

Salmonella in food animals and humans in northern Thailand

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Abstract

A study was conducted to describe the epidemiology of *Salmonella* spp. in chickens, pigs, dairy cows, farm workers with and without livestock contact, and children with diarrhea. Samples were collected in the Chiangmai and Lampoon provinces of northern Thailand during 2000–2003. A total of 2141 samples were processed. The prevalences of *Salmonella* in chickens at the farm, slaughterhouse and chicken meat at the market were 4%, 9% and 57%, respectively. In pigs, the prevalence at the farm, slaughterhouse and pork at the market were 6%, 28% and 29%, respectively. The prevalence of *Salmonella* in dairy cows was 3%. *Salmonella* was isolated from 36% of farm workers with livestock contact and 33% of those with no livestock contact, and from 7% of diarrheal children at the hospital. The longitudinal study of *Salmonella* in pigs showed that the incidences of *Salmonella* isolation at the farm, slaughterhouse, and market were 7%, 50% and 20%, respectively. The most frequently isolated serotypes of *Salmonella* were Weltevreden in chickens and humans, and Rissen in pigs. Serotypes varied between farm, slaughterhouse and market for isolates from chickens and pigs. Antimicrobial resistance was present in isolates from all types of animals and humans in the study, with widespread resistance to tetracycline and nalidixic acid. The proportions of resistant organisms among *Salmonella* from diarrheal children were high, and higher proportions of multi-drug resistant organisms were observed among *Salmonella* isolates from farm workers with livestock contact than among isolates from workers with no livestock contact.

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1. Introduction

In Thailand, reports have shown the relationship between *Salmonella* spp. in foods of animal origin and public health problems (Rasrinual et al., 1988; Boriraj et al., 1997; Boonmar et al., 1998a). *Salmonella* spp. are commonly found in chicken eggs (Saitanu et al., 1994), and chicken (Sasipreeyajan et al., 1996) and pork (Boonmar et al., 1997) meats at market. It has been speculated that the source of the increase in *S. Enterica* infection in humans in Thailand may be associated with the increased prevalence of *S. Enterica* in chickens (Sakai and Chalermchaikit, 1996), but the evidence has not been conclusive.

In addition to its pathogenicity, there has been concern about antimicrobial resistance in *Salmonella*, which has led to failure of treatment for *Salmonella* and other bacterial pathogens

(Travers and Barza, 2002; Butt et al., 2003). Since foods of animal origin are a major source of *Salmonella* spp., it has been suggested that antimicrobial use in food animal production may contribute to the presence of antimicrobial resistance in *Salmonella* spp. that infect humans (Cruchaga et al., 2001; Iovine and Blaser, 2004). Studies in Thailand have found antimicrobial resistance in *Salmonella* spp. isolated from food animals and humans (Boonmar et al., 1998a; Hanson et al., 2002), including resistance to relatively new antimicrobial agents such as azithromycin and ciprofloxacin (Isenbarger et al., 2002). However, there is limited information on associations between the *Salmonella* spp. isolated from food animals and those isolated from humans. The purpose of this study was to further investigate the epidemiology of *Salmonella* spp. in Thailand by determining the prevalence of *Salmonella* spp. in 1) a variety of food animal species and foods, from live animals on the farm to foods available at the market; 2) humans with high and low animal or animal product contact; and 3) farm, slaughterhouse and market environments associated with these animals. In

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addition to determining prevalence, serotypes of *Salmonella* from food animals and humans were compared to identify any common sources of infection for these groups. Finally, antimicrobial susceptibility testing was conducted to describe patterns of antimicrobial resistance in *Salmonella* from food-producing animals from farm to market, and in humans with high and low animal or animal product contact. Results from these studies could form a basis for risk assessment and future interventions intended to reduce the incidence of *Salmonella* in Thailand, and potential hazards from antimicrobial resistance in *Salmonella*.

2. Materials and methods

2.1. Study design

This study used *Salmonella* isolates collected during three phases of a larger project in which the epidemiology of *Salmonella* in food animals and humans in Thailand was examined. The phases were a cross-sectional phase, a longitudinal study phase, and a case-control phase. The cross-sectional study phase was conducted to estimate the prevalence of *Salmonella* in chickens, swine, dairy cows, farm workers, non-farm residents, and children hospitalized with diarrhea. The longitudinal study was conducted to identify potential *Salmonella* spp. contamination points in the pork production system from farm to market. The case-control study was conducted to identify risk factors associated with *Salmonella* spp. infection in children hospitalized with diarrhea. The relationship among *Salmonella* spp. isolated from various sources was determined by serotyping, and comparison of antimicrobial resistance in isolates from various animal species, locations and sample types. The number of individual animal samples collected from each farm was based on a true population prevalence of 50%, a 95% confidence level, and an estimated prevalence within 5% of the true prevalence on the farm (Smith, 1995).

2.2. Sample collection

A total of 2150 samples were collected and processed (Table 1), including 504 samples from humans. Samples were collected and processed during the period May 2000 to July 2003. Due to logistical constraints random sampling was not possible, and all sampling sites were selected to be located in the Chiang Mai and Lamphung provinces of northern Thailand within 3 h of the laboratory. These study locations included farms, slaughterhouses, fresh meat markets, and hospitals. In addition, the farms, slaughterhouses and markets selected needed to keep records so that animals could be tracked from the farm to the slaughterhouse, and subsequently from slaughter to the markets.

Individual animals were randomly selected for sampling based on their age and stage of production. Pigs <1 month old prior to slaughter, chickens <2 weeks old prior to slaughter, and milking cows were included in the on-farm study. The same pigs and chickens were tracked and sampled at the slaughterhouse and again at the markets. All workers on the farms participating in the study, and workers at the slaughterhouses involved in the study were asked to provide stool samples for the study. The parents of all children with diarrhea in the hospitals within the study area were contacted for participation while the children were hospitalized. Due to the low numbers of farm workers, slaughterhouse workers and children with diarrhea, efforts were made to contact and enroll all possible candidates. Approvals to conduct research involving human subjects and animals were given by the Chiang Mai University Committees on Human Subjects and Animal Research, respectively.

For the cross-sectional study, samples were collected once from each study subject. For the longitudinal study, samples were collected from the same animals at the farm, the slaughterhouse and market. At the farm, fecal samples were collected from pigs and dairy cows by rectal evacuation, and from chickens by cloacal swabs. Ten milliliter milk samples were collected from dairy farms.

Table 1
Number of sites and samples collected, by species and location, Thailand 2000–2003

Species	Location	Cross-sectional and longitudinal ^a				Cross-sectional ^a		Case-Control ^a		Total N
		2000 ^b		2001 ^b		2002 ^b		2003 ^b		
		Sites	N	Sites	N	Sites	N	Sites	N	
Chickens	Farm	3	155	3	187	24	192	–	–	534
	Slaughterhouse	–	–	1	148	–	–	–	–	148
	Market	–	–	1	72	–	–	–	–	72
	All locations	3	155	5	407	24	192	0	0	754
Pigs	Farm	3	146	4	285	–	–	–	–	431
	Slaughterhouse	–	–	1	167	–	–	–	–	167
	Market	–	–	1	69	–	–	–	–	69
	All locations	3	146	6	521	0	0	0	0	667
Dairy	Farm	–	–	–	–	25	225	–	–	225
Human	Farm	7	22	8	41	49	136	–	–	199
	Non-farm community	–	–	–	–	40	100	–	–	100
	Hospital	–	–	–	–	–	–	3	205	205
	All locations	7	22	9	45	89	236	3	205	504
Grand total		13	323	19	969	138	653	3	205	2150

^a Study phase.

^b Study year.

Table 2
Prevalence of *Salmonella* in animals by location and sample type, Thailand 2000–2003

Species	Location	Sample type	# Samples	# Positive	Prevalence (%)
Chicken	Farm	Cloacal swab	425	18	4
		Slaughterhouse	Cloacal swab	73	31
			Surface swab	73	2
Pig	Market	Meat	72	41	57
	Farm	Fecal	361	20	6
		Slaughterhouse	Lymph node	70	18
			Surface swab	75	28
		Unspecified	204	51	25
Dairy cows	Market	Meat	69	20	29
	Farm	Fecal	125	4	3
			Milk	25	0

At the slaughterhouse, samples were collected by swabbing an area of approximately 50 cm on each pig carcass with sterile gauze, and cotton swabs were used to swab under the wing and around the cloaca of each chicken carcass. Samples of mesenteric lymph nodes were collected from pigs after carcass dressing. The sample source (carcass swab or lymph node) of pig samples taken from the slaughterhouse in 2000 was lost — data from these samples were included in reports of overall prevalence, but were excluded in comparisons of prevalence of *Salmonella* from different types of samples at slaughter.

The slaughterhouses participating in our study were small-scale facilities providing meat for local market consumption. The poultry slaughterhouses processed 500–800 birds per night. At the slaughterhouse, birds from various farms were kept together in the holding pen while waiting to be slaughtered by hand. After mechanical defeathering, carcasses were chilled in cold water without evisceration, since evisceration is done at the market to provide consumers with viscera for separate purchase. Chicken slaughterhouse samples were collected after defeathering and before chilling. The pig slaughterhouse in this

Table 3
Prevalence of *Salmonella* in the farm environment, by species, location, and sample type, Thailand 2000–2003

Species	Sample type	Number of samples	Number positive	Prevalence (%)
Chicken	Feed	6	1	17
	Feed tray	24	5	21
	Pen floor	29	7	24
	Water	26	3	11
	Water tray	24	7	29
Pigs	Feed	6	1	17
	Feed tray	32	5	16
	Pen floor	32	7	22
Dairy	Feed	25	2	8
	Feed tray	25	7	28
	Pen floor	25	9	36

Table 4
Prevalence of *Salmonella* in humans by group, location and sample type, Thailand 2000–2003

Group	Sample	Number of samples	Number positive	Prevalence (%)
Livestock farm workers	Stool	199	72	36
Slaughterhouse workers	Stool	4	1	25
Non-livestock farm workers	Stool	100	33	33
Hospitalized diarrhea patients	Rectal swab	205	15	7

study did not use machinery for the slaughtering process. After slaughter, the pigs were dehaired, eviscerated, and cut into 6 pieces by hand. Pig carcasses, including visceral organs, were delivered to the market directly after slaughtering. The slaughterhouse samples were collected from the pigs at the end of the butchering process but before shipment to market.

At the market, approximately 100 g of pork from the neck and a thigh from each chicken were purchased. All farm, slaughterhouse, and market environmental samples were collected using a sterile gauze swab soaked in sterile skim milk (Pacific Sciences, Bangkok, Thailand), which was used as a transport medium for these samples. All samples were held in an icebox or refrigerator until further processing, which was completed within 48 h of collection.

Livestock-handling farm workers and farming neighbors who did not have livestock contact were asked to submit 10 g stool samples in sterile cups containing Cary–Blair transport medium (Pacific Sciences, Bangkok, Thailand) provided by the investigator. With parental consent, rectal swabs were collected from children with diarrhea, prior to treatment with any antimicrobial agents, at the study hospitals. Swabs were kept in semi-solid Cary–Blair medium (Pacific Sciences) and held as fecal and environmental samples.

Pre-tested questionnaires were administered by the principal investigator at the farm, to collect information regarding general farm management and risk factors for *Salmonella* infection. To ascertain the exposures of children with diarrhea to food animals, pre-tested questionnaires on food consumption and animal contact were administered to the consenting parents by the same nurse who collected the stool specimen from the hospitalized child.

2.3. Isolation and identification of *Salmonella*

Approximately 10 g of meat were excised from each purchased sample and minced. All environmental swabs and meat samples were placed in 10 ml of buffered peptone water (BPW; Oxoid, Basingstoke, UK) as pre-enrichment media, and incubated at 37 °C for 18 h. After incubation, 0.1 ml of the BPW was added to Rappaport–Varsiliadis broth (Pacific Sciences), an enrichment media, and incubated at 42 °C for 18 h. A swab of the broth was inoculated onto Xylose–Lysine–Tergitol agar (XLT4; Pacific Sciences) selective media. Fecal, cloacal and rectal swabs were

Table 5
Risk factors for *Salmonella* isolation in children hospitalized for diarrhea, Thailand 2000–2003

Risk factor	Level	# Samples	# Positive	% Positive	Odds ratio	<i>p</i>
Hospital	A	25	2	8	1.37	0.661
	B	113	9	8	1.36	0.769
	C	67	4	6	Baseline	
Gender	Female	100	11	11	3.12	0.087
	Male	105	4	4	Baseline	
Age (years)	1	119	10	8	1.21	0.770
	2	50	5	10	–	
	3	22	0	0	–	
	4	7	0	0	–	
	5	5	0	0	–	
Consumed chicken	Yes	34	3	9	1.27	0.721
	No	169	12	7	Baseline	
Consumed pork	Yes	57	6	11	1.79	0.369
	No	140	9	6	Baseline	
Consumed milk	Yes	155	13	8	2.11	0.528
	No	48	2	4	Baseline	

inoculated directly on XLT4. The XLT4 plates were incubated at 37 °C for 24 h. Up to 5 colonies were selected from each plate and were streaked on triple sugar iron agar (TSI; Pacific Sciences), motility-indole-lysine decarboxylase media (MIL; Pacific Sciences) and urea agar (Pacific Sciences) to confirm *Salmonella* (Popoff and LeMinor, 1992). Positive colonies were streaked on tryptic soy agar (TSA; Pacific Sciences) and incubated for 18 h at 37 °C, and then an isolated colony was stabbed into half-strength nutrient agar (Pacific Sciences) in a 1.5 ml tube. After incubation for 18 h at 37 °C of incubation, the tubes were stored at 4 °C.

Table 6
Predominant serotypes of *Salmonella* in farm animals and environments (Env.) and humans, sorted by number of species affected and total number of isolates, Thailand 2000–2003

Serotype	Chicken		Pigs		Dairy cattle		Healthy humans	Diarrheal children
	Animal	Env.	Animal	Env.	Animal	Env.		
Rissen	2	0	62	1	2	0	19	2
Weltevreden	23	3	11	1	1	11	22	1
Anatum			11	0			21	1
Emek	10	6	2	0			2	0
Stanley			4	0	1	1	3	4
Blockley	1	7					2	0
Derby			6	2			2	0
Hadar	4	0					2	0
Hvittingfoss							6	0
Brunei			1	0	1	3		
Eastbourne							5	0
Panama			4	0			0	1
Senftenberg			1	0	0	1	3	0
Virchow	1	3					0	1
Enteritidis	3	0						
Lexington							3	0
Newport			1	0			2	0
Typhimurium			2	0			1	0
Bovismorbificans							2	0
Braenderup							0	2
Give							1	1
Salmonella Rough	2	0						
Total typed isolates	48	19	109	4	6	18	98	15

Serotypes of *Salmonella* isolates were determined by slide agglutination using the Kauffman–White serotyping scheme (Popoff and LeMinor, 1992), by the Department of Medical Service of the Thai Ministry of Health.

2.4. Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done using micro-broth dilution, following guidelines set by the National Committee on Clinical Laboratory Standards (NCCLS, 2000), and using *Staphylococcus aureus* NTCC 25922 and *Escherichia coli* NTCC 29213 as quality control organisms. The antimicrobial agents tested included ampicillin, ceftiofur, ceftriaxone, florfenicol, nalidixic acid, tetracycline, and ciprofloxacin. A 10⁵ CFU/ml bacterial suspension was inoculated into a 96 well microtitre plate containing 2-fold dilutions of antimicrobial agents. Plate counts were conducted to confirm the concentrations of the inocula used in susceptibility testing. After incubating the inoculated plate aerobically for 20 h, the minimum inhibitory concentration (MIC) of each antimicrobial agent was determined by observing bacterial growth in each well, and identifying the minimum concentration needed to inhibit bacterial growth. The breakpoints provided by the US National Antimicrobial Resistance Monitoring System (NARMS, 2003) were used to categorize *Salmonella* isolates as resistant or non-resistant.

2.5. Statistical analysis

Prevalence was calculated by dividing the number of samples positive for *Salmonella* by the total number of samples

Table 7
Proportions of most common serotypes of typed *Salmonella* isolates, by species and location, Thailand 2000–2003

Serotype	Chickens					Pigs				Dairy cows: farm			Crop farm workers (n=32)
	Farm			Slaughter animals (n=14)	Market meat (n=20)	Farm ^a		Slaughter animals (n=80)	Market meat (n=13)	Animals (n=6)	Env. (n=18)	Human (n=29)	
	Animal (n=14)	Env. (n=19)	Human (n=30)			Animals (n=16)	Human (n=6)						
Weltevreden		16	27	86	55		17	5	54	17	61	7	31
Rissen	14		23			94	50	56	15	30		21	9
Stanley			10					5		17	6		
Anatum			3					11	15			28	38
Emek	36	32	7		25				15				
Senftenberg			3					1			6	7	
Blockley	7	37	7										
Derby			3					6					3
Hadar				14	10	0							6
Brunei										17	17		
Panama								5					
Virchow	7	16											
Newport			7					1					
Typhimurium								3				3	
Enteritidis	21												

^a No serotypes for environmental samples from pig farms were determined.

processed. The significance ($p < 0.05$) of differences between prevalences of *Salmonella* spp. in various population, location and sample types were determined using the Chi-square test for independent proportions, or McNemar's Chi-square test for correlated proportions when comparing results of pig carcass swabs with results from lymph nodes; or Fisher's Exact test when the numbers within categories were too small for the Chi-square test. Analysis of risk factors associated with *Salmonella* isolation in children hospitalized with diarrhea was conducted using the Chi-square or Fisher's Exact tests.

3. Results

3.1. Prevalence

The prevalence of *Salmonella* in animals varied between species and between locations (Table 2), but the prevalence of *Salmonella* was not significantly different ($p > 0.05$) between pigs, chickens, or dairy cattle, regardless of sampling location. There was no significant difference ($p = 0.726$) between food animal species in the prevalence of *Salmonella* from environ-

mental samples (Table 3). The prevalence of *Salmonella* in chickens at each farm was found to range from 6% to 75%, with an average prevalence of 13%, and *Salmonella* was found in 12 out of 27 (44.4%) chicken farms sampled. The biggest difference in prevalence by location for chickens was between farm (4%) and slaughter (42%) ($p < 0.01$). At slaughter, the prevalence of *Salmonella* in isolates from chicken cloacal swabs was higher than carcass swab samples (Chi-square $p < 0.01$). Four out of seven pig farms (57%) were found with *Salmonella*, with the prevalences ranging from 2% to 25% (average 7%). At slaughter, the prevalence from lymph node samples was not significantly different from the prevalence observed from carcass swab samples (McNemar $p = 0.055$).

In humans (Table 4), the prevalence of *Salmonella* spp. in healthy adults (livestock farm workers, slaughterhouse workers and non-livestock farm workers) was significantly higher than in children hospitalized with diarrhea ($p < 0.01$). Analysis of risk factors showed that gender, age, and consumption of chicken, pork or milk were not significantly associated with isolation of *Salmonella* from children with diarrhea (Table 5).

Table 8
Proportions of *Salmonella* demonstrating resistance to antimicrobial agents, Thailand 2000–2003

Species	Location	N	Ampicillin	Ceftiofur	Ceftriaxone	Florfenicol	Nalidixic acid	Tetracycline	Ciprofloxacin	Multi-Resistant
Chicken	Farm	11	0	0	0	27	100	100	0	100
	Slaughterhouse	57	0	0	0	0	16	16	2	16
	Market	87	0	1	1	0	43	33	1	35
	All chickens	155	0	1	1	2	37	32	1	32
Pig	Farm	51	0	0	0	6	2	98	0	8
	Slaughterhouse	155	1	0	0	15	39	89	1	48
	Market	48	0	0	0	8	21	60	0	21
	All pigs	254	0.4	0	0	12	28	85	0	35
Humans	Livestock farm workers	52	23	4	0	21	23	69	0	42
	Non-livestock farm workers	19	16	0	0	5	37	32	5	26
	Healthy adults	71	21	2.8	0	17	27	59	1	38
	Diarrheal children	72					31	92	67	85

3.2. *Salmonella* serotypes

The most commonly identified serotypes of *Salmonella* in this study were Rissen and Weltevreden (Table 6), which were found in all food animal species and humans, and Stanley was found in pigs, cattle and humans. When separated by type of sample and location (Table 7), samples from chickens on the farm carried more serotypes than chickens at slaughter or poultry meat, while pigs on the farm carried fewer serotypes than animals at slaughter or meat at the market.

3.3. Antimicrobial resistance

Antimicrobial resistance was found in all types of animal and human samples (Table 8). Resistance to ampicillin was common in *Salmonella* isolated from healthy adult humans, while resistance to ciprofloxacin was high in children with diarrhea. Isolates resistant to more than one antimicrobial agent (multi-resistant) were highest in chickens on the farm and children with diarrhea. Livestock farm workers had higher levels of multi-resistant *Salmonella* than farm workers with no livestock contact.

4. Discussion

In food animals, the prevalence of *Salmonella* was consistently lower from samples collected from live animals on the farm in comparison to samples collected after slaughter and samples from meat purchased at the market. The prevalence of *Salmonella* was low in environmental samples. In humans, the prevalence of *Salmonella* in healthy adults was relatively consistent, regardless of exposure to livestock.

The prevalence of *Salmonella* from chickens on the farm, and the proportion of infected farms in our study, were lower than previously reported in Thailand (Sasipreeyajan et al., 1996). At slaughter, the prevalence of *Salmonella* found in this study was much higher than that reported in a previous study in Thailand (Vadhanasin et al., 2004). However, the slaughterhouse in the earlier study was a large processing plant, while that in our study was small-scale local facility. The carcasses were not eviscerated, as evisceration is commonly done at the market since many consumers purchase the viscera for home consumption. This practice may explain the increase in prevalence at the market over the slaughterhouse, and why our study found a much higher prevalence of *Salmonella* in chicken meat than previous studies in Thailand (8%, Boonmar et al., 1997) and the United Kingdom (8%, Meldrum et al., 2004). However, our prevalence in market chicken was comparable to results of studies in Portugal (60%, Antunes et al., 2003), Spain (60%, Carramiñana et al., 1997), and Greece (69%, Arvanitidou et al., 1998).

In pigs at slaughter, the prevalence of *Salmonella* from carcass swabs in this study was higher than carcass swabs from pigs in the U.K. (5.3%, Davies et al., 2004), but the prevalence from lymph nodes was comparable to caecal contents of pigs at slaughter in the same study (23%). The prevalence of *Salmonella* from meat at the market was slightly higher than

what was previously reported in Thailand (Boonmar et al., 1997). Based on results from the prospective study of pigs from farm to market, the high incidence of *Salmonella* at slaughter, compared to the incidence at the farm and market, may indicate that transport stress may cause pigs to shed *Salmonella* more frequently (Berends et al., 1996).

This study supports other researchers in finding that cattle have relatively low levels of *Salmonella* in comparison to other food animal species. The prevalence of *Salmonella* in individual dairy cattle in this study was comparable to other studies of healthy cattle (Wells et al., 2001; Huston et al., 2002; Fossler et al., 2004). The farm prevalence reported in this study was higher than some studies of *Salmonella* in the U.S. (28%, Wells et al., 2001; 31%, Huston et al., 2002), but was comparable to the prevalences for individual farm visits (31%–54%) reported in a longitudinal study of dairy farms in the midwestern and northeastern U.S. (Fossler et al., 2004).

The proportion of samples yielding *Salmonella* in this study was fairly consistent between the three healthy adult groups, especially between livestock farm workers and farm workers with no livestock contact. These levels were higher than those reported in a study of U.S. military troops in Thailand, where 12% of healthy subjects carried *Salmonella* (Sanders et al., 2002). The percentage of *Salmonella* isolates from children with diarrhea was low, and agrees with findings from other studies that indicate that *Salmonella* is not a significant cause of diarrhea in children (3%, Suwatano, 1997; 7%, Murphy et al., 1993; 8%, Echeverria et al., 1993; 12%, Rasrinal et al., 1988). Differences in the observed prevalence of *Salmonella* in humans within this study may be the result of the different sample collection protocols used for the human samples. Healthy individuals were provided with instructions, and collected and submitted their own stool specimens, while samples from diarrheal children were taken with rectal swabs by nurses at the hospital. Consequently, the samples from healthy patients may have become contaminated during sample collection. Analysis of consumption of foods of animal origin as risk factors for finding *Salmonella* in children with diarrhea were not significant, which may be attributable to the fact that *Salmonella* was not an important cause of diarrhea in this study. Other studies have also failed to connect *Salmonella* infection with locally-produced food consumption (Kapperud et al., 1998).

Factors that may affect prevalence include those that affect detection of the organism, such as sampling procedures, bacterial isolation and identification methods; and factors that directly influence prevalence, such as animal management, slaughter practices, and cross-contamination (Bryan and Doyle, 1995; Uyttendaele et al., 1999). Differences in prevalence may be the result of using different sample types (Hurd et al., 2004), or different methods for detection of *Salmonella* (Pangloli et al., 2003). Study design has an effect on the detection of *Salmonella*. The cross-sectional nature of sample collection for the majority of samples in this study make it difficult to arrive at conclusions about changes in *Salmonella* prevalence, serotype, or antimicrobial resistance over time, but much of the published data on *Salmonella* prevalence was collected through cross-sectional studies.

A variety of different serotypes were reported from human and animals from different locations in this study. Our study found that *S. Weltevreden* was the most common serotype of *Salmonella* in chickens at slaughter and at the market. *Weltevreden* was also the most common serotype of *Salmonella* in healthy humans, which agrees with an earlier study in Thailand from 1993–2002, which found *Weltevreden* to be the most common serotype from human samples (Bangtrakulnonth et al., 2004). While the 1993–2002 study also found that *S. Enteritidis* was the second most common serovar identified in humans, the proportion of *S. Enteritidis* in human samples began declining in 2001 (Bangtrakulnonth et al., 2004), and our study found no *Enteritidis* in humans, and only in a small proportion of isolates from chickens. There was a reported increase in the incidence of *S. Enteritidis* infections in humans in Thailand from 1990 to 1995, (Boriraj et al., 1997), with a concurrent increase in the incidence of *S. Enteritidis* in chickens (Sakai and Chalermchaikit, 1996). Our study found very little *S. Enteritidis*, which may be due to the fact that the source of *Salmonella* infections in humans and chickens may have changed over time.

The most common *Salmonella* serotype found in pediatric diarrhea patients in this study was Stanley, in contrast with findings from sporadic diarrheal patients in hospitals in Thailand from 1993–1996, where *Enteritidis* was the most common serotype identified (Boonmar et al., 1998b). Stanley is one of the *Salmonella* serotypes increasing in importance in Thailand (Bangtrakulnonth et al., 2004), and in the numbers from human samples in the U.S. since 1993 (CDC, 2003). Given the relatively low number of *Salmonella* isolates available for typing (15) from children with diarrhea, the fact that *Salmonella* is not a significant cause of diarrhea in Thailand (Rasrinual et al., 1988; Echeverria et al., 1993; Murphy et al., 1993; Suwatano, 1997), and the low levels of *Enteritidis* found throughout this study, it appears that the importance of *Enteritidis* is waning in pediatric diarrhea cases in Thailand.

The predominant serotype found in dairy cows and in pigs throughout all sampling locations was Rissen, an uncommon serotype in the U.S. (CDC, 2003), and one that was not identified in any food animals in studies in Canada (Guerin et al., 2005). The increasing importance of Rissen in Thailand was also reported in an earlier study, and its presence in water, human, and food products suggested a waterborne or foodborne reservoir (Bangtrakulnonth et al., 2004).

Several interesting trends were present when comparing the serotypes of different types of samples from different location. When comparing environmental source *Salmonella* serotypes with serotypes from samples taken from live animals, the most common serotypes found in dairy cattle environmental samples (*Weltevreden* and *Brunei*) were among the most common serotypes found in dairy cows. However, in chickens, the most common serotype found in environmental samples were *Blockley* and *Emek*, while the most common serotypes found in live chickens were *Emek*, *Enteritidis*, and *Rissen*. Given that the rates of *Salmonella* isolation were higher in environmental samples than from live animals on the farm,

these findings suggest that sources other than livestock may be more important contributors to *Salmonella* in the farming environment.

The most common serotypes found in farm workers from chicken farms were *Weltevreden* and *Rissen*, *Rissen* from pig farm workers, *Anatum* and *Rissen* from dairy farm workers, and *Anatum* and *Weltevreden* in farm workers with no exposure to livestock. Serotype *Anatum* in Thailand appears to be associated with non-poultry food products and water contamination (Bangtrakulnonth et al., 2004). Interestingly, the most common serotypes of chickens (*Emek* and *Enteritidis*) did not match the most common serotypes found in chicken farm workers, suggesting that transmission between chickens and farm workers may not be a significant source of *Salmonella* for either group. One study has shown that the serotype of *Salmonella* in humans may not be related to animals living in close contact with these humans (Kariuki et al., 2002). Serotype *Rissen* was relatively common in farm workers with livestock exposure, which may indicate a common source of this serotype for all farm workers. When comparing farm workers with livestock exposure and those with no livestock exposure, one interesting pattern is the higher levels of serotypes *Anatum* and *Weltevreden* in farm workers with no livestock exposure. These *Salmonella* are probably acquired from farm sources not associated with food animals.

The shift in serotypes from farm to slaughterhouse and market may be indicative of contamination at slaughter and market, when serotypes not found on the farm emerge at slaughter, such as the emergence of *S. Hadar* and *S. Weltevreden* in chickens at slaughter, and the decline in the percentage of *Salmonella* isolates of serotype *Rissen* in pigs as they move from farm to market. Given the higher levels of serotype *Weltevreden* in humans, it would appear that humans might be serving as a source of *Salmonella* contamination for chickens and pigs as they move from farm to market. Other studies have also identified shifts in predominant serotypes as food animals move through different stages from farm to market (Korsak et al., 2003; Bahnson et al., 2005).

Antimicrobial resistance was found in all types of samples from all species investigated in this study. High proportions of *Salmonella* spp. with resistance to tetracycline were found in all host species. Low levels of resistance to ciprofloxacin, ceftriaxone and ceftiofur were observed, which may be due to the limited use of these classes of antimicrobial agents in food animal production, and were in agreement with previous studies conducted in Thailand (Leelarasamee and Tian-Grim, 1994; Boonmar et al., 1998a; Isenbarger et al., 2002). For both pigs and chickens, the proportions of resistant *Salmonella* spp. were highest at the farm, suggesting the higher risk of developing resistance or acquiring *Salmonella* spp. with resistance to antimicrobial agents occurred at the farm (McEwen and Fedorka-Cray, 2002). A high proportion of isolates resistant to nalidixic acid were collected from chickens at the farm, where quinolone use is common. In chickens, the increase in resistance seen from slaughter to market may be due to evisceration at the market rather than at slaughter, or through contamination of the market environment.

Multi-drug resistance has been reported as an emerging problem in poultry isolates from Thailand (Boonmar et al., 1998a). Multi-resistant isolates were highest for chickens at the farm and lowest at slaughter, while multi-resistance was highest for pig isolates at slaughter. The differences in slaughter practices concerning evisceration may explain these patterns, in that multi-resistance was higher in isolates from locations where fecal contamination was highest. For pigs, evisceration occurred at the slaughter plant, and the cleaning of carcasses and meat prior to marketing could impact the carriage of multi-resistant *Salmonella* on meat at the market.

In humans, the proportion of resistant *Salmonella* spp. in those isolates from diarrhea patients was higher than in *Salmonella* spp. isolated from healthy individuals for all antimicrobial agents. High levels of multi-resistant *Salmonella* have been reported in Thai children with diarrhea (Moolasart et al., 1997). The levels of resistance to tetracycline, ciprofloxacin, and nalidixic acid in pediatric cases from this study were higher in comparison to an earlier study in Thailand, where resistance to these agents were 59%, <1% and 21%, respectively (Isenbarger et al., 2002). These differences may be associated with differences in bacterial culture and antimicrobial susceptibility testing techniques, or they may be indicative of increasing antimicrobial resistance in *Salmonella* infecting young diarrhea patients in Thailand. Levels of resistance to ampicillin and tetracycline in healthy adults from this study were comparable to results reported for isolates collected from diarrhea patients and controls in Thailand from 1981–1995 (Hoge et al., 1998). Interestingly, there were high levels of ciprofloxacin resistance in children with diarrhea in our study compared to low levels in the healthy adult population, and little or no ciprofloxacin resistance in *Salmonella* isolates from earlier studies (Hoge et al., 1998; Isenbarger et al., 2002). This may be a reflection of the emergence of ciprofloxacin resistance in *Salmonella* in Thailand, as seen in *Campylobacter* and other enteropathogenic organisms (Hoge et al., 1998; Hanson et al., 2002; Isenbarger et al., 2002).

In conclusion, *Salmonella* was found in all levels of food production systems, from farm to market, in northern Thailand. Antimicrobial resistance was present in *Salmonella* from all stages of food production, and *Salmonella* serotypes changed as animals moved from farm to market. In humans, *Salmonella* was present in farm workers regardless of their level of contact with livestock. For children hospitalized with diarrhea, the prevalence of *Salmonella* was lower than in farm workers, but those isolates had much higher levels of antimicrobial resistance than isolates from healthy adults. The results of this study demonstrate that the epidemiology of *Salmonella* in food animals and humans in Thailand is complicated, and further research is needed to understand this system so that the risks of disease and antimicrobial resistance for both humans and food animals can be minimized.

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