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FECAL EXCRETION OF *YERSINIA ENTEROCOLITICA* IN MICE AND RATS INOCULATED INTRAGASTRICALLY

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Two strains of *Yersinia enterocolitica*, 03 biovar 4 and 06 biovar 1, were inoculated intragastrically into laboratory rats and mice. The 03 organism began to reside in the intestinal tracts and was excreted in the feces in large amounts during a period of 2 to 4 weeks from the rats and 2 to 10 weeks from the mice which had been inoculated with 10^7 or more cells of the organism. In the mice and rats inoculated with 10^9 cells of the 06 strain, however, there was no evidence of the settling of the organism in the intestinal tract. The serum O agglutinin was detected only in half of the animals in which 03 strain became resident.

INTRODUCTION

Human *Yersinia enterocolitica* infections are almost always caused by 03 biovar 4; 05 biovar 2; 08 biovar 1 and 09 biovar 2.

KANEKO et al. (1978) first isolated 03 biovar 4 organism of human pathogen from the intestines of brown rats (*Rattus norvegicus*) captured in a slaughterhouse. The question of whether mice and rats act as reservoirs of the organism was left unclear.

This study was undertaken to investigate the role of rats and mice as reservoirs of *Y. enterocolitica* by noting the presence of the organism in the excreted feces of animals which had been intragastrically inoculated.

MATERIALS AND METHODS

Animals Female, SPF dd-N mice and Wistar rats (Japan CLEA Inc., Fuji, Shizuoka Prefecture, Japan) were used under the normal conventional conditions and fed pellet feed (Lab. MR Standard, Nihon Nohsan Kogyo, Ltd., Yokohama, Japan) and fresh water. Serum agglutinins against the inoculated bacteria were negative in these mice before inoculation.

Strain Two strains of *Y. enterocolitica*, 03 biovar 4 and 06 biovar 1, which had been isolated from the intestinal contents of captured rats,²⁾ were used.

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Preparation of the bacterial suspension for inoculation The organism were grown on Trypticase soy agar (BBL) plates at 25°C for 48 hours and then suspended in physiological saline and centrifuged. The cells were then re-suspended in a solution made up of an equal volume of 10 % lactose in water and calf serum. The bacterial suspension was adjusted to 10 mg bacteria per ml of the solution and stored at -85°C. There was no significant change in the viable cell counts of the solution noted during the study.

Intragastric administration of the bacteria Both the mice and rats were fed intragastrically by means of a stainless steel gastric feeding tube, 7 cm long 1 mm external diameter, which was connected to a syringe.

Enumeration of *Y. enterocolitica* in the feces The feces were suspended in and diluted serially with physiological saline at tenfold, and 0.1 ml of the dilution was plated on SS agar (Eiken, Tokyo). After incubation at 25°C for 48 hours, the colonies suspected to be *Y. enterocolitica* were counted and then tested by slide agglutinin using rabbit 0 antisera. This technique is valid only for count above 100 colony-forming units per g of the feces.

Detection of serum 0 agglutinin The serum was obtained before and 1, 3, 5, 7, 10, 13 weeks after inoculation. All sera from the coccygeal veins or arteries was diluted tenfold with physiological saline and incubated at 56°C for 30 minutes before the test. The agglutinin test was performed as described by WINBLAD et al. (1966). Serial dilutions of serum were employed using a microtiter technique, the lowest being 1:20.

RESULTS

03 and 06 organism recovery in the feces of mice and rats

The fecal excretion of the organism in four mice and four rats inoculated with 10^9 viable cells of each organism is shown in figure 1.

The excretion of 03 organism In all mice, 10^6 to 10^7 viable cells/g feces were excreted on the 1st day after inoculation, and the number of excreted cells increased to 10^8 cells/g on the 3rd day. The mice continued to excrete over 10^7 cells/g for several days. In 2 mice, the number of excreted cells began to decrease 2 weeks after inoculation; the other 2 mice continued to excrete over 10^7 cells/g until 5 to 10 weeks. The excreted cells in the rats reached the maximum number on the 5th or 7th day, after which they began to decrease to less than 10^3 cells/g by the 3rd to the 4th week.

The excretion of 06 organism One mouse did not excrete the organism during the course of the investigation, while the three others excreted 10^4 to 10^6 bacteria/g on day 1. In these 3 cases, the number of excreted cells decreased to about 10^3 cells/g on the 3rd day. The animals ceased to excrete the organism on the 7th day. Two of the four rats inoculated excreted 10^3 to 10^4 cells/g only on the 1st day, and thereafter, these rats did not excrete the cells. The 06 strain failed to grow in the intestines of the mice and rats which had been inoculated with the same dose (10^9 bacteria/animal) as the 03 strain.

FIGURE 1 Excretion of *Y. enterocolitica* in the feces of mice and rats intragastrically inoculated with 10^9 cells

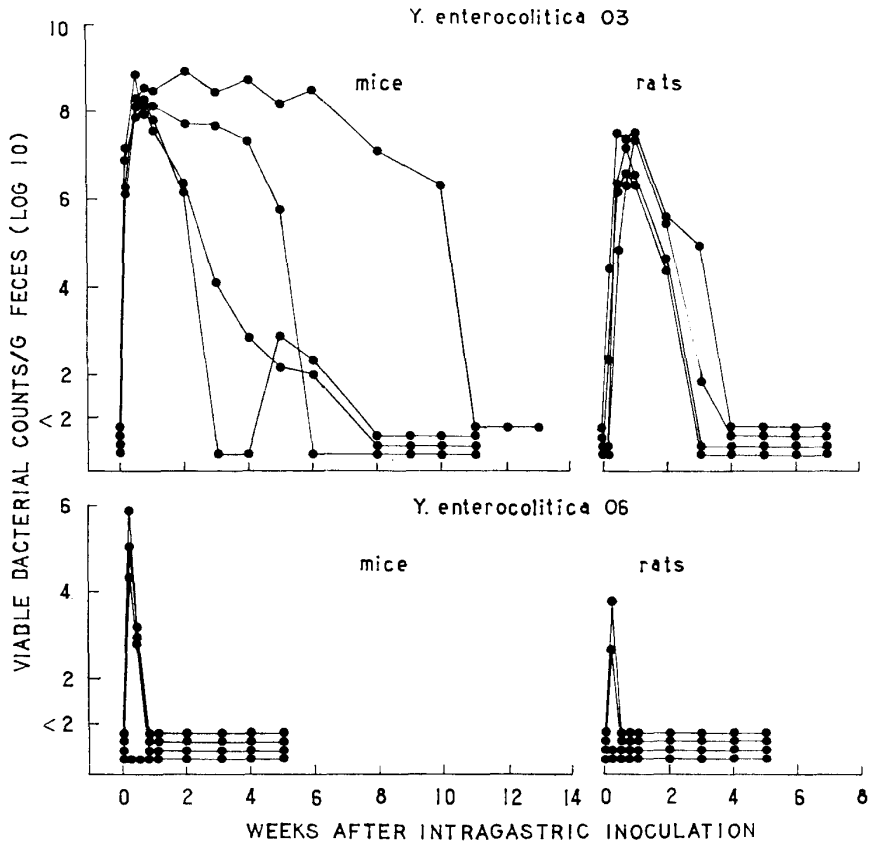


TABLE 1 Relation between settlement in the alimentary tract and serum O agglutinin production of *Y. enterocolitica* strains

ANIMAL	INOCULUM		NO. OF ANIMALS SHOWING SERUM O AGGLUTININ TITER OF 1:20 OR HIGHER	
	0-Group	size	Organism settled* ¹ in intestines	Organism unsettled in intestines
Mouse	03	10^3		0/4* ²
		10^5	0/1	0/3
		10^7	4/8	
		10^9	4/6	
Rat	06	10^9		0/4
	03	10^9	2/4	
	06	10^9		0/4

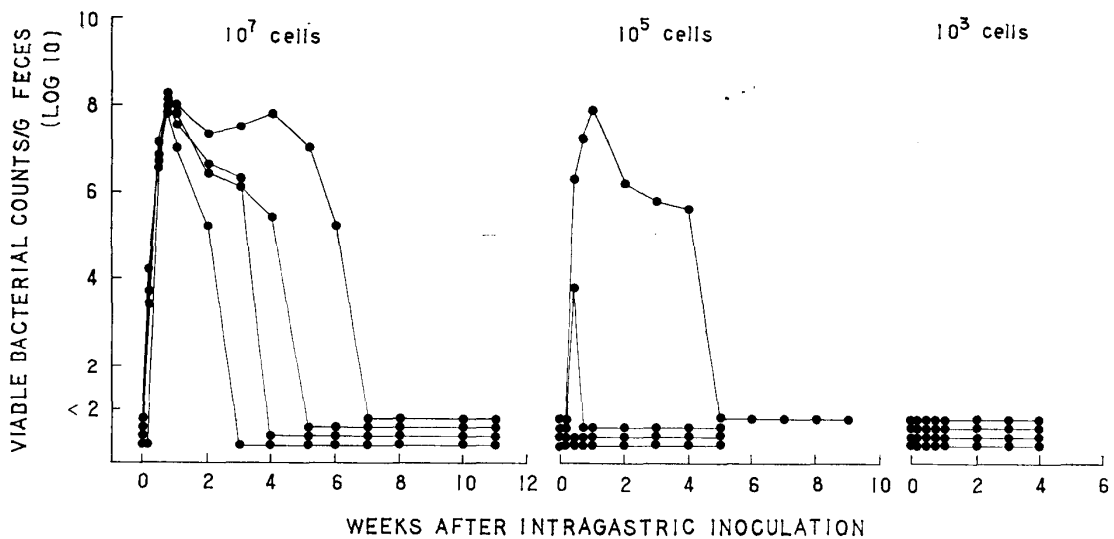
*1: Bacterial settlement in the intestines is defined here as persistent excretion of 10^4 bacteria per g in the feces of mice and rats for at least 2 weeks.

*2: Number of animals showing an agglutinin titer of 1:20 or higher per number of animals studied.

Relation between the number of inoculated cells and the fecal excretion of the 03 organism in mice

The results are shown in figure 2. In the 4 mice inoculated with 10^7 cells, the counts in the feces continued to increase from day 1 (about 10^3 bacilli/g) to day 5 (approximately 10^8 bacilli/g), followed by a continuous excretion for the 2 ensuing weeks or longer and a subsequent rapid disappearance. Of the four mice administered 10^5 cells, two excreted the bacilli at day 3. However, only one mouse from this group showed high levels of bacillary excretion which persisted for a few weeks. None of the mice given the 10^3 cells showed detectable bacterial counts at any period. Thus we concluded that the persistent and high counts in the feces over the several weeks period indicating the presence of *Y. enterocolitica* in the intestines was influenced by the amount of intragastric inoculum.

FIGURE 2 Relation between intragastric inoculum size and the fecal excretion of *Y. enterocolitica* 03 in mice



Relation of intraintestinal *Y. enterocolitica* settlement and serum 0 agglutinin production in intragastrically inoculated mice and rats

The results obtained from 26 mice and 8 rats inoculated with 03 or 06 organism are summarized in table 1. Serum 0 agglutinin was undemonstrable after inoculation in the animals in which the organism (03 or 06) had failed to settle. Of a total of fifteen mice in which the 03 strain had been settled in the intestines, eight (55.3%) were positive for the serum agglutinin. Although it was noted that the percentage of animals positive for agglutinin rose with increasing doses, the positive rate was 66.7% (4/6) even in the group receiving 10^9 bacteria. 03 but not 06 organism became

settled in the alimentary tract of the 4 rats inoculated; however only 2 rats (50 %) inoculated with 03 organism proved positive for the serum agglutinin.

DISCUSSION

In this study, *Y. enterocolitica* 03 became settled in the intestines of all of the mice and rats dosed with 10^7 or more cells intragastrically. This finding indicated that the organism may have entered the animals by the oral route, which was assumed to be the most natural mode of transmission, based on the evidence that the organism was isolated predominantly from the cecum, colon and rectum¹⁾ and that it became colonized in the large intestines of mice inoculated intravenously with a pathogenic strain.³⁾ In this study, after the organism had settled in the intestines of the mice and rats, they excreted large amounts of the organism in the feces (over 10^4 cells/g feces) for a few weeks. This finding suggested that mice and rats may have an important role in the dissemination of the organism into the environment.

Compared with the rats inoculated 10^9 cells of *Y. enterocolitica* 03, the similarly inoculated mice excreted a higher maximum number of the organism in the feces, which revealed a longer duration of the bacterial settlement. This result suggests that a different degree of sensitivity to the organism exists between both species and that the mice are more prone to excrete the organism in the feces than the rats.

The dose for the bacterial settlement in the intestines of 50 % of the mice was estimated to be approximately 10^6 cells from the finding that the organism had settled in all of the 8 mice dosed at 10^7 and in 1 out of the 4 mice administered 10^5 cells (table 1). On the other hand, in 3 of the 9 mice in which the organism had settled, excretions of 10^7 cells/g feces (figs. 1 & 2) above ID_{50} were detected for a few weeks. This finding suggested that the organism might have maintained its population by direct feces-to-mouth transmission.

Although *Y. enterocolitica* 06 biovar 1 has been frequently isolated from rodents,^{1,2)} the non-pathogenic strain (e.g. 06 biovar 1) has not been observed to settle in the intestines of mice by the intravenous route.³⁾ In this study, the strain also failed to settle in the intestines of mice and rats by intragastric administration. Further studies are needed to determine why the 06 organism is prevalent in rodents.

The serum 0 agglutinin titration was performed frequently for each mouse throughout the study. No agglutinin was detected (the titer being less than 1:20) in any of the uninfected animals, and it was found in only half of the infected animals (the titer being over than 1:20). Thus we concluded that a titer ≥ 20 against the organism may be a reliable indicator of the presence of infection in the mice and rats in this experimental system, although it may also be necessary to isolate the organism to determine the same.

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