T. Chu, S.M. Chung, *Helicobacter heilmannii* of the stomach – a case report, Tzu Chi Med. J. 16 (2004) 59–62.
[33] S. Ochoa, J. Ojeda, O.A. Martínez, B. Vidal-Veuthey, L. Collado, Exploring the role of healthy dogs as hosts of enterohepatic *Helicobacter* species using cultivation-dependent and -independent approaches, Zoonoses Public Health 68 (4) (2021 Jun) 344–352.
[34] M.Z. Abidi, M.P. Wilhelm, J.L. Neff, J.G. Hughes, S.A. Cunningham, R. Patel, *Helicobacter* canis bacteremia in a patient with fever of unknown origin: fig 1, J. Clin. Microbiol. 51 (2013) 1046–1048, https://doi.org/10.1128/JCM. 02548-12.
[35] D. Alon, Y. Paitan, Y. Ben-Nissan, M. Chowers, Persistent *Helicobacter* canis bacteremia in a patient with gastric lymphoma, Infection 38 (2010) 62–64, https://doi.org/10.1007/s15010-009-9067-6.
[36] J. Tankovic, M. Smati, D. Lamarque, J.-C. Delchier, First detection of *Helicobacter* canis in chronic duodenal ulcerations from a patient with Crohn's disease, Inflamm. Bowel Dis. 17 (2011) 1830–1831, https://doi.org/10.1002/ibd.21610.
[37] M.A. Sabry, K.A. Abdel-Moein, A. Seleem, Evidence of zoonotic transmission of *Helicobacter* canis between sheep and human contacts, Vector Borne Zoonotic Dis. 16 (2016) 650–653, https://doi.org/10.1089/vbz.2016.1994.
[38] W. Peng, H. Li, Y. Xu, L. Yan, Z. Tang, A. Hossein Mohseni, S. Taghinezhad-S, X. Tang, X. Fu, Association of *Helicobacter* bilis infection with the development of colorectal cancer, Nutr. Cancer 73 (11–12) (2021) 2785–2795, https://doi.org/10.1080/01635581.2020.1862253. Epub 2020 Dec 16. PMID: 33325271.
[39] Y. Gotoh, Y. Atsuta, T. Taniguchi, R. Nishida, K. Nakamura, Y. Ogura, N. Misawa, T. Hayashi, *Helicobacter* cinaedi is a human-adapted lineage in the *Helicobacter* cinaedi/canicola/ 'magdeburgensis' complex, Microb. Genom. 8 (5) (2022 May), https://doi.org/10.1099/mgen.0.000830. PMID: 35536747.
[40] Y. Kawamura, J. Tomida, T. Miyoshi-Akiyama, T. Okamoto, M. Narita, K. Hashimoto, M. Cnockaert, P. Vandamme, Y. Morita, T. Sawa, T. Akaike, Proposal of *Helicobacter* canicola sp. nov., previously identified as *Helicobacter* cinaedi, isolated from canines, Syst. Appl. Microbiol. 39 (5) (2016 Jul) 307–312, https://doi.org/10.1016/j.syapm.2016.06.004. Epub 2016 Jun 25. PMID: 27381809.
[41] M.P. Dore, H.M. Malaty, D.Y. Graham, G. Fanciulli, G. Delitala, G. Realdi, Risk factors associated with *Helicobacter* pylori infection among children in a defined geographic area, Clin. Infect. Dis. 35 (3) (2002 Aug 1) 240–245, https://doi.org/10.1086/341415. Epub 2002 Jul 8. PMID: 12115088.
[42] Liu Daijun, Statistical Application Analysis Report- Analysis on the Characteristics of the Administrative Districts of Taipei City. Department of Budget, Accounting and Statistics, Taipei City Government, 2009. September. (in Chinese).
[43] A.S. Asl, S. Jamshidi, M. Mohammadi, M.H. Soroush, A. Bahadori, A. Oghalaie, Detection of atypical cultivable canine gastric *Helicobacter* strain and its biochemical and morphological characters in naturally infected dogs, Zoonoses Public Health 57 (4) (2010 Jun) 244–248.
[44] H. Fernández, R. Martin, Campylobacter intestinal carriage among stray and pet dogs, Rev. Saude Publica 25 (6) (1991 Dec) 473–475.
[45] E. Lashnits, M. Correa, B.C. Hegarty, A. Birkenheuer, E.B. Breitschwerdt, Bartonella seroepidemiology in dogs from North America, 2008-2014, J. Vet. Intern. Med. 32 (1) (2018 Jan) 222–231, https://doi.org/10.1111/jvim.14890. Epub 2017 Dec 2. PMID: 29197186; PMCID: PMC5787158.
[46] J.C. Shin, A.W. Reyes, S.H. Kim, S. Kim, H.J. Park, K.W. Seo, K.H. Song, Molecular detection of Giardia intestinalis from stray dogs in animal shelters of Gyeongsangbuk-do (Province) and Daejeon, Korea, Korean J. Parasitol. 53 (4) (2015 Aug) 477–481, https://doi.org/10.3347/kjp.2015.53.4.477. Epub 2015 Aug 25. PMID: 26323847; PMCID: PMC4566509.