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## CULTIVATION OF SAMPLES OF HATCHER CHICK FLUFF, FLOOR LITTER AND FECES FOR THE DETECTION OF SALMONELLA INFECTION IN CHICKEN FLOCKS\*

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Salmonella status on a farm was investigated by the cultivation of samples of hatcher chick fluff, floor litter and feces from a small number of chickens of breeder flocks or progeny flocks. Five lots of progeny chicks were found to be infected with *Salmonella typhimurium* or *Salmonella enteritidis* on the basis of the detection of the organisms from litter and/or feces, and 2 of the 5 lots gave Salmonella-positive fluffs. The hatcher-fluff-culturing method could not detect *S. typhimurium* infection in the breeder chickens from which hatching eggs were obtained.

The effect of the import of chicks carrying Salmonella on the Salmonella status of chickens in Japan was discussed.

### INTRODUCTION

It is well known that bacteriological examination of dead embryos is useful in finding Salmonella carriers in stock birds, or in finding Salmonella infections in progeny from the same hatch. Moreover, examination of dead embryos seems to give information on Salmonella contamination of incubators. MIURA et al. indicated that bacteriological examination of hatcher chick fluff samples, especially from a series of hatches, may be used for the measurement of Salmonella contamination in commercial hatcheries, in place of the examination of dead-in-shell chick embryos. Recently SATO et al. described that *Salmonella newington* infection of breeder chickens and of their progeny could be detected on a farm not by the examination of dead embryos, but by that of fluff samples.

SNOEYENBOS et al.<sup>10)</sup> found that isolation of Salmonella from either floor- or nest-litter samples was applicable as the index of flock infection. A formula of the examination procedure to be used for field work has been given<sup>9)</sup>. SNOEYENBOS et al.<sup>11)</sup> applied the litter-sample culturing to identify and maintain Salmonella-free commercial chicken breeding flocks.

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This paper describes isolation of *Salmonella* from samples of hatcher chick fluff, floor litter or feces from breeding chickens and their progeny on a farm. It will also give information on the difference of dependability among the 3 sampling methods for monitoring the *Salmonella* status of the chicken flocks.

## MATERIALS AND METHODS

### Sampling and cultivation of hatcher chick fluff

Eggs from different breeder flocks of New Hampshire chickens and those of a meat-type on the farm were set in separate incubators. One lot of hatching eggs consisted of those set simultaneously into 3~8 incubators. A fluff sample was collected from each incubator after removal of chicks. A dose of about 0.2 g of the sample was inoculated into about 15 ml of selenite broth and incubated for 18 hrs at 37°C. A loopful of the broth culture was streaked on MacConkey agar plate. After the first isolation of *Salmonella* from fluff sample in late 1970, the broth inoculated was incubated for 24 hrs at 43°C and subcultured on brilliant-green agar plate. In 1971, SBG Sulfa broth (Eiken) was used in place of selenite broth. Several *Salmonella*-like colonies from each sample were examined serologically and biochemically.

### Sampling and cultivation of floor litter or feces

Progeny broiler chickens: Each lot of chicks collected from different incubators was divided into 2~4 flocks. Each flock consisting of 2,000~7,000 chicks was kept in one pen. Floor litter samples composed of fine dry feces were collected into plastic bags. Two samples were taken from representative sites of each pen, 2 times on the ages of 2 and 10 weeks in 1969 and early 1970.

From late 1970, floor litter and newly passed feces of a small number of chickens were sampled simultaneously, as a rule. One or 2 floor litter samples were collected from each pen, 1~4 times on the ages of 1~10 weeks. Pieces of feces were sampled with sterile chopsticks from each bird into sterile tube. On an average 5 chickens (1~9 chickens) were selected for sampling arbitrarily from each flock.

A dose of 5 or 10 g of the litter samples was inoculated in tenfold volume of SBG Sulfa broth and incubated at 43°C. Incubation period was 48 hrs until early 1970. Then it was changed to 24~30 hrs. The broth culture was streaked on brilliant-green agar plate. Several *Salmonella*-like colonies were checked from each sample by routine method. Fecal sample (about 5 g) from each bird was cultured into about 20 ml of SBG Sulfa broth and mashed with a glass rod. Further procedure was the same as for litter samples.

Breeder chickens: Each breeder flock occupied one pen. As a rule, 2 floor litter samples containing rice straws and feces were collected from each pen 1 to 6 times at 1~4 weeks intervals from November of 1970 to March of 1971. At the same time, newly passed feces were sampled from about 5 birds per flock as indicated in table 3. Cultivation was done by the procedure described above.

## RESULTS

### Relationship between *Salmonella* isolation from hatcher fluffs and infection

TABLE 1 Relationship between *Salmonella* isolation from hatcher chick fluff and infection of chicken flocks

YEAR	FLUFF	SAMPLES FROM CHICKS HATCHED	
		floor litter	feces
1968	$\frac{0}{230 \text{ samples (from 39 lots)}}$ *1	ND*2	ND
1969	$\frac{0}{117 (18)}$	$\frac{0}{208 \text{ samples (from 52 flocks of 19 lots)*5}}$	ND
1970*3	$\frac{3 (2)}{271 (60)}$	Before the 1st <i>Salmonella</i> isolation from fluff	ND
		$\frac{0}{88 (22-7)*6}$	
		After the 1st <i>Salmonella</i> isolation from fluff	$\frac{28 \text{ samples (from 6 flocks of 2 lots)}}{44 \text{ samples (from 6 flocks of 2 lots)}}$
		Lots*7 containing <i>Salmonella</i> -positive fluffs	
$\frac{4 (2-1)}{4 (2-1)}$			
Lots*7 not containing <i>Salmonella</i> -positive fluffs			
$\frac{2 (2-2)}{40 (28-14)}$	$\frac{7 (2-1)}{125 (31-14)}$		
1971*4	$\frac{0}{33 (11)}$	$\frac{0}{70 (20-9)}$	$\frac{1 (1-1)}{220 (20-9)}$

\*1: No. positives/No. samples tested

\*2: Not done

\*3: *S. enteritidis* was isolated.

\*4: *S. typhimurium* was isolated.

\*5: Sampled from Sept. to Dec.

\*6: Sampled from Jan. to Feb.

\*7: Sampled from late Nov.

Detection of *Salmonella* infection in chicken flocks

TABLE 2 Examples of *Salmonella* detection from the samples of broiler chickens

LOT NO.	FLUFF	FLOCK NO.	1 WK* <sup>1</sup>		2		4		5		7		10		SEROTYPE OF ISOLATES
			floor litter	feces	f. l.	f.	f. l.	f.							
70-46	2/6* <sup>2</sup>	1* <sup>3</sup>	. * <sup>4</sup>	.	.	.	.	.	.	.	.	4/5* <sup>2</sup>	.	.	<i>S. enteritidis</i>
		2* <sup>3</sup>	.	.	.	.	.	.	.	.	.	1/5	.	.	
		3	.	.	.	.	.	.	.	.	.	5/5	2/2	2/5	
		4	.	.	.	.	.	.	.	.	.	3/5	2/2	5/9	
70-55	0/3	1* <sup>3</sup>	0/2	0/1	1/1	0/5	0/1	0/5	.	.	.	.	.	.	<i>S. enteritidis</i>
71- 4	0/3	1* <sup>3</sup>	.	.	0/1	1/5	0/1	0/5	0/2	0/5	0/2	0/5	.	.	<i>S. typhimurium</i>
		2	.	.	0/1	0/5	0/1	0/5	0/2	0/5	0/2	0/5	.	.	

\*<sup>1</sup>: Age of chickens at which samples were taken.

\*<sup>2</sup>: No. positives/No. samples tested

\*<sup>3</sup>: Flocks of the meat-type chickens

\*<sup>4</sup>: Not sampled

of chicks hatched

Cultivation of hatcher chick fluffs had been made to monitor *Salmonella* infection of the flocks. Since late 1968, breeder replacement chicks of a meat type have been introduced periodically from a farm in an eastern state of the United States of America. In order to check the introduction of *Salmonella* by the imported chickens, floor litter samples from broiler chickens which were hatched from the breeders and from New Hampshire breeder chickens which had been kept on the farm were examined in late 1969 and early 1970. No *Salmonella* was isolated as indicated in table 1.

Fluffs from 3 hatches of 2 lots gave *Salmonella enteritidis* twice at interval of 4 weeks in September and October of 1970. Then bacteriological examination of fecal and litter samples from broiler flocks was started. As shown in table 1, samples of litter or feces tested indicated high frequency of *Salmonella* isolation in the lots containing *Salmonella*-positive fluffs. On the other hand, also in 3 lots not containing *Salmonella*-positive fluffs, *Salmonella* infection of chicken flocks was detected by the cultivation of litter and/or feces. Clinical problems due to the *Salmonella* infection were not recorded. Table 2 shows examples of *Salmonella* isolation from litter or fecal samples from broiler chickens. Chickens of the lots not containing *Salmonella*-positive fluffs appeared to be infected slightly with *Salmonella*, though sampling was not made regularly.

TABLE 3 *Detection of Salmonella from floor litter or feces of breeder chickens*

FLOCK NO.	SOURCE OF CHICKENS	IN NOV. OF 1970		SAMPLES* <sup>1</sup>		SEROTYPE OF ISOLATES
		age (months)	No. of chickens	floor litter	feces	
1	New Hampshire chickens produced in the farm	19	500	Removed		
2	"	15	780	0/4 (0/2)* <sup>2</sup>	0/15 (0/3)* <sup>2</sup>	
3	"	11	550	0/3 (0/2)	0/16 (0/3)	
4	"	11	550	0/3 (0/2)	0/16 (0/3)	
5	"	7	560	0/4 (0/2)	0/19 (0/4)	
6	Meat-type chickens imported from U. S. A.	16	800	Removed		
7	"	13	360	0/2 (0/1)	0/15 (0/3)	
8	"	10	600	1/6 (1/3)	3/20 (2/4)	<i>S. typhimurium</i>
9	"	8	700	0/6 (0/3)	0/20 (0/5)	
10	"	6	940	2/12 (2/6)	21/41 (8/8)	<i>S. typhimurium</i>
11	"	3	1,100	10/13 (6/6)	16/38 (6/7)	<i>S. typhimurium</i> <i>S. blockley</i> * <sup>3</sup> <i>S. enteritidis</i> * <sup>3</sup>

\*<sup>1</sup>: Sampled at intervals of 1~4 weeks from Nov. of 1970 to Mar. of 1971

\*<sup>2</sup>: No. positives/No. samples tested (No. positives/No. sampling times)

\*<sup>3</sup>: One isolation

Thus, fluff-sample culturing could indicate in advance Salmonella infection in 2 of the 5 lots of progeny chickens, in which Salmonella was detected by the floor litter and/or fecal cultivation.

#### Detection of Salmonella from samples of floor litter and feces from breeder chickens

As shown in table 3, Salmonella was not isolated from the domestic chickens, but only from the imported breeders, though sample size from the domestic birds was small. There was a flock (No. 10) in which Salmonella was isolated more frequently by the fecal culture compared with the litter-sample culturing. Since, unfortunately, flocks 1 and 6 had been removed when the examination for Salmonella was started, it remained uncertain whether or not *S. enteritidis* isolated from fluffs and chicks originated from the breeder chickens. Eggs from flocks 8 and 10, in which *Salmonella typhimurium* was isolated, had been hatched, but no Salmonella was detected from fluff samples of the hatches corresponding. On the other hand, the organism was isolated from a broiler chicken (Lot 71-4 in table 2). Thus, so far as *S. typhimurium* is concerned, fluff-sample culturing gave no information on Salmonella status of the breeder chickens and their progeny.

*S. typhimurium* cultures obtained during this study belonged to the phage type Ia (Anderson's old scheme)<sup>1)</sup> and to the biotype 10<sup>1)</sup>.

#### DISCUSSION

In this study, chicks of the lots containing Salmonella-positive fluffs were contaminated heavily with the same Salmonella. However, hatcher fluff cultivation could not always detect flock infection with Salmonella. MORRIS et al. reported that hatchery samples including air samples indicated much lower frequency of Salmonella isolation compared with samples from flocks of breeders and broilers. In addition, SNOEYENBOS et al.<sup>11)</sup> described that they could not isolate Salmonella from fluffs of 18 hatches, in which hatching eggs were obtained from Salmonella-infected breeding chickens, and a great part of flocks of their progeny chicks were infected with the same Salmonella as in the breeders. However, the fluff-culturing method appears to be most suitable to monitor continuously the Salmonella status of hatchery environment. Investigation on improvement of the method, for example, cultivation of large dose of fluffs, is needed.

The data of this study indicated that the floor-litter culturing was efficient to know Salmonella infection of chicken flocks. Moreover, it should be noted that the cultivation of feces from a small number of birds gave much information on Salmonella in the flocks.

As indicated in table 3, *S. typhimurium* and other Salmonella were isolated from the imported breeder chickens. *S. typhimurium* had not yet been isolated from any material on this farm<sup>7)</sup>. It was probable that the imported meat-type

chickens carried the *Salmonella*, though record on clinical problem during the quarantine was not available. A report<sup>2)</sup> indicated that the same meat-type chickens which were imported from the United States of America in the spring of 1970 had *S. typhimurium* infection during and after the quarantine.

According to the survey by SAKAZAKI & NAKAYA, a total of 1,680 *Salmonella* cultures belonging to 25 serotypes were isolated from chickens and eggs during the period from 1946 to 1963 in Japan. The serotypes were arranged in descending of isolation frequency as follows; *Salmonella pullorum* (31.8%), *Salmonella senftenberg* (22.7%), *Salmonella thompson* (8.5%), *Salmonella give* (8%), *Salmonella bareilly* (6.1%), *Salmonella potsdam* (5.4%), *Salmonella new brunswick* (4.6%) and so on. Recently SHIMIZU described *Salmonella* isolation from chicks which were imported mainly from the United States of America and died at the time of arrival and during the quarantine for the first 2 weeks of life. During 4 years from 1965 to 1968, 303 *Salmonella* cultures of 20 serotypes were isolated. Eleven of the 20 types had not yet been isolated from chickens in Japan. The cultures included *S. typhimurium* (32%), *Salmonella infantis* (22.4%), *Salmonella heidelberg* (20.5%), *S. thompson* (4.6%), *Salmonella blockley* (2.6%), *Salmonella oranienburg* (2.6%), *S. enteritidis* (2.6%) and so on. No isolation of *S. pullorum* was recorded. Among the serotypes described above, *S. infantis*, *S. heidelberg* and *S. blockley* were the newly added ones. Rearing of imported poultry flocks contaminated with *Salmonella* other than *S. pullorum* has not been restricted in Japan, though clinical problem occurred on the flocks during the quarantine. During the period from 1965 to 1968, about 8.8 million chicks (mainly breeders) were imported. According to a recent survey<sup>6)</sup> on the reports of *Salmonella* isolation from domestic chickens, type distribution of *Salmonella* of chicken origin in Japan has changed remarkably for the past several years, almost corresponding to the distribution in imported chicks shown by SHIMIZU. Excluding *S. pullorum*, *S. typhimurium* or *S. thompson* has been dealt most frequently in the reports. Isolation of *S. infantis* and *S. enteritidis* increased in frequency. *S. blockley* infection was observed among domestic chickens.

Thus, it should be considered more seriously that foreign chicks carrying *Salmonella* have been playing a role in changing the *Salmonella* status in Japan.

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