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Isolation and Serological Survey of *Salmonella* in Pigs in Japan

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ABSTRACT. A total of 267 fecal and serum samples collected from individual pigs reared on a *Salmonella*-positive farm were subjected to bacteriological and serological examinations of *Salmonella*. *Salmonella* was isolated from 47 pigs (17.6%) and prevalence of antibody to lipopolysaccharide (LPS) of *S.* Typhimurium, which was partly common to *S.* O4, 12: d: -, was observed in 90 pigs (33.7%). *Salmonella* was isolated from 26 (28.9%) of 90 antibody-positive pigs and 21 (11.9%) of 177 antibody-negative pigs. Twenty-one of 36 pigs (58.3%) positive for *S.* O4, 12: d: -, five of 10 pigs (50.0%) positive for *S.* Havana, and none for *S.* Anatum had antibodies. Thus, seropositive rates were higher than isolation-positive rates, and antibody prevalence was associated with serovars of the isolates. Then, we analyzed antibody prevalence among pigs on Japanese pig farms. The antibodies to LPS of *S.* Typhimurium were found in 195 of 1,498 pigs (13.0%) and in at least one serum sample on 35 of 52 farms (67.3%). Our results indicate that *Salmonella* does not seem to be so prevalent in pigs though it is widely prevalent among pig farms.

KEY WORDS: antibody, ELISA, *Salmonella*, swine.

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Salmonella is a causative agent of food-borne diseases in humans and its contamination of animal products is a significant implication on public health [4]. To avoid outbreak of food-borne diseases due to meat and eggs, their practitioner and veterinarian should recognize and take care for prevention of infection of domestic animals with food-borne disease related organisms including *Salmonella*. Generally, to estimate the *Salmonella* prevalence in pig herds, bacteriological examinations were performed on fecal and clinical tissue samples of pigs [2, 7, 13, 17]. Serological examinations by enzyme-linked immunosorbent assay (ELISA) of serum and meat juice were also undertaken in several countries [9, 10, 14, 15]. Though serological examination was evaluated in the pigs experimentally infected with *Salmonella* [9, 12], little information on the relation between serological and bacteriological examinations is available in the pigs naturally infected with *Salmonella*. We encountered a herd contaminated with *Salmonella* (mainly of O4, 12: d: -). As the O-antigen O4, 12: d: - is partly common to that of *S.* Typhimurium, the serum antibody prevalence in the herd could be tested by ELISA using lipopolysaccharide (LPS) of *S.* Typhimurium as an antigen. So, under the field conditions, we compared the serological examinations with bacteriological examinations. Furthermore, as previous reports have shown that O4 group is the dominant serovars of *Salmonella* infecting pigs in Japan [1, 2, 7, 17], we conducted surveillance for the antibody prevalence in the serum samples collected from pigs in Japan.

MATERIALS AND METHODS

Serological and bacteriological studies in a pig operation (Experiment 1): The herd we surveyed consisted of 800 sows, under continuous farrow-to-finish operation in Japan. According to the previous health inspection, the herd had been infected with *Salmonella* (O4, 12: d: -, Havana, and Anatum). Sows were replaced by own-producing gilts on the farm. Bloods and feces were collected from each individual pig. After each sample was transported within 24 hr, fecal samples were cultured for isolation of *Salmonella*. After centrifugation, the serum samples were stored at –20°C until use.

Serological surveys on the pig farms (Experiment 2): A total of 1,498 serum samples were randomly collected from 52 pig farms in 2000 and 2001. The serum samples were stored at –20°C until use for serological examinations.

Bacteriological examination: Isolation of *Salmonella* was attempted from fecal samples as previously described [2, 8].

Serological examinations: Serum antibodies to LPS of *S.* Typhimurium were measured by ELISA, according to the method of Nielsen *et al.* [9] with slight modification. In brief, commercially available LPS of *S.* Typhimurium (Sigma, Co, Ltd., U.S.A.) was used as the ELISA antigen. From the results of the preliminary examinations, positive wells were coated with 0.1 m*l* of 60 ng/0.1 m*l* of LPS in the coating buffer and negative wells with 0.1 m*l* of the coating buffer. After incubation overnight at 4°C, the plates were washed three times with phosphate-buffered saline (PBS) containing 0.05% (v/v) Tween 20 (PBS-T). Fetal bovine serum (10%) in PBS-T (PBS-T-FBS) was added to each well for blocking for 1 hr at room temperature (RT) and washed three times. Serum samples were diluted 1:300 in PBS-T-FBS and applied to positive and negative wells for 1 hr at RT. After three-times washings, 0.1 m*l* of diluted horseradish-peroxidase-labeled rabbit anti-pig serum in PBS-T-FBS (Bethyl Laboratories, U.S.A.) was added to each well. The plates were incubated for 1 hr at RT, and then washed three times. One-tenth milliliter of substrate (20 mg of 1–2 orthophenylendiamine dihydrochioride) (Dotide, Co. Ltd., Japan), 0.01 m*l* H_2O_2, 25 m*l* of 0.1 M citrate, and 25 m*l* of 0.2 M NaH_2PO_4 were added to each well. After 15 min, 0.1 m*l* of 1 M H_2SO_4 was added as a stop solu-

Table 1. Isolation of *Salmonella* from pigs on a *Salmonella*-positive farm and antibody pevalence in the *Samonella*-isolated pigs

Stage	Age (month)	No. of pigs tested	*Salmoenlla*-isolated pigs							
			S. O4, 12:d:–		*S.* Havana		*S.* Anatum		One of serovars	
			No. of pigs	No. of pigs positive for antibody	No. of pigs	No. of pigs positive for antibody	No. of pigs	No. of pigs positive for antibody	No. of pigs (%)	No. of pigs positive for antibody
Fattening pigs	1	15	0	0	0	0	0	0	0 (0)	0
	2	35	0	0	0	0	0	0	0 (0)	0
	3	35	0	0	0	0	0	0	0 (0)	0
	4	21	6	2	0	0	1	0	7 (33.3)	2
	5	45	7	5	3	1	0	0	10 (22.2)	6
	6	22	7	5	0	0	0	0	7 (31.8)	5
	subtotal	173	20	12	3	1	1	0	24 (13.9)	13
Gilts		59	13	7	5	2	0	0	18 (30.5)	9
Sows		35	3	2	2	2	0	0	5 (14.3)	4
Total		267	36	21	10	5	1	0	47 (17.6)	26

Table 2. Comparison of isolation and serological examination in pigs on a *Salmonella*-positive farm

Stage	Age (month)	Tested	Antibody positive		Antibody negative	
			No. of pigs (%)	No. of pigs isolated	No. of pigs (%)	No. of pigs isolated
Fattening pigs	1	15	0 (0)	0	15 (100)	0
	2	35	0 (0)	0	35 (100)	0
	3	35	6 (17.1)	0	29 (82.9)	0
	4	21	2 (9.5)	2	19 (90.5)	5
	5	45	14 (31.1)	6	31 (68.9)	4
	6	22	10 (45.5)	5	12 (54.5)	2
	subtotal	173	32 (18.5)	13	141 (81.5)	11
Gilts		59	31 (52.5)	9	28 (47.5)	9
Sows		35	27 (77.1)	4	8 (22.9)	1
Total		267	90 (33.7)	26	177 (66.3)	21

tion. The optical density (OD) was read at 490 nm with a plate reader. ELISA value was calculated as follows; OD of positive well - OD of negative well. In the preliminary study using the sera of primary specific pathogen-free (SPF) pigs, the cut-off value of ELISA value was determined as 0.20 (average + 3SD), since the average ELISA value were 0.05 ± 0.05.

Statistical analysis: The results were evaluated statistically by the Chi-square test, by Yetes' correlation or by Fisher's exact test.

RESULTS

Experiment 1: *Salmonella* was isolated from 47 of 267 pigs (17.6%) on a *Salmonella*-positive farm (Table 1). The age of pigs from which *Salmonella* was isolated was over 4 month. Seven of 21 pigs (33.3%) at 4 months of age, 10 of 45 (22.2%) at 5 months of age, and seven of 22 (31.8%) at 6 months of age were found positive for *Salmonella*, and 18 of 59 gilts (30.5%) and 5 of 35 sows (14.3%) were found positive for *Salmonella*. *Salmonella* isolates were identified as three different serovars. Out of 47 positive pigs, *S.* O4, 12: d: - was isolated from 36 (76.6%), *S.* Havana from 10 (21.3%), and *S.* Anatum from one (2.1%).

Prevalence of antibody to LPS of *S.* Typhimurium was observed in 90 of 267 (33.7%) pigs (Table 2). Six of 35 pigs (17.1%), two of 21 (9.5%), 14 of 45 (31.1%), and 10 of 22 (45.5%) at 3 to 6 months of age, respectively, and 31 of 59 gilts (52.5%) and 27 of 35 sows (77.1%) were positive. Out of 90 antibody-positive pigs, 26 (28.9%) were positive for isolation. Isolation positive rates were 100% (2/2), 42.8% (6/14), and 50.0% (5/10) in antibody-positive pigs at 4, 5 and 6 months of age, respectively. *Salmonella* was isolated also from 21 pigs (11.9%) of 177 antibody-negative pigs. Isolation positive rates were 26.3% (5/19), 12.9% (4/31), and 16.7% (2/12) in antibody-negative pigs at 4, 5 and 6 months of age, respectively.

The antibody prevalence of the *Salmonella*-isolated pigs is shown in Table 1. Out of 47 isolation-positive pigs, serum antibodies were detected in 26 pigs (55.3%). Twenty-one of 36 pigs (58.3%) positive for *S.* O4, 12: d: -, 5 of 10 pigs (50.0%) positive for *S.* Havana, and none for *S.*

Table 3. Prevalence of antibodies to LPS of *S.* Typhimurium in pigs

	No. of farms	No. of pigs
	Positives/tested (%)	Positives/tested (%)
Sows	19/33 (57.6)	77/407 (18.9)
Fattening pigs	25/50 (50.0)	118/1,091 (10.8)
Total	35/52 (67.3)	195/1,498 (13.0)

Table 4. Prevalence of antibodies to LPS of *S.* Typhimurium in pigs

Age (months)	No. of pigs		Positive rates (%)
	Tested	Positives	
1	140	0	0[a]*
2	234	4	1.7[a]
3	118	4	3.4[a]
4	141	16	11.3[b]
5	147	31	21.1[b]
6	311	63	20.3[b]
Total	1,091	118	10.8

* Significant differences between a and b (P<0.01).

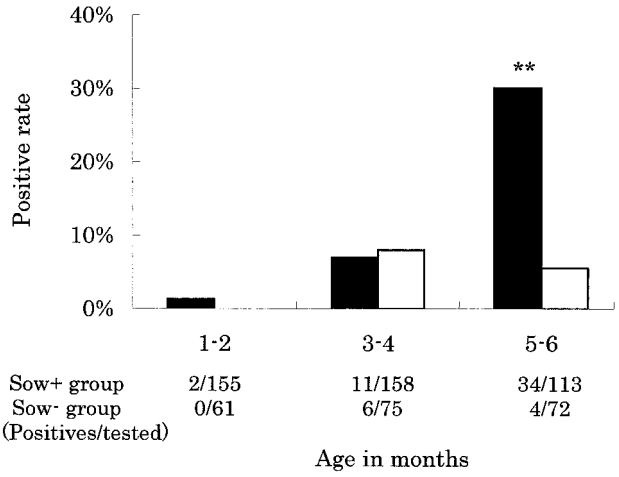

Fig. 1. Relationship of antibody prevalence between fattening pigs and sows. The farms were divided into two groups, based on the results of antibody prevalence in sows. Data represented antibody prevalence in pigs on the farms (■: Sow+ group, n=18) where antibodies were detected from at least one serum sample in sows , and farms where no antibody was detected in sows (□: Sow- group, n=13). **: Significant difference between sow+ and sow- groups at P<0.01.

Anatum had antibodies to LPS of *S.* Typhimurium. Regarding to pigs positive for *S.* O4, 12: d: -, two of six pigs (33.3%), five of seven (71.4%), and five of seven (71.4%) had antibodies at 4, 5 and 6 months of age, respectively.

Experiment 2: The antibodies to LPS of *S.* Typhimurium were detected in 195 of 1,498 pigs (13.0%) and in at least one serum sample on 35 of 52 farms (67.3%; Table 3). Seventy-seven of 407 sows (18.9%) and 118 of 1,091 fattening pigs (10.8%) had antibodies to LPS of *S.* Typhimurium. The positive rate was 0% (0/140), 1.7% (4/234), 3.4% (4/118), 11.3% (16/141), 21.1% (31/147), and 20.3% (63/311) at 1 to 6 months of age, respectively (Table 4). The positive rates of pigs at 4 to 6 month of age were significantly higher than those at 1 to 3 month of age (P<0.01).

Out of 52 farms, serum samples from sows and fattening pigs were collected from 31 farms. The farms were divided into two groups, based on the results of antibody prevalence in sows. One is the group of farms, where antibodies were detected in at least one serum sample of sows (Sow+ group, n=18). The other is the group of farms, where no antibody was detected in sows (Sow- group, n=13). As shown in Fig. 1, the antibody prevalence in pigs at 5 and 6 months of age was higher in the Sow+ group than in the Sow- group (P<0.01).

DISCUSSION

Experiment 1 shows comparison between bacteriological and serological examination under field conditions. *Salmonella* was isolated from about 30% of fattening pigs at 4 to 6 months of age. Besides, the antibody-positive rate increased in the pigs at 5 (31.1%) and 6 (45.5%) months of age. In the breeding stock, the antibody positive rate was higher in sows (77.1%) than in gilts (52.5%), though the isolation-positive rate decreased in sows. As the infected pigs intermittently shed *Salmonella*, it is considered that the antibody-positive rates were higher than the isolation-positive rates in finishers and sows. On the other hand, *Salmonella* was isolated from 11.9% in antibody-negative pigs, and 26.3% pigs at 4 months of age. The antibody response of the pigs may be associated with the time of infection. Nielsen *et al.* [9] have shown that serological tests are not to be used on individual pigs. The serological examinations may be useful to monitor *Salmonella* prevalence in pig herds.

Regarding serovars of *Salmonella*, 58% of isolation-positive pigs for *S.* O: 4,12: d: - and 50% of those for *S.* Havana had antibodies to LPS of *S.* Typhimurium. In the pigs experimentally infected with *S.* Typhimurium, *S.* Livingstone, *S.* Goldcoast, *S.* Panama, or *S.* Brandenburg, efficiency of mixed ELISA to monitor *S.* Typhimurium or *S.* Brandenburg was reported [12]. The O-antigens of *S.* Typhimurium, used as ELISA antigen in the present study, consisted of O: 1, 4, 5, and 12. So, the pigs infected with *S.* O: 4,12: d: - can acquire the antibodies to LPS of *S.* Typhimurium, as it reacts to a part of LPS of *S.* Typhimurium as common antigens. On the other hand, the sera from pigs infected with *S.* Havana might react to O: 1 of *S.* Typhimurium as common antigens. Also, it is considered that the positive pigs, that were in older stages such as gilt or sow, have previously been infected with O4 group of *Salmonella*. Further investigation is needed to clarify this point. As pre-

viously described in the experimental infections [9, 12], the antibody prevalence in pigs may also be associated with serovars of the isolates from them under field conditions.

Salmonella was not isolated from any pig below 3 month of age in the bacteriological examinations. In the results of serological examinations, however, positive results were obtained in six pigs at 3 months of age. *Salmonella* was isolated from pigs at 4 months of age or older, and seropositive rates to LPS of *S.* Typhimurium increased from 5 months of age. Most isolation-positive pigs at 5 to 6 months of age have antibodies to LPS of *S.* Typhimurium, but those at 4 months of age did not always have antibodies to LPS of *S.* Typhimurium. In the herd, transmission of *Salmonella* occurred mainly in the pigs between 4 to 5 months of age. The seropositive pigs at 3 months of age may be false positive due to some factors in the sera. Beside, experimentally infected pigs were intermittently shedding organisms and some of pigs stopped its shedding within a few weeks [12]. Further studies are being made to clarify whether young seropositive pigs play a role as a reservoir.

In the previous reports in Japan, 19, 17, and 12 serovars were isolated from apparently healthy pigs in the 1970s, 1980s, and in 1998, respectively [7, 17]. The isolation rates of *Salmonella* belonging to serovar O4 group were 68.1%, 58.7%, and 58.6% in the 1970s, 1980s and in 1998, respectively [7, 17]. Furthermore, the isolation rates of *Salmonella* belong to serovar O4 group were 51.4% and 88.1% of pigs in disorder [1] or diarrhea [2], respectively. Thereby, our results in Experiment 2 may partially show the *Salmonella* prevalence of pigs in Japan. The antibodies to LPS of *S.* Typhimurium were found among 13.0% of pigs on 67.3% of farms. The antibody prevalence increased in pigs at 4 to 6 months of age (11.3, 21.1, and 20.3%, respectively). In Japan, *Salmonella* was isolated from 5.7% (98/1,717) and 2.3% (58/2,511) of apparently healthy pigs in slaughterhouses in the late 1980s and on farms in 1998, respectively [7, 17]. Experiment 1 shows that the antibody-positive rate was higher than the isolation positive rate. Our results indicated that *Salmonella* does not seem so prevalent in pigs though it is widely prevalent among pig farms.

Experiment 2 shows that there is no difference of antibody prevalence between sows (18.9%) and pigs of 5 (21.1%) to 6 (20.3%) months of age. Van der Wolf *et al.* [15] have reported that antibody prevalence was higher in sows than in finisher pigs. They have shown that the antibody prevalence of finisher and sows in 1999 was 24.5% and 60.4%, respectively. As ELISA for detection of antibody to mixed LPS (O: 1, 4, 5, 6, 7, and 12) of *Salmonella* (mixed ELISA) was used in their reports, it is difficult to compare actually their results with ours.

On the farms with antibody-positive sows, antibodies were highly prevalent among pigs of 5 to 6 months of age. Previous investigations showed that piglets have been infected with *Salmonella* from sows [3, 5, 6, 11]. However, *Salmonella* was rarely isolated from apparently healthy sows on the farms [7]. Low antibody prevalence was observed among pigs of 1 to 4 months of age, when piglets were usually weaned and mingling. The frequency of infection of suckling pigs with *Salmonella* from contaminated feces shed by sows continuously or intermittently is obscure.

The present study showed that antibody detected with LPS of *S.* Typhimurium was prevalent among pig farms and high antibody prevalence was observed in some herds. *Salmonella* infection in apparently healthy pigs indicated the potential risk for food-borne diseases. It is considered that transmission and distribution of *Salmonella* to pig herds occurred by wild carrier animals and pigs, contaminated environments, and transmission vectors such as feed, boots, vehicles and so on [16]. It is important to recognize the *Salmonella* prevalence on pig farms and to practice the health management. We are now continuing to survey *Salmonella* infection among Japanese pigs.

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