The role of roof rats (*Rattus rattus*) in the spread of *Salmonella* Enteritidis and *S.* Infantis contamination in layer farms in eastern Japan

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SUMMARY

The prevalence of *Salmonella* in four layer farms in eastern Japan was investigated between 2004 and 2006 to determine the role of roof rats (*Rattus rattus*) in the epizootology of *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*S.* Enteritidis). Persistent *S.* Enteritidis and *S.* Infantis contamination of the environment and pooled egg samples were detected in three out of four layer farms. A total of 113 (13·3%) and 158 (18·6%) out of 851 rats examined were positive for *S.* Enteritidis and *S.* Infantis, respectively. By pulsed-field gel electrophoresis, only one indistinguishable pulsed-field pattern was yielded by *S.* Enteritidis strains from rats, eggs and environmental samples from each of the two contaminated layer farms. Although, a variety of pulsed-field patterns were generated by *S.* Enteritidis isolates from rats, eggs, and the environment of the other contaminated farms, there are, however, some *S.* Enteritidis strains that are closely related clones. These results suggest that roof rats are carriers of *S.* Enteritidis and *S.* Infantis and that persistent *S.* Enteritidis and *S.* Infantis infections in a rat population may play an important role in the spread and maintenance of these pathogens inside the layer premises.

INTRODUCTION

Microbial pathogens of the genus *Salmonella* are among the leading causes of foodborne illness in the world. In Japan, most cases of bacterial food poisoning have generally been attributed to *Salmonella* in the past decade. From 2004 to 2006, the top two *Salmonella* serovars associated with human illness were *Salmonella* subsp. *enterica* serovar Enteritidis (*S.* Enteritidis) and *Salmonella enterica* subsp. *enterica* serovar Infantis (*S.* Infantis). The total number of human salmonellosis cases recorded in Japan from 2004 to 2006 was 3784 cases. From this, a total of 1617 and 246 cases of human salmonellosis were attributed to *S.* Enteritidis and *S.* Infantis, respectively. Details can be found in the Infectious Agents Surveillance Report [1].

Food poisoning outbreaks caused by *S.* Enteritidis in Japan have been attributed to consumption of food associated with contaminated eggs and egg products [2]. In contrast, human foodborne outbreaks caused by *S.* Infantis has been attributed to consumption of contaminated poultry meat products [3, 4].

Egg consumption in Japan is one of the highest in the world. On average, each Japanese person

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consumes 330 eggs annually [5]. Eggs are consumed as omelettes, custards, and in soups. Moreover, most Japanese consume raw eggs or undercooked eggs as part of their native cuisine, which is one of the major factors that may have contributed to high incidences of salmonellosis in Japan.

The concept of the Hazard Analysis and Critical Control Point (HACCP), as observed by the saying 'from farm to table', is generally known to Japanese farmers. The Japanese layer industry has carried out numerous measures to prevent egg contamination in layer houses [6]. Intervention strategies to control and prevent *Salmonella* contamination in poultry farms include *S.* Enteritidis vaccination (optional), 'all-in all-out' production, regular monitoring of feeds, eggs, the environment and breeder flocks for *Salmonella* [7]. However, the Japanese layer industry has not yet realized the important role of rodents in the spread of *Salmonella* contamination in layer farms.

The main route of transmission of *S.* Enteritidis is vertically by the egg. *S.* Enteritidis organisms may colonize the ovaries and peri-ovarian tissues of different breeds of layer chickens, and thus, poses the threat of vertical transmission from breeders to layers and then to the eggs. Consequently, in integrated poultry organizations, infection of breeder flocks with *S.* Enteritidis can lead to rapid dissemination of the organism to commercial layer flocks, which makes it important to stock layer houses with *Salmonella*-negative birds from uninfected breeders to prevent egg contamination. However, *S.* Enteritidis can also be cultured from insects and animals such as rodents living in and around hen houses [8–11]. Rodents have been considered as the most important vector of *S.* Enteritidis in contaminated layer farms [9, 11]. In Japan, roof rats (*Rattus rattus*) are generally considered as the dominant rodent species in poultry farms [12]. Even though, rats are commonly found in poultry farms in Japan, the role of rats in the transmission of *Salmonella* contamination in layer farms has not been elucidated.

In this present study, prevalence of *Salmonella* spp. in rats found in contaminated layer farms was investigated. Additionally, *Salmonella* isolation rates on environmental samples and different types of eggs were also conducted. Furthermore, pulsed-field gel electrophoresis (PFGE) analysis of *Salmonella* isolates was performed to elucidate the epidemiological role of rats in *Salmonella* contamination in layer farms.

MATERIALS AND METHODS

Poultry farms

Four layer farms consisting of 23 windowless houses in eastern Japan were monitored for *Salmonella* contamination from January 2004 to December 2006. Farms A, B, and C have reported rodent problems. In farm D, no rodents were observed. All of the farms were windowless multiple-house in-line complexes consisting of several hen houses connected by conveyor belts that transport the eggs to the egg-processing facility for grading and packing. The layer houses are environmentally controlled and operated with automated systems. In all, 30 000–45 000 birds were fed. The 'all-in all-out' system was applied on all farms. Farms are restocked after cleaning and disinfection. Layer flocks are replaced by 120-day-old replacement pullets from *Salmonella*-free breeder flocks when the layer hens are about 800 days old. *Salmonella* monitoring was also regularly conducted in the breeder flocks, hatchery, chicks, and rearing houses. No salmonellae were detected from these poultry facilities.

Rat trapping

Rats were trapped using adhesive traps and pipe traps (custom-made traps by poultry workers). Traps were baited with chicken feed and various kinds of grains and seeds and placed where the rats regularly visited. Traps were checked every 24–48 h. A total of 818 dead rats obtained from adhesive traps were examined for *Salmonella* infection. Thirty-three live rats were caught by pipe traps. Live rats were killed by chloroform inhalation. All of the rats trapped were placed in individual plastic bags on ice for transfer to the laboratory, and cultured within 1–3 days after trapping. In total, 851 rats were examined (Table 1). All rodents submitted for examination were identified as roof rats.

Isolation of *Salmonella* spp. from rats

Rats were cultured individually. Each rodent was disinfected by dousing it with a 3:1 solution of 70% ethyl alcohol and 10% iodine, and the abdominal cavity was opened. Approximately 1–2 g of the heart, spleen, liver, kidney and intestine were collected in aseptic conditions and added to a tube containing 8 ml of heart infusion broth separately. This pre-enrichment medium was then incubated at 37 °C for 48 h. Heart infusion broth culture (1 ml) was then

Table 1. Salmonella *spp. isolation from different organs of rats*

Farm	No. of rats	*Salmonella* spp.	No. of positive (%)	Heart (%)	Liver (%)	Spleen (%)	Kidney (%)	Intestine (%)
A	465	*S.* Enteritidis	51 (11·0)*	15	26	21	9	9
		S. Infantis	146 (31·4)	39	62	61	39	67
B	308	*S.* Enteritidis	46 (14·9)†	11	16	22	12	11
		S. Infantis	12 (3·9)	2	2	5	4	3
C	78	*S.* Enteritidis	16 (20·5)	7	8	12	8	2
D	0	*Salmonella* spp.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Total	851	*S.* Enteritidis	113 (13·3)	33 (29·2)	50 (44·2)	55 (48·7)	29 (25·7)	22 (19·5)
		S. Infantis	158 (18·6)	41 (25·9)	64 (40·5)	66 (41·8)	43 (27·2)	70 (44·3)

n.d., Not done.
* Nineteen rats were both positive for *S.* Enteritidis and *S.* Infantis.
† Nine rats were both positive for *S.* Enteritidis and *S.* Infantis

added to 9 ml Hajna Tetrathionate (HTT) broth (Eiken, Kyoto, Japan) and incubated for 24 h at 42 °C. A loopful of the mixture was then streaked on desoxycholate hydrogen sulfide lactose (DHL) agar (Eiken) for 24 h at 37 °C. At least five suspected colonies from each organ were tested for *Salmonella* identification by standard procedures. Confirmed *Salmonella* spp. isolates were further serotyped for agglutination with *Salmonella* O and H antigens (Denka Seiken, Tokyo, Japan).

Environmental survey of poultry houses

Each poultry house was monitored for *Salmonella* contamination at least once a month. Twenty swabs, 4 × 4 inches of cotton gauze, pre-moistened with 25 ml of sterile double-strength skimmed milk, were dragged over the floor litter, egg belts, house dust and manure in each poultry house every month. A total of 14 020 environmental swabs were tested. Approximately 100 ml of double-strength skimmed milk was added to each environmental swab and incubated for 36 h at 37 °C. Then 1 ml of the culture was added to 9 ml HTT broth and incubated for 24 h at 42 °C. Salmonellae were isolated and serotyped as described above.

Egg samples and culture method

Three types of eggs from layer farms were examined for *Salmonella* contamination.

'Unprocessed' eggs or 'dirty' eggs

These are soiled eggs which were directly collected from the poultry houses and were not washed and cleaned. Eggs were pooled for examination. Each batch of 'dirty' eggs consisted of 90 eggs. A total of 1766 batches (158 940 eggs) of dirty eggs were examined for *Salmonella* contamination.

'Processed' eggs

These eggs were considered 'clean' eggs. The eggs were collected from the egg-processing facility after cleaning and washing. Each batch of 'processed' eggs consisted of 40 eggs. A total of 11 280 batches (451 200 eggs) were tested for *Salmonella* contamination.

'Packed' eggs

These eggs were sold in the supermarket. Representative packed egg samples were sent to the laboratory weekly for *Salmonella* testing. Each batch of packed eggs consisted of 10 eggs. A total of 9010 batches (90 010 eggs) were examined for *Salmonella* contamination.

Culture method for unprocessed or dirty eggs

The shell and the contents of 90 eggs were pooled into a sterilized plastic bag (60 cm × 100 cm). The bags containing the whole eggs were sealed and incubated for 48 h at 37 °C; after which 1 ml of the incubated contents was poured into 9 ml of HTT broth and incubated for 48 h at 42 °C. Salmonellae were isolated and serotyped as described above.

Culture method for processed and packed eggs

Any visible adherent material from the shell was removed and eggs were disinfected with a 3:1 solution of 70 % ethyl alcohol and 10 % iodine, this was performed by dipping the eggs in the solution for at least

2 min; the eggs were then air-dried. Following this they were cracked in aseptic conditions, and the contents of several eggs were pooled in one sterile plastic bag. The bag was massaged until the yolks were completely blended with the albumin. The samples were incubated at 37 °C for 48 h; after which 1 ml of the culture was inoculated into 9 ml HTT broth and incubated at 42 °C for 24 h. Salmonellae were isolated and serotyped as described above.

PFGE analysis of *S*. Enteritidis and *S*. Infantis

A total of 278 *S*. Enteritidis and 166 *S*. Infantis isolates were characterized by PFGE analysis. *S*. Enteritidis and *S*. Infantis isolates were obtained from eggs, rats, and from environmental samples. DNA for PFGE analysis was prepared as described previously [13, 14]. Chromosomal DNA in each plug was digested with 20 U *Bln*I (Takara, Kyoto, Japan) at 37 °C for 18 h. PFGE was performed using CHEF-DR III apparatus (Bio-Rad, Tokyo, Japan) in gels of 1% agarose (Bio-Rad) on 0·5× Tris-borate EDTA buffer (Bio-Rad) for 21 h at 200 V and 14 °C with a pulse time ranging from 2 s to 43·2 s. The gels were stained with ethidium bromide (Bio-Rad) and photographed with an UV illuminator (Atto Systems, Osaka, Japan). DNA lambda ladder was used as the molecular marker (Bio-Rad). DNA fragments were analysed visually and roman letters were used for assigning the different pulsed-field patterns generated.

RESULTS

Prevalence of *Salmonella* infection in rats

A total of 851 rats were examined, of which 243 (28·6%) were infected with *Salmonella* spp. *Salmonella*-infected rats appeared to be normal. No significant clinical findings were observed from the rat samples. In total, 113 (13·3%) rats were infected with *S*. Enteritidis and 158 (18·6%) of the rats were infected with *S*. Infantis. There were 28 (3·3%) rats that were infected by *S*. Enteritidis and *S*. Infantis concurrently. Rodents in farms A, B, C were infected by *S*. Enteritidis, while *S*. Infantis infections among the rodents were detected in farms A and B only. *S*. Enteritidis was the predominant serovar in rodents in farms B (14·9%) and C (20·5%). In contrast, *S*. Infantis was the predominant serovar in farm A (33·9%). There were no rodents caught in farm D.

The highest isolation rates for *S*. Enteritidis-positive rats were obtained from the spleen (48·7%) and liver (44·2%). In contrast, highest isolation rates for *S*. Infantis-positive rats were observed from the intestines (44·3%) and spleen (41·8%) (Table 1).

Isolation of *Salmonella* from environmental samples

Salmonella was isolated from farms A, B, and C. In farm A, *S*. Infantis, *S*. Enteritidis, and *S*. Potsdam strains were isolated. The predominant *Salmonella* serovar in farm A was *S*. Infantis. A total of 165 out of 6480 (2·5%) environmental swabs were positive for *S*. Infantis. In contrast, only nine (0·1%) environmental samples were positive for *S*. Enteritidis. However, *S*. Enteritidis was the only serotype isolated from environmental samples in farm B (1·7%) and farm C (2·1%). Moreover, *S*. Enteritidis contamination on the farm environment significantly decreased after implementation of a rodent control programme, repairing of rat-damaged structures, and stocking with new flocks from September 2005 to March 2006 (Table 2). There were no *Salmonella* isolated from environmental samples in farm D.

Isolation of *Salmonella* from eggs

S. Enteritidis and *S*. Infantis strains were isolated from processed, dirty and packed eggs (Table 2). In farm A, a total of 59 (0·0038%, single egg equivalent) out of 3888 batches of pooled processed eggs were positive for *S*. Enteritidis but it was also detected in unprocessed eggs (0·005%, single egg equivalent) and packed eggs (0·002%, single egg equivalent). In comparison, *S*. Infantis isolates were mainly detected from dirty eggs (0·025%, single egg equivalent) but were also isolated from processed eggs (0·003%, single egg equivalent) and packed eggs (0·002%, single egg equivalent). In contrast, *S*. Enteritidis was the only *Salmonella* serotype isolated from egg samples from farms B and C. For farm B, *S*. Enteritidis strains were only isolated from pooled processed eggs (0·024, single egg equivalent), and dirty eggs (0·08%, single egg equivalent). In farm C, *S*. Enteritidis were detected in pooled processed eggs (0·02%, single egg equivalent), dirty eggs (0·003%, single egg equivalent) and packed eggs (0·006%, single egg equivalent). There were no *Salmonella* isolated from pooled egg samples in farm D.

PFGE analysis

Several *S*. Enteritidis and *S*. Infantis isolates from different farm were characterized by PFGE analysis.

Table 2. *Comparison of* Salmonella *isolation from different samples from layer farms*

Samples	Year	Farm A (9 layer houses)					Farm B§ (4 layer houses)				Farm C (6 layer houses)		Farm D (4 layer houses)	
		No. of samples	Total *Salmonella*	*S.* Enteritidis	*S.* Infantis	Other *Salmonella*	No. of samples	Total *Salmonella*	*S.* Enteritidis	*S.* Infantis	No. of Samples	*S.* Enteritidis	No. of samples	Total *Salmonella*
Environment	2004	2160	43 (2·0)*	4 (0·19)	39 (1·8)	0 (0)	720	19 (2·6)	19 (2·6)	0 (0)	1440	29 (2·0)	480	0 (0)
	2005	2160	76 (3·5)	3 (0·03)	73 (3·4)	0 (0)	480	11 ((2·3)	11 (2·3)	0 (0)	1440	55 (3·8)	480	0 (0)
	2006	2160	56 (2·6)	2 (0·01)	53 (2·4)	1 (0·005)	540	0 (0)	0 (0)	0 (0)	1440	6 (0·4)	480	0 (0)
	Total	6480	175 (2·7)	9 (0·1)	165 (2·5)	1 (0·01)	1740	30 (1·7)	30 (1·7)	0 (0)	4320	90 (2·1)	1440	0 (0)
Unprocessed eggs†	2004	324	0 (0)	0 (0)	0 (0)	0 (0)	120	2 (0·02)	2 (0·02)	0 (0)	120	1 (0·01)	48	0 (0)
(1 batch = 90 eggs)	2005	324	6 (0·02)	0 (0)	6 (0·02)	0 (0)	80	0 (0)	0 (0)	0 (0)	120	0 (0)	48	0 (0)
	2006	324	21 (0·07)	4 (0·014)	16 (0·055)	1 (0·003)	90	0 (0)	0 (0)	0 (0)	120	0 (0)	48	0 (0)
	Total	972	27 (0·03)	4 (0·005)	22 (0·025)	1 (0·001)	290	2 (0·08)	2 (0·08)	0 (0)	360	1 (0·003)	144	0 (0)
Processed eggs‡	2004	1296	0 (0)	0 (0)	0 (0)	0 (0)	1152	24 (0·05)	24 (0·05)	0 (0)	1152	4 (0·01)	384	0 (0)
(1 batch = 40 eggs)	2005	1296	36 (0·07)	34 (0·066)	2 (0·004)	0 (0)	768	3 (0·01)	3 (0·01)	0 (0)	1152	18 (0·04)	384	0 (0)
	2006	1296	27 (0·05)	25 (0·05)	2 (0·004)	0 (0)	864	0 (0)	0 (0)	0 (0)	1152	4 (0·01)	384	0 (0)
	Total	3888	63 (0·04)	59 (0·038)	4 (0·003)	0 (0)	2784	27 (0·024)	27 (0·024)	0 (0)	3456	26 (0·02)	1152	0 (0)
Packed eggs	2004	1680	0 (0)	0 (0)	0 (0)	0 (0)	600	0 (0)	0 (0)	0 (0)	600	0 (0)	240	0 (0)
(1 batch = 10 eggs)	2005	1680	0 (0)	0 (0)	0 (0)	0 (0)	400	0 (0)	0 (0)	0 (0)	600	0 (0)	240	0 (0)
	2006	1680	2 (0·012)	1 (0·006)	1 (0·006)	0 (0)	450	0 (0)	0 (0)	0 (0)	600	1 (0·02)	240	0 (0)
	Total	5040	2 (0·004)	1 (0·002)	1 (0·002)	0 (0)	1450	0 (0)	0 (0)	0 (0)	1800	1 (0·006)	720	0 (0)
Rats	2004	9	3 (33·3)	0 (0)	3 (33·3)	0 (0)	113	21 (18·6)	17 (15·0)	4 (3·5)	6	1 (16·7)	0	0 (0)
	2005	146	50 (34·2)	12 (8·2)	38 (26·0)	0 (0)	165	32 (19·4)	24 (14·6)	6 (3·6)	49	9 (18·4)	0	0 (0)
	2006	310	144 (46·4)	39 (12·6)	105 (33·8)	0 (0)	30	7 (23·3)	5 (16·6)	2 (6·7)	23	6 (26·1)	0	0 (0)
	Total	465	178 (38·3)‖	51 (10·9)	146 (31·4)	0 (0)	308	49 (19·5)¶	46 (14·9)	12 (3·9)	78	16 (20·5)	0	0 (0)

* No. of positive (%), in eggs (single egg equivalent).

† Dirty and soiled eggs.

‡ Clean and washed eggs.

§ Farm B temporarily stopped operation from September 2005 to March 2006 to change layer flocks, control rodents, and for reconstruction of layer houses.

‖ Farm A: *Salmonella* spp.-positive rats includes 19 rats that were concurrently infected with *S.* Enteritidis and *S.* Infantis.

¶ Farm B: *Salmonella* spp.-positive rats includes 9 rats that were concurrently infected with *S.* Enteritidis and *S.* Infantis.
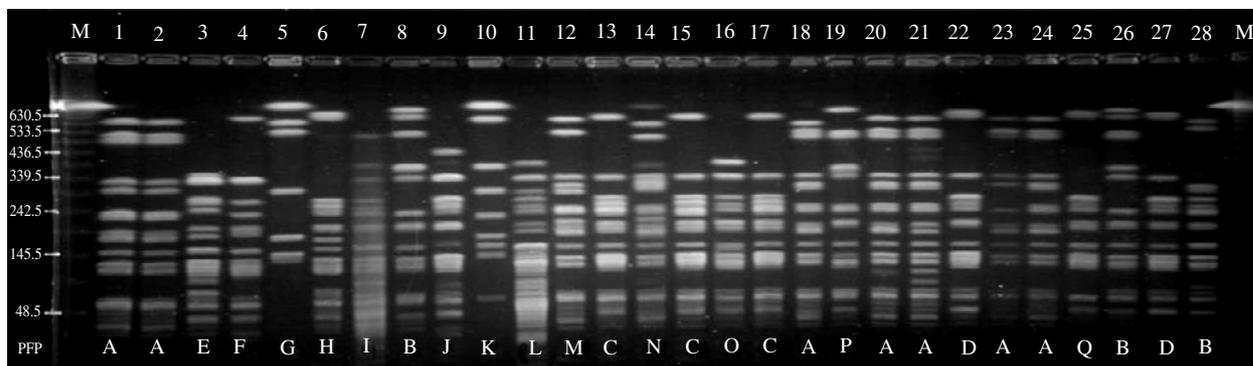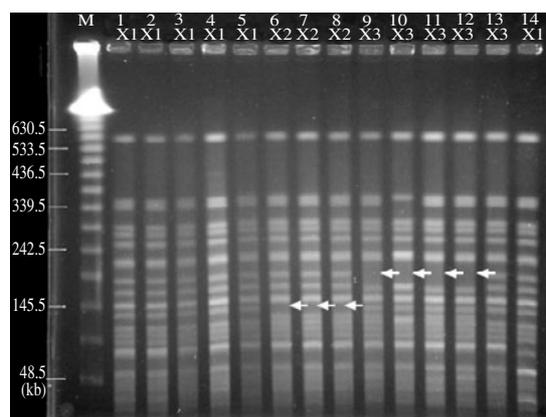
Fig. 1. Representative PFGE patterns of *Bln*I-digested chromosomal DNA of *Salmonella* Enteritidis isolates from farm A. Lanes 1–13, *S.* Enteritidis isolates from rats; lanes 14–22, *S.* Enteritidis isolates from egg samples; lanes 23–28, *S.* Enteritidis isolates from environmental samples; M, marker which shows the DNA size standard used was a bacteriophage lambda concatemer starting at 48·5 kb pairs. Letters below the figure indicate the pulsed-field patterns assigned.
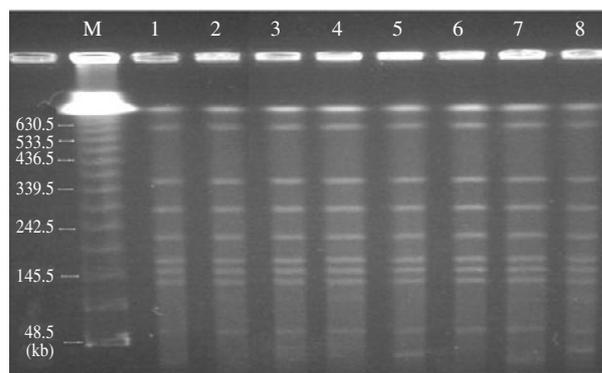
Fig. 2. Representative PFGE patterns of *Bln*I-digested chromosomal DNA of *S.* Infantis isolates from rats in farm A. Lanes 1–4, *S.* Infantis isolates from rats; lanes 5–6, *S.* Infantis isolates from farm environmental samples; lanes 7–8, *S.* Infantis isolates from eggs; M, marker which shows the DNA size standard used was a bacteriophage lambda concatemer starting at 48·5 kb pairs.

Fig. 3. Representative patterns of *Bln*I-digested chromosomal DNA of *S.* Enteritidis isolates from farm B. Lanes 1–5, *S.* Enteritidis from farm environmental samples; lanes 6–10, *S.* Enteritidis, from rats; lanes 11–14, *S.* Enteritidis from eggs; M, marker which shows the DNA size standard used was a bacteriophage lambda concatemer starting at 48·5 kb pairs. Letters below the figure indicate the pulsed-field patterns assigned.

*Bln*I-digested chromosomal DNA of *S.* Enteritidis from rats, eggs and environmental samples of farm A yielded 17 distinct pulsed-filed patterns (Fig. 1). Some of the *S.* Enteritidis isolates from rats, eggs and the environment shared similar patterns (A, B, C, and D). In addition, *Bln*I-digested chromosomal DNA of *S.* Infantis isolated from rats, eggs, and the environment yielded only one identical pattern (Fig. 2). In farm B, *S.* Enteritidis strains generated three closely related pulsed-field patterns, X1, X2, and X3 (Fig. 3). In addition, *S.* Infantis isolates from farm B yielded only one distinct pattern after *Bln*I digestion of their chromosomal DNA (data not shown). For farm C, *Bln*I-digested chromosomal DNA of *S.* Enteritidis isolates from rats, eggs and the environment also shared indistinguishable pulsed-field patterns (Fig. 4).

However, pulsed-field patterns of *S.* Enteritidis isolates from each farm are distinct from each other. In contrast, *Bln*I-digested chromosomal DNA of *S.* Infantis isolates from farms A and B generated similar pulsed-field patterns.

DISCUSSION

S. Enteritidis and *S.* Infantis are the most predominant serotypes associated with human salmonellosis in Japan since 2004 [1]. The primary contamination of eggs occurs at poultry premises because most of the confirmed egg-associated outbreaks can be traced back to contaminated layer farms [15–18]. Henzler &
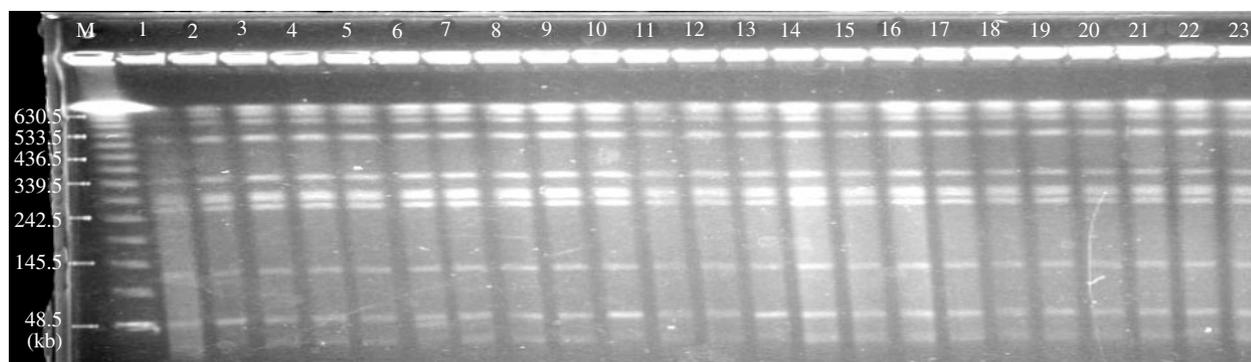
Fig. 4. Representative PFGE Patterns of *Bln*I-digested chromosomal DNA of *S.* Enteritidis isolates from farm C. Lanes 1–8, *S.* Enteritidis isolates from rats; lanes 9–16, *S.* Enteritidis isolates from the environment; lanes 17–23, *S.* Enteritidis isolates from eggs; M, marker which shows the DNA size standard used was a bacteriophage lambda concatemer starting at 48·5 kb pairs.

Opitz [11] have previously suggested that the high incidences of *S.* Enteritidis food poisoning was due to the presence of *S.* Enteritidis-infected rodents in poultry farms but this has not yet been confirmed.

In this investigation, *S.* Enteritidis and *S.* Infantis strains were persistently detected from roof rats found in the three contaminated farms (A, B, and C). Salmonellae were isolated from the heart, liver, spleen, kidneys, and intestines. For *S.* Enteritidis-positive rodents, spleen and liver were the organs with the highest isolation rates. In contrast, intestines were the organs with the highest isolation rate for *S.* Infantis-positive rats. These findings suggest that rats are highly susceptible not only to *S.* Enteritidis as previously reported [6, 19] but also to *S.* Infantis. Interestingly, lower isolation rates of *S.* Enteritidis from intestines compared with spleen and liver were observed. This may be due to post-mortem contamination of *Salmonella* organisms from the intestines to other intestinal organs such as liver, spleen, and kidneys that may have possibly occurred during rodent collection and transportation prior to necropsy procedures. In addition, it is also possible that the intestinal microbial flora of the rodents may have inhibited the growth of *Salmonella* organisms during isolation procedures resulting in lower isolation rates. However, it is also possible that *Salmonella* organisms detected in parenchymal organs did not originate from the intestines, but were already present in those organs, since most of the rodents examined were not in the advanced stage of decomposition (R. Lapuz, personal observation).

Salmonellae were also isolated from environmental and pooled egg samples. *S.* Enteritdis strains were primarily isolated from processed eggs (egg contents), which means that the contamination was via vertical transmission (in-egg contamination). In contrast, *S.* Infantis isolates were mainly obtained from dirty eggs (egg shell and egg content pool). This result suggests that *S.* Infantis contamination on eggs is probably by horizontal transmission (on-egg contamination) because of the highly *S.* Infantis-contaminated environment including the egg belts in farm A. This is probably due to the presence of the *Salmonella*-infected resident rat population in farm A. The *S.* Infantis infection rate in rats found in farm A was high (33·9 %). However, much lower *Salmonella* contamination rates were observed in packed eggs compared with processed eggs, even though, both types of eggs are considered 'clean' and cultured similarly. It is possible that smaller pool size used in packed eggs may have resulted in lower *Salmonella* isolation rates.

Higher isolation rates of *Salmonella* from eggs and environmental samples of contaminated farms correlated with the high infection rates of *S.* Enteritidis and *S.* Infantis in rats inside the layer houses. Moreover, PFGE analyses of *S.* Enteritidis and *S.* Infantis isolates from rats, environmental samples, and eggs revealed that most of the isolates obtained from the same farm were very closely related. Although, *S.* Enteritidis isolates from egg samples of farm B yielded two additional pulsed-field patterns, X2 and X3, and these patterns resembled pulsed-field pattern X1 having only 1–2 band differences (at about 190 kb and 145 kb) (Fig. 3). According to Tenover *et al.* [20], these differences in fragment patterns can be attributed to inversions or transpositions in the restriction enzyme recognition sites. Moreover, *F* values of these three pulsed-field patterns were of $\geqslant 0.80$

similarity, which means that patterns X1, X2 and X3 are very closely related. Even though, there were identical pulsed-field patterns (A, B, C, and D) that were shared by *S*. Enteritidis isolates from rats, eggs, and the environmental samples of farm A, a variety of unique pulsed-field patterns were also generated from these samples (Fig. 1). These findings suggest that there was significant genetic diversity of *S*. Enteritidis isolates existing in farm A. This genetic diversity of *S*. Enteritidis (in farm A) may be caused by the numerous exposures of the farm to previously contaminated layer flocks, or contaminated feed, or even due to *S*. Enteritidis-infected rodents. Furthermore, it cannot be ruled out that the persistence of *S*. Enteritidis in a rodent population over a long period of time can lead to some changes in the genetic make up of the organism.

In this study, roof rats may be important carriers and amplifiers of *S*. Enteritidis and *S*. Infantis on the contaminated layer farms investigated, because higher isolation rates for *S*. Enteritidis and *S*. Infantis organisms from eggs and environmental samples were persistently detected for 3 years. In addition, similar pulsed-field patterns shared by isolates from rats, environmental and egg samples from the same farm were generated. Since *Salmonella* isolation rates from rat samples were higher compared with environmental and egg samples, monitoring of *Salmonella* infection in resident rodent populations may serve as an additional tool in assessing the status of *Salmonella* contamination on layer flocks.

Salmonella contamination in poultry farms can be attributed to various factors such as contaminated feed [21], chicks [22], poultry practices [23, 24], and insects [8]. However, although taking into consideration, that the 'all-in all-out' system was applied, adequate cleaning and disinfection was performed during re-stocking, and replacement flocks were obtained from *Salmonella*-negative parent stocks in these layer farms, *Salmonella* contamination still persisted. One possible explanation for this is the presence of a *Salmonella*-infected resident rat population in layer houses. The infection cycle of *Salmonella* in the hen houses may have been re-introduced by the resident rat population that was not excluded during the cleaning and disinfection period. The rat population provides the opportunity for environment–rat–chicken interaction via ingestion of *Salmonella*-infected rodent faecal droppings from the new replacement flocks which increases the risk of re-introducing *Salmonella* infection in the layer houses. The rodents may have not only amplified the transmission of *Salmonella* contamination but also may be responsible for maintaining the infection cycle of *Salmonella* in layer houses. It is difficult, however, to determine whether the rodents introduce *Salmonella* into a poultry house or, if they pick up the bacteria from an already infected house. It is probable that both scenarios occur. The frequency at which rodents cause primary salmonellosis infection in poultry premises is not known.

Rodent control measures such as trapping and rodenticide baiting to eradicate resident rodent populations may help decrease the *Salmonella* contamination level inside the layer houses, such as in the case of farm B. Eggs and environmental samples from farm B were negative for *Salmonella* contamination after resumption of its operation (Table 2). However, residual *S*. Enteritidis-infected rats were still observed, which poses a potential problem once the rodent population builds up again and might even re-introduce *Salmonella* infection in layer flocks in the future.

In this study, roof rats are carriers of *S*. Enteritidis and *S*. Infantis organisms and may have played a major role in the spread and maintenance of these pathogens inside the layer houses. Poultry farmers should not underestimate the important role of rats in the *Salmonella* infection transmission cycle in poultry farms. Prevention of *Salmonella* contamination on eggs should start at the farm level by implementation of a comprehensive rodent monitoring and control programme in conjunction with thorough cleaning and disinfection during re-stcocking, use of *Salmonella*-negative replacement flocks, regular monitoring of feeds, the environment, and eggs for salmonellae, and in some cases vaccination.

DECLARATION OF INTEREST

None.

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