[1] Food Safety Research Activities in the frame of PRISE: http://www.prise-pcp.org/en/research_areas/food_safety_issues_for_foods_of_animal_origin

[2] Mainly tetrodotoxin, http://www.fao.org/DOCREP/003/T1768F/T1768F04.htm

[3] http://www.thepigsite.com/swinenews/12874/dangerous-levels-of-growth-hormones-found-in-vietnamese-pork ; http://english.vietnamnet.vn/social/2006/12/641318/ ; http://iasvn.org/uploads/files/bai15-food%20safety_0827160350.pdf

[4] http://www.thanhniennews.com/healthy/?catid=8&newsid=25036

[5] http://www.monstersandcritics.com/news/health/news/article_1432546.php/Chinese_milk_containing_melamine_found_in_Vietnam_ ; http://english.vietnamnet.vn/social/2008/10/807450/ ; http://www.cbc.ca/world/story/2008/10/03/tainted-milk.html

[6] http://www.thanhniennews.com/healthy/?catid=8&newsid=46649

[7] http://www.wto.org/english/thewto_e/acc_e/a1_vietnam_e.htm

[8] http://www.wto.org/English/tratop_e/sps_e/spsund_e.htm
http://www.wto.org/English/docs_e/legal_e/15-sps.pdf
http://www.wto.org/English/res_e/booksp_e/analytic_index_e/sps_01_e.htm#article2B1a

[9] SPS Balance Sheet for Vietnam, Strengthening links between supply and demand of SPS-related technical assistance in a sub-group of ASEAN countries (Stage 2) Research work for the Standards and Trade Development Facility, http://www.standardsfacility.org/New_files/Briefings/Final_Viet%20Nam.pdf

[10] "The use of chemical substances in livestock production": The consumer is buying diseases (http://www.hanoimoi.com.vn/vn/46/65112/); "Eat 'extra lean' pork meat may change the gender (http://www.hanoimoi.com.vn/vn/46/65218/)

[11] NATISCIENCE DAILY 28/08/03 Study Shows Agent Orange Contamination Of Food In Vietnamese City, http://www.sciencedaily.com/releases/2003/08/030828073513.htm

[12] http://www.cpv.org.vn/cpv/Modules/News/NewsDetail.aspx?co_id=30181&cn_id=110339

13 Vietnam Official Gazette, Nr4, March 2006, http://www.nea.gov.vn/luat/luat_eng/toanvan/43_2006_QD-TTg.pdf

[14] 2002a. Capacity Building for the prevention of foodborne diseases - Legislation report. In Institution, Institution, ed. (Hanoi, World Health Organization), 44 p.; Review of regulations related to food safety in Vietnam, from 2002 to March 2007, http://www.prise-pcp.org/en/publications/thesis_collection

[15] "Vietnamese farming sector needs modernization" http://www.thepoultrysite.com/poultrynews/12294/vietnamese-farming-sector-needs-modernization

[16] http://web.worldbank.org/external/projects/main?menuPK=228424&pagePK=64283627&piPK=73230&theSitePK=40941&Projectid=P090723

[17] http://devex.com/projects/food-and-agriculture-products-quality-project-in-vietnam

[18] http://aimp.apec.org/Documents/2008/SCSC/SCSC1/08_scsc1_007.doc

[19] http://www.ambhanoi.um.dk/en/menu/AboutUs/News/NewsArchives2008/DanidaSupports-NewTechnicalRegulationsForFoodSafety.htm?WBCMODE=AuthoringPre

[20] http://www.adb.org/Documents/TARs/VIE/39421-VIE-TAR.pdf

SALMONELLA ENTERICA SUBSP. ENTERICA ALONG THE PIG COMMODITY CHAIN IN VIETNAM

C. Le Bas[a,*], T. T. Hanh[b], N. T. Thanh[b], Nguyen Manh Cuong[c], Hoang Vu Quang[c], Vu Trong Binh[c], N. B. Minh[d], C. Gardon[e], A. Patin[e], Chu Ky Son[f], Le Thanh Mai[f], L. Bily[g], A Labbé[g], M. Denis[g], P. Fravalo[g]

[a]CIRAD, Campus International de Baillarguet, TA30/A, 34398 Montpellier Cedex 5, France
[b]NIVR, National Institute of Veterinary Research, 86, Truong Chinh, Hanoi, Vietnam
[c]IPSARD, 6, Nguyen Cong Tru, Hai Ba Trung, Hanoi, Vietnam
[d]NIHE, National Institute of Hygiene and Epidemiology, 1, Yersin Street, Hanoi, Vietnam
[e]IUT Amiens, Institut Universitaire de Technologie, Avenue des Facultés, Le Bailly, 80025 AMIENS Cedex 1, France
[f]HUT, Hanoi University of Technology, 1, Dai Co Viet, Hanoi, Vietnam
[g]AFSSA, Agence Française de Sécurité Sanitaire des Aliments, Unité HQPAP, BP53, 22440 Ploufragan, France

ABSTRACT

Foodborne diseases are a particularly important concern in the current Vietnamese context, shortly after accession to the WTO, not only for public health reasons, but also because of the global evolution of consumer demand and habits, the production chain and state regulations. Salmonella enterica subsp. enterica (Salmonella) is known as one of the most frequent foodborne zoonoses in the world and has been isolated in human and pork products in Vietnam, where pork represents 77% of total meat consumption.

The aim of this paper is to describe Salmonella prevalence and epidemiology results along the pig commodity chain, at farm, slaughterhouses (both in Hanoi suburb, 206 samples and in slaughter plants connected with Nam Sach farmers in Hai Duong Province, 126 samples,) and for traditional raw meat fermented Vietnamese sausages 'nem chua'. The prevalences by fattening pigs at farm (19%) and in caecal content before slaughter (52% and 40%) were comparable to other studies, whereas the carcass contamination rates were much higher (95.7% and 67%). 35.7 % of 213 'nem chua' samples were positive. Serotyping of these isolates suggested that Salmonella contamination is mainly originated from the pig meat itself, with a smaller contribution from the sausages' processing steps. The results confirm that slaughterhouses in Vietnam are the key point to be focused on for improving food safety in the pork commodity chain. The potential public health threat of Salmonella in pork products has been proven by the high prevalence found on 'nem chua' sausages.

Further serotyping and genotyping of Hanoi slaughterhouses' isolates aimed to understand the ways of contamination of Salmonella at this important key step. The direct contamination of carcasses through faecal material from the same pig was not clear, but a direct faecal contamination was observed for the tank and the well water. Thus, our results suggested that the main source of carcass contamination was indirect, through the slaughtering environment. Moreover, the results may indicate that live pigs could be infected during lairage through contaminated water and environment, leading to a persistence of certain clones over longer periods.

Since the majority (about 90%) of the pig production in Vietnam goes through small slaughter plants, we propose priority economical hygienic control measures adapted to small plants, that could largely decrease the carcass contamination rate.

Keywords: Salmonella; epidemiology; pig;

farm; nem chua; serotyping; slaughterhouse; pig; serotyping; pulsed-field gel electrophoresis; Vietnam

INTRODUCTION

Food safety issues have become a priority in Vietnam over the last five years, because of the need for a general public health improvement (2002b; 2006), the increasing consumer demand for food quality and safety (Ginhoux, 2001), and the recent integration of Vietnam into the WTO (2006). 1 421 herds, 36 904 cases and 400 deaths were officially reported from 2000 to 2008. According to the WHO, reported cases are widely underestimated (2002a). In Vietnam, nontyphoidal Salmonella has been isolated on human patients with diarrhoea (Isenbarger, et al., 2002; Vo, et al., 2006b) and in various food products (Dao and Yen, 2006; Quang, 1999). Pork has been described in many countries as an important source of Salmonella (Berends, et al., 1997; Chang, et al., 2005; Chiu, et al., 2002; Murugkar, et al., 2005; Padungtod and Kaneene, 2006), with a determinant role of slaughterhouses and slaughtering practices (Berends, et al., 1998; Botteldoorn, et al., 2003; Hurd, et al., 2005). Pork represents about 77% of the meat products consumed in Vietnam. Studies on retailed pork revealed contamination with Salmonella in 33 to 40% of samples (Dao and Yen, 2006; Thuy, et al., 2006). Recent studies in South Vietnam described very variable Salmonella prevalence (from 5.2 to 69.9%) among pig faeces, carcasses and meat (Phan, et al., 2005a; Vo, et al., 2006b). Nevertheless, data on Salmonella contamination and epidemiology along the pig commodity chain in Vietnam are scarce. Small, traditional slaughterhouses (10 to 20 pigs/day/unit) are still the most common structures for pig slaughtering in Vietnam (Wegener, 1999). The aim of this paper is to give an overview of Salmonella contamination and epidemiology along the pig commodity chain through various studies at farm, during slaughter and in a traditional Vietnamese sausage 'nem chua', made out of raw pork meat, fermented in a banana leaf and consumed without cooking. Serotyping and pulsed-field gel electrophoresis analyses were used for some of the isolates, in order to understand the possible means of Salmonella contamination.

MATERIAL AND METHODS

Sampling

- At farm: Small pig farms, from 4 to 10 sows, member of the quality improvement program from the Nam Sach cooperative in Hai Duong province, were sampled in 2006. A total of 180 samples, rectal and environment swabs in fattening boxes, water and feed, were taken in 10 randomly selected farms, among the 128 farms of Nam Sach Breeders' Association.
- At slaughter: In small slaughterhouses, 206 samples -caecal contents, carcass swabs, rinsing and well water- were taken in Hanoi suburban area in 2004 and 2005, and 126 samples -caecal contents, carcass and environment swabs, rinsing water- in slaughterhouses connected with Nam Sach sampled farmers in 2006.
- 'Nem chua': From 36 different sellers in 19 markets in Hanoi, 213 samples of Vietnamese traditional fermented sausage 'nem chua', were randomly taken in 2007.

Detection method

The bacterial analysis included a 1/10 pre-enrichment in buffered peptone water (sample of 25g for faeces), selective enrichment in Rappaport-Vassiliadis broth, and isolation through streaking onto XLT4 and Rambach agar. One Salmonella characteristic colony on XLT4 and on Rambach was inoculated to Kligler-Iron agar tubes. Each characteristic Kligler-Iron was confirmed through additional biochemical tests: manitol, motility, urease, indol, Lysin decarboxylase, ONPG, ADH, ODC.

Salmonella strains were further serotyped and some of the slaughterhouse isolates were genotyped.

Slaughter practices

In Hanoi, slaughter practices were observed during the entire slaughtering process in several slaughtering units, between 11am and 2pm. All the units observed were contiguous, with identical slaughter practices. For all sampled pig batches, the duration of transportation and lairage, the province of origin and the farming type were recorded.

Serotyping

All Salmonella isolates were serotyped according to the Kauffmann-White scheme (Popoff, 2001), using slide agglutination tests with Salmonella-O and H sera (Diagnostics Pasteur, Paris, France). Throughout this paper, Salmonella enterica subsp. enterica will be abbreviated to Salmonella. The serotype name, for instance Salmonella enterica subsp. enterica serotype Typhimurium, will be abbreviated to S. Typhimurium.

Genotyping

The bacteria were obtained from an overnight culture in BHI and DNA agarose plugs were prepared as described by Ragimbeau et al. (Ragimbeau, et al., 1998). A quarter plug was used for restriction endonuclease digestions in separate reactions using 40 U of either Xba1 or Bln1 (Boehringer) under the manufacturer's conditions, in a final volume of 100 µl for five hours' incubation at the appropriate temperature. PFGEs were done using the CHEF-DRIII system (Biorad Laboratories, USA).

Macrorestriction profile analysis

Electrophoretic patterns were compared by BioNumerics® (Applied Maths). Similarities between profiles, based on band positions, were derived from the Dice correlation coefficient with a maximum position tolerance of 1%. A dendrogram of the analysis of the combined Xba1- and Bln1-digested DNA was constructed to reflect the similarities between the strains in the matrix. Strains were clustered by the Unweighted Pair-Group Method using the Arithmetic Mean UPGMA (Struelens, 1996).. The use of a combination of two enzymes enabled an increase in the discriminatory power of the method. When any differences in PFGE patterns were observed, the patterns were reported as different (Barret, et al., 2006). In case of two indistinguishable PFGE patterns with the combination of the two enzymes, the isolates were considerate as clonally related and thus belonging to the same chain of transmission (Struelens, 1998).

RESULTS

Slaughter practices in Hanoi suburban area

The practices observed in traditional abattoirs in Hanoi, represented in Figure 1, are the same from one unit to another. Slaughter usually takes place during the night and at lunchtime, in order to supply Hanoi markets with fresh meat in the morning and in the afternoon. The sampled pigs came from family smallholdings and bigger state farms. Most of the farms were situated in the provinces of Ha Nam (about 50 km from Hanoi), Ha Tay (about 20 km) or Thanh Hoa (about 150 km). The duration of transportation and lairage together varied between 2 and 24 hours. All slaughtering steps took place on the floor without mechanical equipment. The pigs were unloaded in the front and were moved through the slaughter hall to reach the lairage box (Figure 1). Bleeding was done without stunning. Workers used boiling water for scalding. Experienced workers did the evisceration by hand. Well water was stored in a tank and then used for rinsing carcasses and offal or for other purposes, such as rinsing tools or cleaning the floor. Offal was rinsed in the front of the hall, not in a separate place. After work, tank, ground and tools were rinsed and cleaned without a defined disinfection programme. There was no specific wastewater management. Pig carcasses and offal were weighed, bought by wholesalers or retailers and transported to Hanoi markets on motorbikes. All steps between bleeding and transportation lasted about 10 to 30 min.

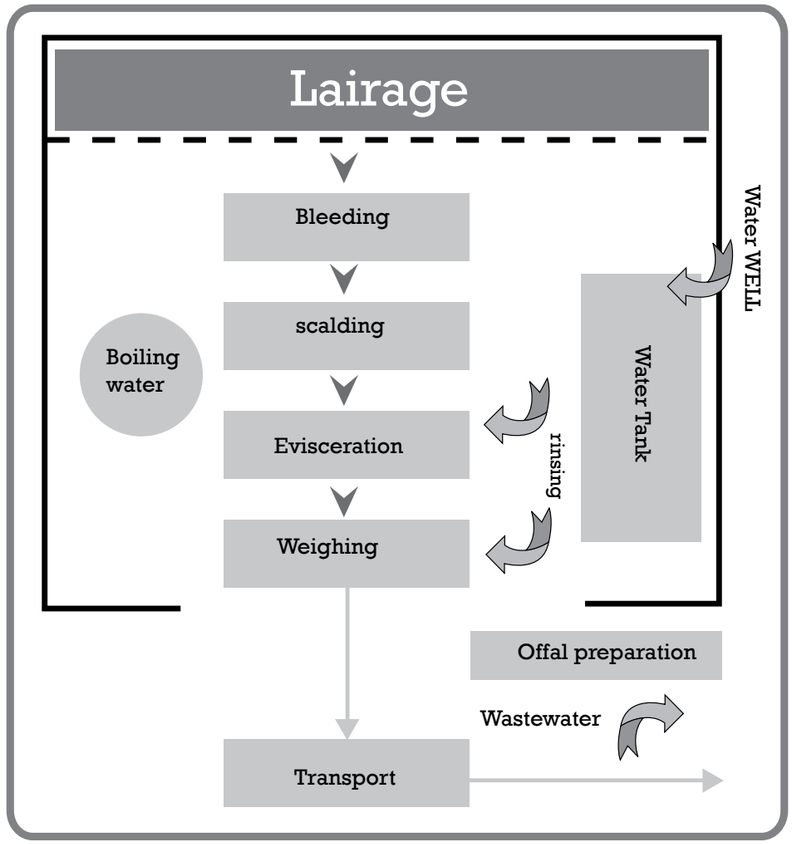


Figure 1: Schematic floor diagram of processing steps in small slaughtering units in Hanoi

Proportion of positive results

- At farm: Results are presented in Table 1. 50% [20,2-79,8%] of the farms have at least one positive sample. 19% of the fattening pigs and 27% of the environment swabs were positive. Only one water sample used as drinking water for the pigs was found positive.

Table 1: Salmonella Positive samples at farm

Sample type	*Positive samples*	*Confidence Interval at 95%*
Rectal Swabs (fattening pigs)	*19% (17/90)*	*10,9-27,1%*
Environment Swabs (fattening box)	*27% (8/30)*	*11,4-42,6%*
Water	*3% (1/30)*	
Feed	*0% (0/30)*	

- At slaughterhouse: The proportions of positive results for Salmonella in Hanoi slaughterhouses were 52.1% for caecal contents, 95.7% for carcass swabs, 62.5% in tank water (Le Bas, et al., 2006) and 45.5% in well water samples (Table 2).

Table 2: Salmonella Positive samples in Hanoi suburban slaughterhouses

Sample type	*Positive samples*	*Confidence Interval at 95%*
Caeca	*52.1% (61/117)*	*43.1-61.2%*
Swabs	*95.7% (44/46)*	*89.8-100%*
Water Tank	*62.5% (20/32)*	*45.7-79.3%*
Water Well	*45.5 (5/11)*	

The proportions of positive samples in the slaughterhouses connected with the Nam Sach producers sampled, are summarized in Table 3: 40% in caeca, 67% on carcass swabs, 58% in tank water and in lairage environment, and 92% in bleeding environment.

Table 3: Salmonella Positive samples in slaughterhouses connected with the sampled farms belonging to the Nam Sach association.

Sample type	*Positive samples*	*Confidence Interval at 95%*
Caeca	*40% (18/45)*	*7.3-28.7%*
Carcass Swabs	*67% (30/45)*	*53.9-80.1%*
Water Tank	*58% (7/12)*	
Lairage Environment	*58% (7/12)*	
Bleeding Environment	*92% (11/12)*	

- In Vietnamese sausages 'nem chua': The prevalence of Salmonella positive 'nem chua' sampled in 36 distribution shops in 19 different markets in Hanoi was 35.2% (IC= 28.8-41.6%). A significant difference was found between the prevalence in bigger (100g) and in smaller (20g) sausages ($p<0,001$). No correlation was found between the prevalence and the size of the selling point (number of sausages sold per day), nor the origin of the meat for the sausages' manufacturing (slaughterhouse or market).

Serovar distribution

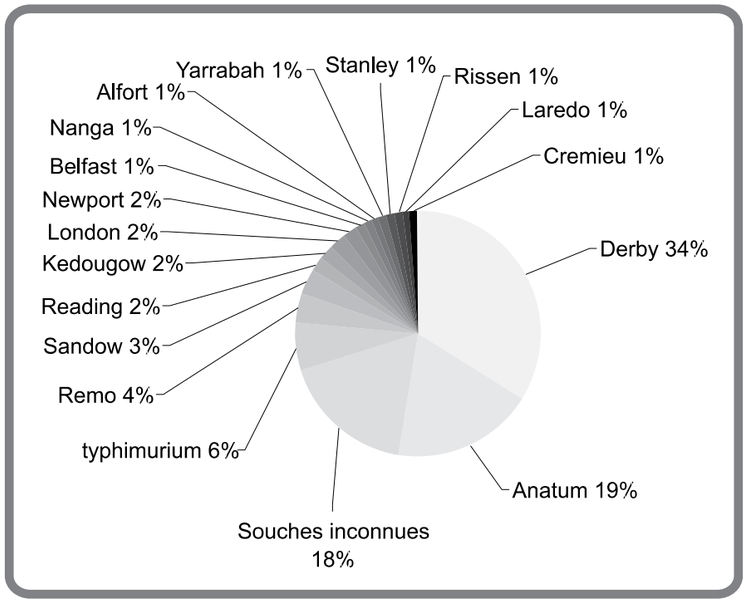
The serovar distribution of Salmonella strains isolated in Hanoi slaughterhouses is presented in Table 4. In total, the main serovars were S. Derby (51%), S. Typhimurium (12%) and S. Saint-Paul (8%). Similar proportions were found in caecal samples, with respectively 47%, 13% and 6%. On swabs, the proportion was 61%, 11% and 16%, with more S. Saint-Paul than S. Typhimurium. S. Saint-Paul was not found in tank or well water. S. Agona and Salmonella enterica subsp. salamae (II ssp.) were only isolated in tank water and S. Kedougou and S. Lamberhurt only on carcass swabs. The highest serovar diversity, with 11 different serovars, was found in caecal content, decreasing in swabs, 7 serovars, and in tank water, 6 serovars. S. London was found twice during the study, in caeca and in tank water in the same sampling batch, during the same slaughtering day.

Table 4: Serovar distribution of Salmonella isolates in pig samples from Hanoi slaughterhouses

Number of isolates per serovar					
Serovar	*Total*	*Caecal content*	*Swabs*	*Tank Water*	*Well*
Water					
Derby	*70 (51%)*	*30 (47%)*	*27 (61%)*	*11 (48%)*	*2 (40%)*
Typhimurium	*16 (12%)*	*8 (13%)*	*5 (11%)*	*2 (9%)*	*1 (20%)*
Saint-paul	*11 (8%)*	*4*	*7*		
Anatum	*5*	*3*	*1*	*1*	
Enteritidis	*4*	*4*			
Wetervreden	*2*	*2*			
Lamberhurt	*2*		*2*		
Stanley	*2*	*1*	*1*		
Kedougou	*1*		*1*		
Dumfries	*1*	*1*			
Weston	*1*	*1*			
London	*2*	*1*		*1*	
Agona	*2*			*2*	
Newport	*1*	*1*			
II spp.(1)	*1*			*1*	
Untypable strains	*15*	*8*		*5*	*2*
Total	*136*	*64*	*44*	*23*	*5*

(1) Salmonella enterica subsp. salamae (II spp.)

Figure 2: Serovar distribution of Salmonella isolated from 'nem chua' in Hanoi markets



The serovars' distribution of Salmonella strains isolated in 'nem chua' is summarized in Figure 2. The main serovars were S. Derby (34%), S. Anatum (19%) and S. Typhimurium (6%). Some less frequent serovars were also found in pork samples in Hanoi slaughterhouses: S. Kedougou, S. London, S. Newport and S. Stanley, whereas S. Remo, S. Sandow, S. Reading, S. Belfast, S. Nanga, S. Alfort, S. Yarrabah, S. Rissen, S. Laredo and S. Cremieu were not found in slaughterhouses.

Genotyping

For isolates from Hanoi slaughterhouses, we compared the PFGE profiles of S. Typhimurium and S. Derby strains between different samples (Table 6). A batch corresponds to one slaughtering period (half-day). The indices of discrimination with the combined use of Xba1 and Bln1 for S. Typhimurium and S. Derby were respectively 0.900 and 0.962. Xba1 analyses of 16 S. Typhimurium isolates produced 5 profiles. The TX1 profile was found for 9 out of the 16 isolates. The use of Bln1 provided 9 profiles with 5 out of the 16 strains presenting the type TB6. The combination of both enzymes resulted in 10 different definitive types (Figure 3). Five isolates had indistinguishable pulsotypes, i.e. the profile TX1TB6, Table 6, coming from two separate pig batches. Specifically, it was found on carcass samples in November 2004 and 6 months later in the well water and in caeca, but no longer on the carcass. TX1TB1 and TX2TB2 were found on the carcass in batch 2, October 2004, and in caeca in batch 3, November 2004, but then also no longer on the carcass.

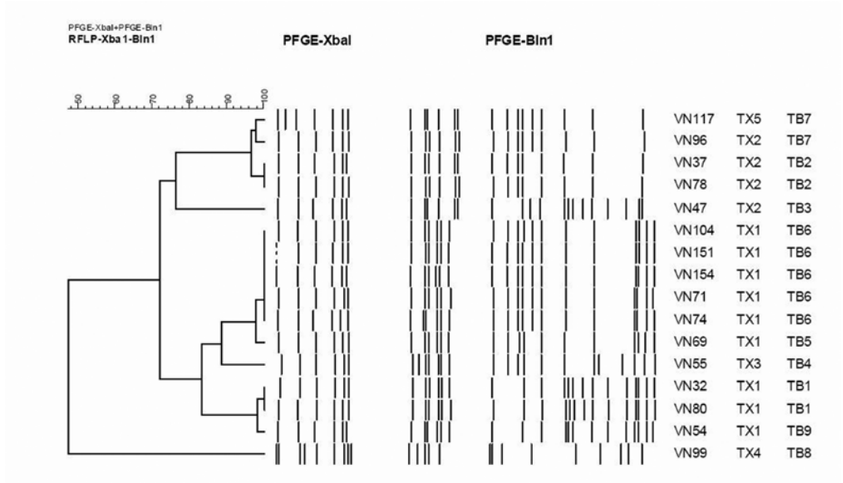
Out of the 32 S. Derby isolates, Xba1 analyses produced 11 profiles. X13 profile was found for 12, X12 for 6 and X8 for 5 isolates (Table 6). Bln1 provided 16 pulsotypes and B18 was found for 11 strains. With both enzymes, 21 different combined profiles were established. In total, 16 strains presented a similar profile and were therefore considered as clonally related. The pulsotype X13B18 was found in the same batch in caecal and water samples several times: in batch 4 in December 2004 and in batch 5 in May 2005 (Table 6). The pulsotype X12B18 was found in batch 3 in carcass swabs and caecal content. In this batch, the carcasses presented many different pulsotypes, whereas in the caecal sample the S. Derby pulsotype X12B18 was the only one detected.

Table 6: S. Typhimurium (TX) and S. Derby (X) PFGE profiles per slaughter batch in different sample types

Batch number	Date	Samples			
		Carcass	Caeca	Water Tank	Water Well
Batch 1	9.7.04	X06B12 X17B13			
Batch 2	10.25.04	TX1TB1 TX2TB2 X07B14 X08B15(2)	TX2TB3 TX1TB9 TX3TB4 X08B17 X09B18		
Batch 3	11.22.04	TX1TB6 (2) TX1TB5 X08B20 X10B18 X11B18 X12B18 X12B22	TX1TB1 TX2TB2 X12B18	X08B19	
Batch 4	12.8.04		TX2TB7 X12B23 X12B24 X12B25 X13B27 X13B18	TX4TB8 X13B18	
Batch 5	5.16.05		TX1TB6 (2) X13B18(2) X13B28(4) X14B29 X15B30(2)	TX5TB7 X13B18(2)	TX1TB6
Batch 6	6.9.04				X13B31

Numbers in parenthesis represent strains with the same profile in the same batch and sample type.

Figure 3: Macrorestriction profiles of S. Typhimurium isolates. DNA was digested with Xba1 and Bln1 and the restriction patterns analyzed. Dendrogram of the cluster analysis based on macroprofiling analysis.



DISCUSSION

Prevalence

• At farm

The precision of the Salmonella positive farms is to low to be compared with another context. Besides, farm prevalence assessments vary considerably in literature: 14.8% in the UK in 1997, 22% in Danemark in 1993-4, 23% in Holland in 1999, 27% in Germany in 1996, 51% en Ireland in 2003, 58% in France in 1999 or from 38% to 83% in the United States in 1999, for instance (Rajic, et al., 2005; Wray, 2001). Salmonella prevalence can vary depending on the season or the time of the sampling: in Alberta in 2005, 90 farms were sampled, 25.6% were found positive during the first visit, 34.4% during the second and 26.7% during the third (Rajic, et al., 2005).

The variability is also high for the proportion of Salmonella positive fattening pigs: 5.9% in Ireland, 14.3% for faecal material and 20.1% for environment samples in Alberta, 12% from faecal material in the UK (Rajic, et al., 2005; Wray, 2001).

In South-Vietnam, Vo et al reported a global Salmonella prevalence of 49.4%, in pig faecal and carcass swabs (Vo, et al., 2006b). In 1g faecal samples, another study reported 5.2% positif samples (Phan, et al., 2005b). These very various results emphasize the importance of the sampling protocol, like using pool faeces, 1g, 10g or 25g samples or the use of rectal swabs, for instance. The context of each study also contributes to the variability. Our results are closer to the findings of Vo et al than Phan et al.

The use of rectal swabs in our study can lead to less positive samples than the method using faeces pooling or caecal contents at slaughter. But despite the less sensitivity of swab method, the percentage of positive samples is quite high. The advantage of swabbing is to be able to take a sample for each pig without the interference of non-pig salmonella contamination possible in the environment.

The only positive water sample was found in a farm using the pond water for the pigs, compared to the other farms sampled using well water. This farm didn't have a biogas system and was spreading the dung directly on the fields and in the fish ponds around the farm. 2/9 rectal swabs and 3/3 fattening box's environment swabs were positive in this farm. There may be a link between the use of pond water as drinking

water for the pigs, the lack of good waste management practices and the Salmonella positive pigs in the farm.

We didn't find any positive sample in industrial feed. Other studies report very variable prevalence in industrial feed, from 0 to 34%, depending on the manufacturing process, for instance (Sauli, et al., 2005), or until 86% of some meat meals in the USA. In average, the proportion of contaminated industrial feed is around 2% (Wray, 2001). In our study, we probably stayed under the detection threshold, since we initially estimated the prevalence at 10%.

• At slaughterhouse

Prevalence results showed that carcass contamination prior to expedition to market was very high. Indeed, by comparison, recent studies in the region found different prevalence values for carcass swabs: in South Vietnam, 55.9% Salmonella positive carcass swabs among pig samples (Vo, et al., 2006a; Vo, et al., 2006b); in Thailand, 33.1% (Chantong, 2005) or 37% (Padungtod and Kaneene, 2006); in Lao PDR, 47% to 66% (Inthavong, 2005). These variations could be due to the use of different swabbing protocols between the studies, namely the entire carcass (Vo, et al., 2006b), 4x100 cm2, i.e. ham, back, belly and jowl (Chantong, 2005; Inthavong, 2005), 50 cm2 (Padungtod and Kaneene, 2006) or the entire half-carcass in our study. Nevertheless, although a larger surface was swabbed in the study in South Vietnam (Vo, et al., 2006b), the percentage of Salmonella positive swabs was still lower than in our study. The protocol was therefore not the only factor. This was confirmed by the study in slaughterhouses connected with the Nam Sach farmers, using the same carcass sampling protocol than in Hanoi suburb. The average prevalence on carcasses (67%) was found lower than in Hanoi, although this percentage remains high compared to other studies.

A high prevalence of Salmonella on the carcasses represents a high contamination pressure for later stages in the commodity chain, like persistence in the environment, cross-contamination with raw products or infection through insufficiently cooked meat (Korsak, et al., 2003). Pork is therefore a potentially significant Salmonella source into the human food chain in Vietnam. Since the surface samples of carcass give information about hygiene during the slaughter process (Swanenburg, et al., 2001a), the carcass prevalence data confirmed the general lack of hygiene during slaughter.

This is particularly remarkable because, both in Hanoi and in Nam Sach related slaughterhouses, the faecal sample contamination rate (52% and 40% respectively), which gave us an estimate of the infection of live pigs before slaughter, was comparable with or lower than in other studies: in South Vietnam, 49.3% in 25g (Vo, et al., 2006a) of rectal faecal samples on the farm, in Thailand, from 62.5% of faecal samples positive on the farm (Dorn-In, 2005) to 82.4% at slaughter. In Europe, the prevalence data for faecal samples before slaughter are also not very different from our results. Indeed, 23% in 25g caecal content in Great Britain (Davies, et al., 2004), 23.7% in Germany (Kasbohrer, et al., 2000), 25.6% in rectal contents in the Netherlands (Swanenburg, et al., 2001b), 45% (Boudry, et al., 2002) or 47.3% (Korsak, et al., 2003) in colon content in Belgian studies were described.

Salmonella prevalence was also high in the rinsing water samples in all slaughterhouses studied (62.5% around Hanoi and 58% for Nam Sach related), which made us first suppose that rinsing was partly responsible for carcass contamination and what we were trying to confirm through serotyping and genotyping. The presence of Salmonella in the few samples of water from the well, used as the water source for the plant, could be due to an infiltration of pig faecal material into the soil. This emphasizes the need for wastewater management.

Serotyping and genotyping are relevant tools for clarifying the means of Salmonella contamination during slaughter. Since slaughter is a key stage in the production chain for Salmonella contamination (Berends, et al., 1998; Hurd, et al., 2005), and since we found high prevalence values at this level, analytic epidemiology should provide precious information for Salmonella control and management programmes suited to local conditions. To our knowledge, this is the first study comparing serotypes and genotypes of different sample types at slaughter in Vietnam.

• In 'nem chua'

In total, 35.2% sample found positive for Salmonella is a high prevalence. According to the European legislation, processed products made out of raw meat should not content Salmonella in 5 samples from 25g each (Regulation 2073/2005). Compared with our result, following percentages were found in fermented sausages: A cumulative 3-years Salmonella prevalence study in the USA reported for dry and semidry fermented sausages 1.43% positive samples (Levine, et al., 2001); 8.6% in chilled sausages in UK (Mattick, et al., 2002); in a Turkish fermented meat sausage called 'soudjouck' or in Turkish camel sausages, the prevalence was 7% (Ozbey, et al., 2005; SirIken, et al., 2006); a prevalence of 12% was found in fermented sausages in Iran (Mehrabian and jaberi, 2007). In Thailand in a 'nem chua' similar traditional fermented product cold 'nham', 20% Salmonella prevalence was found (Osiriphun, et al., 2004); in French dry sausage 'Chorizo', a study showed a contamination of 20% before starting the processing, and from 1.3% after 21 days of drying (Christieans, et al., 2006).

The size of the sausage was correlated to the Salmonella prevalence, higher in smaller nems. This may support the hypothesis that the manufacturing process including fermentation plays an important role in the bacterial presence. The initial bacterial load being important in the raw meat, the process can influence the survival and/or multiplication of the bacterial in the meat. This process difference between small and big sausages should be further examined.

Serotyping

• Hanoi slaughterhouses' isolates

S. Derby and S. Typhimurium have often been reported as the most frequent serovars among pigs in Europe and North America (Baggesen, et al., 1996; Bouvet, et al., 2003; Davies, et al., 2004; Guerin, et al., 2005; Hald, et al., 2003; Korsak, et al., 2004; Letellier, et al., 1999; Wonderling, et al., 2003). Vo et al reported in South Vietnam S. Anatum (26.1%), S. Typhimurium (20.7%), S. Weltevreden (15.3%), S. Derby (11.7%) and S. Rissen (11.7%) as most prevalent by pig samples on the farm and at the slaughterhouse and assumed that pigs could be considered as an important reservoir of S. Typhimurium (Vo, et al., 2006b). Among 25 isolates from pig faecal samples on the farm, Phan et al. found a predominance of S. Saviana (21.3%), S. Weltevreden (12.5%) and S. Derby (6.3%) (Phan, et al., 2004), but in 2005, the authors reported a predominance in pork of S. Derby, S. Weltevreden and S. London (Phan, et al., 2005a). In Taiwan, on pork carcasses, the most frequent serovars were S. Derby, S. Anatum, S. Typhimurium and S. Schwarzengrund (Chen, et al., 2006). In Thailand on faecal samples, Dorn-In et al found S. Rissen (45.4%), S. Typhimurium (18.6%), S. Stanley (11.2%), S. Weltevreden (3.7%), S. Krefeld (3.1%) and S. Anatum (2.4%) (Dorn-In, 2005).

Thus, our findings are in accordance with others in Southeast Asia, where S. Weltevreden, S. Anatum and S. Stanley, for instance, are present in larger numbers than on other continents (Galanis, et al., 2004). The S. Typhimurium prevalence is usually high among pigs, as also confirmed by our data. S. Derby is also often associated to pig production and was already mentioned as predominant in Taiwan (Chen, et al., 2006) and in South Vietnam (Phan, et al., 2005a). S. Saint-Paul was not so frequently isolated in Asia. Our finding could be due to the specific conditions of the slaughtering area and should be supplemented by sampling on the farm before tran sportation and lairage of the pigs. Among humans in Thailand between 1993 and 2002, S. Derby (6.6%) and S. Typhimurium (5.3%) have also been isolated and the 3 most frequently isolated serovars, i.e. S. Weltevreden (12.5%), S. Enteritidis (11.4%) and S. Anatum (7.4%), were also found in our study (Bangtrakulnonth, et al., 2004). This emphasizes the potential

role of pigs in human salmonellosis, which should be further investigated in Vietnam.

During the whole sampling period, the global homogeneity of the serovars between caecal and swab samples and their decreasing diversity from caecal to swab and water samples tends to confirm that all isolates were coming primarily from pig faeces. This is in accordance with previous results, where the origin of carcass contamination with Salmonella was mainly attributed to live pigs. Specifically, it has been described that Salmonella strains found on carcasses and pork cuts come mainly from pig faeces, following bacterial spread due to the ineffective cleaning process and procedures (Giovannacci, et al., 2001). 48% (11/23) of the positive pigs (positive caecal samples) presented the same serotype on the carcass and in the caecal sample, which is lower than in other studies, where 69% (Vieira-Pinto, et al., 2005) or 70% (Berends, et al., 1997) of the carcass contaminations were attributed to a contamination from the same pig. Moreover, in our study, the serotypes found in common between carcasses and caeca were S. Derby (8 pigs out of 11), S. Saint-Paul (2 pigs) and S. Typhimurium (1 pig), which represent the most frequent serovars found in all samples. Of the less frequent serovars, none was found at the same time on the carcass and in caecal content of the same pig. We found the same serovar in caeca and on a carcass within the same batch only once, i.e. S. Anatum in caecal samples of pig 3 and 14 and on the swab sample of pig 15 from batch 3. In addition, S. Agona and Salmonella enterica subsp. salamae were isolated only in the tank water, and S. Lamberurt only on swabs, not in caecal samples of any batch. Since the serovar S. London was only found in one caecal and one water sample during the same slaughtering day, there should be an epidemiological relationship between tank water and pig faeces.

Thus, serotyping results indicate that the contamination of carcasses and water originates primarily from live pigs, but that a direct contamination of the carcass by faecal material from the same pig or even within a pig batch is a rare event. Consequently, these results emphasize the significant role of the slaughtering environment as a potential origin of carcass contamination. Previous publications already described floor and environment as possibly significant sources of Salmonella contamination for pig carcasses, especially after scalding, through contact with soiled ground (Bolton, et al., 2002; Bouvet, et al., 2003; Hald and Wegener, 1999; Mafu, et al., 1989).

• ***In 'nem chua'***

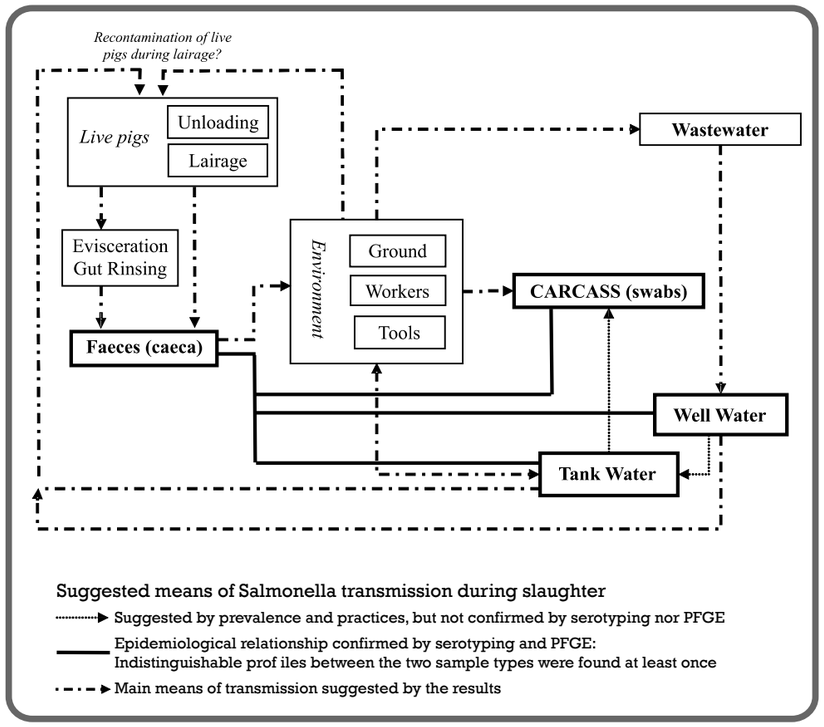
The most frequent serovars in 'nem chua' isolates, S. Derby, S. Anatum and S. Typhimurium, are also among the most frequent isolted in slaughterhouses in our study, and S. Derby and S. Anatum are usually not so frequent in human isolates, S. Derby being often associated with pork (Galanis, et al., 2004; Giovannacci, et al., 2001; Valdezate, et al., 2005). This indicates that the original load of raw pork meat may be the main contributor to the Salmonella contamination in the sausage. Some of the contamination may also result from the processing, namely during the forming of the sausages into the guava and banana leaves, usually done by hand. Genotyping would be necessary to confirm the origin of the contamination.

Genotyping

Genotyping of S. Typhimurium and S. Derby strains was used for the strains isolated in Hanoi slaughterhouses in order to obtain supplementary information on the origins of carcass contamination and more generally, on the ways Salmonella is transmitted during the slaughter process, in order to assist in the development of prevention and control strategies.

Indistinguishable isolates between carcass and caecal samples within a batch were found only once, i.e. the S. Derby pulsotype X12B18 in batch 3, Table 3. The carcass sample of this batch presented 5 different pulsotypes of S. Derby strains, whereas only one type was found in caecal samples. Thus, the epidemiological relationship between caecal and carcass within a batch is not clear. This is in accordance with serotyping results and confirms the important role of the slaughtering environment in carcass contamination. Moreover, genotyping results suggested the persistence of clonally related isolates over at least a six-month period in the slaughter environment, like the S. Typhimurium pulsotype TX1TB6 isolated on carcass of batch 3 and six months later in batch 5 in caecal and water samples, or the Derby type X13B18 found in caecal and water samples of batch 4 and five months later in caecal and water samples of batch 5. Since previous epidemiological studies (Giovannacci, et al., 2001) usually reported that Salmonella clones are not likely to persist for lengthy periods in slaughter or processing plant environments, this finding could be explained by the lack of cleaning and disinfection measures and of wastewater management in the slaughter plants, and/or by a possible reinfection of live pigs by the same clone before slaughter. However, the persistence of Salmonella clones in the slaughter environment should be further investigated over a longer period. The presence of TX1TB6 S. Typhimurium type on carcass and in caeca and well water indicates a probable contamination of the well water with faeces from pigs for slaughter, confirming the importance of wastewater management. Similarly, the S. Derby type X13B18 was isolated several times in different batches in caecal and tank water samples, which seems to confirm the relationship between pig faeces and tank water, suggested by the prevalence (Le Bas, et al., 2006) and serotyping results. This emphasizes the need to clean and disinfect the tank after each slaughtering period and the necessity of following good hygiene procedures during slaughter. Moreover, our results suggest that live pigs could be orally infected during lairage from the environment or drinking water contaminated by faecal material from previously slaughtered pigs, since we isolated the same pulsotype several times on carcass or in water and later in pig caeca of a subsequent batch. Indeed, even short lairage periods of 2 to 4 hours have been described as determinant for the contamination of pigs before slaughter (Beloeil, et al., 2004; Gebreyes, et al., 2004; Hurd, et al., 2001; Korsak, et al., 2003; Rostagno, et al., 2003; Swanenburg, et al., 2001c). The main means of Salmonella transmission during slaughter suggested by this study are summarized in Figure 4.

Figure 4: Schematic representation of Salmonella means of transmission in a small slaughter plant



Slaughtering practices: towards specific recommendations

The workers in small abattoirs have a general lack of knowledge about good hygienic practices. There is also a lack of space and equipment. Although these constraints would be difficult to overcome without switching to bigger and more industrial plants, better knowledge of hygienic slaughtering and focused changes in practices would probably considerably reduce the Salmonella contamination of the environment and carcasses. Indeed, a study in small (80 pigs/day) slaughter plants in Europe showed that Salmonella prevalence on carcasses was highest after bleeding (50%), probably due to contact with the floor before this step, and the prevalence decreased to 0% after scalding and dehairing, and remaining at 0% after evisceration, washing and chilling (Bolton, et al., 2002). These data show that the respect of good hygiene practices, especially after scalding, can avert most carcass cross-contaminations, even in small slaughter plants. Contact with the floor after scalding operations seems to be a decisive factor, and this has been proven by the high percentage of bleeding environment samples in our study. Evisceration by hand can be considered as advantageous, if done carefully. Indeed, evisceration has been described as a critical stage for carcass contamination (Berends, et al., 1997) and the most important factor would be the training of operatives. This explains why Bolton et al, 2002 report 0% carcass contamination after evisceration in the small plant versus 7% in the large plant.

Therefore, slaughtering practices should be improved, with an initial focus on working area separation and on avoiding the soil contact of carcasses, especially after scalding. Then, the implementation of targeted and adapted good hygienic practices, wastewater management and cleaning and disinfection protocols should decrease the Salmonella contamination rate of the carcasses (Table 7). And since Vietnam is in a process of modernizing slaughtering structures that may last several years, so that the pork production for the next decade will still mainly go through small slaughterhouses, it is an urgent matter to test and undertake these specific good hygienic practices for small slaughter plants, in a simple and economical way adapted to the actual context.

Subsequently, as described by Alban et al, 2005, a combination of measures at slaughter and on farms would be the most effective way to reduce the Salmonella prevalence in swine carcasses (Alban and Stark, 2005).

Table 7: Recommendations for good hygiene practices in small slaughterhouses in Vietnam in order of priority

- Separate gut rinsing and carcass dressing
- Separate lairage and carcass dressing
- Use specific working surface for the carcass during slaughter
- Cleaning and disinfection of these surfaces after work
- Give clean water to the pigs at lairage (well water, not tank water)
- Clean and disinfect tanks and tools after work
- Waste management avoiding contamination of clean water sources by faecal material

Conclusions

The Salmonella prevalences at farm and in caecal contents before slaughter are comparable to other studies, whereas the carcass prevalence, during the slaughter process is higher than in other contexts. This has been shown by our results in North Vietnam in different slaughtering environment and seems to be linked to the slaughtering practices in small slaughterhouses in Vietnam, where hygienic practices are very poorly respected. The key efforts in this context have to be put on the slaughtering process and hygiene, especially in small slaughterplants, where a majority of the pork production (about 90%) is being processed.

Serotyping and genotyping of the strains isolated in Hanoi slaughterhouses allowed to better understand the ways of contamination during the slaughtering process. Pigs before slaughter were carrying numerous different Salmonella strains, which appear to contaminate the slaughtering environment and water, and some of which could be reisolated six months later. However, during the same slaughtering day, Salmonella strains in caecal samples could rarely be reisolated on the carcass samples. Although carcasses are usually rinsed with contaminated tank water after evisceration, we did not find an epidemiological link between carcass and tank water. Thus, we can state that carcass contamination mainly resulted from a contact with the floor soiled by previous evisceration, and not specifically by the carcass rinsing itself. It appears also that a contamination cycle exists between live pigs and the environment or water, explaining the presence of some clones over a long period alternatively in water or on carcass and in faecal material.

The prevalence in 'nem chua' found in our study represents a potential public health threat for the consumer. Nevertheless, a quantification of the bacterial load would be necessary to calculate the ingested doses for a risk assessment. Further studies should work on the manufacturing process to help producers to improve the fermentation process regarding food safety, in order to both preserve the Vietnamese traditional culinary culture and assure safe products to the consumers. An improvement of the fermentation process could also avoid the use of toxic and forbidden additives like borax as preservatives. But overall, the microbial load of raw meat needs to decrease by specific control measures at slaughter.

Serotyping of 'nem chua' strains suggesting that they mainly may be originated from the pork meat itself, this emphasizes the importance of focusing the good hygiene practice on the slaughterhouses first, by improving the hygiene with adapted and economical focused control measures, as suggested in this study. This is even more priority, since in Vietnam, more and more products go through the cold chain, increasing the storage delays, which requires a good microbial status of the meat.

ACKNOWLEDGEMENTS

This study was co-founded by CIRAD and AFSSA, and performed through a partnership between Vietnamese (NIVR, NIHE, HUT, IPSARD) and French (CIRAD, AFSSA, IUT Amiens) research institutes within the framework of the PRISE consortium in Vietnam. The authors would like to thank all the staff involved and also abattoir owners and veterinary officials.

REFERENCES

2002a. Capacity building for the prevention of foodborne diseases - Foodborne disease surveillance report (Hanoi, World Health Organization), 58.

2002b. Restitution of food quality and hygiene management activities in 2001 - Action plan of year 2002. In: Inter-sectorial conference, Hanoi, 10/01/2002.

2006. Vietnam food safety and agricultural health action planHanoi, World Bank: 102 p. http://www-wds.worldbank.org/external/default/WDSContentServer/IW3P/IB/2006/03/15/000090341_20060315085256/Rendered/PDF/352310VN.pdf.

ALBAN, L., STARK, K.D., 2005. Where should the effort be put to reduce the Salmonella prevalence in the slaughtered swine carcass effectively? Preventive Veterinary Medecine. 68, 63-79.

BAGGESEN, D.L., WEGENER, H.C., BAGER, F., STEGE, H., CHRISTENSEN, J., 1996. Herd prevalence of Salmonella enterica infections in Danish slaughter pigs determined by microbiological testing. Preventive Veterinary Medicine. 26, 201-213.

BANGTRAKULNONTH, A., PORNREONGWONG, S., PULSRIKARN, C., SAWANPANYALERT, P., HENDRIKSEN, R.S., LO FO WONG, D.M.A., AARESTRUP, F.M., 2004. Salmonella Serovars from Humans and Other Sources in Thailand, 1993–2002. Emerging Infectious Diseases. 10, 131-136.

BARRET, T.J., GERNER-SMIDT, P., SWAMINATHAN, B., 2006. Interpretation of Pulsed-Field Gel Electrophoresis Patterns in Foodborne Disease Investigations and Surveillance. Foodborne Pathogens and Disease. 3, 20-31.

BELOEIL, P.-A., CHAUVIN, C., PROUX, K., MADEC, F., FRAVALO, P., ALIOUM, A., 2004. Impact of the Salmonella status of market-age pigs and the pre-slaughter process on Salmonella caecal contamination at slaughter. Veterinary Research. 35, 513-530.

BERENDS, B.F., VAN KNAPEN, F., MOSSEL, D.A., BURT, S.A., SNIJDERS, J.M., 1998. Salmonella spp. on pork at cutting plants and at the retail level and the influence of particular risk factors. International Journal of Food Microbiology. 44, 207-217.

BERENDS, B.R., VAN KNAPEN, F., SNIJDERS, J.M.A., MOSSEL, D.A.A., 1997. Identification and quantification of risk factors regarding Salmonella spp. on pork carcasses. International Journal of Food Microbiology. 36, 199-206.

BOLTON, D.J., PEARCE, R., SHERIDAN, J.J., 2002. Risk based determination of critical control points for pork slaughter., The National Food Research p. http://www.teagasc.ie/research/reports/foodprocessing/4667/eopr-4667.pdf, http://www.teagasc.ie/research/reports/foodprocessing/4667/eopr-4667.pdf.

BOTTELDOORN, N., HEYNDRICKX, M., RIJPENS, N., GRIJSPEERDT, K., HERMAN, L., 2003. Salmonella on pig carcasses: positive pigs and cross contamination in the slaughterhouse. Journal of Applied Microbiology. 95, 891-903.

BOUDRY, C., KORSAK, N., JACOB, B., ETIENNE, G., THÉWIS, A., DAUBE, G., 2002. Ecologie de Salmonella dans le tube digestif du porc à l'abattage et étude de la contamination des carcasses. Ann. Méd. Vét. 146, 353-360.

BOUVET, J., BAVAI, C., ROSSEL, R., LE ROUX, A., MONTET, M.P., MAZUY, C., VERNOZY-ROZAND, C., 2003. Evolution of pig carcass and slaughterhouse environment contamination by Salmonella. Revue de Médecine Vétérinaire. 154, 775-779.

CHANG, C.C., LIN, Y.-H., C.-F., C., YEH, K.-S., C.-H., C., C., C., CHIEN, M.-S., HSU, Y.-M., TSAI, L.-S., C.-S., C., 2005. Epidemiologic Relationship between Fluoroquinolone-Resistant Salmonella enterica Serovar Choleraesuis Strains Isolated from Humans and Pigs in Taiwan (1997 to 2002). Journal of Clinical Microbiology. 43, 2798-2804.

CHANTONG, W., 2005. Salmonella isolation from slaughter pigs and carcasses in a slaughterhouse in Chiang Mai, Thailand (Chiang Mai University and Freie Universitat Berlin), 84.

CHEN, T.H., WANG, Y.C., CHEN, Y.T., YANG, C.H., YEH, K.-S., 2006. Serotype occurrence and antimicrobial susceptibility of Salmonella isolates recovered from pork carcasses in Taiwan (2000 through 2003). Journal of Food Protection. 69, 674-678.

CHIU, C.-H., WU, T.-L., SU, L.-H., CHU, C., CHIA, J.-H., KUO, A.-J., CHIEN, M.-S., LIN, T.-Y., 2002. The Emergence in Taiwan of Fluoroquinolone Resistance in Salmonella enterica Serotype Choleraesuis. New England Journal of Medicine. 346, 413-419.

CHRISTIEANS, S., DORCHIE, G., CHACORNAC, J.P., TALON, R., 2006. Microbial ecology of French Chorizo: Influence of technological parameters on the safety of the products [French]. In: 12th JSMTV.

DAO, H.T.A., YEN, P.T., 2006. Study of Salmonella, Campylobacter, and Escherichia coli Contamination in Raw Food Available in Factories, Schools, and Hospital Canteens in Hanoi, Vietnam. Annals of the New York Academy of Sciences. 1081, 262-265.

DAVIES, R.H., DALZIEL, R., GIBBENS, J.C., WILESMITH, J.W., RYAN, J.M.B., EVANS, S.J., BYRNE, C., PAIBA, G.A., PASCOE, S.J.S., TEALE, C.J., 2004. National survey for Salmonella in pigs, cattle and sheep at slaughter in Great Britain (1999–2000). Journal of Applied Microbiology. 96, 750-760.

DORN-IN, S., 2005. Prevalence of Salmonella in pre-slaughter pigs in Chiang Mai. (Thailand, Chiang Mai University and Freie Universitat Berlin, dissertation.), 87.

GALANIS, E., LO FO WONG, D., PATRICK, M., WEGENER, H.C., 2004. Characterizing the Worldwide Distribution of Salmonella Serotypes. The role of WHO Global SalmSurv, Danish Institute for Food and Veterinary Research p., http://www.who.int/salmsurv/activities/bulletin_board/en/GSSMessage0404dataposterICEID2004.pdf.

GEBREYES, W.A., DAVIES, P.R., TURKSON, P.K., MORROW, W.E.M., FUNK, J.A., ALTIER, C., 2004. Salmonella enterica Serovars from Pigs on Farms and after Slaughter and Validity of Using Bacteriologic Data To Define Herd Salmonella Status. Journal of Food Protection. 67, 691-697.

GINHOUX, V., 2001. Etude de la sensibilité des consommateurs urbains de viande porcine. (Programme Fleuve Rouge - GRET-VASI-VSF-CIRAD), 79.

GIOVANNACCI, I., QUEGUINER, S., RAGIMBEAU, C., SALVAT, G., VENDEUVRE, J.L., CARLIER, V., ERMEL, G., 2001. Tracing of Salmonella spp. in two pork slaughter and cutting plants using serotyping and macrorestriction genotyping. Journal of Applied Microbiology. 90, 131-147.

GUERIN, M.T., MARTIN, S.W., DARLINGTON, G.A., RAJIC, A., 2005. A temporal study of Salmonella serovars in animals in Alberta between 1990 and 2001. Canadian Journal of Veterinary Research. 69, 88-99.

HALD, T., WEGENER, H.C., 1999. Quantitative assessment of the sources of human salmonellosis attributable to pork. In: Symposium on the Epidemiology and Control of Salmonella in Pork, Washington, DC, USA. 200-205.

HALD, T., WINGSTRAND, A., SWANENBURG, M., VON ALTROCK, A., THORBERG, B., 2003. The occurrence and epidemiology of Salmonella in European pig slaugh-

terhouses. Epidemiology and Infection. 131, 1187-1203.

HURD, H., GAILEY, J., MCKEAN, J., GRIFFITH, R., 2005. Variable abattoir conditions affect Salmonella enterica prevalence and meat quality in swine and pork. Foodborne Pathogens and Disease. 2, 77-81.

HURD, H.S., MCKEAN, J.D., WESLEY, I.V., KARRIKER, L.A., 2001. The Effect of Lairage on Salmonella Isolation from Market Swine. Journal of Food Protection. 64, 939-944.

INTHAVONG, P., 2005. Microbiological quality of pig carcass at Dorn Du slaughterhouse in Ventiane Municipality. (Lao PDR, Chiang Mai University and Freie Universitat Berlin, dissertation), 77.

ISENBARGER, D.W., HOGE, C.W., SRIJAN, A., PITARANGSI, C., VITHAYASAI, N., BODHIDATTA, L., HICKEY, K.W., CAM, P.D., 2002. Comparative antibiotic resistance of diarrheal pathogens from Vietnam and Thailand, 1996-1999. Emerging Infectious Diseases. 8, 175-180.

KASBOHRER, A., PROTZ, D., HELMUTH, R., NOCKLER, K., BLAHA, T., CONRATHS, F.J., GEUE, L., 2000. Salmonella in slaughter pigs of German origin: an epidemiology study. European Journal of Epidemiology. 16, 141-146.

KORSAK, N., CLINQUART, A., DAUBE, G., 2004. Salmonella spp. dans les denrées alimentaires d'origine animale : un réel problème de santé publique ? Annales de Médecine Vétérinaire. 148, 174-193.

KORSAK, N., JACOB, B., GROVEN, B., ETIENNE, G., CHINA, B., GHAFIR, Y., DAUBE, G., 2003. Salmonella contamination of pigs and pork in an integrated pig production system. Journal of Food Protection. 66, 1126-1133.

LE BAS, C., HANH, T.T., THANH, N.T., THUONG, D.D., THUY, N.C., 2006. Prevalence and Epidemiology of Salmonella spp. in Small Pig Abattoirs of Hanoi, Vietnam. Annals of the New York Academy of Sciences. 1081, 269-272.

LETELLIER, A., MESSIER, S., PARÉ, J., MÉNARD, J., QUESSY, S., 1999. Distribution of Salmonella in swine herds in Québec. Veterinary Microbiology. 67, 299-306.

LEVINE, P., ROSE, B., GREEN, S., RANSOM, G., HILL, W., 2001. Pathogen Testing of Ready-to-Eat Meat and Poultry Products Collected at Federally Inspected Establishments in the United States, 1990 to 1999. Journal of Food Protection. 64, 1188-1193.

MAFU, A.A., HIGGINS, R., NADEAU, M., COUSINEAU, G., 1989. The incidence of Salmonella, Campylobacter and Yersinia enterocolitica in swine carcasses and the slaughterhouse environment. Journal of Food Protection. 52, 642-645.

MATTICK, K.L., BAILEY, R.A., JØRGENSEN, F., HUMPHREY, T.J., 2002. The prevalence and number of Salmonella in sausages and their destruction by frying, grilling or barbecuing. Journal of Applied Microbiology. 2002, 541-547.

MEHRABIAN, S., JABERI, E., 2007. isolation, Identification and Antimicrobial Resistance Patterns of Salmonella from Meat Products in Teheran. Pakistan Journal of Biological Sciences. 10, 122-126.

MURUGKAR, H.V., RAHMAN, H., KUMAR, A., BHATTACHARYYA, D., 2005. Isolation, phage typing and antibiogram of Salmonella from man and animals in northeastern India. Indian Journal of Medical Research. 122, 237-242.

OSIRIPHUN, S., PONGPOOLPONSAK, A., TUITEMWONG, K., 2004. Quantitative Risk Assessment of Salmonella spp. in Fermented Pork Sausage (Nham). Kasertsart J. (Nat. Sci.). 38, 52-65.

OZBEY, G., KOK, K., MUZ, A., 2005. Isolation of Salmonella spp. in Camel Sausages from Retail Markets in AydÝn, Turkey, and Polymerase Chain Reaction (PCR) Confirmation. Turk. J. Vet. Anim. Sci. 31, 67-71.

PADUNGTOD, P., KANEENE, J.B., 2006. Salmonella in food animals and humans in northern Thailand. International Journal of Food Microbiology. 108, 346-354.

PHAN, T.T., KHAI, L.T.L., OGASAWARA, N., TAM, N.T., OKATANI, A.T., AKIBA, M., HAYASHIDANI, H., 2005a. Contamination of Salmonella in Retail Meats and Shrimps in the Mekong Delta; Vietnam. Journal of Food Protection. 68, 1077-1080.

PHAN, T.T., KHAI, L.T.L., TAN, N.T., AKIBA, M., OGASAWARA, N., SHINODA, D., OKATANI, A.T., HAYASHIDANI, H., 2004. Prevalence of Salmonella spp. in Pigs, Chicken and Ducks in the Mekong Delta, Vietnam. Journal of Veterinary Medicine and Science. 66, 1011-1014.

PHAN, T.T., TAM, N.T., KHAI, L.T.L., OGASAWARA, N., NAKADAI, A., IWATA, T., KAMADA, T., HAYASHIDANI, H., 2005b. Prevalence of Salmonella in Rice-Field Rats in the Mekong Delta, Vietnam. Journal of Veterinary Epidemiology. 9, 85-88.

POPOFF, M.Y., 2001. Antigenic formulas of the Salmonella serovars, 8th edition (WHO Collaborating Centre for Reference and Research on Salmonella, Institut Pasteur, Paris, France), 156.

QUANG, N.D., 1999. Pre case study of food supply and distribution to Hanoi. In (Hanoi, FAO), 33.

RAGIMBEAU, C., SALVAT, G., COLIN, P., ERMEL, G., 1998. Development of a multiplex PCR gene fingerprinting method using gyrA and pflA polymorphisms to identify genotypic relatedness within Campylobacter jejuni species. Journal of Applied Microbiology. 85, 829-838.

RAJIC, A., KEENLISIDE, J., MCFALL, M.E., DECKERT, A.E., MUCKLE, A.C., O'CONNOR, B.P., MANNINEN, K., DEWEY, C.E., MCEWEN, S.A., 2005. Longitudinal study of Salmonella species in 90 Alberta finishing farms. Veterinary Microbiology. 105, 47-56.

ROSTAGNO, M.H., HURD, H.S., MCKEAN, J.D., ZIEMER, C.J., GAILEY, J.K., LEITE, R.C., 2003. Preslaughter Holding Environment in Pork Plants Is Highly Contaminated with Salmonella enterica. Applied and Environmental Microbiology. 69, 4489-4494.

SAULI, I., DANUSER, J., GEERAERD, A.H., VAN IMPE, J.F., RUEFENACHT, J., 2005. Estimating the probality and level of contamination with Salmonella of feed for finishing pigs produced in Switzrland- the impact of the production pathway. interbational Journal of Food microbiology Institution. 21, 289-310.

SIRIKEN, B., PAMUK, S., ÖZAKIN, C., GEDIKOGLU, S., EYIGÖR, M., 2006. A note on the incidences of Salmonella spp., Listeria spp. and Escherichia coli O157:H7 serotypes in Turkish sausage (Soudjouck). Meat Science. 72, 177-181.

STRUELENS, M.J., 1996. Consensus guidelines for appropriate use and evaluation of microbial epidemiologic typing systems. Clinical Microbiology and Infection. 2, 2-11.

STRUELENS, M.J., 1998. Molecular Epidemiologic Typing Systems of Bacterial Pathogens: Current Issues and Perspectives. Memorias do Instituto Oswaldo Cruz. 93, 581-585.

SWANENBURG, M., BERENDS, B.F., URLINGS, H.A.P., SNIJDERS, J.M.A., VAN KNAPEN, F., 2001a. Epidemiological investigations into the sources of Salmonella contamination of pork. In: Proceedings of the 4th International Symposium on the Epidemiology and Control of Salmonella and other food pathogens in Pork., 2-5 september 2001, Leipzig, Germany. 301-303.

SWANENBURG, M., URLINGS, H.A., SNIJDERS, J.M., KEUZENKAMP, D.A., VAN KNAPEN, F., 2001b. Salmonella in slaughter pigs: prevalence, serotypes and critical control points during slaughter in two slaughterhouses. International Journal of Food Microbiology. 70, 243-254.

SWANENBURG, M., URLINGS, H.A.P., KEUZENKAMP, D.A., SNIJDERS, J.M.A., 2001c. Salmonella in the Lairage of Pig Slaughterhouses. Journal of Food Protection. 64, 12-16.

THUY, D.N., PHU, C.H., HUONG, V.T., HAO, D.T., HUYEN, N.X., HUE, N.B., 2006. Danh gia tinh hinh nhiem mot so loai vi khuan gay benh trong thit tuoi tren dia ban Ha Noi. Criteria and quantity standards of microorganisms in raw meat in Hanoi area (in Vietnamese, summary in english). Khoa hoc ky thuat thu y - Veterinary Sciences and Techniques. 13, 48-54.

VALDEZATE, S., VIDAL, A., HERRERA-LEÓN, S., POZO, J., RUBIO, P., USERA, M.A., CARVAJAL, A., ECHEITA, M.A., 2005. Salmonella Derby Clonal Spread from Pork. Emerging Infectious Diseases. 11, 694-698.

VIEIRA-PINTO, M., OLIVEIRA, K., BERNARDO, F., MARTINS, C., 2005. Evaluation of fluorescent in situ hybridization (FISH) as a rapid screening method for detection of Salmonella in tonsils of slaughtered pigs for consumption: a comparison with conventional culture method. Journal of Food Safety. 25, 109-119.

VO, A.T.T., TUAN, N.N., NGOC, L.H., 2006a. Tình hình nhiem Salmonella trong phan va than thit (bo, heo, ga) tai mot so tinh phia Nam - Prevalence of Salmonella in excrements and in carcasses (beef, pork and chickens) in some provinces of the South Vietnam (In Vietnamese, summary in english). Khoa hoc ky thuat thu y - Veterinary Science and Techniques. 13, 37-42.

VO, A.T.T., VAN DUIJKEREN, E., FLUIT, A.C., HECK, M.E.O.C., VERBRUGGEN, A., MAAS, H.M.E., GAASTRA, W., 2006b. Distribution of Salmonella enterica Serovars from humans, livestock and meat in Vietnam and the Dominance of Salmonella Typhimurium Phage Type 90. Veterinary Microbiology. 113, 153-158.

WEGENER, H.C., 1999. The Hygiene Inspection System of the Veterinary Services in Vietnam. Consultancy Mission, 6-10 June 1999. Strengthening of Veterinary Services in Vietnam (SVSV). In (Hanoi, Ministry of Agriculture and Rural Development, European Commission), 73.

WONDERLING, L., PEARCE, R., WALLACE, F.M., CALL, J.E., FEDER, I., TAMPLIN, M., LUCHANSKY, J.B., 2003. Use of pulsed-field gel electrophoresis to characterize the heteroganeity and clonality of salmonella isolates obtained from the carcasses and faeces of swine at slaughter. Applied and Environmental Microbiology. 69, 4177-4182.

WRAY, C., 2001. Review of research into Salmonella infections in pigs. In Institution, Institution, ed. (Meat and Livestock Commission), 63 p.

* *Corresponding author. Tel.: +84-4-869-33-06; fax: +84-4-869-40-82. E-mail address: cedric.le_bas@cirad.fr*

ANALYSIS OF ANTIBIOTIC RESISTANCE AMONG SALMONELLA STRAINS ISOLATED FROM PIG IN VIETNAM

Luu Quynh Huong[1]*, Eric Jouy[2], Cédric Le Bas[3]

[1] National Institute of Veterinary Research, 86 Truong Chinh road, Dong Da district, Hanoi, Vietnam
[2] Agence Française de Sécurité Sanitaire des Aliments, AFSSA Ploufragan – LERAP, Zoopole, Les Croix, 22440 Ploufragan, France
[3] Unité Animal et Gestion Intégrée des Risques (AGIRs), Centre de coopération Internationale en Recherche Agronomique pour le Développement (CIRAD), Campus International de Baillarguet, TA C-22, 34398 Montpellier Cedex 5, France

SUMMARY

This study aimed to examine the susceptibility to 16 antimicrobial agents of a total of 102 Salmonella strains isolated from slaughter pig in Vietnam. No strain was found resistant to Amoxicillin clavulanic acid, Cefalexin, Ceftazidime, Cefotaxime, Ceftiofur nor Cefoxitin. 53% of the strains were resistant to at least one antibiotic, 48% of the strains were found resistant to Tetracycline, 39.2% to Sulfamide and 35.3% to Streptomycine. Multiresistance to six antibiotics (AM, TE, S, GM, SSS, TMP) was found for both S. Derby and S. Typhimurium.

KEY WORDS: Salmonella, S. Derby, S. Typimurium, Antibiotic, Antimicrobial resistance, slaughterhouse, pig, Vietnam.

*Corresponding author: Department of Animal Hygiene, National Institute of Veterinary Research, 86 Truong Chinh road, Dong Da district, Hanoi, Vietnam. Tel: 00 84 4 8695544; Fax: 00 84 4 8694082. E-mail: lqhuongvet@yahoo.com

INTRODUCTION

Salmonellosis is one of the most frequent foodborne disease, representing an important public health problem worldwide (D'Aoust, J.Y., 1997). Foods most often associated with the transmision of Salmonella include those of animal origin, such as beef, pork and poultry (Jay, 2000).

Contamination can occur at multiple steps along the food chain, including production, processing, distribution, and retail marketing, and handling preparation (Shaohua et al., 2003). Infected pigs are usually asymptomatic carriers and may shed Salmonella through their feces during the whole fattening period. Contamination of swine carcasses and the slaughter line with Salmonella via the intestinal content and cut lymph nodes poses a potential health risk for humans (Swanenburg et al., 2001). In Vietnam, Salmonella has been described as an important issue in pig slaughterhouses with a very high carcass prevalence (Vo et al. 2006; Le Bas et al. 2008).

The use of antibiotics for animal disease treatment and prevention, as well as for growth promoting feed additives, has led to a serious increase in multiple antibiotic-resistant bacteria, including zoonotic pathogens, which can be transmitted to human via the food chain (Moelleing, 1998, Tollefson and Miller, 2000; WHO, 1997).

The aim of this study was to determine the incidence of antimicrobial resistance among Salmonella strains isolated from slaughter pig in Vietnam.

MATERIALS AND METHODS

Bacterial strains:

A total of 102 Salmonella strains representing 15 serotypes (Table 1) were analyzed for antimicrobial resistance. The strains were taken out of 125 Salmonella strains