Environmental health and socio-economic risks associated with livestock intensification

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 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 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.

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

Keywords: Salmonella; epidemiology; pig;...
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 (Ginhon, 2001), and the recent integration of Vietnam into the WTO (2006). 1 421 herds, 36 904 cases (2002a). In Vietnam, nontyphoidal Salmonella has been isolated on human patients with diarrhoea (Jeanberger, 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; Chu, et al., 2005; Murugkar, et al., 2005; Padungtod and Kanee, 2006), with a determinant role of slaughterhouses and slaughtering practices (Berends, et al., 1998; Bot-teldoorn, et al., 2003; Hurd, et al., 2005). Pork represents about 72% of the meat products consumed in Vietnam. Studies on retail pork revealed contamination with Salmonella in 33 to 40% of samples (Dow and Yen, 2006; Thuy, et al., 2008). Recent studies in South Vietnam described very variable Salmonella prevalence (5.2 to 69.4%) 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 30 plugs/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 slaughter-houses 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 XLT74 and Rambach agar. One Salmonella characteristic colony on XLT74 and on Rambach was inoculated to Kligler-Iron agar tubes. Each characteristic Kligler-Iron was confirmed through additional biochemical tests: mannitol, molity 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 (Diagnostic Pasteur, Paris, France). Throughout this paper, Salmonella enterica subs. enterica will be abbreviated to Salmonella. The serotype name, for instance Salmonella enterica subs. 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 10 µl for five hours’ incubation at the appropriate temperature. PFGEs were done using the CHEF-DRIII system (Biorad Laboratories, USA).

Macrolist restriction profile analysis

Electrophoretic patterns were compared by BioNumerics® (Applied Matha). 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. Clusters were by the Unweighted Pair-Group Method using the Arithmetic Mean (UPGMA (Struclen, 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 considered as clonally related and thus belonging to the same chain of transmission (Struclen, 1996).

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) and 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 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 waste-water 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.
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

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Positive samples</th>
<th>Confidence Interval at 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rectal Swabs (fattening pigs)</td>
<td>19% (12/60)</td>
<td>10.6-27.1%</td>
</tr>
<tr>
<td>Environment Swabs (fattening box)</td>
<td>27% (8/30)</td>
<td>11.4-42.6%</td>
</tr>
<tr>
<td>Water</td>
<td>3% (1/30)</td>
<td></td>
</tr>
<tr>
<td>Feed</td>
<td>0% (0/30)</td>
<td></td>
</tr>
</tbody>
</table>

- At slaughterhouse: The proportions of positive results for Salmonella in Hanoi slaughterhouses were 52.1% for caecal contents, 65.7% for carcase 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

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

The proportions of positive samples in the slaughterhouses connected with the Nam Sach producers sampled, are summarized in Table 3: 40% in caeca, 87% on carcase swabs, 68% 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.

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Positive samples</th>
<th>Confidence Interval at 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caeca</td>
<td>40% (18/45)</td>
<td>7.3-80.7%</td>
</tr>
<tr>
<td>Carcase Swabs</td>
<td>87% (20/23)</td>
<td>53.3-90.1%</td>
</tr>
<tr>
<td>Water Tank</td>
<td>59% (7/12)</td>
<td></td>
</tr>
<tr>
<td>Lairage Environment</td>
<td>59% (7/12)</td>
<td></td>
</tr>
<tr>
<td>Bleeding Environment</td>
<td>92% (11/12)</td>
<td></td>
</tr>
</tbody>
</table>

- 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 (50g) 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 subspp. salamae (II ssp.) were only isolated in tank water and S. Kedougou and S. Lambertii only on carcase 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

<table>
<thead>
<tr>
<th>Serovar</th>
<th>Total</th>
<th>Caecal content</th>
<th>Swabs</th>
<th>Tank Water</th>
<th>Well</th>
<th>Untypable strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>136</td>
<td>64</td>
<td>44</td>
<td>23</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>70</td>
<td>30</td>
<td>27</td>
<td>11</td>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>Derby</td>
<td></td>
<td>(51%)</td>
<td>(47%)</td>
<td>(61%)</td>
<td>(48%)</td>
<td>(40%)</td>
</tr>
<tr>
<td>Typhimurium</td>
<td>16</td>
<td>9</td>
<td>5</td>
<td>2</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>Saint-paul</td>
<td>11</td>
<td>4</td>
<td>7</td>
<td></td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Anatum</td>
<td>5</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
<td>4</td>
</tr>
<tr>
<td>Enteritidis</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td>3</td>
</tr>
<tr>
<td>Watereden</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Lamberhurt</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>Stanley</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Kedougou</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Dumfries</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Weston</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>London</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Agona</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Newport</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Il spp. (1)</td>
<td>1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Untypable</td>
<td>15</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td></td>
<td>1</td>
</tr>
</tbody>
</table>

(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 (8%). 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 XbaI and BlnI for S. Typhimurium and S. Derby were respectively 0.900 and 0.962. XbaI analyses of 16 S. Typhimurium isolates produced 5 profiles. The TX1 profile was found for 9 out of the 16 isolates. The use of BlnI provided 9 profiles with 5 out of the 16 strains presenting the type TB1. The combination of both enzymes resulted in 10 different definitive types (Figure 3). Five isolates had indistinguishable pulsotypes, i.e. the profile TX1TB1, 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, XbaI analyses produced 11 profiles. X13 profile was found for 12, X12 for 6 and X18 for 5 isolates (Table 6). BlnI 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 2 in November 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

<table>
<thead>
<tr>
<th>Batch number</th>
<th>Date</th>
<th>Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Carcass</td>
</tr>
<tr>
<td>Batch 1</td>
<td>9.7.04</td>
<td>X08B13</td>
</tr>
<tr>
<td>Batch 2</td>
<td>10.25.04</td>
<td>TX1TB1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TX2TB4</td>
</tr>
<tr>
<td>Batch 3</td>
<td>11.22.04</td>
<td>TX1TB6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X11B18</td>
</tr>
<tr>
<td>Batch 4</td>
<td>12.5.04</td>
<td>TX1TB7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X13B33</td>
</tr>
<tr>
<td>Batch 5</td>
<td>5.16.05</td>
<td>TX1TB6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>X13B18</td>
</tr>
<tr>
<td>Batch 6</td>
<td>6.9.04</td>
<td></td>
</tr>
</tbody>
</table>

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

DISCUSSION

Prevalence

• At farm

The precision of the Salmonella positive farms is low to be compared with another context. Besides, farm prevalence assessments vary considerably in literature: 14.8% in the UK in 1997, 22% in Denmark 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, 28.8% were found positive during the first visit, 34.4% during the second and 28.7% during the third (Rajic, et al., 2005).

The variability is also high for the proportion of Salmonella positive fattening pigs: 8.9% 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 48.4% in pig faecal and carcass swabs (Vo, et al., 2006b). In 1g faecal samples, another study reported 8.2% positif samples (Phan, et al., 2005b). These 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 only positive water sample was found in a farm using 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, 2008) 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 Sac farmers, using the same carcass sampling protocol than in Hanoi suburb. The average prevalence on carcasses (87%) 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 Sac related slaughterhouses, the faecal sample contamination rate (55% 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, et al., 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), 23.3% in Germany (Kasbohrer, et al., 2000), 25.6% in rectal contents in the Netherlands (Swanenburg, et al., 2001b), 45% (Boudry, et al., 2005) 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 Sac 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 prevalence 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., 2006), 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 contain 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 semidyremed sausages 1.43% positive samples (Levine, et al., 2001); 8.6% in chilled sausages in UK (Matrick, et al., 2003); in a Turkish fermented meat sausage called ‘sudjouck’ or in Turkish camel sausages, the prevalence was 7% (Ozbev, et al., 2006; Sirven, et al., 2006); a prevalence of 12% was found in fermented sausages in Iran (Mehrabi and Jaberi, 2007). In Thailand in a ‘nem chua’ similar traditional fermented product cold ‘sham’, 20% Salmonella prevalence was found (Osiriphun, et al., 2004); in French dry sausage ‘Chorizo’, a study showed a contamination of 10% before starting the processing, and from 1.3% after 21 days of drying (Christoza, et al., 2000).

The size of the sausage was correlated to the Salmonella prevalence, higher in smaller ones. 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 (Bagnessen, et al., 1998; Bouvet, et al., 2003; Davies, et al., 2004; Guerin, et al., 2001; Hald, et al., 2005; Korsak, et al., 2004; Letellier, et al., 1998; Wonderling, et al., 2005; Vo, et al., 2006b). 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 2008, the authors reported a predominance in pork of S. Derby, S. Weltevreden and S. London (Phan, et al., 2005a). In Taiwan, on pork carcases, 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. Kentucky (3.5%) 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 (Galania, 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 transport 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 (Bangtrakulnorm, 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% (Berenda, 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 and S. Typhimurium, are also among the most frequent isolated 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 (Galán, 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 the contamination of carcasses. 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 (Beloel, et al., 2004; Gebreyes, et al., 2004; Hurd, et al., 2001; Korsak, et al., 2003; Rostagno, et al., 2003; Swansenburg, et al., 2001c). The main means of Salmonella transmission during slaughter suggested by this study are summarized in Figure 4.
Environmental health and socio-economic risks associated with livestock intensification

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 hygienic 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

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 consumer. 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 consumer.
Serotyping of ‘nem cha’ 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.

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Environmental health and socio-economic risks associated with livestock intensification

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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, Ceftazidim, Ceftoxime, Cefotaxime, Cefloflor nor Cefotaxin. 93% of the strains were resistant to at least one antibiotic, 48% of the strains were found resistant to Tetracycline, 35.3% to Sulfamethoxazole and 35.3% to Streptomycin. Multiresistance to six antibiotics (AM, TE, S, GM, SSS, TMP) was found for both S. Derby and S. Typhimurium.


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INTRODUCTION
Salmonellosis is one of the most frequent foodborne disease, representing an important public health problem worldwide (D’Arcy, J.Y., 1997). Foods most often associated with the transmission 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 (Shachou et al., 2003). Infected pigs are usually asymptomatic carriers and may shed Salmonellla 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, Tolleson 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 16 serotypes (Table 1) were analyzed for antimicrobial resistance. The strains were taken out of 125 Salmonella strains

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

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