SALMONELLOSIS AND THE FOOD CHAIN IN KHON KAEN, NORTHEASTERN THAILAND

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Abstract. Non-typhoidal salmonellosis is a major cause of food-borne illness in Thailand. Specific serotyping of Salmonellae, linked with certain foods, can be used to identify outbreaks, transmission, and for surveillance. We aimed to identify the chain of non-typhoidal *Salmonella* transmission from food to humans in five slums, two open markets, four supermarkets and an abattoir in the municipality of Khon Kaen. During three months representing the cool-dry, hot-dry, and rainy seasons of 2002, culture samples were collected from water, food, pork, and chicken. Stool cultures of food venders, and others in the same area, were performed. Serological typing was done by the WHO National Salmonella and Shigella Center in Thailand. Of the food, drinking water, and stool samples from food handlers and healthy persons, 18, 7, 11, and 5%, respectively, were positive for *Salmonella*. Nearly all (96-98%) of the fresh pork and chicken, both from the open markets and supermarkets, were positive for *Salmonella*. The major *Salmonella* serovars were *S.* Anatum, *S.* Rissen, *S.* Virchow, *S.* Enteritidis and *S.* Panama, similar throughout the food chain and to the other reports that year. To reduce the incidence of human salmonellosis, several preventative measures must be taken where animals are produced, slaughtered and processed, and at home and in eateries. Vulnerable groups, such as infants, the elderly and the immuno-compromised, should be made aware of their increased susceptibility to food-borne disease.

INTRODUCTION

Food-borne diseases caused by non-typhoidal salmonellosis persists despite progress in public education (Gomez *et al*, 1997). The primary reservoir of *Salmonella* is the intestinal tract of infected or colonized humans and domestic and wild animals. Livestock and farm animals may be infected through contaminated feed, animal-to-animal transmission or by a contaminated environment. Humans can be infected by ingestion of contaminated food and water (Guthrie, 1992). Food of animal origin, such as poultry, pork, beef, eggs, and milk, are the main vectors of infection. Contamination of these foods can occur during production, processing, distribution, and preparation. Humans can be infected person-to-person (Stone *et al*, 1993) and via contact with animals, including pets (CDC, 1995; Friedman *et al*, 1998)

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There are 2,449 known serotypes of *Salmonella* (Breuner *et al*, 2000). Serotyping is a useful classification scheme that allows identification of outbreaks, transmission, and trends for *Salmonella* surveillance. Recent data indicate that specific serotyping is linked with certain foods or types of exposure. Outbreaks of *S.* Enteritidis are repeatedly associated with raw or undercooked eggs and poultry (St Louis *et al*, 1988; Mishu *et al*, 1991; NSSC, Thailand, 2002), and *S.* Marina with exposure to reptiles (CDC, 2003).

Investigations in Thailand over the last two decades reveal that salmonellosis is the leading cause of infective bacterial diarrhea (Phan-Urai, 1978; NIH Thailand, 2001). In the province of Khon Kaen (population 2 million), Northeast Thailand, *Salmonella* is the leading reported bacteria isolated from stool cultures of diarrhea (Table 1).

The present study was undertaken to determine the chain of non-typhoidal *Salmonella* transmission in the food supply and the carrier status of food handlers in the Municipality of Khon Kaen, and to develop control measures to reduce related morbidity and economic losses in the region.

MATERIALS AND METHOD

Six slum communities, two food markets, three supermarkets and a slaughter house in the Municipality of Khon Kaen were identified as the study sites. During the months of February, June, and October, 2002, months representing the hot-dry, rainy, and cool-dry seasons, respectively, we took swab cultures at random from various foods from street venders in the slums, from fresh pork and chicken at an abattoir, open markets and supermarkets. Stool samples of food handlers and healthy persons in the same area were cultured. All swabs were transported, in Blair transport medium, at room temperature, to a microbiological laboratory within three hours of sampling. Isolation of bacteria was accomplished by the Microbiological Unit of the Department of Public Health. The Salmonellae were identified by routine biochemical tests.

Serological testing was performed at the National Salmonella and Shigella Center, Thailand. The swab culture was obtained after fully explaining the objectives and obtained informed consent from participants. The identity of participants and localities (eateries) in the study were kept confidential.

Table 1
Annual (2000-2002) numbers and percentages in parentheses of bacterial isolates from stool/rectal swab cultures of diarrhea cases at health service centers in Khon Kaen, Thailand.

Organisms	Year		
	2000	2001	2002
Salmonella spp	345 (62.8)	112 (29.5)	336 (32.0)
Vibrio cholerae 01, Inaba	43 (7.8)	35 (9.2)	235 (22.4)
Vibrio cholerae 01, Ogawa	6 (1.1)	0	0
Vibrio cholerae 0139	1 (0.2)	0	0
V. parahaemolyticus	73 (13.3)	204 (53.6)	188 (17.8)
Aeromonas spp	29 (5.3)	12 (3.2)	120 (11.4)
Plesiomonas spp	15 (2.7)	1 (0.3)	94 (8.9)
Shigella spp	29 (5.3)	7 (1.8)	29 (2.8)
Others	8 (1.5)	9 (2.4)	49 (4.7)
Total	549	380	1,051

Table 2
Number of strains of *Salmonella* isolated from food and drinking water in five communities in Khon Kaen, 2002.

Community	Food		Drinking water	
	Specimens	*Salmonella* isolation (%)	Specimens	*Salmonella* isolation (%)
1	84	12 (14.3)	16	2 (12.5)
2	74	14 (18.9)	18	0
3	69	16 (23.2)	16	0
4	105	20 (19.0)	14	2 (14.3)
5	71	12 (16.7)	18	2 (11.1)
Total	403	74 (18.4)	82	6 (7.3)

Table 3
Number of *Salmonella* strains by sex and age, isolated from food handlers and others in five communities in Khon Kaen 2002.

	Food handlers		Healthy population	
	Specimens	*Salmonella* isolation (%)	Specimens	*Salmonella* isolation (%)
Sex				
Males	14	6	235	11 (4.7)
Females	76	4 (3.3)	168	8 (4.8)
Age (year)				
<30	14	2 (14.3)	96	0
31-40	20	4 (20.0)	170	12 (7.1)
41-50	35	3 (8.6)	95	5 (5.3)
51-60	21	1 (4.8)	32	2 (6.3)
>60	-	-	10	0
Total	90	10 (11.0)	403	19 (4.7)

Table 4
Salmonella serogroups isolated from food handlers and others in Khon Kaen 2002.

Salmonella serogroup	Food handlers Strains (%)	Others Strains (%)
Group B	0	3 (15.7)
Group C	4 (40)	4 (21.1)
Group D	5 (50)	4 (21.1)
Group E	1 (10)	8 (42.1)
Total	10	19

RESULTS

Salmonella isolation from food and drinking water.

During the three study months, we took 403 samples from various kinds of ready-to-eat foods and 82 samples of drinking water or food from street venders in five slums. Seventy-four (18.4%) of food and six (7.3%) of drinking water samples were positive for *Salmonella* (Table 2). The majority of positive cultures from food were hot salads made of raw vegetables, chili, and

Table 5
Number of strains and serogroups of *Salmonella* isolated in Khon Kaen 2002 from pork and chicken from fresh markets during the months of February, June and October.

Month	Pork			Chicken		
	Specimen	*Salmonella*		Specimen	*Salmonella*	
		Positive(%)	Serogroup[a]		Positive (%)	Serogroup[a]
February	32	29 (90.6)	E 18 C 13 D 7 B 2	28	28 (100)	D 17 C 18 B 6 E 6
June	34	34 (100)	E 31 C 9 D 7 B 3 G 2 I 1	30	28 (93.3)	C 21 D 10 E 7 B 5 G 1 I 2
October	25	24 (96.0)	E 13 C 10 D 5 B 2 G 1	26	25 (96.2)	C 16 B 5 D 3 E 3 G 1
Total	91	87 (95.6)	124	84	81 (96.4)	121

[a]some specimens yielded more than one *Salmonella* serogroup.

Table 6
Salmonella serovars isolated from pork and chicken from fresh markets in Khon Kaen, Thailand.

Pork		Chicken	
Serovar	Strains (%)	Serovar	Strains (%)
S. Anatum	57 (45.9)	*S.* Virchow	31 (25.6)
S. Rissen	30 (24.2)	*S.* Enteritidis	21 (17.4)
S. Panama	17 (13.7)	*S.* Panama	9 (7.4)
S. Albany	2 (1.6)	*S.* Hadar	9 (7.4)
S. Amsterdam	2 (1.6)	*A.* Anatum	7 (5.8)
S. Derby	2 (1.6)	*S.* Blockley	6 (4.9)
S. Subspecies I $_{9, 12, 1v}$:-	2 (1.6)	*S.* Agona	4 (3.3)
S. Worthington	2 (1.6)	*S.* Albany	3 (2.5)
S. Subspecies I $_{4, 5, 12:i}$:-	2 (1.6)	*S.* Saintpaul	3 (2.5)
S. Saintpaul	2 (1.6)	*S.* Typhimurium	3 (2.5)
Others	6 (5.0)	*S.* Stanley	3 (2.5)
		Others	22 (18.2)
Total	124	Total	121

Table 7
Salmonella serovars and serogroups isolated in 2002 from pork and chicken from Khon Kaen supermarkets.

Pork		Chicken	
Serovar (serogroup)	Strains (%)	Serovar (serogroup)	Strains (%)
S. Anatum (E)	43 (32.6)	*S.* Rissen (C)	12 (15.8)
S. Rissen (C)	34 (25.8)	*S.* Virchow (C)	12 (15.8)
S. Panama (D)	10 (7.6)	*S.* Anatum (E)	12 (15.8)
S. Weltevreden (E)	5 (3.8)	*S.* Panama (D)	5 (6.6)
S. Worthington (G)	5 (3.8)	*S.* Stanley (B)	5 (6.6)
S. Derby (B)	4 (3.0)	*S.* Hadar (C)	4 (5.3)
S. Stanley (B)	4 (3.0)	*H.* Havana (G)	4 (5.3)
S. Virchow (C)	3 (2.5)	*S.* Enteritidis (D)	3 (3.9)
S. Cerro (K)	3 (2.5)	*S.* Scwarzengrund (B)	2 (2.6)
S. Albany (C)	2 (1.5)	*S* Blockley (C)	2 (2.6)
S. Scwarzengrund (B)	2 (1.5)	*S.* Senftenberg (E)	2 (2.6)
S. Typhimurium (B)	2 (1.5)	*S.* Weltevreden (E)	2 (2.6)
S. Havana (G)	2 (1.5)		
Others	13 (9.8)	Other	11 (14.5)
Total	132 (100)	Total	76 (100)

partially cooked pork or chicken. Nearly all of the well-cooked foods were negative for *Salmonella*.

Salmonella isolation from food handlers and healthy persons

During the same period and in the same communities, ninety stool samples from food handlers (mostly street venders) were sent for *Salmonella* isolation. Ten of these specimens (11%) were positive. The *Salmonella* serogroups are shown in Tables 3 and 4. Stool samples from the other adults in the same area were sent to the same laboratory. Of the 403 specimens, 19 (4.7%) were positive for *Salmonella*. All *Salmonella* strains were in serogroups B, C, D, and E.

Table 8
Salmonella strains, serogroup and serovars isolated in 2002 from pork and chicken and internal organs from the Khon Kaen abattoir.

	Salmonella isolated from chicken				*Salmonella* isolated from pork			
	Specimens	Strains (%)	Serogroup	Serovars (No.)		Specimens	Strains (%)	Serogroup	Serovars (No.)
Meat	20	14 (70)	B	*S.* Derby (2) *S.* Brandenburg (1)	Meat	20	17 (85)	C	*S.* Rissen (4)
			C	*S.* Virchow (3) *S.* Blockley (1)				E	*S.* Anatum (12) *S.* London
			D	*S.* Enteritidis (2) *S.* Panama (1)	Liver	20	14 (70)	B	*S.* Derby (3)
			E	*S.* Amsterdam (4)					*S.* Anatum (11)
Internal organs	20	17 (85)	B	*S.* Agona (2) *S.* Schwarzengrund (1)	Internal organs	20	11 (55)	E	*S.* Anatum (10)
			C	*S.* Virchow (2) *S.* Blockley (2)				E	*S.* Senftenberg (1)
				S. Rissen (2)					
			D	*S.* Enteritidis (2)					
			E	*S.* Anatum (6)					
Total	40	31 (77.5)			Total	60	42 (70)		

Salmonella isolation from pork and chicken in fresh markets and supermarkets

Ninety-one and 84 swab cultures were taken from fresh market pork and chicken, respectively. Eighty-seven (95.6%) and 81 (96.4%) of the pork and chicken swabs, respectively, tested positive for at least one serogroup of *Salmonella* (Table 5). The chances of getting a positive culture were comparable, irrespective of the season of the year. The *Salmonella* serogroups E and C predominated in the pork, while D and C predominated in the chicken. The most common serovars for pork *vs* chicken were *S.* Anatum, *S.* Rissen and *S.* Panama *vs S.* Virchow, *S.* Enteritidis and *S.* Panama, respectively (Table 6).

Similar results were obtained when we conducted the study in chain supermarkets, which had better sanitation than the open markets. *Salmonella* isolation was positive in 98% of 66 pork and 92% of 48 chicken specimens. The majority of the serovars for pork *vs* chicken were *S.* Anatum, *S.* Rissen and *S.* Panama *vs S.* Anatum, *S.* Rissen and *S.* Virchow (Table 7); comparable to what was found in the open markets.

Salmonella from pork and chicken at a slaughter house

The only registered abattoir in Khon Kaen still uses unhygienic slaughter and dressing procedures. For example, there is no *ante mortem* veterinary inspection, no appropriate evisceration area and no refrigeration. Twenty swabs were taken randomly from pork, pork liver and pork intestinal organs and 20 from chicken and their intestinal organs. Seventeen (85%) of the cultures from the pork swabs yielded strains of *Salmonella,* 14 (70%) from pork liver and 11 (55%) from the pork intestinal organs. The predominant serovars were *S.* Anatum and *S.* Rissen (Table 8). By comparison, 14 strains (70%) of *Salmonellae* were isolated from the chicken and 17 (85%) from their intestinal organs; the predominant serovars being *S.* Virchow, *S.* Enteritidis and *S.* Anatum (Table 8).

DISCUSSION

Pork and chicken are the main animal-proteins consumed by people in this area. The study

Table 9
Annual report of serogroups and serovars of *Salmonella* isolated from stool/rectal swab cultures of diarrheal cases submitted from hospitals in Khon Kaen and Region 6 Northeast Thailand, 2002.

Region 6			Khon Kaen		
Serovar	Serogroup	Strains (%)	Serovar	Serogroup	Strains (%)
S. Anatum	E	120 (27.9)	*S.* Rissen	C 1	22 (16.5)
S. Rissen	C	62 (14.4)	S. Weltevreden	E 1	12 (9.0)
S. Virchow	C	46 (10.7)	*S.* Enteritidis	D 1	11 (8.2)
S. Enteritidis	D	32 (7.4)	*S.* Anatum	E 1	10 (7.5)
S. Panama	D	29 (6.7)	*S.* Derby	B	9 (6.7)
S. Hadar	C	17 (3.9)	S. Stanley	B	9 (6.7)
S. Derby	B	10 (2.3)	*S.* Panama	D 1	7 (5.2)
S. Amsterdum	E	9 (2.1)	S. 14, 12, I	B	6 (4.5)
S. Saintpaul	B	9 (2.1)	*S.* Braenderup	C 1	5 (3.7)
S. Weltevreden	E	8 (1.8)	*S.* Virchow	C 1	5 (3.7)
31 Other serovars		87 (20.2)	23 Other serovars		37 (2.7)
Total		429	Total		133

demonstrated epidemic *Salmonella* contamination of the food chain, especially on these meats, from the abattoir, to market and ready-to-eat foods. Contamination is sufficient to affect the end-of-the-line consumers. This perhaps explains why 10% of food handlers and 5% of other people in the area are *Salmonella* carriers.

The serovars and serogroups of *Salmonella* isolated along the food chain were similar. The major serogroups were B, C, D and E, while the majority of serovars were *S.* Anatum, *S.* Virchow, *S.* Rissen, *S.* Enteritidis and *S.* Panama. That year, the Northeast Public Health Station also reported these same serovars as infecting humans (Table 9).

The increasing incidence of non-typhoidal salmonellosis may be the result of industrialization and urbanization. Higher incomes and standards of living have led to an increase in consumption of animal protein. The extensive breeding and raising of animals has resulted in subclinical infection of these animal with *Salmonella* and *Campylobacter* (WHO, 2000).

During the economic transition, increased unemployment and changes in eating habits led to a rapid expansion of street food-vending. Persons involved in the preparation and sale of food often lack knowledge of food safety and hygiene. Street food has been recognized as an important vehicle for the transmission of food-borne disease in Asia (Swaddiwudhipong *et al*, 1992; WHO, 1995).

Inadequate knowledge and poor implementation of standards, unhygienic slaughter, dressing processes, and transportation have led to massive contamination and infection among both food handlers and consumers in Northeast Thailand.

A large proportion of food-borne salmonellosis is potentially preventable. Several measures should be taken during the production, processing, distribution, retail marketing, and handling of food to prevent the introduction and multiplication of *Salmonella* (Gomez *et al*, 1997). Food industries, including slaughterhouses, packers and processors, should be encouraged to develop Hazard Analysis and Critical Control Point (HACCP) programs (WHO, 1988). The consumers, the public, and especially food handlers, need education regarding safe food handling. Vulnerable groups, such as infants, the elderly, and immuno-compromised, should be made aware of their increased susceptibility to food-borne salmonellosis (Gomez *et al*, 1997). Control measures and effective surveillance programs should be supported.

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