Rôle of Rats in the Spread of Food Poisoning Bacteria of the Salmonella Group*

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THE fate of *Salmonella enteritidis* when fed to rats under laboratory conditions was demonstrated in a previous publication.[1] Transmission of infection with *Salmonella enteritidis* from an infected rat to cage mates and the existence of the carrier state in a small number of infected rats as long as the 40th day after infection was also shown. The absence of *Salmonella* infection and agglutinins for organisms of the *Salmonella* group in 800 normal laboratory animals was reported. In the present investigation these studies have been extended to include the longevity of *Salmonella enteritidis* in rat pellets, the minimum dose of this organism necessary to provoke infection in rats and mice and the distribution of *Salmonella* in rats and mice pellets collected throughout the United States.

Attempts to isolate organisms of the *Salmonella* group from rat or mouse excreta have usually been made because of food poisoning outbreaks. As far as can be determined from the literature no attempt has been made previously to study rodent pellets collected over a large area without regard to history of intestinal disease. Savage and White[2] isolated *Salmonella enteritidis* from the intestines of rats but failed to demonstrate these organisms in their excreta while Salthe and Krumweide[3] were successful in isolating *Bacillus pestis-caviae* from rodent excreta obtained in a bakery where prepared cream filling was shown to be the cause of a food poisoning outbreak. Our preliminary[1] studies appeared to indicate that infected rats excrete the infecting organism intermittently. Because of this, it would seem that a study of the incidence of *Salmonella* in rat or mouse excreta would be of more significance from the standpoint of food poisoning potentialities than a similar study of the organs of the rodents. Isolation of *Salmonella* from the organs of rats has been demonstrated by Meyer and Matsumura,[4] Verder,[5] Kerrin,[6] Khabil,[7] and Hatta,[8] in from 0.7 per cent to approximately 13 per cent of the animals examined. In each instance these investigators studied rats obtained from a relatively small locality and, in view of the apparent ease with which infection is transmitted within colonies of these animals, a false impression of the incidence of infection in rodents as a whole might be obtained by a study of the

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figures presented. Furthermore, the significance of rats as vectors of food poisoning organisms similarly might be overemphasized, particularly in view of the known intermittency of excretion of the *Salmonella* organisms by infected animals. Meyer and Matsumura (*loc. cit.*) recognized the possibility of misinterpreting the incidence of *Salmonella* in rats when they point out that although Savage and Reed found an incidence of 8.5 per cent their studies were confined to a slaughterhouse. Meyer and Matsumura, on the other hand, made their studies in large districts of a growing city. In studies on the relation of virulence of *Bacterium aertrycke* and epidemic potency Topley, *et al.*[9] have demonstrated an apparent relationship between virulence and power of infection to spread within colonies of mice. Webster[10] and his coworkers, however, in a long series of carefully controlled studies describe the virulence of any single strain of *Salmonella aertrycke* as but slightly variable, considering that any single strain maintains a constant level of virulence for an indefinite period of time under the usual methods of cultivation. Topley[11] later showed that when susceptible mice are added continuously and at a constant rate to an infected population, the spread of infection, as judged by a mortality curve, is propagated in regularly recurring waves. There is little doubt that, in laboratory studies on the infectivity of *Salmonella* for rats or mice, consideration must be given to the strain used. It will be shown later that the strain of *Salmonella enteritidis* used in this study was highly infective for both rats and mice. In contrast to these results, Kligler and Olitzki[12] report that rats are highly resistant to infection with *Salmonella enteritidis* and because of this massive doses and infant rats had to be used in their studies.

EXPERIMENTAL

To determine the longevity of *Salmonella enteritidis* in rat excreta a single animal was fed 0.5 cc. of a broth culture by stomach tube and placed with 4 normal rats for a period of 10 days. On the 10th day all feces were collected and stored in Petri dishes at room temperature. At intervals specimens of these stored feces were examined for *Salmonella enteritidis* by emulsifying 2 gm. in 10 cc. of tetrathionate broth from which decimal dilutions were made in the same medium. The broth tubes were incubated at 37° C. for 24 hours, after which bismuth sulfite agar plates were heavily streaked from the broth tubes and incubated at 37° C.

TABLE 1

Longevity of Salmonella enteritidis in Rat Feces Stored at Room Temperature

Amount of Feces Examined (Grams)	*Days Feces Examined*											
	1	*10*	*14*	*21*	*29*	*50*	*74*	*92*	*121*	*148*	*182*	*189*
2.0	+	+	+	+	+	+	+	+	+	+	O	O
0.2	+	+	+	+	O	+	+	+	+	+	O	O
0.02	+	+	O	O	O	O	O	+	+	O	O	O
0.002	O	O	O	O	O	O	O	O	O	O	O	O

NOTE—One rat fed 0.5 cc. 24 hr. broth culture. Fed rat caged with 4 normal rats for 10 days. On 10th day all feces collected and stored at room temperature.

METHOD OF EXAMINATION—Two grams feces emulsified in 10 cc. tetrathionate broth and decimal dilutions made in tetrathionate broth. Tubes incubated at 37° C. for 24 hrs. Bismuth sulfate agar plates streaked then incubated at 37° C. for 2 days. Suspicious colonies fished and identified biochemically and serologically.

+ = sal. ent. isolated.
O = negative.

for 48 hours. Suspicious colonies were isolated and identified biochemically and serologically. The results are given in Table 1.

At no time was *Salmonella enteritidis* isolated from the dilution representing 0.002 gm. of feces while in every instance *Salmonella enteritidis* was isolated from 2 gm. portions of feces up to and including the 148th day. All specimens examined on the 182nd day were negative.

Preliminary studies had shown that extremely small inocula (*per os*) of the strain of *Salmonella enteritidis* used in this investigation were necessary to bring about infection and subsequent excretion of the organism in both rats and mice. To determine the minimum numbers of *Salmonella enteritidis* neces-

TABLE 2

Minimum Numbers of Salmonella enteritidis Necessary to Produce Infection in Rats and Mice by Stomach Tube Feeding

Rats									Mice								
Average Number Organisms		Animal Number	Excretion of S. enteritidis in Feces; on Days After Isolation 1	3	7	9	13	15	Number Positive	Average Number Organisms		Animal Number	Excretion of S. enteritidis in Feces; on Days After Isolation 1	3	7	9	13	15	Number Positive
F	15	1	O	O.	O	O	O	O		F		1	O	O	O	O Dead			
e	"	2	+	O	O	O	O	O		e		2	O	O	O	O	O	O	
d	"	3	+	+	O	+	O	O		d		3	O	O	O	+ Dead			
	"	4	+	O	O	+	+	O	3			4	O	O	O	O	O Dead		
												5	O	+	* Dead				4
o										o									
n	6	5	O	O	O	O	O	O		n		6	+	O	O	O	O	O	
e	"	6	O	O	O	+	O	O		e		7	O	O	O	O	+ Dead		
	"	7	O	+	O	+	O	O				8	+	+	+	+	O	O	
c	"	8	+	+	O	+ Dead				h		9	O	O	O	O Dead			
c	"	9	+	O	O	O	O	O	4	a		10	O	O	+	+	+ Dead		5
										l									
										f									
a																			
m	4	10	+	O	O	O	O	O		c		11	O	O	O	O	+	O	
o	"	11	+	O	O	O	O	O		c		12	O Lost						
u	"	12	O	O	O	O	O	O				13	O	O	O Dead				
n	"	13	O	O	O	O	O	O				14	*	* Dead					
t	"	14	O	O	O	O	O	O	2			15	O	O	O	O	+	O Dead	4
s																			
										a									
	2	15	O	+	O	O	O	O		m		16	O	O	O	O Dead			
	"	16	O	O	O	O	O	O		o		17	O	O	+	O	O Dead		
	"	17	O	O	O	O	O	O		u		18	O	O	O Dead				
	"	18	+	+	O	O	O	O		n		19	O	O	+	O	O	O	
	"	19	O	O	O	O	O	O	2	t		20	+	+	+ Dead				5
										s									
	1	20	O	O	O	O	O	O				21	* Dead						
	"	21	O	O	O	O	O	O				22	O	+	O	+ Dead			
	"	22	O	O	+	+	O	O				23	O	O	O	O Dead			
	"	23	+	+	O	O	O	O				24	O	O	O Dead				
	"	24	+	O	O	O	O	O	3			25	O	O	O	O	O	O	4

Average number organisms—based on triplicate plate counts of amounts fed
Dead animals positive for *S. enteritidis* on autopsy
+ = Isolation of *S. enteritidis*
O = Negative
* = No feces available

sary to bring about infection in these animals a 24 hour broth culture was diluted 10–8 and 1 cc. amounts fed by stomach tube to each of four rats. The same amount of this dilution was inoculated into dextrose agar plates in triplicate. The average plate count was 15 organisms per cc. Further dilutions of 1:2, 1:4, 1:8, and 1:16 of the 10–8 dilution were made and each dilution in 1 cc. amounts was fed to each of 5 rats. Triplicate plate counts were made of each dilution. These results are given in Table 2.

Included in Table 2 also are the results obtained on feeding 0.5 cc. amounts of the above dilutions to each of 5 mice. The mice thus received approximately half the number of organisms fed to the rats. Three of the 4 rats fed with 15 organisms (based on plate counts) were infected and one-half this amount fed to mice resulted in infection of 4 out of 5 fed. Similarly, infection was obtained in rats with dilutions of broth culture which were shown by plate counts to contain an average of one organism per cc. In mice the infecting dose averaged less than one organism per cc. Although the plate counts may not reflect the exact numbers of organisms fed these animals, these results would indicate that very few organisms of the strain of *Salmonella enteritidis* used were necessary to bring about infection in both rats and mice by stomach tube feeding.

In studying the natural transfer of *Salmonella enteritidis* in rats, 8 attempts at transfer into colonies of normal rats have been made. Five of the transfers were successful and 3 resulted in failures. For transfer purposes the animals were either infected by stomach tube feeding or by intravenous injection. After inoculation the treated ani-

TABLE 3

Natural Transfer of Salmonella Enteritidis in Rats

	Positive Fecal Specimens on Days After Isolation											*Agglutinin Titers*
	2	*3*	*5*	*6*	*7*	*8*	*9*	*10*	*14*	*21*	*28*	
☒ Fed 100 cells												
☒ □ ☒ Composite fecal specimen		+	+	+								1:320 (30 Day)
☒ □ ☒ →		+			+			+				
☒ □ ☒ →	+			+			+		O			1:640 (14 Day)
☒ □ ☒ →		+		+		+						1:80 (20 Day)
☒ □ ☒ →		+				O						1:80 (10 Day)
☒ □ ☒ →		+		+								1:80 (9 Day)
☒ □ ☒ →		+		+		+						1:160 (8 Day)
□ □ □ →											O	1:40 (28 Day)
□ □ □ →												1:40 (21 Day)

NOTE—One rat fed, by stomach tube then placed in a colony of normal rats for 7 days
One rat was then isolated to determine infection. The other rat was placed in a new colony of normal rats
□ = Uninfected rat
☒ = Infected rat
\+ = *S. enteritidis* isolated
O = Negative

TABLE 4

Natural Transfer of Salmonella enteritidis in Mice

	Examination of Fecal Specimens on Days After Isolation									
Animal Number	*2*	*3*	*5*	*7*	*9*	*12*	*14*	*16*	*20*	*22*
1	O	+	+	O	+ Dead					
2	+ Dead									
3	+	O	O	O	O Dead					
4	O	+		O		O	O	O	O	O
5	+		O	O		O	O	O Dead		
6	+		O Dead							
7	O		O	O		O	O	O	+ Dead	
8	O		O	O		O	O	O	O	O
9	O		O	O		O	O	+	O Dead	
10	+		O (Placed in a colony with 10 normal mice for 7 days)							
→ 10		Dead								
31			+		O	O		+ Dead		
32			O		O	O		+	O	
33			+		+	C		+	+ Dead	
34			+		O	O		+ Dead		
35			+		O (Placed in a colony with 4 normal mice for 7 days)					
36			O		O	O Dead				
37			O		O	+ Dead				
38			O		O	O Dead				
39			O		O Dead					
40		Dead								
→ 35	O		O Dead							
41										
42	O		O							
43	+		O (Placed in a colony with normal mice)							
44	+		O " " " " " " "							

NOTE—Animal number 1 injected intravenously 0.1 cc. 24 hr. broth culture
Infected animal caged with 9 normal mice for 7 days
All animals then isolated to determine infection
Transfer colonies 10 & 35 as above
+ = *S. enteritidis*
O = Negative

mal was isolated until *Salmonella enteritidis* could be demonstrated in the excreta, following which the infected animal was placed with a group of normal animals. The colonies of normal animals varied in number from a minimum of 2 to a maximum of 10. Although one of the 3 failures occurred in a colony containing 10 animals, it is of interest that all 3 failures occurred in those colonies in which the infected animals had been injected intravenously. In each instance where the infected rat was fed by stomach tube, colony transfer of *Salmonella enteritidis* was successful. Intravenous injections consisted of 0.1 cc. of broth culture (approximately 10^7 organisms), while stomach tube feeding was accomplished with approximately 100 bacterial cells in 1 cc. of salt solution. The composite feces from colonies in which transfer of infection from one infected animal to cage mates had been accomplished were examined for *Salmonella enteritidis* at intervals for 1 month before being discarded.

The results of natural transfer of *Salmonella enteritidis* through 7 rat colonies are given in Table 3.

In this experiment one rat was fed 100 bacterial cells by stomach tube, identified with dye, and placed with a colony of 5 normal rats for a period of 7 days. During this interval composite samples of excreta were examined for

Salmonella enteritidis by emulsifying in tetrathionate broth followed by streaking on bismuth sulfite agar. For the sake of simplicity 3 rats only in each colony are shown in the table. At the end of 7 days one of the rats in the first colony was isolated to determine whether passage had been successful, and another was placed with a fresh colony. This procedure was repeated with 9 colonies. It will be seen from the table that natural transfer occurred in 7 colonies. Both the 8th and 9th colonies failed to show *Salmonella enteritidis* in their excreta. In spite of failure to get transfer of infection in colonies 8 and 9, the blood sera of rats isolated from these colonies showed agglutinin titers of 1:40. Since studies of a large number of our normal laboratory rats have failed to reveal agglutinins for the *Salmonella enteritidis* strain used, a titer of 1:40 probably indicates transfer of infection in both colonies 8 and 9.

Similar studies of natural transfer of *Salmonella enteritidis* in mice have been made. The results are given in Table 4 which shows that to date only 3 colony transfers have been completed. This work, however, is being continued.

In contrast to the natural infection in rats which seemed to withstand infection with *Salmonella enteritidis* quite well, many of the mice so far studied have died shortly after becoming infected.

The relative ease with which infection and, in some instances, death, resulted in mice following stomach tube feeding, coupled with the observation that massive doses of organisms were necessary to bring about this result in rats and mice by intravenous injection, made it desirable to determine the minimum lethal dose of organisms for these animals when the latter route of injection was used. Accordingly, 100 rats and 100 mice were divided into groups of 20 each and increasing numbers of *Salmonella enteritidis* were injected intravenously in the median vein of the tail, and the number of animals dying of acute infection determined over a period of 5 days. These results are shown in Table 5.

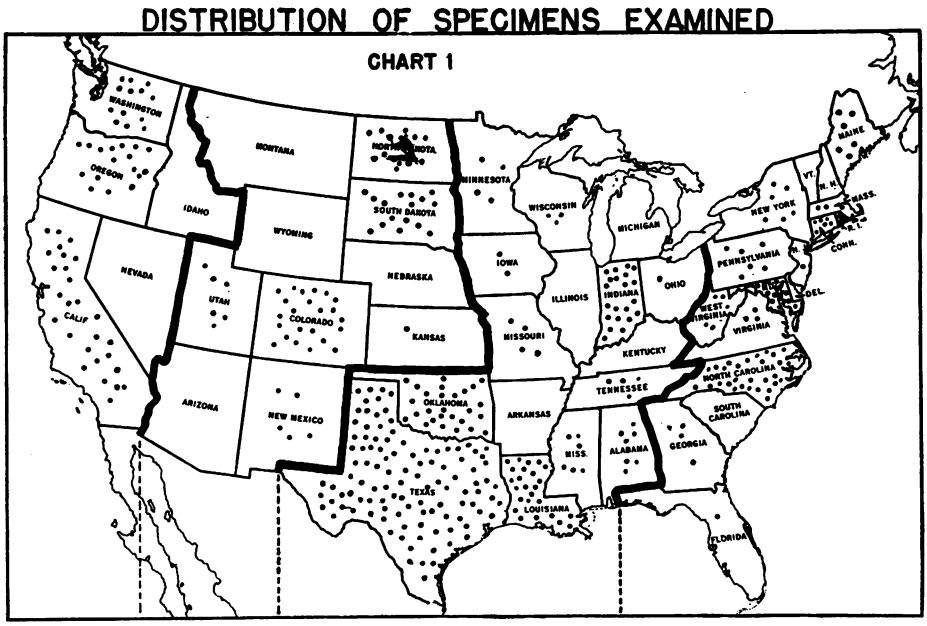
TABLE 5

Mortality in Rats and Mice Following Intravenous Injection of Massive Doses of Salmonella enteritidis

Mice								*Rats*							
		Mortality in Days								*Mortality in Days*					
Number Animals	*Number of Organisms*	*1*	*2*	*3*	*4*	*5*	*Total Number Dead*	*Number Animals*	*Number of Organisms*	*1*	*2*	*3*	*4*	*5*	*Total Number Dead*
20	850,000,000	8	7	4	0	1	20	20	4,000,000,000	4	2	3	1	0	10
20	425,000,000	1	5	9	2	2	19	20	3,000,000,000	1	2	4	3	3	13
20	212,000,000	1	3	7	7	0	18	20	2,000,000,000	2	1	4	4	2	13
20	42,500,000	0	0	2	3	0	5	20	1,000,000,000	1	0	1	1	3	6
20	21,000,000	0	0	1	1	2	4	20	200,000,000	0	0	0	0	2	2

It will be noted that more than 212 million organisms were required to cause 90–100 per cent mortality in mice and that only 65 per cent of the rats were killed with 2 billion organisms by this method of injection. Nearly 50 per cent of the mice injected with 21 million organisms lived for 28 days, while those injected with twice this number lived for 11 days. These results are in marked contrast to those obtained by feeding this organism (see Table 2). The results shown in Table 2 indicate that infinitely small numbers of organisms will infect both mice and rats and that fatalities in mice occur in 13 days or less when only approximately 7 organisms are fed by stomach tube.

To determine the incidence of *Salmonella* in rat and mice excreta col-



lected from widespread areas, samples have been collected throughout the United States through coöperation of the Division of Predator and Rodent Control, Fish and Wildlife Service, U. S. Department of Interior. To date, some 420 samples have been examined. The areas from which samples have been obtained and their distribution throughout the country are given in Chart 1. With each sample collected a brief history was obtained, giving the source of the material and whether a rat virus had been used in the area involved. The source of the specimens are given in Table 6.

Of the 420 specimens examined, 340 were samples of rat excreta and 80 were mice excreta. Seven samples, 1 from mice and 6 from rats, contained *Salmonella*. Two of the rat samples contained *Salmonella morgani* while a total of 5 (1.2 per cent), 1 from mice and 4 from rats, contained food poisoning types of *Salmonella*. Through the coöperation of Dr. K. M. Wheeler, Connecticut State Department of Health, Bureau of Laboratories, Hartford, Conn., and Dr. P. R. Edwards, Kentucky Agricultural Experiment Station, University of Kentucky, Lexington, Ky., an antigenic analysis of these strains has been made. Strain 31, isolated from mouse excreta obtained in Baltimore, Md., was classi-

TABLE 6

Sources and Numbers of Rat and Mice Pellets Examined and Number Found Positive for Salmonella

Sources	*Number of Specimens*	*Number Positive*
Bakery	10	0
Bottling Plant	2	0
Candy Plant	66	1 (Maryland) mouse
Cotton Gin	3	0
Crab Meat Plant	2	0
Creamery	13	0
Dump	9	1 (California) rat
Farm	31	0
Field	7	0
Granary	37	0
Grocery	36	0
Home	30	0
Mdse. Store	19	0
Meat Packing Plant	29	1 (Colorado) rat
Restaurant	21	* Rat
Warehouse	95	2 (Texas) rat
Unknown	10	0
Total	420	5

Mice 80 Rats 340

* 2 strains *Sal. morgani.* N. M. & Miss.

fied as *Salmonella typhimurium;* strain 151, isolated from rat excreta obtained in Grasy Horse Canyon, Calif., appeared to be *Salmonella sandiego* although because of conflicting results this strain is still being studied; strain 110, isolated from rat excreta obtained in Denver, Colo., was classified as *Salmonella sp.* (Newington type); and strains 127 and 211, both isolated from rat excreta obtained in Houston, Tex., are classified as strains of *Salmonella anatum.* As far as could be determined, rat virus had not been used in the areas from which samples of excreta containing *Salmonella* were obtained.

DISCUSSION

For a great many years considerable emphasis has been placed on the significance of rats and mice as vectors of food poisoning organisms of the *Salmonella* group; yet only an extremely small number of outbreaks of food infection have been established beyond reasonable doubt as traceable to rodents infected with these organisms. Although the difficulty in obtaining the necessary evidence implicating rodents in an outbreak might be caused by hazards of sampling and limitations of laboratory technic, as pointed out by Staff and Grover,[13] we have had little difficulty in the isolation of *Salmonella enteritidis* from the excreta of naturally infected rats and mice. These studies have shown the strain used is extremely infective for both rats and mice and that it is transmitted from infected animals to cage mates rapidly and in the absence of cannibalism. Very few organisms were required to bring about infection in rats and mice injected by stomach tube while massive doses were necessary to bring about infection and death by intravenous injection. These findings relating to stomach tube feeding are in contrast to those reported by Kligler and Olitzki (*loc. cit.*) who fed massive cultures of *Salmonella enteritidis* to young rats to bring about infection, and concluded that rats are highly resistant to infection with this organism. We feel that undoubtedly these differences in results are related to the invasiveness or virulence of the strain of *Salmonella enteritidis* used. The particular strain used in these studies was originally isolated by Staff and Grover [13] from an epidemic of food poisoning involving 208 cases and 3 deaths in which infected rats had apparently contaminated a cooked cream filling for bakery products.

This investigation has shown that excreta from naturally infected rats may contain living *Salmonella enteritidis* for at least 148 days when kept at room temperature. The small number of virulent *Salmonella* organisms necessary to bring about infection in rats and mice, the ease and rapidity with which infected animals transmit the disease to cage mates in colonies, and the longevity of *Salmonella* in rat excreta cannot be correlated with the paucity of food poisoning outbreaks known to involve these animals. The results of examining some 420 specimens of rat and mice pellets, from which only 5 strains of *Salmonella* of the food poisoning type were isolated, collected throughout the country, however, might explain the dearth of food poisoning outbreaks proved to be the result of infected rodents. It is true that much higher incidences of food poisoning organisms have been reported in rats and mice, but invariably the investigators reporting such figures gathered their animals in a small area, or from a definite source, such as a packinghouse or a bakery and in several instances the studies were carried out because of a recent outbreak. Under such conditions with the apparent ease of transmission of infection in rat colonies higher incidences of infection in a given area might be expected.

Although a much larger series of specimens of rodent excreta need to be

examined from many more areas in the United States before a definite conclusion can be reached, our studies to date indicate that relatively few rodents are infected with food poisoning organisms of the *Salmonella* group. It would appear that this would explain, in part at least, the relatively few outbreaks of food poisoning in which rodents were proved to be vectors of the causative organisms. Nevertheless, since some few rats or mice may be infected with food poisoning organisms, they all must be considered potentially dangerous to health, and every effort should be made to eliminate them from establishments where human food is prepared or stored.

SUMMARY—CONCLUSIONS

Excreta of rats naturally infected with *Salmonella enteritidis* held at room temperature may contain living organisms for at least 148 days.

Infection of rats and mice with very few organisms is possible when a virulent strain of *Salmonella enteritidis* is fed them by stomach tube.

Transfer of infection from an infected animal to cage mates has been carried through 7 colonies with rats and through 3 colonies with mice.

A study of rat and mouse excreta collected in areas throughout the United States indicates that only a small percentage (1.2 per cent) of these animals are excreting food poisoning organisms of the *Salmonella* type.

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