Prevalence and Persistence of *Salmonella* in Broiler Chicken Flocks

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ABSTRACT. Cecal contents of 2,345 broiler chickens consisting of 28 flocks originated from 12 farms were examined for the prevalence of *Salmonella* to know the actual status of infection with *Salmonella* in the chicken flocks. *Salmonella* was isolated from 336 (14.3%) samples. From these isolates, eight serovars were identified. Of the 336 *Salmonella* isolates, 242 (72.0%) were serotyped as *S.* Blockley, 60 (17.9%) *S.* Hadar, 15 (4.5%) *S.* Bredeney, nine (2.7%) *S.* Schwarzengrund, four (1.2%) *S.* Anatum, three (0.9%) *S.* Enteritidis, two (0.6%) *S.* Ohio, and one (0.3%) *S.* Livingstone. The same serovars of *Salmonella* were repeatedly found in the chickens from the same farms. *S.* Typhimurium and *S.* Enteritidis were detected in pooled broken eggshell samples collected from the hatchery. Analysis of plasmid profiles revealed 11 patterns of *S.* Blockley and seven patterns of *S.* Hadar. Strains of the same plasmid profiles of *S.* Blockley were isolated repeatedly from the same farm over one year after the first isolation.—KEY WORDS: broiler, hatchery, poultry farm, prevalence, *Salmonella.*

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In the past decade, the incidence of systemic infection with *Salmonella* such as *Salmonella* Typhi and *S.* Paratyphi has declined markedly all over the world, but food-borne infection such as food poisoning due to other *Salmonella* serovars has increased [16, 27]. Recently, human infection with *S.* Enteritidis due to the consumption of chicken meat and egg products has increased dramatically around the world including Japan [2, 19, 27]. Broilers are widely accepted as an important reservoir for human salmonellosis. Although reports on the contamination of the broiler chicken meat in slaughterhouses and retail shops have been published all over the world [3, 4, 7, 11–14, 28], little is known about the incidence of *Salmonella* in broiler chickens in Japan. This study was designed to investigate the incidence of *Salmonella* in broiler farms to assess the status of infection with *Salmonella* in flocks of broiler chickens.

MATERIALS AND METHODS

Sample collections: During the period from April 1995 to March 1997, a total of 2,345 commercial broiler chickens sent from 12 farms to a slaughterhouse in Tochigi Prefecture in Japan were examined for prevalence of *Salmonella* in the cecal contents. In the present investigation, a flock was defined as the chickens sent from one farm in one day. The cecal contents were collected aseptically after evisceration. In May and June 1996, samples were also collected in the hatchery supplying chicks to the farms. A total of 27 samples consisting of eight pooled broken eggshell samples taken from a broken eggshell reservoir on different days, 15 broken eggshell samples taken from different hatcher trays and four lining paper samples of transport boxes from breeder farms taken on different days were examined for *Salmonella*. All the samples were put into plastic bags, cooled in an icebox and immediately transported to our laboratory.

Isolation and identification of Salmonella : To each bag of cecal contents, added was 9 volumes of selenite cystine broth (SCB, Nissui). These samples were incubated at 37°C for 48 hr, then each sample was inoculated onto a brilliant green agar (BBL) plate supplemented with 20 μg novobiocin/m*l* (BGN) and a desoxycholate hydrogen sulfide lactose (DHL) agar (Nissui) plate. The plates were incubated at 37°C for 24 hr. Three suspicious colonies morphologically similar to *Salmonella* from each plate were subcultured for biochemical examinations. Biochemical characteristics were examined on triple sugar iron medium (Eiken) and lysine indole motility medium (Nissui). When reactions of typical *Salmonella* were seen, additional biochemical tests were performed as described by Barrow and Feltham [5]. The serotyping of *Salmonella* was done according to Popoff and Le Minor [25].

Each of pooled broken eggshell and broken eggshell samples from hatcher trays was mixed thoroughly and approximately 200 g was placed in a sterile flask containing 9 volumes of Enterobacteriaceae Enrichment Mannitol broth (EEM, Nissui). Each box lining paper was cut into pieces with scissors and immersed in one liter of phosphate-buffered saline (PBS, pH 7.2), which was then centrifuged at 3,500 rpm for 30 min to collect the feces on the paper. The feces was added to 9 volumes of EEM, which was incubated at 37°C for 24 hr. One ml of each EEM culture was transferred to 9 m*l* of SCB, which was then incubated and plated as described previously.

Plasmid extraction and detection: Plasmids were extracted from *S.* Hadar and *S.* Blockley according to the method previously described by Nakamura *et al.* [24]. Plasmid DNA extracts were electrophoresed in 0.6% (wt.:vol.) agarose gels in Tris-acetate, EDTA buffer. The

molecular weight of plasmids was determined by referring to the plasmid markers of a supercoiled DNA ladder (GIBCOBRL, Gaithersburg, MD, USA).

RESULTS

Salmonella was isolated from 336 (14.3%) of 2,345 samples and 18 of 28 flocks (64.3%) in nine of 12 farms (Table 1). From these 336 isolates, eight serovars were identified. Each of *Salmonella* -positive samples yielded a single serovar. Of 336 *Salmonella* isolates, 242 (72.0%) were serotyped as *S*. Blockley, 60 (17.9%) *S*. Hadar, 15 (4.5%) *S*. Bredeney, nine (2.7%) *S*. Schwarzengrund, four (1.2%) *S*. Anatum, three (0.9%) *S*. Enteritidis, two (0.6%) *S*. Ohio and one (0.3%) *S*. Livingstone. Of 28 flocks examined, 13 flocks were positive with a single *Salmonella* serovar and five other flocks were positive with two serovars. *S*. Enteritidis was detected in only one flock from farm B. The individual flock isolation rate varied from negative-farms (farms J, K and L) to high prevalence of infection (49.7%) in farm A (flock No. 5). Although only one serotype of *S*. Blockley was found in farm A, *Salmonella*-positive rate in this farm varied from 5% to 49.7%. *S*. Hadar and *S*. Bredeney were isolated from flock No. 14 in farm C and again from flock No. 16 about five months later. *S*. Hadar was also isolated again in two flocks

Table 1. Isolation of *Salmonella* and plasmid patterns of isolates in broiler chicken flocks at a slaughterhouse

Farm	Flock No.	Date of sampling	No. of samples (%)[a)]	Serovars (No. of isolates)	Plasmid patterns[b)]
A	1	96/02/01	38/120 (31.7)	*S*. Blockley (38)	a:2, b:9, c:9, d:8, i:2, NT:8
	2	96/02/09	29/100 (29.0)	*S*. Blockley (29)	c:23, d:3, j:1, NT:2
	3	96/02/10	23/ 70 (32.9)	*S*. Blockley (23)	c:21, j:1, NT:1
	4	96/04/24	8/ 70 (11.4)	*S*. Blockley (8)	a:1, c:3, d:2, e:2
	5	96/09/25	72/145 (49.7)	*S*. Blockley (72)	a:2, c:30, d:2, e:8, j:5, k:25
	6	96/09/27	35/150 (23.3)	*S*. Blockley (35)	c:27, d:1, e:1, f:1, j:4, NT:1
	7	97/02/27	5/100 (5.0)	*S*. Blockley (5)	c:1, d:4
	8	97/0304	32/140 (22.9)	*S*. Blockley (32)	c:15,d:11, f:4, g:1, h:1
	Subtotal		242/895 (27.0)		a:5, b:9, c:129, d:31, e:11, f:5, g:1, h:1, i:2, j:11, k:25, NT:12
B	9	95/12/28	8/ 50 (16.0)	*S*. Hadar (5), *S*. Enteritidis (3)	o:1, p:2, q:1, NT:1
	10	96/01/12	0/ 70 (0.0)		
	11	96/03/05	0/ 70 (0.0)		
	12	96/03/16	0/ 70 (0.0)		
	13	96/04/09	0/ 70 (0.0)		
	Subtotal		8/330 (2.4)		o:1, p:2, q:1, NT:1
C	14	95/10/25	30/ 70 (42.9)	*S*. Hadar (18), *S*. Bredeney (12)	n:8, o:6, r:4
	15	95/12/28	0/ 40 (0.0)		
	16	96/03/16	5/ 70 (7.1)	*S*. Hadar (2), *S*. Bredeney (3)	o:1, p:1
	Subtotal		35/180 (9.4)		n:8, o:7, p:1, r:4
D	17	95/06/22	3/100 (3.0)	*S*. Hadar (3)	l:3
	18	96/04/09	2/ 70 (2.9)	*S*. Hadar (2)	o:1, q:1
	Subtotal		5/170 (2.9)		l:3, o:1, q:1
E	19	95/10/25	2/ 70 (2.9)	*S*. Hadar (2)	r:2
	20	95/12/28	0/ 50 (0.0)		
	Subtotal		2/120 (1.7)		r:2
F	21	96/01/12	6/ 70 (8.6)	*S*. Hadar (2), *S*. Anatum (4)	r:2
G	22	95/10/14	26/120 (21.7)	*S*. Hadar (26)	m:26
H	23	95/07/20	10/130 (7.7)	*S*. Schwarzengrund (9), *S*. Livingstone (1)	
I	24	96/03/05	2/ 70 (2.9)	*S*. Ohio (2)	
J	25	95/06/22	0/ 50 (0.0)		
	26	95/04/24	0/ 70 (0.0)		
	Subtotal		0/120 (0.0)		
K	27	96/03/05	0/ 70 (0.0)		
L	28	96/02/10	0/ 70 (0.0)		
	Total		336/2345 (14.3)		

a) No. of positive samples/No. of samples examined (%).
b) Plasmid pattern of *S*. Blockley of *S*. Harder (refer to Fig. 1 and Fig. 2 respectively): No. of strains examined.
NT=Not tested.

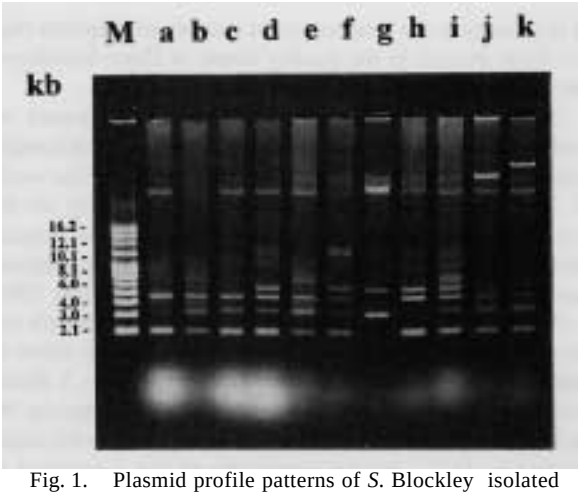

Fig. 1. Plasmid profile patterns of *S.* Blockley isolated from chicken. Lane M = Molecular weight marker in kilobase pairs (GIBCOBRL, Gaithersburg, MD); a to k = Type a to type k of *S.* Blockley respectively. Numbers at the left are molecular weight markers in kilobase pairs (kb).

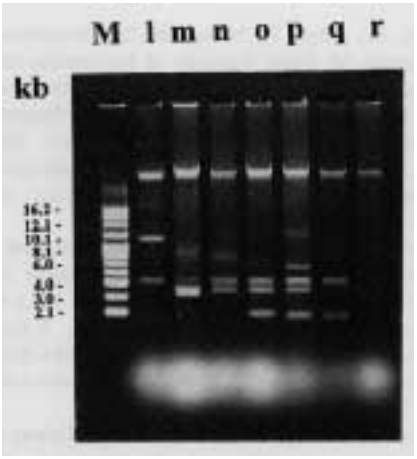

Fig. 2. Plasmid profile patterns of *S.* Hadar isolated from chicken. Lane M = Molecular weight marker in kilobase pairs (GIBCOBRL, Gaithersburg, MD); l to r = Type l to type r of *S.* Hadar. Numbers at the left are molecular weight markers in kilobase pairs (kb).

of farm D 10 months after the first isolation.

In Figs. 1 and 2, plasmid DNA profiles of *S.* Blockley and *S.* Hadar are shown, respectively. *S.* Blockley could be categorized into 11 patterns (Types a to k). These types were similar to each other only with a difference in a few bands (Fig. 1). In order of prevalence, the first was type c, the second type d and then type k (Table 1). *S.* Blockley strains having the same plasmid profile patterns c and d were continuously isolated for over one year. *S.* Hadar was categorized into seven patterns (Types l to r). A few common bands were seen in types l to q (Fig. 2). In addition to reisolation in farm C, *S.* Hadar strain showing pattern o was found also in farm B (flock No. 9) and farm D (flock No. 18) (Table 1). Moreover, the strain of the same plasmid profile type r of *S.* Hadar was also found in different flocks from farms C (flock No. 14), E (flock No. 19) and F (flock No. 21).

Table 2 shows that only *S.* Typhimurium and *S.* Enteritidis were recovered from two of eight samples of pooled broken eggshell. *Salmonella* was not isolated from the broken eggshell from hatcher trays (15 samples) nor from young chick delivery box lining papers (four samples).

DISCUSSION

The high detection rates of *Salmonella* in chicken meat in retail shops and poultry processing plants were reported in Thailand [13], Malaysia [3], Japan [12, 22] and Spain [7]. In Japan, previous studies showed that the contamination rate was higher in chicken meat samples than pork and beef samples [11, 28]. Recently, Carraminana *et al.* [7] reported that cross contamination of *Salmonella* in a Spanish poultry slaughterhouse elevated the contamination rate of air-chilled carcasses to 60% during the processing. This finding suggested also the severeness of the cross contamination of *Salmonella* in poultry products. Jones *et al.* [14] also reported that any increase in *Salmonella* isolation rate in subsequent stages of processing could be a result of cross contamination.

In our study, cecal samples were examined for prevalence of *Salmonella* by conventional culture methods as an indicator of the infection in broilers from the farms. Although prevalence of *Salmonella* in pooled cecal contents

Table 2. Detection of *Salmonella* in a hatchery

No.	Sample type	No. of samples examined	No. of positive samples (%)	Serovars (No. of isolates)
1	Pooled broken eggshell	8	2 (25.0)	*S.* Typhimurium (1) *S.* Enteritidis (1)
2	Broken eggshell from hatcher trays	15	0	
3	Box lining paper	4	0	

in spent hens has been reported [29], none has been reported on the prevalence of *Salmonella* in cecal contents of chickens in Japan. Since *Salmonella* detection of 14.3% in cecal contents in the present study was lower than 65.4% of cecal pools from spent laying hens reported in the U.S.A. [29], 25% of cloacal swab collected from broilers in Thailand [26], and 60 to 80% of poultry carcasses examined in Spain [7], actual infection rate of Japanese chickens with *Salmonella* might be lower than those of foreign countries. This lower infection rate might be due to the difference in incubation temperature, types of enrichment media and selecting plating media used for isolation of *Salmonella* in our study. Nakamura *et al.* (23) have reported isolation of *Salmonella* from the cecal contents of spent hens increased by using Hajna's tetrathionate enrichment broth incubated at 41.5°C for 24 hr.

S. Blockley infection was found only in farm A with a high infection rate. Analysis of plasmids revealed repeatedly the same plasmid profile in these strains. The persistence of *S.* Blockley in farm A was observed for more than one year, although the management of all-in all-out, cleansing, disinfection of poultry house had been applied in this farm. This suggests that such ordinary sanitation and control measures may fail to prevent reinfection with *Salmonella.* Persistent bacterial infectious sources may have been present in the environment of farm A, as the reisolation of the same serotypes of *Salmonella* in empty chicken houses has been reported by Lahellec *et al.* [18]. Elimination of *Salmonella* from broiler chicken flocks is difficult due to many sources of contamination including chicks, feed, rodents, wild birds and insects. From the finding of persistence of *Salmonella* in empty poultry houses for 52 weeks [8], the reinfectious sources might be present in the environment of poultry houses of farm A.

The same plasmid profile patterns of *S.* Hadar types o and r were found in three different farms. The reisolation of *S.* Hadar was observed in two farms. These results implied that the common sources of infection might be present outside these poultry houses. In this case, the hatchery may act as the common source of infection, or if the sources of infection persist inside broiler farms, the grow-out period may be at a greater risk in these farms rather than introduction from hatchery. Because the present study shows further that mixed infection with different serovars such as *S.* Enteritidis, *S.* Bredeney and *S.* Anatum was observed in four out of eight *S.* Hadar-positive flocks, there might be another source in these four farms. Although something in the farm environment might serve as a source of *Salmonella* , the source was not clarified in the present study. Previous study [6] has demonstrated dissemination of *Salmonella* from hatchery to broiler farm. However, association of hatchery with the contamination of *Salmonella* in these broiler farms was not indicated in our study, since the predominant serovars, *S.* Blockley and *S.* Hadar, were not detected in the hatchery which supplied young chicks to the farms examined. This result might agree with the previous observations suggesting that serotypes originating in the hatchery are less important sources of infection than are those present in the poultry house or those introduced into the poultry house during rearing [9, 17, 18].

A number of outbreaks and sporadic cases of gastroenteritis caused by *S.* Hadar and *S.* Blockley in humans and isolation from chickens were reported all over the world [1, 3, 7, 10, 13, 19, 21]. *S.* Hadar and *S.* Blockley are the first and fifth most predominant serovars in the human-imported cases in Japan respectively [20]. It was shown that during the period from April 1985 to December 1994, *S.* Hadar (4.6%) and *S.* Blockley (3.0%) were the fourth and seventh major serovars responsible for sporadic cases of diarrhea in Yamanashi Prefecture [15]. Moreover, *S.* Hadar was also reported as the most common serovar among the isolates from sporadic diarrheal cases reported in Shizuoka Prefecture [21]. Future investigations are warranted to clarify the relationship of *S.* Hadar and *S.* Blockley strains between human and poultry origins to know the significance of chickens as the source of human infection.

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