A Longitudinal Epizootiological Study of *Salmonella* Infection on a Piggery: A Study on the Mode of Contamination by Biotyping of *Salmonella typhimurium* and by the Antibigram

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Abstract. The distribution of *Salmonella* on a pig farm and the mode of spreading of *Salmonella* infection were studied for 2 years. The possibilities of exotic fresh infection and cross-infection within the farm were investigated by dividing serovars of *Salmonella* isolates or biovars of *S. typhimurium*. During the present investigation, 745 (22.0%) out of 3,374 samples of pen fecal samples and various environmental specimens examined were positive for *Salmonella*. The serovar strains isolated were *S. typhimurium* 582, *S. anatum* 153, *S. senftenberg* 65, *S. livingstone* 7 and *S. infantis* 6. Of the 5 serovars *S. typhimurium* was persistently found for 2 years. These isolates were classified into 2 different biovars (3 and 19, and 1 and 2) according to Duguid's primary tests and the method of Brandis, respectively. Furthermore, 11 different sub-biovars were found within 2 different biovar by Duguid's secondary tests. Appearance of different serovars and the change of biovars in *S. typhimurium* indicated the occurrences of different exotic infection sources on this farm. The isolation rate of *Salmonella* from manure and sewage samples and pen fecal samples of market pigs indicated that this farm was contaminated heavily with *Salmonella*. Antibiotic susceptibility of 317 isolates of the 5 *Salmonella* serovars from pig pen fecal samples and 163 isolates from various environments was examined. Over 50% of *S. typhimurium* isolates tested and each one of *S. anatum* and *S. senftenberg* were resistant to TC, SM and SA. However, the antibiogram was not useful as an epidemiological marker on this farm.

Meats, eggs, and their products of contaminated pigs and poultry have recognized to be the commonest source of human salmonellosis [4, 8, 18, 19]. Buxton [3] stated that the majority of clinical infection in pigs is due to *S. choleraesuis* and *S. typhimurium* and mesenteric lymph nodes are common site for latent *Salmonella* infection in clinically normal pigs. Katsube et al. [11] reported that 18 animals (45%) of 40 ap-

parently healthy slaughtered pigs were *Salmonella* carriers in Japan. The data indicated that pigs being housed by the intensive farming method may be contaminated with *Salmonella*.

However, little work has been done in analysing the results of repeated examinations over a period of time concerning the perpetuation of *Salmonella* infection within a conventional pig farm.

We attempted to demonstrate the distribution and mode of spread of *Salmonella* on a farm by differentiation of serovars of *Salmonella* isolates, biotyping of *S. typhimurium* and their drug susceptibility. This paper deals with a longitudinal study of detection of *Salmonella* from fecal samples, rectal swab samples and various environmental specimens within a pig farm during 1972–1974.

Materials and Methods

1. The farm investigated

Farm K was an intensive pig breeding-fattening unit in Hokkaido of northern Japan. It contained consistently about 2,000 pigs, and a part of piglets for fattening were repeatedly introduced from markets until Sept. 1973, but the introduction of piglets decreased gradually, after sows were mass-introduced at the end of Sept. 1973. The farm was comprised of several subunits such as farrowing house, weanling house, fattening house I, -II, sow house, boar house, isolation house, sow house yard, fattening house II yard, equipment stores and office. The new weanling house and sow house were built in Oct. 1973. Animals were housed in different buildings divided into pens, with exception of boar house and farrowing house. Pens had concrete floors, dividing walls and a communal gatter. The drain from different pig houses was collected in a sewage pool, and the manure from the pens were stored in concrete tanks. The stored manure was semidried to use as fertilizer.

Outdoor housing in both the sow yard and fattening house II yard was carried out for a period from the end of Apr. to early Nov. every year. Sows were housing in the sow yard until the approach of parturition and they were moved into the farrowing house. Pigs were moved from house to house and pen to pen as they grew. Hygienic status on the pig farm was relatively good. The feed used on the farm was commercial meal which was purchased from a feeding factory. No information was available about the origin and the feeding stuff ingredients in the meal. There was no history of salmonellosis in this pig farm. The first outbreak of clinical salmonellosis occurred in Dec. 1972. *Salmonella typhimurium* was isolated from dead pigs at postmortem examination. After this isolation of *S. typhimurium*, the present investigation was carried out during the period from Jan. 1973 to Dec. 1974.

2. Collection of specimens for *Salmonella* isolation

Either a composite fecal sample or a rectal swab sample per pen was collected from the different pig houses (farrowing house, weanling house, fattening house I, -II, sow house, boar house, isolation house) every week between Jan. 1973 and Dec. 1974. The samples of pig manure were put into sterile polyethylene bags and the sewage samples were collected from a sewage pool with cotton swab during the period indicated in Table 4. All samples from gum boots of attendants were obtained with sterile cotton swabs. Also, feed samples and wood shavings being used as piglet bedding were put into polyethylene bags. The soil samples from sow yard, fattening house II yard and the aisles in or between pig houses were collected into polyethylene bags at random during 2 years. In order to check *Salmonella* contamination, the samples of rectal fecal and pig skin (shoulder or abdomen) swab were obtained from marker pigs, just before being shipped to the abattoirs from the farm. Three dead wild rats were examined for *Salmonella*. Also, 19 dead pigs were examined bacteriologically at necropsy during this survey.

3. Isolation of salmonellas

Throughout this survey, 0.5 to 10 g composite fecal samples and rectal fecal samples of market pigs were incubated in 10 ml or about tenfold amount of selenite brilliant green (SBG) broth (Nissui) at 43°C. The rectal and environmental swab samples, sewage samples, 1 to 2 g semi-dried manure samples, feeding stuffs and wood shaving samples were enriched in SBG in similar way. After 24 and 48 hrs incubation, a loopful of the SBG broth cultures was plated on brilliant green (BG) agar plates (Eiken) or MacConkey agar plates (Eiken). The plates were examined after 24 hr incubation at 37°C. Two or three *Salmonella*-like colonies on BG plates were examined biochemically according to the method of Edwards and Ewing [6] and were identified serologically by the slide agglutination test with *Salmonella* polyvalent O and group O antisera, and by the tube agglutination test with H antisera. The organs or a part of intestinal tract of the dead three rats were separately inoculated into SBG broth and incubated at 43°C. The heart, spleen, kidney, liver, lung, mesenteric lymph nodes and rectal contents were obtained from each 19 dead pig. Each tissue sample was directly plated onto blood agar and MacConkey agar plates. The suspicious colonies on these plates were identified biochemically and serologically.

4. Biotyping of *S. typhimurium*

Biotyping was done by the method described by Duguid et al. (Method of Duguid) [5], in addition

to the method of Brandis [2, 14]. A total 174 isolates of *S. typhimurium* from various fecal and environmental samples were used in this test (Fig. 1 and Table 6). These isolates were picked up from different pen samples obtained for a long time. Also, for a few isolates failing to grow on the minimal medium, or for isolates to be tested which were auxotrophic and failed to grow on the minimal medium supplemented with nicotinamide (25 µg/ml) or L-cysteine (25 µg/ml), m-tartrate inhibition tests were done according to Alfredsson et al. [1], using the turbidity test of a liquid medium instead of the plate test. Turbidity between m-tartrate containing and m-tartrate free medium was compared by the naked eye and then by measurement with spectrophotometer model 101 (Hitachi Ltd., Tokyo) at 660 nm.

5. Antibiotic susceptibility tests

Antibiotic susceptibility testing of the isolates was performed by the agar dilution method using 12 kinds of antibiotics at the following final concentrations: ampicillin (APC) 25 µg/ml, streptomycin (SM) 12.5 µg/ml, tetracycline (TC) 25 µg/ml, chloramphenicol (CP) 25 µg/ml, kanamycin (KM) 25 µg/ml, gentamicin (GM) 12.5 µg/ml, colistin (CL) 12.5 µg/ml, cephaloridine (CER) 25 µg/ml, furatrizine (FT) 6.3 µg/ml, nalidixic acid (NA) 25 µg/ml, rifampicin (RFP) 25 µg/ml, sulfadimethoxine (SA) 800 µg/ml. Heart infusion agar (Eiken) was used for the tests except in the test with SA, in which Mueller Hinton agar (Eiken) was used. An isolate was recorded as resistant if its growth was not inhibited by these concentrations of the drugs.

Results

1. Isolation of different serovars of *Salmonella* and its epidemiological significance

Over 2 years, 745 (22.0%) out of 3,374 of the samples tested were positive for salmonellas and 5 serovars were isolated (Table 1). As can be seen in Tables 1 and 2, the greatest number of *Salmonella* isolates was *S. typhimurium* among 5 serovars and they are isolated on the farm for a long term.

S. livingstone was obtained from pig houses in Jun. and Aug., 1974, and *S. infantis* was isolated only from sow house and environmental samples in Jan., 1974.

Table 3 shows the incidence of 3 serovars of salmonellas grown from pen samples of two pig houses and the soil from their yards during outdoor housing. As shown in Table 2, *S. anatum* had not been isolated from any pig houses until May, 1973. As can be seen, this serovar was found from farrowing house for the first time in May, and it was isolated frequently from sow house yard in Aug. and Sep., 1973. The serovar was also isolated from pen fecal samples in fattening house II later in Aug., 1973 (Table 3). Since *S. anatum* was found from farrowing house and fattening house II yard simultaneously with starting of outdoor housing, it can be thought that the occurrence of *S. anatum* in the pig houses and their yards was associated with the outdoor housing in this piggery.

Afterwards, this serovar was repeatedly found from pen fecal samples and the environmental samples from different sources (Tables 1 and 2). This would suggest that cross-infection between pig houses and their yards and the consecutive movements of pigs caused the prevalence of *S. anatum*, though it was not apparent how the yards were contaminated with this serovar. *S. senftenberg* was also persistently found (Tables 2 and 3).

The *Salmonella* isolations from semi-dried manure and sewage samples for 7 months are shown in Table 4. During this period, 56 (88.9%) out of 63 manure samples and 33 (97.0%) out of 34 sewage samples were positive for salmonellas. The results indicate that this farm was heavily contaminated with *Salmonella*. *S. anatum* was obtained from the part of intestinal tract of one of three dead rats. *S. typhimurium* was isolated from the organs and rectal contents of 6 of the 19 dead pigs examined at necropsy (Table 1).

Despite repeated examinations, only 2 of

Table 1. The isolation frequency of different serovars of *Salmonella* on K farm from Dec., 1972 to Dec., 1974

Source	No. of samples examined	No. of <i>Salmonella</i> -positive samples	Serovars*				
			S. t	S. a	S. s	S. l	S. i
Farrowing house	660	112 (17.0)	74	47	3	4	
Weanling house	460	18 (3.9)	14	3	1		
Fattening house I	929	243 (26.2)	231	11	4	1	
Fattening house II	250	95 (38.0)	87	8	12		
Boar house	43	14 (32.6)	9		5		
Sow house	158	12 (7.6)	8		8	2	2
Isolation house	45	15 (33.3)	13	2			
Sow house yard	65	27 (41.5)	5	25			
Fattening house II yard	98	53 (54.1)	11	38	13		
Manure	63	56 (88.9)	45	9	7		1
Sewage pool	34	33 (97.1)	27	2	8		
Gum boots	46	16 (34.8)	13	2			1
Aisle in or between pig houses	80	26 (32.5)	19	5	4		2
Feed stuffs	197	2 (1.0)	2				
Wood shavings (Bedding materials)	24	0					
Wild rats	3	1 (33.3)		1			
Dead pigs	19	6 (31.6)	8**				
Market pigs	300	16 (8.0)	16				
Total	3374	745 (22.0)	582 (71.6)	153 (18.8)	65 (8.0)	7 (0.9)	6 (0.7)

* S. t, *S. typhimurium*; S. a, *S. anatum*; S. s, *S. senftenberg*; S. l, *S. livingstone*; S. i, *S. infantis*.

** Two or more isolates were isolated from one sample.

Figures in parentheses are percentages.

Table 2. Isolation of *Salmonella* from various samples for a 2-year period

Serovar isolated	Month (1972-1974)																				Total										
	'72										'73											'74									
	12	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	4	5	6	7		8	9	10	11	12					
<i>S. typhimurium</i>	1*	12	28	3	31	81	16	15	46	51	46	36	79	44	20	24	20	21	2	5	1					582					
<i>S. anatum</i>						7	3	5	39	50	17	17	2	11				1	1							153					
<i>S. senftenberg</i>			7	4	2	2	1		5	9		3		29	2		1									65					
<i>S. livingstone</i>																		4		3						7					
<i>S. infantis</i>														6												6					

* The total number of *Salmonella* isolates from various samples in each month.

the 197 feed stuff samples were positive for *S. typhimurium* (Table 1). On loading for transport to abattoirs from the farm, *S. typhimurium* alone was isolated from the market pigs examined (Table 5).

2. Epizootiological study of *S. typhimurium* infection by the biotyping

In order to study in detail infection of *S.*

typhimurium which was most prevalent on this farm, biovars of this serovar was used as epizootiological marker. The distribution of biovar of *S. typhimurium* is shown in Fig. 1 in which biovars obtained by the method of Duguid and that of Brandis were comparatively indicated.

The biovar 3 (Duguid) and biovar 1

Table 3. Frequency of *Salmonella* isolations from pen fecal samples and environmental samples during outdoor housing

Source	Salmonella seorvar	Month (1973-1974)												
		'73 Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	'74 Jan
														← Outdoor housing →
Farrowing house	<i>S. typhimurium</i>	—*	9/60**	—	—	37/73	3/43	—	7/36	5/69	1/39	1/39	4/39	3/39
	<i>S. anatum</i>	—	0/60	—	—	7/73	2/43	—	3/36	23/69	9/39	3/39	0/39	0/39
	<i>S. senftenberg</i>	—	0/60	—	—	1/73	0/43	—	1/36	1/69	0/39	0/39	0/39	0/39
Sow house yard	<i>S. typhimurium</i>	—	—	—	—	—	—	—	1/19	4/15	—	—	—	—
	<i>S. anatum</i>	—	—	—	—	—	—	—	17/19	5/15	—	—	—	—
Fattening house II	<i>S. typhimurium</i>	—	0/51	0/15	3/24	23/25	2/26	11/30	21/24	16/22	8/30	3/19	—	—
	<i>S. anatum</i>	—	0/51	0/15	0/24	0/25	0/26	0/30	1/24	5/22	2/30	0/19	—	—
	<i>S. senftenberg</i>	—	6/51	4/15	1/24	0/25	0/26	0/30	0/24	0/22	0/30	0/19	—	—
Fattening house II yard	<i>S. typhimurium</i>	—	—	—	1/1	2/13	2/3	1/4	2/40	3/19	0/3	0/13	—	0/15
	<i>S. anatum</i>	—	—	—	0/1	4/13	1/3	3/4	16/40	3/19	1/3	7/13	—	3/15
	<i>S. senftenberg</i>	—	—	—	0/1	1/13	0/3	0/4	4/40	8/19	0/3	0/13	—	0/15

* Not tested.

** No. of positive specimens/No. of specimens examined.

Table 4. Frequency of *Salmonella* isolations from manure and sewage samples on K farm

Sampling date	No. of positives/ No. of samples examined	Manure					Sewage pool							
		No. of samples yielding different serovars*					No. of positives/ No. of samples examined	No. of samples yielding different serovars*						
		S. t	S. a	S. s	S. l	S. i		S. t	S. a	S. s	S. l	S. i		
Aug. 22, '73	3/3	2	1	—**	—	—								
Sep. 28, '73	2/5	—	2	—	—	—								
Nov. 7, '73	5/5	4	1	—	—	—								
Nov. 21, '73	5/5	5	—	—	—	—								
Nov. 29, '73	5/5	4	1	—	—	—	3/3	3	—	—	—	—	—	—
Dec. 5, '73	4/5	4	—	—	—	—	5/5	5	—	—	—	—	—	—
Dec. 12, '73	5/5	3	2	—	—	—								
Dec. 20, '73	4/5†	3	2	—	—	—	4/5	4	—	—	—	—	—	—
Dec. 27, '73	4/5	4	—	—	—	—	3/3	3	—	—	—	—	—	—
Jan. 16, '73	5/5	2	—	—	3	—	5/5†	3	—	4	—	—	—	—
Jan. 22, '73	5/5	5	—	—	4	1	5/5†	1	2	4	—	—	—	—
Jan. 30, '73	4/5	2	2	—	—	—								
Feb. 12, '73	5/5	5	—	—	—	—	8/8	8	—	—	—	—	—	—
Total	56/63 (88.9) ‡ 43	11			7	1	33/34 (97.0)	27	2	8				
		(69.3)	(17.8)		(11.3)	(1.6)		(73.0)	(5.4)	(21.6)				

* S. t, *S. typhimurium*; S. a, *S. anatum*; S. s, *S. senftenberg*; S. l, *S. livingstone*; S. i, *S. infantis*.

** Not tested.

† Including samples showing multiple-serovars contamination.

‡ Figures in parentheses are percentages.

Table 5. Frequency of *Salmonella* isolations from market pigs

Sampling date	Rectal samples	Skin swab samples	Pen fecal samples	Serovar and sub-biovar
May 8, '74	0/10*	2/10	1/4	<i>S. typhimurium</i> hz (3) **
May 24, '74	0/10	0/10	1/3	<i>S. typhimurium</i> h (1)
June 5, '74	0/13	1/13	2/4	<i>S. typhimurium</i> h (3)
June 13, '74	2/9	1/9	3/4	<i>S. typhimurium</i> h (3)
				<i>S. typhimurium</i> hz (3)
June 19, '74	0/11	0/11	1/4	<i>S. typhimurium</i> hz (1)
June 26, '74	0/9	0/9	1/3	<i>S. typhimurium</i> hz (1)
July 10, '74	0/3	0/3	0/3	
July 18, '74	0/9	0/9	0/9	
Aug. 9, '74	0/3	0/3	0/1	
Aug. 16, '74	1/8	0/8	0/3	<i>S. typhimurium</i> hz (1)
Total	3/85 (3.5) ***	4/85 (4.7)	9/30 (30.0)	

* No. of positives/No. of samples examined.

** Figures in parentheses are No. of *Salmonella* isolates.

*** Figures in parentheses are percentages.

Fig. 1. The comparison of biotyping between Duguid's scheme and Brandis' scheme in *S. typhimurium* isolates

Remarks.

Biovar symbols: Duguid's primary scheme

Brandis' scheme

□ Biovar 3

▤ Biovar 1

▤ Biovar 19

▤ Biovar 2

Table 6. Distribution of the different sub-biovars of *S. typhimurium* isolates by the secondary tests of Duguid's scheme

Source	Month of sampling (1973-1974)																			
	'73												74'							
	1	2	3	4	5	6	7	8	9	10	11	12	1	2	3	5	6	7	8	12
Farrowing house		a-2*			a-2 f-2	a-2		a-2	a-3 b-1	h-1	a-1	a-1 i-1	a-2					i-1 hz-1	hz-1	
Weanling house										a-1 f-1	f-1		a-2	h-1			hz-1 fhz-2			
Fattening house I	a-4	a-3 f-1	a-1	a-3 i-1	a-1 f-1 bf-1	a-1	a-2	a-2	a-1 h-1	a-1 h-3		a-2 h-2	a-2 h-2	a-1 h-2	a-1 h-1	z-1 hz-1	h-2 hz-2	hz-1	hz-1	hz-1
Fattening house II				d-2	a-3	a-1	a-2	a-1 cf-1	a-2	a-2	a-2									
Boar house												h-1	h-2		hz-1				hz-2	
Sow house												h-1	h-2							
Isolation house				a-1		a-1		a-2	a-2 h-1											
Fattening house II yard					a-2	f-2	a-1	a-2	a-2											
Manure							a-1					a-1 h-5	h-8	h-7	h-2					
Sewage pool												h-2	h-4	h-4	h-2					
Gum boots												a-3	h-3	h-2						
Aisle in or between pig houses								h-2		h-2	a-1	a-1	a-1	h-2	h-2					

Remarks.

* Marker(s) of sub-biovar—No. of isolate biotyped.

Marker(s) used are the same as described by Duguid et al. [5].

a: Isolate gave a positive reaction in all secondary tests.

b: Non-haemagglutinating (Fim⁻).d: 1-Tartrate not utilized (IT⁻).f: Trehalose not fermented (Tre⁻).

h: L-rhamnose not fermented in Bitter's medium though fermented in peptone water.

i: meso-Inositol not fermented at 25°C.

z: No growth without growth factor other than nicotinamide or cysteine.

bf: Non-haemagglutinating and trehalose not fermented.

cf: Non-motile and trehalose not fermented.

hz: L-rhamnose not fermented in Bitter's medium though fermented in peptone water and no growth without growth factor other than nicotinamide or cysteine.

fhz: Trehalose not fermented and L-rhamnose not fermented in Bitter's medium though fermented in peptone water and no growth without growth factor other than nicotinamide or cysteine.

(Brandis) changed simultaneously in May, 1974 (Fig. 1). The changes in biovars indicate the occurrence of new infection source on this farm. Moreover, these 2 biovars determined by the primary tests of Duguid's method were divided further into 11 different sub-biovars (a, b, d, f, h, i, z, bf, cf, hz and fhz) by the secondary tests, as shown in Table 6, indicating frequent introductions of new infection sources. Sub-biovar

a was found dominantly at half of 1973, except that sub-biovar f was sporadically present in May and June, 1973. Since sub-biovar f was found from weanling house and fattening house I, the appearance of sub-biovar f may be related with the introduction of piglets from the markets. Table 6 shows that the change from sub-biovar a to h occurred in Sept. and Oct., 1973, and sub-biovar h was found consistent-

Table 7. Drug resistance patterns of *Salmonella* isolates from various pig houses

Source of isolates tested	Serovar*	No. of isolates examined	Drug resistance patterns**							(S)†
			TC-SM-SA-KM	TC-SM-SA	Tc-SM	CP-TC	SM-SA	TC	SM	
Farrowing house	S. t	50		22	1		2	19		6
	S. a	24			1			3	8	12
	S. s	1								1
	S. l	4								4
Weanling house	S. t	13		8				5		
	S. a	3			1			1	1	
	S. s	1								1
Fattening house I	S. t	114	1	66	10		11	21		5
	S. a	5				1			3	1
	S. s	3			1			1		1
Fattening house II	S. t	44		23	2		1	17		1
	S. a	5				1				4
	S. s	9								9
Boar house	S. t	5		3						2
	S. s	5								5
Sow house	S. t	7		5			1			1
	S. s	7								7
	S. l	2								2
	S. i	2								2
Isolation house	S. t	11		7	1		2			1
	S. a	1							1	
Total	S. t	245	1 (0.4) ‡	134 (54.7)	15 (6.1)		17 (7.0)	64 (25.3)		16 (6.5)
	S. a	33			2 (5.3)	2 (5.3)		4 (10.5)	13 (34.2)	20 (44.7)
	S. s	26			1 (3.9)			1 (3.9)		24 (92.2)
	S. l	6								6
	S. i	2								2

* S. t, *S. typhimurium*; S. a, *S. anatum*; S. s, *S. senftenberg*; S. l, *S. livingstone*; S. i, *S. infantis*.

** TC, tetracycline; SM, streptomycin; SA, sulfadimethoxine; CP, chloramphenicol; KM, kanamycin.

† Susceptible to drugs used.

‡ Figures in parentheses are percentages.

ly afterwards.

Sub-biovar z found in 1974 means that the isolates tested were auxotrophic and failed to grow even of the minimal medium added glycerol instead of citrate or growth factors such as nicotinamide and L-cysteine originally prescribed for the secondary tests of Duguid et al. But, sub-biovar z isolates in present study utilized m-tartrate in the turbidity test.

3. Antibiotic resistance of *Salmonella* isolates

The antibiotic susceptibility of *Salmonella* isolates from various pig houses or various environmental sources is shown in Tables 7 and 8. All of isolates tested were susceptible to following drugs: APC, GM, CER, CL, FT, NA, RFP. Of the 5 serovars, 135 (54.7%) out of 245 *S. typhimurium* isolates from various pig houses and 58 (57.8%) of 88 *S. typhimurium* isolates from various environmental samples were resistant to TC, SM and SA (Tables 7 and 8). Only one isolate was resistant to four anti-

Table 8. Drug resistance patterns of *Salmonella* isolates from various environmental samples

Source	Serovars*	No. of isolates examined	Drug resistance patterns**					
			TC-SM-SA	TC-SM	SM-SA	TC	SM	(S)†
Boar house yard	<i>S. t</i>	5	2			1		2
	<i>S. a</i>	10						10
Fattening house II yard	<i>S. t</i>	10	6	1	1		2	
	<i>S. a</i>	23		1		1	8	13
	<i>S. s</i>	8				1	3	4
Manure	<i>S. t</i>	28	18	2		6	1	1
	<i>S. a</i>	7	1				1	5
	<i>S. s</i>	4	1					3
	<i>S. i</i>	1						1
Sewage pool	<i>S. t</i>	18	11		2	4		1
	<i>S. a</i>	2						2
	<i>S. s</i>	4						4
Gum boots	<i>S. t</i>	11	4		3	2		2
	<i>S. a</i>	2						2
	<i>S. i</i>	1						1
Aisle in or between pig houses	<i>S. t</i>	14	10		1	1		2
	<i>S. a</i>	5						5
	<i>S. s</i>	4				2		2
	<i>S. i</i>	2						2
Feed stuffs	<i>S. t</i>	2						2
Wild rat	<i>S. a</i>	1						1
Total	<i>S. t</i>	88	58 (57.8) ‡	3 (3.4)	7 (8.0)	14 (16.0)	3 (3.4)	10 (11.3)
	<i>S. a</i>	50	1 (2.0)	1 (2.0)		1 (2.0)	9 (18.0)	38 (76.0)
	<i>S. s</i>	20	1 (5.0)			3 (15.0)	3 (15.0)	13 (65.0)
	<i>S. i</i>	4						4 (100)

* *S. t*, *S. typhimurium*; *S. a*, *S. anatum*; *S. s*, *S. senftenberg*; *S. l*, *S. livingstone*; *S. i*, *S. infantis*.

** TC, tetracycline; SM, streptomycin; SA, sulfadimethoxine.

† Susceptible to drugs used.

‡ Figures in parentheses are percentages.

biotics. There is no difference of drug resistance patterns of *Salmonella* isolated from among various pig houses and various environmental samples. Most of isolates of *S. anatum* were susceptible to drugs used in this study, but two isolates were resistant to CP and TC (Table 7). All of *S. livingstone* and *S. infantis* isolates tested were sensitive to the drugs used.

Discussion

This 2-year study was conducted to know

the mode of dissemination and perpetuation of *Salmonella* infection on a piggery. Since the isolation of salmonellas from pen fecal samples of various pig houses and environmental samples on the piggery is considered to be useful to check the incidence of salmonellas in pigs [7, 9, 20], pen fecal samples and environmental specimens were examined as much as possible for 2 years in this study. During the period, *S. typhimurium* isolates were divided into 2 biovars each (1 and 2, and 3 and 19) by the biotyping

method of Brandis and Duguid's primary tests, respectively (Fig. 1). The change from biovar 3 to 19 in 1974 coincided with that of sub-biovar (Fig. 1 and Table 6). Since piglets had been introduced for fattening or sows for breeding from different markets in this farm, there were many possibilities of introduction of *Salmonella*-carrier pigs from external sources. This farm became an intensive pig breeding-fattening unit completely, after mass-introduction of sows was done at the end of Oct. 1973. Since *Salmonella* excretor boars and sows are responsible for the spread and perpetuation of infection in the farm [7], the sows introduced may have played an important role in the dissemination and spread of salmonellas on this farm latter stage of this study. Therefore, it is thought that symptomless excretors carrying *S. typhimurium* which was biotyped as h or hz in sub-biovar might be introduced sows. It is well recognized that the method of biotyping has been useful for identifying different isolates within serovar in respect to assess the epidemiology of this organism [14, 15]. However, there is little information on the evaluation for an epizootiological marker of biotyping of Duguid, in particular concerning its application to the spread of *S. typhimurium* infection within the closed community. These data indicate that biotyping of the primary and secondary tests of Duguid are more useful on investigating the sources and perpetuation of *S. typhimurium* infection than that of Brandis. It should be noted that 15 sub-biovar z isolates required supplement of medium with growth factors other than nicotinamide or L-cysteine which were recommended by Duguid et al. [5] (Table 6). However, growth tests on glucose-ammonium medium supplemented with different amino acid or other factors according to Maynell and

Maynell [12] are not yet examined.

During this investigation, only 2 out of 152 samples of commercial animal feed stuffs were positive for *S. typhimurium* (sub-biovar α). Since 5 different serovars of *Salmonella* were detected on this farm, it was thought that contaminated feed stuffs might be another probable exotic source of *Salmonella*, and also potential spread of new serovars [16, 19].

Appearance of *S. anatum* in two pig houses and their yards was a characteristic feature of *Salmonella* transmission in the present study (Table 3). The data obtained indicate that the dissemination of this serovar is initiated by the outdoor housing.

The detection of *Salmonella* from gum boots of attendants and aisles shows that cross-infection was caused by the mechanical transmission of infection by farm attendants as well as by movements of pigs. In this study, *S. anatum* was isolated from 1 out of 3 dead rats. Wild rats have been estimated as a main carrier of *Salmonella* in a closed community [13].

It is of interest to note that the manure and sewage samples from various pig houses were contaminated heavily with *Salmonella* serovars which were prevalent on the farm (Tables 1 and 4). This indicates that repeated examinations of manure or sewage must be useful in detection of *Salmonella* infection in pig herds, as described by Jones and Hall [10]. Moreover, the contamination of the pasture spread pig manure and the effluent of pig farm wastes which are contaminated with *Salmonella* would be a potential hazard for man and grazing animals.

The market pigs being shipped to abattoirs were estimated as symptomless excretor by the examination of their fecal samples, their skin swab samples and pen fecal samples, as shown in Table 5. This indicates that the market pigs are potential

sources of the environmental contamination with *Salmonella* in the abattoirs. In order to prevent transmission of *Salmonella* infection in man and animals, it is important to survey persistently the incidence of pig farms and to eliminate symptomless excretors from the farms. It has been indicated that *S. typhimurium* is carried by market pigs more frequently than other non-host adapted serovars in Japan [11]. Also, the same tendency was observed in this study.

About 90% or more of *S. typhimurium* isolates tested were singly or multiply resistant, and over 50% of the isolates were resistant to TC, SM and SA, as shown in Table 7 and 8. No relationship was found between drug resistance patterns and subbiovars in *S. typhimurium* isolates tested. From these results, antibiogram was not useful as a marker in this epizootiological study. However, it is necessary to assay the genetic properties of R plasmids derived from the present *Salmonella* isolates on an epidemiological viewpoint, as Sato and Terakado showed on a feedlot [15]. Detection of R plasmids from these resistant isolates and properties of the plasmids are now under study.

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要 約

1 養豚場におけるサルモネラ感染症の疫学的長期観察: ネズミチフス菌の生物型と薬剤耐性面からの伝播様式の検討: 石黒直隆・佐藤義平 (帯広畜産大学獣医公衆衛生学教室), 竹内孔二 (名古屋市南生協病院), 中山 明 (旭川保健所)——1 養豚場内のサルモネラの分布及びその伝播様式について, 2 年間観察を行ってきた。分離サルモネラの血清型とネズミチフス菌の生物型別の面から, 新たな汚染源の導入及び交差感染に関して, 疫学的検討も加えた。観察期間中, 豚糞便材料及び環境材料の総検索数 3,374 中 745 (22.0%) 試料からサルモネラが分離された。分離サルモネラの血清型は, 以下のとおりである。 *S. typhimurium* 582 株, *S. anatum* 153 株, *S. senftenberg* 65 株, *S. livingstone* 7 株 および *S. infantis* 6 株であった。5 血清型の内, *S. typhimurium* は, 2 年間の観察中, 終始分離された。これらネズミチフス菌は, Duguid の生物型別の一次分類と Brandis の型別法によりそれぞれ, 3 と 19, それに 1 と 2 の 2 生物型に大別された。さらにこれら 2 種の生物型は, Duguid の二次分類により, 11 種の亜生物型に型別された。異なった生物型の出現や生物型の変化は, 外部からの汚染源の導入を強く示唆した。糞便, 汚水及び出荷豚の直腸糞便試料等よりサルモネラの分離された事は, 本養豚場でのサルモネラの濃厚汚染を意味している。糞便由来 317 株と環境材料由来 163 株について, 薬剤感受性試験を行った結果, 5 血清型の内, *S. typhimurium* の 50% 以上の株と, *S. anatum*, *S. senftenberg* それぞれ 1 株ずつが, TC, SM, SA 3 剤に耐性であった。しかし, 本研究においては, 上記薬剤耐性型は疫学マーカーとしての有効性はみとめられなかった。