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Transmission and Shedding Patterns of *Salmonella* in Naturally Infected Captive Wild Roof Rats (*Rattus rattus*) from a *Salmonella*-Contaminated Layer Farm

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SUMMARY. Rodents play a major role in the transmission and maintenance of *Salmonella* contamination cycles in poultry facilities. However, very limited field data are available regarding the transmission routes, infection cycle, and shedding patterns of *Salmonella* by naturally infected wild rodents from commercial layer farms. In this study, a total of 128 resident wild roof rats (*Rattus rattus*) were captured from a *Salmonella*-contaminated layer facility. All roof rats were divided into 51 laboratory cages, and weekly monitoring of *Salmonella* fecal shedding patterns was conducted for 53 wk. Seven roof rats from cages that were observed to frequently shed *Salmonella* were isolated in individual cages, and daily *Salmonella* monitoring was performed for 35 days. At the end of monitoring, each roof rat was euthanatized, and isolation of *Salmonella* from different organs was performed. Results of weekly monitoring of *Salmonella* showed that 21 of 51 cages (41.2%) were positive for *Salmonella* Infantis, while two cages (3.92%) were positive for *Salmonella* Enteritidis. Moreover, 11 cages were positive for *Salmonella* for at least two sampling weeks. Isolation of *Salmonella* from fecal droppings was mainly observed during the first 12 wk of captivity. The longest interval between two *Salmonella*-positive fecal dropping was 24 wk. In the daily *Salmonella* monitoring, only *Salmonella* Infantis was isolated from fecal droppings, in which the highest number of *Salmonella* Infantis organisms per fecal dropping was at 1×10^8 colony-forming units (cfu), while the lowest measured quantity was 1×10^3 cfu. It was noted that the frequency of *Salmonella* shedding in fecal droppings appeared to have a linear correlation ($r = 0.85$) with the number of *Salmonella* organisms (cfu) per fecal pellet ($P < 0.05$). Moreover, pulsed-field gel electrophoresis analysis of *Salmonella* Infantis isolates revealed a single identical pulsed-field pattern. *Salmonella* Enteritidis isolates from fecal droppings and internal organs also generated a single identical pulsed-field pattern. Interestingly, *Salmonella* Infantis was not isolated from any of the organs examined, while *Salmonella* Enteritidis was isolated from the spleen and liver of one roof rat. These results may indicate that wild roof rats could persistently carry *Salmonella* and contaminate commercial poultry facilities through intermittent fecal shedding. Moreover, *Salmonella* Enteritidis in wild roof rats appears to be more of a systemic infection, in which isolation is most likely to occur in internal organs, whereas *Salmonella* Infantis is more likely an enteric type of infection, in which isolation is most likely to occur in the intestinal contents. It is very plausible that layer chickens could become infected with *Salmonella* through ingestion of *Salmonella*-positive fecal droppings or feeds contaminated with these fecal droppings from infected resident roof rats. This is likely one of the major reasons why layer houses can be persistently infected by *Salmonella* even if the facilities are thoroughly cleaned and disinfected and if replacement stocks are obtained from *Salmonella*-free breeders and rearing units. It is therefore a noteworthy suggestion that rodent control programs inside poultry premises comprise an essential and effective tool in the management and control of *Salmonella* contamination in layer flocks.

RESUMEN. Patrones de transmisión y eliminación de *Salmonella* en ratas negras o de tejado (*Rattus rattus*) provenientes de una granja de gallinas de postura contaminada con *Salmonella,* infectadas de forma natural y mantenidas en cautiverio.

Los roedores desempeñan un papel importante en la transmisión y el mantenimiento de los ciclos de la contaminación por *Salmonella* en las instalaciones avícola. Sin embargo, son muy limitados los datos de campo disponibles con relación a las vías de transmisión, el ciclo de infección, y los patrones de eliminación de *Salmonella* por los roedores en libertad infectados de forma natural de las granjas de ponedoras comerciales. En este estudio, un total de 128 ratas negras en libertad (*Rattus rattus*) fueron capturadas en una granja de gallinas de postura contaminada con *Salmonella*. Todas las ratas se dividieron en 51 jaulas de laboratorio, y se realizó un seguimiento semanal de los patrones de eliminación fecal de *Salmonella* durante 53 semanas. Siete ratas en las que se observó eliminaban con frecuencia *Salmonella* fueron aisladas en jaulas individuales, y el seguimiento diario de *Salmonella* se realizó durante 35 días. Al final del periodo de observación, cada rata fue sometida a la eutanasia, y el aislamiento de *Salmonella* a partir de diferentes órganos se realizó. Los resultados del monitoreo semanal de *Salmonella* mostró que 21 de 51 jaulas (41.2%) fueron positivas para *Salmonella* Infantis, mientras que dos jaulas (3.92%) fueron positivos para *Salmonella* Enteritidis. Además, 11 jaulas fueron positivas para *Salmonella* durante al menos dos semanas de muestreo. El aislamiento de *Salmonella* a partir de las muestras fecales se observó principalmente durante las primeras 12 semanas de cautiverio. El intervalo más largo entre dos muestras fecales positivas para *Salmonella* fue de 24 semanas. En el seguimiento diario de *Salmonella*, sólo *Salmonella* Infantis fue aislada de heces, en la que el mayor número de organismos de *Salmonella* Infantis en la muestra fecal fue de 1×10^8 unidades formadoras de colonias (UFC), mientras que la menor cantidad medida fue de 1×10^3 UFC. Se observó que la frecuencia de eliminación de *Salmonella* en muestras fecales parecía tener una correlación lineal ($r = 0.85$) con el número de organismos de Salmonella (UFC) por muestra fecal ($P < 0.05$). Por otra parte, el análisis de electroforesis en gel con campos de pulsaciones de los aislamientos de *Salmonella* Infantis reveló un patrón de campo pulsado único e idéntico. Los aislamientos de *Salmonella* Enteritidis de muestras fecales y de los órganos internos también se generó un patrón de campo pulsado único e idéntico. Curiosamente, la *Salmonella* Infantis no fue aislada de cualquiera de los órganos examinados, mientras que *Salmonella* Enteritidis se aisló a partir del bazo y el hígado de una rata negra. Estos resultados pueden indicar que las ratas negras en libertad, acarrean persistentemente *Salmonella* y contaminar las instalaciones comerciales de aves de corral a través de la excreción fecal intermitente. Además,

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Salmonella Enteritidis en ratas de techo silvestres parece ser más de una infección sistémica, en la que el aislamiento es más probable que ocurra en los órganos internos, mientras que *Salmonella* Infantis es más probable un tipo entérico de la infección, en el que el aislamiento es más probable que ocurra a través de la ingestión en los contenidos intestinales. Es muy probable que pudiera convertirse en gallinas ponedoras infectadas por Salmonella a través de la ingestión de positivas a *Salmonella* de heces o alimentos contaminados con muestras fecales de estas ratas infectadas residentes en el techo. Esto es probablemente una de las razones principales por las casetas pueden persistir infectadas por *Salmonella*, incluso si las instalaciones se limpian y desinfectan, y si las poblaciones de reemplazo se obtienen de los criadores libres de *Salmonella* y las unidades de cría. Por tanto, se sugiere destacar que los programas de control de roedores dentro de las instalaciones de aves de corral constituyen una herramienta esencial y eficaz en la gestión y el control de la contaminación por *Salmonella* en parvadas de ponedoras.

Key words: chicken, commercial layer farm, fecal shedding patterns, *Salmonella* Enteritidis, *Salmonella* Infantis, roof rats, rodents, pulsed-field gel electrophoresis, poultry, transmission patterns

Abbreviations: BHI = brain heart infusion; CFU = colony-forming units; DHL = desoxycholate hydrogen sulfide lactose; HTT = Hajna tetrathionate broth; PFGE = pulsed-field gel electrophoresis

Elimination of *Salmonella* contamination in poultry facilities has been one of the most difficult problems to address in poultry production because of numerous factors associated with its transmission. Aside from feed, water, source of replacement stock, and environmental contamination, resident rodent population has been implicated (2) as one of the major contributors to *Salmonella* contamination.

Salmonella has been reported (1,4,5,7,8,9,14) to be associated with rodents since 1913, and it has been isolated from commensal rodents in urban, rural, and agricultural habitats worldwide. In poultry, researchers (1,3,5,7,8,9) have shown that rodents play a major role in the transmission and maintenance of *Salmonella* contamination cycles in poultry farms. Rodents reproduce rapidly in poultry premises because of abundance of food and ideal shelter. In addition, rodent population in poultry facilities occurs in clusters, and transmission between infected and uninfected clusters is more likely as rodent density increases. Moreover, the transmission of *Salmonella* within rodent colonies is horizontal; thus, the *Salmonella* infection cycle in a rodent colony can persist without the reintroduction of infection (14).

Recently, Lapuz *et al*. (7,8,9) have reported the epidemiologic link between roof rats (*Rattus rattus*) and *Salmonella* from contaminated layer farms and egg processing facilities in Eastern Japan. In addition, Henzler and Opitz (5) observed that detectable *Salmonella* environmental contamination declines in the absence of rodents. Both studies concluded that high rodent population in a poultry facility is an important vector and amplifier of transmission and infection of *Salmonella* (5,7,8,9). McKiel *et al*. (11) have also isolated *Salmonella* from roof rats and Norway rats. They reported that *Salmonella* Enteritidis, *Salmonella* Typhimurium, *Salmonella* Montivideo, and *Salmonella* Derby were the predominant *Salmonella* serotypes for this species. In mice, Davies and Wray (1) studied the persistence and excretion of *Salmonella* Enteritidis and found that *Salmonella* organisms in naturally infected mice were shed intermittently. In the study of Monack *et al.* (12), which used mouse models, the authors concluded that *Salmonella* could persist in the mesenteric lymph nodes and/or spleen of chronically infected, apparently healthy mice and that shedding varied from day to day, indicating that shedding occurs in waves as *Salmonella* replicates. Moreover, Lawley and co-workers (10) reported that about 27% of experimentally infected mice may shed high levels of *Salmonella* in their feces ($>10^8$ colony-forming units [CFU]/g) and may efficiently serve as reservoirs to transmit these organisms to naive individuals. However, no studies have yet been conducted that investigate the pattern of transmission of *Salmonella* from naturally infected wild roof rats, the predominant rodent species in poultry facilities in Japan (7). Understanding the transmission routes, infection cycle, and fecal shedding patterns of *Salmonella* from wild roof rats may provide invaluable insights in formulating effective control and preventive strategies against rodent-related *Salmonella* contamination in poultry facilities, especially here in Japan.

In this study, wild roof rats from a confirmed *Salmonella*-contaminated commercial layer farm were captured, and the frequency, quantity, and *Salmonella* fecal shedding patterns of these wild rodents were investigated. In addition, molecular and epidemiologic analyses conducted through pulsed-field gel electrophoresis (PFGE) analysis of *Salmonella* isolates were performed to determine if changes in the genetic make-up of *Salmonella* in captive roof rats could occur over time.

MATERIALS AND METHODS

Poultry farm. All roof rats used in this study were captured from a commercial layer farm with three window-less house complexes that were connected by conveyor belts that transport eggs to the egg processing facility for grading and packing. All houses were high-rise houses, wherein feces were dropped and collected on the first story, while the flock was located at the second story. All houses were environmentally controlled and operated with automated systems. Each house had a population of 40,000 adult layer chickens. Monthly monitoring of *Salmonella* contamination showed that environmental samples and some egg samples from this farm were frequently contaminated with *Salmonella* Infantis and Enteritidis. Complete details on *Salmonella* isolation from this farm was reported previously (7,8,9).

Capture and maintenance of wild roof rats. Rodents were trapped using customized pipe traps designed by farm staff, as reported previously (7). Traps were located along the posts and ceiling of the first story, where roof rats frequently traveled. Traps were baited with chicken feed and various kinds of grains and seeds. Traps were checked every 24–48 hr. In total, 128 roof rats were caught and were housed in 51 individual rodent cages with two to three roof rats per cage. Each individual cage was supplied with an adequate amount of wood chips. Each roof rat was fed with approximately 40 g of *Salmonella*-free commercial rodent feed pellets daily. Sterilized water was supplied *ad libitum*.

Weekly monitoring of *Salmonella* from roof rat feces. A total of five fresh fecal droppings from each cage were collected weekly and pooled into a sterile plastic tube containing 8 ml of brain heart infusion (BHI) broth (Eiken, Kyoto, Japan). This pre-enrichment medium was incubated at 37 C for 48 hr. One milliliter from this culture was inoculated to 9 ml of Hajna tetrathionate broth (HTT; Eiken) and incubated for 18 hr at 37 C. A loopful from the HTT culture was then streaked onto a desoxycholate hydrogen sulfide lactose (DHL) agar (Eiken) and incubated for 18 hr at 37 C. *Salmonella* suspect colonies were confirmed and identified by biochemical tests and were serotyped using *Salmonella* O and H antigens (Denkaseiken, Tokyo, Japan).

Daily monitoring of *Salmonella* from roof rat feces. Seven roof rats from cages that were frequently observed to have *Salmonella*-positive

Table 1. Weekly isolation of *Salmonella* from fecal droppings of roof rats.[A]

Cage	Weeks in confinement																																																			
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[A]Numbers indicate *Salmonella* O-antigen grouping (7 = *Salmonella* Infantis; 9 = *Salmonella* Enteritidis); D indicates death of roof rats; X indicates start of daily *Salmonella* monitoring.

Table 2. Daily isolation of *Salmonella* from fecal droppings of roof rats.[A]

Rodent ID	Days in confinement																																	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35
35	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
25-1	0/4	0/4	0/4	0/4	0/4	1/4	0/4	2/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
25-2	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
6	0/4	0/4	2/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	4/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
36-1	1/4	1/4	4/4	2/4	3/4	4/4	4/4	1/4	2/4	1/4	1/4	1/4	1/4	4/4	1/4	1/4	0/4	0/4	2/4	1/4	2/4	4/4	2/4	3/4	1/4	1/4	2/4	0/4	3/4	1/4	1/4	1/4	1/4	2/4	1/4
36-2	1/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	1/4	1/4	1/4	0/4	0/4	0/4	1/4	0/4	0/4	0/4	0/4	0/4	0/4
31	2/4	1/4	2/4	1/4	1/4	2/4	1/4	3/4	1/4	1/4	1/4	4/4	1/4	4/4	1/4	3/4	1/4	1/4	2/4	4/4	0/4	0/4	0/4	0/4	1/4	0/4	0/4	1/4	1/4	1/4	1/4	4/4	2/4	1/4	1/4

[A]All positive samples were *Salmonella* Infantis; numerator = number of positive samples; denominator = number of fecal samples.

fecal droppings during weekly monitoring were selected and isolated in individual laboratory cages. Four fresh fecal droppings from each cage were collected daily for 35 days. Each fecal dropping was individually placed in a sterile tube containing 1.8 ml of sterile normal saline water. A semihomogeneous solution was prepared by shaking the mixture vigorously. Tenfold serial dilutions of the fecal dropping solution were made, and 100 µl from each dilution was spread evenly on a DHL agar and incubated at 37 C for 24 hr. Colony counts of suspected *Salmonella* colonies on each plate of each dilution were performed. CFU per fecal dropping were calculated. *Salmonella* identification and serotyping were done as previously described.

Isolation of *Salmonella* from roof rats. After 35 days of observation, the seven roof rats monitored daily for *Salmonella* infection were euthanatized by chloroform inhalation. Roof rats were aseptically necropsied individually. Each roof rat was disinfected by dousing it with a 3:1 solution of 70% ethyl alcohol and 10% iodine. The abdominal cavity was opened. Approximately 1–2 g of the heart, spleen, liver, and kidney were collected in aseptic conditions and were added separately to a tube containing 8 ml of HI broth (Eiken). *Salmonella* identification and serotyping were conducted as previously described.

PFGE analysis of *Salmonella* isolates. A total of 24 *Salmonella* Infantis and two *Salmonella* Enteritidis isolates were characterized by PFGE analysis. *Salmonella* Infantis isolates were obtained from weekly *Salmonella* monitoring of fecal droppings, while *Salmonella* Enteritidis were from the weekly monitoring and from internal organs of roof rats. DNA for PFGE analysis was prepared as described previously (13). Chromosomal DNA in each plug was digested with 20 U of B*ln*I (Takara, Kyoto, Japan) at 37 C for 18 hr. PFGE was performed using the CHEF-DR III apparatus (Bio-Rad, Tokyo, Japan) in gels of 1% agarose (Bio-Rad) on 0.5× Tris-borate ethylene diamine tetraacetic acid buffer (Bio-Rad) for 21 hr at 200 V and 14 C with a pulse time ranging from 2 to 43.2 sec. The gels were stained with ethidium bromide (Bio-Rad) and photographed with an ultraviolet illuminator (Atto Systems, Osaka, Japan). DNA lambda ladder was used as molecular marker (Bio-Rad). DNA fragments were analyzed visually.

Statistical analysis. The Pearson product moment correlation coefficient was computed to determine whether a relationship exists between the number of *Salmonella* organisms (CFU) per fecal pellet and the frequency of *Salmonella* shedding in roof rat droppings. Results were considered significant at $P < 0.05$.

RESULTS

Weekly monitoring of *Salmonella* from roof rat feces. Table 1 shows the results of weekly isolation of *Salmonella* from fecal droppings of roof rats from each cage. Twenty-one of 51 cages (41.2%) were positive for *Salmonella* Infantis. In addition, fecal droppings from two cages (cage nos. 25 and 41) were positive for *Salmonella* Enteritidis. Fecal droppings from these two cages were previously positive for *Salmonella* Infantis. Furthermore, fecal droppings from 11 cages were positive for *Salmonella* for at least two weekly sampling dates. The longest interval between *Salmonella*-positive results was 24 wk (cage 18). Isolation of *Salmonella*-positive droppings was mainly observed during the first 12 wk of captivity; positive droppings then started to decline thereafter. In some cases, cages that yielded positive results eventually became negative over time. Roof rats from cage 31 recorded the highest number of *Salmonella*-positive samples.

Daily monitoring of *Salmonella* from roof rat feces. *Salmonella* Infantis was the only serotype isolated from fecal droppings of roof rats from daily *Salmonella* monitoring. No *Salmonella* Enteritidis strains were recovered, even though droppings from cages 25 and 35 were previously positive during the weekly isolation of *Salmonella*. As expected, roof rats from cages 31 and 36 showed higher *Salmonella* isolation rates; roof rat 36-1 had *Salmonella* Infantis–positive droppings on 32 out of 36 days during which *Salmonella*

Table 3. Colony-forming units (CFU) of *Salmonella* Infantis per fecal droppings of roof rats.

	Average *Salmonella* CFU per rodent fecal droppings										
Rodent ID	1	2	3	4	5	6	7	8	9	10	11
35	0/4	1×10^3	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
25-1	0/4	0/4	0/4	0/4	0/4	2×10^3	0/4	2×10^5	1×10^2	7×10^3	0/4
25-2	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
6	0/4	0/4	3×10^5	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2×10^4
36-1	1×10^4	1×10^5	2.5×10^5	1×10^6	1.3×10^5	4×10^5	2×10^5	1×10^8	2×10^3	2×10^5	1×10^6
36-2	2×10^6	0/4	1×10^4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	1×10^4
31	1×10^4	3×10^7	1.5×10^5	1×10^5	1×10^5	1×10^5	3×10^5	3×10^3	1×10^5	8×10^5	2×10^6

isolation was performed. The results of daily isolation of *Salmonella* from roof rat fecal droppings are summarized in Table 2. In comparison, fecal droppings from rat 25-2 yielded only *Salmonella*-negative results. The highest number of *Salmonella* organisms per fecal dropping was 1×10^8 CFU from rat 36-1, while the lowest CFU per fecal dropping was 1×10^3 from rat 35 (Table 3). The frequency of *Salmonella* shedding in roof rat droppings appears to have a correlation ($r = 0.85$) with the number of *Salmonella* organisms (CFU) per fecal pellet ($P < 0.05$).

Life span of roof rats in captivity. In this study, the life spans of the captive wild roof rats monitored ranged from 2 wk to more than 50 wk. On average, the life span of wild roof rats in this study was noted to be approximately 6 mo.

Isolation of *Salmonella* from roof rats. Interestingly, *Salmonella* Infantis was not isolated from any of the internal organs examined. Surprisingly, *Salmonella* Enteritidis was isolated from the spleen and liver of rat 25-1. Results of *Salmonella* isolation from individual organs of wild roof rats were summarized in Table 4.

PFGE analysis of *Salmonella* isolates. PFGE analysis of *Salmonella* Infantis strains isolated from fecal droppings of wild roof rats generated only one pulsed-field pattern. *Salmonella* Enteritidis isolates from roof rat droppings and internal organs also generated one identical pulsed-field pattern (Fig. 1).

DISCUSSION

The presence of a resident rodent population in a poultry facility is a serious public health risk. Previous studies (1,5,6,7,8,9) have implied an important role of rodents in the spread and maintenance of *Salmonella* contamination on poultry premises. However, data on the exact mechanism for how wild resident rodents contaminate poultry facilities with *Salmonella* is lacking. Hence, a better understanding of the transmission routes, infection cycles, and fecal shedding of *Salmonella* by naturally infected wilds rodent from *Salmonella*-contaminated poultry facilities may lead to better strategies for the control and prevention of this public health risk.

Table 4. Isolation of *Salmonella* spp. from internal organs of roof rats.

Rodent ID	Heart	Liver	Spleen	Kidney
35	–	–	–	–
25-1	–	+[A]	+[A]	–
25-2	–	–	–	–
6	–	–	–	–
36-1	–	–	–	–
36-2	–	–	–	–
31	–	–	–	–

[A] *Salmonella* Enteritidis.

In this study, we could not determine the initial age of each roof rat, which may be one of the factors involved in the variability of the results. However, the data showed that the life span of wild roof rats in captivity ranged from 2 wk to more than a year. Average life span was approximately 6 mo. These data are probably the first to reveal the survivability of wild roof rats in captivity in Japan.

Salmonella Infantis was the predominant serotype isolated from roof rat fecal droppings during the weekly and daily *Salmonella* monitoring. *Salmonella* Infantis is also the most prevalent *Salmonella* serovar in commercial layer farms in Japan (6). *Salmonella* Infantis was also the prevalent *Salmonella* serovar in the commercial layer farm from which the roof rats used in this study were obtained (7,8,9). In comparison, *Salmonella* Enteritidis was only recovered from two of the 51 cages.

Isolation of *Salmonella* from individual organs only yielded *Salmonella* Enteritidis strains, particularly from the spleen and liver of rat 25-1. Surprisingly, *Salmonella* Infantis was not recovered from any internal organs of frequently *Salmonella*-shedding roof rats. One possible reason is that only a portion of each organ was obtained and later enriched in BHI broth; thus, low numbers of *Salmonella* Infantis were probably not detected. In contrast, *Salmonella* Enteritidis was occasionally recovered from the droppings of rat 25-1 during the weekly monitoring of *Salmonella* from feces but never during the individual monitoring. However, during necropsy, this roof rat's spleen and liver were *Salmonella* Enteritidis–positive. These findings may indicate that *Salmonella* Infantis infection in roof rats may occur as 'intestinal carriage,' while *Salmonella* Enteritidis may occur as a 'systemic type' of infection with intermittent shedding patterns, but further studies are recommended to support this point. These findings may also indicate that isolation of *Salmonella* Enteritidis from roof rats should be recovered from internal organs such as spleen and liver, aside from fecal droppings. *Salmonella* Infantis, on the other hand, can more likely be recovered from the intestinal contents. Unfortunately, isolation of *Salmonella* from the intestines was not performed in this study. Further studies on the colonization of *Salmonella* in intestinal segments of wild roof rats are recommended.

The PFGE results revealed that *Salmonella* Infantis isolates from roof rat droppings were genetically related because only one indistinguishable pulsed-field pattern was generated. Similarly, *Salmonella* Enteritidis isolates from droppings and internal organs (spleen and liver) were also genetically related. No changes in the PFGE profile were noted during the entire 52 wk, which may indicate that no significant changes in the genetic make-up of *Salmonella* strains were observed during the entire study period.

Davies and Wray (1) have observed that *Salmonella* Enteritidis were shed intermittently in artificially inoculated wild mice. In this investigation, *Salmonella* Infantis were also observed to be excreted intermittently in the weekly *Salmonell*a isolation experiment (Table 1). In one particular case, roof rats in cage 18 had an

Table 3. Extended.

Average *Salmonella* CFU per rodent fecal droppings											
12	13	14	15	16	17	18	19	20	21	22	23
0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
1×10^6	3×10^4	2×10^5	1×10^7	2×10^5	0/4	0/4	1×10^4	3×10^4	2×10^5	7×10^6	5×10^4
3×10^2	0/4	0/4	0/4	0/4	0/4	0/4	1×10^5	0/4	0/4	0/4	5×10^6
2.5×10^6	2×10^3	2×10^4	1×10^6	6×10^5	2×10^5	1×10^6	5×10^5	3×10^4	0/4	0/4	0/4

interval of 24 wk in between *Salmonella*-positive fecal droppings. It was noted that the frequency of *Salmonella* shedding appears to have a linear relationship ($r = 0.85$) with the number of *Salmonella* organisms (CFU) per fecal pellet ($P < 0.05$). A higher number of *Salmonella* organisms (CFU) present on a fecal pellet corresponds to a higher frequency of *Salmonella* shedding in fecal droppings (Tables 2, 3). These results may indicate that if roof rats become severely infected with *Salmonella* Infantis, the frequency and the length of time during which the *Salmonella* are being shed in feces are also increased.

In conclusion, these findings provide a better understanding of the role of roof rats in the transmission and maintenance of the *Salmonella* contamination cycle on poultry premises.

This study revealed that roof rats can shed *Salmonella* Infantis and *Salmonella* Enteritidis intermittently in their feces and that the number of *Salmonella* organisms per fecal pellet can be as high as 1.0×10^8. This is probably one of the major reasons why layer houses can be persistently infected by *Salmonella* even if the facilities were thoroughly cleaned and disinfected, the prescribed downtime for restocking was followed, and replacement stocks were obtained from *Salmonella*-free breeders and rearing units. It is very plausible that layer chickens could become infected with *Salmonella* through ingestion of *Salmonella*-positive fecal droppings or feeds contaminated with these fecal droppings from infected resident roof rats. It is therefore noteworthy to suggest that rodent control programs inside poultry premises comprise an essential and effective tool in the management and control of *Salmonella* contamination in layer flocks.

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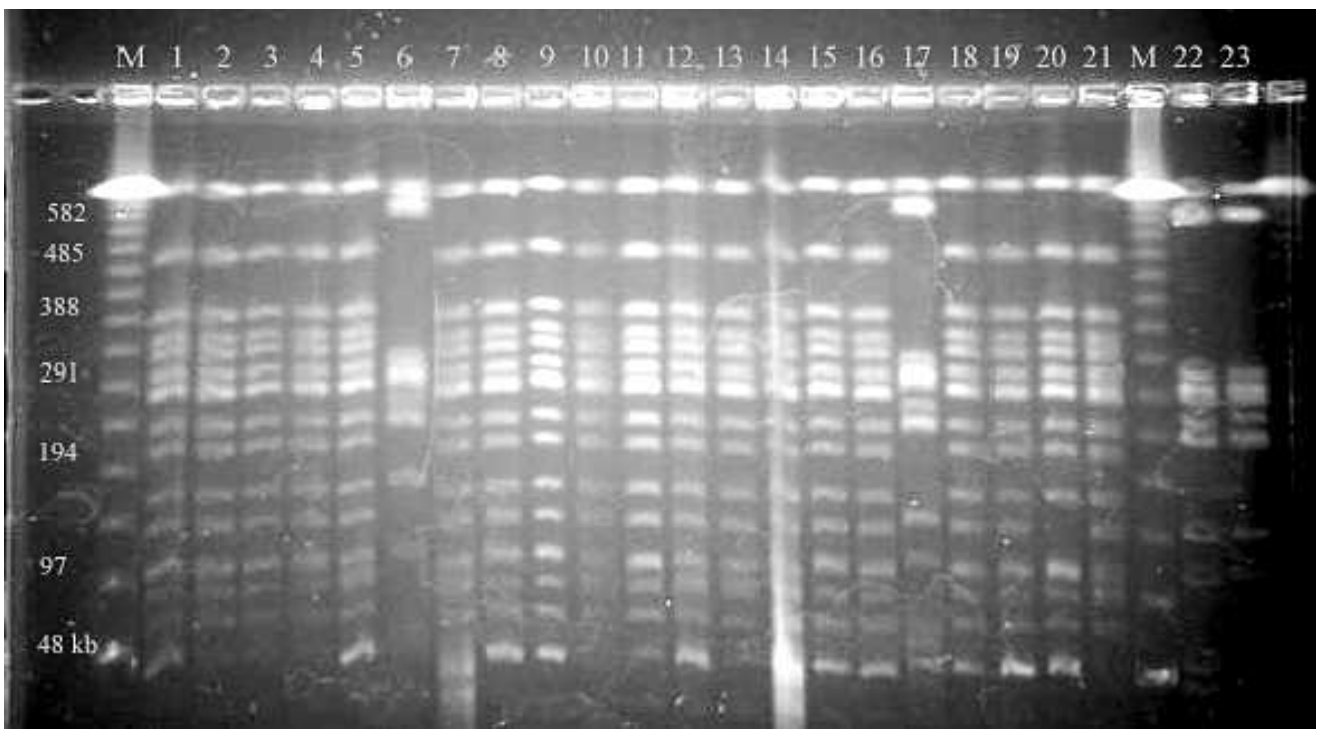

Fig. 1. PFGE patterns of *Salmonella* isolates from fecal droppings and internal organs of roof rats. Lanes 1–5, 7–16, and 18–21: *Salmonella* Infantis isolates from fecal droppings of roof rats showing only one identical PFGE pattern. Lanes 6, 17, and 22–23: *Salmonella* Enteritidis isolates from fecal droppings and internal organs of roof rats showing similar PFGE patterns.

Table 3. Extended.

Average *Salmonella* CFU per rodent fecal droppings											
24	25	26	27	28	29	30	31	32	33	34	35
0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4	0/4
1×10^5	2×10^6	1×10^4	1×10^6	0/4	3×10^5	1×10^5	1×10^5	1×10^8	2×10^6	1×10^5	1×10^5
1×10^4	3×10^7	0/4	0/4	0/4	7×10^5	0/4	0/4	0/4	0/4	0/4	0/4
0/4	1×10^5	0/4	0/4	1×10^7	3×10^5	1×10^4	2×10^5	2×10^3	1×10^3	3×10^4	1×10^3

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