เชียงใหม่สัตวแพทยสาร 2546;1:33-38.

Original article

# PRE-SLAUGHTER INFECTION OF *Salmonella spp.* AND CONSIDERATION OF USING THE DANISH MIX-ELISA® FOR MONITORING *SALMONELLA* IN PIGS

Prapas Patchanee,[1] Karl-Hans Zessin,[3] Christian Staak,[3] Lertrak Srikijkarn,[1] Prasit Taravijitkul,[2] Ted Tesaprateep[4]

[1]*Department of Food Animal Clinic, Faculty of Veterinary Medicine,*
[2]*Department of Microbiology, Faculty of Medicine, Chiang Mai University,*
[3]*Department of Epidemiology and Veterinary Public Health, Faculty of Veterinary Medicine, Free University, Berlin Germany,*
[4]*President Office, Mae FaLuang University, Chiang Rai*

**Abstract** Serogroups of Salmonella recovered by microbiological culture were analyzed for evaluation the possibilities of using the Danish Mix-ELISA® for monitoring Salmonella infective situations in pigs. Based on representative sample, proportionally distributed between two slaughterhouses and slaughter-unit strata within each slaughterhouse, 616 samples were cultured for Salmonella, using the ISO 6579 method. Mesenteric lymph node and fecal samples were taken from each study slaughter pig at the two slaughterhouses and were processed within 24 hours. The study revealed that pre-slaughter Salmonella prevalence of slaughtered pigs at farm of origin ranged from 50-83.3%, with an overall prevalence of 69.5%; this farm level prevalence of 69.5% increased to 82.5% prior to slaughter as a result cross-infections during transportation and during the waiting phase at slaughterhouse lairages. Serogroups of Salmonella, determined from microbiological cultures, revealed that the Danish Mix-ELISA® would miss to detect about 30-40% of infected cases because of lacking of antigenic identity.

**Keywords :** *Salmonella spp.,* Danish Mix-ELISA®, monitoring, pigs

Address requests for reprints : Prapas Patchanee, D.V.M., Department of Food Animal Clinic, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand, E-mail:Prapasp@chiangmai.ac.th
Date received : 28 October 2002.

## Introduction

*Salmonella* has long been regarded as a major food borne pathogen to cause human Salmonellosis.[1-3] At present, the pork industry adopts the monitoring and control program to reduce contamination in pork. It has been suggested that controlling *Salmonella* at herd level can reduce contamination in pork.[3] In Denmark a *Salmonella* control program to decrease the level of *Salmonella* was started in 1993. The ultimate goal of these programs is to reduce level of *Salmonella* in pork.[4] One possible method for monitoring *Salmonella* level is microbiological culture method. The Enzyme Linked Immunosorbent Assay (ELISA) is another way to determine if a pig has been exposed to *Salmonella*. Because of its practical property regarding time consumed, economic, and amount of testing samples, ELISA was used in testing herds for the presence of *Salmonella* in Denmark. The Danish Mix-ELISA® was designed for monitoring *Salmonella* in slaughter pig.[5] The Danish Mix-ELISA® uses O-antigen 1,4,5,6,7, and 12 to detect *Salmonella* antibody in the serum or meat juice.[3-5] Therefore, any serogroup that has at least one of these O-antigens can be monitored by this assay. The objectives of this study are to monitor the prevalence of *Salmonella* infective situation of delivering herd and and the possibility of using the Danish Mix-ELISA® for *Salmonella* antibody detection.

## Materials and Methods

This study was conducted in Chiang Mai, the northern region of Thailand. The mesenteric lymph nodes and fecal samples were collected from two pig slaughterhouses, Muang and Sansai slaughterhouse. The total of 616 test samples obtained from 308 slaughter pigs was proportional distributed between two slaughterhouses and slaughter-unit strata within each slaughterhouse. Selecting of pigs per stratum (delivering herd) has been done randomly. Mesenteric lymph nodes, fecal samples from rectum were collected into plastic bags and transferred into icebox until processing. Samples were cultured within 24 hours. ISO 6579 is the reference method for microbiological investigation, regulates in detail the stages, standards and quality assurances for the detection of Salmonella in products for human consumption or animals feeding. Twenty-five grams of sample were incubated in 225 ml in Buffered Peptone Water (BPW) at 37 °C for 16-24 hours. One hundred microlitre of BPW were then incubated in 10 mL Rappaport-Vassiliadis (RV) Broth at 42 °C for 24 hours. Ten microlitre of RV Broth was streaked onto XLD (Xylose Lysine Desoxycholate) Agar and BG (Brilliant Green) Agar. A single colony positive colony showing red color with a black center on XLD and red color on BG Agar were further examined for microbiological and serological confirmation. According to Salmonella groups with identical O-antigens,

**Table 1.** *Salmonella* detection in both slaughter pigs (Muang and Sansai Slaughterhouse)

| ***Salmonella* detection** | **Prevalence(%)** | **95%Confidence Interval (CI)** |
|---|---|---|
| Mesenteric lymph nodes | 69.5 | 64.3-74.6 |
| Feces | 54.9 | 49.3-60.4 |
| Negatives | 13.6 | 9.8-17.5 |

the following group sera were available (Sifin®, Berlin Germany):

Anti-Salmonella A-E
Anti-Salmonella F-67
Anti-Salmonella B (O 4,5,27)
Anti-Salmonella C (O 6,7,8,20)
Anti-Salmonella D (O 9,46,Vi)
Anti-Salmonella E (O 3,10,15,19,34)

Cross-tabulation and percentage calculation were analyzed to assess the chances of using the Danish Mix-ELISA®. The percent of different *Salmonella* serogroup recovered from microbiological culture were compared with the combination of antigen (Salmonella Thyphimurium O-antigen 1, 4, 5, 12 and Salmonella Cholerasuis O-antigen 6,7) in the Danish Mix-ELISA®.

## Results

### *Salmonella* bacteria prevalence

In total, Salmonella were detected in 82.5% (254/308) of the total samples (mesenteric lymph node and/or fecal samples). The overall sample-specific prevalence for mesenteric lymph nodes and fecal samples were 69.5% (214/308), and 54.9% (169/308), respectively (Table 1).

### Herd level pre-slaughter infection of infection of pigs with Salmonella bacteria

If the percentage of *Salmonella* isolation from mesenteric lymph nodes is considered an estimate of the herd-level prevalence, pre-slaughter infection at individual farms ranged from 50% to 83.3%, with an overall prevalence of 69.5% for the total of farms. The overall prevalence of pre-slaughter *Salmonella* infection at the slaughterhouse level on the other hand was as high as 82.5%. This suggests that about 13% cross-infection occurred during transportation and at the lairage (% found from fecal samples, as shown in Table 2).

**Table 2.** Account on *Salmonella* isolations from mesenteric lymph nodes, and faeces samples in individual pigs

| **Mesenteric Lymph nodes** | **Faeces** | **Percent** |
|---|---|---|
| + | + | 41.9 |
| + | - | 27.6 |
| - | + | 13 |
| - | - | 17.5 |

+ = *Salmonella* isolation

**Applicability of Danish Mix-ELISA for Salmonella bacteria screening test**

Based on the results in Table 3, the serogroup E1 which contains the antigens O: 3,10 and D2 (O: 9,46) only was prevalent in 32.1% and 9.4% of cases in each of the two slaughterhouses respectively. The Danish Mix-ELISA, which contains the antigens O 1,4,5,6,7 and 12, therefore would be unable to recover all the antigens detected in this investigation for Thailand

## Discussion

**Pre-slaughter Salmonella infections of pigs at farm and slaughter levels**

The overall prevalence estimate of *Salmonella* infections of pigs at the farm level of 69.5% and at the slaughterhouse level of 82.5% constitute the first report of its kind on the infection status of pigs herd in Thailand from the farms of origin of animals to slaughter. So far, no other comparable investigations on *Salmonella* prevalences at the farm and slaughterhouse levels have been carried out in the country. In Europe, any farm found of having *Salmonella* prevalences at the herd level higher than 50% is categorized under the highest level (level 3) and is placed under special control measures at the expense of owners.[6-9] Results of this study showed that none of the study pig farm had prevalence less than 50%. Hence, these farms could not be spared punitive measures if they were located in Europe. However, it worth noting that at present there is no such management system for monitoring and controlling *Salmonella* infections and contaminations in the production chain of pigs and pork products in place in Thailand yet. The study results though strongly point to the need to start introducing such systems. Without that, *Salmonella* bacteria easily will continue to be introduced into the farms through feed, pest and birds, workers contaminated soil and water without the farmer being aware of the problem.

Based on *Salmonella* identifications from mesenteric lymph nodes, it can be estimated that in fact close to 70% of slaughter pigs were already infected at their farms. Isolating Salmonella from lymph nodes identifies chronic infections.

During transportation and holding of pigs at the lairages, pre-slaughter cross-

**Table 3.** The percent of *Salmonella* recovered by serogrouping

| Serogroup | Total | Percent | Detectabality by the Danish Mix-ELISA |
|---|---|---|---|
| B | 99 | 25.85 | All |
| C | 123 | 32.11 | All of C1, most of C2-C3 (via O6) |
| D | 36 | 9.4 | All of D1, D3 (via O1, 2,27), none of D2 |
| E | 123 | 32.11 | All of E4 (via O1), none of E1 |
| Others | 2 | 0.52 | Depend on serogroup |
| Total | 383 | 100 | |

infection/contamination is possible and probable. Finding of this study supports this by investigations of the fecal samples prior to slaughter. An additional prevalence of new *Salmonella* infections of at least 13% was detected in pigs between farm and slaughter. Berend *et al*. (1997) has indicated that shedding of *Salmonel la* bacteria often is triggered by stress factors, for example transportation and crowding.[10]

**Applicability of Danish Mix-ELISA for screening Salmonella infections in pigs**

The Danish Mix-ELISA is used as a mass-screening test in support of Salmonella monitoring and control program in several countries.[11] It is rapid, inexpensive and simple to conduct. In this study, the Danish Mix-ELISA would have been able to only detect up to 59.5% of the *Salmonella* positive samples. Modification of this test or development of a new comparable one for local use in Thailand is strongly recommended.

**Acknowledgement**

We would like to acknowledge the officers and staff members of Muang and Sansai slaughterhouse for their support on specimen collection. We also would like to thank all of the officers and colleagues of the Faculty of Veterinary Medicine, Chiang Mai University for facilitating in the laboratory working.

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# การติดเชื้อ *Salmonella spp.* ในสุกรก่อนฆ่าชำแหละ และการพิจารณาความเป็นไปได้ในการใช้ DANISH MIX-ELISA®สำหรับการตรวจติดตามภาวะการติดเชื้อ *Salmonella spp*. ของสุกร

**ประภาส พัชนี**,[1] Karl-Hans Zessin,[3] Christian Staak,[3] **เลิศรัก ศรีกิจการ**,[1] **ประสิทธิ์ ธราวิจิตรกุล**,[2] **เทอด เทศประทีป**[4]

*[1]สาขาวิชาคลินิกสัตว์บริโภค คณะสัตวแพทยศาสตร์, [2]ภาควิชาจุลชีววิทยา คณะแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่, [3]ภาควิชาระบาดวิทยาและสัตวแพทย์สาธารณสุข คณะสัตวแพทยศาสตร์ มหาวิทยาลัยฟรี เบอร์ลิน ประเทศเยอรมันนี, [4]สำนักงานอธิการบดี มหาวิทยาลัยแม่ฟ้าหลวง จังหวัดเชียงราย*

**บทคัดย่อ** ทำการซีโรกรุ๊ปของเชื้อแบคทีเรียซัลโมเนลลา เพื่อนำมาวิเคราะห์ความเป็นไปได้ในการใช้ชุดตรวจสอบสำเร็จรูป Danish Mix-ELISA® ในการตรวจติดตามภาวะการติดเชื้อแบคทีเรียซัลโมเนลลา ในสุกร การประมาณขนาดตัวอย่างในการวิจัย ทำโดยการประมาณสัดส่วนของสุกร ที่คาดว่าจะติดเชื้อและจัดลำดับชั้นตามจำนวนสุกรที่ส่งเข้าโรงฆ่าสัตว์ของแต่ละฟาร์มตัวอย่างที่ใช้เป็นต่อมน้ำเหลืองลำไส้ (Mesenteric lymph node) และตัวอย่างอุจจาระ จำนวน 616 ตัวอย่าง ได้นำมาเพาะเลี้ยงตามมาตรฐาน ISO 6579 เพื่อหาเชื้อแบคทีเรียซัลโมเนลลาภายใน 24 ชั่วโมง หลังจากนำการเก็บตัวอย่างจากการศึกษาพบว่า ความชุกของการติดเชื้อแบคทีเรียซัลโมเนลลา ของสุกรระดับฟาร์มอยู่ในช่วงระหว่างร้อยละ 50-83.3 โดยมีค่าเฉลี่ยเท่ากับร้อยละ 69.5 และความชุกของการติดเชื้อแบคทีเรียซัลโมเนลลาของสุกรในโรงฆ่าสัตว์ เพิ่มขึ้นจากระดับฟาร์มโดยมีค่าอยู่ที่ระดับร้อยละ 80.5 ซึ่งเป็นผลจากการติดเชื้อข้ามในระหว่างการขนส่งและความเครียดในช่วงระหว่าง ก่อนฆ่า และจากซีโรกรุ๊ปของเชื้อแบคทีเรียซัลโมเนลลา ที่ได้จากการเพาะเชื้อพบว่า ชุดทดสอบสำเร็จรูป Danish Mix-ELISA® ไม่เหมาะสมในการครวจติดตามภาวะการติดเชื้อแบคทีเรียซัลโมเนลลาของสุกร โดยมีความผิดพลาดที่เกิดจากการไม่คลอบคลุมซีโรไทป์ที่พบประมาณร้อยละ 30-40 **เชียงใหม่สัตวแพทยสาร** 2546;1:30-36.

**คำสำคัญ** : เชื้อแบคทีเรียซัลโมเนลลา, Danish Mix-ELISA®, การตรวจติดตาม, สุกร