

***Salmonella* Prevalence in Slaughtered Buffaloes and Cattle in Champasak Province, Lao People's Democratic Republic**

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ABSTRACT

This is the first local study of *Salmonella* prevalence in slaughtered buffaloes and cattle in Champasak province, Lao PDR. In total, 269 animals (225 buffaloes and 44 cattle) were examined for the presence of *Salmonella* in mesenteric lymph nodes, caecum, abdominal and diaphragmatic muscles. The proportion of slaughtered buffaloes and cattle identified as positive for *Salmonella* was 6.69%, with 7.11% in buffaloes and 4.54% in cattle. The highest proportion was identified in the Houayset (9.67%), followed by the Nasaykham (6.25%) abattoir, and the lowest proportion was found in the KhanGneng abattoir (5.37%). No *Salmonella* was found in the Houayphek abattoir. Only 3.25% (22 of 676) of all samples collected were identified as contaminated with *Salmonella*. Out of 22 isolates, four serotypes and three untypable *Salmonella*-attributed to serogroups B, C and E, were identified. *S. Weltevreden* accounted for 45.45% (10 of 22) of the total isolates, followed by *S. Brunei* 22.72% (5 of 22) and *S. 8,20:-* 13.63% (3 of 22), while a similar level of 4.54% was found for *S. Typhimurium*, *S. Bovismorbificans*, *S. 4.5.12:b:-* and *S. 8,20:y:-*. The results indicated that slaughtered buffaloes and cattle sampled in this study served as sources of *Salmonella* in humans. Hence, slaughterhouse surveillance of *Salmonella* and other food-borne diseases is needed in order to prevent *Salmonella* from reaching foodstuffs meant for human consumption.

Keywords: buffaloes and cattle, *Salmonella* serotypes, Champasak Province, Lao PDR

INTRODUCTION

Salmonella species are one of the most important food-borne bacteria in the world and a major cause of diarrhea in children and young adults in developing countries (Al-Abri *et al.*, 2005). The primary reservoir of these organisms is the intestinal tract of carrier animals and humans

and these organisms are easily isolated from feces (Foley and Lynne, 2008). Globally, animal meat and animal products are the main sources of *Salmonella* infection in humans. Next to poultry, beef is the most important source of *Salmonella* food-borne infection. The process of evisceration is regarded as one of the most important sources of contamination of carcasses with *Salmonella* in

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abattoirs (Samuel *et al.*, 1980; Adesiyun and Oni, 1989).

In Southeast Asian countries, *Salmonella* spp. are commonly found in chicken eggs, chicken meat, pork and beef sold in markets (Boonmar *et al.*, 1998; Nakamura *et al.*, 2004; Tran *et al.*, 2004; Padungtod and Kaneene, 2006).

Previous studies conducted in Thailand on *Salmonella* in animals, animal products and humans indicated the presence of 127 *Salmonella* serotypes, with the predominant serotypes *S. Weltevreden*, *S. Stanley*, *S. Enteritidis*, *S. Rissen*, *S. Anatum* and *S. Covallis* (Bangtrakulnonth *et al.*, 2004; Bangtrakulnonth *et al.*, 2005; Padungtod *et al.*, 2007). In Vietnam, 38 *Salmonella* serotypes were found in humans, animals and animal products, with the predominant serotypes *S. Typhimurium*, *S. Anatum*, *S. Weltevreden*, *S. Emek* and *S. Rissen* (Vo *et al.*, 2006).

In Lao PDR, 15 *Salmonella* serotypes were found in humans in 2009 and 12 serotypes in 2010. The predominant serotypes are *S. Stanley* (9 of 27) and *S. Give* (4 of 27) (National Center for Laboratory and Epidemiology, 2011). However, little is known about *Salmonella* serotypes found in meat and animal products and that negatively affect humans. Moreover, such serotypes have only been found in Vientiane, Lao PDR (Nakamura *et al.*, 2004; Inthavong *et al.*, 2006; Boonmar *et al.*, 2008).

In Champasak province, Lao PDR, cases of human *Salmonella* infection have been reported (National Center for Laboratory and Epidemiology, 2009). However, no further details on the serotypes that cause human diseases are available. Moreover, there is still an absence of pertinent information on *Salmonella* contamination in meat, especially in beef, buffalo meat and animal products, in Champasak province.

In the present study, *Salmonella* serotypes were identified among *Salmonella* isolates from diverse samples of buffaloes and cattle. Those serotypes can be used for future protective and diagnostic purposes. This local study revealed the

potential role of buffaloes and cattle as sources of infection not only in their own species, but also for other animals as well as humans.

The objective of this study was firstly, to determine the prevalence of *Salmonella* spp. in buffaloes and cattle at four small abattoirs in Champasak province, Lao PDR and secondly, to identify the serogroups and serotypes of the various isolates.

MATERIALS AND METHODS

Slaughterhouses

Four small slaughterhouses were chosen in this study. Two slaughterhouses (Houayphek and Khan-Gneng) in Phonethong district, one slaughterhouse in Pathoumphone (Nasaykham) and one slaughterhouse in Bachieng district (Houayset) were chosen on the basis of their convenient accessibility to the provincial veterinary laboratory as well as to Champasak University. The slaughterhouses were all located within a radius of 4–25 km. All the slaughterhouses were small scale and consisted of a roof and a concrete floor. The equipment used in all slaughterhouses included large knives, axes and wooden tables, but no other modern or mechanized equipment. Both electricity and water were available in all slaughterhouses, however, a waste treatment system did not exist in any of the slaughterhouses. Among the four slaughterhouses, only the Houayphek abattoir had walls (1 m high). In all the slaughterhouses, electrical stunning was applied. The slaughtering process (stunning, bleeding, dressing, eviscerating and splitting of carcasses) was performed on the floor and in the same location within each slaughterhouse. Only meat cutting was performed on the wooden tables. In the Houayset and Khan-Gneng slaughterhouses, pigs were slaughtered in the same abattoir, but in a separate area. The equipment solely used for pig slaughtering and the wooden table solely for pork cutting were separate from the beef and buffalo meat processing areas. There were also workers who were allocated solely

to pig slaughtering.

Animals

In total, 269 slaughtered buffaloes and cattle in four slaughterhouses were sampled. Of these, 225 were buffaloes and 44 were cattle. The animals were aged approximately 1–7 years and were usually fed roughage (grasses and rice straw). The animals were supplied by a multitude of farms in various districts of Champasak province and freely shipped by truck to the diverse slaughterhouses. The animals were kept in lairage for approximately 1–5 d prior to slaughtering.

Samples

Diaphragmatic and abdominal muscle samples from 269 animals were aseptically collected. In addition, mesenteric lymph nodes and caecal samples were collected from 69 animals.

Collection and preparation of tissue samples

Samples were collected during the period December 2010 to January 2011. Samples were collected immediately after slaughter in the slaughtering area and placed in individual labeled plastic bags. Approximately 25 g of each tissue sample (mesenteric lymph node, diaphragmatic and abdominal muscles) was collected with a clean knife. The caecum samples (approximately 1g) were collected with a sterile swab and subsequently stored in 9 mL of semisolid Cary-Blair transport medium. Then, these samples were placed in an ice box and transported within 1 hr to the veterinary laboratory of Champasak province, for storage in a freezer for 1 wk. Subsequently, the samples were transported to the WHO National *Salmonella* and *Shigella* Center, Bangkok, Thailand for further processing. In the laboratory, samples were aseptically cut into small pieces, weighed and then placed in separate sterile plastic bags with buffered peptone water (BPW; Merck KGaA; Darmstadt, Germany).

Isolation and Identification

Techniques recommended by the International Organization for Standardization, 6579:2002/Amd 1:2007 were used to isolate and identify the *Salmonella*. Briefly, the following procedures were employed.

Amounts of 25 g of diaphragmatic and abdominal muscle samples from each of the 269 animals were separately pre-enriched in 225 mL of BPW and incubated at 37 °C for 24 hr. Approximately 1 mL of the caecum sample in semisolid Cary-Blair transport medium was added to 10 mL of BPW and subsequently incubated at 37 °C for 24 hr. Approximately 0.1 mL and 0.2 mL of the culture was then transferred into 10 mL of Rappaport-Vassiliadis soy (RVS) broth (Merck KGaA; Darmstadt, Germany) and modified semisolid Rappaport Vassiliadis (MSRV) agar (Merck KGaA; Darmstadt, Germany), respectively, and incubated at 42 °C for 24 hr. On the third day, the inoculum was separately streaked on selective CHROMagar™ *Salmonella* medium (CHROMagar, Paris, France) and xylose lysine desoxycholate (XLD) agar medium (Merck KGaA; Darmstadt, Germany) and incubated at 37 °C for 24 hr.

The plates were examined for growth of *Salmonella* colonies. Approximately five suspected colonies (translucent, red with jet black center on XLD and mauve on CHROMagar) were selected from each plate, streaked onto the nutrient agar and incubated at 37 °C for 24 hr. Suspect colonies were tested biochemically with the standard methods (Ewing, 1986) and putative *Salmonella* isolates were examined for agglutination with polyvalent O antiserum (S&A Reagents Lab Ltd.; Bangkok, Thailand). *Salmonella* serotyping was also performed at the WHO National *Salmonella* and *Shigella* Center, Bangkok, Thailand.

For serotyping, the somatic (O) antigens of the *Salmonella* isolates were determined with the application of the slide agglutination tests as described by Ewing (1986). The flagellar (H) antigens were identified by means of the Sven

Gard technique (Koehn, 1970). The antigenic formulae of the *Salmonella* serovars, as described by Grimont and Weill (2007), were used to name the serovars.

The chi-square (goodness of fit) test was applied to determine the differences in the positivity rate among slaughterhouses.

RESULTS

The distribution of slaughtered buffaloes and cattle that tested positive for *Salmonella* listed by abattoir is shown in Table 1. The overall positivity rate was 6.69% (18 of 269 buffaloes and cattle) with 7.11% (16 of 225) in buffaloes and 4.54% (2 of 44) in cattle. The highest positivity rate for *Salmonella* was identified in the Houayset (9.67%), followed by the Nasaykham (6.25%) abattoir and lowest rate was in the Khan Gneng abattoir (5.37%). No *Salmonella* was found in the Houayphek abattoir (Table 1). There was a significant difference ($P < 0.05$) in the proportion

of animals which tested positive for *Salmonella* in the Houayset and Houayphek abattoirs.

It was found that 3.25% (22 of 676) of all samples collected were contaminated with *Salmonella* (Table 1). The contamination rate of *Salmonella* in all samples was highest in the Houayset abattoir (3.91%).

Table 1 also shows the contamination rate of *Salmonella* in the mesenteric lymph nodes was the highest (7.24%), followed by in the diaphragm muscle (4.08%) and the abdominal muscle (2.23%). No *Salmonella* was found in the caecal samples.

Out of 22 isolates, 4 serotypes and 3 untypable *Salmonella*-attributed to serogroups B, C and E were identified. These were: *S. Weltevreden*, *S. Brunei*, *S. Bovismorbificans*, *S. Typhimurium*, *S. 8, 20:-:-*, *S. 4.5.12:b:-* and *S. 8,20:y:-*. *S. Weltevreden* accounted for 45.45% (10 of 22) of total isolates, followed by *S. Brunei* 22.72% (5 of 22), *S. 8,20:-:-* 13.63% (3 of 22), *S. Typhimurium* 4.54% (1 of 22), *S. Bovismorbificans*

Table 1 *Salmonella* prevalence of buffalo and cattle samples in the four abattoirs.

Abattoir	Species	Positive animals	Sample ¹				Total
			AM	DM	MLN	Caecum	
Houayset	Buffalo	11/93(11.8) ²	5/93(5.37)	4/93(4.3)	4/43(9.3)	0/43(0)	13/272(4.78)
	Cattle	1/31(3.2)	0/31(0)	1/31(3.22)	0/12(0)	0/12(0)	1/86 (1.16)
	subtotal	12/124(9.67)	5/124(4.03)	5/124(4.03)	4/55(7.27)	0/55(0)	14/358(3.91)
KhanGneng	Buffalo	4/85(4.7)	1/85(1.17)	3/85(3.52)	1/14(7.14)	0/14(0)	5/198(2.52)
	Cattle	1/8(12.5)	0/8(0)	2/8(25.00)	0/0(0)	0/0(0)	2/16(12.5)
	subtotal	5/93(5.37)	1/93(1.07)	5/93(5.37)	1/14(7.14)	0/14(0)	7/214(3.27)
Nasaykham	Buffalo	1/15(6.66)	0/15(0)	1/15(6.66)	- ³	-	1/30(3.33)
	Cattle	0/1(0)	0/1(0)	0/1(0)	-	-	0/2(0)
	subtotal	1/16(6.25)	0/16(0)	1/16(6.25)	-	-	1/32(3.12)
Houayphek	Buffalo	0/32(0)	0/32(0)	0/32(0)	-	-	0/64(0)
	Cattle	0/4(0)	0/4(0)	0/4(0)	-	-	0/8(0)
	subtotal	0/36(0)	0/36(0)	0/36(0)	-	-	0/72(0)
Total	Buffalo	16/225(7.11)	6/225(2.66)	8/225(3.55)	5/57(8.77)	0/57(0)	19/564(3.36)
	Cattle	2/44(4.54)	0/44(0)	3/44(6.81)	0/12(0)	0/12(0)	3/112(2.67)
	subtotal	18/269(6.69)	6/269(2.23)	11/269(4.08)	5/69(7.24)	0/69(0)	22/676(3.25)

¹ AM = Abdominal muscle, DM = Diaphragmatic muscle, MLN = Mesenteric lymph nodes

² All data are shown as Number of positive samples / Number of samples (%).

³ No samples.

4.54% (1 of 22), S. 4,5,12:b:- 4.54% (1 of 22) and S. 8,20:y:- 4.54% (1 of 22) as shown in Table 2.

In this study, three serotypes (*S. weltevreden*, *S. Brunei* and *S. Bovismorbificans*) and *S. untypable* (*S. 8,20:y:-*) were identified in the KhanGneng slaughterhouse. Similarly, two serotypes (*S. Weltevreden* and *S. Brunei*) and two *untypable* (*S. 8,20:-:-* and *S. 4,5,12:b:-*) were found in the Houayset slaughterhouse. However, only one serotype (*S. Typhimurium*) was detected in

the Nasaykham abattoir and no *Salmonella* was isolated in the Houayphek slaughterhouse. *S. Weltevreden* and *S. Brunei* were found in two of the four slaughterhouses.

Table 2 shows that three serotypes (*S. Weltevreden*, *S. Brunei* and *S. Bovismorbificans*) and two *untypable* (*S. 8,20:-:-* and *S. 4,5,12:b:-*) were identified in the mesenteric lymph nodes. Similarly, three serotypes (*S. Weltevreden*, *S. Brunei* and *S. Typhimurium*) and two *untypable* (*S. 8,20:-:-* and

Table 2 *Salmonella* serovars of buffalo and cattle samples in the four abattoirs.

Slaughter-house	Animal species	Sample	No. of positive	Serovar(s)
Houayset	Buffalo	Adbominal muscle	5	<i>S. Weltevreden</i> (4); <i>S. 8,20:-:-</i> (1)
		Diaphragmatic muscle	4	<i>S. Weltevreden</i> (3); <i>S. 8,20:-:-</i> (1)
		Mesenteric lymph nodes	4	<i>S. Weltevreden</i> (1); <i>S. Brunei</i> (1); <i>S. 8,20:-:-</i> (1); <i>S. 4,5,12:b:-</i> (1)
	Cattle	Caecum	0	-
		Adbominal muscle	0	-
		Diaphragmatic muscle	1	<i>S. Weltevreden</i> (1)
		Mesenteric lymph nodes	0	-
		Caecum	0	-
KhanGneng	Buffalo	Adbominal muscle	1	<i>S. Brunei</i> (1)
		Diaphragmatic muscle	3	<i>S. Brunei</i> (2); <i>S. Weltevreden</i> (1)
		Mesenteric lymph nodes	1	<i>S. Bovismorbificans</i> (1)
		Caecum	0	-
	Cattle	Adbominal muscle	0	-
		Diaphragmatic muscle	2	<i>S. Brunei</i> (1); <i>S. 8,20:y:-</i> (1)
		Mesenteric lymph nodes	0	-
		Caecum	0	-
Nasaykham	Buffalo	Adbominal muscle	0	-
		Diaphragmatic muscle	1	<i>S. Typhimurium</i> (1)
		Mesenteric lymph nodes	0	-
		Caecum	0	-
	Cattle	Adbominal muscle	0	-
		Diaphragmatic muscle	0	-
		Mesenteric lymph nodes	0	-
		Caecum	0	-
Houayphek	Buffalo	Adbominal muscle	0	-
		Diaphragmatic muscle	0	-
		Mesenteric lymph nodes	0	-
		Caecum	0	-
	Cattle	Adbominal muscle	0	-
		Diaphragmatic muscle	0	-
		Mesenteric lymph nodes	0	-
		Caecum	0	-

S.8,20:y:-) were found in diaphragmatic muscle samples. However, no *Salmonella* was isolated in the caecal samples. Two serotypes (*S. Weltevreden* and *S. Brunei*) and *S. untypable* (*S. 8,20:-:-*) were found in three types of samples (mesenteric lymph nodes, abdominal and diaphragmatic muscles).

DISCUSSION

In the present study, the prevalence of *Salmonella* in the animals was 6.69% (18 of 269), with a more pronounced prevalence in buffaloes (7.11%) than in cattle (4.54%), although the difference was not statistically significant (at $P = 0.05$). In this study, the contamination of *Salmonella* identified in slaughtered buffalo and cattle was low. This might have been due to the free range system of animal rearing, without the use of concentrated feed or ingredients of animal origin that have the potential to be contaminated with *Salmonella*. It is speculated that the lack of stress prior to slaughter is likely to decrease the number of animals shedding *Salmonella*.

Out of four selected slaughterhouses in this study, the *Salmonella* contamination rate was revealed to be highest in the Houayset abattoir, while there was no contamination at the Houayphek abattoir. Such a divergence in findings might have been due to the dissimilar levels of hygiene, quality of meat inspection and facilities present in the abattoirs at dissimilar periods.

In this study, *Salmonella* was isolated from 5 of 69 (7.24%) of the mesenteric lymph node samples, 11 of 269 (4.08%) of the diaphragmatic muscle sample and 6 of 269 (2.23%) of the abdominal muscle samples. However, the difference in the rate of *Salmonella* contamination among these tissue types was not statistically significant (at $P = 0.05$). In the present study, the contamination rate of *Salmonella* in the mesenteric lymph node samples was low. This finding was in accordance with the results of previous studies conducted in different countries. Wray and Sojka (1977) found 0.30% to 11.60%

Salmonella prevalence in apparently healthy cattle at abattoirs. Similar findings were reported in the Philippines (Tacal and Menez, 1968), in Pakistan (Rehman *et al.*, 1987), in Canada (Abouzeed *et al.*, 2000) and in Ethiopia (Molla *et al.*, 2003). In addition, Alemayehu *et al.* (2003) reported 4.5% *Salmonella* contamination in the mesenteric lymph node samples of slaughtered cattle in Ethiopia. However, compared to other countries, *Salmonella* contamination rates in mesenteric lymph node samples were high in Australia where Frost *et al.* (1988) and Samuel *et al.* (1979) reported contamination rates of 46.67% and 54% respectively. Similarly, Moo *et al.* (1980) reported a contamination rate as high as 71.76%.

The present study revealed that *Salmonella* contamination in the diaphragmatic and abdominal muscle samples of slaughtered buffalo and cattle was low. This finding was in accordance with the results of Alemayehu *et al.* (2003). However, the contamination of *Salmonella* in beef and buffalo meat varies in different nations. In Iran, the contamination of *Salmonella* in buffalo and bovine meat was 7% and 12.2%, respectively (Bahiraei and Moghaddam, 2004). In Nepal, the prevalence of *Salmonella* in buffalo meat was 13.50%, (Maharjan *et al.*, 2006), whereas, in India the prevalence of contamination exceeded that of Nepal (Sharma *et al.*, 1989). On the other hand, in Senegal, *Salmonella* contamination in beef samples at slaughterhouses was 43% (Stevens *et al.*, 2006). Furthermore, in Thailand, *Salmonella* prevalence in beef samples was in the range 22.3–66.7% (Angkititrakul *et al.*, 2011).

A previous study conducted in Vientiane, Lao PDR by Boonmar *et al.* (2008) revealed the contamination rate of *Salmonella* in caecal samples of buffaloes to be 8%. However, in the present study, no *Salmonella* was found in the caecal samples. This was in accordance with the work of Tacal and Abellanosa (1965) conducted in the Philippines. None of the 300 individual fecal samples of carabao were found to be contaminated with *Salmonella*. Hence, the

present study shows that faecal material was not the source of the *Salmonella* contamination. However, the contamination of carcasses may have resulted from the cutting knives and chopper axe used for slaughtering and from the water used for washing the slaughterhouse floor. Aftab *et al.* (2012) reported that the abattoir floor had a higher *Salmonella* contamination than did the chopper axe and knife. Peel and Simmons (1978) also found that knives used for slaughtering and for dressing beef carcasses were factors in spreading *Salmonella* in meatworks in Australia. In the present study, all slaughtering processes were performed on unclean abattoir floors which could have been an abundant source of contamination. In addition, *Salmonella* contamination may have resulted from the unclean hands and clothing of workers.

In this study, seven *Salmonella* serotypes were identified, with *S. Weltevreden* and *S. Brunei* as the predominant serotypes which are of public health importance and non-host adapted. Three of these serotypes (*S. Weltevreden*, *S. Brunei* and *S. Typhimurium*) are known to negatively affect human health in Lao PDR (National Center for Laboratory and Epidemiology, 2011). In addition, *S. Typhimurium* and *S. Weltevreden* were also found in swine carcasses and caecal samples at the Don Du abattoir, Vientiane, Lao PDR (Inthavong *et al.*, 2006; Boonmar *et al.*, 2008). The assessed animals constituted a potential reservoir of infection and thus might have infected other animals. The results of the present study indicate that *S. Weltevreden* is likely to be widely distributed among animals in Laos. This serotype also was found in buffalo meat in India (Singh *et al.*, 2010). Similarly, *S. Typhimurium* was a predominant serotype found in buffalo and bovine meat in Iran (Bahiraei and Moghaddam, 2004). However, serotypes identified in buffaloes in the course of the present study were found to be divergent from those serotypes found in buffaloes slaughtered at the Don Du abattoir, Vientiane, Lao PDR. At Don Du abattoir, the serotypes were

established to be *S. 9,12:-: 1,5*, *S. derby* and *S. Javiana* (Boonmar *et al.*, 2008).

CONCLUSION

The proportion of slaughtered buffaloes and cattle identified as positive for *Salmonella* was 6.69%, with 7.11% in buffaloes and 4.54% in cattle. The highest proportion was identified in the Houayset (9.67%) and the lowest proportion was in the KhanGneng abattoir (5.37%). No *Salmonella* was found to be present in the Houayphek abattoir. Only 3.25% (22 of 676) of all samples collected were identified as contaminated with *Salmonella*. The contamination in the mesenteric lymph node samples was revealed to be the highest (7.24%, 5 of 69), followed by in the diaphragm muscle samples (4.08%, 11 of 269) and in the abdominal muscle samples (2.23%, 6 of 269). No *Salmonella* was found to be present in the caecal samples.

Out of 22 isolates, four serotypes and three untypable *Salmonella*-attributed serogroups B, C and E were identified. These consisted of: *S. Weltevreden*, *S. Brunei*, *S. Bovismorbificans*, *S. Typhimurium*, *S. 8,20:-:-*, *S. 4.5.12:b:-* and *S. 8,20:y:-*. *S. Weltevreden* accounted for 45.45% (10 of 22) of the total isolates, followed by *S. Brunei* 22.72% (5 of 22) and *S. 8,20:-:-* 13.63% (3 of 22). Similarly, 4.54% (1 of 22) was found for *S. Typhimurium*, *S. Bovismorbificans*, *S. 4.5.12:b:-* and *S. 8,20:y:-*.

The findings of the present study suggest firstly, a need for measures for appropriate control of *Salmonella* and other food-borne diseases in abattoirs, in order to prevent *Salmonella* from reaching foodstuffs meant for human consumption; secondly, the need for further research on meat and meat products at several markets in order to detect the extent of *Salmonella* in the province assessed in the course of the present study.

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