Original Article

Distribution of Salmonella spp. and serovars isolated from captive reptiles in four zoos of the Zoological Park Organization, Thailand, between 2017 and 2018

Sompoth Weerakhun[1]* Paweena Kongsanan[2] Wichit Kongkham[3]

Abstract

Salmonellosis is the main reason for food borne diseases in many countries globally and it has been found that reptiles are carriers of the disease. Therefore, this study was performed to determine the distribution of *Salmonella* spp. and their serovars from reptiles in zoos in Thailand between 2017 and 2018. A total of 257 reptile samples were collected from four zoos which are Dusit (128 samples), Chiangmai (32 samples), Songkhla (34 samples) and Nakhonratchasima (63 samples). *Salmonella* was isolated and identified, serotyping of those isolates was investigated using a panel of Salmonella antisera and serotypes were characterized according to the Kauffman-White classification scheme. *Salmonella* isolates were found in 93 (36.19%) of all samples, 51 (39.84%), 25 (39.68%), 9 (28.13%) and 8 (23.53%) isolates in Dusit, Nakhonratchasima, Chiangmai, and Songkhla, respectively. *Salmonella* was found mostly in snakes (70.13%) followed by lizards (57.57%) and turtles (13.70%). Sixty-two *Salmonella* isolates were selected for serotyping study and there were 4 subspecies and 32 serovars, *S. diarizonae* being the most common followed by *S. enterica*, *S. houtenae*, and *S. arizonae*, respectively. Dusit was the most common variety with 18 serovars followed by Nakhonratchasima (10 serovars), Songkhla (5 serovars) and Chiangmai (4 serovars). *Salmonella* from snakes showed the greatest diversity of serovars. The study showed that these reptiles were the sources of *Salmonella* spp. especially *S. enterica* which causes disease in humans with a high ratio (43.55%). This revealed a risk of *Salmonella* infection. It is necessary to carefully observe and emphasize the risk of contamination within the zoos in order to prevent the spread of *Salmonella* from reptiles to other animals and the community.

Keywords: *Salmonella*, Surveillance, Reptile, Zoonosis, Zoo

[1]*Faculty of Veterinary Medicine, Khon Kaen University, Khon Kaen 40002, Thailand*
[2] *National Institute of Health, Department of Medical Sciences, Ministry of Public Health, Nonthaburi, 11000, Thailand*
[3]*Bureau of Conservation and Research, The Zoological Park Organization of Thailand Under the Royal Patronage of H.M. the King, Bangkok, 10800, Thailand*
***Correspondence**: somwee@kku.ac.th* (S. Weerakhun)
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Introduction

Salmonella is considered an important health problem around the globe for both humans and animals (Woodward *et al*., 1996). It is estimated that there have been three million infectious patients each year. Nowadays, infection by *Salmonella* is the main reason for food borne diseases in many countries globally. The Center for Disease Control and Prevention (CDC) has estimated the number of patients infected with *Salmonella* in the USA to be 1.2 million people annually (Scallan *et al*., 2011). A survey of the spread of food borne diseases in USA has found *Salmonella* contributed 34% to the cause of the spread (Center for Disease Control and Prevention, 2015a). Moreover, in 2014, a study in Thailand found that 26.04% of food borne diseases was caused by *Salmonella* (Bureau of Epidemiology, 2015). From the previous study, it was found that 90% of reptiles have *Salmonella* but without any symptoms (Chiodini and Sundberg, 1981; Woodward, 1996; Robinowitz and Conti, 2010; Whiley, 2017). *Salmonella* is a rod-shaped negative-gram bacterium in Enterobacteriaceae. There are two species of *Salmonella* which are *Salmonella enterica* and *Salmonella bongori*. There are also six subspecies of *S. enterica* which include *S. enterica* subsp. *enterica* (subspecies I), *S. enterica* subsp. *salamae* (subspecies II), *S. enterica* subsp. *arizonae* (subspecies IIIa), *S. enterica* subsp. *diarizonae* (subspecies IIIb), *S. enterica* subsp. *houtenae* (subspecies IV), and *S. enterica* subsp. *indica* (subspecies VI). They can be categorized into different serovars from the attributes of their antigens. Nowadays, there are more than 2,500 serovars (Grimont and Weill, 2007).

There has been a report of patient infection from pets, however, there is a limitation of information about the situation of the spread because of the investigation and report systems in each area. There have been reports in many countries about patients with Salmonellosis who have had physical contact with turtles. For instance, there was a baby with meningitis symptoms and septicemia caused by their pet turtle (Meervenne *et al*., 2009; Ricard *et al*., 2015). The latest connection of *S.* Enteritidis between a patient and a turtle was in the Netherlands (Bosch *et al*., 2016) in 2017. There has also been the spread of *S.* Agbeni in 13 states in the USA due to physical contact with small turtles (Center for Disease Control and Prevention, 2017). Even though the U.S. Food and Drug Administration (FDA) has set a restriction on selling and distributing turtles smaller than four inches since 1975 (US Food and Drug Administration, 2015), there has still been a spread of *Salmonella* due to physical contact with turtles in the USA yearly (Walters *et al*., 2016). Normally, most animals are reservoir hosts of all species of *Salmonella* except *S.* Typhi and *S.* Paratyphi which are specific to humans only. Even so, it has been found that reptiles are one of the main causes of the spread of *Salmonella*; n0% of the reptiles have *Salmonella* but without symptoms (Chiodini and Sundberg, 1981; Woodward, 1996; Robinowitz and Conti, 2010; Whiley, 2017). However, from a study of the prevalence of reptiles in New Zealand, it was found that captive reptiles in different places have *Salmonella* with an 11.4% prevalence; it has been found in lizards more than turtles and there are 6 serovars in total which include *S.* Onderstepoort (30.2%), *S.* Thompson (20.9%), *S.* Potsdam (14%), *S.* Wangata (14%), *S.* Infantis (11.6%), and *S.* Eastbourne (2.3%) (Kikillus et al. 2011). *S.* Infantis is a serovar with the human pathogen report (Kikillus *et al*., 2011). Additionally, there was also a report of examination in 57 species of reptiles comparing 294 animals from the wild and 210 captive animals in Australia and it was found that 14% of the wild animals and 47% of the captive ones have *Salmonella* (Scheeling *et al*., 2011). Prapasarakul *et al*. (2006), examined *Salmonella* in snakes caught in the wild and in farms in Thailand (Prapasarakul *et al*., 2006) and also did a study in 2012 of a serovar of *Salmonella* in cobras, snake food and individuals working in the Pasteur Institute at the Thai Red Cross Society (Prapasarakul *et al*., 2012). Moreover, *Salmonella* has been found in captive king cobras and cobras at 75.86% which is considered very high (Viwanmetanon *et al*., 2003). For this reason, in order to observe the *Salmonella* disease in zoo areas where there are captive reptiles which might cause disease and becoming reservoir hosts, the researchers agreed that it is vital to do research on the surveillance, prevalence and distribution of *Salmonella* spp. and serovars in captive reptiles from four of the country's major zoos, which represents all regions of the Zoological Park Organization in Thailand.

Materials and Methods

Sample collection: This study was a sample collection from reptiles including snakes, turtles, lizards and crocodiles from four zoos, Dusit, Chiangmai, Songkhla and Nakhonratchasima between 2017 and 2018. The samples were collected by cloacal swab then put in Cary-Blair transport medium (Oxoid, UK) and the specimens transported to the laboratory at room temperature within 24 hours. Sample collection was done at the annual health check of the zoo animals, carried out under ethical guidelines, USDA pain and distress categories: Category C. The animals experienced no pain and stress or, if at all, only occurring for a short period of time without the use of analgesic or anesthetic drugs.

Bacterial isolation and identification: The sample cases were cultured in 9 ml aliquots of pre-enrichment media, buffer peptone water (Merck, Germany) and incubated at a temperature of 37°C for 24 hours. After that, the buffer peptone water was put in Rappaport-Vassiliadis enrichment (Merck, Germany) broth with 1:9 ratio and incubated at a temperature of 42°C for another 24 hours. The water was then brought to culture on Salmonella-Shigellla agar and Xylose Lysine Desoxycholate agar (Merck, Germany). Typical Salmonella colonies with the construction of H2S were selected where there were black spots to be tested using standard procedures as previously described (Mikoleit, 2014). Afterwards, the bacterium was preserved in 20% Glycerol typtic soy broth at atemperature of -20 °C for other investigations.

Serological testing for Salmonella spp.: The selection of 62 species of *Salmonella* from 93 species was from a

representative of each species of an animal and their living area: from 14 species of turtles, 35 species of snakes and 13 species of lizards. Salmonella serotyping was performed at the Department of Medical Sciences, Ministry of Public Health, Thailand, using a panel of Salmonella antisera (S & A Reagent Lab Ltd., Thailand) and serotypes were characterized according to the Kauffman-White classification scheme (Grimont and Weill, 2007).

Results

The data for the examination of *Salmonella* spp. in four zoos collected via cloacal swab approach included 257 sample cases: 63 cases from Nakhonratchasima Zoo, 34 cases from Songkhla Zoo, 128 cases from Dusit Zoo and 32 cases from Chiangmai Zoo. The information about the isolation in accordance with the locations and types is as shown in Table 1. From all the sample cases, 36.19% of *Salmonella* spp. was isolated (93/257). When categorizing the data in accordance with each zoo, *Salmonella* was found most in Dusit Zoo (39.84%) followed by Nakhonratchasima Zoo (39.68%), Chiangmai Zoo (28.13%) and Songkhla Zoo (23.53%) respectively as shown in Table 2. It was revealed that *Salmonella* spp. was found in snakes the most (70.13%: 54/77) followed by lizards (57.58%: 19/33) and turtles (13.7%: 20/146), however, the bacterium was not found in one case of the Caiman crocodile.

There was a selection of 62 isolates of *Salmonella* spp. for serological testing for *Salmonella* serotyping and four subspecies with 32 serovars were found. The subspecies called *diarizonae* was found in most subspecies (29 isolates) followed by *enterica* (27 isolates), *houtena*e (5 isolates) and *arizonae* (1 isolate), respectively. The results of the isolation of the bacterium samples by areas showed that Dusit Zoo had the most serovars (18 serovars) followed by Nakhonratchasima Zoo (10 serovars), Songkhla Zoo (5 serovars) and Chiangmai (4 serovars), respectively as shown in Table 3. It showed that 35 isolates of *Salmonella* spp. that were from snakes comprised four subspecies and 22 serovars which are *S. arizonae* (1 serovar), *S. diarizonae* (11 serovars), *S. enterica* (8 serovars) and *S. houtenae* (2 serovars). *Salmonella* spp. from turtles revealed three subspecies with 11 serovars which are *S. diarizonae* (3 serovars), *S. enterica* (7 serovars) and *S. houtenae* (1 serovar) and lizards comprised three subspecies and 9 serovars which are *S. diarizonae* (3 serovars), *S. enterica* (5 serovars), and *S. houtenae* (1 serovar) as shown in Table 4. Details of the results of the isolation of the bacterium and the results of serovars for each animal are as shown in Table 5-7.

Table 1 Reptiles and number of samples divided by zoo in Thailand between 2017 and 2018

Animal Sources	Number of Samples				Total
	Nakhonratchasima	Songkhla	Dusit	Chiangmai	
Turtles	33	26	63	24	**146**
Snakes	22	8	43	4	**77**
Lizards	8	0	21	4	**33**
Crocodile	0	0	1	0	**1**
Total	**63**	**34**	**128**	**32**	**257**

Table 2 Reptiles and isolated *Salmonella* spp. divided by zoo in Thailand between 2017 and 2018

Animals	Number of isolated *Salmonella* spp. / sample sizes (%)				
	Nakhonratchasima (n=63)	Songkhla (n=34)	Dusit (n=128)	Chiangmai (n=32)	Total (n=257)
Turtles (n=146)	8/33 (24.25)	5/26 (19.23)	4/63 (6.35)	3/24 (12.50)	**20 (13.70)**
Snakes (n=77)	11/22 (50.00)	3/8 (37.50)	37/43 (86.05)	3/4 (75.00)	**54 (70.13)**
Lizards (n=33)	6/8 (75.00)	0	10/21 (45.46)	3/4 (75.00)	**19 (57.58)**
Crocodile (n=1)	0	0	0/1	0	**0**
Total (%)	**25 (39.68)**	**8 (23.53)**	**51 (39.84)**	**9 (28.13)**	**93 (36.19)**

Table 3 Isolated *Salmonella* and serovars from reptiles divided by zoo in Thailand between 2017 and 2018

Sources (Zoos) (Sample sizes)	Number of *Salmonella* (Number of serovars)	Serovars	Animals and number
Nakhonratchasima (n=69)	25 (25)	Agona	Turtle 1, Snake 1
		Anatum	Turtle 1, Snake 1
		Enteritidis	Turtle 3
		Give	Turtle 1
		Kedougou	Turtle 1, Lizard 1
		Tennessee	Lizard 2
		38:z_4z_{23}:-	Snake 1, Lizard 1
		48:i:z_{35}	Snake 4, Lizard 2
		58:r:z_{53}	Snake 3
		65:lv:z	Turtle 1, Snake 1
Songkhla (n=34)	8 (8)	Tennessee	Turtle 1
		4,5,12:b:-	Turtle 1
		6,7:y:-	Snake 1
		43:z_4,z_{23}:-	Turtle 1, Snake 1
		60:r:z	Turtle 2, Snake 1
Dusit (n=133)	52 (26)	Corvallis	Lizard 1
		Dublin	Snake 1
		Rissen	Lizard 1
		Newport	Snake 2
		Uganda	Snake 3
		9,12:-:z_6	Snake 2
		17:-:enxzl$_5$	Snake 1
		38:lv:-	Snake 1
		38:z_4z_{23}:-	Snake 1
		47:k:z_{35}	Snake 1, Lizard 3
		48:i:-	Snake 1
		48:lv:1,5	Snake 1
		48:z:-	Lizard 1
		50:k:z	Snake 1
		58:r:z_{53}	Snake 1
		60:r:enxzl$_{15}$	Snake 1
		65:lv:z	Snake 1
		O:47 H: untypeable	Snake 1
Chiangmai (n=32)	9 (4)	Cerro	Snake 1
		47:k:z_{35}	Snake 1
		48:k:1,5	Turtle 1
		50:k:-	Lizard 1
Total (n=**257**)	**93 (62)**		

Table 4 Subspecies and serovars of *Salmonella* isolation divided by animal species in four zoos of the Zoological Park Organization, Thailand between 2017 and 2018

***Salmonella* spp.**		**Number of Isolation**			
		Turtles	**Snakes**	**Lizards**	**Total**
subspecies *arizonae*			**1**		**1**
serovar	O:47 H:untypeable		1		1
subspecies *diarizonae*		**4**	**19**	**6**	**29**
serovar	17:-:enxz$_{15}$		1		**1**
	38:lv:-		1		**1**
	47:k:z$_{35}$		2	3	**5**
	48:i:-		1		**1**
	48:i:z$_{35}$		4	2	**6**
	48:k:1,5	1			**1**
	48:lv:1,5		1		**1**
	50:k:-			1	**1**
	50:k:z		1		**1**
	58:r:z$_{53}$		4		**4**
	60:r:enxz$_{15}$		1		**1**
	60:r:z	2	1		**3**
	65:lv:z	1	2		**3**
subspecies *enterica*		**9**	**12**	**6**	**27**
serovar	Agona	1	1		**2**
	Anatum	1	1		**2**
	Cerro		1		**1**
	Corvalis			1	**1**
	Dublin		1		**1**
	Enteritidis	3			**3**
	Give	1		1	**1**
	Kedougou	1			**2**
	Newport		2		**2**
	Rissen			1	**1**
	Tennessee	1		2	**3**
	Uganda		3		**3**
	4,5,12:b:-	1			**1**
	6,7:y:-		1		**1**
	9,12:-:z$_{6}$		2		**2**
	48:z:-			1	**1**
subspecies *houtenae*		**1**	**3**	**1**	**5**
serovar	38:z$_{4}$z$_{23}$:-		2	1	**3**
	43:z$_{4}$,z$_{23}$:-	1	1		**2**

Table 5 Number of *Salmonella* spp. and serovars isolated from captive reptiles **(snakes)** in four zoos of the Zoological Park Organization, Thailand between 2017 and 2018

Animal Sources	Number of *Salmonella* per sample sizes (%)	Number of serotyping	Serovar (number)
Snakes (n = 77)			
Malayan pit viper	1/2 (50)	-	-
Stripe tailed rat snake	2/2 (100)	2	65:lv:z (1), 48:lv:1,5 (1)
Red tailed rat snake	2/2 (100)	2	48:i:z_{35} (1), 65:lv:z (1)
Yellow rat snake	0/1 (0)	-	-
Golden tree snake	1/1 (100)	1	47:k:z_{35} (1)
Green pit viper	2/4 (100)	2	48:i:z_{35} (2)
Large-eyed pit viper	1/1 (100)	-	-
White-lipped pit viper	1/1 (100)	-	-
Corn snake	3/4 (75)	2	Uganda (1), Cerro (1)
Desert kingsnake	1/1 (100)	1	Newport (1)
California kingsnake	1/1 (100)	1	9,12:-:z_6 (1)
King cobra	1/3 (33)	1	58:r:z_{53} (1)
Red cat snake	1/1 (100)	-	-
Brazilian rainbow	2/2 (100)	1	38:z_4z_{23}:- (1)
Black rat snake	1/1 (100)	1	50:k:z (1)
Albino black rat snake	1/1 (100)	1	Uganda (1)
Gold-ringed cat snake	1/2 (50)	1	38:lv:- (1)
Mangrove pit viper	1/1 (100)	1	17:-:enxz_{15} (1)
Yellow spotted keelback	1/1 (100)	1	Dublin (1)
Banded krait	0/1 (0)	-	-
Oriental rat snake	1/5 (20)	1	Uganda (1)
Dog-toothed cat snake	1/1 (100)	1	O:47 H:untypeable (1)
Burmese python	2/2 (100)	-	-
Golden python	4/4 (100)	2	38:z_4z_{23}:- (1), 48:i:z_{35} (1)
Macklot python	2/2 (100)	1	Newport (1)
Ball python	5/5 (100)	3	43:z_4,z_{23}:-(1), 9,12:-:z_6 (1), 47:k:z_{35} (1)
White ball python	2/2 (100)	-	-
Carpet python	0/2 (0)	-	-
Green Burmese python	2/2 (100)	-	-
Masked water snake	2/2 (100)	1	58:r:z_{53} (1)
Albino masked water snake	1/1 (100)	-	-
Reticulated python	3/4 (75)	3	Agona (1), Anatum (1), 6,7:y:- (1)
Green tree python	2/2 (100)	-	-
Siamese cobra	0/2 (0)	-	-
Albino Siamese cobra	2/2 (100)	2	58:r:z_{53} (1), 60:r:z (1)
Monocled cobra	1/2 (50)	1	58:r:z_{53} (1)
Anaconda	2/3 (33)	1	48:i:- (1)
Amazon tree boa	1/1 (100)	1	60:r:enxz_{15} (1)

Table 6 Number of *Salmonella* spp. and serovars isolated from captive reptiles **(turtles)** in four zoos of the Zoological Park Organization, Thailand between 2017 and 2018

Animal Sources	Number of *Salmonella* per sample sizes (%)	Number of serotyping	Serovar (number)
Turtles (n = 146)			
Alligator turtle	0/2 (0)	-	-
American sideneck turtle	0/1 (0)	-	-
Snapping turtle	0/2 (0)	-	-
African spurred tortoise	0/5 (0)	-	-
Albino Chinese soft-shelled turtle	0/1 (0)	-	-
Flap shelled turtle	0/1 (0)	-	-
Radiated tortoise	0/1 (0)	-	-
Marsh turtle	0/1 (0)	-	-
Red eared slider turtle	0/11 (0)	-	-
Snake necked turtle	1/1 (100)	1	Enteritidis (1)
Eastern long-necked turtle	0/2 (0)	-	-
Brisbane short-necked turtle	0/1 (0)	-	-
Pig nose turtle	0/2 (0)	-	-
Spiny turtle	1/4 (25)	1	Tennessee (1)
Chinese stripe necked turtle	0/5 (0)	-	-
Burmese star tortoise	0/4 (0)	-	-
Indian star tortoise	0/2 (0)	-	-
Black marsh turtle	2/5 (20)	1	60:r:z (1)
Impressed tortoise	0/1 (0)	-	-
Red footed tortoise	0/1 (0)	-	-
Red bellied short necked turtle	0/2 (0)	-	-
New Guinea snake necked turtle	0/2 (0)	-	-
Spotted pond turtle	0/2 (0)	-	-
Yellow headed temple turtle	3/11 (27)	2	Anatum (1), Enteritidis (1)
Albino yellow headed temple turtle	0/1 (0)	-	-
Asian leaf turtle	0/6 (0)	-	-
Florida softshell turtle	0/1 (0)	-	-
Reeves turtle	0/2 (0)	-	-
Painted terrapin	0/4 (0)	-	-
Four eye spotted turtle	0/1 (0)	-	-
Asian black tortoise	2/4 (50)	2	Agona (1), 43:z_4,z_{23}:- (1)
Asian forest tortoise	0/1 (0)	-	-
Asian brown tortoise	1/5 (20)	-	-
African helmeted turtle	0/1 (0)	-	-
Giant Asian pond turtle	3/9 (33)	2	Enteritidis (1), 60:r:z (1)
Siamese (Asian) box turtle	3/11 (27)	3	Kedougou (1),65:lv:z (1), 4,5,12:b:-(1)
Yellow margined (Chinese) box turtle	0/1 (0)	-	-
Eastern box turtle	0/1 (0)	-	-
Elongated tortoise	3/10 (30)	1	48:k:1,5 (1)
Aldabra giant tortoise	0/7 (0)	-	-
Bornean river turtle	0/1 (0)	-	-
Big headed turtle	0/2 (0)	-	-
Northern river terrapin	0/6	-	-
Unclassified turtle	1/2 (50)	1	Give (1)

Table 7 Number of *Salmonella* spp. and serovars isolated from captive reptiles **(lizards and crocodile)** in four zoos of the Zoological Park Organization, Thailand between 2017 and 2018

Animal Sources	Number of *Salmonella* per sample sizes (%)	Number of serotyping	Serovar (number)
Lizards and Crocodile (n = 33,1)			
Bearded dragon lizard	2/3 (33)	1	47:k:z_{35} (1)
Blue tongued skink	2/4 (50)	1	Corvallis (1)
Sailfin dragon lizard	0/2 (0)	-	-
Basilisk (Jesus) lizard	0/1 (0)	-	-
Rhinoceros iguana	1/1 (100)	1	48:z:- (1)
Uromastyx lizard	0/1 (0)	-	-
Agama lizard	1/1 (100)	1	47:k:z_{35} (1)
Common iguana	7/12 (58)	4	Tennessee (1), 48:i:z_{35} (1) 47:k:z_{35} (1), 50:k:- (1)
Monkey tailed skink	0/1 (0)	-	-
Savannah monitor	2/2 (100)	1	Rissen (1)
Nile monitor	0/1 (0)	0	0
Chinese water dragon	2/2 (100)	2	Tennessee (1), Kedougou (1)
Dumeril's monotor	1/1 (100)	1	38:z_4z_{23}:- (1)
Black water monitor	1/1 (100)	1	48:i:z_{35} (1)
Common caiman (*Caiman crocodylus*)	0/1 (0)	-	-

Discussion

The result of the examination of *Salmonella* spp. from four species of reptiles showed that the greatest *Salmonella* isolation was in snakes followed by lizards and turtles (Table 2). It was found that rate of the isolation of *Salmonella* in each zoo was very similar. Dusit had the highest rate (39.84%)and was followed by Nakhonratchasima (39.68%), Chiangmai (28.13%) and Songkhla Zoo (23.53%) (Table 2). Sixty-two of the *Salmonella* spp. isolates from 93 isolates were serotyped and there were four subspecies and 32 serovars of *Salmonella* spp. (Table 3). The previous report of the prevalence of *Salmonella* in Thailand revealed that it was 80% (16/20), from cobras in the Pasteur Institute at the Thai Red Cross Society and 39.2% (20/51) isolated from wild caught snakes (Prapasarakul *et al*., 2006) and this increased to 100% (128/128) in both captive (70/70) and wild caught snakes (58/58) in 2012 (Prapasarakul *et al*., 2012), while a study in Malaysia found only 38% (16/42) of infection from captive and wild snakes (Abatcha *et al*., 2013). There was 67.37% (62/92) of *Salmonella* in common iguanas from Dusit zoo and an animal hospital in Thailand (Bangtrakulnonth *et al*., 1998) and 87.5% (14/16) in iguanas from Chiangmai Zoo in the same year (Nimtrakul *et al*., 2003); while this study could isolate 57.58% (19/33) in lizards whereas 58% (7/12) in iguanas which is fewer. Turtles in this study revealed 13.70% (20/146) of *Salmonella* while a study in Korea found 50% (17/34) (Back et al. 2016) and China found 18.9% (31/164) (Zhang *et al*., 2016). Various isolations in previously mentioned studies were caused by multiple factors such as geography, environment, animal species and approaches used to collect the samples and culture the bacterium. Isolation of a high percentage of *Salmonella* in reptiles may be done directly from feces and water contaminated with feces (Back *et al*., 2016). The selection of more than one colony of the bacteria similar to *Salmonella* to be biochemically tested will increase the chance of the isolation with more than one serovar in the same sample (Bauwens *et al*., 2006).

The results of the serological tests categorized *Salmonella* into 32 serovars. It was discovered that the most found subspecies was *S. diarizonae* with 29 serovars followed by *S. enterica*, *S. houtenae*, and *S. arizonae*. (Table 4). *S. diarizonae* was found the most subspecies (54.28%) followed by *S. enterica* (34.28%) from snakes in this study which is different from the examination in the Pasteur Institute at the Thai Red Cross Society which found 95.09% (155/163) of *S. enterica* and only 4.91% (8/163) of *S. diarizonae* in cobras (Prapasarakul *et al*., 2012). While Malaysia found *S. enterica* in all cases of pythons (Abatcha *et al*., 2013). This resulted in various ratios of the isolation. The 48:i:z_{35} and 58:r:z_{53} as the most found serovars (6.45%:4/62) (Table 4). It reported the isolation of 58:r:z_{53} in cobras from the examination previously (Prapasarakul *et al*., 2012).

S. enterica as a subspecies was most found (64.28%:9/14) in turtles corresponding with the examination in China (Zhang *et al*., 2016). The subspecies was relative to diseases in humans following by *S. diarizonae* (4/14%:28.57). The results showed that the samples from turtles consisted of 11 serovars and the most found serovar was Enteritidis (21.42%) (Table 4). It was reported that *S.* Enteritidis was a cause of multi-country outbreak in humans (National Institute of Health of Thailand, 2016). *S.* Kedougou was the same serovar that was isolated from turtles in China (Zhang *et al*., 2016). Reports in multiple countries showed that the patients with Salmonellosis had direct contact with turtles. A baby was diagnosed with meningitis and septicemia after having a physical contact with their pet turtles (Meervenne *et al*., 2009; Ricard *et al*., 2015). There was a spread of *S.* Agbeni in 13 states in the USA and the cause was from direct contact with small turtles (Center for Disease Control and Prevention, 2017). Even though the U.S. Food and Drug Administration has set a restriction on selling and distributing turtles smaller than 4 inches since 1975 (US Food and Drug Administration, 2015), the spread of *Salmonella* from the contact with turtles still happens in the country every year (Walters *et al*., 2016). However, in this study no such *S.* Agbeni infection was found.

In lizards, *S. enterica* and *S. diarizonae* were equally found (46.15%), *Salmonella* could be categorized into 9 serovars, and the most found serovar was 47:i:z_{35} (23.07%) (Table 4). This serovar was also reported to cause human disease (Center for Disease Control and Prevention, 2015b). The isolation of *Salmonella* from iguanas in Thailand in 1998 showed *Salmonella* with 20 serovars (Bangtrakulnonth *et al*., 1998) and in Chiangmai Zoo in 2001 six serovars (Nimtrakul *et al*., 2003) were found. Nevertheless, the results of this study did not show any similar serovar to the previous study (Table 7). The difference of the results might be because of the smaller number of the samples from iguanas, despite of the fact that it was conducted in the same areas which were Chiangmai and Dusit Zoos. There was also a difference in serovars in the collected samples from this examination because the previous two studies were conducted with iguanas only while this examination was conducted with various species of lizards which resulted in the variety of serovars. However, this study found *S.* Rissen in Savannah monitors from Dusit which was the same serovar found in iguanas from Dusit in the previous study (Table 7). In addition, it cannot be identified as a local bacterium or that there was a co-transmission; it was only an observation to prevent contamination and transmission between species.

The results showed that animals in the same species and area could have the same serovar such as 48:i:z_{35} which was found in two Green Pit vipers in Nakhonratchasima Zoo. It was also possible that the same animal species may have different serovars. For instance, 65:lv:z and 48:lv:1,5 could be found in Striped-tailed rat snakes, and *S.* Uganda and *S.* Cerro could be found in Corn snakes. Additionally, different serovars can be found in the same animal species which were captive in the same area, such as two Golden pythons from Nakhonratchasima could have both serovar 38:z_4z_{23}:- and 48:i:z_{35} (Table 5). It was also found that the same serovar can be found in more than one species of animals in the same zoos. *S.* Agona was found in Asian Black tortoises and Reticulated Pythons in Nakhonratchasima; serovar 60:r:z was found in

Albino Siamese cobras, Asian Black tortoises and Giant Asian Pond turtles in Dusit, and 47:k:z_{35} in Bearded Dragon lizards, agama, iguana, and Golden Tree snakes (Table 5-7). Some serovars can be found in more than one zoo such as 48:k:z_{35} which can be found in Nakhonratchasima and Dusit and 47:k:z_{35} in Chiangmai and Dusit. This study showed that each serovar can have a reservoir host in many different animals and disperse of the serovars in many areas. The result of this study was different from previous studies because the samples were collected from various animals, and some animals had never been examined before which resulted in a variety.

The comparison among the subspecies isolated showed that these reptiles are the resource of *S. enterica* (43.55%), and are related to diseases in mammals and humans. *Salmonella* can disperse via an animal's feces or eggs and can be contaminated in the soil, water, and the environment, and it can live for more than two years (Chiodini and Sundberg, 1981). Although the report of the occurrence of Salmonellosis from direct contact with reptiles in Thailand might not be much different from other countries for there has never been a serious study, Thailand is a source of *Salmonella*. While the data about *Salmonella* studies in the aspect of zoonosis is mostly about looking for a connection between livestock development sector and community, there is still a lack of information about the connection of reptile-associated human Salmonellosis. This study showed that the reptiles are the source of *Salmonella* and indicates that it is essential to carefully observe and emphasize the risk of the infection to humans in order to raise awareness of those who are involved to set preventive measures and regulations to prevent the spread of *Salmonella* from reptiles to the community.

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