SALMONELLA LAMPHUN: FIRST ISOLATION OF A NEW *SALMONELLA* SEROVAR IN THAILAND

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Abstract. Two isolates of a new *Salmonella* serovar, *Salmonella* Lamphun were discovered from animal feeds in Thailand, in 2003, which belongs to group C, with antigenic formula 6,8:y:1,2. Both isolates were susceptible to all antimicrobials tested. The pulsed field gel electrophoresis pattern of both isolates comprises 11 DNA fractions sized 48, 65, 77, 105, 110, 170, 244, 330, 337, 453 and 1,135 kbp. Up to April 2005, no human or animal infection by this new *Salmonella* serovar was reported.

INTRODUCTION

Non-typhoidal *Salmonella* is a major food-borne pathogen that affects people worldwide. It can be found in various animal reservoirs, including pigs, poultry, chicken, and animal feeds. The National Salmonella and Shigella Center, Thailand (NSSCT) in Nonthaburi reports more than 100 *Salmonella* serovars isolated from various sources throughout the country each year. It was found that *Salmonella* serovars isolated from human beings seemed to be related to serovars distributed in animals and food products (Bangtrakulnonth *et al*, 2004). The NSSCT also discovered new *Salmonella* serovars; *S*. Bangkok and *S*. Ratchaburi, isolated from gastroenteritis patients in 1972 and 1999, respectively (Bangtrakulnonth *et al*, 1999; Phan-Urai and Bangtrakulnonth, 1995). To assess the salmonellosis situation in Thailand, several surveillance activities are carried out under the collaboration between the Ministry of Agriculture and Cooperatives and the Ministry of Public Health. In 2003, a change in serovar distribution to the *S*. Enteritidis infection in some animal species and animal feeds was observed. This serovar was previously rare, but has now become one of the most commonly isolated. Increasing uncommon characteristics of *Salmonella* isolates were found and further determined. This study reports the phenotypic and genotypic patterns of a new *Salmonella* serovar.

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MATERIALS AND METHODS

Bacterial isolation and identification

The *Salmonella* isolates collected from all over the country in 2003 were confirmed at NSSCT using a conventional method (Ewing, 1986). Two *Salmonella* strains detected from animal feed at a local veterinary laboratory of the Department of Livestock Development, Ministry of Agriculture and Cooperatives, in Lamphun Province, northern Thailand, were confirmed by the NSSCT. *Salmonella* were identified according to Popoff and Le Minor (2001).

Serological testing of *Salmonella*

All strains identified as *Salmonella enterica* were serotyped according to the Kauffman-White serotyping scheme (Popoff and Le Minor, 2001) by Serosystem Antisera (S & A Reagent Lab, Thailand).

Antimicrobial susceptibility testing

The studied isolates were tested for susceptibility to ampicillin (10 µg), chloramphenicol (30 µg), cefotaxime (5 µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), norfloxacin (10 µg), trimethoprim-sufamethoxazole (25 µg), and tetracycline (30 µg), by disk diffusion method, as defined by the National Committee for Clinical Laboratory Standards (NCCLS, 2004).

Pulsed Field Gel Electrophoresis (PFGE)

PFGE of the two strains of *Salmonella* was performed by the method described by the Center for Disease Control and Prevention, USA (Susan *et al*, 2005). Briefly, *Salmonella* cell suspension was adjusted for cell concentration by spectrophotometer at 610 nm with OD 1.3-1.4. One percent sodium dodecyl sulfate

agarose was prepared in TE buffer and mixed with the cell suspension to make plugs for PFGE. The cells in the plugs were treated with lysis solution and then DNA was digested 10-15 minutes with *xba* I. The CHEF DR III system was used with switch times 2.16 S and 63.8 S for 18 hours at 6 v/cm. The gels were stained with ethidium bromide and photographed under UV light. The DNA of *Salmonella* serovar Braenderup H9812 was used as reference marker. Isolates with indistinguishable patterns were considered to belong to the same PFGE patterns.

RESULTS

Bacterial isolates

In 2003, 4,916 Salmonella were isolated from humans, foods, animals, animal feeds and other sources from all over the country. Of these, 257 were detected from animal feeds. Among these, 2 *Salmonella* group C were detected from animal feeds from a factory in Lamphun Province, northern Thailand, under the routine surveillance program on animal-feed quality control of the regional laboratory of the Department of Livestock Development, Ministry of Agriculture and Cooperatives. The major components of the feeds were corn and soy beans. Both strains were sent to NSSCT for confirmation. According to Kauffman-White scheme serotyping, the two isolates were confirmed as *Salmonella enterica* subspecies *enterica* with antigenic formula 6,8:y:1,2, which has never been reported in the antigenic formulas of the *Salmonella* serovars (Popoff and Le Minor, 2001). The strains were sent to the WHO Collaborating Center for Reference and Research on Salmonella, Institute Pasteur, Paris, France; the WHO Collaborating Center for Salmonella, Atlanta, USA; and the Salmonella Zentrale Hygienischen Institute, Hamburg, Germany, for further clarification. Both isolates were confirmed to be a new *Salmonella enterica* subsp *enterica*. The name *Salmonella* Lamphun was registered in 2005, based on the location where the organisms were first recovered. Since the organisms had been isolated in 2003, the occurrence of S. Lamphun was further investigated from 2004 to April 2005. Although *Salmonella* serovars were detected in 7,987 humans, 2,498 foods, 351 animals, 724 animal feeds, and 642 environment sources, *S.* Lamphun was not found in this investigation.

Table 1
Biochemical characterization of *Salmonella* serovar Lamphun (6,8:y:1,2).

Test	Reaction
Triple sugar iron(TSI)	Acid butt/alkaline slant(K/A), gas
H_2S	Positive
Motility	Positive
Indole	Negative
Citrate	Positive
Urease	Negative
LDA	Negative
LDC	Positive
D-tartrate	Positive
Dextrose	Positive, gas
Lactose	Negative
Mannitol	Positive
Malonate	Negative
Dulcitol	Positive
Sorbitol	Positive
Salicin	Negative
Mucate	Positive
ONPG	Negative

Table 2
Antigenic formular of *Salmonella* serovar Lamphun (6,8:y:1,2).

Test	Result
0.85% NaCl	-
Antiserum OMA	-
Antiserum OMB	++
Antiserum OMC	-
Antiserum OMD	-
Antiserum Group C	++
Antiserum Group F	-
Antiserum Group G	-
Antiserum Group H	-
Antiserum O:6	++
Antiserum O:7	-
Antiserum O:8	++
Antiserum O:14	-
Antiserum O:20	-
Antiserum HMA	-
Antiserum HMB	-
Antiserum HMC	++
Antiserum H: k	-
Antiserum H: y	++
Antiserum H: z	-
Antiserum H: L complex	-
Antiserum H: Z_4 complex	-
(Continued) Table 2	
Antiserum H: r	-
Antiserum H: 1 complex	++
Antiserum H: 2	++
Antiserum H: 5	-
Antiserum H: 6	-
Antiserum H: 7	-

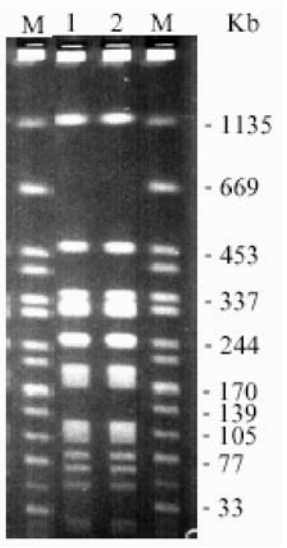

Fig 1- Representative PFGE patterns of *Salmonella* serovar Lamphun (6,8:y:1,2). Lanes 1 and 2 were the studied isolates (SO 988/03, SO 989/03). Both lanes M were *Salmonella* serovar Braenderup H9812. Numbers on the right indicates the positions of the molecular mass markers.

Biochemical characteristics

The two isolates were gram-negative rod, motile bacteria, which reduced nitrate to nitrite and utilized glucose with gas production, including hydrogen sulfide on triple sugar iron (TSI). The biochemical characteristics of *Salmonella* serovar Lamphun are summarized in Table 1.

Serological patterns

The two isolates were positive to OMC, group C and subdivided C_{2-3} with a presence of O antigens 6 and 8. They contained H antigen y and 1, 2, as detected by Svengard technique for phase invasion. The isolates were confirmed to be *Salmonella* group C with a clearly distinguishable antigenic formula,as 6,8:y:1,2 (Table 2).

Antimicrobial susceptibility pattern

Antimicrobial susceptibility patterns of 2,057 *Salmonella* isolated from humans in 2003 were determined. Resistance to ampicillin, chloramphenicol, cefotaxime, gentamicin, kanamycin, nalidixic acid, norfloxacin, trimethoprim-sufamethoxazole and tetracycline was 30.7, 19.1, 0.7, 8.6, 5.4, 39.3, 0.4, 28.7, and 49.8%, respectively. The two new serovar *S.* Lamphun isolates were susceptible to all tested antimicrobial agents.

Pulse Field Gel Electrophoresis (PFGE) pattern

Both isolates demonstrated the same PFGE pattern, with 12 discrete fragments of DNA sized between 33 to 1,135 kbp (Fig 1).

DISCUSSION

To the author's knowledge, the *Salmonella* Lamphun, *Salmonella enterica* subspecies *enterica* is a new *Salmonella* serovar, which was discovered for the first time from poultry feed in Thailand. Two isolates of this new serovar were susceptible to all nine antimicrobial agents tested. No differences in PFGE patterns between the two studied isolates were seen, which suggests the same source for these bacteria. As *Salmonella enterica* subspecies *enterica* mostly colonizes the enteric tract of warm-blooded animals, including humans, it is one of the most important causes of food-borne gastrointestinal infections in humans (Bangtrakulnonth *et al*, 2004). Contamination of *Salmonella* in the food chain can create serious health and economic effects. The PFGE pattern can be used as the prototype of the *Salmonella* serovar Lamphun in epidemiological studies of the isolates from various sources, not only in Thailand but also other parts of the world. Although *S.* Lamphun was not reported in the ensuing years, it is kept under surveillance.

Only a few studies have been reported on the occurrence of *Salmonella* in animal feed. A study in Australia in 2003 showed that *S.* Agona and *S.* Anatum were the most frequent isolates found in feedstuffs, and accounted for 19.8 and 14.9%, respectively (Anonymous, 2004; Powling, 2004.). Since animal feeds play important roles in food-chain hygiene, the surveillance system for *Salmonella* contamination in animals and animal feed should be conducted routinely in food production communities.

S. Lamphun was discoverd in a routine survey as part of an animal-feed quality-control program run by a regional laboratory of the Department of Livestock, Ministry of Agriculture and Cooperatives, in co-operation with the central reference laboratory of the Department of Medical Sciences. The Salmonella surveillance network in Thailand was a successful collaboration at both national and international levels in demonstrating detection of the contaminated pathogens and in discovering new strains of microorganisms. Discovery of this new serovar reconfirms the importance of networking cooperation between local and central laboratories, both within and between agencies.

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