

## Salmonella Prevalence in Slaughtered Buffaloes and Pigs and Antimicrobial Susceptibility of Isolates in Vientiane, Lao People's Democratic Republic

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**ABSTRACT.** This is the first report regarding isolation of *Salmonella* from cecum samples of buffaloes and pigs and characterization of the isolates in Laos. The organisms were isolated from 8% (4/50) of buffaloes and 76% (37/49) of pigs. In buffaloes, 3 animals harbored serotype 9,12:–,1,5, and 1 animal harbored both *S. Derby* and *S. Javiana*. In pigs, the most predominant serotypes were *S. Derby* (51%) followed by *S. Anatum* (45%), *S. Weltevreden* (15%) and *S. Stanley* (5%). The buffalo isolates were susceptible to the antimicrobials tested, whereas the pig isolates showed 10 resistance patterns to 1–5 antibiotics. Of the 59 pig isolates, the resistance rates to tetracycline, streptomycin, ampicillin, sulfamethoxazole-trimethoprim, chloramphenicol, amoxicillin-clavulanic acid and nalidixic acid were 24%, 22%, 14%, 5%, 2%, 2% and 2%, respectively. The results suggest that pigs and buffaloes harbor *Salmonella*, with a higher prevalence especially in pigs, and all the isolates showed sensitivity to cefotaxime, norfloxacin and ciprofloxacin.

**KEY WORDS:** buffaloes, cecum contents, Lao People's Democratic Republic (Laos), *Salmonella*, swine.

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*Salmonella enterica* subspecies *enterica* is one of the most important foodborne bacteria in the world. Infection with *Salmonella* spp. is a major cause of diarrhea in children and young adults in developing countries [2, 17]. The reservoirs of these organisms are considered to be animals, particularly chickens and pigs, and these organisms are easily isolated from the feces [8]. The main source of infection in humans seems to be consumption of foods from animal origin such as contaminated eggs, unpasteurized milk, cross-contaminated foods and drinking water [5, 6, 9, 17]. In Southeast Asian countries, *Salmonella* spp. are commonly found in chicken eggs, chicken meat, and pork sold in markets [4, 8, 9, 11, 12].

In Lao People's Democratic Republic (Laos), there are very few reports of *Salmonella* in humans and livestock. Previous reports have suggested that the prevalence of *Salmonella* in Laotians presenting with diarrhea is only 0.6% (5/880) [16]. However, Inthavong *et al.* [10] reported that the prevalence of *Salmonella* on pig carcass swabs at slaughterhouses is relatively high (66.1%) and indicated that a large amount of contaminated meat is consumed daily. Laotians prefer to eat buffalo, pig and chicken over other meat sources; however, there are no report available concerning the prevalence of *Salmonella* in live animals or the antimicrobial susceptibility of isolates from animal feces.

In the present study, we examined the prevalence of *Salmonella* in buffaloes and pigs at a slaughterhouse in Vientiane, Laos, and determined the antimicrobial susceptibility of the various isolates obtained.

Cecum swab samples were collected from 50 buffaloes and 49 pigs in February 2007 at the Dorn Du slaughterhouse in Vientiane, Laos. The buffaloes were approximately 3–10 years of age, and were usually fed roughage (not grain). The pigs were approximately 8 months of age, and were bred on personal farms. The buffaloes were shipped to the slaughterhouse from 6 livestock brokers and a few farmers, and the pigs were from 6 livestock brokers. We could not confirm the number of farms from which the pigs were shipped.

Each cecum sample was collected using 2 commercial swab sets (BD BBL Culture Swab Plus, BD, NJ, U.S.A.), stored at 4°C and immediately transported to the microbiology laboratory of the WHO International Salmonella & Shigella Center, National Institute of Health, Nonthaburi, Thailand; the samples were analyzed within 30 hr of collection. Briefly, for isolation of *Salmonella* spp., each swab sample was placed in 9 ml of buffered peptone water (Merck, Darmstadt, Germany), thoroughly mixed and then incubated at 37°C for 18 hr. Afterwards, 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, the RV cultures were streaked onto modified semi-solid Rappaport Vassiliadis (MSRV) agar (Merck) and Desoxycholate-Hydrogen-Sulfide-Lactose (DHL) agar (Nissui, Tokyo, Japan) and were incubated at 37°C for 18

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hr. Typical *Salmonella* colonies (1–3 colonies) were selected from each specimen for confirmation based on biochemical characteristics [7] using triple sugar iron (TSI) agar (Nissui), lysine indole motility (LIM) agar (Nissui), catalase, oxidase tests and other biochemical tests. Serotyping with anti-O and anti-H immune sera was then carried out, and the antimicrobial susceptibility of the serotypes was examined by the disk diffusion method [3] using BD Sensi-Disks (BD) with Mueller-Hinton agar plates (BD). Ten types of antimicrobial disk, namely, 10 µg of ampicillin (ABPC), 20 µg of amoxicillin with 10 µg of clavulanic acid (AMPC-CVA), 30 µg of cefotaxime (CTX), 10 µg of streptomycin (SM), 30 µg of tetracycline (TC), 30 µg of chloramphenicol (CP), 30 µg of nalidixic acid (NA), 30 µg of norfloxacin (NFLX), 5 µg of ciprofloxacin (CPFX) and 23.75 µg of sulfamethoxazole with 1.25 µg of trimethoprim (SMX-TMP) were used for the tests. In these tests, *Escherichia coli* ATCC25922, were used as quality control strains. The choice of the 10 antimicrobial disks was based on the guidelines of BD Sensi-Disks for Enterobacteriaceae (<http://www.bdj.co.jp/micro/products/1f3pro00000qho5o-att/54-sd-hantei-CLSI.pdf>).

As shown in Table 1, all *Salmonella* strains isolated in this study were identified as *S. enterica* subspecies *enterica*. *Salmonella* spp. were isolated from 4 (8%) of the 50 buffalo samples and 37 (76%) of the 49 pig samples. In the buffa-

loes, serotype 9,12:–:1,5 was isolated from 3 animals, and both *S. Derby* and *S. Javiana* were isolated from 1 animal. In the 37 positive pigs, *S. Derby* and *S. Anatum* were found in 12 samples, *S. Derby* and *S. Weltevreden* were found in 8 samples, *S. Anatum* was found in 8 samples, *S. Derby* was found in 5 samples, *S. Stanley* and *S. Anatum* were found in 3 samples, *S. Stanley* was found in 1 sample and *S. Weltevreden* was found in 1 sample. In the present study, the most predominant serotypes isolated were *S. Derby* (51%; 25/49), *S. Anatum* (45%; 22/49), *S. Weltevreden* (15%; 9/49) and *S. Stanley* (5%; 3/49).

We examined the antimicrobial resistance of 5 isolates from the 4 buffaloes and 59 isolates from the 37 pigs. All the isolates from the buffaloes were susceptible to the 10 antimicrobials tested. Table 2 shows the proportion of *Salmonella* isolates from the pigs demonstrating antimicrobial resistance by serotype. The resistance rates to ABPC, AMPC-CVA, SM, TC, CP, NA and SMX-TMP of the pig isolates were 14%, 2%, 22%, 24%, 5%, 2% and 12%, respectively; all the isolates were susceptible to CTX, NFLX and CPFX.

Table 3 shows the profile of antimicrobial resistance of the *Salmonella* isolates from the pigs. Among the 21 resistant isolates, there were 8 multidrug resistance patterns, and the ABPC/SM/TC/CP/SMX-TMP pattern was the most frequent (3 strains; all *S. Derby*). Monodrug resistance to SM

Table 1. Prevalence of *Salmonella* spp. in cecum samples obtained from buffaloes and pigs at a slaughterhouse in Lao PDR

Animal species	No. of animals	No. of positive samples (%)	Serotype	No. of samples <sup>a)</sup>
Buffalo	50	4(8)	9,12:–:1,5	3
			Derby and Javiana	1
			Derby and Anatum	12
Pig	49	37 (76)	Derby and Weltevreden	8
			Anatum only	8
			Derby only	5
			Stanley and Anatum	2
			Stanley only	1
			Weltevreden only	1

a) *Salmonella* 9,12:–:1,5, *S. Derby* and *S. Javiana* were isolated from 6% (3/50), 2% (1/50) and 2% of the buffaloes (1/50), respectively. *S. Derby*, *S. Anatum*, *S. Weltevreden* and *S. Stanley* were isolated from 51% (25/49), 45% (22/49), 18% (9/49) and 6% of the pigs (3/49), respectively.

Table 2. Proportion of *Salmonella* isolates from pigs demonstrating antimicrobial resistance by serotype

Serotype	No. of strains examined	Antimicrobial agents <sup>a)</sup>									
		ABPC	AMPC-CVA	CTX	SM	TC	CP	NA	NFLX	CPFX	SMX-TMP
Derby	25	20	4		36	20	12	4			12
Anatum	22	14			9	27					14
Weltevreden	9										
Stanley	3				67	67					33
Total	59	14	2		22	24	5	2			12

a) ABPC: ampicillin. AMPC-CVA: amoxicillin with clavulanic acid. CTX: cefotaxime. SM: streptomycin. TC: tetracycline. CP: chloramphenicol. NA: nalidixic acid. NFLX: norfloxacin. CPFX: ciprofloxacin. SMX-TMP: sulfamethoxazole with trimethoprim.

Table 3. Numbers of *Salmonella* isolates from pigs with different resistance profiles

Profiles <sup>a)</sup>	No. of isolates	<i>Salmonella</i> serotype			
		Derby	Anatum	Weltevreden	Stanley
Noresistance demonstrated	38	12	16	9	1
Resistance to one agent					
TC only	3	1	2		
SM only	6	6			
Total	9	7	2	0	0
Resistance to two agents					
ABPC/AMPC-CVA	1	1			
ABPC/TC	1	1			
SM/TC	2		1		1
TC/NA	1	1			
Total	5	3	1	0	1
Resistance to three agents					
ABPC/TC/SMX-TMP	2		2		
SM/TC/SMX-TMP	1				1
Total	3	0	2	0	1
Resistance to four agents					
ABPC/SM/TC/SMX-TMP	1	0	1	0	0
Resistance to five agents					
ABPC/SM/TC/CP/SMX-TMP	3	3	0	0	0
Total	59	25	22	9	3

a) ABPC: ampicillin. AMPC-CVA: amoxicillin with clavulanic acid. CTX: cefotaxime. SM: streptomycin. TC: tetracycline. CP: chloramphenicol. NA: nalidixic acid. NFLX: norfloxacin. CPFX: ciprofloxacin. SMX-TMP: sulfamethoxazole with trimethoprim.

(6 strains; all *S. Derby*) and TC (3 strains; 2 strains of *S. Anatum* and 1 strain of *S. Derby*) was predominant in the isolates from pigs. Multiantimicrobial resistance was shown by *S. Derby*, *S. Anatum* and *S. Stanley*.

In the present study, the prevalence of *Salmonella* in the buffaloes was 8% (4/50), and the most predominant serotype identified was 9,12:–:1,5. To the best of our knowledge, there are no reports available concerning the prevalence of *Salmonella* in buffaloes in Asia. The prevalence of *Salmonella* among domestic buffaloes in Laos may generally be around 8%, and serotype 9,12:–:1,5 may particularly be observed in buffaloes in Laos.

The prevalence of *Salmonella* in the pigs was 76% (37/49), and the most predominant serotypes identified were *S. Derby* and *S. Anatum*. The prevalence and serotypes of the isolates in the pigs were thought to depend on geographic location; for example, in a previous study, 12% (7/110 heads) of slaughtered pigs in Gunma Prefecture, Japan, harbored *Salmonella*, and the predominant serotypes were *S. Typhimurium* and *S. Derby* [13]. On the other hand, in a study conducted in Vietnam, *Salmonella* spp. were isolated from 5% (23/439 heads) of pigs, and the predominant serotypes were *S. Javiana* (9/25 strains), *S. Derby* (4/25 strains) and *S. Weltevreden* (3/25 strains) [15]. In a study conducted in Northern Thailand, the overall prevalence of *Salmonella* in pigs in slaughterhouses was 28% (97/349 strains), and most of the serotypes isolated were *S. Rissen*, *S. Weltevreden*

and *S. Anatum* [11]. The prevalence of *Salmonella* in Laos obtained in the present study was higher than that in other Asian countries. We could not, however, confirm the number of farms from which the pigs were shipped by the 6 livestock brokers, and the present study was conducted in only 1 slaughterhouse. In Laos, large-scale commercial pig production is not common and therefore many pigs are bred on personal farms. In this regard, additional surveys of different pig farms are necessary. *S. Derby* and *S. Anatum* are the most common serotypes found in animals and humans throughout the world; however, *S. Weltevreden* has recently been reported to be a frequent and increasing cause of human salmonellosis and is the predominant serotype in Southeast Asian countries [1, 4, 14, 15]. The results of the present study indicate that *S. Weltevreden* might be widely distributed in pigs in Laos.

In general, high sensitivity to most antimicrobial agents was observed among the buffalo and pig isolates. In Laos, the use of antimicrobial agents as a feed additive to animals is not regulated; however, nobody gives feed additives to animals because most pigs and buffaloes are bred on personal farms. The unpopularity of using antimicrobial agents as feed additives may be one of the predisposing factors for many *Salmonella* strains with high sensitivity to antimicrobial agents.

The cecum contents of pigs and buffaloes may be potential vehicles for contaminating edible products at slaughter-

houses. A survey of the prevalence of *Salmonella* spp. in other food animals, such as chickens, and other foods should be performed to determine the important sources of *Salmonella* infection in Laos.

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