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Salmonella prevalence in meat at retail markets in Pakse, Champasak Province, Laos, and antimicrobial susceptibility of isolates

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ABSTRACT

Non-typhoidal *Salmonella* is one of the most common causes of human gastroenteritis worldwide and most human outbreaks are associated with the consumption of contaminated food. However, there are no reports on *Salmonella* contamination in market meat in Laos. The objective of this study was to determine the prevalence of *Salmonella* in meat samples in Pakse, Champasak Province, Laos, as well as the antimicrobial susceptibility of isolates. The prevalence of *Salmonella* was 82% in beef, 93% in pork and 80% in buffalo meat. In total, 80 isolates and 11 serovars were found, including serovars Stanley ($n = 15$), Anatum ($n = 14$), Derby ($n = 11$), Rissen ($n = 9$) and Amsterdam ($n = 7$). The drug susceptibility of 60 strains against 10 antimicrobial agents was tested. The 60 isolates examined were sensitive to ciprofloxacin (100% susceptible), norfloxacin (100%), cefotaxime (95%), nalidixic acid (90%) and chloramphenicol (88%), but were resistant to streptomycin (67% resistant), tetracycline (67%) and ampicillin (63%). Of the isolates, 73% were multidrug-resistant. These findings indicate a high *Salmonella* prevalence in market meat in Pakse. Therefore, programmes to control *Salmonella* contamination are needed.

1. Introduction

Diarrhoeal diseases are a primary social and health problem in developing countries, particularly those in tropical zones. Non-typhoidal *Salmonella* is one of the most common causes of human gastroenteritis worldwide and most human outbreaks are associated with the consumption of contaminated food [1]. In Laos, there have been few reports of *Salmonella* in humans and livestock. Of the 880 cases of diarrhoea in Laotians between October 1996 and August 1997, only 5 (0.6%) were attributed to *Salmonella* [2]. Other reports showed the resistance rate of six isolates of *Salmonella enterica* serovar Weltevreden in Laotians to be 50% and 33.3% for streptomycin and tetracycline, respectively [3]. We have previously reported the *Salmonella* prevalence in caecum samples obtained from slaughtered buffalo and pigs to be 8% and 76%, respectively [4]. The prevalence of *Salmonella* in pig carcass swabs at slaughterhouses is relatively high (66%) and a large amount of contaminated meat is consumed daily [5]. However, there are no reports on *Salmonella* contamination in market meat in Laos. The objective of this study was therefore to determine the prevalence of *Salmonella* in meat samples in Pakse, Champasak Province, Laos, as well as the antimicrobial susceptibility of isolates.

2. Materials and methods

2.1. Sample collection and treatment laboratory

In December 2011, 49 meat samples (17 beef, 27 pork and 5 buffalo meat) were purchased from five local markets in Pakse.

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Meat samples were purchased from a local market with no refrigeration or freezing system, stored in a box at 1–4 °C and immediately transported to Champasak Animal Health Service Center (Champasak Province, Laos) for *Salmonella* isolation. Samples were analysed within 3 h of collection. Suspected isolates were then sent to the World Health Organization (WHO) National *Salmonella* and *Shigella* Center (National Institute of Health, Nonthaburi, Thailand) for biochemical and serological testing.

2.2. Isolation methods

Briefly, for isolation of *Salmonella* spp., each 25 g meat sample was placed in 225 mL of buffered peptone water (Merck, Darmstadt, Germany), thoroughly mixed and incubated at 37 °C for 18 h. Then, 1 mL of pre-enrichment culture was added to 5 mL of Rappaport–Vassiliadis (RV) broth (Merck) and incubated at 42 °C for 1 day. After incubation, RV cultures were streaked onto modified semisolid RV agar (Merck) and deoxycholate–hydrogen sulphide–lactose agar (Nissui, Tokyo, Japan) and incubated at 37 °C for 18 h. Typical *Salmonella* colonies (n = 1–3) were selected from each specimen for confirmation based on biochemical characteristics [6] using triple sugar iron agar (Nissui), lysine indole motility agar (Nissui), and catalase and oxidase tests. Serotyping of 80 *Salmonella* isolates was performed on the basis of somatic O and phase 1 and phase 2 flagellar antigens by agglutination testing with antisera (S&A Reagents, Bangkok, Thailand) according to the Kauffman–White Scheme of the '*Antigenic formulae of the Salmonella serovars (9th ed)*' by the WHO Collaborating Centre for Reference and Research on *Salmonella* (http://www.scacm.org/free/Antigenic%20Formulae%20of%20the%20Salmonella%20Serovars%202007%209th%20edition.pdf).

2.3. Antimicrobial susceptibility testing

Sixty strains of 80 *Salmonella* isolates were tested for antimicrobial susceptibility by the disk diffusion method of the Clinical and Laboratory Standards Institute (CLSI) [7] using BD Sensi-Discs™ (BD, Franklin Lakes, NJ) with Mueller–Hinton agar plates (BD). Ten antimicrobial agents in the form of disks were employed for susceptibility testing of 60 *Salmonella* isolates at the following concentrations: ampicillin (AMP), 10 μg; amoxicillin/clavulanic acid (AMC), 30 μg; chloramphenicol (CHL), 30 μg; ciprofloxacin (CIP), 5 μg; cefotaxime (CTX), 30 μg; nalidixic acid (NAL), 30 μg; norfloxacin (NOR), 10 μg; streptomycin (STR), 30 μg; tetracycline (TET), 30 μg; and sulfamethoxazole/trimethoprim (SXT) (23.75/1.25 μg). *Escherichia coli* ATCC 25922 was used as the quality control strain. The choice of the 10 antimicrobials was based on the guidelines of BD Sensi-discs for Enterobacteriaceae (http://www.bdj.co.jp/micro/products/1f3pro00000qho5o-att/54-sd-hantei-CLSI.pdf). Isolates that were resistant to two or more classes of antimicrobial agents were considered multidrug-resistant, with SXT counting as one agent.

3. Results

3.1. Salmonella *prevalence in samples examined*

All *Salmonella* strains isolated in this study were identified as *S. enterica* subspecies *enterica*. As shown in Table 1, *Salmonella* specimens were isolated from 14 (82%) of 17 beef samples, 25 (93%) of 27 pork samples and 4 (80%) of 5 buffalo meat samples. Eleven *Salmonella* serovars were isolated from all meat samples examined.

Analysis of beef samples identified nine *Salmonella* serovars. *Salmonella* Stanley (n = 8) was the most prevalent, followed by *S.*

Table 1
Number of samples positive for *Salmonella* obtained from beef, pork and buffalo meat and their serotypes.

Sample	No. of samples examined	No. (%) of positive samples	Serotype(s)	No. of samples
Beef[a]	17	14 (82)	*S.* Rissen and *S.* Stanley	2
			S. Stanley only	2
			S. Amsterdam, *S.* Derby and *S.* Stanley	1
			S. Anatum and *S.* Stanley	1
			S. Derby and *S.* Rissen	1
			S. Give, *S.* Stanley and *S.* Weltevreden	1
			S. Newport and *S.* Stanley	1
			S. Anatum only	1
			S. Derby only	1
			S. Give only	1
			S. Rissen only	1
			Salmonella 3,10:-:1.7 only	1
Pork[b]	27	25 (93)	*S.* Anatum only	8
			S. Derby only	4
			S. Amsterdam and *S.* Derby	2
			S. Amsterdam and *S.* Anatum	1
			S. Amsterdam, *S.* Anatum and *S.* Rissen	1
			S. Amsterdam, *S.* Orion, *S.* Rissen and *S.* Stanley	1
			S. Anatum and *S.* Orion	1
			S. Anatum and *S.* Derby	1
			S. Derby, *S.* Rissen and *S.* Stanley	1
			S. Orion and *S.* Panama	1
			S. Panama, *S.* Rissen and *S.* Stanley	1
			S. Amsterdam only	1
			S. Rissen only	1
			S. Stanley only	1
Buffalo meat[c]	5	4 (80)	*S.* Stanley only	2
			S. Orion only	1
			S. Stanley and *S.* Newport	1

[a] *Salmonella enterica* serovar Stanley (8 strains), *S.* Rissen (4), *S.* Derby (3), *S.* Anatum (2), *S.* Give (2), *S.* Amsterdam (1), *S.* Newport (1), *S.* Weltevreden (1) and *Salmonella* 3,10:-:1.7 (1) were isolated from beef.
[b] *Salmonella enterica* serovar Anatum (12 strains), *S.* Derby (8), *S.* Amsterdam (6), *S.* Rissen (5), *S.* Stanley (4), *S.* Orion (3) and *S.* Panama (2) were isolated from pork.
[c] *Salmonella enterica* serovar Stanley (3 strains), *S.* Orion (1) and *S.* Newport (1) were isolated from buffalo meat.

Table 2
Proportion of *Salmonella* isolates from beef, pork and buffalo meat demonstrating antimicrobial resistance.

Sample	No. of strains examined	No. (%) of strains resistant to antibiotic									
		AMP	AMC	CHL	CIP	CTX	NAL	NOR	STR	TET	SXT
Beef	20	14 (70)	9 (45)	3 (15)	0	1 (5)	1 (5)	0	16 (80)	15 (75)	6 (30)
Pork	35	21 (60)	4 (11)	4 (11)	0	2 (6)	5 (14)	0	20 (57)	22 (63)	13 (37)
Buffalo meat	5	3 (60)	0	0	0	0	0	0	4 (80)	3 (60)	0
Total	60	38 (63)	13 (22)	7 (12)	0	3 (5)	6 (10)	0	40 (67)	40 (67)	19 (32)

AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; NAL, nalidixic acid; NOR, norfloxacin; STR, streptomycin; TET, tetracycline; SXT, sulfamethoxazole/trimethoprim.

Table 3
Antimicrobial resistance rates of 11 *Salmonella* serotypes isolated from meat.

Serotype	No. of strains examined	Resistance rate (%)									
		AMP	AMC	CHL	CIP	CTX	NAL	NOR	STR	TET	SXT
S. Stanley	14	100	57	0	0	7	0	0	100	100	7
S. Anatum	13	92	15	15	0	0	15	0	54	100	23
S. Derby	10	50	30	30	0	10	0	0	90	60	80
S. Rissen	6	83	0	17	0	17	0	0	67	83	100
S. Orion	5	5	0	0	0	0	0	0	80	0	0
S. Amsterdam	4	0	0	0	0	0	100	0	0	0	0
S. Panama	2	100	0	50	0	0	0	0	0	50	50
S. Newport	2	0	0	0	0	0	0	0	0	0	0
S. Give	2	0	0	0	0	0	0	0	50	0	0
S. Weltevreden	1	0	0	0	0	0	0	0	0	0	0
3,10:-:1,7[a]	1	0	0	0	0	0	0	0	100	100	0
Total	60	63	22	12	0	5	10	0	67	67	32

AMP, ampicillin; AMC, amoxicillin/clavulanic acid; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; NAL, nalidixic acid; NOR, norfloxacin; STR, streptomycin; TET, tetracycline; SXT, sulfamethoxazole/trimethoprim.
[a] *Salmonella enterica* subsp. *enterica* serovar 3,10:-:1,7.

Rissen ($n = 4$), *S.* Derby ($n = 3$), *S.* Anatum ($n = 2$) and *S.* Give ($n = 2$). *Salmonella* Amsterdam, *S.* Newport, *S.* Weltevreden and 3,10:-:1.7 were isolated from one sample each. Multiple serovars were isolated from 7 of 14 positive samples.

Analysis of pork samples identified seven *Salmonella* serovars. *Salmonella* Anatum ($n = 12$) was the most common, followed by *S.* Derby ($n = 8$), *S.* Amsterdam ($n = 6$), *S.* Rissen ($n = 5$), *S.* Stanley ($n = 4$), *S.* Orion ($n = 3$) and *S.* Panama ($n = 2$). Multiple serovars were isolated from 10 of 25 samples.

Analysis of buffalo meat samples identified three *Salmonella* serovars. *Salmonella* Stanley ($n = 3$) was the most frequent, followed by *S.* Orion ($n = 1$) and *S.* Newport ($n = 1$). Multiple serovars (*S.* Stanley and *S.* Newport) were isolated from one sample.

3.2. Antimicrobial resistance of isolates

Next, the antimicrobial resistance of 20 isolates from 12 beef samples, 35 isolates from 23 pork samples and 5 isolates from 4 buffalo meat samples was examined. Table 2 shows the resistance rates of *Salmonella* isolates from beef, pork and buffalo meat, which serve as an indicator of antimicrobial resistance. All 60 isolates examined in this study were susceptible to CIP and NOR. In the 20 isolates from beef samples, resistance to STR, TET, AMP, AMC, SXT, CHL, CTX and NAL was 80%, 75%, 70%, 45%, 30%, 15%, 5%, and 5%, respectively. In the 35 pork isolates, resistance to TET, AMP, STR, SXT, NAL, AMC, CHL and CTX was 63%, 60%, 57%, 37%, 14%, 11%, 11%, and 6%, respectively. In the five buffalo meat isolates, resistance to STR, TET and AMP was 80%, 60%, and 60%, respectively, and all five buffalo meat isolates were susceptible to AMC, CHL, CIP, CTX, NAL, NOR and SXT.

Table 3 shows the antimicrobial resistance of all 11 isolated *Salmonella* serovars. Among four of the serovars examined, all *S.* Stanley isolates showed resistance to AMP, STR and TET, *S.* Anatum isolates showed resistance to TET, *S.* Rissen showed resistance to SXT, and *S.* Amsterdam showed resistance to NAL.

3.3. Antimicrobial resistance profiles of isolates

The resistance profiles of isolated *Salmonella* samples are shown in Table 4. The 55 resistant isolates comprised 21 resistance profiles. The AMP–STR–TET profile ($n = 9$) was the most frequent, followed by AMP–AMC–STR–TET ($n = 7$), STR ($n = 5$) and STR–SXT ($n = 5$). A total of 73% of the isolates (44/60) were identified as multidrug-resistant. The most predominant profile in beef was AMP–AMC–STR–TET (five strains of *S.* Stanley and one strain of *S.* Derby), in pork was STR–SXT (four strains of *S.* Derby and one strain of *S.* Rissen) and in buffalo meat was AMP–STR–TET (three strains of *S.* Stanley).

4. Discussion

This study showed an overall *Salmonella* prevalence of 82% (14/17) in beef, 93% (25/27) in pork and 80% (4/5) in buffalo meat samples collected from retail meat shops in Pakse. To the best of our knowledge, this is the first report regarding isolation of *Salmonella* from retail meat shops in Laos. In retail meat shops in Vietnam, ca. 40–62% of beef [8–10] and 33–70% of pork [8,10–12] meat are *Salmonella*-positive. In retail meat shops in Thailand, ca. 41% of beef [13] and 29–96% of pork [13–17] meat were found to be contaminated with *Salmonella*. The isolation rates in Laos in the current study are the same or higher than those reported in neighbouring countries such as Vietnam and Thailand; however, several factors must be considered when making such comparisons, including differences in sampling season, sanitation and isolation method. Previous reports showed that *S.* Anatum and *S.* Derby were the common serovars in pigs in Laos [4,5]. Interestingly, no *S.* Infantis was isolated in two previous studies

Table 4
Resistance profiles of *Salmonella* isolates.

Antimicrobial resistance profile[a]	No. of isolates			
	Beef	Pork	Buffalo meat	Total
No resistance demonstrated	3	1	1	5
Resistance to one agent				
STR	2	2	1	5
NAL		4		4
TET		1		1
AMP		1		1
Resistance to two agents				
STR–TET	1			1
AMP–TET		4		4
CTX–TET		1		1
STR–SXT		5		5
Resistance to three agents				
AMP–STR–TET	2	4	3	9
AMP–TET–SXT	1	1		2
AMP–AMC–TET		1		1
Resistance to four agents				
AMP–AMC–STR–TET	6	1		7
AMP–STR–TET–SXT		3		3
AMP–NAL–STR–TET		1		1
AMP–CHL–TET–SXT		1		1
Resistance to five agents				
AMP–CHL–STR–TET–SXT	1	2		3
AMP–CTX–STR–TET–SXT	1			1
AMP–AMC–STR–TET–SXT	1			1
AMP–AMC–CTX–STR–TET		1		1
Resistance to six agents				
AMP–AMC–CHL–STR–TET–SXT	1	1		2
Resistance to seven agents				
AMP–AMC–CHL–NAL–STR–TET–SXT	1			1
Total	20	35	5	60

STR, streptomycin; NAL, nalidixic acid; TET, tetracycline; AMP, ampicillin; CTX, cefotaxime; SXT, sulfamethoxazole/trimethoprim; AMC, amoxicillin/clavulanic acid; CHL, chloramphenicol.
[a] SXT counted as one agent.

in Laos or in the present study [4,5], whereas *S.* Infantis was frequently found in humans, pigs and pork in European countries [18], the USA (http://www.cdc.gov/nationalsurveillance/salmonella_surveillance.html) [19] and Japan [20,21]. The high prevalence of *S.* Anatum and *S.* Derby in pigs and pork, with no *S.* Infantis, might be characteristic of Laos. The current isolates from beef, pork and buffalo meat in Laos showed no resistance to new quinolones such as CIP and NOR, but ≥50% of predominant *Salmonella* isolates such as *S.* Stanley, *S.* Anatum, *S.* Derby and *S.* Rissen were resistant to AMP, STR and TET. To date, reports have shown that all *Salmonella* isolates from meat and animals in Laos lack resistance to new quinolones [3,4], which is in agreement with the present results. In general, many isolates are resistant to AMP, STR and TET, which constitute some of the most widely used antibiotics in human medicine and veterinary fields. Thus, the present results were expected and reflect those reported in neighbouring countries such as Vietnam [9,10,12,15] and Thailand [15,16].

Percentages of multidrug resistance to more than two of the examined antibiotics in beef *Salmonella* isolates ($n = 15$) and in pork *Salmonella* isolates ($n = 26$) were not statistically different (χ^2 test, $P = 0.953$). Furthermore, the current results suggested that 73% of isolates (44/60) were resistant to multiple antibiotics, which is in good agreement with the findings of previous studies in Vietnam [9,10,12,22] and Thailand [16].

When consuming meat in the study area, cooking with sufficient heat to prevent cross-contamination from raw meat to unheated food and cooking equipment is very important. The present findings may provide useful information for treatment of patients and infected domestic animals. In May 2010, the World Health Assembly approved the following resolution on food safety: 'Advancing food safety initiatives' (WHA63.3; http://apps.who.int/gb/ebwha/pdf_files/WHA63/A63_R3-en.pdf). In Laos, programmes for food safety should be implemented as soon as possible. Moreover, education on food hygiene and other hygiene practices should also be promoted.

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Competing interests

None declared.

Ethical approval

Not required.

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