INFECTION OF *SALMONELLA PULLORUM*, *SALMONELLA NEWINGTON* OR *SALMONELLA ENTERITIDIS* IN LABORATORY RATS BY ORAL INOCULATION*

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INTRODUCTION

Laboratory rats have been used for many studies of salmonella infection, which were intended to obtain knowledge of the role of wild rats in the epidemiology of salmonellosis in man and animals. However, in the field of avian salmonellosis, studies of this type can be found only in case of pullorum disease[10], although a number of reports are available on experimental infection with *Salmonella enteritidis* in the fields of public health[1,12] or laboratory animal care[2,6,8]. Moreover, there is no information on experimental infection of rats with non-host-adaptive salmonella types such as *Salmonella newington* as pointed out by BUXTON & FIELD.

In order to obtain more reliable information on the role of wild rats in the epidemiology of salmonellosis in chickens, the present author conducted experimental infections in wild rats which had been bred under laboratory conditions. The results obtained will be presented in another publication[9]. Before the above mentioned study, pilot experiments were made using laboratory rats.

This paper describes the propagation of 3 serotypes of salmonella in experimentally infected laboratory rats and duration of the carrier state. In addition, differences in infection status between laboratory and wild rats (*Rattus norvegicus*) will be discussed.

MATERIALS AND METHODS

Rats Albino rats of Wistar strain were obtained from Dr. MAKINO's laboratory of Hokkaido University, Sapporo. Usually female rats were used; however, in some experiments, rats of both sexes were used. Each rat was placed into a single cage 22×13×11 cm

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and given commercial pelleted feed (Oriental Yeast Co. Ltd., Tokyo) in a petri-dish and tap water in a bottle with a spout. In order to check latent infection with salmonella, the rats were inspected a few times by fecal culture before use. Through the present study, no salmonella was isolated from control rats and salmonella types other than those used in the experiment were not recovered.

Test strain A strain of *S. pullorum* was isolated from yolk material of a naturally infected hen. The yolk material was preserved at −30°C. Day-old chicks fed several cells of this culture died within 15 days after inoculation. The *S. newington* strain was isolated from the cecum of a dead chick and maintained in a cooked meat medium. Virulence of this strain was checked in baby chicks according to the method of MILNER & SHAFFER. That is, 5 doses ranging from approximately 5.5~5.5×10^8 organisms were instilled, respectively, in the mouth of 3 baby chicks. All chicks shed the infected organism and harbored it at necropsy 8 days post inoculation. A strain of *S. enteritidis* (411 D) was obtained from the heart blood of a dead chick and maintained in a cooked meat medium. A virulent laboratory strain (No. 11) was obtained from Dr. T. WATANABE of Keio University, Tokyo. Feeding of approximately 5.5×10^6 cells from plate cultures of both strains killed mice regularly (tab. 1).

TABLE 1 *Mouse virulence of 2 strains of Salmonella enteritidis*[*1]

STRAIN	NO. OF DEAD MICE / NO. OF MICE INOCULATED IN EACH INOCULUM[*2]				
	5.5×10^8	5.5×10^6	5.5×10^4	5.5×10^2	5.5
411 D	3/3	3/3	1/3	0/3	0/3
No. 11	3/3	3/3	0/3	1/3	

*1 Observation period was 3 weeks.
*2 Inocula were instilled in the mouth of mice.

Preparation of inocula *S. pullorum* used for inoculation was obtained from a culture propagated on an agar medium containing yeast extract (5 g), poly-peptone, Takeda (15 g), glucose (2 g), l-cystine (0.2 g), anhydrous sodium sulfite (0.2 g) and sodium chloride (4 g), in a total amount of 1,000 ml. *S. newington* and *S. enteritidis* were propagated on horse meat infusion agar. After 20 hours incubation the cultures were washed from the agar and suspended in normal saline. The bacterial suspensions were diluted to contain one mg of wet cells per 0.05 ml of saline. One mg of *S. pullorum* contained approximately 6×10^8 organisms, and those of *S. newington* and *S. enteritidis* approximately 5.5×10^8 organisms. From the original suspensions a series of 10-fold dilutions were made. The number of bacterial cells was determined by direct count on agar following log dilution.

Inoculation and handling of inoculated rats Rats which had been fed earlier were anesthetized with ether and 0.05 ml of inoculum was instilled in their mouths. In some of the experiments with *S. pullorum*, an inoculum of 0.1 ml was instilled without anesthesia. In one experiment, the culture of *S. pullorum* was fed with a piece of bread. Each inoculated rat was transferred to another sterile cage a few hours after inoculation in order to avoid cage contamination with the organisms present in its mouth. The single cage had a wire

floor of 1.5×1.5 cm mesh to allow the excreta to fall into a dropping pan. The procedure for transfer of a rat from one cage to another was as follows: Two cages were placed door to door so that the rat could move to the second cage through the vertically sliding doors that were opened simultaneously.

Recovery of salmonella from rat feces Once daily, several pellets of fresh feces from each rat were suspended into 15 ml of selenite broth and mashed with a sterile glass rod. Following inoculation, the cultures were incubated overnight and then seeded on MacConkey or brilliant green agar plates. MacConkey agar was used in experiments with *S. pullorum* only.

Cages and dropping pans were changed daily and sterilized in boiling water after having been washed in solution of disinfectant. Other equipment was also changed regularly.

Necropsy and cultivation of the rat tissues Rats were bled under anesthesia by heart puncture. Their sera were examined by O (alcohol-treated antigen) and H (formalized antigen) agglutinations. All of the submandibular (submaxillary), axillar and inguinal lymph nodes, the entire urinary bladder, almost all of the mesenteric nodes, and portions of the heart, lungs, spleen, liver, kidneys were removed aseptically, cut into pieces and placed on MacConkey agar or brilliant green agar plates. The same tissues were inoculated into nutrient broth of the same components described in preparation of inocula for *S. pullorum*. During the experiments with *S. pullorum*, the gonads were cultured instead of the urinary bladder. Approximately one cm of the duodenum and cecum were cut lengthwise and placed into selenite broth. The broth cultures were incubated overnight and seeded onto MacConkey or brilliant green agar. Suspicious colonies were checked by serological and biochemical procedures.

TABLE 2 *Fecal excretion and infection of laboratory rats*[*1] *fed Salmonella pullorum*

AGE	INOCULUM	SALMONELLA IN FECES TAKEN IN WEEKS AFTER INOCULATION			NECROPSY		
		1	2	3	On days post infection	No. of salmonella-positive rats/No. of rats examined	Positive organs
days							
95~134	6×10^9	5/7×4 (4)[*2][*3]	0/7×4	0/7×4	21	4/4	Sn[*4]
	6×10^8	5/7×4 (2)	5/7×4 (1)	0/7×4	21	4/4	Sn
	6×10^6	0/7×4	0/7×4	0/7×4	21	1/4	Sn
29	6×10^9	19/7×3 (3)	13/7×3 (3)	2/7×3 (2)	28	1/3 2/3	Sn Lungs
	6×10^8	13/7×3 (3)	6/7×3 (3)	2/7×3 (2)	28	2/3 1/3	Sn Sn, Lungs
	6×10^6	3/7×3 (3)	0/7×3	0/7×3	28	0/3	

[*1] These rats were fed each dose of *S. pullorum* culture in 0.1 ml without anesthesia.

[*2] Total number of positive fecal cultures in each group in each week/total number of fecal cultures taken per week (7×number of rats examined)

[*3] Figures in parentheses indicate number of rats shedding salmonella in each week.

[*4] Sn—submandibular nodes

RESULTS

Experiments with *S. pullorum*

Excretion of *S. pullorum* in rats inoculated orally without anesthesia As indicated in table 2, young rats inoculated with a dose of 6×10^8 or 6×10^9 *S. pullorum* showed continuous excretion of the organism.

In young rats, 29 to 54 days of age, fed a dose of 6×10^9 organisms resulted in a wide distribution of salmonella in the body 2 days post infection (tab. 3). It should be noted that the submandibular nodes were uniformly infected.

TABLE 3 *Distribution of Salmonella pullorum in different organs of rats*[*1] *fed the organism*

INOCULUM	ORGANS[*2]	CULTIVATION ON DAYS AFTER INFECTION	
		2	7
6×10^9	Submandibular nodes	6/6[*3]	8/8[*3]
	Axillar nodes	2/6	
	Heart	1/6	1/8
	Lungs	2/6	2/8
	Mesenteric nodes	3/6	3/8
	Spleen	4/6	
	Liver	1/6	
	Kidneys	2/6	
	Duodenum	2/6	
	Cecum	2/6	1/8
	No. of rats of salmonella-positive / No. of rats examined	6/6	8/8
6×10^6	Submandibular nodes	1/5	2/6
	Mesenteric nodes	1/5	
	Spleen		2/6
	Duodenum	1/5	
	No. of rats of salmonella-positive / No. of rats examined	3/5	4/6

[*1] Rats of 29~54 days of age were fed each dose in 0.1 ml without anesthesia.
[*2] Organs of negative culture are not listed.
[*3] No. of salmonella-positive cultures/No. of rats examined

Comparison of 2 methods of oral inoculation of *S. pullorum* Feeding the organism with a piece of bread and instilling it in the mouth of rats under anesthesia were compared. As shown in table 4, the submandibular nodes were infected at a high frequency in the latter case. Although feeding of infected bread is more natural than the instillation method,

TABLE 4 *Excretion and infection of Salmonella pullorum in rats*[*1] *inoculated by 2 oral methods*

MANNER OF INOCULATION	INOCULUM	NO. OF RAT	POSITIVE FECAL CULTURES ON DAYS AFTER INOCULATION							NECROPSY	
			1	2	3	4	5~9	10	11~28	On days after inoculation	Positive organs[*2]
Feeding (bread)	6×10^8	1	+		+			+		21	
		2	+							"	Sn, Mn
		3	+							"	Mn
		4	+							"	
		5								"	
"	6×10^8	6	+	+		+				28	Sn, An
		7	+							"	
		8	+							"	
		9	+							"	
		10								"	
"	6×10^6	11	+							21	
		12	+							"	Heart
		13								"	Mn
		14								"	
"	6×10^6	15	+	+						28	
		16	+							"	
		17								"	Sn, Mn
		18								"	
Instillation (under anesthesia)	6×10^8	19	+	+						21	Sn
		20	+	+						"	Sn
		21	+							"	Sn, An
		22	+							"	Sn, An, Kidneys
		23								"	

[*1] Rats of 57~150 days of age were used.
[*2] An = axillar nodes Mn = mesenteric nodes

it was more time consuming. Thus, only the latter method was applied in subsequent experiments.

Distribution of *S. pullorum* in the body of laboratory rats following oral inoculation Rats of 2 different age groups were fed a dose of 6×10^8 organisms of *S. pullorum* (tab. 5). Salmonella was recovered almost regularly from the submandibular nodes of inoculated rats. It was of interest that *S. pullorum* was isolated from the spleen, liver, and even from the heart of these rats. *S. pullorum* was recovered from more different organs of older rats than the younger age group.

TABLE 5 *Distribution of Salmonella pullorum in rats fed a dose of 6×10^8 cells*

AGE	ORGANS	CULTIVATION OF DAYS AFTER INFECTION	
		7	14
days			
62	Submandibular nodes	5/5	5/5
	Axillar nodes	2/5	
	Mesenteric nodes		2/5
	Spleen	1/5	1/5
	No. of rats of salmonella-positive / No. of rats examined	5/5	5/5
132	Submandibular nodes	3/5	4/4
	Axillar nodes	2/5	1/4
	Inguinal nodes		1/4
	Heart		1/4
	Mesenteric nodes	2/5	2/4
	Spleen	3/5	1/4
	Liver	2/5	
	No. of rats of salmonella-positive / No. of rats examined	5/5	4/4

TABLE 6 *Infection in laboratory rats of 2 ages on 7 and 14 days after oral inoculation with different doses of Salmonella newington*

AGE	ORGANS	CULTIVATION OF INOCULATED RATS					
		5.5×10^8		5.5×10^6		5.5×10^4	
		7 days	14 days	7 days	14 days	7 days	14 days
days							
33	Submandibular nodes	2/3	1/3	2/3	2/3		
	Axillar nodes	2/3					
	Mesenteric nodes	1/3	3/3				
	Cecum	3/3	1/3				
	No. of rats of salmonella-positive / No. of rats examined	3/3	3/3	2/3	2/3	0/3	0/3
103	Submandibular nodes	2/3	2/4	1/3	1/3		
	Mesenteric nodes	2/3	3/4	2/3	2/3	1/3	
	Spleen	1/3		1/3			
	Cecum	2/3	3/4				
	No. of rats of salmonella-positive / No. of rats examined	3/3	4/4	3/3	2/3	1/3	0/3

TABLE 7 *Presence of Salmonella newington in feces obtained daily from rats fed different doses of the organism*

AGE	INOCULUM	RESULTS OF FECAL CULTIVATION ON WEEKS AFTER INOCULATION					NECROPSY					
		1	2	3	4	5	On days post inoculation	Organs*				No. of positive rats/No. of rats examined
								Sn	Mn	K	C	
days												
103	5.5×10^8	16/7×4 (4)	9/7×4 (3)	10/7×4 (4)	7/7×4 (2)	4/7×4 (1)	35	3/4	1/4	1/4	1/4	3/4
	5.5×10^6	15/7×4 (4)	11/7×4 (2)	8/7×4 (2)	7/7×4 (1)	5/7×4 (1)	35	2/4			1/4	2/4
	5.5×10^4	4/7×4 (3)	0/7×4	0/7×4			21					0/4
33	5.5×10^8	22/7×4 (4)	10/7×4 (3)	6/7×4 (2)	6/7×4 (2)	6/7×4 (2)	35	1/4	1/4		2/4	2/4
	5.5×10^6	6/7×4 (4)	1/7×4 (1)	4/7×4 (1)	2/7×4 (1)	2/7×4 (1)	35	1/4	1/4			1/4
	5.5×10^4	3/7×4 (2)	0/7×4	0/7×4			21		1/4			1/4

* K = kidneys C = cecum

Agglutinin production in inoculated rats Clinical or pathological changes were not observed in inoculated rats. However, the sera of 6 out of 24 rats (25%) which were killed 3~4 weeks post infection showed positive agglutination in dilution of 1 : 20 or more. When only rats which harbored or excreted *S. pullorum* were counted, 4 out of 18 had agglutinins.

Experiments with *S. newington*

Distribution of *S. newington* in the body of rats following oral inoculation Most of the rats fed a dose of 5.5×10^8 cells of *S. newington* yielded salmonella in the mesenteric or submandibular nodes as well as in the cecum as shown in table 6. No marked difference of infection was found between different age groups.

The presence of *S. newington* in feces of rats fed different doses of the organism As shown in table 7, 2 out of 8 rats of 103 days of age and 3 of the 33 days of age inoculated with a dose of 5.5×10^6 organisms of *S. newington* shed salmonella intermittently or continuously for 35 days. Cecal cultures from 4 of the 5 rats yielded salmonella at necropsy 35 days post infection. The greatest number of isolations were made from the submandibular nodes at necropsy. It should be noted that a rat fed a dose of 5.5×10^4 cells of *S. newington* harbored salmonella 3 weeks after infection.

None of the rats in this experiment had O agglutinins in dilution of 1 : 10.

Experiments with *S. enteritidis*

Distribution of *S. enteritidis* in rats following oral inoculation with the organism As shown in table 8, rats fed a dose of 5.5×10^8 organisms of *S. enteritidis* resulted in systemic infection 7 and 14 days after inoculation. The infection was more severe in younger rats. However, infection was not evident in either age group when inoculated with a dose of 5.5×10^4 organisms.

The presence of *S. enteritidis* in feces of rats fed different doses of the organism The similar experiments were made using two strains of *S. enteritidis* (411 D & No. 11). Rats inoculated with a dose of 5.5×10^8 or 5.5×10^6 of either strain shed the infecting organism continuously or intermittently and most of them harbored salmonella about 5 weeks after inoculation (tab. 9 & 10). Infection of the submandibular nodes was regular in these cases. Salmonella was occasionally isolated from organs of these rats even when the inocula were less than 5.5×10^4. Excretion of salmonella was more active in the rats fed 5.5×10^4 organisms of strain No. 11 than in strain 411 D. However, no evident difference of virulence could be found between the two strains. A similar finding was obtained in the mouse inoculation test (tab. 1).

Clinical signs, pathological findings and serological response in inoculated rats Rats inoculated with large doses of *S. enteritidis*, showed severe clinical signs of anorexia and ruffled coat at the initial stage of infection. However, septicemic death was uncommon. One of 20 rats inoculated with a dose of 5.5×10^8 and one of 4 fed a dose of 5.5 organisms died of septicemia. Rats fed larger doses showed swollen spleens or livers, white flecks on the liver, and edema of the lymph nodes. Macroscopic changes of the intestine was observed

TABLE 8 *Infection in laboratory rats fed different doses of Salmonella enteritidis*

ORGANS	180-DAY-OLD RATS				31-DAY-OLD RATS			
	5.5×10^8		5.5×10^4		5.5×10^8		5.5×10^4	
	7 days*1	14 days	7 days	14 days	7 days	14 days	7 days	14 days
Submandibular nodes	3/3	3/3			3/3	3/3		
Axillar nodes	1/3				1/3	3/3		
Inguinal nodes		2/3				1/3		
Heart						2/3		
Lungs	2/3				2/3	2/3		
Mesenteric nodes	3/3	3/3			3/3	3/3		
Spleen	3/3	3/3			3/3	3/3		
Liver	3/3	2/3			3/3	3/3		
Kidneys	2/3	1/3			3/3	3/3		
Urinary bladder					2/3	3/3		
Duodenum						3/3		
Cecum	3/3	2/3			3/3	2/3		
No. of rats salmonella-positive / No. of rats examined	3/3	3/3	0/3	0/3	3/3	3/3*2	0/3	0/3

*1 Days post infection

*2 One of the 3 rats died from septicemia 14 days after inoculation.

TABLE 9 *Presence of Salmonella enteritidis in feces obtained daily from rats*[*1] *fed different doses of the organism (Strain 411 D)*

INOCULUM	RESULTS OF FECAL CULTIVATION ON WEEKS AFTER INOCULATION					NECROPSY												
	1	2	3	4	5	On days post infection	Organs[*2]											No. of positive rats/No. of rats examined
							Sn	An	In	Lu	Mn	S	L	K	U	D	C	
5.5×10^8	26/7×4 (4)	19/7×4 (4)	5/7×4 (4)	21/7×4 (4)	7/2×4 (4)	34	4/4	1/4	1/4	1/4	2/4	2/4	3/4	1/4	1/4	1/4		4/4
5.5×10^6	17/7×4 (4)	2/7×4 (2)	4/7×4 (3)	1/7×4 (1)	2/2×4 (2)	34	3/4	1/4		1/4	2/4	1/4						3/4
5.5×10^4	3/7×4 (3)	0/7×4	0/7×4	0/7×4	0/2×4	33												0/4
5.5×10^2	2/7×4 (2)	0/7×4	0/7×4	0/7×4	0/2×4	33												0/4
5.5	1/7×4 (1)	0/7×4	0/7×4	0/7×3	0/2×3	33 24[*3]	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	0/3 1/1

[*1] About 180-day-old rats were used.
[*2] In = inguinal nodes Lu = lungs S = spleen L = liver U = urinary bladder D = duodenum
[*3] Died

TABLE 10 *Presence of Salmonella enteritidis in feces obtained daily from rats* fed different doses of the organism (Strain No. 11)*

INOCULUM	RESULTS OF FECAL CULTIVATION ON WEEKS AFTER INOCULATION					NECROPSY								
	1	2	3	4	5	On days post infection	Organs							No. of positive rats/No. of rats examined
							Sn	In	Lu	Mn	S	L	C	
5.5×10^8	11/7×4 (4)	16/7×4 (4)	7/7×4 (3)	2/7×4 (1)	2/7×4 (1)	35	4/4			1/4			1/4	4/4
5.5×10^6	9/7×4 (4)	11/7×4 (3)	10/7×4 (3)	10/7×4 (2)	10/7×4 (4)	35	3/4	1/4	2/4		1/4	1/4		3/4
5.5×10^4	5/7×4 (4)	3/7×4 (3)	1/7×4 (1)	0/7×2	0/7×2	36 23, 27 Died								0/2 0/2
5.5×10^2	1/7×4 (1)	0/7×4	0/7×4	0/7×4	0/7×4	36				1/4				1/4

* About 180-day-old rats were used.

in cases of septicemic death.

Seven rats which were inoculated with a dose of 5.5×10^8 or 5.5×10^6 of 411 D strain and which harbored the organism at necropsy about 5 weeks post infection showed positive O agglutination in dilution of 1 : 20~1 : 320. Moreover, 6 out of 7 rats fed the same doses of No. 11 strain developed O agglutinin titers of 1 : 20~1 : 160 but the remaining one rat was negative at a dilution of 1 : 10. The H agglutinin titers were relatively higher than O agglutinins. No agglutination was found either in rats which were inoculated with doses of 5.5×10^6~5.5 organisms of *S. enteritidis* and which yielded no salmonella at necropsy or in a salmonella-positive rat fed a dose of 5.5×10^2 of the organism.

DISCUSSION

It has been reported that rats are not susceptible to oral inoculation with *S. pullorum*. RETTGER et al. described that laboratory rats fed large doses of *S. pullorum* did not show any symptoms. SCHULZE stated that rats inoculated perorally with large doses of *S. pullorum* resulted in transient excretion of the organism, but he could not isolate the infecting organism from visceral organs of the rats. However, he did not examine the lymph nodes. In the present study, it was found that rats inoculated perorally with *S. pullorum* in different manners excreted the organism in their feces transiently or intermittently; further, the organism was localized regularly in the lymphoid tissues such as the submandibular or mesenteric nodes and occasionally in the spleen or liver. This finding was observed frequently in rats inoculated with a dose of 6×10^8 or more and occasionally with a dose of 6×10^6 organisms. From these results, it is evident that rats could be possible carriers of *S. pullorum*. In another study made by the present author[9], it was shown that wild rats fed 1 or 2 baby chicks dead from pullorum disease excreted *S. pullorum* transiently and also harbored the organism in their lymph nodes.

Young rats inoculated with a dose of 6×10^9 of *S. pullorum* resulted in invasion of various organs in the early stages of infection. This observation is similar to that of GERICHTER who obtained a high isolation rate of salmonella from different organs of white mice inoculated orally with a dose of 5×10^9 *S. typhi*.

So as far as the present author knows, there has been no information on the infection of rats infected experimentally with non-host-adaptive salmonella types. The most evident findings in rats inoculated with *S. newington* were localized infection of their submandibular nodes, mesenteric nodes and ceca. These infections persisted for at least 5 weeks, at which time the rats were necropsied. Rats inoculated with a dose of 5.5×10^8 or 5.5×10^6 organisms showed active excretion of *S. newington* in their feces for many days, although the frequency of shedding of the infecting organism was paralleled with the size of the inocula,

to a certain extent. It should be noted that although agglutinin production was not observed in the rats inoculated with *S. newington*, persistence of the carrier state was rather similar to that of *S. enteritidis*.

BARTRAM et al. and WELCH et al. stated that a strain of *S. enteritidis* employed by them was highly infective to both mice and rats when the organism was inoculated into the stomach. Even rats inoculated with a few organisms excreted salmonella. It is natural that the most virulent strain should be used for infection experiments. Therefore, the present author attempted to determine the virulence of strain 411 D of *S. enteritidis* by comparing it with the virulent strain No. 11. An intraperitoneal dose of several organisms of the latter strain can kill mice, although increased numbers of cells are needed for intrastomach inoculation (USHIBA et al.) In the present experiments using mice and rats, there appeared to be no marked difference of virulence between the two strains.

Rats fed a dose of 5.5×10^8 organisms of *S. enteritidis* showed systemic infection 7~14 days after inoculation, and distribution of the organism in the body became narrow about 5 weeks post infection. The course of infection is similar to the descriptions of PRICE-JONES who fed salmonella-infected bread to rats. Septicemic death was uncommon even in rats inoculated with a dose of 5.5×10^6 or 5.5×10^8 *S. enteritidis* while all mice inoculated with the same doses died (tab. 1). WELCH et al. also described a higher fatality rate of infected mice compared with that of rats.

One of the most important findings of this study was that salmonella could be isolated frequently from the submandibular nodes of inoculated rats. The infection of the submandibular nodes had a tendency to persist for many days and direct smear of such tissues on agar plates frequently yielded salmonella. Infection of the mesenteric nodes was detected generally by enrichment cultures, especially at the later stages of infection. These findings were common with the three serotypes of salmonella used in this study. The cultivation of the submandibular nodes seems to be important in examination of rats infected with salmonella, although the frequency of infection of these nodes may be influenced, to some extent, by the method of inoculation as can be seen in table 4.

The present author has conducted infection experiments with salmonella using wild rats bred in cages following the present study. It is interesting and important to know differences of the susceptibility to salmonella infection between wild rats and laboratory rats. This will make it possible to use more accurately the knowledge obtained from the study of laboratory rats for explanation of the role of wild rats in the epidemiology of salmonellosis. Although the susceptibility of both laboratory and wild rats to salmonella infection was not compared directly to each other in this study, some information on differences of the susceptibility

of both kinds of rats may be obtained from the experiments conducted under very similar conditions.

Three of 7 laboratory rats instilled with a dose of 6×10^6 organisms of *S. pullorum* shed the organism in their feces and another one of them harbored the organism at necropsy 3 weeks after inoculation (tab. 2). In an experiment with 6 wild rats, inocula of 6×10^7 and 6×10^6 did not cause any systemic infection, although one of them showed transient excretion of the organism. Eleven (92%) of 12 laboratory rats fed a dose of 6×10^8 cells of *S. pullorum* harbored salmonella 3～4 weeks after inoculation (tab. 2 & 4), while 10 (48%) out of 21 wild rats inoculated similarly resulted in infection over the same interval. Four (25%) of 16 laboratory rats fed doses of 5.5×10^4～5.5×10^6 organisms of *S. newington* showed infection in the lymph nodes 3～5 weeks post infection (tab. 7), while 7 wild rats given the similar dose did not yield salmonella from their tissues 4 weeks after infection. Six (75%) out of 8 laboratory rats inoculated with 5.5×10^6～5.5×10^8 cells of *S. enteritidis* (strain 411 D) excreted the organism in their feces on the 5th week after inoculation (tab. 9). On the other hand, only one (14%) of 7 wild rats infected similarly yielded salmonella in their feces when culturing at the same interval. These data seem to indicate that wild rats are somewhat less susceptible to salmonella infection than laboratory rats.

SUMMARY

Albino rats were inoculated orally with different doses of *Salmonella pullorum*, *Salmonella newington*, or *Salmonella enteritidis*.

The rats fed a dose of approximately 6×10^8 or more cells of *S. pullorum* shed the organism transiently in most instances or sometimes intermittently for a few weeks. Agglutinins were detected in inoculated rats in the absence of any clinical and pathological changes. *S. pullorum* was recovered during the early stage of infection from different organs including spleen and liver of the rats. Rats became infected transiently when they were inoculated with a dose of 6×10^6 of the organism.

A part of the rats inoculated with a dose of approximately 5.5×10^8 or 5.5×10^6 cells of *S. newington* shed the organism continuously or intermittently at least for 5 weeks after inoculation. The organism was localized in the submandibular, and mesenteric nodes, and ceca of the rats in the early stages of infection without resulting in any symptom, pathological change and agglutinin production. In rats fed a dose of 5.5×10^4 organisms, transient excretion of salmonella in feces and occasional infection in the lymph nodes were observed.

The rats infected with approximately 5.5×10^8 or 5.5×10^6 cells of *S. enteritidis* excreted salmonella intermittently or continuously and a part of them did so for

at least 5 weeks after inoculation. In this case, systemic infection occurred along with clinical and pathological changes and development of agglutinins. Rats inoculated with 5.5×10^4 or less of *S. enteritidis* transiently excreted the organism with an occasional infection occurring in their organs.

Laboratory rats appear to be somewhat more susceptible to salmonella infection than wild rats.

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