NOTE *Public Health*

Enrichment for Isolating *Salmonella* Choleraesuis and other *Salmonella* spp. from Pigs

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ABSTRACT. The growth of *Salmonella* Choleraesuis was examined in Rappaport Vassiliadis broth (RV) and Hajna-tetrathionate broth (HTT) at 37 and 42°C. As the enrichment in RV at 37°C was satisfactory for isolating *S.* Choleraesuis, we used this enrichment for isolation from the samples collected from 15 asymptomatic pigs reared on a *S.* Choleraesuis contaminated farm. *S.* Choleraesuis was frequently isolated from six pigs (40.0%) under field conditions. The isolation of other *Salmonella* serovars than *S.* Choleraesuis was attempted by using both RV enrichment at 37°C and HTT enrichment at 42°C. *Salmonella* organisms were isolated from 156 (44.8%) of 348 fecal samples and more frequently with HTT at 42°C (43.4%) than with RV at 37°C (20.9%). If other serovars in addition to *S.* Choleraesuis are to be surveyed, HTT enrichment should be used in combination with RV enrichment.
KEY WORDS: enrichment, *Salmonella*, swine.

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Salmonella infection in pigs frequently leads to sepsis and enteritis, causing great economic loss [17]. *Salmonella* Choleraesuis is a causative agent of swine salmonellosis, characterized by septicemia, pneumonia, or sometimes enteritis [16, 17]. The serovar is also of importance for public health, as it causes human systemic infection [8]. Many *Salmonella* serovars other than *S.* Choleraesuis cause food poisoning in human. Previous studies have shown that several serovars of *Salmonella* are prevalent among pigs [2, 4, 6, 11–13]. Hence, *Salmonella* prevalence among pigs causes a significant health problem for pigs and for people.

S. Choleraesuis has directly been isolated from the lung, liver, spleen, and other organs of diseased pigs [15, 16]. In several recent studies of experimental infection of pigs with *S.* Choleraesuis, Rappaport Vassiliadis broth (RV) [19] was used as the enrichment broth [1, 10, 16]. Besides, Hajna-tetrathionate broth (HTT) has widely been used as an enrichment broth for *Salmonella* in Japan [2, 4, 13, 14]. In this report, we assess the enrichment for isolation of *S.* Choleraesuis and *Salmonella* serovars other than *S.* Choleraesuis from pigs.

Firstly, the growth of several isolates of *S.* Choleraesuis was examined in RV and HTT at 37 and 42°C. Four strains of *S.* Choleraesuis isolated from pig in Japan were used for the growth tests in RV (Oxoid Ltd., England) and HTT (Eiken Chemical Co., Ltd., Japan). *S.* Choleraesuis cultivated in TS broth (Eiken Chemical Co., Ltd.) at 37°C for 20 hr was diluted in phosphate buffered saline (PBS). One milliliter of 10-fold diluted culture was inoculated into 9 m*l* of RV and HTT. After incubation at 37 or 42°C for 20 hr, the bacteria were recovered in DHL (Eiken Chemical Co., Ltd.) and brilliant green (BG) (BBL, Becton Dickinson and Company, U.S.A.) agar plates containing 20 μg/m*l* of novobiocin (Sigma-Aldrich Co., U.S.A.) (DHLN and BGN, respectively). Out of the 4 strains of *S.* Choleraesuis we studied, 2 and all 4 strains grew poorly in HTT at either 37°C or 42°C. Though 1 strain grew poorly in RV at 42°C, all 4 strains grew in RV at 37°C (Table 1). Our study showed that some strains of *S.* Choleraesuis grew poorly in an enrichment broth incubated at 42°C and grew well in RV compared with HTT. Some of *Salmonella* serovars, including *S.* Typhi and *S.* Pullorum grow poorly in tetrathionate broth [7]. A study by Anderson *et al.* [1] supported the use of GN-Hajna rather than tetrathionate broth as a pre-enrichment medium for *S.* Choleraesuis isolation.

We then examined the enrichment for isolating *S.* Choleraesuis under field conditions. Four clinical samples, small and large intestinal swabs of 2 naturally infected pigs necropsied, were obtained on a farm where clinical salmonellosis had occurred. Each sample was inoculated into 10 m*l* of RV and HTT, followed by incubation for 20 hr at 37°C. After incubation, each culture was streaked onto DHLN and BGN agar plates. Colonies suspected to be *Salmonella* on each plate were identified biochemically and serologically by standard methods [4, 14]. Out of the 4 samples, *S.* Choleraesuis was isolated from 3 with RV, and not isolated with HTT. Similarly, Morozumi *et al.* (personal communication) found that RV enrichment of *S.* Choleraesuis is better than HTT enrichment, isolating *S.* Choleraesuis from experimentally infected pigs. Thus, RV enrichment at 37°C may be more suitable for isolating *S.* Choleraesuis.

Additionally, we examined the prevalence of *S.* Choleraesuis among pigs on the farm by RV enrichment at 37°C. Blood, nasal and rectal samples were collected from 15 pigs reared on the farm. Blood clots obtained by centrifuging their blood served as specimens for isolation (Table 2). *S.* Choleraesuis was isolated from 1 (20.0%) and 4 (100%) of the pigs at the age of 7 and 9 weeks. Of the 6 isolation-positive pigs, *S.* Choleraesuis was isolated from 4 blood clots

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Table 1. Growth of *S.* Choleraesuis in RV and HTT at 37 and 42°C

Strain	Region (Year)	Enrichment broth	Temperature of incubation: 37°C, Inoculation dose (cfu Log10) 4	3	2	1	42°C, Inoculation dose (cfu Log10) 4	3	2	1
B-90	Kyoto	RV	+*	+	+	+	–	–	–	–
	(1984)	HTT	+	+	+	+	–	–	–	–
C-00	Chiba	RV	+	+	+	+	+	+	+	+
	(2000)	HTT	–	–	–	–	–	–	–	–
S-01	Saitama	RV	+	+	+	+	+	+	+	+
	(2001)	HTT	+	+	+	+	–	–	–	–
S-02	Saitama	RV	+	+	+	+	+	+	+	+
	(2002)	HTT	+	+	–	–	–	–	–	–

* +: Growth, –: No-growth.

Table 2. Isolation of *S.* Choleraesuis from naturally infected pigs

Group No.	Age of pig (Weeks)	Number of pigs tested	Sample: BLood clot	Nasal swab	Rectal swab	Total*
1	5	5	0 (0)**	0 (0)	0 (0)	0 (0)
2	7	5	1 (20.0)	0 (0)	0 (0)	1 (20.0)
3	9	5	3 (60.0)	1 (20.0)	3 (60.0)	5 (100)
Total		15	4 (26.7)	1 (6.7)	3 (20.0)	6 (40.0)

* Number of samples in which *S.* Choleraesuis was detected in either of 3 kinds of sample.
** Number of pigs yielding *S.* choleraesuis (%).

(66.7%), 3 rectal swabs (50.0%), and 1 nasal swab (16.7%). As *S.* Choleraesuis infection in pigs might lead to septicemia, pneumonia, and enteritis [17, 18], the blood clots, nasal swabs, and rectal swabs were examined for the bacteria. In pigs infected with pneumonia, pneumonia-associated pathogens have been detected in the nasal swabs [3, 5]. Roof *et al.* [16] have reported that neutrophils of *S.* Choleraesuis-infected pigs are useful materials for isolation, though they failed to isolate the bacteria from feces. In several experimental infections, fecal samples were used to monitor the infection [1, 10]. Our results also showed that *S.* Choleraesuis was isolated from blood clots and feces at relatively high ratios.

On an *S.* Choleraesuis-contaminated farm, *S.* Choleraesuis was isolated from 1 pig at the age of 7 weeks and then slowly spread among the pig herd. While such remarkable clinical signs as loss of appetite, lethargic, and febrile symptoms were present in pigs at the age of 10 weeks or older, infection of pigs with *S.* Choleraesuis occurred at least 3 weeks before development of clinical signs. We are now surveying for *S.* Choleraesuis prevalence among pigs on contaminated farms by the present procedures.

Several studies have shown that the difference in results depends on the enrichment culture [6, 7, 9, 11, 20]. The discrepancy in isolation results between RV and HTT may occur in the case of isolation from feces or drag swabs, contaminated with other bacterial pathogens. Then, we compared enrichment for isolation of *Salmonella* serovars in porcine feces. A total of 348 fecal samples of apparently healthy pigs at 3 to 5 months of age were obtained from 8 pig farms, which had been confirmed to be *Salmonella* contaminated in previous investigations. Out of 348 fecal samples, *Salmonella* was isolated from 156 (44.8%), being more frequently with HTT at 42°C (151 samples, 43.4%) than with RV at 37°C (73, 20.9%)(Table 3). Eighty-three samples were positive for *Salmonella* only by HTT enrichment at 42°C, and 5 samples were only by RV enrichment at 37°C. Of 68 fecal samples positive in both enrichments, different serovars were isolated from 7 samples. Nine different *Salmonella* serovars were isolated as follows: O4, 12: d: - (67 samples), Havana (37), Livingstone (21), Typhimurium (12), London (9), Corvallis (7), Infantis (7), Anatum (6), and Virchow (5)(Table 4). Two *Salmonella* serovars were isolated from 6 samples (4 samples: O4, 12: d: - and Livingstone; 1 sample: O4, 12: d: - and Infantis; one sample: O4, 12: d: - and Virchow) in RV and from 2 samples (O4, 12: d:

Table 3. *Salmonella* isolation from porcine fecal samples by using two enrichment procedures

Enrichment	Isolation	Samples (%)
RV (37°C)/HTT (42°C)	+/+	68 (19.5)
	+/–	5 (1.4)
	–/+	83 (23.9)
	–/–	192 (55.2)
	Total	348 (100)

Table 4. Distribution of *Salmonella* serovars isolated from enrichment culture in HTT and RV broth

Serovars	Enrichment		Total*
	RV	HTT	
O4,12:d:–	27	65	67
Havana	17	31	37
Livingstone	12	16	21
Typhimurium	5	11	12
London	7	8	9
Corvallis	4	6	7
Infantis	3	6	7
Anatum	1	6	6
Virchow	3	4	5
Total	79**	153**	171***

* Number of samples in which *Salmonella* was detected in either of two enrichment.
** Two *Salmonella* serovars were isolated from 6 and 2 individual fecal samples in RV and HTT, respectively.
*** Different serovars were isolated from seven samples.

- and Anatum) in HTT. The recovery of the serovars isolated in this study was slightly better in HTT than in RV. Harvey *et al.* [11] reported that pre-enrichment in tetrathionate broth for isolation of *Salmonella* serovars other than *S.* Choleraesuis is better than pre-enrichment in GN-Hajna. Our result suggests that RV at 37°C is not a suitable procedure for isolating *Salmonella* serovars other than *S.* Choleraesuis from porcine feces.

In conclusion, enrichment in RV at 37°C is effective for isolating *S.* Choleraesuis. However, enrichment in HTT at 42°C was better than RV at 37°C for isolating *Salmonella* serovars other than *S.* Choleraesuis. If other serovars than *S.* Choleraesuis are also to be surveyed, HTT enrichment should be used in combination with RV enrichment.

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