Original Article

Prevalence of *Salmonella* spp. and *Escherichia coli* in dogs with diarrhoea in Western Taiwan

Chiu-Huang Ting[1] Chia-Ying Lin[2] Chia-Wen Chung[1]

Perng-Chih Shen[3] Ching-Dong Chang[1] Hung-Yi Wu[1*]

Abstract

Salmonella spp. can be widely spread by air or contaminated food. There are six common pathogenic *Escherichia coli* which account for 4–8% of all *E. coli* pathogens. Amongst them, *E. coli* O157 is the most commonly known. In this study, we investigated the prevalence of diarrhoea pathogens, referred to as *Salmonella* spp. and *Escherichia coli* (*E. coli*), in pet dogs in western Taiwan using the conventional polymerase chain reaction (PCR). The related analysis between the prevalence rates and the epidemiological data of pet dogs was correlated with age, season, area and breed. *Faecal* samples were collected from *240* dogs which had symptoms of diarrhoea and had been examined by *veterinary* hospitals from January 2015 to January 2017. The *PCR sensitivity* of the total deoxyribonucleic acids (DNAs) extracted from 0.1g faecal samples ranging from 10 fg to 100 ng was examined. The prevalence of *Salmonella* spp. and *E. coli* infections was 19.2% (46/240) and 3.3% (8/240), respectively. Results showed that *Salmonella* spp. was at its most prevalent during spring, whilst *E. coli* was most prevalent in summer, and the highest proportions might occur in puppies in suburban dogs. Since both diseases are zoonotic, the more humans are exposed to it, the higher its occurrence will be. This study has provided clinical veterinarians with the advanced ability to diagnose both diseases and with crucial information for the prevalence of *Salmonella* spp. and *E. coli* diseases in dogs with diarrhoea in Taiwan.

Keywords: dog, *E. coli*, faecal sample, PCR, *Salmonella*, Taiwan

[1]*Department of Veterinary Medicine, College of Veterinary Medicine, National Pingtung University of Science and Technology. No 1, Shuefu Road, Neipu, Pingtung, 91201 Taiwan*

[2]*Managing Director of Da Dian Biotechnology Company Limited*

[3]*Department of Animal Science, National Pingtung University of Science and Technology*

***Correspondence**: wuhy@mail.npust.edu.tw* (H. Wu)

Introduction

Diarrhoea is a very common problem in dogs which is usually manifested through loose or liquid faeces. Diarrhoea can be acute or chronic; acute diarrhoea is more common in puppies and young dogs, although it can affect dogs of any age. Bacteria and viruses are the most common causes of diarrhoea. However, for infectious diarrhoea, the most common cause is bacteria. This study will focus on two bacterial causes of diarrhoea in pet dogs in Western Taiwan: *Salmonella* spp. and *E. coli*.

Salmonella spp. is widely found in humans and animals (Luk-In S *et al*., 2018), and is one of the main pathogens of a global zoonotic infectious disease, among which *Salmonella enteritidis* and *Salmonella* Typhimurium (Hung YT *et al*., 2017. Thierry M. Work, *et al*., 2019) are the most common. The clinical and pathological characteristics of salmonellosis are indistinguishable from canine parvovirus and coronavirus infections and are often misdiagnosed. It should be diagnosed with more detailed haematology, molecular biology identification, bacteriology, serology and other tests.

E. coli is a normal environment flora (González Garcia EA., 2002; Bumunang EW *et al*., 2019). According to the modified Kauffman scheme, *E. coli* are serotyped on the basis of their O (somatic), H (flagella) and K (capsular) surface antigen profiles (Nataro J P *et al*., 1998). A total of 170 different O antigens, each defining a serogroup, are currently recognised. Due to its multiantigens and the lack of a vaccine available for epidemic prevention, it can only be used to eliminate external pathogens, reducing contact as a precaution. Pathogenic *E. coli* strains are categorised into pathotypes. Six pathotypes are associated with diarrhoea and are collectively referred to as diarrhoeagenic *E. coli* (Nataro J P *et al*., 1998; Nataro *et al*., 1998; Nandre R. *et al*., 2018). *E. coli* O157: H7 is one of the Shiga toxin types. It is usually transmitted through the faecal–oral route by raw milk and food contamination (Gally *et al*., 2017; Karch *et al*., 2005; Bauwens A *et al*., 2017).

This study will investigate the prevalence of diarrhoea in Taiwanese dogs. It can be used to explore related factors of *Salmonella* spp. and *E. coli* infection and the differences in symptoms caused by the different categories of age, season, breed, suburbs and urban areas.

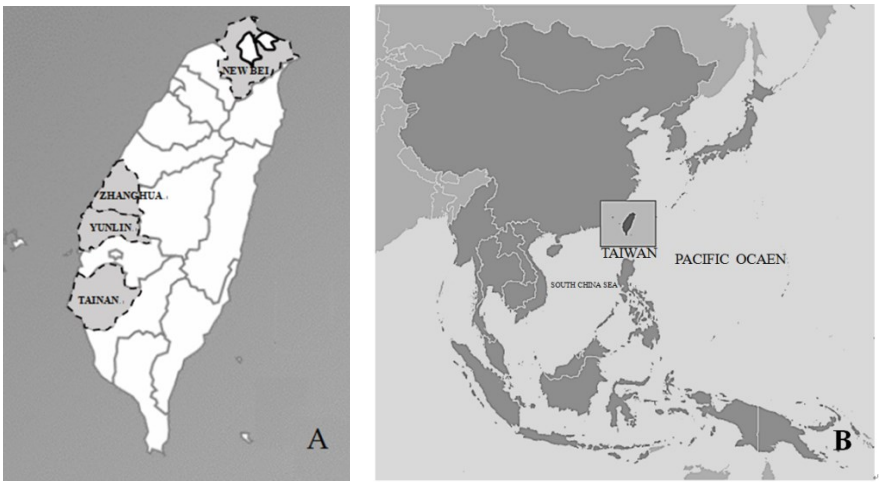

Figure 1 (A): Sampling regions (New Taipei, Zhanghua, Yunlin, Tainan) in western Taiwan. (B): Regional map of Taiwan (From the website of the Ministry of Foreign Affairs of Taiwan)

Materials and Methods

Experimental design: The study was designed to be conducted for two years (spring, summer, autumn and winter in two rounds) from January 2015 to January 2017, screening animal hospitals in western Taiwan to investigate 240 cases (one faecal sample for each dog) for suspected bacterial enteritis as a statistic to understand the main cause of bacterial enteritis and its infection in relation to seasonal, age, dog breed, suburbs and urban areas. The epidemiology of both *Salmonella* spp. and *E. coli* canine bacterial enteritis was investigated under a molecular biology polymerase chain reaction (PCR) in combination with clinical diagnosis.

Sampling: This study included 240 dogs with diarrhoea from animal hospitals in western Taiwan; ten samples were tested each month, including New Taipei, Zhanghua, Yunlin and Tainan (Figure 1). Dogs from ten hospitals (New Taipei, 3 hospitals; Zhanghua, 2; Yunlin, 2 and Tainan, 3) were picked, with 24 dogs per hospital. Faecal samples were collected directly upon defecation. To prevent contamination, clean clothes, gloves and masks were worn; 5–10 g of faeces were collected with a sterile spoon and stored in a clean, sealed, sterile plastic sampling tube. Specimens were kept cool at 4–10°C and processed in the laboratory within two hours of collection. All animal experiments were approved by the ethics committee of the National Pingtung University of Science and Technology and care was taken to comply with the 3R concept. Clinical consultation: After a detailed examination (including dog breed, age, vaccine application, symptoms, water consumption, feed type and feed volume) in each animal hospital, the canine faecal samples were obtained after the consent of the owner. Diarrhoea criteria were based on The

WALTHAM™ Faeces Scoring System. Samples were graded from grades 4 to 5: in grade 4, most or all form was lost and not real; in grade 4.5, a liquid stool with slight consistency; in grade 4.5, the entire liquid stool. The age, breed, gender and date had to be specially marked. Age was divided into < 1 year old, 1–6 years old, 6–11 years old and > 11 years old. Whilst the breed was distinguished by physical characteristics only, it could be identified as mixed breed in cases where the breed could not be determined through its appearance. The seasons were divided into spring from February to April, summer from May to July, autumn from August to October and winter from November to January.

DNA extraction and PCR: DNA was extracted from 240 dogs' diarrhoea faecal samples using the Gene PlusTM Genomic DNA Extraction Miniprep System Kit (VIOGENE, GG2002). The corresponding PCR conditions were amplified by the DNA extracted from the specific primer. *Salmonella* spp. using the primer pair **5′-** TGCGGCTGGATCACCTCCTT **-3′** and **5′-** ACAAGCGCTATCCTGCCAAACG**-3′.** *E. coli* using the primer pair **5′-** TATAGCCCCATCGTGTAGTCAG AAC**-3′** and **5′-**TCACTATCGGTCAGTCAGGAG**-3′** (Table 1) (El-Baradei *et al*., 2007; Lu *et al*., 2014). Conventional PCR was performed using 0.625 mM dNTPs, and the specific primers (Forward, Reverse) were 0.4 µM, 5U Taq DNA polymerase, and 20 U RNase inhibitor and buffer solution. The PCR reaction programme protocol was as follows: *Salmonella* spp. target gene, 312 bp. Step 1, pre-denaturation at 95°C for 15 mins, one cycle; step 2, 95°C for 30 seconds (denaturation) and another 58°C for 30 secs (annealing); step 3, extension at 72°C for 1 minute, performed for 30 cycles; step 4, final extension at 72°C for 10 minutes; final temperature, 4°C. *E. coli* target gene, 232 bp. Step 1, pre-denaturation at 95°C for 5 minutes, one cycle; step 2, 95°C for 30 seconds (denaturation) and 64°C for 30 seconds (annealing); step 3, extension at 72°C for 40 seconds, performed for 35 cycles; step 4, final extension at 72°C for 10 minutes; final temperature, 4°C. The electrophoresis of amplicons was achieved in a 1.5% agarose gel (stained with 0.5 mg/mL ethidium bromide) and visualised in an MS UVCI Image Capture transilluminator (Major Science, USA).

Table 1 Primers used in this study for the species-specific PCR assays

Pathogens	Gene	Oligonucleotide sequence (5′-3′)	Product length (bp)
Salmonella spp.	ITS	F 5′- TGCGGCTGGATCACCTCCTT -3′	312
	ITS	R 5′- TATAGCCCCATCGTGTAGTCAGAAC-3′	
Escherichia coli	Eco-223	F 5′-ATCAACCGAGATTCCCCCAGT-3′	232
	Eco-455	R 5′-TCACTATCGGTCAGTCAGGAG-3′	

Sensitivity and specificity of PCR: DNA was extracted from canine *Salmonella* spp. infected with the DNA of the detection of the target gene at 312 bp. The minimum amount of DNA was 10^{-4}ng. Its can be certain that *S.* Typhimurium. *E. coli* infection detected by PCR increases with a primer, which shows that the minimum DNA amount of the target gene at 232 bp can be detected by the primer as 10^{-5}ng. Its accession number is CP050862.1.

Results

Prevalence of Salmonella spp. and E. coli enteritis in diarrhoeic dogs (Table 2): The prevalence rate of *Salmonella* spp. and *E. coli* were 19.2% (46/240) and 3.3% (8/240), respectively, which indicates that the prevalence rate of *Salmonella* spp. in diarrhoeic dogs is higher than *E. coli* (Table 2). There was 1.25% (3/240) co-infection with both *Salmonella* spp. and *E. coli* in this study, which occurred to mixed-breed puppies in summer in the suburbs.

Age distribution: *Salmonella* spp. prevalence rate: <1 year, 10.8% (26/240); 1–6 years, 5% (12/240); 6–11 years, 0.8% (2/240); > 11 years, 2.5% (6/240). *E. coli*: < 1 year, 2.5% (6/240); 1–6 years, 0% (0/240); 6–11 years, 0% (0/240); > 11 years, 0.8% (2/240). This shows a higher proportion of infection with *E. coli* in puppies but not much difference in other ages and puppy cases, whilst the incidence of *Salmonella* spp. in puppies is significantly different from other age cases.

The Proportion of positive detection samples of all ages: The prevalence rate of *Salmonella* spp. at all ages: <1 year, 57% (26/46); 1–6 years, 26% (12/46); 6–11 years, 4% (2/46); > 11 years, 13% (6/46), indicating a higher proportion of salmonella infection in puppies. *E. coli* prevalence rates: <1 year, 75% (6/8); 1–6 years 0% (0/8); 6–11 years, 0% (0/8); > 11 years, 25% (2/8), showing a higher proportion of young dogs infected with *E. coli*. Based on the prevalence rate for all ages, Salmonella enteritis and *E. coli* have the most cases in puppies, and the incidence of *Salmonella* spp. in puppies is higher compared with *E. coli*.

Seasonal distribution: *Salmonella* spp. prevalence rate: February–April (Spring) was 10% (24/240), May–July (Summer) was 5% (12/240), August–October (Autumn) was 0.8% (2/240) and November–January (Winter) was 3.3% (8/240). *E. coli*: February–April (Spring) is 0.8% (2/240), May–July (Summer) is 2.5% (6/240), August–October (Autumn) was 0% (0/240), and November–January (Winter) was 0% (0/240). *E. coli* had the highest prevalence rate in May–July (Summer). The results shows that *Salmonella* spp. and *E. coli* have the highest occurrence in spring and summer, respectively.

The Proportion of positive detection samples of each season: Both of the positive bacteria relied on the season to distinguish the prevalence rate. *Salmonella* spp. the prevalence rate in each season was found to be as follows: February–April (Spring), 52% (24/46); May–July (Summer), 26% (12/46); August–October (Autumn), 4% (2/46) and November–January

(Winter), 18% (8/46). Thus, for *Salmonella* spp. the prevalence rate was the highest in February–April (Spring). The *E. coli* prevalence rate in each season was found to be as follows: February–April (Spring), 25% (2/8) and May–July (Summer), 75% (6/8). No positive results for *E. coli* were obtained in August–October (Autumn) and November–January (Winter). Thus, the highest prevalence rate of *E. coli* was in May–July (Summer).

Suburban and urban area distribution: Of the 240 samples, 96 were from the suburbs and 144 from the urban area. On differentiating the prevalence rates of the two positive bacteria by region, the prevalence rate of *Salmonella* spp. was higher in the suburbs [33.3% (32/96)] than in the urban areas [9.7% (14/144)]. Similarly, the prevalence rate of *E. coli* was higher in the suburbs [6.3% (6/96)] than in the urban area [1.4% (2/144)]. Therefore, most cases of both *Salmonella* spp. and *E. coli* enteritis occurred in the suburbs.

Table 2 Enteritis prevalence rates due to *Salmonella* spp. and *E. coli* in pet dogs

Items	Diversity	Kinds of bacteria Various prevalence rates			
		Positive		*Proportion of positive**	
Total		*Salmonella* spp.	*E. coli*	*Salmonella* spp.*	*E. coli**
		19.2% (46/240)	3.3% (8/240)	-	-
Age	<1 year	10.8% (26/240)	2.5% (6/240)	57% (26/46)	75% (6/8)
	1- 6 years	5% (12/240)	0% (0/240)	26% (12/46)	0% (0/8)
	6-11 years	0.8% (2/240)	0% (0/240)	4% (2/46)	0% (0/8)
	>11 years	2.5% (6/240)	0.8% (2/240)	13% (6/46)	25% (2/8)
Season	Spring	10% (24/240)	0.8% (2/240)	52% (24/46)	25% (2/8)
	Summer	5% (12/240)	2.5% (6/240)	26% (12/46)	75% (6/8)
	Autumn	0.8% (2/240)	0% (0/240)	4% (2/46)	0% (0/8)
	Winter	3.3% (8/240)	0% (0/240)	18% (8/46)	0% (0/8)
Area	Suburbs	33.3% (32/96)	6.3% (6/96)	70% (32/46)	75% (6/8)
	Urban	9.7% (14/144)	1.4% (2/144)	30% (14/46)	25% (2/8)
Breed	Mixed	2.78% (30/108)	5.6% (6/108)	65% (30/46)	75% (6/8)
	Poodle	17.9% (10/56)	3.6% (2/56)	22% (10/46)	25% (2/8)
	Maltese	5.6% (2/36)	0 (0/36)	5% (2/46)	-
	Pomeranian	9.1% (2/22)	0 (0/22)	4% (2/46)	-
	Other	11.1% (2/18)	0 (0/18)	4% (2/46)	-

**Proportion of positive samples*

The Proportion of positive detection samples in suburban and urban areas: Among The prevalence rate of *Salmonella* spp. in the suburbs was 70% (32/46), 30% in the urban area (14/46), and the incidence of *Salmonella* spp. in the suburbs was higher. *E. coli* positive, the prevalence rate was 75% in the suburbs (6/8), the urban area was 25% (2/8), and the incidence of *E. coli* in the suburbs was higher.

Breed distribution: Among the canine species positive for *Salmonella* spp., the prevalence rate in each breed was as follows: mixed dogs, 2.78% (30/108); Poodles, 17.9% (10/56); Marzis, 5.6% (2/36); Boomerangs, 9.1% (2/22) and others, 11.1% (2/18). *E. coli* prevalence rates were as follows: mixed-breed dogs, 5.6% (6/108) and Poodles, 3.6% (2/56). Marzis, Boomerangs and other dogs were not detected with *E. coli*. *Salmonella* spp. had a high prevalence rate in mixed dogs and Poodles, whereas *E. coli* had a high prevalence rate in mixed dogs in various canine breeds; hence, *Salmonella* spp. and *E. coli* enteritis had the highest prevalence rate in mixed-breed dogs. The bacteria infecting each dog is the only independent statistic, X^2: chi-square test for independence $P< 0.05$, the number of samples does not affect the positive rate.

The Proportion of positive samples in the breed: Among the positive breed, the *Salmonella* spp. prevalence rate, mixed-breed dogs were 65% (30/46), Poodles were 22% (10/46), Marzis 5% (2/46), Boomerang 4% (2/46), others 4% (2/46), indicating that the proportion of mixed-breed dogs infected with *Salmonella* spp. is higher than other canine species. For *E. coli* prevalence rate, mixed-breed dogs accounted for 75% (6/8), Poodles accounted for 25% (2/8), showing a higher ratio of *E. coli* than was detected in mixed-breed dogs.

Discussion

Most bacterial enteropathogens are associated with self-limiting diarrhoea, and injudicious administration of antimicrobials can be more harmful than beneficial. Salmonella is a well-documented zoonotic but antimicrobial administration is not routinely advocated in uncomplicated cases, hence supportive therapy is recommended. Basic practices of isolation, the use of appropriate protective equipment and proper cleaning and disinfection are the the mainstays of control. Handwashing with soap and water is preferred over the use of alcohol-based hand sanitizers because spores of some bacteria are alcohol-resistant (Marks, 2011).

In order for any preventative or controlling strategy to be effectively put in place, it first requires zoonosis to be recognised. Recognition most likely does not occur until the disease or infection has presented itself within the human population, even though an animal may have been the first to show any symptoms (Chomel, 2003).

In this study, there were 26 puppies infected with *Salmonella* spp. enteritis and most of the cases were suburban cases. It should be mentioned that in previous references Salmonella was widely found in the intestines of humans, rodents, amphibians, reptiles, insects and domestic animals. The way to raise dogs in the suburbs is to adopt free-feeding instead of cage-type puppies to eat everywhere; that is why it is easy to get infected with diarrhoea caused by salmonella. It is related to the infection of dogs with *Salmonella* spp. because the chance of exposure to these animals in suburban areas is relatively high. Therefore, the highest incidence rate of *Salmonella* spp. enteritis in puppies is similar to a previous study that reported an internal case in Taiwan. The results of *Salmonella* spp. that occurs in spring and summer are similar to those in the past that reported that *Salmonella* spp. is a common pathogen that occurs during the hot season from May to July (Houseknecht, 1992; Bondo *et al*., 2016; Dang-Xuan S, 2019). There were six puppies and two dogs more than 11 years old which were positive for *E. coli*. Results have been confirmed that the products can be producing *E. coli* non-O157 fragments [(enterohemorrhagic *E. coli* (EHEC)] in addition to fragments of *E. coli* O157. *E. coli* O157 is commonly found in puppies but *E. coli* enteritis can be found in all ages. In particular, there were four dog samples from cattle farms and it can be surmised that the source of infection might have been cattle. The specificity of the enzyme-linked immunosorbent assay (ELISA) was improved by a modified test protocol incorporating immunocapture (Johnson *et al*., 1995). In Taiwan, from 2006 to 2009, ELISA was used to detect EHEC cases in domestic milk and dysentery stools. Two cases were isolated from 1674 milk samples (0.001%), and five cases were isolated from 24 dysentery samples (20.8%). Infected dogs need have special attention due to the high overlap between the dog and the living environment of the owner, which easily causes human infection. *E. coli* occurs in summer and spring, with the latter being more prevalent. This result is not consistent with previous studies. In the past, the infection caused by *E. coli* had obvious seasonality and occurred mostly in summer and autumn and peaked in July–August. However, in global regional distribution, it occurs mostly in developed countries, mainly in sporadic infections (Gibbons *et al*., 2014; Williams *et al*., 2013). The favourite seasons are similar in our results. In the suburbs or urban areas, investigation of the occurrence of *E. coli* in dogs has not been reported in past studies, nor has it been investigated for dog breed. In this study, *E. coli* was found to be the most common in the suburbs. The proportion of mixed-breed dogs had the highest prevalence rate. *E. coli* and *Salmonella* spp. have a *high percentage* in suburban, mixed-breed dogs, which may be associated with the tendency of most of Taiwan's suburbs to raise mixed-breed dogs; the cleanliness of urban and suburban environments is very different. Moreover, in X^2: chi-square tests for independence $P< 0.05$, the prevalence was not affected by sample size.

In conclusion, Puppy infection from *Salmonella* spp. and *E. coli* causes the most cases of enteritis but there is not much difference between *E. coli* enteritis and puppy cases at other ages. *Salmonella* spp. and *E. coli* enteritis favours the suburbs, mixed-breed dogs and the spring and summer seasons. *Salmonella* spp. enteritis has a higher incidence in spring than in the summer; the incidence of *E. coli* enteritis in summer is higher than that in spring.

Acknowledgements

Special thanks is offered to Dr. Hung-Yi Wu and Chia-Wen Chung for their outstanding technical support.

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