The Thai Journal of Veterinary Medicine

Volume 41
Issue 3 *September, 2011*

Article 9

9-1-2011

Prevalence and Genotype of Salmonella Choleraesuis in Gunma Prefecture, Japan

Chieko Sakano

Yukio Morita

Koichi Goto

Yoko Yokota

Hideyuki Annaka

See next page for additional authors

Prevalence and Genotype of Salmonella Choleraesuis in Gunma Prefecture, Japan

Authors

Chieko Sakano, Yukio Morita, Koichi Goto, Yoko Yokota, Hideyuki Annaka, Masahiro Fujita, Satoshi Kobatake, Taisei Ishioka, Toshie Hoshino, Sumalee Boonmar, Chaiwat Pulsrikarn, Atsuyoshi Nishina, Kunihisa Kozawa, Shigeki Yamamoto, and Hirokazu Kimura

Original Article

Prevalence and Genotype of *Salmonella* Choleraesuis in Gunma Prefecture, Japan

Chieko Sakano[1] Yukio Morita[1,2] Koichi Goto[1] Yoko Yokota[1] Hideyuki Annaka[1] Masahiro Fujita[1] Satoshi Kobatake[1] Taisei Ishioka[1] Toshie Hoshino[1] Sumalee Boonmar[1]* Chaiwat Pulsrikarn[3] Atsuyoshi Nishina[4] Kunihisa Kozawa[1] Shigeki Yamamoto[5] Hirokazu Kimura[1,6]

Abstract

We studied the prevalence of swine salmonellosis and PFGE genotype of isolates in Gunma Prefecture, Japan. Between 2005 and 2008, swine salmonellosis was confirmed in 430 of 2,707,402 (0.02%) swine at slaughterhouses. All isolates were identified as deriving from *Salmonella* Choleraesuis, biotype Choleraesuis (negative for H_2S production). We used 30 bacterial strains from 15 farms that had experienced outbreaks in 2006 and 2007. All strains were susceptible to various antibiotics such as cephems (cefotaxime), fluoroquinolones (norfloxacin and ciprofloxacin), and fosfomycin. On the other hand, all strains were resistant to tetracycline (TC), and 29 of 30 (97%) strains were resistant to streptomycin (SM). The most predominant profiles were those of SM-TC (26 strains). During *Bln* I digestion, 30 strains showed 6 profiles on PFGE as G1 to G6, and each profile was assigned into 1 of 4 clusters (I to IV). The most prevalent profile was G1 (22 strains), followed by G3 (3 strains), and G2 (2 strains). Strains showing the same antimicrobial resistance profiles (SM-TC) and the same PFGE profiles (G1) were isolated from 5 of 15 farms (A to E) during the 2006 and 2007 outbreaks. In conclusion, the prevalence of swine salmonellosis caused by SM-TC resistant-*S.* Choleraesuis biotype Choleraesuis is around 0.02%, as determined by infection rate at pig farms between 2005 and 2008 in Gunma prefecture. *S.* Choleraesuis usually causes systemic infections in swine and humans and antimicrobial treatment is necessary. The antimicrobial susceptibility of *Salmonella* in swine should be surveyed further.

Keywords: antimicrobial resistance, genotyping, pig, *Salmonella*

[1]*Gunma Prefectural Institute of Public Health and Environmental Sciences, 378 Kamioki, Maebashi, Gunma 371-0052, Japan*
[2]*Tokyo Kasei University, 1-18-1 Kaga, Itabashi-ku, Tokyo 173-8602, Japan*
[3]*WHO International Salmonella & Shigella Center, National Institute of Health, Nonthaburi 11000, Thailand*
[4]*Yamagata Prefectural Yonezawa Women's Junior College, 6-15-1Torimachi, Yonezawa, Yamagata 992-0025, Japan.*
[5]*National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan*
[6]*National Institute of Infectious Disease, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan*
***Corresponding author E-mail**: sumaleeb@th.cdc.gov*

บทคัดย่อ

ความชุก จีโนทัยป์ของเชื้อ *Salmonella* Choleraesuis ในจังหวัด Gunma ประเทศญี่ปุ่น

Chieko Sakano[1] Yukio Morita[1,2] Koichi Goto[1] Yoko Yokota[1] Hideyuki Annaka[1] Masahiro Fujita[1]
Satoshi Kobatake[1] Taisei Ishioka[1] Toshie Hoshino[1] Sumalee Boonmar[1] Chaiwat Pulsrikarn[3]*
Atsuyoshi Nishina[4] Kunihisa Kozawa[1] Shigeki Yamamoto[5] Hirokazu Kimura[1,6]

การศึกษาความชุกของโรคซัลโมเนลลาในสุกรและจีโนทัยป์ของเชื้อซัลโมเนลลาโดยวิธี PFGE ในจังหวัด Gunma ประเทศญี่ปุ่น ผลการศึกษา พบว่าระหว่างปี ค.ศ. 2005-2008 มีความชุกของโรคนี้ในสุกร ประมาณร้อยละ 0.02 คือ พบว่าสุกรจำนวน 430 ตัว (จาก 2,707,402 ตัว) ที่โรงงานฆ่าสัตว์ มีการติดเชื้อซัลโมเนลลา โดยเชื้อทั้งหมดเป็น *Salmonella* Choleraesuis ชนิดไม่สร้าง H2S ทำการทดลองโดยนำ เชื้อซัลโมเนลลาจำนวน 30 ตัวอย่าง ที่เพาะแยกจากฟาร์มสุกร 15 ฟาร์มในปีที่มีการระบาดของโรคนี้ คือปี ค.ศ. 2006-2007 มาศึกษาหาความไวของเชื้อต่อยาปฏิชีวนะ พบว่าเชื้อทั้งหมดไวต่อยาจำนวนมาก เช่น cephems (cefotaxime) quinolones (norfloxacin และ ciprofloxacin) และ fosfomycin ในทางตรงข้ามพบว่าเชื้อทั้งหมดดื้อต่อยา tetracycline (TC) และร้อยละ 97 ของเชื้อดื้อต่อยา streptomycin (SM) โดยมี แบบ(profile) การดื้อยาเป็นแบบ SM-TC (จำนวน 26 ตัวอย่าง) เมื่อนำเชื้อมาย่อยด้วยเอนไซม์ *Bln* I ในขบวนการ PFGE พบว่า สามารถแบ่งเชื้อเป็น 6 แบบ (profile) คือ แบบ G1 ถึง G6 และแบ่งย่อยได้เป็น 4 cluster (I ถึง IV) โดยแบบ G1 ประกอบด้วย เชื้อ 22 ตัว แบบ G3 ประกอบด้วย เชื้อ 3 ตัว และแบบ G2 ประกอบด้วย เชื้อ 2 ตัว เชื้อที่มีรูปแบบการดื้อยาเป็นแบบ SM-TC และมีแบบ PFGE อยู่ในกลุ่ม G1 นั้นมาจากฟาร์มสุกร 5 ฟาร์ม (ในจำนวน 15 ฟาร์ม) คือ ฟาร์ม A ถึง E ระหว่างที่มีการระบาดของโรคนี้ในปี ค.ศ. 2006-2007 จากการศึกษาสามารถสรุปได้ว่า ความชุกของโรคซัลโมเนลโลซีสในฟาร์มสุกร คิดเป็นร้อยละ 0.02 และเป็นเชื้อ *Salmonella* Choleraesuis ที่ดื้อต่อยา SM-TC ในระหว่างปี 2005-2008 ในจังหวัด Gunma ประเทศญี่ปุ่น เชื้อนี้ทำให้เกิดโรคทั้งในสุกรและคนและมีความจำเป็นต้องใช้ยาปฏิชีวนะในการรักษา ดังนั้นควรมีการศึกษาเรื่องการดื้อยาของเชื้อนี้ในสุกรต่อไป

คำสำคัญ: การดื้อต่อยาปฏิชีวนะ จีโนทัยป์ สุกร เชื้อซัลโมเนลลา

[1]*Gunma Prefectural Institute of Public Health and Environmental Sciences, 378 Kamioki, Maebashi, Gunma 371-0052, Japan*
[2] *Tokyo Kasei University, 1-18-1 Kaga, Itabashi-ku, Tokyo 173-8602, Japan*
[3]*WHO International Salmonella & Shigella Center, National Institute of Health, Nonthaburi11000, Thailand*
[4]*Yamagata Prefectural Yonezawa Women's Junior College, 6-15-1Torimachi, Yonezawa, Yamagata 992-0025, Japan.*
[5]*National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan*
[6]*National Institute of Infectious Disease, 4-7-1 Gakuen, Musashimurayama, Tokyo 208-0011, Japan*
****ผู้รับผิดชอบบทความ E-mail:*** *sumaleeb@th.cdc.gov*

Introduction

More than 2,500 serovars of non-typhoid *Salmonella* (NTS) have been confirmed (Callaway et al., 2008). NTS is a significant food-borne agent with worldwide distribution. In general, NTS persists by using animal intestines as reservoirs where it survives in a dormant state. However, some serovars such as Gallinarum-Pullorum, Dublin, Enteritidis, Typhimurium, and Choleraesuis can cause serious disease in domestic animals (Shivaprasad, 2000; Callaway et al., 2008; Pullinger et al., 2008). Many serovars, such as Enteritidis, Typhimurium, and Infantis, of *S. enterica* subsp. *enterica* are frequently isolated from both swine and humans. In particular, *S.* Choleraesuis is a host-adapted, facultative, intracellular pathogen that causes swine paratyphoid fever (Wilcock and Scwarts, 1992).

There are two biotypes of *S.* Choleraesuis. Choleraesuis is H_2S-negative and Kunzendorf is H_2S-positive in Triple Sugar Iron (TSI) agar (Sato, 1987). In Japan, *S.* Choleraesuis was first isolated in 1928 and swine salmonellosis caused by *S.* Choleraesuis occurred sporadically in the 1970s (Sato, 1987). In the past 10 years from 2010, swine salmonellosis caused by *S.* Cholaraesuis has increased (Asai et al., 2010).

In humans, *S.* Choleraesuis infection is responsible for salmonellosis, particularly in the elderly and in immunocompromised patients (Chiu et al., 2004). In the United State and United Kingdom,

S. Choleraesuis was sporadically isolated from humans (Barrel, 1987; Vugia et al., 2004). In Asian countries, especially in Taiwan, *S.* Choleraesuis is a significant serovar isolated from both humans and animals. In humans, it is frequently identified as the main cause of salmonellosis (Chiu et al., 2004). In Japan, the number of humans infected by
S. Choleraesuis remains very low in spite of increased outbreaks in swine. Only a few reports regarding swine salmonellosis caused by *S.* Choleraesuis in Japan have been published. Asai et al. (2010) reported that both biotypes of *S.* Choleraesuis such as Choleraesuis and Kunzendorf exist in Japan, especially west of Japan. Biotype Choleraesuis was classified as one big cluster, while biotype Kunzendorf was divided into two big clusters according to the results of pulsed-field gel electrophoresis (PFGE) comparing biotype Choleraesuis and Kunzendorf. However, the results of field epidemiological studies were obscure. In Gunma prefecture, located in center of Japan geographically, there is no *Salmonella* pig cases in meat inspection, but we began to find the *Salmonella* cases on viscera inspection from 2005. Here, we report the field epidemiological study in Gunma prefecture.
In Japan, according to the report of the local infectious disease survey center, the National Institute of Infectious Disease (http://idsc.nih.go.jp/iasr/virus/bacteria-j.html), isolation of *S.* Choleraesuis from humans is not common and bacteria have been isolated only once each year in 2004, 2005, 2007, and 2008. In Taiwan, however, human systemic infection by *S.* Choleraesuis is common and emergence of fluoroquinolone-resistant salmonella has become a serious problem (Chiu et al., 2002). Considering such aspects, we studied the prevalence, genotyping and antimicrobial susceptibility of *Salmonella enterica* subsp. *enterica* serovar Choleraesuis isolated in Gunma Prefecture, Japan.

Materials and Methods

Samples: Between 2005 and 2008, a total of 2,707,402 swine were inspected for salmonellosis by viscera inspection at G slaughterhouse in Gunma Prefecture. When swine with suspicious *Salmonella* systemic disease were detected during viscera inspection by a veterinary meat inspector, the swine samples of liver, lung and/or lymph nodes lesions were taken for bacteriological examination.

Bacteriological examination: Swine samples of liver, lung and/or lymph nodes lesions collected in viscera inspection were stored at 4°C and analyzed within 4 hours of sampling. One to two grams of each swine sample was inoculated into 10 ml of tetrathionate broth (Oxoid, Hampshire, UK) and rappaport-vassiliadis (RV) enrichment broth (Oxoid). The broth was incubated at 42°C for 20 hours under aerobic conditions. After incubation, a loopful of broth culture was streaked across brilliant green sulpha agar (BGS agar) (Oxoid) and double modified lysine iron agars (dmLIA : own composition; pH 6.7, containing 34 g of lysine iron agar, 1.5 g of bile salts No. 3, 10 g of lactose, 10 g of sucrose, 6.76 g of sodium thiosulfate, 0.3 g of ferric ammonium citrate, 0.015 g of sodium novobiocin, and 1 liter of distilled water) and incubated at 37°C for 24 hours under aerobic conditions. One to three presumptive *Salmonella* colonies such as red color colony on BGS agar and purple color colony on dmLIA were selected and sub cultured onto triptosoy agar (Oxoid) for 24 hours at 37°C under aerobic conditions. Colonies growing on the agar were selected according to the Laboratory Guidebook of the United State Department of Agriculture (http://www.fsis.usda.gov/PDF/MLG_4_04.pdf). Each *Salmonella* isolate was serotyped by a combination of O and H reactions using commercial antiserum (Denka, Tokyo), and the resulting serotype was identified by the Kauffman-White serotyping scheme. The isolates were classified into biotypes by confirming their H_2S production capability in sulfide-indole-motility (SIM) media (Nissui, Tokyo). When the slaughtered pig has suspicious salmonellosis systemic infection and *Salmonella* were isolated from lesions using bacteriological examination, we defined as salmonellosis.

Antimicrobial susceptibility of the isolates: We examined 30 strains of antimicrobial susceptibility test. The 30 strains were *S.* Choleraesuis from 15 pig farms that harbored swine salmonellosis both 2006 and 2007. Antimicrobial susceptibility of the isolates was examined by the disk diffusion method using Mueller-Hinton agar (Oxoid) plates (Bauer et al., 1966). Twelve types of antimicrobial disks (BD, NJ, USA) were used for the tests: 10 μg of ampicillin (AMP); 25 μg of amoxicillin (AMPC); 30 μg of cefotaxime (CTX); 30 μg of kanamycin (KM); 10 μg of streptomycin (SM); 30 μg of tetracycline (TC); 30 μg of chloramphenicol (CP); 30 μg of nalidixic acid (NA); 10 μg of norfloxacin (NOR); 5 μg of ciprofloxacin (CIP); 50μg of fosfomycin (FOM); 23.75 μg of sulfamethoxazole; and 1.25 μg of trimethoprim (ST). Next, we determined isolate resistance to AMP and AMPC using 10 μg of ampicillin with 10 μg of sulbactam sodium/ampicillin sodium (ABPC/SBT) disk: BD to investigate β-lactamase production. In these tests, *Escherichia coli* ATCC25922 was used for the control. The breakpoint for the antimicrobial drugs was based on the guidelines provided by the National Committee on Clinical Laboratory Standard (2002).

Pulsed-field gel electrophoresis: PFGE was performed according to the Pulse Net standardized protocol (Ribot et al., 2006). Briefly, plugs were digested for 3 hours with 25 U of *Bln* I (Takara, Tokyo). DNA fragments were separated by 1% agarose gel electrophoresis (Takara) using a CHEF DR-III PFGE system (Bio-Rad, CA, USA). CHEF DNA size standards lambda ladder (Bio-Rad) was used as a molecular size marker.

DNA fingerprint analysis: DNA fingerprints were analyzed with Fingerprinting II Software (Bio-Rad). After an automatic band search and a band-based analysis using Dice's coefficient with 1.5% band position tolerance, cluster analysis was performed by using the un weighted pair-group method with

arithmetic averages (UPGMA) with 85% similarly index.

Results

Prevalence of swine salmonellosis: Examination revealed that 430 (0.02%) of 2,707,402 inspected swine were infected with salmonellosis. Swine salmonellosis was found in 35 (0.01%) of 662,270 swine in 2005, 198 (0.03%) of 655,203 in 2006, 115 (0.02%) of 686,613 in 2007, and 82 (0.01%) of 703,316 in 2008. Isolates from all the infected swine were identified as *S.* Choleraesuis and were classified as biotype Choleraesuis (H_2S negative). We were unable to isolate biotype Kunzendorf (H_2S positive) in this study.

Antimicrobial susceptibility of the isolates: Table 1 shows antimicrobial resistance profiles of isolated *S.* Choleraesuis. By using 12 types of antimicrobial drugs, we determined that not all strains were resistant to CTX, NOR, CIP, and FOM, whereas all strains were resistant to TC and 29 of 30 (97%) strains showed resistance to SM. The 30 strains were classified into 4 groups and a profile was established for each. The profiles were based on the resistance to the 8 antimicrobial agents. The most predominant profiles were that of SM-TC (26 strains), followed by SM-TC-NA (2 strains), AMP-AMPC-KM-SM-TC-ST (1 strain), and AMP-AMPC-KM-TC-CP-ST (1 strain). Two strains with resistance to AMP-AMPC were noted as capable of β-lactamase production as they demonstrated susceptibility to the ABPC/SBT disk.

Table 1 Antimicrobial resistance profiles of isolated S. Choleraesuis

Antimicrobial agent [a)]	No. of Strain	Isolation year	
		2006	2007
SM-TC	26	13	13
SM-TC-NA	2	1	1
AMP-AMPC-KM-SM-TC-ST	1[b)]	0	1
AMP-AMPC-KM-TC-CP-ST	1[b)]	1	0
Total	30	15	15

a) SM: streptomycin, TC: tetracycline, NA: nalidixic acid, AMP: ampicillin, AMPC: amoxicillin, KM: kanamycin, CP: chloramphenicol, and ST: sulfamethoxazole with trimethoprim. b) β-lactamase production

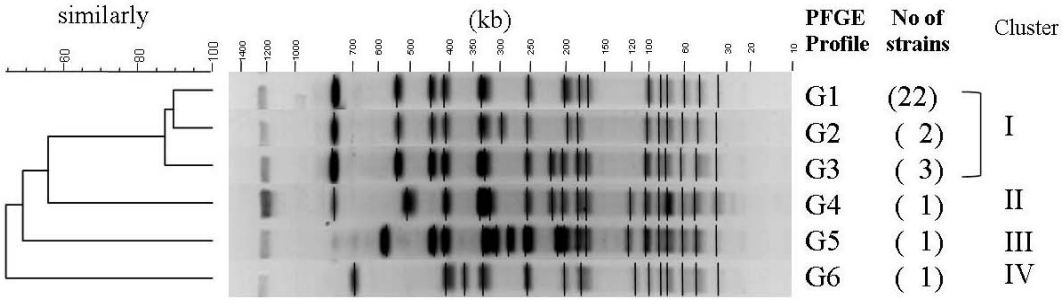

Figure 1 Diagram of PFGE profiles according to UPGMA algorithms with 85% similarly index in *S.* Choleraesuis biotype Choleraesuis by *Bln I*-digested chromosomal DNA of the 30 strains.

Table 2 Profiles of antimicrobial resistance and PFGE of S. Choleraesuis isolated in 2006 and 2007.

No.	Farm	2006		2007	
		Antimicrobial agent [a)]	PEGE profile	Antimicrobial agent [a)]	PEGE profile
1	A	SM-TC	G1	SM-TC	G1
2	B	SM-TC	G1	SM-TC	G1
3	C	SM-TC	G1	SM-TC	G1
4	D	SM-TC	G1	SM-TC	G1
5	E	SM-TC	G1	SM-TC	G1
6	F	SM-TC	G1	SM-TC	G2
7	G	SM-TC	G1	SM-TC	G3
8	H	SM-TC	G1	SM-TC	G5
9	I	SM-TC	G2	SM-TC	G6
10	J	SM-TC	G3	SM-TC	G1
11	K	SM-TC	G3	SM-TC	G1
12	L	SM-TC	G1	AMP-AMPC-KM-SM-TC-ST	G1
13	M	SM-TC-NA	G1	SM-TC	G1
14	N	SM-TC	G1	SM-TC-NA	G1
15	O	AMP-AMPC-KM-TC-CP-ST	G4	SM-TC	G1

a) SM: streptomycin, TC: tetracycline, NA: nalidixic acid, AMP: ampicillin, AMPC: amoxicillin, KM: kanamycin, CP: chloramphenicol, ST: sulfamethoxazole with trimethoprim.

Diagram of PFGE profiles: Figure 1 shows a diagram of PFGE profiles according to UPGMA algorithms of the Choleraesuis biotype by *Bln* I-digested chromosomal DNA of the 30 strains. During *Bln* I digestion, the strains showed 6 profiles (G1 to G6), each of which was assigned to 1 of 4 clusters (I to IV). Strains of G1 to G3 were assigned to cluster I and showed 85% similarity. The most predominant profile of cluster I was G1 (22 strains) followed by G3 (3 strains) and G2 (2 strains). G4 strains were assigned to cluster II, G5 to cluster III, and G6 to cluster IV; each profile having only one strain.

Relationship between isolation year, farm, result of antimicrobial resistance, and PFGE profiles: Table 2 shows the profiles in relation to antimicrobial resistance and PFGE of *S.* Choleraesuis isolated at 15 farms during the 2006 and 2007outbreaks. Strains showing the same antimicrobial resistance profiles (SM-TC) and same PFGE profiles (G1) were most predominant and the strain was isolated from 14 of 15

farms during the experiment period. In both 2006 and 2007, the same strains were isolated from 5 farms (A to E). Strains isolated from 6 farms (F to K) had the same antimicrobial resistance profile (SM-TC), but different PFGE profiles in each year. In contrast, 4 farms (L to O) showed different antimicrobial resistance and PFGE profiles in each year. There is no relationship between antimicrobial resistance and PFGE profiles in our study.

Discussion

It is reported that swine salmonellosis caused by *S. enterica* subsp. *enterica* serovar Choleraesuis has increased in Japan (Asai et al., 2010). In Gunma prefecture, we first detected swine salmonellosis during meat inspection in 2005. Since the first case, the disease has been sporadically observed between 2005 and 2008, and 0.02% (430/2,707,402) of swine were infected salmonellosis caused by *S.* Choleraesuis biotype Choleraesuis (H_2S negative) strains. Despite the widespread proliferation, no human cases or incidents of food poisoning caused by the strains have been observed to date in the prefecture.

With regard to the antimicrobial susceptibility of the isolates of 2006 and 2007, all examined strains were susceptible to various antibiotics such as cephems (CTX), fluoroquinolones (NOR and CIP), and FOM, while all strains showed resistance to TC, and 29 of 30 (97%) strains were resistant to SM. Asai et al.(2010) showed that various *S*. Choleraesuis strains isolated from 2001 to 2005 in Japan were resistance to dihydrostreptomycin (100%), oxytetracycline (69.2%), trimethoprim (40.4%), and AMP (34.6%). Although the antimicrobials examined by Asai et al. (2010) were slightly different to those of ours, most *S.* Choleraesuis isolated in Japan may show resistance to SM and TC. As a possible reason, many types of antibiotics such as penicillin, streptomycin, tetracycline, and methoprims are frequently used in Japan for treating bacterial infections in swine. Multidrug-resistant isolates, including those with fluoroquinolone and cephalosporin resistance, were found in *S.* Choleraesuis in Taiwan and biotype Kunzendorf (H_2S positive) was predominant in these countries (Chiu et al., 2004; Chang et al., 2005; Kulwichit et al., 2007). However, our strains and the previous report by Asai et al. (2010) indicate that no strains exist with resistance to fluoroquinolones (NOR, CIP, and enrofloxacin) or cephems (CTX and cefazolin). Swine-spread *S.* Choleraesuis strains in Japan are different from those of Taiwan and Thailand. However, 2 strains in the present study demonstrated multidrug resistance (AMP-AMPC-KM-SM-TC-ST and AMP-AMPC-KM-TC-CP-ST).

In diagram of PFGE profiles by using *Bln* I enzyme, examined 30 strains were divided to 6 profiles (G1 to G6) and 4 clusters (I to IV). The most predominant profile were G1 (22 strains), followed by G3 (3 strains) and G2 (2 strains), and G1 to G3 belong to cluster I. Our study shows that genetically close *S.* Choleraesuis were isolated from the infected swine in Gunma Prefecture.

When swine salmonellosis caused by *S.* Choleraesuis was detected, a veterinarian must notify the local government of the infected animals as stipulated by the Act on Domestic Animal Infectious Diseases Control of 1951, Japan. Infected animals are then treated and the animal quarters are thoroughly disinfected. However, a complete elimination of the *Salmonella* might be difficult, because of strains with the same profiles of antimicrobial susceptibility and because PFGE were isolated from the infected swine from 5 of the 15 farms in the 2006 and 2007 outbreaks.

In Japan, we usually use Salmonella Shigella agar (SS agar), deoxycholate hydrogen sulfide lactose agar (DHL agar), or mannitol lysine crystal violet brilliant green agar (MLCB agar) to isolate *Salmonella* from samples. These selective agars are able to isolate H_2S-producing *Salmonella* colonies. However, we may not be able to isolate non-H_2S-producing *Salmonella* like *S.* Choleraesuis biotype Choleraesuis. Thus, we should use another type of selective agar, such as BGS agar, Xylose lysine deoxycholate agar (XLD agar), or dmLIA which target lysine decarboxylase, or chromogenic agar.

S. Choleraesuis usually causes systemic infections in swine and humans and antimicrobial treatment is necessary. In Japan, human cases caused by *S.* Choleraesuis are very rare. However, in swine, salmonellosis occurred in swine that is usually caused in present times by *S.* Choleraesuis biotype Choleraesuis (H_2S negative), and has spread to many swine farms in Gunma prefecture, Japan. In this study, isolated *S.* Choleraesuis were not resistant to fluoroquinilones. However, in Taiwan, *S.* Choleraesuis isolated from swine and humans were resistant to fluoroquinolones and other antibiotics (Chang et al., 2005; Kulwichit et al., 2007). The antimicrobial susceptibility of *Salmonella* in swine should be surveyed further, and the selective method for isolating *Salmonella* should be reconstructed in Japan.

References

Asai, T., Namimatsu, T., Osumi, T., Kojima, A., Harada, K., Aoki, H., Sameshima, T. and Takahashi, T. 2010. Molecular typing and antimicrobial resistance of *Salmonella* enterica subspecies enterica serovar Choleraesuis isolates from diseased pigs in Japan. Comp Immun Microbiol Infect Dis. 33: 109-119.

Barrel, R.A. 1987. Isolations of *Salmonella* from humans and foods in the Manchester area: 1981-1985. Epidemiol Infect. 3: 277-284.

Bauer, A.W., Kirby, W.M., Sherris, J.C. and Turck, M. 1966. Antibiotic susceptibility by a standardized single disk method. Am J Clin Pathol. 45: 493-496.

Callaway, T.R., Edrington, T.S., Anderson, R.C., Byrd, J.A. and Nisbet, D.J. 2008. Gastrointestinal microbial ecology and the safety of our food supply as related to *Salmonella*. J Anim Sci. 86 (14 Suppl): E163-172.

Chang, C.C., Lin, Y.H., Chang, C.F., Yeh, K.S., Chiu, C.H., Chu, C., Chien, M.S., Hsu, Y.M., Tsai, L.S. and Chiou, C.S. 2005. Epidemiologic

relationship between fluoroquinolone-resistant *Salmonella* enterica Serovar Choleraesuis strains isolated from humans and pigs in Taiwan (1997 to 2002). J Clin Microbiol. 43: 2798-2804.

Chiu, C.H., Su, L.H. and Chu, C. 2004. *Salmonella enterica* serotype Choleraesuis: Epidemiology, pathogenesis, clinical disease, and treatment.Clin Microbiol Rev. 17: 311-322.

Chiu, C.H., Wu, T.L, Su, L.H., Chu, C., Chia, J.H., Kuo, A.J., Chien, M.S. and Lin, T.Y. 2002. The emergence in Taiwan of fluoroquinolone resistance in *Salmonella enterica* serotype choleraesuis. N Engl J Med. 346: 413-419.

Kulwichit, W., Chatsuwan, T., Unhasuta, C., Pulsrikarn, C., Bangtrakulnonth, A. and Chongthaleong, A. 2007. Drug-resistant nontyphoidal *Salmonella* bacteremia, Thailand. Emerg Infect Dis. 13: 501-502.

National Committee for Clinical Laboratory Standards, 2002. Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from animals, 2nd ed. Approved Standard M31-A2.Wayne, PA.

Pullinger, G.D., Dziva, F., Charleston, B., Wallis, T.S. and Stevens, M.P. 2008. Identification of *Salmonella* enterica serovar Dublin-specific sequences by subtractive hybridization and analysis of their role in intestinal colonization and systemic translocation in cattle. Infect Immun. 76: 5310-5321.

Ribot, E. M., Fair, M.A., Gautom, R., Cameron, D.N., Hunter, S.B., Swaminathan, B. and Barrett, T. J. 2006. Standardization of pulsed-field gel electrophoresis protocols for the subtyping of *Escherichia coli* O157:H7, *Salmonella*, and Shigella for Pulse Net. Foodborne Pathog Dis. 3: 59-67.

Sato, S. 1987. Salmonellosis in Disease of Swine. 3rd ed. T. Kumagai, M. Kashiwazaki, Y. Shimizu, T. Yoshimoto, T. Azuma, K. Kawada, S. Namioka, S. Watanabe (eds.), Kindai syuppan. 385-387 (in Japanese).

Shivaprasad, H.L. 2000. Fowl typhoid and pullorum disease. Rev Sci Tech. 19: 405-424.

Vugia, D.J., Samuel, M., Farley, M.M., Marcus, R., Shiferaw, B., Shallow, S., Smith, K. and Angulo, F.J. 2004. Invasive *Salmonella* infections in the United States, FoodNet, 1996-1999: Incidence, serotype distribution, and outcome. Clin Infect Dis. 38 (Suppl 3): S149-156.

Wilcock, B.P. and Scwartz, K.J. 1992. Salmomellosis in Diseases of Swine. A.D. Leman, B.E. Straw, W.L. Mengeling, S. D'Allaire, D.J. Taylor (eds), Iowa State University Press: 570-583.