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Rodents as a Source of *Salmonella* Contamination in Wet Markets in Thailand

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Abstract

Background: Few studies have been conducted on the presence of *Salmonella* in the rodents that inhabit the wet markets that play an important role in daily life in Southeast Asia. The results of studies of rodents as carriers of *Salmonella* vary greatly, ranging from an absence of *Salmonella* to high prevalences. Previous studies investigated habitats such as farms and urban and wild areas where there is less rodent-human interaction than in wet markets. Consequently, the potential role of rodents as reservoirs and transmitters of *Salmonella* in wet markets is of great interest.
Methods: Rodents were trapped in eight traditional wet markets in Thailand and identified to species level. Subsequently, they were screened for *Salmonella* and isolates were serotyped.
Results: A total of 110 rats (*Rattus norvegicus* and *Rattus exulans*) were examined. Overall, the prevalence of *Salmonella* in rats was 49.10%, but varied between 0% and 73.3% among markets. Three serovars were identified: *Salmonella* Typhimurium (30%), *S.* Weltevreden (12.7%), and *S.* 4,[5],12:i:- (6.4%).
Conclusions: Our results show that rodents in wet markets are a potential reservoir of *Salmonella* due to the close contact they have with humans and food. The three isolated serovars, of which serovar *S.* 4,[5],12:i:- is reported for the first time in rodents, are among the 10 commonest serovars isolated from humans in Thailand. Thus, more attention should be paid to rodents as potential reservoirs of *Salmonella*.

Key Words: Norway rat—Pacific rat—*S.* 4,[5],12:i:-—*Salmonella*—Typhimurium—Weltevreden—Wet market—Zoonoses.

Introduction

Salmonella is regarded as one of the most prevalent foodborne and diarrheal diseases in the world (Meerburg et al. 2006), and is a major public health problem in tropical countries such as Thailand (Minami et al. 2010).

Traditional wet markets are part of daily life in Southeast Asian countries such as Thailand. They primarily sell fresh products such as meat, vegetables, and fruit, although cooked food can also be found. Markets are undercover and most vendors have a permanent stall where they display their products on a cloth placed on the ground. The presence of commensal rodents is common and so any study of possible rodent-borne zoonoses will be of great relevance to public health.

Rodents have been found to be responsible for salmonellosis outbreaks (Lee et al. 2008). However, few studies on commensal rodents as sources of infection in humans have ever been conducted. Some attempts to examine the role of rodents as reservoirs of *Salmonella* have been made for certain food animals (mainly pigs and poultry) on farms (reviewed by Meerburg and Kijlstra 2007). Recently, a further study by Andrés-Barranco et al. (2014) on pig farms has been published. Although a few studies have tested rats for *Salmonella* in urban habitats (Hilton et al. 2002, Yokoyama et al. 2007, Antoniou et al. 2010), little work has been conducted on rodents in the wild (reviewed by Meerburg and Kijlstra 2007). To the best of our knowledge, no studies have ever examined the presence of *Salmonella* in wet markets despite the proximity between rodents and the food on sale.

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Rodents are a public health concern as transmitters of several zoonoses, especially in Southeast Asia (Chaisiri et al. 2015). However, no efforts have been made to test for *Salmonella* in wet markets, even though rodents are known reservoirs for pathogens and can contaminate food. Such research is imperative since in tropical countries high food availability gives rise to stable rodent populations that breed continuously throughout the year.

Wet markets are ideal environments for commensal rats as food, water, and shelter are readily available throughout the year. Rats have few predators and so can exist in high densities. Rodent-to-rodent transmission of *Salmonella* is very likely to occur in this environment. Stable rat populations, unregulated by any extrinsic factors, will guarantee that persistent contamination occurs in these market environments. Consequently, the presence of *Salmonella* is maintained in these markets by rodents.

The aim of this study was thus to examine the possible occurrence of *Salmonella* in rodents collected in wet markets and to characterize the serovars that could be isolated from them.

Materials and Methods

The survey was conducted in mid-July–mid-August 2014. Traps for rodents were set in eight traditional wet markets in Udon Thani City (Udon Thani Province, Thailand). These pitfalls were made of wire mesh and baited with food to capture rodents alive. They were set by the research team with the help of local vendors in the afternoon and then collected at about 05.00, when sellers redisplay their goods and market activities restart. Once collected, traps were covered using black plastic and immediately transported to our laboratory at the Center of Science and Technology for Research and Community Development, Udon Thani Rajabhat University. The journey time from all surveyed markets was less than 30 min.

Trapped animals were euthanized and dissected following an international standard (American Veterinary Medical Association Council on Research). Rodent protocols that maximize animal care, guarantee the health and safety of field parasitologists, and allow the generation of quality data were used (Herbreteau et al. 2011). The study was approved by the Udon Thani Rajabhat University Animal Care and Ethical Use Committee.

A piece of muscle tissue from each rodent was preserved in absolute ethanol and used for molecular identification. The total genomic DNA of individual samples was extracted using the E.Z.N.A® Tissue DNA kit (Omega Bio-Tek) following the manufacturer's instructions. The mitochondrial CO1 region was amplified using primers and PCR conditions, as detailed by the Barcoding Tool/RodentSEAsection of the CERoPath project web site: www.ceropath.org.phology. All PCR products were gel-purified using an E.Z.N.A Gel Purification Kit (Omega Bio-Tek). The purified PCR products were cycle-sequenced using ABI BigDye v3.1 chemistry and run on an ABI Prism 377 automated sequencer (Applied Biosystems).

To examine for *Salmonella*, we followed protocol ISO 6579: 2002, modified according to the procedures of the National *Salmonella* and *Shigella* Center of the National Institute of Health of Thailand. About one gram of the content of the large intestine was pre-enriched in 10 mL Buffered Peptone Water 2.5% and incubated at 37°C ± 1°C for 18 ± 2 h. The pre-enriched samples were transferred onto Semi-Solid Modified Rappaport Vassiliadis agar plates (MSRV) and incubated at 41.5°C ± 1°C for 24–48 h. The culture obtained on the MSRV was inoculated onto xylose-lysine-desoxycholate (XLD) and incubated at 37°C ± 1°C for 24–48 h. Red colonies with a black spot in their centers were subcultured (one for plate) on XLD and incubated at 37°C ± 1°C for 24–48 h. Biochemical tests including Triple Sugar Iron, Lysine Iron Agar (LIA), and Sulfur-Indole-Motility medium were conducted. The purified colonies were transferred onto nutrient agar before serotyping by the National *Salmonella* and *Shigella* Center of the National Institute of Health of Thailand using the Kauffmann–White technique.

Results

A total of 110 rodents were trapped in the eight wet markets. Molecular tests showed that the rats belonged to two species: Norway rats (*Rattus norvegicus*) ($n = 99$) and Pacific rats (*Rattus exulans*) ($n = 11$). The number of rodents collected in each market varied from 7 to 22 (Table 1).

Rats from seven of the eight studied markets were positive for *Salmonella* (Table 1). The overall prevalence of *Salmonella* was 49.10%, but varied between 0% and 73.3% among markets. A total of three serovars were identified: *Salmonella* Typhimurium (30%), *S.* Weltevreden (12.7%), and *S.* 4,[5],12:i:- (6.4%) (Table 1). The serotyps *S.* Typhimurium and *S.* Weltevreden were detected in both *R. exulans* and *R. norvegicus*, while *S.* 4,[5],12:i:- was only found in Norway rats.

Discussion

These two rodent species are synanthropic and act as reservoirs in the spread of several diseases (Morand et al. 2015; Wells et al. 2015). Studies on the presence of *Salmonella* in rodents show great variability in prevalences, which range from absence (Meerburg and Kijlstra 2007), low prevalence (Meerburg et al. 2006, Runge et al. 2013, Himsworth et al. 2015) to high prevalence (Antoniou et al. 2010; Andrés-Barranco et al. 2014; Himsworth et al. 2015).

However, the aforementioned studies were performed in scattered areas with highly variable sanitary conditions and so comparisons between studies are difficult. Additionally, sampling habitats were highly variable, varying from natural areas to organic farms, thereby making comparisons even more of a challenge. The present study sampled sites in which rodents live in great proximity to humans and where, due to constant human presence and high rodent density, the probability of contact between rodents and/or their feces with food and environment is greater than in previous studies. To the best of our knowledge, this is the first study of *Salmonella* from rodents captured in wet markets; to date, none of the few studies of zoonotic parasites carried out on rodents in wet markets have dealt with *Salmonella* (Claveria et al. 2005; Paramasvaran et al. 2009; Tung et al. 2013).

The three reported serovars are among the 10 most commonly isolated serovars in humans in Thailand (Pulsrikarn and Tishyadhigama 2008). Therefore, we suspect that rodents play a role as a source of contamination in wet markets due to the high prevalences and wide distributions found in this study.

Table 1. Frequency of Isolation of *Salmonella* Serotypes from Rats in Wet Markets

Market	Rattus norvegicus	Rattus exulans	*S. Typhimurium*		S. *Weltevreden*		*S. I. 4,5,12:i:-*		*General prevalence*
			R. norvegicus	R. exulans	R. norvegicus	R. exulans	R. norvegicus	R. exulans	
1	13	2	2 (13.33%)	1 (6.67%)	3 (20.00%)	0 (0%)	3 (20.00%)	0 (0%)	60% (CI:32.3–83.7)
2	14	4	2 (11.11%)	2 (11.11%)	2 (11.11%)	0 (0%)	1 (5.56%)	0 (0%)	38.89% (CI:17.3–64.3)
3	19	3	8 (36.36%)	0 (0%)	4 (18.18%)	1 (4.55%)	1 (4.55%)	0 (0%)	63.64% (CI:40.7–82.8)
4	8	0	4 (50%)	0 (0%)	0 (0%)	0 (0%)	1 (12.50%)	0 (0%)	62.5% (CI:24.5–91.5)
5	15	0	7 (46.67%)	0 (0%)	4 (26.67%)	0 (0%)	0 (0%)	0 (0%)	73.33% (CI:44.9–92.2)
6	12	0	2 (16.67%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	16.67% (CI:2.1–48.4)
7	5	2	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0% (CI:0–41.0)
8	13	0	5 (38.46%)	0 (0%)	0 (0%)	0 (0%)	1 (7.69%)	0 (0%)	46.15% (CI:19.2–74.9)
Subtotals	99	11	30 (30.30%)	3 (27.27%)	13 (13.13%)	1 (9.09%)	7 (7.07%)	0 (0%)	—
Totals	110		33 (30% CI:21.6–39.5)		14 (12.7% CI:7.1–20.4)		7 (6.4% CI:2.6–12.7)		49.10% (CI:39.4–58.8)

S. Typhimurium and *S.* Enteriditis are the two serovars, most frequently associated with human disease (Hendriksen et al. 2011). Norway and Pacific rats have spread worldwide due to anthropogenic factors and are common in China (Songet al. 2014) and Southeast Asia (Thomson et al. 2014), respectively. This spread cannot explain the origin of this serovar in Southeast Asia and China as other vertebrates (e.g: migrant birds or poultry) could have a role. Future studies aiming to determine the origin should target *Salmonella* in rodents and other vertebrates (wild and domestic) in Southeast Asia.

The second isolated serovar, *S.* Weltevreden, has been reported as a frequent and increasingly common cause of human *Salmonella* infection in Southeast Asia (Bangtrakulnonth et al. 2004, Galanis et al. 2006). Outside Southeast Asia, an outbreak on Réunion Island was recorded as causing acute gastroenteritis, vomiting, and fever (D'Ortenzio et al. 2008). The source of this food poisoning was never identified despite the epidemiological surveillance carried out. Nevertheless, water was also postulated as the possible source of this serovar (D'Ortenzio et al. 2008).

The third isolated serovar, *S.* 4,[5],12:i:-, is reported here for the first time in rodents. This serovar is considered either to be a variant of *S.* Typhimurium or to have a close common ancestor (de la Torre et al. 2003). We cannot assess the role of rodents in the rapid spread of this serovar over the past few years (de la Torre et al. 2003) as several possible sources should be explored. As a complement to our study, future studies of *Salmonella* in rodents should focus on wild rodents in Southeast Asia as a means of improving knowledge of the distribution of serotypes in nonanthropogenic habitats taking in account that other vertebrates as source of *Salmonella* should not be neglected.

Our results show that fecal contamination of food by rodents in wet markets could play a major role in *Salmonella* transmission. Even though several preventive measures have been taken to reduce the incidence of human salmonellosis, none will be of any value if the final sales points are not controlled. Thus, special regard should be paid to wet markets since a large proportion of food-borne salmonellosis is preventable. All prevention efforts will be futile if food markets continue to be contaminated.

Conclusions

Even though rodents have never previously been included in studies of the sources of *Salmonella*, we show here the potential of rodents to act as vectors of salmonellosis in Asian wet markets, which are part of the daily lives of many people.

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Author Disclosure Statement

No competing financial interests exist.

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