

# MICROBIAL CONTAMINATION OF PIG CARCASSES AT A SLAUGHTERHOUSE IN VIENTIANE CAPITAL, LAO PDR

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**Abstract.** A cross-sectional study was conducted to determine microbial contamination of pig carcasses at a slaughterhouse in Vientiane, capital of Lao People Democratic Republic (Lao PDR). Between November 2004 and April 2005, 62 pig carcasses were randomly selected. From each carcass, pooled swabs (from "1" prior to and "2" after evisceration) and 25 g of tissue of mesenteric lymph nodes (MLN) were collected. The swab samples were examined for Aerobic Plate Count (APC) and Enterobacteriaceae Counts (EBC) and cultured for *Salmonella*. The lymph nodes were cultured for *Salmonella* only. Swabs 1 and 2 had mean APC of 4.70 and 4.85 log<sub>10</sub>CFU/cm<sup>2</sup>, respectively. These two means were significantly ( $p=0.0001$ ) different. The means of EBC were 2.81 log<sub>10</sub>CFU/cm<sup>2</sup> for Swab 1, and 2.98 log<sub>10</sub>CFU/cm<sup>2</sup> for Swab 2. The difference were also statistical significant ( $p=0.0001$ ). The frequency of *Salmonella* isolation from Swab 1 was 46.8%, for Swab 2 was 66.1%, and from mesenteric lymphnodes was 53.2%. Eight different *Salmonella* serotypes were identified. The most frequent (29.1%) serotype was *S. Rissen*, followed by *S. Anatum* (26.2%), *S. Derby* (18.4%), and *S. Elisabethville* (8.7%). The other serotypes identified were *S. Amsterdam* (7.8%), *S. Typhimurium* (4.9%), *S. Agona* (2.9%), and *S. Enteritidis* (1.9%). Results of this study showed the levels of contamination with aerobic bacteria and Enterobacteriaceae were higher than recommended standards, and the carcasses were contaminated with *Salmonella*.

## INTRODUCTION

The slaughtering process is important to assure meat safety. To prevent microbial contamination, which affects consumer health and causes serious public health problems, appropriate slaughterhouse design and internal control are necessary. The level and type of microbial contamination are monitored for maintaining and improving the hygienic status and quality of meat produced by a slaughterhouse. This report is the first study in Lao PDR reflecting the hygiene of pigs in a slaughterhouse.

Aerobic Plate Count (APC) and Enterobacteriaceae Counts (EBC) are generally used as hygienic indicators in the food chain (Anonymous, 2001; Warriner *et al*, 2002; Nel *et al*, 2004; Zweifel *et al*, 2005). The APC depicts general microbial contamination. The EBC is a marker for possible fecal contamination. Feces are a main source of pathogens, such as *Escherichia coli* O157:H7, *Salmonella* or *Campylobacter* (WHO, 1995).

The specific objectives of this pork hygiene study in Lao PDR were: (1) to determine the contamination level using APC and EBC of pig carcasses prior to and post-evisceration in slaughtering process; (2) to estimate the *Salmonella* prevalence and serotypes from the carcass surfaces and mesenteric lymph nodes.

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## MATERIALS AND METHODS

Samples were taken from the largest slaughter house in Vientiane in the Dorn-Du District. Sixty to eighty pigs per day are processed. After mechanical stunning, the animals are suspended on a rail, bled, then submerged in a 62-68°C water container (water changed daily prior to commencing slaughter). Dehairing is done using a machine with additional manual treatment if necessary. After spraying with tap water, the slaughtered animals are eviscerated, dropped on the floor for splitting and suspended on the rail again. After another washing, the split carcasses are left to dry in another room, then sent to market.

During 7 visits, 62 pig carcasses were randomly selected. From each carcass, two swab samples (prior to and after evisceration) and mesenteric lymph nodes were collected. The samples were stored in a cooling box and transported to the laboratory, where the microbiological analysis was performed the same day. The samples were taken by sponge swabs from the back, jowl, ham and belly of 62 pig carcasses and pooled for each carcass.

Swab samples were kept in plastic bags (Stomacher bag) for at least two minutes in 100 ml of buffered peptone water and 250 cycles per minutes of a peristaltic Stomacher was performed. Thereafter, serial ten-fold dilutions for APC and EBC were performed.

Mesenteric lymph nodes (MLN) were placed in boiling water for 3 seconds to eliminate superficial contamination (Swanenburg *et al*, 2001), and cut into small pieces with sterile devices. Thereafter, 25 g was transferred into a stomacher bag, 225 ml of BPW was added and homogenized at about 250 cycles per minute for two minutes to detect *Salmonella*.

### Aerobic Plate Count and Enterobacteriaceae Counts

For enumeration of APC, the pour-plating procedure was performed. One milliliter of a series of dilutions ( $10^{-2}$ ,  $10^{-3}$  and  $10^{-4}$ ) of the

sample homogenate was mixed with agar medium (Nutrient agar, Merck®) and incubated at 37°C for 24 hours.

EBC was performed following the guidelines given in the standard operating procedure "Enumeration of Enterobacteriaceae by the colony count technique" issued by the Health Protection Agency, UK (Anonymous, 2002). One milliliter of a series of dilutions ( $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$ ) was mixed with melted violet red bile glucose agar (VRBGA).

### *Salmonella* isolation and identification

*Salmonella* isolation and identification followed the procedure of the Institute of Meat Hygiene (Freie Universitat Berlin, 2004) and ISO 6579 (2002). The procedure included pre-enrichment (BPW), selective enrichment (Muller-Kauffmann Tetrathionate (MKTT) and Rappaport-Vassiliadis (RV) broths, selective plating (XLD and BPLS (Merck) and finally a serological identification using *Salmonella* antisera of SIFIN®, Berlin, Germany.

## RESULTS

### Aerobic Plate Count

The APC was calculated as  $\log_{10}$ CFU of APC per  $\text{cm}^2$  (Table 1). Generally, the APC ranged from 4.4 to 4.9 with a mean of 4.70  $\log_{10}$ CFU/ $\text{cm}^2$  for Swab 1. In Swab 2, the results ranged from 4.5 to 5.3 with a mean of 4.85  $\log_{10}$ CFU/ $\text{cm}^2$ . Overall, the difference between the  $\log_{10}$ CFU/ $\text{cm}^2$  of the two swabs was significant ( $p=0.0001$ ).

### Enterobacteriaceae Counts

The EBC was obtained by culturing the pooled swab samples like the APC (Table 1) and calculated in  $\log_{10}$ CFU of EBC per  $\text{cm}^2$ . In Swab 1, the EBC ranged from 2.3 to 3.1 with a mean of 2.81. Swab 2 values ranged from 2.1 to 3.3 with a mean of 2.98. Overall, the  $\log_{10}$ CFU/ $\text{cm}^2$  of EBC was significantly ( $p=0.0001$ ) different between Swab 1 and Swab 2.

### *Salmonella*

Of 186 samples (2 swabs and 1 MLN per

Table 1  
Means and ranges of Log<sub>10</sub>CFU/cm<sup>2</sup> of APC and EBC in Swab 1 and Swab 2.

Indicator	Samples	Mean Log <sub>10</sub> CFU/cm <sup>2</sup>	Range Log <sub>10</sub> CFU/cm <sup>2</sup>	Sign
APC	Swab 1	4.70	4.3-4.9	p = 0.0001
	Swab 2	4.85	4.4-5.3	
EBC	Swab 1	2.81	2.3-3.1	p = 0.0001
	Swab 2	2.98	2.1-3.3	

Table 2  
Prevalence of *Salmonella* in Swab 1 and Swab 2, and MLN of pig carcasses.

Sample type	N	Positive	Prevalence (%)	95% CI
Swab 1	62	29	46.8	34.2 - 59.8
Swab 2	62	41	66.1	52.9 - 77.4
MLN	62	33	53.2	40.2 - 65.8
Over all	186	103	55.4	47.93 - 62.6

carcass), 103 (55.4%) were positive for *Salmonella*. Table 2 shows the prevalence of *Salmonella* obtained from each type of sample. *Salmonella* was found more frequently (66.1%, 95% CI: 52.9 - 77.4) in Swab 2, followed by the MLN (53.2%, 95% CI: 40.2 - 65.8) then swab 1 (46.8%, 95% CI: 34.2 - 59.8). No significant (p=0.088) difference was observed among these data.

**Serotyping**

Eight serotypes were identified from 103 isolates (Fig 1). The most frequent (29.1 %) serotype was *Salmonella* Rissen, followed by *S. Anatum* (27.2%), *S. Derby* (19.4%), and *S. Elisabethville* (7.8%). The other serotypes identified were *S. Amsterdam* (7.8%), *S. Typhimurium* (3.9%), *S. Agona* (2.9%), and *S. Enteritidis* (1.9%). These serotypes were found in all types of samples, with the exception of *S. Enteritidis*, which was isolated from Swab 2 only.

**DISCUSSION**

In this study, the APC ranged from 4.4 log<sub>10</sub>CFU/cm<sup>2</sup> to 5.3 log<sub>10</sub>CFU/cm<sup>2</sup>. These findings are similar to those obtained by Pearce *et al* (2004). Those authors obtained an APC of 4.46 log<sub>10</sub>CFU/cm<sup>2</sup> (belly) and 4.75 log<sub>10</sub>CFU/

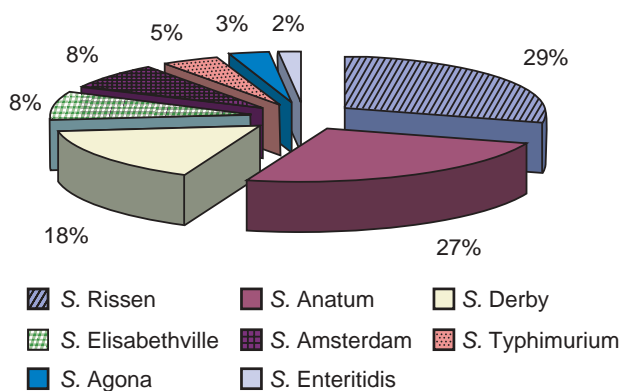


Fig 1–*Salmonella* serotypes from Swab 1 and Swab 2 and MLN.

cm<sup>2</sup> (neck) at dehairing. Nevertheless, the mean APC in Swab 2 (at the end of the process) of 4.85 log<sub>10</sub>CFU/cm<sup>2</sup>, was much higher than the results obtained by Pearce *et al* (2004) with were 3.65 log<sub>10</sub>CFU/cm<sup>2</sup> (belly) and 3.53 log<sub>10</sub>CFU/cm<sup>2</sup> (neck). The decrease could be due to the singeing. According to other studies (Rivas *et al*, 2000; Warriner *et al*, 2002) in this step the microbial load on the surface of carcass usually decreases. In the slaughterhouse investigated here, no singeing took place.

Similar results were obtained for EBC. All samples had EBC above the acceptable value

of 2.0 log<sub>10</sub>CFU set by Decision 2001/471/EC of the EU Commission (Anonymous, 2001). This study found levels ranging from 2.3 to 3.1 and 2.1 to 3.3 log<sub>10</sub>CFU/cm<sup>2</sup> for Swab 1 and Swab 2, respectively. The overall numbers of EBC were significantly different ( $p = 0.0001$ ) between Swab1 and Swab 2. Increase in number of EBCs was probably caused by contamination during subsequent operations, such as evisceration, washing and cutting. In this slaughterhouse, after evisceration, the carcasses were laid down on the floor for cutting.

Sources of *Salmonella* in pork carcasses and products have been investigated over the years in many developed countries. For example, the SALINPORK Project (Danilo *et al*, 2000) explored various epidemiological and economic aspects of *Salmonella* in pork.

The occurrence of *Salmonella* in Swab 2 was higher (66.1%) than in Swab 1 (46.8%). Such an increase on carcass surfaces during processing is well documented (Gill and Jones, 1997). It has been attributed to the contamination of carcasses in the gastrointestinal tract, mouth and tonsils during the course of processing. *Salmonella* can also come from the slaughterhouse environment as well as from humans, if hygienic standards are extremely low (Warriner *et al*, 2002). Therefore, the high proportion of positive Swab 2 samples compared to that obtained from Swab 1 samples is due to increased contamination of carcasses along the slaughter line. The rotating flails that are used to remove hairs may squeeze feces from the anus, potentially contaminating the equipment with fecal microorganisms, including *Salmonella*, and hence contaminate the carcass (Borch *et al*, 1996). Thus, the presence of *Salmonella* in Swab 1 samples strongly suggests carcass contamination during dehairing and/or during earlier stages of the procedure (Berends *et al*, 1997).

In particular the evisceration process, including bung dropping, and the removal of the pluck-set have been identified as critical con-

trol points. The cutting process is not normally considered to be an important source of carcass contamination (Berends *et al*, 1997; Gill and Jones, 1997). In this study, Swab 2 was taken at the splitting stage after washing the carcass. This was done to monitor for the presence of *Salmonella* on the final carcass.

With particular reference to the serotypes obtained from the lymphnodes, *S. Rissen* (9), *S. Anatum* (9), *S. Derby* (9), *S. Typhimurium* (2), *S. Elisabethville* (2), *S. Amsterdam* (1) and *S. Agona* (1) were obtained. In three cases, three serotypes were found per slaughtered pig: 1. *S. Rissen*, *S. Anatum* and *S. Rissen*; 2. *S. Anatum*, *S. Rissen* and *S. Anatum*; 3. *S. Derby*, *S. Anatum* and *S. Anatum*

Finally, in 4 cases both swabs were positive with the same serotype (2 with *S. Rissen*, 1 with *S. Anatum*, and 1 with *S. Derby*).

In Lao PDR, *Salmonella* is commonly found in all types of meat and cooking materials (Nakamura *et al*, 2004). To date, there is no information regarding the *Salmonella* serotypes in Lao PDR. The findings given here are similar to the serotypes obtained from neighboring countries. Here, the most common serovars from all sources (human, pig, poultry) were *S. Weltevreden*, *S. Enteritidis*, *S. Anatum*, *S. Derby*, *S. Typhimurium*, *S. Rissen*, *S. Stanley*, *S. Panama*, *S. Agona*, and *S. Paratyphi B var Java* (Bangtrakulnonth *et al*, 2004). From a study in the Mekong Delta in Vietnam, Tran *et al* (2004) found the most predominant *Salmonella* serotypes were *S. Javiana*, *S. Derby*, and *S. Weltevreden*.

The results obtained here indicate that microbiological contamination of pork carcasses during the slaughter processing in the abattoir Dorn-Du was high. The carcasses were contaminated with aerobic and Enterobacteriaceae bacteria, as well as with *Salmonella*. *Salmonella* Enteritidis, which is considered a risk to human health, was among the *Salmonella* isolates found. Microbiological contamination could be due to various aspects

that include environmental slaughterhouse conditions, as well as pig and farm managerial factors.

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