

Original Article

Transition of *Salmonella* Prevalence in Pork Value Chain from Pig Slaughterhouses to Markets in Hung Yen, Vietnam

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Summary

In Vietnam, pork is the most consumed meat, and contamination of pork with *Salmonella* spp. is a serious public health problem. This study aimed to trace the value chain forward from pig slaughterhouses to markets in order to elucidate the dynamics of *Salmonella* contamination on the pork value chain in Hung Yen, Vietnam. This survey was conducted between January and February 2014 in two randomly selected slaughterhouses in Hung Yen. Swab samples were collected from 88 carcasses and of them, 21 carcasses were traced to the markets and pork samples were collected from these samples. Microbial tests were performed to detect the presence of *Salmonella* from carcass samples at slaughterhouses. MPN was determined in addition to the presence of *Salmonella* for pork at markets.

The *Salmonella* prevalence on carcasses was 25.0% (22/88, 95%CI : 16.7%–35.6%), and on pork at markets was 28.6% (6/21, 95%CI : 12.2%–52.3%). There was no significant difference in prevalence between carcasses (25.0%) and pork (28.6%, $\chi^2 = 0.0034$, $df = 1$, $p = 0.95$). There was no significant difference in the prevalence of *Salmonella* on pig carcass samples between the two slaughterhouses (22.2% (10/45) and 27.9% (12/43), $\chi^2 = 0.18$, $df = 1$, $p = 0.71$). The transition of *Salmonella* contamination status on pork was observed, and the κ value 0.53, and attributable risk percent 53.3% calculated suggested that more than half of the *Salmonella* prevalence in marketed pork can be attributable to contamination in slaughterhouse. MPN of positive pork samples ranged from < 0.3 to 1.5 MPN/g. The prevalence remained high at the same level between the slaughterhouse and market, and the contamination largely occurs before the end of slaughtering due to inadequate hygiene, but cross-contamination during transportation and marketing also poses a risk to humans.

Keywords : Pork value chain, *Salmonella*, Vietnam

1. Introduction

Salmonella spp., which lives in the intestines of livestock, is a pathogen that can cause food poisoning. Salmonellosis in humans is usually mild, but it can be fatal in young children, the elderly and immunocompromised people⁽⁶⁾. Although the actual

number of infections is unknown because many mild cases are not reported, it has been estimated that there are approximately 93.8 million cases of salmonellosis annually with 155,000 deaths globally from acute salmonellosis⁽¹⁾. Most human *Salmonella* outbreaks occur due to consumption of contaminated food, such as meat and eggs⁽⁵⁾.

In Vietnam, pork is the most consumed meat, accounting for over 70% of total meat consumption^(5,8), and contamination of pork with *Salmonella* spp. in the pork value chain is a serious public health problem. Some previous studies in Vietnam reported that prevalence of *Salmonella* in pig carcass at slaughterhouse

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takes wide range from 15% to 95%^{4,10,13}. Moreover, *Salmonella* in pork at market was also reported as a high prevalence which varied from 33 to 69%^{4,12,13}. For these reasons, elucidating the dynamics of *Salmonella* contamination in the pork value chain is urgently required in Vietnam. In June 2012, a research project funded by the Australian Center for International Agricultural Research (ACIAR) and coordinated by the International Livestock Research Institute (ILRI), called 'Reducing Disease Risks and Improving Food Safety in Smallholder Pig Value Chain in Vietnam (PigRISK)' started, and is expected to be finalized in June, 2017. The project proposes the new method of intervention for food safety based on the quantitative assessment of health risks and economic incentives for farmers and actors along the pork value chain.

The present study was conducted as a part of above mentioned project, aiming (1) to trace the value chain forward from pig slaughterhouses to the markets in order to elucidate the dynamics of *Salmonella* contamination in the pork value chain, (2) to determine the prevalence of *Salmonella* on pork in pig slaughterhouses and markets, and (3) the most probable number (MPN) in pork sold at markets. These findings are used in the quantitative risk assessment model which is the main component of the project, and will be reported elsewhere.

In Vietnam, slaughterhouses are characterized into four types. Commercial type slaughterhouses deal with more than 100 pigs per day, and have a standard hygienic condition and regular veterinary inspection. Small and medium scale slaughterhouses slaughter less than 10 and 10–100 pigs per day, respectively, and

might occasionally operate under veterinary inspection. Mobile slaughtering applies to slaughter at pig farms or at home on special events (such as weddings and funerals). In this study, we focused on the medium scale type (slaughter 10–100 pigs/day) which provides most of the pork to the consumers.

II. Materials and Methods

2.1. Study sites

The study sites were in Hung Yen Province, which is located in the northern part of Vietnam. Hung Yen is about 30 km from the center of Hanoi and is an important pork supply base. This study was conducted in Van Giang district, Hung Yen province between January and February, 2014. Two slaughterhouses, called A and B in this paper, were selected randomly from the list of slaughterhouses. These are medium scale slaughterhouses with a capacity from 10–40 pigs per day. They apply the floor slaughtering style where all slaughtering activities take place on the floor with relatively poor hygienic practices.

Pig carcasses were traced from these slaughterhouses to the markets located in the urban area of Hanoi (Dao Tan and Gia Lam markets) or in the peri-urban areas of Hung Yen province (Van Giang and Van Lam districts) (Fig. 1).

2.2. Sampling methods

Sample size (n) was determined using following formula¹⁴.

$$n = \frac{1.96^2 \times P_{\text{exp}} \times (1 - P_{\text{exp}})}{d^2}$$

Where P_{exp} is the expected prevalence and d is the desired preci-

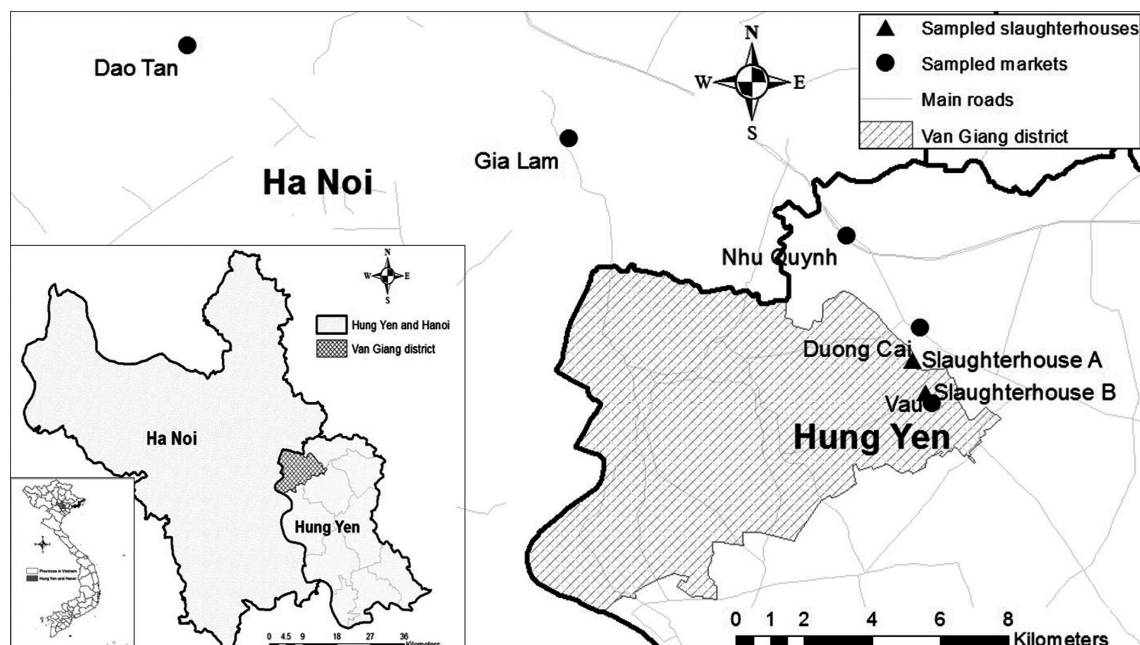


Fig. 1 Maps showing the locations of Hung Yen province, sampled slaughterhouses and markets

sion. P_{exp} was set as 0.35 referring from Dang-Xuan et al. (2013)⁴⁾, and d was set to be 10% error. Sample size n was calculated to be 88.

Sampling was conducted at each slaughterhouse, and 45 and 43 carcass samples were collected from four visits to slaughterhouse A and three visits to slaughterhouse B, respectively. To trace the carcasses from slaughterhouses to markets, we purposively selected three swabbed carcasses per visit, which were transported to different markets. In total, 21 pork samples were collected. To obtain carcass samples, a carcass-swabbing method using cotton surgical gauze and a 100-cm² steel frame was applied, and four sites on the medial surface of the carcass were swabbed. Pork collected in the markets included the parts of shoulder, belly and loin, which were swabbed in slaughterhouses, and totaled 200–300 g per sample. The collected samples were kept in a cool box and then microbiological tests were conducted within 24 hr. For carcass samples at slaughterhouses, the presence of *Salmonella* was analyzed, while for pork at markets, MPN was examined in addition to the presence of *Salmonella*.

2.3. Microbiological test

2.3.1. *Salmonella* qualitative determination

For qualitative determination for presence of *Salmonella*, three steps were conducted (enrichment, isolation and confirmation) following ISO 6579 : 2002⁹⁾. For non-selective enrichment, buffered peptone water (BPW ; Merck KGaA, Germany) was used. Twenty-five grams of pork were added to 225 ml BPW, while carcass swabs were added to 100 ml BPW. The BPW containing sample was then homogenized and incubated at 37°C for 24 hr. For selective enrichment, modified semi-solid Rappaport Vassiliadis (MSRV ; Merck KGaA, Germany) medium base and Muller-Kauffman Tetrathionate-Novobiocin broth (MKTTn ; Merck KGaA, Germany) were used. Three drops of incubated medium were dispensed to MSRV agar plate and were incubated at 42°C for 24 hr, and 1 ml of incubated medium was added to 9 ml of MKTTn and incubated at 37°C for 24 hr. The enriched bacteria on MSRV agar plate and MKTTn were inoculated to Xylose-Lysine-Tergitol 4 (Merck KGaA, Germany) agar and Rambach agar (Merck KGaA, Germany) and then were incubated at 37°C for 24 hr. After incubation, each medium was examined to identify the presence of typical and atypical colonies of *Salmonella*. After *Salmonella* isolation, the typical or suspect colonies were streaked onto pre-dried nutrient agar (NA ; Merck KGaA, Germany) plates and incubated at 37°C for 24 hr. The pure colonies from the NA agar plates were picked up and inoculated onto Triple Sugar Iron agar (Merck KGaA, Germany), Motility Indole Lysine agar (Merck KGaA, Germany) and into Urea broth (Sigma-Aldrich, USA). All biochemical tests were incubated at 37°C for 24 hr.

2.3.2. *Salmonella* quantitative determination

The number of *Salmonella* was also quantified from positive pork samples. The three tube-MPN technique was used for the

enumeration. Triplicates of three ten-fold dilutions (10^{-1} , 10^{-2} and 10^{-3}) were pre-enriched with BPW and incubated at 37°C for 24 hr. After incubation, two other steps were performed following the same procedure as described above, however only MSRV was applied at selective enrichment step. The number of *Salmonella* was confirmed based on the MPN table^{2,3,6)}.

2.4. Checklist survey

Hygiene practice was assessed using check lists based on observation at slaughterhouses and markets. Modes of business operation for both slaughterhouses and markets were studied by observation, and informal interviews were conducted in addition to that. In slaughterhouses, the items assessed for the hygiene practice of workers during slaughtering were wearing an apron and a uniform, washing floor, knife, hook and hands after slaughtering each pig. At markets, hygiene practice of sellers were checked and recorded for wearing gloves, using a cutting board, wiping hands with a cloth used for wiping surface of pork and equipment, and transporting pork in a basket from slaughterhouse to the shop at market.

2.5. Statistical analysis

Statistical analysis was conducted using statistical software R version 3.0.2. The prevalence was calculated by dividing the number of positive samples by the total number of samples. The Chi-square test or Fisher's exact test was used for a comparison of prevalence. The *kappa* value was calculated to understand the degree of maintenance of contamination and cross-contamination by the agreement between the prevalence in slaughterhouses and in markets. Attributable risk percent¹⁴⁾ was calculated to see the proportion of contaminated pork for sale attributable to the contamination at the slaughterhouse level. An MPN table for three tube tests was used as a reference for measuring the MPN per gram in pork samples. For checklist survey, statistical analysis was conducted only for the market survey results, as only two slaughterhouses were assessed. Chi-square test or Fisher's exact test was performed to analyze the data from markets.

III. Results

3.1. Observational descriptions of slaughterhouses and markets

Slaughtering in the visited slaughterhouses included following stages : restraining, bleeding, scalding and dehairing, evisceration, washing, wiping and splitting. After pigs were moved from a pen, they were restrained by the trunk using iron fixer with a stand on cement floor, until bleeding finishes. Carcasses were then placed on the floor, and hot water was poured on the skin from a kettle (scalding). Hairs were removed using a knife (dehairing), and carcasses were washed with water taken from a water tank which stored pumped water from underground. At evisceration, internal organs were either put on the cement floor or in a bucket, and in most of the case, they were transferred in a bucket to retailer's home to be washed and processed for sale. Other

butchers (retailers) carried out washing internal organs including large and small intestines in a corner of the same slaughtering area with carcasses. Inside surface of the carcasses were then washed with water taken from the same water tank, and were wiped with a towel. Carcasses were split into two along the spine, and were weighed for pricing. All stages were conducted directly on the floor at both slaughterhouses. In slaughterhouses, both slaughterhouse workers and traders worked together in slaughtering pigs and people walked freely in all slaughterhouse areas without any sanitary restrictions. Water used for various purposes, such as cleaning intestines, carcasses and floor, was taken from above said same water tank placed in the slaughtering area. Traders transported one to four halves of carcasses on a motorbike, without any chilling facility, directly to the markets.

According to the informal interviews in these two slaughterhouses, slaughterhouse owners collected and bought pigs from different villages in the same district, or sometimes even from the other provinces. Pig traders (in fact they are butchers as well after all) came to select and buy pigs from the slaughterhouse owner, and the traders did all stages of slaughtering by themselves with the help of slaughterhouse workers. As most of traders act as a family business, they sold pork by themselves in the markets as retailers.

In all the pork markets accessed, pork was sold at specialized pork sales places in the compound areas where other foods (vegetable, dried food stuffs, and fish) were also sold in an open environment. Pork was usually sold from 5 to 11 am in the morning. Pork was kept on the table at ambience temperature where any buyer could touch it.

3.2. *Salmonella* prevalence in slaughterhouses and markets

The overall *Salmonella* prevalence on carcasses was 25.0% (22/88, 95% confidence interval (CI) : 16.7%–35.6%), and that of pork at markets was 28.6% (6/21, 95%CI : 12.2%–52.3%). In a comparison between slaughterhouse and market levels, the prevalence of *Salmonella* was not significantly different between at the slaughterhouse level (25.0%) and market level (28.6%, $\chi^2 = 0.0034$, $df = 1$, $p = 0.95$).

Fig. 2 shows the *Salmonella* prevalence on carcasses in each slaughterhouse, and marketed pork sourced by these slaughterhouses. Overall, the prevalence in slaughterhouse B tended to be higher than that in slaughterhouse A. However, there was no significant difference in the prevalence of *Salmonella* on carcass samples between the two slaughterhouses (A : 22.2% (10/45) and B : 27.9% (12/43), $\chi^2 = 0.18$, $df = 1$, $p = 0.71$, Fig. 2). When the prevalence in marketed pork was compared based on the sourcing slaughterhouses, there was again no significant difference in the prevalence of *Salmonella* between two slaughterhouses (A : 23.1% (3/13) and B : 37.5% (3/8), $p = 0.63$ (Fisher's exact test)). Moreover, even when the results from carcasses at slaughterhouses and pork at markets are combined, the prevalence of *Salmonella* on all pork products were not different between the two

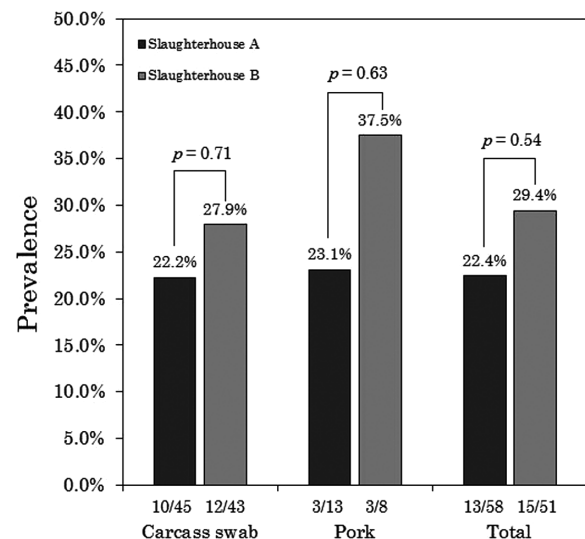


Fig. 2 *Salmonella* prevalence on pig carcasses and pork in each slaughterhouse

slaughterhouses (22.4% (13/58) and 29.4% (15/51), $\chi^2 = 0.38$, $df = 1$, $p = 0.54$, Fig. 2).

3.3. MPN in markets

The MPN was measured for six pork samples from which *Salmonella* was isolated. Out of these six samples, the mean and confidence interval was determined for two samples : 0.92 (95%CI : 0.14–3.8), and 1.5 (95%CI : 0.37–4.2) MPN/g. The MPN/g of the rest of four samples was less than 0.3 (95%CI : –0.95).

3.4. The transition of *Salmonella* contamination status

The transition of *Salmonella* contamination status for pork is shown in Fig. 3. Out of six positive carcass samples in slaughterhouses, only four pork samples were positive and the other two samples were negative at the markets. On the other hand, out of 15 negative carcass samples in slaughterhouses, two samples changed to positive. The kappa value calculated from the transition of contamination status was 0.53, suggesting substantial agreement between the prevalence at the slaughterhouse and market levels. Attributable risk percent was 53.3%, and this suggested that 53.3% of contaminated pork at sales at markets is attributable to the contamination at the slaughterhouse level.

3.5. Checklist survey

In these slaughterhouses, workers didn't wear an apron and a uniform and moreover they didn't wash floor after slaughtering each pig. Furthermore, they didn't wash knife, hook and hands with soap after slaughtering each pig. As above, most of the hygiene practices checked was not performed in these slaughterhouses.

Table 1 shows risk factor analysis results using checklist. There was no significant factor associated with prevalence of *Sal-*

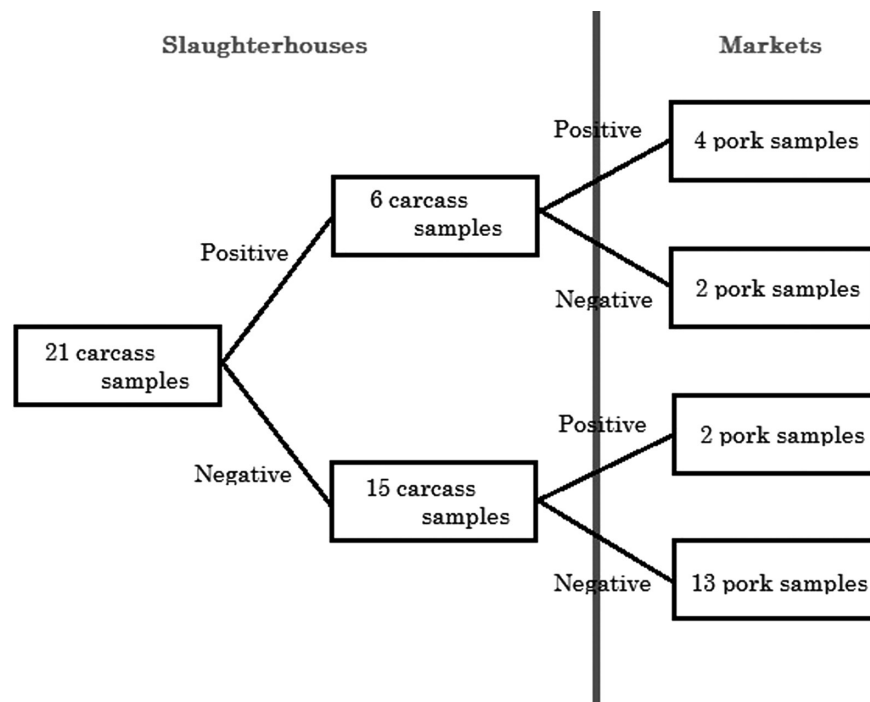
Fig. 3 Transition of *Salmonella* contamination status on pork

Table 1 The results of the checklist survey at markets

Observations at markets	Positive samples	Negative samples	Prevalence (%)	<i>p</i> -value
Seller wears gloves				
Yes	0	3	0	0.53
No	6	12	33.3	
Seller uses a cutting board				
Yes	2	8	20.0	0.64
No	4	7	36.4	
Seller wipes hands with a cloth used for wiping pork and/or equipment				
Yes	6	9	40.0	0.12
No	0	6	0	
Seller transport pork in a basket to the shop at market				
Yes	0	6	0	0.12
No	6	9	40.0	

monella in marketed pork.

IV. Discussion

From the results, *Salmonella* prevalence was maintained at considerable level from slaughterhouses in carcasses to markets on pork, and the contamination occurred both at slaughterhouse and during transportation/marketing. In the present study in Hung Yen, the *Salmonella* prevalence in slaughterhouses and markets, with confidence intervals, was within a range of previ-

ous reports : from 15.5% to 95.7%^{4, 10, 13)} in slaughterhouses, and 33 to 69%^{4, 12, 13)} in markets, as mentioned in the introduction. Sampling was conducted in a cool season (January and February) in the present study, and the prevalence can be higher in hotter season, as in the previous study. The MPN/g in a previous study in Vietnam ranged from less than 0.3 to 15⁴⁾, which included much larger value than those shown in the present study. However, the number of samples of which MPN/g was measured was small in the present study, and bacterial concentration of *Salmo-*

nella on pork sold in markets can be as high as the previous report, considering the mode of sales observed.

Studies on the modes of business revealed the informal and mutually-aiding activities between slaughterhouse workers and traders, who are in fact at the same time pork sellers at the markets. Hanoi is the second largest city in Vietnam, but even at the markets located in urban areas of Hanoi, pork sold are sourced through such informal value chains. This fact elucidated in the present study is suggesting a challenge in improvement of hygiene in pork value chains in Vietnam, if intervention is to be targeted only at formal and large scale slaughterhouses.

The *kappa* value suggested that the prevalence at the slaughterhouse substantially affects the prevalence at the market, which was quantified as more than half by the attributable risk percent. This suggests that contamination can largely occur before the end of slaughtering because of the inadequate hygiene during slaughtering and meat processing. However, on the other hand, cross-contamination during transportation and marketing also can occur substantially. As the sample size of carcasses traced was small, the attributable risk percent calculated in this study has a limited accuracy. Nevertheless, informal mutual-aiding relationship between slaughterhouse workers, traders and sellers endorsed the high chance of cross-contamination along the value chain¹⁷⁾. Maintenance of *Salmonella* prevalence between slaughterhouse and market mentioned here does not necessarily mean that this study proved that identical isolates on carcasses are maintained to the market. In this study, detailed sero-typing or molecular identification for *Salmonella* strains was not performed. However, several different types of *Salmonella* isolates can contaminate a single pig carcass in a slaughterhouse, considering these strains harboring in the environment of slaughterhouse come from different pig farms. What epidemiologically important in terms of understanding food safety risks is degree of change in *Salmonella* prevalence from carcasses in slaughterhouse to pork in market, which was described in this paper.

From the results of checklist survey, unhygienic practices were observed at slaughterhouses. Moreover, 'wiping' practice of carcasses after washing using the same cloth, which was observed during slaughtering process. The previous studies reported different management practices in the slaughterhouses could be associated the *Salmonella* prevalence⁷⁾ and "dirty polishing equipment" and "faulty techniques and sloppy hygiene during evisceration" were risk factors in slaughterhouses¹⁾. In the present study, use of water from the same tank for various purposes, and placing intestines on the floor near carcasses was observed. This qualitatively supported our hypothesis of *Salmonella* cross-contamination by unhygienic practices in the processes in a slaughterhouse.

In considering effective improvement of hygiene, introduction of a table to place carcasses is started in several small scale slaughterhouses in Vietnam, where introduction of expensive in-

frastructure such as hanging hooks is financially difficult. Microbiological studies comparing *Salmonella* prevalence in slaughterhouses between processing on the floor and using a table was not conducted in the present study. If the effect of use of a table in hygiene improvement would be proved, such affordable intervention may be adopted easily. On the other hand, this study also showed that *Salmonella* contamination greatly occurs during transportation and marketing as well. Future studies should focus on affordable effective intervention options at slaughterhouses, transportation, and markets in Vietnam, where small to medium scale informal business is dominating along pork value chains.

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原 著

ベトナム・フンイエンのと畜場から市場への豚肉の
バリューチェーンにおけるサルモネラ属菌の汚染率の変遷横澤輝美¹・Sinh DANG-XUAN²・Hung NGUYEN-VIET^{2,3,4}・Lucila LAPAR³・蒔田浩平^{1,3*}¹〒069-8501 北海道江別市文京台緑町 582 番地, 酪農学園大学獣医学ユニット²138 Giang Vo, Ba Dinh, Hanoi, Vietnam, ハノイ公衆衛生大学³B1- Van Phuc Diplomatic Compound, 298 Kim Ma, Ba Dinh, Hanoi, Vietnam,
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要旨

ベトナムでは豚肉が食肉消費量の約 70% 以上を占めるため、豚肉のサルモネラ属菌による汚染は消費者に甚大な健康被害をもたらすことが考えられる。本研究はベトナム国フンイエンのと畜場から市場への豚肉のバリューチェーンにおけるサルモネラ属菌の汚染状況を調べるため、と畜場でのと体と、市場での豚肉における汚染率、ならびに市場販売豚肉における菌数の定量化を目的に実施された。調査はフンイエンのと畜場 2 か所を無作為に抽出し実施した。2014 年 2 月にこれら 2 つのと畜場において、計 88 と体からスワブサンプルを採取し、そのうち 21 と体について市場まで追跡し、解体後に市場で販売されている豚肉から再度採取した。と体スワブにおいてはサルモネラ属菌の汚染率を、市場の豚肉においては汚染率と最確数 (MPN) を算出した。

と体におけるサルモネラ汚染率は 25.0% (22/88, 95%CI : 16.7%-35.6%) であり、市場での豚肉の汚染率は 28.6%

(6/21, 95%CI : 12.2%-52.3%) であった。2 つのと畜場の比較では、汚染率に有意な差は見られず (22.2% (10/45) と 27.9% (12/43), $\chi^2=0.18$, $df=1$, $p=0.71$), また、と体と豚肉の汚染率にも有意な差が見られなかった (25.0% (22/88) と 28.6% (6/21), $\chi^2=0.0034$, $df=1$, $p=0.95$)。と畜場から市場にかけてのサルモネラ汚染状況の変化について、カッパ係数 0.53, 寄与リスク割合は 53.3% であり、市場での販売豚肉汚染の半数以上がと畜場で起きている可能性が示唆された。豚肉における菌濃度は <0.3-1.5 MPN/g であった。ベトナムでの豚肉のサルモネラ属菌の汚染率はと畜場から市場にかけて高く維持されており、不衛生な加工によると畜場での汚染とともに、不衛生な輸送・販売管理についても、人の健康リスクを考える上で問題であることが示唆された。

キーワード：サルモネラ, 豚肉バリューチェーン, ベトナム

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