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Epidemiology and Antimicrobial Resistance of *Salmonella* sp. Isolated from Dogs and Cats in Northeastern Thailand

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Abstract: During the period December 2009-November 2010, 600 fecal samples were collected from 250 diarrheal and 250 non-diarrheal dogs, 50 diarrheal and 50 non-diarrhea cats. It was found that 11.6 and 13.2% of diarrheal and non-diarrheal dogs and 8.0 and 10.0% of diarrheal and non-diarrheal cats were infected with Salmonella, respectively. The five most common serovars in dogs were *S. stanley*, *S. rissen*, *S. enterica* ser 4, 5, 12 :i:-, *S. weltevreden* and *S. tryphimurium* (14.5, 12.9, 11.3, 11.3 and 9.7%, respectively). The five most common serovars in cats were *S. weltevreden*, *S. eastbourne*, *S. typhimurium*, *S. virchow* and *S. hvittingfoss* (44.4, 22.2, 11.1, 11.1 and 11.1%, respectively). Isolates from dogs were resistant to amoxicillin (43.5%), gentamicin (8.1%), nalidixic acid (9.7%), sulphamethoxazole/trimethoprim (12.9%) and tetracycline (43.5%). The isolates from cats were resistant to amoxicillin (25%) and tetracycline (25%). Detection of *Salmonella* sp. in dogs and cats without clinical signs indicated that the animals were in carrier stage and potentially able to pass the disease to their owners.

Key words: Antimicrobial resistance, Salmonella, dogs, cats, epidemiology, Thailand

INTRODUCTION

Salmonella sp. is an important foodborne pathogen that causes human gastroenteritis worldwide and has long been a serious public health concern. Estimated case number of human salmonellosis was 1.4 million year^{-1} in the United States (Voetsch *et al*., 2004). In Khon Kaen, Thailand, *Salmonella* sp. was the most common pathogen found in diarrheal patients (Vaeteewootacharn *et al*., 2005). It also accounted for 7% of diarrheal children in hospitals in Thailand (Padungtod and Kaneene, 2006). *Salmonella* sp. can cause enteritis and diarrhea in dogs although, some dogs showing no signs of the disease may shed the bacteria in their feces (Cantor *et al*., 1997). Transmission of the disease from pets to humans occurs by contacting with fecal contaminated environments. Sato *et al*. (2000) reported that *Salmonella* sp. isolated from diarrheal child and the dog living in the same house were of similar strain. Impacts of antimicrobial resistance of *Salmonella* sp. on treatment in human patients included higher chance of hospitalization, prolonged treatment duration and higher mortality rate (Varma *et al*., 2005). In Thailand, multidrug resistance was observed in poultry and swine isolates (Chuanchuen *et al*., 2008). Angkititrakul *et al*. (2005) reported that *Salmonella* sp. isolated from human patients in Khon Kaen, Thailand were resistant to amoxicillin, chloramphenicol, gentamicin, sulfamethoxazole/trimethoprim and tetracycline at 27.8, 20.4, 5.6, 31.5 and 92.6%, respectively.

Since, having dogs and cats living closely at home has become more common in Thailand, epidemiological data and patterns of antimicrobial resistance are needed in order to prevent and control *Salmonella* sp. in pets. The information is essential for control management for the diseases affecting animals, humans and public health in Thailand.

MATERIALS AND METHODS

Sample collection: Fecal samples from dogs and cats were collected by using a rectal swab at Khon Kaen University Veterinary Teaching Hospital and 4 private practices in Khon Kaen municipality, Thailand during August 2008-May 2010. The population number in 2009 was

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approximately 103,000 for dogs and 17,000 for cats in the town of Khon Kaen. The sample size calculation yielded a total 600 fecal samples of which 250 were from non-diarrheal dogs, 250 from diarrheal dogs, 50 from non-diarrheal cats and 50 from diarrheal cats.

***Salmonella* sp. isolation and identification:** Isolation and identification of *Salmonella* sp. from the samples was done according to ISO 6579 (2002) Amd1: 2007. The isolates were then precipitated by O-antigen and sent to Regional Medical Sciences Center, Khon Kaen, Thailand for identification of the serovars.

Antimicrobial susceptibility test: The Salmonella isolates were tested for antimicrobial susceptibility against Amoxicillin (AML) 10 μg, Ciprofloxacin (CIP) 5 μg, Gentamicin (GN) 10 μg, Nalidixic Acid (NA) 30 μg, Norfloxacin (NOR) 10 μg, Sulphamethoxazole/ Trimethoprim (SXT) 25 μg and Tetracycline (TE) 30 μg (Oxoid, Basingstoke, Hants, UK) by Disc Diffusion Method. The interpretive standards for resistance were based on CLSI (2011) guidelines.

Statistical analysis: Chi-square analysis in Epi-Info Version 6.01 (Centers for Disease Control and Prevention, Atlanta, GA, USA) was used to find significant difference between dogs and cats.

RESULTS AND DISCUSSION

Salmonella sp. were isolated from diarrheal dogs (11.6%), non-diarrheal dogs (13.2%), diarrheal cats (8%) and non-diarrheal cats (10%). The prevalence among dogs (12.4%) and cats (9.0%) are not significantly different in this study ($p>0.05$). Predominant serovars identified in dogs were *S. stanley*, *S. rissen*, *S. enterica* ser 4,5,12 :i:, *S. weltevreden* and *S. typhimurium* (14.52, 12.90, 11.29, 11.29 and 9.68%, respectively) (18 and 13 serovars were identified in diarrheal and non diarrheal dogs, respectively with 9 serovars being in common) and those in cats were *S. weltevreden*, *S. eastbourne*, *S. typhimurium*, *S. virchow* and *S. hvittingfoss* (44.44, 22.22, 11.11, 11.11 and 11.11%, respectively) (3 and 4 serovars were identified in diarrheal and non diarrheal cats, respectively with 2 serovars being in common). Only one serovar was found in each infected animals (Table 1).

The antimicrobial susceptibility test showed that *Salmonella* sp. isolated from diarrheal dogs were resistant to AML (34.5%), NA (10.3%), SXT (10.3%) and TE (37.9%). All isolates from diarrheal dogs were susceptible to CIP, GN and NOR. Isolates from non-diarrheal dogs were resistant to AML, GN, NA, SXT and TE at 51.5, 15.2, 9.1, 15.2 and 48.5%, respectively. All isolates non-diarrheal dogs were susceptible to CIP and NOR. *Salmonella* sp. isolated from diarrheal cats were resistant to AML (25%) and TE (25%). Isolates from non-diarrheal cats were susceptible to all the tested antimicrobial agents (Table 2). Patterns of antimicrobial resistance are shown in Table 3.

Risk of salmonella infections in humans caused by their pets is highlighted in this study as some animals were infected without showing any clinical signs and they were in close contact to their owners. The overall incidence of *Salmonella* sp. infection in dogs was 12.4% in this study. The percentage of Salmonella isolation from dogs exceeded that of Taiwan (4.3%) (Tsai *et al*., 2007), Trinidad (3.6%) (Seepersadsingh *et al*., 2004) and Italy (2.4%) (Nastasi *et al*., 1986) but was lower than that of USA (20.8%) (Frye and Fedorka-Cray, 2007). Lower proportion of infection found in cats (9.0%) compared to dogs (12.4%) was in agreement with Weber *et al*. (1995) by which the incidence was reported at 1.9% in cats and 3.5% in dogs.

In this study, *Salmonella* sp. isolated from dogs were more likely to be resistant to the tested antimicrobial agents and at higher percentages. The isolates from dogs were resistant to amoxicillin (43.5%), gentamicin (8.1), nalidixic acid (9.7), sulphamethoxazole/trimethoprim (12.9%) and tetracycline (43.5%) while the cat isolates were resistant to AML (25%) and TE (25%). Higher chance of access to veterinarians and antimicrobial treatment in dogs as compared with cats in Thailand may

Table 1: Prevalence and serovars of *Salmonella* sp. isolated from dogs and cats

Animal	No. of isolates (%)	Serovars (Number of isolates)
Diarrheal dogs (250)	29 (11.60)	Weltevreden (4), Stanley (3), Typhimurium (3), Emek (2), Enterica ser. 4,5,12, :i:- (2), Panama (2) and Virchow (2)
		Bovismorbificans (1), Brunei (1), Eastbourne (1), *enterica* ser. 8,20, :-:-(1), Enteritidis (1), Give (1), Hvittingfoss (1), Kedougou (1), Lexington (1), Rissen (1) and Wentworth (1)
Non-diarrheal dogs (250)	33 (13.20)	Rissen (7), Stanley (6), *enterica* ser. 4, 5, 12, :i:- (5), Typhimurium (3), Weltevreden (3), Aberdeen (2), Brunei (1), enterica ser. 9,12,:-:1,5 (1), Havana (1), Hvittingfoss (1), Kedougou (1), Schwarzengrund (1) and Virchow (1)
Diarrheal cats (50)	4 (8.00)	Weltevreden (2), Eastbourne (1) and Typhimurium (1)
Non-diarrheal cats (50)	5 (10.00)	Weltevreden (2), Eastbourne (1), Hvittingfoss (1) and Virchow (1)
Overall dogs (500)	62 (12.40)	
Overall cats (100)	9 (9.00)	
Total (600)	71 (11.83)	

Table 2: Antimicrobial resistance of *Salmonella* sp. isolated from dogs and cats by animal groups and salmonella serovars

		Number (%)						
Groups	Serovar (n)	AML	CIP	GN	NA	NOR	SXT	TE
Diarrheal dogs								
B	*S. stanley* (3)	0.0	0	0.0	0.0	0	0.0	0.0
	S. typhimurium (3)	3 (100.0)	0	0.0	0.0	0	0.0	3 (100.0)
	S. enterica ser. 4, 5, 12,:i:- (2)	2 (100.0)	0	0.0	0.0	0	0.0	2 (100.0)
C	*S. emek* (2)	1 (50.0)	0	0.0	2 (100.0)	0	1 (50.0)	1 (50.0)
	S. virchow (2)	0.0	0	0.0	0.0	0	0.0	0.0
	S. bovismorbificans (1)	1 (100.0)	0	0.0	0.0	0	0.0	1 (100.0)
	S. brunei (1)	0.0	0	0.0	0.0	0	0.0	0.0
	S. enterica ser. 8, 20,:-:- (1)	0.0	0	0.0	0.0	0	0.0	0.0
	S. rissen (1)	1 (100.0)	0	0.0	0.0	0	1 (100.0)	1 (100.0)
D	*S. panama* (2)	1 (50.0)	0	0.0	0.0	0	1 (50.0)	1 (50.0)
	S. eastbourne (1)	0.0	0	0.0	0.0	0	0.0	0.0
	S. enteritidis (1)	0.0	0	0.0	0.0	0	0.0	0.0
E	*S. weltevreden* (4)	0.0	0	0.0	0.0	0	0.0	1 (25.0)
	S. give (1)	0.0	0	0.0	1 (100.0)	0	0.0	0.0
	S. lexington (1)	0.0	0	0.0	0.0	0	0.0	0.0
F	*S. wentworth* (1)	0.0	0	0.0	0.0	0	0.0	0.0
G	*S. kedougou* (1)	1 (100.0)	0	0.0	0.0	0	0.0	1 (100.0)
I	*S. hvittingfoss* (1)	0.0	0	0.0	0.0	0	0.0	0.0
	Total (%)	10 (34.5)	0 (0)	0 (0.0)	3 (10.3)	0 (0)	3 (10.3)	11 (37.9)
Non-diarrheal dogs								
B	*S. stanley* (6)	3 (50.0)	0	0.0	0.0	0	0.0	2 (33.3)
	S. enterica ser. 4, 5, 12, :i:- (5)	4 (80.0)	0	2 (40.0)	0.0	0	0.0	5 (100.0)
	S. typhimurium (3)	3 (100.0)	0	2 (66.7)	1 (33.3)	0	0.0	1 (33.3)
	S. schwarzengrund (1)	1 (100.0)	0	1 (100.0)	1 (100.0)	0	0.0	0.0
C	*S. rissen* (7)	5 (71.4)	0	0.0	0.0	0	5(71.4)	7 (100.0)
	S. brunei (1)	0.0	0	0.0	0.0	0	0.0	0.0
	S. enterica ser. 9, 12,:-:1,5 (1)	0.0	0	0.0	0.0	0	0.0	0.0
	S. virchow (1)	0.0	0	0.0	0.0	0	0.0	0.0
E	*S. weltevreden* (3)	0.0	0	0.0	0.0	0	0.0	0.0
F	*S. aberdeen* (2)	0.0	0	0.0	0.0	0	0.0	0.0
G	*S. havana* (1)	0.0	0	0.0	1 (100.0)	0	0.0	0.0
	S. kedougou (1)	0.0	0	0.0	0.0	0	0.0	1 (100.0)
I	*S. hvittingfoss* (1)	0.0	0	0.0	0.0	0	0.0	0.0
	Total (%)	17 (51.5)	0 (0)	5 (15.2)	3 (9.1)	0 (0)	5 (15.2)	16 (48.5)
Diarrheal cats								
B	*S. typhimurium* (1)	1 (100.0)	0	0.0	0.0	0	0.0	1 (100.0)
D	*S. eastbourne* (1)	0.0	0	0.0	0.0	0	0.0	0.0
E	*S. weltevreden* (2)	0.0	0	0.0	0.0	0	0.0	0.0
	Total (%)	1 (25.0)	0 (0)	0 (0.0)	0 (0.0)	0 (0)	0 (0.0)	1 (25.0)
Non-diarrheal cats								
C	*S. virchow* (1)	0.0	0	0.0	0.0	0	0.0	0.0
D	*S. eastbourne* (1)	0.0	0	0.0	0.0	0	0.0	0.0
E	*S. weltevreden* (2)	0.0	0	0.0	0.0	0	0.0	0.0
I	*S. hvittingfoss* (1)	0.0	0	0.0	0.0	0	0.0	0.0
	Total (%)	0 (0.0)	0 (0)	0 (0.0)	0 (0.0)	0 (0)	0 (0.0)	0 (0.0)

AML: Amoxicillin, CIP: Ciprofloxacin, GN: Gentamicin, NA: Nalidixic Acid, NOR: Norfloxacin, SXT: Sulfamethoxazole/Trimethoprim, TE: Tetracycline

Table 3: Resistance patterns of *Salmonella* sp. isolated from dogs and cats

	Dogs (%) (n/N)		Cats (%) (n/N)	
Resistance	Diarrheal	Non-diarrheal	Diarrheal	Non-diarrheal
AML	0.0	3.0 (1/33)	0.0	0
NA	6.9 (2/29)	3.0 (1/33)	0.0	0
TE	3.5 (1/29)	9.1 (3/33)	0.0	0
AML-GN	0.0	3.0 (1/33)	0.0	0
AML-TE	24.1 (7/29)	18.2 (6/33)	25.0 (1/4)	0
AML-GN-NA	0.0	6.1 (2/33)	0.0	0
AML-GN-TE	0.0	6.1 (2/33)	0.0	0
AML-SXT-TE	6.9 (2/29)	15.2 (5/33)	0.0	0
AML-NA-SXT-TE	3.5 (1/29)	0.0	0.0	0

AML: Amoxicillin, GN: Gentamicin, NA: Nalidixic Acid, SXT: Sulfamethoxazole/Trimethoprim, TE: Tetracycline

account for this difference. Tsai *et al*. (2007) reported higher resistance percentages in isolates from dogs in Taiwan (gentamicin (5%), nalidixic acid (43%), sulphamethoxazole/trimethoprim (37.5%) and tetracycline (77.5%)) except for amoxicillin (22%) and ciprofloxacin (0%).

Proportions of non-diarrheal dogs and cats infected with *Salmonella* sp. were higher than those of diarrheal dogs and cats in this study although, not statistically significant. This finding indicated that pets without diarrhea or other clinical signs can be salmonella carriers. The owners should be aware of this finding and pay attention to children playing with dogs and cats since, they are more susceptible than adults. Washing hands after contacting with pets especially before cooking and eating should be strictly practiced.

CONCLUSION

Salmonella sp. can be detected in dogs and cats without any clinical signs. This correlates the risk of *Salmonella* sp. spreading from pets to their owners especially children. To reduce the chance of infection in pets and avoid transmission of the bacteria from pets to humans, hygienic pet care and pet handling are crucial for the owners.

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