

PREVALENCE AND ANTIMICROBIAL RESISTANCE OF *SALMONELLA* ISOLATED FROM CARCASSES, PROCESSING FACILITIES AND THE ENVIRONMENT SURROUNDING SMALL SCALE POULTRY SLAUGHTERHOUSES IN THAILAND

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Abstract. *Salmonella* is a major food-borne pathogen worldwide, including Thailand, and poultry meat plays a role as a vehicle for the spread of the disease from animals to humans. The prevalence and characteristics of *Salmonella* isolated from 41 small scale poultry slaughterhouses in Chiang Mai, Thailand were determined during July 2011 through May 2012. *Salmonella*'s prevalence in live poultry, carcasses, waste water, and soil around processing plants were 3.2%, 7.3%, 22.0% and 29.0%, respectively. Eighteen different serotypes were identified, the most common being Corvallis (15.2%), followed by Rissen (13.9%), Hadar (12.7%), Enteritidis (10.1%), [I. 4,5,12 : i : -] (8.8%), Stanley (8.8%), and Weltevreden (8.8%). Antimicrobial susceptibility tests revealed that 68.4% of the *Salmonella* spp were resistant to at least one antimicrobial while 50.6% showed multiple drug resistance (MDR). Specifically, 44.3% of *Salmonella* were resistant to nalidixic acid, followed by streptomycin (41.8%), ampicillin (34.2%), tetracycline (34.2%), and sulfamethoxazole/trimethoprim (20.3%). *Salmonella* contamination was found in processing lines, carcasses, and in the environment around the processing stations. These findings indicate that improving hygiene management in small scale poultry slaughterhouses as well as prudent use of antimicrobial drugs is urgently needed if *Salmonella* contamination is to be reduced.

Keywords: *Salmonella*, small scale poultry slaughterhouse, prevalence, antimicrobial resistance

INTRODUCTION

The foodborne salmonellosis is an important disease which is spreading

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worldwide, resulting in an important public health problem. In the US, *Salmonella* was the second largest cause of food poisoning from 2009 to 2010 (CDC, 2013). In the EU countries in 2010 approximately 100,000 patients suffered from food poisoning caused by *Salmonella* (EFSA, 2012). In Thailand, *Salmonella* was found to be the second largest cause of food poisoning, following *Rotavirus* (Padungtod *et al*, 2008). There have also been reports of antimicro-

bial resistant *Salmonella* which would affect patient care (Padungtod *et al*, 2008).

A major cause of human salmonellosis is the consumption of meat such as chicken which has been contaminated with *Salmonella* (Foley *et al*, 2008). A previous report has indicated a bacterial contamination rate of 48% in chicken sold at fresh-food markets and retail meat shops in Thailand (Minami *et al*, 2010). Processing in poultry slaughterhouses is an important source of *Salmonella* contamination in chicken meat (Rasschaert *et al*, 2007), especially in small scale poultry slaughterhouses where traditional slaughtering processes are commonly followed (Padungtod *et al*, 2008).

Almost 90% of poultry slaughterhouses in Thailand are small scale and are not licensed by the government (Kueylaw *et al*, 2008), suggesting an urgent need to improve the management of small scale slaughterhouses to address food safety issues. However, any interventions or guidelines for improvement should be developed based on a detailed understanding of the current situation. To date, there have been no studies of pathogen contamination in the slaughtering process or of the effects of contamination on public health and the environment around small scale poultry slaughterhouses in Thailand. In order to quantify the significance of small scale poultry slaughterhouses in spreading *Salmonella*, this study determined the prevalence and antimicrobial resistance of *Salmonella* isolated from carcasses, processing facilities, and the environment around small scale poultry slaughterhouses.

MATERIALS AND METHODS

Study site

Chiang Mai Province, located in the northern part of Thailand, was characterized as a dense area of poultry produc-

tion in the 2010 annual report of the Thai Department of Livestock Development, more than three million chickens were produced in the province (Department of Livestock Development, 2010). For that reason, Chiang Mai Province was selected as the study site.

Sample collection

Forty-one small-scale poultry slaughterhouses out of a total of 55 such facilities were visited during the period July 2011-May 2012. At each slaughterhouse, 10 samples from live poultry were collected using cloacal swabs plus 10 samples from carcasses collected using the rinsing method. In addition, samples from stations along the processing line, including holding pens, were collected using sock swabs. In addition, samples from workers' hands, slaughtering knives, defeathering machines, cutting boards, meat cleaning buckets, carcass storage boxes, and slaughterhouse floors were collected using surface swabs from a 25 cm² area. One liter of water used in processing and one liter of waste water drained from the plants were also collected using sterile bottles as well as 500 gram soil samples collected using plastic bags. All samples were kept in ice boxes and transported within 12 hours of collection to the Diagnostic Center, Faculty of Veterinary Medicine, Chiang Mai University, for laboratory testing.

Bacterial isolation, serotyping and antimicrobial susceptibility testing

Isolation of *Salmonella* spp was performed following the FDA *Salmonella* culture method (USFDA, 2007). Isolated *Salmonella* were serotyped and antimicrobial susceptibility was determined at the Department of Medical Science, National Institute of Health of Thailand, Ministry of Public Health. Antimicrobials tested

Table 1
The prevalence of *Salmonella* in small scale slaughterhouses.

Sampling points	Slaughterhouses testing positive	Positive samples from slaughterhouses
Live poultry	14.6% (6/41)	3.2% (13/410)
Holding pens	12.2% (5/41)	12.2% (5/41)
Worker hands	0.0% (0/41)	0.0% (0/41)
Knives	2.4% (1/41)	4.7% (2/43)
Defeathering machines	7.3% (3/41)	7.3% (3/41)
Cutting boards	13.0% (3/23)	13.0% (3/23)
Meat cleaning buckets	9.8% (4/41)	9.8% (4/41)
Carcass storage boxes	2.4% (1/41)	9.8% (4/41)
Slaughterhouse floors	29.3% (12/41)	29.3% (12/41)
Water used in plants	4.9% (2/41)	2.4% (1/41)
Waste water	22.0% (9/41)	22.0% (9/41)
Soil around the plants	29.3% (12/41)	29.3% (12/41)
Carcasses	39.0% (16/41)	7.3% (30/410)

consisted of ampicillin (AMP, 10 µg), chloramphenicol (C, 30 µg), ciprofloxacin (CIP, 5 µg), cefotaxime (CTX, 30 µg), nalidixic acid (NA, 30 µg), norfloxacin (NOR, 10 µg), streptomycin (S, 10 µg), tetracycline (TE, 30 µg), and sulfamethoxazole/trimethoprim (SXT, 23.75/1.25 µg). *Salmonella* strains which were resistant to two or more antimicrobials were defined as a multidrug resistant (MDR) (Elgroud *et al*, 2009).

RESULTS

The prevalences of *Salmonella* in chickens before slaughtering, during the slaughtering and dissecting process, and in the local environment are shown in Table 1. *Salmonella* was isolated from 3.2% of live poultry and 7.3% of carcasses. *Salmonella* was also found on utensils used in the slaughtering process, slaughterhouses floor (29.3%), in both water used in the slaughterhouses (2.4%) and in waste water (22.0%), as well as in the soil around the processing plants (29.3%), but it was not found on workers' hands. The serotypes of *Salmonella* identified are

shown in Table 2. Eighteen serotypes were identified. The most common serotype was Corvallis (15.2%) followed by Rissen (13.9%), Hadar (12.7%), Enteritidis (10.1%) [I. 4,5,12 : i : -] (8.8%), Stanley (8.8%) and Weltevreden (8.8%).

Drug resistance of the *Salmonella* serotypes is shown in Table 3. It was found that 44.3% of samples of *Salmonella* (35 of 79) were resistant to nalidixic acid, followed by streptomycin (41.8%, 33/79), ampicillin (34.2%, 27/79), tetracycline (34.2%, 27/79), and sulfamethoxazole/trimethoprim (20.3%, 16/79). The isolated pathogens were sensitive (100%) to chloramphenicol, ciprofloxacin, norfloxacin and cefotaxime. In addition, 68.4% (54/79) of the pathogens were resistant to at least one antimicrobial, while 50.6% (40/79) of the pathogens were multidrug resistant (Table 4).

DISCUSSION

Over 90% of poultry slaughterhouses in Thailand are small scale, producing for local consumption. A previous study has

Table 2
Serotypes of *Salmonella* isolated from
small scale slaughterhouses.

Serotypes	Number (%)
S. Corvallis	12 (15.2)
S. Rissen	11 (13.9)
S. Hadar	10 (12.7)
S. Enteritidis	8 (10.1)
S. I. 4,5,12 : i : -	7 (8.8)
S. Stanley	7 (8.8)
S. Weltevreden	7 (8.8)
S. Braenderup	3 (3.8)
S. Mbandaka	3 (3.8)
S. Weltevreden var.15+	2 (2.5)
S. Brunei	2 (2.5)
S. Agona	1 (1.3)
S. Bovismorbificans	1 (1.3)
S. Hvitittingfoss	1 (1.3)
S. Muenchen	1 (1.3)
S. Poona	1 (1.3)
S. Singapore	1 (1.3)
S. Typhimurium	1 (1.3)
Total	79 (100.00)

indicated that small scale poultry slaughterhouses are a key source of the spread of *Salmonella* (Padungtod *et al*, 2008), demonstrating an urgent need for improvement in poultry processing. This study of *Salmonella* contamination in the slaughtering process as well as contamination in the environment provides additional information for the upgrading of food safety in small scale poultry slaughterhouses.

The prevalence of *Salmonella* in carcasses and in final products was 7.3%, similar to the 9% prevalence reported in one previous study of poultry slaughterhouses in Thailand (Padungtod and Kaneene, 2006), but lower than the 43% reported in another study (Kueylaw *et al*, 2008). Studies conducted around the world have found differences in *Salmonella* prevalence isolated from poultry slaughterhouses: Elgroud *et al* (2009) reported a prevalence of *Salmonella* in the

slaughterhouses in Algeria of over 53%; Fuzihara *et al* (2000) found a 42% prevalence of *Salmonella* in chicken carcasses from small scale poultry slaughterhouses in Brazil; Bohaychuk *et al* (2009) reported a 37% *Salmonella* prevalence in poultry slaughterhouses in Alberta, Canada; and Capita *et al* (2007) reported that the prevalence of *Salmonella* in chicken from slaughterhouses in Spain in 2006 was 17.9%. Results of the present study conform more closely to the findings of Cotez *et al* (2006) which reported a 10% prevalence of the pathogen in slaughterhouses in Brazil. A possible reason for the lower pathogen contamination in carcasses in Chiang Mai may be that, as this study found, processors dip carcasses in hot water for a short time to firm up the skin.

Several previous studies have looked at different steps in the preparation of chickens for market. Those studies have found *Salmonella* contamination during the transport of live birds, in the process of slaughtering and in the waste from small scale poultry slaughterhouses (Fuzihara *et al* 2000; Corry *et al*, 2002; Barros *et al*, 2007; Reiter *et al*, 2007). However, this is the first comprehensive study in Thailand to evaluate all the steps involved in preparing chicken for the market. This study found contamination in every step, including transport, slaughter, and in waste material in small scale poultry slaughterhouses in Thailand. *Salmonella* was isolated from live poultry, from poultry buildings before slaughtering, from the slaughterhouse floor, from the slaughtering utensils, and from environmental samples taken around the slaughtering facility. This study supports research done in Brazil which reported that *Salmonella* could be isolated from transportation (transport cages) as well as during the process of slaughtering and dissecting including

Table 3
Antimicrobial resistance in *Salmonella* serotypes isolated from small scale slaughterhouses.

Serotype	No. of isolates	No. (%) of <i>Salmonella</i> isolates resistant to each antimicrobial									
		AMP	C	CIP	CTX	NA	NOR	S	TE	SXT	
<i>S. Corvallis</i>	12	0	0	0	0	12	0	0	0	0	0
<i>S. Rissen</i>	11	11	0	0	0	0	0	11	11	11	0
<i>S. Hadar</i>	10	0	0	0	0	10	0	10	5	0	0
<i>S. Enteritidis</i>	8	7	0	0	0	8	0	2	1	0	0
<i>S. I. 4,5,12 : i :-</i>	7	5	0	0	0	1	0	7	7	4	0
<i>S. Stanley</i>	7	1	0	0	0	0	0	1	1	0	0
<i>S. Weltevreden</i>	7	0	0	0	0	0	0	0	0	0	0
<i>S. Braenderup</i>	3	2	0	0	0	2	0	0	0	0	0
<i>S. Mbandaka</i>	3	0	0	0	0	0	0	0	0	0	0
<i>S. Weltevreden var.15+</i>	2	0	0	0	0	0	0	0	0	0	0
<i>S. Brunei</i>	2	0	0	0	0	0	0	0	0	0	0
<i>S. Agona</i>	1	0	0	0	0	0	0	0	0	0	0
<i>S. Bovismorbificans</i>	1	0	0	0	0	0	0	0	0	0	0
<i>S. Hvitittingfoss</i>	1	0	0	0	0	0	0	0	0	0	0
<i>S. Muenchen</i>	1	0	0	0	0	1	0	1	1	0	0
<i>S. Poona</i>	1	1	0	0	0	0	0	1	1	1	0
<i>S. Singapore</i>	1	0	0	0	0	1	0	0	0	0	0
<i>S. Typhimurium</i>	1	0	0	0	0	0	0	0	0	0	0
Total	79	27 (34.2)	0	0	0	35 (44.3)	0	33 (41.8)	27 (34.2)	16 (20.3)	0

AMP, ampicillin; C, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; NA, nalidixic acid; NOR, norfloxacin; S, Streptomycin; TE, tetracycline; SXT, sulfamethoxazole/trimethoprim.

Table 4
Multidrug resistance patterns in Salmonella serotypes.

Serotypes	Isolates tested	Resistant	Multidrug resistant	Antimicrobial resistance profile
S. Corvallis	12	12	0	12 [NA]
S. Rissen	11	11	11	11 [AMP,S,TE,SXT]
S. Hadar	10	10	10	10 [NA,S]
S. Enteritidis	8	8	7	1 [NA] 5 [AMP,NA] 1 [AMP,NA,S] 1 [AMP,NA,S,TE]
S. I. 4,5,12 : I : -	7	7	7	2 [S,TE] 1 [AMP,S,TE] 3 [AMP,S,TE,SXT] 1 [AMP,NA,S,TE,SXT]
S. Stanley	7	1	1	1 [AMP,S,TE]
S. Braenderup	3	2	2	2 [AMP,NA]
S. Poona	1	1	1	1 [AMP,S,TE,SXT]
S. Muenchen	1	1	1	1 [NA,S,TE]
S. Singapore	1	1	0	1 [NA]
Total	61	54	40	

scalding water and chilled water used in processing (Reiter *et al*, 2007). It also supports a study of small scale slaughterhouses in Brazil which found that the pathogen could be isolated from chicken carcasses after slaughtering as well as in the process of slaughtering where the pathogens were found in utensils, water, freezers and refrigerators (Fuzihara *et al*, 2000). Moreover, the present results are in line with a 2003-2004 study of poultry and swine slaughterhouses in Brazil where *Salmonella* was isolated from waste water treatment systems, indicating another potential source of microorganism dissemination into environment (Barros *et al*, 2007). That study also found that pathogen contamination in slaughtering and dissecting utensils was a potential cause of contamination in the meat after processing (Corry *et al*, 2002).

The most common serotype in this study was *S. Corvallis*, followed by *S.*

Rissen, *S. Hadar*, and *S. Enteritidis*. This finding is consistent with a previous study which reported that in Thailand the pathogens most frequently found in chicken were *S. Weltevreden* and *S. Rissen* (Padungtod and Kaneene, 2006) as well as a study in Algeria which found the most common serotype in poultry slaughterhouses to be *S. Hadar* (Elgroud *et al*, 2009). A study in Vietnam showed that one of the most common serotypes found in poultry meat and pork in that country was *S. Rissen* (Thai *et al*, 2012). A study in Spain reported that *Salmonella* isolated from poultry meat in slaughterhouses in 2006 was mostly *S. Enteritidis* (Capita *et al*, 2007), while Henry *et al* (2012) reported that the pathogens isolated in Reunion Island were mostly *S. Blockley*, *S. Typhimurium* and *S. Brancaster*. Bodhidatta *et al* (2013) reported that *S. Anatum*, *S. Corvallis*, and *S. Derby* were the most common serotypes found in a survey of food samples including

fresh chicken and eggs in Thailand. Furthermore, another report revealed that the most common serotype of *Salmonella* isolated from patients with *Salmonella* in Thailand during the years 2002-2007 was *S. Enteritidis*, followed by *S. Rissen*, *S. Weltevreden*, *S. Anatum*, *S. Stanley*, *S. Corvallis*, and *S. Typhimurium*, in that order (Domingues *et al*, 2014). Thus this study suggests the importance of the public health threat as five out of the top ten serotypes of *Salmonella* isolated were among those most frequently found in humans.

In this study, 100% of the pathogens were sensitive to chloramphenicol, ciprofloxacin, norfloxacin and cefotaxime. This finding is consistent with the results of antimicrobial resistant surveillance of food-borne pathogens in the EU countries which found that *Salmonella* was also susceptible to new antimicrobial agents such as cefepime, cefotaxime, and ciprofloxacin (Bywater *et al*, 2004). This study further found that 44.3% of pathogens were resistant to nalidixic acid, which conforms to a previous study in Thailand by Padungtod and Kaneene (2006) which reported that *Salmonella* isolated from pigs and chickens in northern Thailand in 2002 and 2003 was resistant to tetracycline and nalidixic acid. It also conforms to the study by Akbar and Anal (2013) which reported that *Salmonella* isolated from chicken in Bangkok was resistant to tetracycline and nalidixic acid. It has been reported that resistance to nalidixic acid might reduce the efficacy of members of the fluoroquinolone drug group such as enrofloxacin which has been widely used to control animal diseases (Malorny *et al*, 1999). Moreover, de Jong *et al* (2012) also reported that the extensive use of antimicrobials in both humans and animals was a fundamental cause of drug resistance. From these findings, it appears that antimicrobial resistant *Salmonella*

might affect the use of antimicrobial agents for treatment of bacterial diseases both in humans and in animals.

In this study, 50.6% of the isolates were found to be multidrug resistant, which agrees with previous reports from around the world, *eg*, Spain (Bohaychuk *et al*, 2009; de Jong *et al*, 2012), Vietnam (Thai *et al*, 2012), Algeria (Elgroud *et al*, 2009), China (Li *et al*, 2013), United States (Elgroud *et al*, 2009), and Brazil (Dias de Oliveira *et al*, 2005; Costa *et al*, 2013). In Thailand, Chuanchuen *et al* (2008) reported that 67% of *Salmonella* were MDR. Because the resistance of *Salmonella* to antimicrobial agents could affect both livestock production and public health, it is essential to closely monitor the problem of drug resistance and to urgently encourage the prudent use of antimicrobial agents in the EU countries and elsewhere as recommended by various studies (Finch and Hunter, 2006; Earnshaw *et al*, 2013; Lecky and McNulty, 2013).

In conclusion, this is the first comprehensive study describing the prevalence of *Salmonella* contamination and the antimicrobial resistance of this pathogen on the processing lines and in the environment surrounding small scale poultry slaughterhouses in Thailand. The study found contamination of *Salmonella* in raw poultry meat, on utensils used in processing, in waste water and in soil around the slaughterhouses, indicating they are sources of the spread of *Salmonella* both in raw poultry meat and also in the surrounding environment and local communities. The five most common *Salmonella* serotypes found in this study were among the top 10 serotypes causing human salmonellosis in Thailand, signifying a significant public health threat. In addition, the serotypes had a high rate of multidrug resistance. These findings highlight the importance of and

the urgent need for controlling the use of antimicrobials in animal production and for improving management of small scale poultry slaughterhouses.

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