Salmonella and Campylobacter in Broiler Carcasses In Vietnam
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Abstract
From November 2004 to May 2005, 319 chicken carcass-rinse samples from 15 abattoirs, classified as large and small size, were examined for the presence of Salmonella and Campylobacter. Nearly half of the samples were Salmonella positive. In the small abattoirs, a prevalence of 48% positive carcasses was obtained, whereas in the large abattoirs a significantly lower prevalence of 34.2% was recorded. S. Emek (32.8%), S. Haardt (19.0%), S. Derby (8.6%), S. Typhimurium (7.8%) and S. London (6.9%) were the most prevalent serotypes. Campylobacter spp. was isolated from 35.1% of the carcass samples with 36.6% in the large abattoirs and 34.2% in the small abattoirs. Campylobacter jejuni and Campylobacter coli were identified in 67.9% and 23.2%, respectively of the 112 Campylobacter isolates. Nearly one fifth of the samples were contaminated by both, Salmonella and Campylobacter. Effective hygienic standards along the poultry slaughter line should be implemented.

Keyword: Salmonella, Campylobacter, chicken carcasses

Introduction
Poultry and poultry products are important vehicles for human food-borne illnesses. Among the causes found serovars of Salmonella enterica (Tauxe, 1992) and thermophilic Campylobacter spp. (Altekruse, 1999; Oosterom, 1984) are the most common ones. Almost all serovars of Salmonella cause a self-limiting gastroenteritis in healthy people but, some occasionally cause fatal bacteremia in young and elderly individuals (Yang, 2002).

Campylobacter spp. are usually associated with self-limiting diarrhoea caused by C. jejuni and C. coli (Anonymous, 1993). C. jejuni and C. coli have also been implicated in extra-intestinal diseases such as meningitis, endocarditis, septic arthritis, osteomyelitis, and neonatal sepsis. The most important post-infection complication of C. jejuni is the Guillain-Barre syndrome (Allos, 1998). Studies carried out in slaughterhouses have indicated that the main sources of spread of Salmonella and Campylobacter on carcasses are the intestinal tracts of live birds (Oosterom, 1983; Rivoal et al., 1999). Improper hygienic processing procedures in abattoirs tend to enhance cross-contamination of the carcasses. In almost all developing countries, low hygienic standards in poultry slaughterhouses coupled with old processing facilities have been associated with significant contamination rates of market chicken products with serovars of Salmonella enterica and Campylobacter spp (Boonmar et al., 1998). In Vietnam, there is paucity of information about the Salmonella contamination of chicken carcasses (Tran, 2005). And, no publication of Campylobacter in food producing animal is so far available although in 11% of specimens from diarrhoeal patients in Vietnam Campylobacter spp. had been isolated (Phung, 2001).

The present study intended (1) to assess the prevalence of Salmonella and Campylobacter in chicken carcasses from abattoirs in Ho Chi Minh City, Vietnam and (2) to identify Salmonella serovars and the most important Campylobacter spp. in these carcasses.

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Materials and methods

The abattoirs and sample collection

This study was carried out during the dry season in 15 abattoirs of Ho Chi Minh City. Ho Chi Minh City with a population of 8.5 million inhabitants is considered one of the most developed cities in Vietnam. More than 60,000 chicken are slaughtered every day in 55 poultry abattoirs of the city. The abattoirs were categorized as large, if the daily slaughter figure was between 1,200-2,000 chickens and if automated machinery for stunning, scalding and evisceration was used. Small abattoirs were abattoirs in which less than 1,200 chickens were slaughtered and only manual procedures were applied. Sources of slaughter chickens were farms within Ho Chi Minh City and the surrounding 8 southeastern provinces (Dong Nai, Binh Duong, Binh Phuoc, Long An, Tien Giang, Tay Ninh, and Vung Tau). Slaughtering was done at night.

A total of 319 study samples of broiler carcasses were collected from 15 abattoirs (3 large abattoirs and 12 small abattoirs). Samples were collected twice to three times from each abattoir. Carcass-rinse samples were obtained from the final product after "inside-outside wash", following the procedure described by USDA (Sparling, 2002).

Salmonella, Campylobacter isolation and identification

Salmonella isolation and identification was based on ISO 6579 (2002). Campylobacter isolation followed ISO 10272 (1995). Isolates were confirmed using Gram-staining and biochemical tests (catalase, Triple Sugar Iron, oxidase, Hippurat-Hydrolysis). A test of resistance against nalidixic acid was performed. Campy Latex Agglutination test (Oxoid, Dryspot Campylobacter test) containing Campylobacter jejuni, Campylobacter coli, Campylobacter lari, Campylobacter upsaliensis antiserum was used.

Species identification for Campylobacter isolates using PCR

DNA preparation was performed using the boiled lysate method (van de Giessen, 1998). Colonies of Campylobacter spp. were inoculated in Brain Heart Infusion (BHI) -broth and incubated micro-aerobically at 37 °C for 24 h. One ml of the culture was centrifuged, washed in 500 µl of saline solution and re-suspended in 500µl of distilled water. Then the suspension was heated to 100°C for 3 minutes and centrifuged. The supernatant was adjusted by diluting in distilled water. Five µl were used in the amplification reaction.

The selected isolates were identified using a multi-primer PCR technique for identification of C. jejuni and C. coli (van de Giessen, 1998). In this assay, specific PCR amplifications of C. jejuni and C. coli were performed with primers based on the nucleotide sequences selected for specificity from C. jejuni and C. coli DNA fragment libraries. The DNA sequences of the primers used are shown in Table 1.

Table 1: The DNA sequences of the primers

<table>
<thead>
<tr>
<th>Primer</th>
<th>DNA sequences</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primer COL1</td>
<td>5’- AGG CAA GGG AGC CTT TAA TC- 3’</td>
</tr>
<tr>
<td>Primer COL2</td>
<td>5’- TAT CCC TAT CTA CAA ATT CGC- 3’</td>
</tr>
<tr>
<td>Primer JUN3</td>
<td>5’- CAT CTT CCC TAG TCA AGC CT- 3’</td>
</tr>
<tr>
<td>Primer JUN4</td>
<td>5’ AAG ATA TGG CAC TAG CAA GAC- 3’</td>
</tr>
</tbody>
</table>
Primer sets, COL1-COL2 and JUN3-JUN4, were combined into a *C. jejuni* and *C. coli* multi-primer PCR assay. The reaction mixtures (25µl) had concentrations of 20 mM Tris/HCl pH 8.3, 50 mM KCl, 3 mM MgCl₂, 0.01% gelatin and 0.1 mM of deoxyribonucleotide triphosphate. The reaction mixtures each contained 1.0 unit of *Thermus aquaticus* (Taq) DNA polymerase, 50 pmol of each primer and 5 µl of DNA-extracts. All reactions were performed in a Perkin Elmer DNA thermal cycles model 480, using a touch-down protocol. PCR products were separated on 1.6% agarose gels and stained with ethidium bromide. The species classification was deduced from the size of the amplification product. The PCR product for *C. jejuni* is 773 bp in length, and that of *C. coli* is 363 bp.

**Statistical analysis:**

The prevalence estimates of *Salmonella* and *Campylobacter* contaminated carcasses were determined using standard formula (i.e. the number of positive carcasses divided by the number of samples examined). The exact binomial confidence limits of prevalence were determined using the Fishers exact Chi-square in NCSS software (version 1997).

**Results**

*Salmonella*

Out of all 319 samples, 136 were found to be contaminated with *Salmonella* giving an overall prevalence of 42.6% (Table 2). The prevalence of *Salmonella* contamination in large abattoirs (34.2%) was significantly lower (p = 0.0152 than in small abattoirs (47.96%). These two prevalences were significant different).

The distribution of serovars isolated from chicken carcasses are listed in Table 2. Serotypes such as S. Alminko, S. Bardo, S. Mbandaka, S. Nchanga, S. Galiena, and S. Virchow had only one isolate. Overall, S. Emek and S. Haardt were the most dominant serovars in both small and large abattoirs.

<table>
<thead>
<tr>
<th>Somatic (O) serogroups</th>
<th>Serovar</th>
<th>No. of serovars by abattoir size</th>
<th>Total</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Large</td>
<td>Small</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>S. Agona</td>
<td>1</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td>S. Derby</td>
<td>4</td>
<td>6</td>
<td>10</td>
</tr>
<tr>
<td>B</td>
<td>S. Schwarzengrund</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td>S. Stanley</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>B</td>
<td>S. Typhimurium</td>
<td>2</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>B</td>
<td>O 4,5,12:b:</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>C₁</td>
<td>S. Galiena</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>C₁</td>
<td>S. Mbandaka</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C₁</td>
<td>S. Virchow</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>C₃</td>
<td>S. Corvallis</td>
<td>0</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>C₃</td>
<td>S. Alminko</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C₃</td>
<td>S. Bardo</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>C₃</td>
<td>S. Emek</td>
<td>13</td>
<td>25</td>
<td>38</td>
</tr>
</tbody>
</table>

Table 2: Distribution of Serovars of *Salmonella* isolates from chicken carcasses by abattoir size in Ho Chi Minh City, Vietnam, November 2004 to May 2005
The biochemical test and Campy Latex Agglutination Assay enabled the determination of *Campylobacter* in chicken carcasses. Of 319 carcass-rinse samples examined, 112 *Campylobacter* isolates were recovered giving a *Campylobacter* contamination rate of 35.1%. In large abattoirs the overall sample prevalence was 36.6% and in small abattoirs 34.2%; no significant difference was found between the two proportions.

Using multiplex PCR assay, specific PCR products for *C. jejuni* were obtained in 76 isolates (67.9%), and for *C. coli* in 26 isolates (25.9%). Furthermore, *Campylobacter* spp detected included this assay (Campy Latex Agglutination) were *C. lari* and *C. upsaliensis*. (6.3% with 3 isolations in large and 4 isolations in small abattoirs).

**Campylobacter and Salmonella in the samples**

The prevalence of *Campylobacter* and *Salmonella* in the carcass-rinse samples are given in Table 3. Nearly one fifth (17.9 %) of the samples were contaminated with both *Salmonella* and *Campylobacter*.

Table 3: Prevalence of *Campylobacter* and *Salmonella* in chicken carcasses from abattoirs in HoChi Minh City, Vietnam

<table>
<thead>
<tr>
<th>Code</th>
<th>Salmonella</th>
<th>Campylobacter</th>
<th>No. of samples positive</th>
<th>Sample Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>+</td>
<td>+</td>
<td>57</td>
<td>17.9</td>
</tr>
<tr>
<td>2</td>
<td>+</td>
<td>-</td>
<td>136</td>
<td>42.6</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>+</td>
<td>112</td>
<td>35.1</td>
</tr>
</tbody>
</table>

(1): Positive carcasses for both *Campylobacter* and *Salmonella*
(2): Positive carcasses for *Salmonella*
(3): Positive carcasses for *Campylobacter*

**Discussion and conclusions**

This study indicates a high prevalence of *Salmonella* contamination of chicken carcasses. However, a lower rate was reported from other provinces of Vietnam, where 21.0% of chicken meat were *Salmonella* contaminated (Tran *et al.*, 2005). The difference might be due to different sampling methods: in this study, the carcass-rinse of chicken was used to isolate the *Salmonella* whereas in the study of Tran *et al.* a meat sample of 25g was used.
The rate of *Salmonella* contamination in Vietnam was lower than in other southeast Asian countries such as Thailand and Malaysia. In Thailand, Boonmar *et al.* (1998) reported *Salmonella* findings in 72% of retail chicken meat samples, in 80% of samples from open markets and in 64% of samples in supermarkets. In Malaysia, *Salmonella* was isolated from 50% of broilers from processing plants (Rusul *et al*., 1996).

Prevalence surveys performed in developed and some developing countries indicate that the percentages of *Salmonella* contaminated chicken carcasses range between 0% to 36% (Bailey *et al*., 2001). For example in Japan, *Salmonella* was found in 14.3% of the cecal contents of broiler carcasses from commercial farms (Limawongranee *et al*., 1999). In Argentina, the prevalence of *Salmonella* in chicken carcasses after evisceration in commercial slaughter practice ranged between 20% and 20.8% (Jimenez *et al*., 2002). However, *Salmonella* was found in 40.4% of chicken neck skin samples after the defeathering step in Germany (Fries, 2002).

Nevertheless these contamination rates of *Salmonella* prove that chicken meat continues to be an important carrier of *Salmonella* infection leading possibly to infection of consumers. Our findings in this study suggest that the conditions at large abattoirs may contribute to a lower rate of *Salmonella* in chicken carcasses compared to small abattoirs: The automatic machinery used for stunning, scalding and evisceration in large abattoirs might explain this observed low *Salmonella* contamination of the chicken carcasses. Thus, automated machinery operations coupled with improved hygienic levels in the abattoir environments may significantly reduce or eliminate this contamination.

Nineteen serovars of *Salmonella* were identified from 116 *Salmonella* isolates. The most common serovars were *S. Emek*, *S. Haardt*, *S. Typhimurium* and *S. Derby*. However, the *Salmonella* serovar distribution in this study is different from that reported from other countries. In Thailand, the most common serovars reported are *S. Enteritidis*, *S. Muenchen*, *S. Blockley* and *S. Montevideo* from retail chicken meat (Boonmar *et al*., 1998) while in Malaysia the predominant serovars are *S. Enteritidis*, *S. Muenchen*, and *S. Kentucky* (Rusul *et al*., 1996). In Japan, the predominant serovars in broiler chicken include *S. Blockey*, *S. Hadar*, and *S. Bredeney* (Akiba *et al*., 1996). From 1153 *Salmonella* isolates from poultry in Australia, the most frequently isolated serovars are *S. Sofia* (36.6%), *S. Virchow* (11.3%), *S. Infantis* (10.9%), and *S. Typhimurium PT64* (3.4%), *S. Typhimurium PT108* (3.2%) (Sumner *et al*., 2004). *S. Enteritidis* has become the predominant serovar worldwide (Popoff *et al*., 2000). However, *S. Enteritidis* was not isolated in chicken carcasses from abattoirs in Ho Chi Minh City in the present study but the patterns of the most common *Salmonella* serotypes in the present study is in accordance with the findings of Tran *et al* (2005) who found the predominant serotypes to be *S. Emek*, *S. Typhimurium*, *S. Dessau*, and *S. Derby*.

This study is the first to document on the high rate of *Campylobacter* isolated from chicken meat in Vietnam although many reports do exist of *Campylobacter* contaminated chicken meat elsewhere. Stern and Line (1992) detected *Campylobacter spp* in 98% of retail-packaged broiler samples from grocery stores. Comparatively, the prevalence of *Campylobacter* spp. in poultry and poultry meat products reported in Germany was 41.1% (Atanassova and Ring, 1999).

The present study also confirms the predominance of *C. jejuni* and *C. coli* (91%) among the isolates obtained from chicken carcasses. In a study done in Vietnam with patients from hospitals, the diarrhoeal rate caused by *Campylobacter* spp. was 11% among all diarrheal cases (Phung and Nguyen, 2001). Chicken meat in Vietnam should be considered a potential source of this hazard for public health. *Campylobacter jejuni* was the most predominant species and was present in 68% of
chicken carcasses. Other reports on *Campylobacter* spp. in chicken products also show that the species *C. jejuni* is the more frequently isolated species than *C. coli*; *C. lari* is seldom found (Tauxe, 1992).

Various studies carried out in slaughterhouses have shown that the main source of spread of *Salmonella* and *Campylobacter* on poultry carcasses came from intestinal contents (Bailey, 1990; Oosterom *et al.*, 1983; Berndtson *et al.*, 1992). Furthermore, trucks, pellets, crates, catchers and equipments of the processing procedure were identified as potential sources of *Salmonella* and *Campylobacter* for broilers (Fries, 2002; Ramabu *et al.*, 2004). The high prevalence of *Salmonella* and *Campylobacter* in chicken carcasses in both types of abattoirs in Vietnam suggests that further studies should be done to set up establish hygienic standards for abattoirs and clarify main factors of contamination in poultry processing.

References


