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A LONG-TERM EPIZOOTIOLOGICAL STUDY OF CHICKEN SALMONELLOSIS ON A FARM WITH REFERENCE TO ELIMINATION OF PARATYPHOID INFECTION BY CLOACAL SWAB CULTURE TEST

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A 14-year study of the epizootiology of chicken salmonellosis was described on a broiler farm keeping a closed flock of New Hampshire breeder chickens and conducting a breeder-hatcher plan. Pullorum disease was eradicated by the serological method. The agglutination test adopted for breeder chickens was no value for elimination of *Salmonella enteritidis*, *Salmonella newington* or *Salmonella senftenberg* infection. The single cloacal swab culture test was carried out every year for the detection of carriers from breeder replacement chickens by the age of about 50 days on the basis of results from an experiment on shedding Salmonella in feces and the duration of infection in growing chickens naturally infected with *S. newington*. Thus no Salmonella of the certain types was recovered from the specimens of dead chickens, dead embryos and hatcher chick fluff 3 years after the onset of the eradication plan. The significance of wild rat (*Rattus norvegicus*) as a carrier in chicken salmonellosis was indicated and discussed.

INTRODUCTION

It has been described that the cloacal swab culture method is unreliable as a diagnostic procedure for paratyphoid infection in poultry, because fecal excretion of Salmonella is intermittent in carrier birds[19]. The method has also been considered to be impractical on a commercial scale. However, the method for the detection of paratyphoid infection in a flock appears to be more reliable than serological testing[14]. To overcome the difficulties created by the intermittent excretors, 3-consecutive-day swabbing was adopted for turkey flocks infected with *Salmonella typhimurium* in combination with blood testing by GORDON & TUCKER. The investigators could eradicate the infection from the flocks.

This report deals with the selection of the age suitable in chickens for the cloacal swab culture method and with the application of the method for eradicating paratyphoid infection from chickens on a farm. The efficacy of the method was evaluated on the basis of a long-term epizootiological investigation on the Salmonella status of the farm.

MATERIALS AND METHODS

1 Isolation of Salmonella

The following specimens from T farm were examined bacteriologically. Since this study was conducted over a long period, there were changes of isolation media employed.

Dead chickens Portions of the heart, lungs, spleen, liver, kidneys and retained yolk from carcasses were cultured directly on MacConkey's agar plates and the pooled materials of the tissues were placed in selenite broth. A segment of the cecum was cultured in the broth. The broth was incubated overnight at 37°C and subcultured on MacConkey's agar plate. Brilliant green agar plate was used in place of MacConkey's agar from 1963. In 1967, only the enrichment culture was adopted for the organs or intestines. Salmonella like colonies on the media were checked biochemically and serologically.

Chickens sacrificed The whole of the intestine was cut into pieces by scissors and homogenized in a high speed blender in a large amount of selenite broth. The entire heart, lungs, liver, spleen, kidneys and pancreas were pooled and treated similarly. The overnight incubated broth cultures were subcultured on MacConkey's agar or brilliant green agar plates.

Dead embryos A loopful of yolk was cultured directly on MacConkey's agar plate.

Hatcher chick fluff This specimen was examined using the procedure described by MIURA et al.[8]

Rats and other animals Visceral organs including lymph nodes and intestine were cultured according to the procedures used for dead chickens.

Other specimens including feed samples These specimens were enriched in selenite broth and subcultured onto agar plates.

Cloacal swab culture A sterile cotton swab on the tip of a matchstick of about 5 cm was inserted into the rectum and the swab was transferred into 5 ml selenite broth in serology tube. After overnight incubation, a loopful of the broth was streaked onto an SS agar plate. From 1960 brilliant green agar plates were employed in place of SS agar. One brilliant green agar plate was used for 2~4 specimens.

2 Agglutination test

Whole blood test A stained antigen of *Salmonella enteritidis*, *Salmonella newington* or *Salmonella senftenberg* was employed for the slide test. The antigen contained carbolized cells at 50-time concentration on the McFarland scale No. 1. The mixed antigen of the serotypes was also used. The manner of the slide test was the same as that for the pullorum whole blood test.

Tube test For O agglutination test the alcoholized antigen of the three Salmonella types was used and the formalized broth culture for H agglutination. A reading of O agglutination was made after the reactants had been left overnight at room temperature following a 2 hr incubation period at 37°C. Flagellar agglutination was read after 2 hr incubation in a water bath at 50°C.

Results

1 Salmonella status of chickens in the early stage of T farm

The farm is located in the central district of Hokkaido Island. In 1952, eggs of New Hampshire and White Rock chickens were imported from U.S.A. The eggs were hatched in a commercial hatchery in Sapporo city. Then the chickens were introduced into the farm established in 1952 as parent stock for broiler production. The New Hampshire stock has been kept as a closed flock. This farm took a complete breeder-hatcher plan from 1953. About 500 stock birds were raised in 1955. The number of stock birds has increased to several thousands in the recent several years.

Outbreak of pullorum disease and its eradication In 1953, *S. senftenberg*, *Salmonella bareilly* and unidentified C group Salmonella were isolated from 21 of 24 dead baby chicks examined. *Salmonella pullorum* was recovered for the first time from 10-day-old chicks in April of 1954. During this year, 63 dead or diseased chicks were examined bacteriologically and Salmonella was isolated from 46 of them as in dicated in table 1.

Eradication by means of the rapid whole blood test for pullorum disease was repeated on the breeders without success. Then the eradication plan was applied to the breeder replacement chickens at the ages of 2~3 months with intervals of 1~1.5 months. Thus at the 7th test, no reactor was detected. Annual mean viability rate, 24.6% in 1954 increased to 80.4% in 1955 as shown in table 6. As seen in table 1, no isolation of *S. pullorum* from dead chicks has been recorded on the farm from 1955. Detailed data on the eradication process of pullorum disease was reported by MIURA et al.[7]

Suspected introduction of *S. newington* infection by wild rats In July of 1955, the first outbreak of *S. newington* infection was recorded in a broiler flock. Loss of more than 50% occurred in the flock consisting of 1,670 chicks in the 5 weeks after hatching. The highest mortality rate was observed during the 3rd week of life. *S. newington* was isolated from 10 of 13 chicks examined which died in the 3rd week and from 3 of 6 in the 4th week. During 8 months after the first outbreak, the Salmonella type was obtained twice from dead chickens. Efforts were made to find out the source of the infection. As above mentioned, *S. newington* was detected in the farm for the first time in 1955. Although an increase in *S. newington* agglutination titers was observed in the stock birds after the outbreak, agglutination positive birds did not yield Salmonella on cloacal swabs or at necropsy. Possbility of egg transmission from an outside source should be disregarded, because chicks had been hatched only from breeders of that farm itself. Moreover, no isolation of *S. newington* had been recorded in Hokkaido as indicated in the data by SATO et al.[12] The Salmonella type had not been isolated from birds in Japan[10]. *S. newington* was isolated from the intestine of a wild rat (*Rattus norvegicus*) of 9 caught in a broiler house of this farm as shown in table 2.

From these findings, wild rats were suspected as a possible source of the infection.

Infections with multiple Salmonella types and source of the infections In 1957 mixed infections of Salmonella such as *S. enteritidis*, *S. newington* and *S. senftenberg* were found among chicks in the farm as indicated in table 1. From April to December of the year, a total of 1,161 birds from 35 hatches which died at 0~6 weeks of age were examined

TABLE 1 *Isolation of Salmonella from dead chicks and other specimens from T farm*

YEAR	DEAD CHICKS*1										DEAD EMBRYOS	HATCHER CHICK FLUFF
	No. of hatches sampled/Total hatches through year	No. of Salmonella-positive hatches	No. of Salmonella-positive chicks /No. examined	Salmonella isolates*2							No. positives /No. examined	No. positives /No. examined
				P	T	E	A	N	S			
1954	8/23	8	46/63	35*3 (55.6%)	3 (0.5%)	0	0	0	8 (12.7%)		0/12	ND*4
'55	8/17	2	14/132	0	0	0	0	14 (10.6%)	0		0/352	ND
'56	12/18	1	3/109	0	0	0	0	3 (2.8%)	0		0/183	0/2
'57	35/42	23	143/1,161	0	0	40 (3.4%)	0	54 (4.7%)	49 (4.2%)		0/829	0/21
'58	33/34	16	146/870	0	0	0	0	144 (16.6%)	2 (0.2%)		0/1,473	4/22*5 (18.2%)
'59	33/34	20	198/624	0	0	13 (2.1%)	0	161 (25.8%)	7 (1.1%)		0/2,340	5/31*5 (16.1%)
'60	41/44	11	83/672	0	0	1 (0.1%)	0	82 (12.2%)	0		0/582	1/52*5 (1.9%)
'61	41/43	1	1/345	0	0	0	0	0	1 (0.3%)		0/248	0/25
'62	26/44	0	0/472	0	0	0	0	0	0		0/412	0/37
'63	7/44	0	0/170	0	0	0	0	0	0		0/197	0/16
'67	44/48	1	3/759	0	0	0	3 (0.4%)	0	0		ND	0/136

*1 1~42 day old chicks except for those in 1954 and 1955

*2 P-*S. pullorum*,T-*S. thompson*, E-*S. enteritidis*, A-*S. anatum*, N-*S. newington*, S-*S. senftenberg*

*3 No. of Salmonella positive chicks and % to total chicks examined

*4 Not done

*5 *S. newington*

TABLE 2 *Isolation of Salmonella from wild rats (Rattus norvegicus) and other materials on T farm*

YEAR	SALMONELLA TYPES*1 FROM DEAD CHICKS	NO. POSITIVES /NO. RATS EXAMINED	NO. POSITIVES /NO. ANIMALS EXAMINED	NO. POSITIVES /NO. FEED STUFF SAMPLES EXAMINED
1954	P, T, S	ND*2	ND	ND
'55	N	1/9 N	Cat 0/1, Owl 0/1	ND
'56	N	1/19 N	ND	ND
'57	E, N, S	1/14 E, 1/9*3N	Swine 1/2*3E	0/54
'58	N, S	2/42 N, 0/1*3	ND	0/58
'59	E, N, S	0/13	ND	ND
'60	E, N	5/37 E	ND	ND
Total		Rats 10/134 (E-6, N-4) 1/10*3 N	Cat 0/3 Owl 0/1 Swine 1/2*3 E	0/112

*1 P-*S. pullorum*, T-*S. thompson*, S-*S. senftenberg*, N-*S. newington*, E-*S. enteritidis*

*2 Not done

*3 Fecal sample

for Salmonella. *S. enteritidis* was isolated from 8 hatches within a period of 86 days, *S. newington* from 11 hatches twice within a period of 88 days and then within a period of 9 days after a 3 months interval, and *S. senftenberg* from 18 hatches within a period of 208 days. That is, *S. enteritidis* infection indicated a mass outbreak, while infections with *S. newington* and *S. senftenberg* occurred diffusively.

S. newington infection occurred again in 15 hatches in 1958, while *S. senftenberg* in only one hatch.

As indicated in table 2, *S. enteritidis* and *S. newington* were isolated from wild rats and swine feces. However the following facts indicated the existence of a more significant source of infection, carrier breeders. That is, in 1957, one of 88 dead day old chicks from 13 hatches not fed gave *S. newington*. In 1958, 21 (6 hatches) of 187 day old chicks not fed from 18 hatches yielded the same Salmonella. Moreover fluff samples from 2 of the 6 hatches gave *S. newington*. The facts appeared to indicate that *S. newington* spread in the incubator, possibly on account of egg transmission, though no Salmonella isolation had been recorded on dead embryos examined.

2 Detection of abult breeder carriers by the cloacal swab culture

As will be described later, the whole blood agglutination test by *S. newington* antigen was carried out from 1955 to detect carrier birds. However, the serological test was found unreliable for the purpose. Thus the cloacal swab culture method was adopted for the detection of carriers.

As shown in table 3, positive cloacal swabs were obtained from 4 adult birds which hatched in 1957 and one from young breeders hatched in 1958. Thus possible egg trans-

TABLE 3 *Salmonella isolation from cloacal swabs of young or adult stock birds*

YEAR	AGE AT TEST	NO. POSITIVES /NO. CHICKENS TESTED	NO. POSITIVES /NO. FLOCKS OR HATCHES TESTED	NO. OF SHEDDERS OF		
				S. enteritidis	*S. newington*	others
1958	old stocks (hatched in 1957)	4/827 (0.5%)	3/7 (0.2~0.9%)*	0	4	0
	pullets and cockerels	1/1,468 (0.07%)	1/5 (0.7%)	0	1	0
'59	old stocks	0/1,194	0/6	0	0	0
'59	32~48 days	52/2,159 (2.4%)	7/7 (0.3~11.4%)	1	51	0
'60	37~63	9/3,130 (0.3%)	1/12 (2.6%)	0	9	0
'61	40~51	0/2,463	0/6	0	0	0
'62	39~44	0/3,873	0/6	0	0	0
'63	38~50	0/2,756	0/6	0	0	0

* (minimal~max. % of positives)

mission of *S. newington* on egg was proved on this farm. Two of the 5 positive birds were kept for 52 days for daily cloacal swabbing. One of them gave again positive swab on the 47th day of the test. This bird was sacrificed for cultivation of the entire intestine and visceral organs. The intestine of the bird gave Salmonella, but visceral organs did not. The eggs obtained from the bird gave no Salmonella at cultivation. Another bird examined yielded no Salmonella from the organs and intestine and also from her 7 eggs.

The breeder chickens hatched in 1958 were again examined in 1959 by the cloacal swab method. All birds were negative, while Salmonella was as prevalent in their progeny chickens as in 1958, indicating that the carrier birds still existed. The fact suggested that little efficacy of the single cloacal swab culture test for the detection of adult carriers.

3 Fecal excretion of *S. newington* in naturally infected growing chickens

Under the circumstances of this farm, stock birds for replacement had to be obtained from young chickens probably infected with Salmonella. It is reasonable that Salmonella infected birds are eliminated as early as possible from young stocks for minimizing the spread of infection. The following experiment was conducted to know the appropriate age of growing chickens at which the single cloacal swab culture method can effectively detect carriers.

Ninety-two chickens aged 33 days were isolated from a flock infected with *S. newington* and employed for the experiment. Cloacal swabs were taken at 7-day intervals from each bird. Six of the birds were sacrificed and examined to detect Salmonella by the cultivation of the entire intestine and visceral organs at the age of 37 days, 18 birds at 110 days, 20 at 131 days and 39 at 184 days respectively.

As indicated in table 4, 18 birds gave positive cloacal swabs once at several ages between 33 and 96 days and only one (No. 89) twice at the ages of 40 and 96 days. Ten of 83 birds necropsied showed positive post mortem cultures of *S. newington* and 7 of the 10 birds which had not yielded Salmonella by cloacal swab cultivation harbored Salmonella in the intestines.

From the above mentioned results, it will be seen that Salmonella infected growing chickens excreted Salmonella most frequently by the age of about 100 days, though weekly cloacal swab cultures could not detect those found to be carriers at necropsy. In addition, by that time, 424 dead growing birds aged more than 6 weeks were cultured on this farm. Seventeen (4%) of the birds were Salmonella positive. Thirteen (76%) of the 17 birds were 7 weeks old. This indicates that Salmonella infection seems to be active in infected birds up to about 50 days of age.

Thus the cloacal swab culture method appeared to be more effective for the detection of carriers when the swabs are taken from growing chickens by about 50 days of age.

4 The cloacal swab culture test for the detection and elimination of Salmonella carriers from young stock birds

The birds aged 5~9 weeks were examined every year by the single cloacal swab culture test as seen in table 3. The test was carried out at the time as the pullorum whole blood test. Birds for breeders were selected from many growing chickens of different hatches, wingbanded and tested. Then tested birds were moved to clean colony houses. After

TABLE 4 *S. newington in cloacal swabs from naturally infected chickens*[*1] *at different ages*

NO. OF CHICKEN	SALMONELLA ISOLATION ON DAYS AFTER HATCHING (7-DAY INTERVALS)															SALMONELLA ISOLATION AT NECROPSY[*2]	NO. POSITIVES /NO. CHICKENS EXAMINED
	33	37	40	47	54	61, 68	75	82, 89	96	103	110	117 ~131	138, 145	152	184		
1~ 3	—	N[*3]														—	3/6
4~ 6	—	N														O, (I)	
7~ 9	—	Discarded															
10	—		—	Discarded													
11~12	—		—	—	—	Discarded											
13	—		—	—	—	—	Discarded										
14~22	—		—	—	—	—	—	—	—	—	—N					—	
23~25	+		—	—	—	—	—	—	—	—	—N					—	
26	—		+	—	—	—	—	—	—	—	—N					—	5/18
27~28	—		—	—	—	—	—	—	—	—	—N					(O)	
29~30	—		—	—	—	—	—	—	—	—	—N					(O, I)	
31	—		—	—	—	—	—	—	—	—	—N					(I)	
32	+		—	—	—	—	—	—	—	—	—	Discarded					
33~46	—		—	—	—	—	—	—	—	—	—	—N				—	
47	—		+	—	—	—	—	—	—	—	—	—N				—	1/20
48~49	—		—	—	+	—	—	—	—	—	—	—N				—	

50～51	—	—	—	—	—	—	—	+	—	—	—N			—		
52	—	—	—	—	—	—	—	—	—	—	—N			(I)		
53	—	—	—	—	—	—	—	+	—	—	—	—	— Discarded			
54～84	—	—	—	—	—	—	—	—	—	—	—	—	— —N	—		
85～88	+	—	—	—	—	—	—	—	—	—	—	—	— —N	—		
89	—	+	—	—	—	—	—	+	—	—	—	—	— —N	—		1/39
90	—	—	—	+	—	—	—	—	—	—	—	—	— —N	—		
91	—	—	—	—	—	+	—	—	—	—	—	—	— —N	—		
92	—	—	—	—	—	—	—	—	—	—	—	—	— —N	(O)		
No. of positives /No. of samples examined	8/92 (8.7%)	3/83 (3.6%)		3/82 (3.7%)		1/79 (1.3%)		4/79 (5.1%)								10/83 (12%)

*1 These 92 birds were kept in a battery cage in two groups.

*2 O: Salmonella was isolated from organs by direct cultivation.
(O): Salmonella was isolated from the pooled culture of the entire organs by enrichment.
(I): Salmonella was isolated from the enrichment culture of the entire intestines.

*3 N: Necropsied and cultured

a few days, birds of positive swab were removed from the houses.

An evident decrease in Salmonella isolation from dead chicks and no isolation from other specimens were recorded in 1961 showing results parallel to those from the cloacal swab test (tables 1 & 3). Since 1962 Salmonella types which were prevalent previously have not been recovered from any materials, though a sporadic outbreak of *Salmonella anatum* infection was observed in 1967.

The above mentioned data indicate that the single cloacal swab culture method applied to young stock birds effected the eradication of paratyphoid infection from this farm.

5 Epizootiology of chicken salmonellosis in T farm

Reactors to the pullorum whole blood test in pullorum free flock As described previously, pullorum disease disappeared from the farm since 1955. Under this circumstance, 23 (0.09%) reactors were recovered from a total of 25,677 young stocks (about 44~70 days old) which were tested during 8 years from 1957 to 1964. Sixteen of the reactors were examined bacteriologically by the cultivation of the entire intestine and visceral organs. Two of them gave *S. enteritidis* alone (in 1957) or together with *S. newington* (in 1959). Moreover 72 reactors (0.5%) were detected from 14,372 adult breeders in 6 years from 1956 to 1961. Forty-eight of the reactors examined, however, did not yield Salmonella.

As indicated in table 1, *S. enteritidis* was isolated from dead chickens in 1957, 1959 and 1960. Therefore the Salmonella type might have an influence upon the incidence of the nonspecific pullorum reactors.

The whole blood agglutination test for the detection of Salmonella carrier birds During the period from 1955 to 1960, a total of 15,217 adult birds were tested by the whole blood test using the single antigen of *S. newington* or *S. senftenberg*, or mixed antigen of *S. newington* and *S. enteritidis*. Out of the birds, 1,865 reactors (12.3%) were detected. Twenty-eight of the reactors necropsied gave no Salmonella. On the other hand, 1,884 chickens were tested by single *S. enteritidis* antigen in 1957 and 1958, and only 2 reactors (0.1%) were found.

In the early stage of this study, reactors to antigens of E group Salmonella were separated from non reactors and their eggs were set and hatched in separate incubators. No difference of occurrence of Salmonella infection of chicks was observed between the hatches from reactors and those from non-reactors.

A total of 98 reactors aged about 7~10 weeks were examined bacteriologically during the period 1957 to 1959. Fourteen of them (14.3%) gave Salmonella (*S. newington*-12 birds, *S. enteritidis*-2) while 9 of 25 non-reactors (36%) of the same flocks were positive for Salmonella (*S. newington*-8 birds, *S. enteritidis*-1). The above described data indicate that the whole blood test is no value for the detection of Salmonella infection other than pullorum disease in chickens.

In case of the *S. enteritidis* antigen, results in the whole blood test were parallel to those in the tube test. On the other hand non-reactors to the whole blood test by *S. newington* or *S. senftenberg* antigen frequently became reactors to the tube test using O antigen. Positive agglutination in the tube test to *S. newington* or *S. senftenberg* was not indicative of a caarier in many cases. However the reactors of 5~6 weeks old, showing

high agglutination titres (1 : 1,600～12,800) gave Salmonella at necropsy with considerably high frequency. Only a small number of birds showed positive but weak H agglutination (1 : 12.5～25).

Pathogenicity of different Salmonella types and effects of their infection on viability of broiler chickens Table 5 indicates the distribution of Salmonella organisms in young chicken carcasses cultured directly on agar plates. From this table it will be seen

TABLE 5 *Distribution of Salmonella in the bodies of dead chicks*[*1]

SEROTYPE	HEART-BLOOD	SPLEEN	LIVER	KIDNEY	LUNG	RETAINED YOLK	CECUM[*2]	TOTAL SALMONELLA POSITIVE BIDRS
S. enteritidis	14/40[*3]	22/40	20/40	11/40	14/40	1/23	29/40	40
	35%	55%	50%	28%	35%	4%	73%	
S. newington	14/54	15/54	14/54	10/54	9/54	0/23	50/54	54
	26%	28%	26%	19%	17%		93%	
S. senftenberg	3/49	2/49	1/49	1/49	0/49	0/29	46/49	49
	6%	4%	2%	2%			94%	
S. pullorum	124/139	124/140	126/139	125/138	127/139	106/126	78/85	140
	89%	88%	83%	83%	83%	85%	92%	

[*1] Results were obtained from direct cultivation on McConkey agar plate of dead chicks in 1957. *S. pullroum* data were obtained from cultivation of dead chicks in a flock other than T farm.

[*2] Cecal materials were applied to only enrichment cultivation.

[*3] No. positives/No. samples examined

that *S. senftenberg* was found in the visceral organs less frequently, compared with *S. enteritidis* or *S. newington*. *S. seftenberg* was localized in the intestinal tract. However, invassiveness of *S. enteritidis* and *S. newington* was lower than that of *S. pullorum*.

Chicks infected with each of the above described Salmonella types of non host-specific died most frequently in the 2nd or 3rd week of life. However, deaths within the 4th week or later occurred more often in case of *S. enteritidis* or *S. newington* than in that of *S. senftenberg*. These findings indicate that there is a difference in the pathogenicity of the three Salmonella types prevalent in 1957 on the farm.

As shown in tables 1 and 3, only *S. newington* of the 3 serotypes which were almost equally prevalent in 1957 indicated increasing frequency of infection among chickens and persistent incidence of carriers after 1957. It is difficult to explain whether or not the ability of producing a carrier state varies among the 3 serotypes.

Table 6 shows that pullorum disease prevalent in 1954 caused severe loss in broiler production. Regarding other Salmonella types, effects of their infections on the viability of broiler chickens were variable. A specific Salmonella type showed variation in the viability flock from flock in one year or from year to year, though *S. enteritidis* caused heavier

TABLE 6 *Viability of broiler chickens at the 42nd day of life*

YEAR	ANNUAL TOTAL*1			HATCHES CONTAMINATED WITH SALMONELLA		
	No. of hatches	Average	Minimum ~max.	No. of hatches	Average	Minimum ~max.
1954	23	24.6%*2	0.8~71.3%	8-P,T,S*3	21.0%	0.8~55.5%
'55	17	80.4*2	48.8~96.9	2-N	59.6	48.8~70.4
'56	18	76.1	70.3~96.3	1-N	96.3	
'57	42	80.0	35.5~98.5	23-E,N,S	74.5	35.5~97.1
'58	34	91.7	74.8~98.3	16-N,S	90.0	74.8~98.0
'59	34	91.2	64.3~97.8	20-E,N,S	92.5	84.1~97.8
'60	44	89.5	59.6~95.7	11-E,N	91.0	84.4~95.7
'61	43	91.0	66.4~97.6	1-S	66.4*4	
'62	44	93.6	87.2~99.9	0		

*1 Feeding of commercial feed and the use of sulfonamides for the control of coccidiosis was started from 1957.
Furazolidone (0.01%) medication was started late in 1960.
Each hatch consisted of 1,000~3,000 chickens

*2 Viability rate at the 35th day

*3 P-*S. pullorum*, T-*S. thompson*, S-*S. senftenberg*, N-*S. newington*, E-*S. enteritidis*

*4 Sulfa drug poisoning

loss than *S. newington* or *S. senftenberg* in 1957. As a general rule, the viability rate of the annual total increased at the latter stage of this study.

Wild rats and other animals as carriers When the first outbreak of *S. newington* infection in chickens occurred in 1955, wild rats (*Rattus norvegicus*) were suspected of introducing the Salmonella into the farm in consideration of the farm conditions. However, it was impossible to decide whether the rats had been carriers of *S. newington* before the outbreak or whether they had become infected from the chickens, because the rats were caught about 2 months after the outbreak.

After the outbreak, *S. newington* or *S. enteritidis* was isolated from the rodents and chickens as indicated in table 2. From these data, it was evident that rats and chickens had an interrelationship in the Salmonella infection.

There was one pig giving Salmonella positive feces in 1957. This pig may have been infected by feeding on dead embryos or chicken carcasses infected with Salmonella as pointed out by HINSHAW et al.[5]

Maintenance of a Salmonella free status in the farm Hatchery sanitation had been improved since the initial Salmonella problem on the farm. Although the New Hampshire chickens studied here had been a closed flock, day old chicks of White Leghorn or other breeds had been introduced almost every year into the farm from domestic or foreign hatcheries. In order to check Salmonella infection, cloacal swab culture was done on the chicks of a day old and the Salmonella negative chicks were maintained in isolation

buildings.

Two of 8 flocks introduced and checked during the period from 1957 to 1961 gave *S. bareilly* (in 1957) and *S. pullorum* (in 1959) respectively. The Salmonella positive flocks were rechecked later bacteriologically or serologically. The same Salmonella organisms, however, have not been isolated from dead chicks and other specimens from this farm (tab. 1).

S. anatum was recovered from 3 chicks aged 3~10 days of a hatch in 1967. No effort was made to search for the infection source.

Discussion

Snoeyenbos stated recently, in a short review of the Salmonella problem, that it is his opinion that transmission of Salmonella via the hatching eggs constitutes the major day-to-day source of flock infection. He emphasized also that improved methods to derive and identify a Salmonella-free stock are urgently needed. Moreover, maintenance of a Salmonella-free poultry flock by commercially practical isolation management is an important problem.

The present study indicates that serological procedure was no value for the eradication of salmonellosis of chickens, excepting the pullorum disease. Thus, it is generally accepted that a routine method for preventing paratyphoid infection is to obtain replacement stock and hatching eggs from a source that is known to be paratyphoid free.

The culture of cloacal swabs has been considered an impractical procedure for the detection of paratyphoid infection as described by Williams. However, he stated also that the procedure may be employed as a general measure in the detection of supply flocks that may contain Salmonella carriers. The data given here indicate that the single cloacal swab culture test for growing chickens produced a Salmonella free status after 3 years.

A number of reports have indicated that Salmonella organisms which invaded naturally baby chick bodies disappeared according to the lapse of time after hatching. Hamada et al.[2] described that *S. senftenberg*, *Salmonella thompson* or *S. bareilly* were seldom detected from naturally infected chicks sacrificed more than 5 days after hatching. The frequency of the detection of the organisms from chicks decreased after 5 days of age. Moreover, Hamada et al.[3] stated that *S. senftenberg* naturally invading newly hatched chick bodies in the incubator was detectable with considerable frequency during the first 7 days, and afterwards the frequency of detection decreased, though the organism itself was carried for 20~30 days and it tended to lurk in the digestive and respiratory organs especially. Some reports[6,17] indicate that the decline in the isolation percentage from cloacal swabs coincides with the increasing age of the birds infected artificially when they were babies. Milner & Shaffer described that only one of 40 chicks infected orally with a strain of *Salmonella montevideo* was found to be eliminating

Salmonella in the feces after 50 days, and about half of the chicks had become negative by the end of the third week.

On the other hand, there appears to be little information on the duration of infection or shedding or both in growing chickens, which were naturally infected at earlier age. In the present study (tab. 4), any bird giving a positive cloacal swab did not yield Salmonella at necropsy. This indicates that transient intestinal infection and spontaneous recovery occurred frequently in the flock of growing chickens infected. The present authors adopted the cloacal swab culture test on growing chickens by the age of about 50 days in order to increase the frequency of Salmonella isolation. Recently, MORRIS et al. reported that a flock of 3-week-old breeder chickens heavily infected with Salmonella indicated the infection rate of 76%, and the rate decreased to 10% at 9 weeks of age. The infection rate at 7 weeks of age was about two times as high as that at 9 weeks of age.

In this study, stock birds were taken from many young birds of different hatches. This means that the frequency with which Salmonella infected chickens were selected for breeders would be reduced.

SIEBURTH reported that a 0.01% feed level of furazolidone reduced mortality in day-old chicks orally inoculated with *S. typhimurium*, but failed to decrease the incidence of intestinal carriers. The present authors (unpublished data) also observed that the same level of the drug did not inhibit the incidence of natural carrier chickens. *Salmonella potsdam* was isolated from dead day-old chicks and cloacal swabs from chicks in a flock of broilers. Chicks of the flock were fed furazolidone-medicated feed until the 10th week of life. During this period, several birds yielded Salmonella positive cloacal swabs. About 10% of the birds necropsied and examined bacteriologically at 10 weeks of age were intestinal carriers, and gave the Salmonella positive organ pools on the cultivation of the entire organs or intestines, Thus, the low level of furazolidone does not reduce the incidence of carriers, but it seems to minimize the spread of infection in a flock. Therefore, the successful result in the eradication of paratyphoid infection from the farm would be enhanced, to some degree, by the use of furazolidone-medicated feed from the latter half of 1960.

In the present experiment on the duration of shedding Salmonella, the isolation percentage of Salmonella from cloacal swabs was low. This seems to be due to the cultivation method used. SNOEYENBOS et al.[17] incubated enrichment broth inoculated for 48 hrs at 43°C before subculturing on brilliant green agar and they obtained a high detection percentage in Salmonella isolation from cloacal swabs. In the experiment (tab. 4) birds harboring Salmonella at necropsy were not detected by the cloacal swab culture at 7-day intervals. SNOEYENBOS et al.[17] indicated that Salmonella was isolated from gut sections with higher frequency

than that of isolation from cloacal swabs.

The authors made a cultural examination of dead chicks, dead embryos, hatcher chick fluff samples and cloacal swabs for detection of Salmonella infection in T farm. As pointed out by SNOEYENBOS et al.[17] these approaches seem to have only limited dependability. In a previous paper[8], the present authors stated that Salmonella serotypes in the fluff samples from a series of hatches represent, to a considerable extent, those occurring in the dead embryos from the same hatchery. However, in this study, no Salmonella was isolated from dead embryos, but only from fluff samples. Therefore, to obtain accurate information on Salmonella infection of a flock, combined samples should be used for examination. In addition, the number of samples should be as large as possible. From this view point, the litter sample culturing method described by SNOEYENBOS et al.[16] seems to be easy and to detect Salmonella infection of a chicken flock with accuracy.

In 1967, *S. anatum* infection was observed in a broiler flock. No effort was made to ascertain the source of the infection. It remains unsolved whether or not *S. anatum* isolated in 1967 was a spontaneous mutant[18] from *S. newington* which had been prevalent previously and might have been still in existence. However, it is reasonable to presume that this type of Salmonella was introduced newly to the farm, because *S. newington* was not detected in the same year in spite of the examination of many dead chicks and hatcher chick fluff samples. HASHIMOTO et al.[4] found recently *S. anatum* in domestic fish meal. Therefore, the above mentioned *S. anatum* infection may have been caused by contaminated commercial feed.

Wild rats apparently had a role in the Salmonella infection of chickens on the farm as can be seen from table 2. It is logical to believe that the first outbreak of *S. enteritidis* infection of chickens was caused by wild rats acting as carriers according to the description by WILLIAMS. The senior author[11] investigated the response of wild rats to oral inoculation with *S. pullorum*, *S. newington* or *S. enteritidis*, and the pathogenesis, transmission and carrier state were evaluated. From the study, it was found that *S. enteritidis* appeared to be transmitted from rat to rat more readily than *S. newington*. However, *S. newington* caused a longer carrier state in the rats infected at an earlier age. From these findings, it may be concluded that wild rats play an important role in the dissemination of Salmonella infection among chickens by harboring Salmonella for a long time in their bodies and in their colonies. However, wild rats appear to need more frequently the existence of infected chickens in the transmission of *S. newington* than in *S. enteritidis*. A more intimate interrelationship between chickens and wild rats seems to be necessary for *S. newington* infection than *S. enteritidis*.

Wild rats acquire the Salmonella infection from infected chickens and continue to disseminate it to chickens.

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