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Author(s)	UENO, Hiroshi; KANEKO, Ken-ichi; HASHIMOTO, Nobuo
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RELATION OF THE SERUM AGGLUTININ AND
THE FECAL EXCRETION OF *YERSINIA*
ENTEROCOLITICA 03 IN MICE

Hiroshi UENO,* Ken-ichi KANEKO
and Nobuo HASHIMOTO

Department of Veterinary Public Health
Faculty of Veterinary Medicine
Hokkaido University, Sapporo 060, Japan

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The organism settled in the intestines of all of the mice inoculated at a dose of 10^7 to 10^9 cells. However, most of the serum O agglutinin detected was as low as 1:40, and the highest titer reached was 1:80. In some mice, the organism continued to colonize in the gut and to produce serum agglutinin. These results suggested that the serum antibody did not affect the termination of the fecal excretion. After second inoculation, no excretion of the organism was observed in any of the 4 mice in which the excretion of the first inoculation in the feces had already been terminated.

INTRODUCTION

Using human pathogens of *Y. enterocolitica*, many experiments^{1-5,8)} had been aimed at creating an animal model of human yersiniosis. Some of these reports^{3-5,9)} have demonstrated the successful colonization of the organism in the intestines, and as most of these have dealt primarily with the pathological and clinical findings observed during a brief period, the mechanism of the fecal excretions has not been explained.

As described in a previous report⁷⁾, the colonization of *Y. enterocolitica* 03 in the intestines of mice and rats was achieved after intragastric inoculation of the organism. This study also showed that after a few weeks of chronic fecal excretion, a rapid termination of the excretion occurred. The present study was undertaken this phenomenon by investigating the correlation between the bacterial counts in the feces and the serum O agglutinin titer after a single inoculation. In addition, 10 weeks after the initial inoculation, the mice which demonstrated the termination of fecal excretion were inoculated with the same dose (10^7 cells) of the same strain (*Y. enterocolitica* 03).

MATERIALS AND METHODS

Mice and bacteria used The mice and bacteria used in this study were the same as those described in the previous report⁷⁾.

* Present address: Department of Veterinary Public Health, School of Veterinary Medicine, College of Dairying, Rakunogakuen University, Ebetsu 069-01, Japan

The procedures for the preparation of the bacterial suspension for inoculation, and the intragastric administration of the bacteria, and the enumeration of *Y. enterocolitica* in the feces and serum O agglutinin titration were the same as those described in the previous report⁷.

RESULTS

Detection of *Y. enterocolitica* in the feces

The 22 mice were administered intragastrically 10^7 to 10^9 of viable *Y. enterocolitica* 03 strain. Figure 1 shows the number of mice which excreted the organism in the feces post-infection. The rate of the fecal excretions was 99.5% (21/22) 1 day after the inoculation. The organism was detected in the feces of all of the mice from the 3rd day to the 14th day. Successful colonization of the organism in the intestines was considered to have occurred if the organism could be continuously detected in the feces (10^3 cells/g) over a period of more than 2 weeks after inoculation. The number of mice in which the organism had settled began to decrease gradually, and 9.1% (2/22) were positive for the organism at the 7th week. In one mouse, however, persistent fecal excretion over 14 weeks observed.

Relation between the terms of the fecal excretion of 03 organism and the serum O agglutinin

The relation obtained from 15 mice in which the 03 strain had settled is shown in figure 2. O agglutinin was detected in 8 out of the 15 mice, and in all but one of the mice, it was detectable at 3 to 5 weeks after inoculation. The titer of O agglutinin

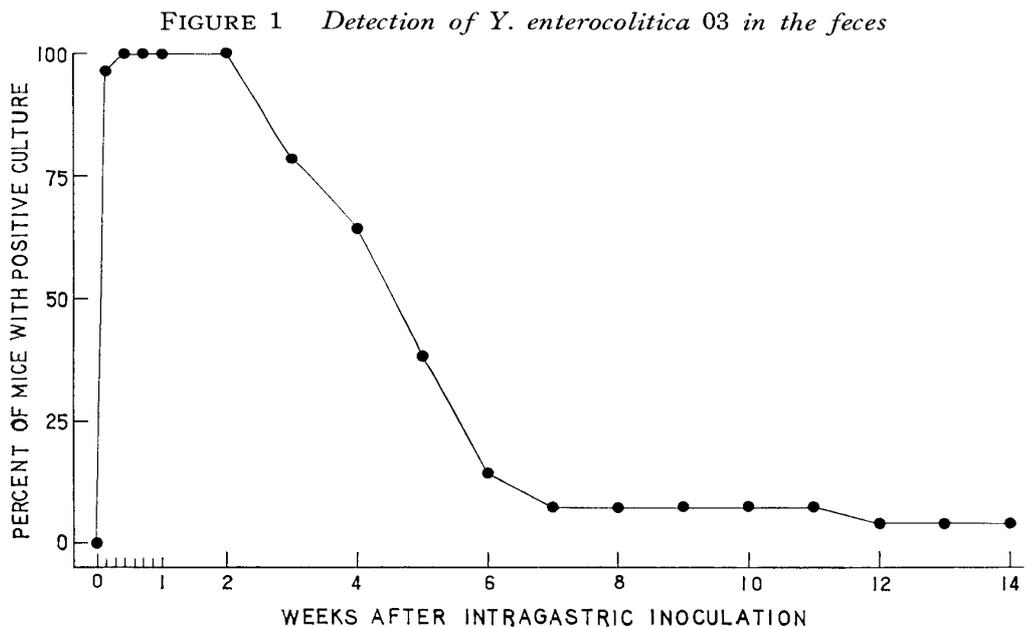
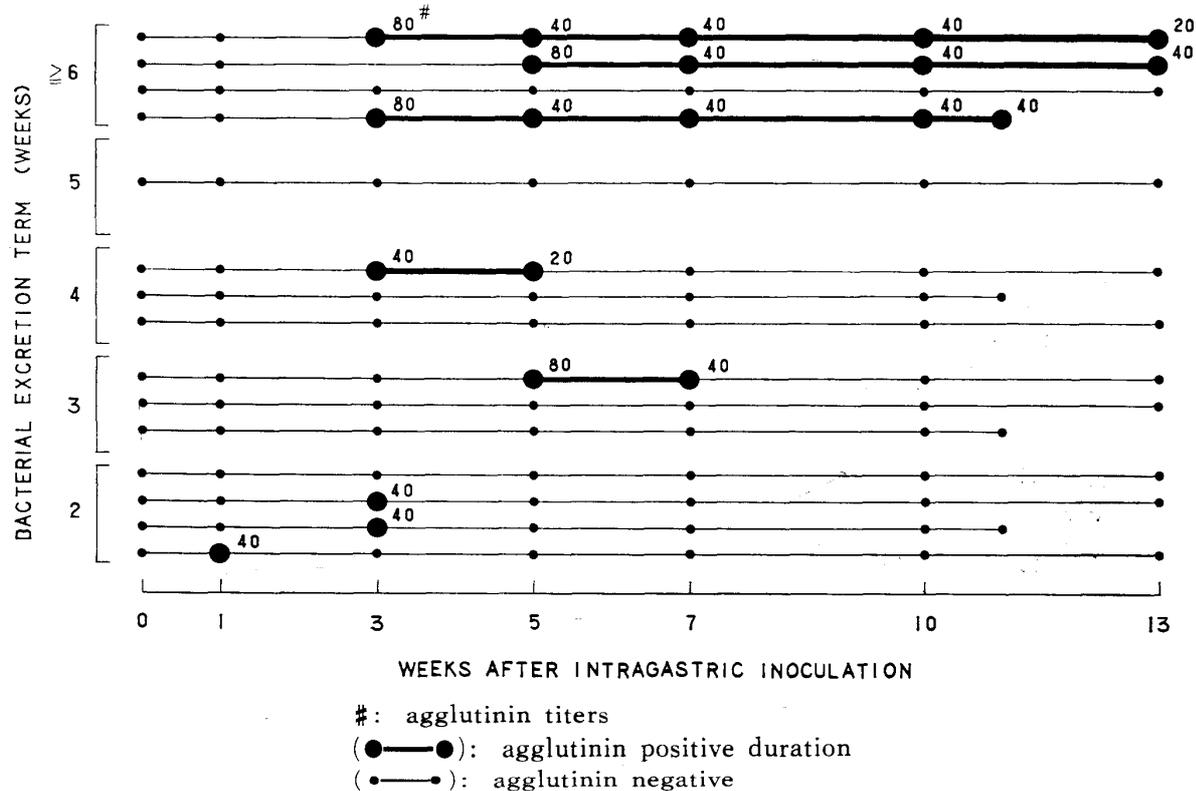


FIGURE 2 Relation between the terms of fecal excretion of *Yersinia enterocolitica* 03 and serum agglutinin detectable in mice inoculated intragastrically with the organism



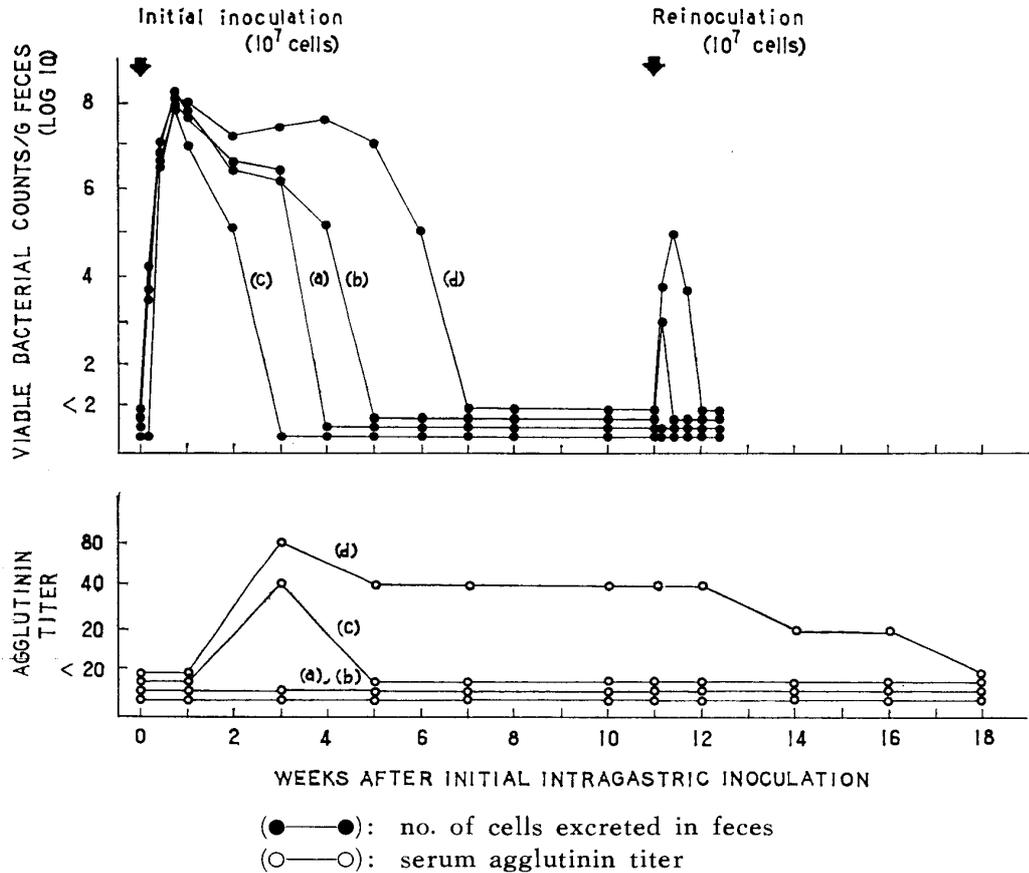
was 1:40 in most cases, and the highest titer was 1:80.

The serum 0 agglutinin was detected in 5 out of the 11 mice which had excreted the organism for less than 6 weeks. In these animals, the duration of agglutinin production was 2 weeks or less. And of the 4 mice which had excreted the organism for up to 6 weeks, 3 proved to be agglutinin positive during a period of 8 weeks or longer. In the mouse which showed the longest duration of agglutinin production, the organism was excreted for over 14 weeks after the inoculation.

Second inoculation test with *Y. enterocolitica* 03 strain

Figure 3 illustrates the number of excreted cells in the feces and the serum agglutinin titers in 4 mice inoculated twice with 03 strain.

The mice were inoculated for a second time with the organism 77 days after the first inoculation. Of these 4 mice, no cells of *Y. enterocolitica* were found in the feces of 2 mice after the second inoculation. In the other 2 mice, a small number of cells were excreted in the feces followed by a complete disappearance of the excretion on the 7th day.

FIGURE 3 *Second inoculation test with Y. enterocolitica* 03

There was no elevation of the agglutinin titer after the second inoculation in any of the mice. Mice (a) and (b), which had demonstrated persistent excretion of the organism following initial inoculation, showed no agglutinin. And mouse (c), which had shown a transient response for agglutinin at 3 weeks after the initial inoculation, also did not develop agglutinin by the second inoculation. The agglutinin titer did not rise after the second inoculation in mouse (d), which showed continuously a titer of 1:40.

DISCUSSION

CARTER (1975) and CARTER & COLLINS (1974) reported that the mice which had been infected intragastrically with a large amount of the virulent strain (WA strain) of 08 biovar 1 of *Y. enterocolitica* showed severe clinical symptoms. UNE (1977) observed that rabbits which had been inoculated with organisms 03 or 09 directly in the intestines developed intestinal disorders, and the organisms were recovered in the feces for about a month. During the period in which the intestinal disorders were observed, there was a high serum agglutinin value (1:256 to 1:2,048) 1-4 weeks after

the inoculation.

In this study, however, there were no apparent clinical signs of infection. There were no inflammatory changes in the animals observed macroscopically on days 5 to 7 when the animals showed the heaviest fecal excretions of the organism. And approximately only 50 % of the mice with intestinal colonization of 03 bacteria became agglutinin positive with a low titer (fig. 2). These results may indicate that the degree of invasiveness of the organism in the intestinal tissues was low, which contradicts the reports^{1-2,8)} in which there was evidence that *Y. enterocolitica* invaded pervasively even into the visceral organs. However, the longer the bacteria remained in the intestines, the longer the agglutinin was detected. In 3 mice, the organism continued to colonize in the gut and to produce serum agglutinin, which suggests that the serum antibody did not effectively inhibit intraintestinal settlement of the organism. This suggestion may be supported by the result that the organisms from the second inoculation failed to re-settle in the gut of the mice with no rise in the serum agglutinin titer. The above-mentioned phenomena and the reported evidence that the human pathogens of *Y. enterocolitica* have the ability to penetrate the epithelial linings of the intestinal mucosa⁸⁾ and to infect the macrophage^{9,10)} may indicate that in animals with chronic proliferation of the organism in the intestines, the intestinal tissues may have been invaded previously by some of the organisms which acted as antigenic stimulants. Therefore the termination of the fecal excretions and the inhibition of the organism inoculated secondly to settle might have been due to the establishment of local immunity in the gut. Further investigations, however, are necessary to demonstrate the mechanism of these phenomena.

RICCIARDI et al. (1978) reported that the mice inoculated intraperitoneally with the 09 strain exhibited long term fecal excretions for up to 135 days. In this study also one mouse displayed continuous excretions of the 03 strain in the feces for more than 14 weeks. Therefore, it was considered that mice might possess the capability to retain the organism for long period, the mechanism of the chronic colonization of the organism remains unknown.

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