

Original article

Epidemiological Survey of *S. Enteritidis* Pulsotypes from Salmonellosis Outbreak in Chiang Mai and Samut Songkhram Provinces, Thailand

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Abstract *Salmonella* serotype Enteritidis (SE) is one of the most common *Salmonella* serotypes reported worldwide. An outbreak of *Salmonella* Enteritidis was emerged in Chiang Mai, Thailand in July 2012. Boiled eggs which had been kept for three days before being eaten were considered to be the cause of the outbreak. In another province, Samut Songkhram, an *S. Enteritidis* outbreak was reported in March 2009. The cause of that outbreak is not known. This study used Pulse-field Gel Electrophoresis (PFGE) to investigate the genetic association between *S. Enteritidis* recovered from human cases in the July 2012 outbreak ($N=20$), from human cases in the March 2009 outbreak ($N=12$) and from *S. Enteritidis* strains isolated from chicken slaughterhouses in Chiang Mai in 2011 ($N=8$). Thirty-eight of the 40 *S. Enteritidis* strains tested were grouped into two main clusters. Fourteen clonal strains were observed in from 12 strains from the March 2009 cases outbreak and two strains from samples collected from chicken slaughterhouses in Chiang Mai in 2011. An emerging *S. Enteritidis* pulsotype, descended from a common ancestral strain recovered from chicken slaughterhouses was identified from the July 2012 outbreak. Preventive measures, including more effective hygiene education as well as improved production line control programs, need to be developed to help control salmonellosis in this region. **Chiang Mai Vet J 2015; 13(2):73-80**

Keyword: *Salmonella* Enteritidis, PFGE, Chiang Mai, Samut Songkhram, Thailand

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Introduction

Salmonella enterica serotype Enteritidis is an important causative agent of non-typhoidal salmonellosis worldwide (Helmuth et al., 1994; Ngoi and Thong, 2013). Clinical signs in the infected-human cases may start with fever, headache, nausea, vomiting and profuse diarrhea within 8 to 48 hours after ingestion of contaminated products, e.g., meats which have not been properly cooked (Jawetz et al., 1984). In Thailand, an outbreak of *Salmonella* Enteritidis emerged in a school in Mae Rim District, Chiang Mai Province, in July 2012 resulting in a total of 460 cases including 123 hospitalizations and 8 seriously ill individuals. The investigation report by the Provincial Public Health Office indicated that boiled eggs which had been stored for three days were the probable cause (Bangkok Post, 2012). That remains the first report and only the



major outbreak of *S. Enteritidis* in Chiang Mai, although a prior outbreak caused by similar organisms occurred in March 2009 in Samut Songkhram Province in the central region of Thailand in which several cases of *S. Enteritidis* infection were reported. In the Samut Songkhram case, neither the origin nor the transmission route of the disease could be determined.

Advances in DNA-based identification methods have been improving the discriminatory ability and reproducibility of *Salmonella* typing (Torpdahl et al., 2005). Pulse-field Gel Electrophoresis (PFGE) is now considered the “gold standard” method for *Salmonella* surveillance and investigation (Patchanee et al., 2010; Campioni et al., 2012). PFGE was used to determine the genetic association between *S. Enteritidis* recovered from human cases in the July 2012 outbreak, the March 2009 outbreak and strains isolated from chicken slaughterhouses in Chiang Mai Province in 2011. Potential salmonellosis transmission routes and infection sources were also investigated to help improve monitoring and control programs in the region.

Materials and Methods

Salmonella strains

A total of 40 *Salmonella* strains, initially identified as *S. Enteritidis*, recovered in Chiang Mai and Samut Songkhram provinces were tested. These were included strains isolated from the July 2012 outbreak ($N=20$), the March 2009 outbreak ($N=12$) as well as strains isolated from samples collected in Chiang Mai in 2011 from slaughtered chicken feces ($N=3$), slaughtered chicken carcasses ($N=1$), slaughter house drainage waters ($N=2$), slaughterhouse cooling storage box ($N=1$) and slaughterhouse floor ($N=1$).

Antimicrobial resistance ability tests

S. Enteritidis strains were submitted to antimicrobial susceptibility testing with agar disk diffusion and to DNA-fingerprinting with PFGE by the WHO National *Salmonella* and *Shigella* Center Laboratory (NSSC), Nonthaburi, Thailand. All strains, except those recovered in Samut Songkhram Province, were tested with ten panels of antimicrobial agents including ampicillin (AMP) 10 µg, amoxicillin-clavulanic acid (AUG) 30 µg, sulfamethoxazole-trimethoprim (SXT) 25 µg, ciprofloxacin (CIP) 5 µg, chloramphenicol (C) 30 µg, streptomycin (S) 30 µg, nalidixic acid (NA) 30 µg, norfloxacin (NOR) 10 µg, cefotaxime (CTX) 30 µg and tetracycline (TE) 30 µg.

Pulse-field Gel Electrophoresis (PFGE)

The PFGE testing process was performed following the US Center for Disease Control and Prevention's standardized PulseNet protocol for *Salmonella* (Ribot et al., 2006). *Salmonella enterica* serotype Braenderup H9812 and *Xba* were used as the reference marker and the digestion enzyme, respectively. DNA patterns generated by PFGE in gel images were transferred to BioNumerics software ver. 7 for analysis. Dendrograms were developed using the unweighted pair group method with arithmetic averages, 5.0 % band position tolerances and 2.5% optimization values. Strains with a similarity index above 90% were grouped in the same cluster.

Results

The PFGE profiles from the 40 *Salmonella Enteritidis* strains tested are shown in Figure 1. Using a similarity index cutoff of 90%, thirty-eight strains could be grouped in two main clusters (cluster A and cluster B), while the remaining two strains could not be identified with any cluster.



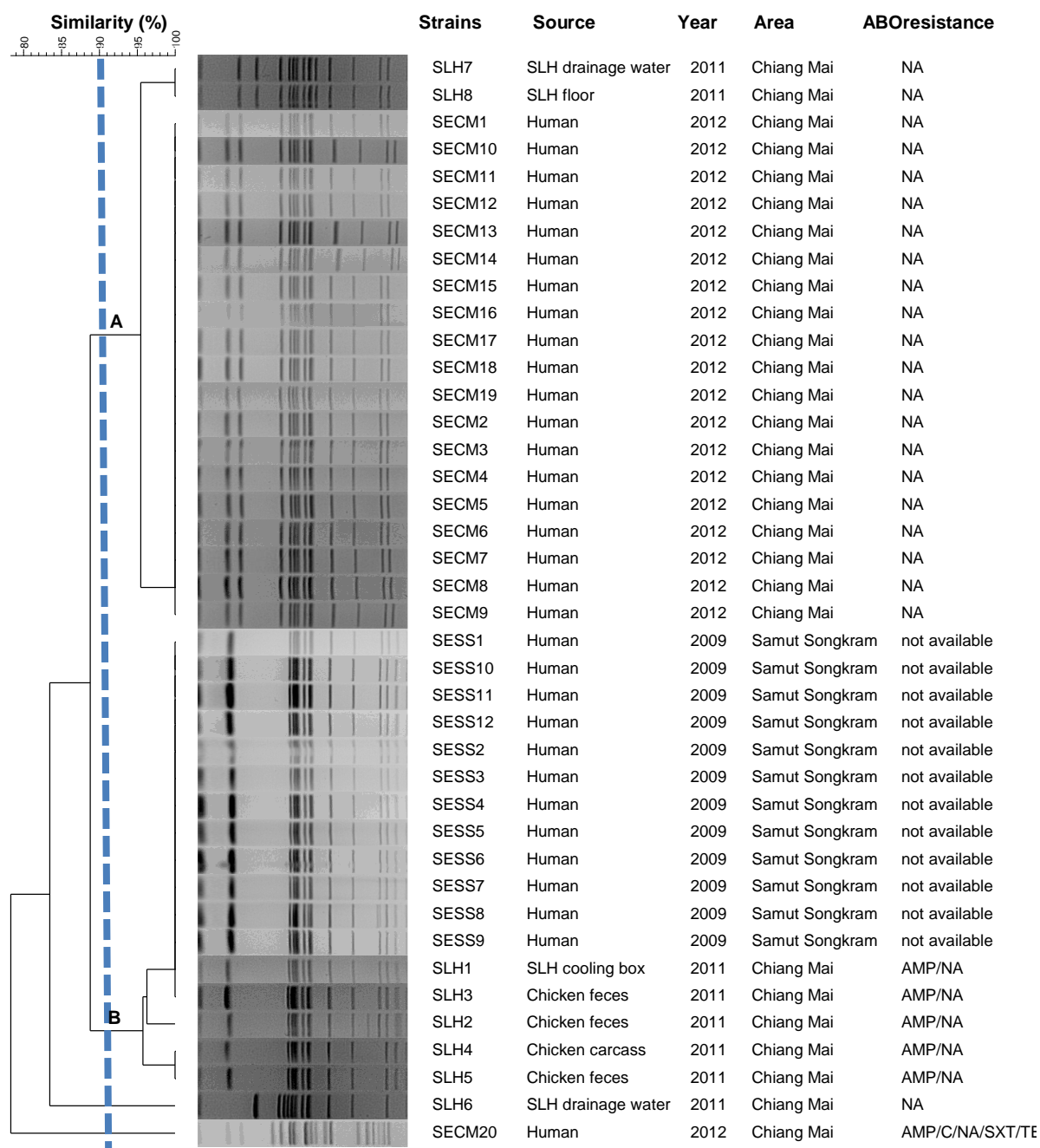


Figure 1. Dendrogram of *S. Enteritidis* strains recovered in Chiang Mai and SamutSongkhramprovinces in 2009-2012(Similarity of genetic relatedness criteria (%) to 90% clustering indicated by dashed line.) ampicillin (AMP); chloramphenicol (C); nalidixic acid (NA); sulfamethoxazole-trimethoprim (SXT) and tetracycline (TE).

All strains in cluster A were classified in two pulsotypes, which were resistant only to nalidixic acid. Group of nineteen strains recovered from the July 2012 outbreak (SECM1-19) were identified as the common pulsotype. Two additional indistinguishable strains obtained from the chicken slaughterhouse environment in 2011 (SLH7, 8) were also observed. Differences in the two DNA-fragments from the



dominant pulsotype presented in Figure 2 which shows a 95% similarity among the strains in that cluster. Additionally, in cluster B, seventeen *S. Enteritidis* strains were identified. Interestingly, fourteen clonal strains were recovered from twelve strains obtained from the March 2009 outbreak (SESS1-12) and two additional strains were obtained from the chicken slaughterhouse line in 2011 (SLH1, 3). Moreover, three strains isolated from chicken slaughterhouses in 2011 (SLH 2, 4, 5) were also found to have a fingerprint-pattern similarity of 95% to 96%, in which only 1 to 3 DNA-fragment bands differed from the dominant pulsotype in this cluster.

Of the two *S. Enteritidis* which could not be grouped into any cluster, the first (SLH 6), which was recovered from a chicken slaughterhouse in 2011, was resistant to nalidixic acid. The second (SECM 20), was obtained from humans in the July 2012 outbreak, and was resistant to five antimicrobial agents.

Analysis of the antimicrobial resistance profiles of the 28 *S. Enteritidis* strains tested found that groups of clonal strains had similar antimicrobial patterns. Remarkably, all strains were resistant to nalidixic acid. The strain with the broadest multidrug resistance (with AMP/C/NA/SXT/TE antimicrobial resistance patterns) was detected in *S. Enteritidis* and showed a genetic relatedness lower than 80% to the other strains.



Figure 2. DNA fingerprints of the strains in each cluster. Solid white lines indicate band similarities; dashed white lines indicate band differences, comparing in each cluster.

Discussion

In recent years, *Salmonella* Enteritidis has been considered the most common serotype causing human salmonellosis in Thailand (Domingues et al., 2013), an indication that the serotype is an important public health concern in the area. In this study, PFGE was used to type 40 strains of *S. Enteritidis* from both human and veterinary sources. All of those sources showed a high genetic similarity (>75%), close to the >80% value found in a study of 96 *S. Enteritidis* isolated from humans and from food in Brazil (Campioni et al., 2012). This similarity indicates a high level of genetic relatedness among the strains tested in the two studies.

Two major pulsotypes (SECM1-19 and SESS1-12) were identified in both Chiang Mai and Samut Songkhram, confirming that the *S. Enteritidis* in both areas was caused by exposure to a common source and that the two groups of people had been infected by a similar organism. Groups of clonal strains were



observed from *S. Enteritidis* recovered from the March 2009 outbreak in Samut Songkhram province and from chicken slaughterhouse lines in 2011 in Chiang Mai province. Cross contamination by *S. Enteritidis* between the two provinces might have occurred. Some aspect of the supply chain, e.g., chicks, pullets, feed or feed ingredients or some other common sources could have allowed the spread of the organism over a wide area (Prendergast et al., 2009). Furthermore, second group with high genetic relatedness (>95%) was also identified. This group was composed of strains recovered from the July 2012 outbreak cases and from chicken slaughterhouse environments in 2011, both in Chiang Mai province. Only two DNA fragment differences were observed in this group. A possible explanation is that the strains might have evolved from a common ancestor. Point mutation or recombination in some DNA bases is considered to be a key factor for slight changes in DNA fingerprint patterns (Liu et al., 2011). Thus it is concluded that the outbreak of salmonellosis in July 2012 might be caused by a new emerging *S. Enteritidis* pulsotype in Thailand.

Testing of samples of *S. Enteritidis* found that 100% were resistant to Nalidixic acid, a finding quite similar to several other studies which also reported high resistance rates of *Salmonella* to nalidixic acid (Mishra et al., 2008; Campioni et al., 2012; Tadee et al., 2014). Based on these findings, this antimicrobial agent is not recommended for salmonellosis treatment. However, nalidixic acid resistant-*Salmonella* is recognized as the cause of serious difficulties for antibiotic treatment in humans. Nalidixic acid-resistant-*Salmonella* strains can also have decreased susceptibility to members of the fluoroquinolone group such as norfloxacin and ciprofloxacin (Kownhar et al., 2007; Michael et al., 2011), thus limiting the choices for treatment and further reducing the performance of some first-line antibiotics in human cases (Van Boxstael et al., 2006).

Two non-clustered strains were found in this study. The first strain, (SLH6), which exhibited a unique pulsotype, was obtained from chicken slaughterhouse lines in 2011. It is not uncommon to find several different pulsotype organisms in each batch of samples from a slaughterhouse. Cross contamination can occur from contaminated carcasses, contaminated equipment or inadequate routine hygiene practices in slaughterhouses (Olsen et al., 2003, Capita et al., 2007). The second strain, (SECM20), was recovered from only one individual in the July 2012 outbreak. Based on the PFGE pattern, that strain is not associated with the common source of infection in that outbreak. Infection of that one individual might have occurred from another, unrelated exposure that just happened to become symptomatic at the time of the 2012 outbreak. Remarkably, that strain was found to be multidrug-resistant. Additional effort is needed to investigate the origin of that strain to help reduce the menace of multidrug-resistant strains in the region.

Conclusions

The PFGE technique can provide useful information for disease surveillance and outbreak investigation. Groups of clonal strains recovered from different areas and a suspected- new emerging *S. Enteritidis* pulsotype were identified. The upshot is that hygienic education for end consumers should be improved. In addition, disease control programs for entire production lines should to be evaluated to identify additional preventive measures to help control salmonellosis in the region.



Declaration of potential conflict of interest

There are no conflicts of interest.

Acknowledgements

We would like to thank the staff of the WHO National *Salmonella* and *Shigella* Center Laboratory (NSSC), Nonthaburi, Thailand, for their assistance in conducting the laboratory testing. We would also like to thank our colleagues from the Bureau of Epidemiology, Ministry of Public Health, for their help with sampling and our colleagues at Chiang Mai University for their significant contributions.

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เชียงใหม่สัตวแพทยสาร 2558; 13(2): 73-80

บทความต้นฉบับ

การสำรวจทางระบาดวิทยาพัลส์ฟิลด์ของเชื้อ *S. Enteritidis* จากการระบาดของโรคซัลโมเนลโลซิสใน จังหวัดเชียงใหม่และสมุทรสงคราม ประเทศไทย

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บทคัดย่อ เชื้อ *Salmonella* ซีโรไทป์ Enteritidis (SE) เป็นหนึ่งในซีโรทัยป์ที่พบได้บ่อยและมีรายงานการเกิดโรคทั่วโลก การระบาดของ *Salmonella* Enteritidis ได้เกิดขึ้นที่จังหวัดเชียงใหม่ ประเทศไทย ในเดือน กรกฎาคม ค.ศ. 2012 ซึ่งสาเหตุของการระบาดมาจากการบริโภคไข่ต้มค้าง 3 คิน และได้มีการระบาดของเชื้อเดียวกันนี้ในจังหวัดสมุทรสงคราม ในเดือนมีนาคม ค.ศ. 2009 โดยไม่ทราบสาเหตุของการระบาด สำหรับในการศึกษานี้ ได้ใช้วิธี Pulse-field Gel Electrophoresis (PFGE) ในการสำรวจความสัมพันธ์ในระดับพันธุกรรม ระหว่างเชื้อ *S. Enteritidis* ที่แยกได้จากผู้ป่วยจากการระบาดในเดือน กรกฎาคม ค.ศ. 2012 ($N=20$) จากการระบาดในเดือน มีนาคม ค.ศ. 2009 ($N=12$) และ *S. Enteritidis* ที่แยกได้จากโรงฆ่าและชำแหละในจังหวัดเชียงใหม่ ค.ศ. 2011 ($N=8$) พบว่า 38 ตัวอย่าง จากทั้งหมด 40 ตัวอย่างของเชื้อ *S. Enteritidis* ที่ทำการศึกษานี้สามารถแยกได้เป็น 2 กลุ่มตามความหลากหลายของพันธุกรรม โดยที่กลุ่มของเชื้อเหมือนกันทางพันธุกรรม 14 สายพันธุ์ได้รับการค้นพบจาก 12 สายพันธุ์ที่แยกได้จากการระบาดในเดือนมีนาคม ค.ศ. 2009 และสองสายพันธุ์จากตัวอย่างที่แยกได้จากโรงฆ่าและชำแหละในจังหวัดเชียงใหม่ ค.ศ. 2011 ส่วนสายพันธุ์ของเชื้อที่ระบาดในเดือน กรกฎาคม ค.ศ. 2012 เป็นสายพันธุ์ที่มาจากบรรพบุรุษร่วมกันของเชื้อที่แยกได้จากโรงฆ่าและชำแหละในจังหวัดเชียงใหม่ ค.ศ. 2011 ซึ่งมาตราบการในการป้องกันประกอบไปด้วยการให้ความรู้ด้านสุขลักษณะที่ดี รวมไปถึง การปรับปรุงการควบคุมห่วงโซ่การผลิต ควรที่จะถูกนำมาพัฒนาเพื่อช่วยในการควบคุมโรคซัลโมเนลโลซิสในภูมิภาคนี้ เชียงใหม่สัตวแพทยสาร 2558; 13(2): 73-80

คำสำคัญ: *Salmonella* Enteritidis, PFGE, เชียงใหม่, สมุทรสงคราม, ประเทศไทย

ผู้รับผิดชอบบทความ: ภาคภูมิ ตาดี ภาควิชาคลินิกสัตว์บริโภค คณะสัตวแพทยศาสตร์ มหาวิทยาลัยเชียงใหม่จังหวัดเชียงใหม่ อำเภอเมือง จังหวัดเชียงใหม่ Email address: td_pakpoom@hotmail.com

