

Experimental Cholera in Mice

I. First Report on the Oral Infection

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Introduction

Many works on the experimental cholera have been carried out in guinea pig, rabbit and monkey, however there have been few works on the oral infection in mouse¹⁾. Kamen (1895) produced a fatal enteric infection in two commensal mice which had been fed with bread contaminated with a cholera-suspect vibrio culture.

However, Karlinski (1896) was failed to develop infection in mice by feeding some authentic cholera strains, and assumed that Kamen's strain was not of a true nature, but was an aberrant species of vibrios.

Koesoemadilaga (1939) stated that he had obtained success when orally infecting mice with *V. cholerae*.

However, those experiments were not systematically studied and seemed not to be satisfied.

Authers tried to produce a cholera in mice and obtained the results that experimental cholera could be produced only in suckling mice by oral challenge of *V. cholerae*.

Materials and Methods

Mouse : ICR mouse was used.

Bacterial strain : *Vibrio cholerae* var. eltor, Inaba type strain V86, was used for the challenge.

The strain was subcultured with the semi-synthetic solid medium, prepared by adding 0.1% of yeast extract (Difco's) as final concentration to Davis' synthetic

medium, and a overnight culture on the same medium was used for the challenge.

Infection : Mice, which had been starved for one day, were orally administered using a thin vinyl tube connecting to the injection syringe, as seen in Photograph 1, with about 0.02ml of vibrio-suspension in 5% glucose solution.

Infant mice which had been separated from their mothers for one day starvation were pooled, and after challenge they were randomly returned to their mothers for breeding.

The mice were sacrificed at different intervals of time after challenge and the intestine was removed for the quantitative cultivation of challenge organisms

and pathological sampling.

For the quantitative cultivation, whole intestine was divided into three parts- upper part of small intestine, lower part of small intestine and cecum & colon and these parts were homogenized respectively within the blender which harboured a 10ml of saline (ca 5,000 r. p. m., 60 seconds).

The homogenized material was serially diluted with saline and 0.1ml each of the dilutions was plated on the alkaline nutrient agar (pH 8.6) and TCBS agar "Eiken" (Nihon Eiyo Kagaku Co., Ltd.), and viable count was done after overnight cultivation.

Results

The multiplication of vibrios in the intestine of the suckling mice was illus-

trated in Figures 1 and 2.

From the results it was recognized that

Figure 1 Multiplication of vibrios in the intestine of 8-day-old ICR mouse (counted with alkaline nutrient agar)

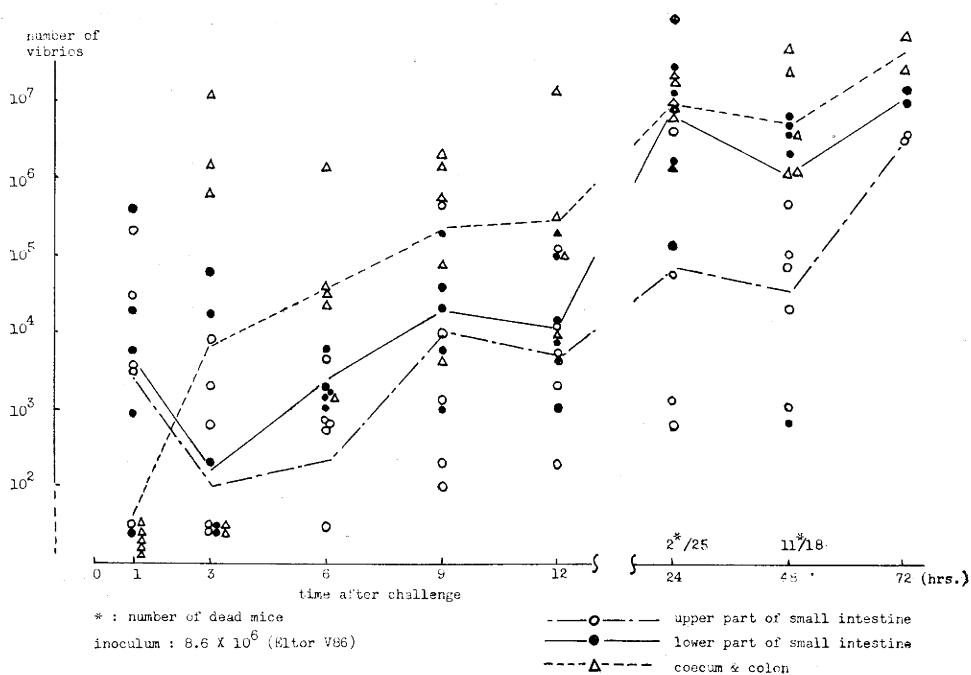
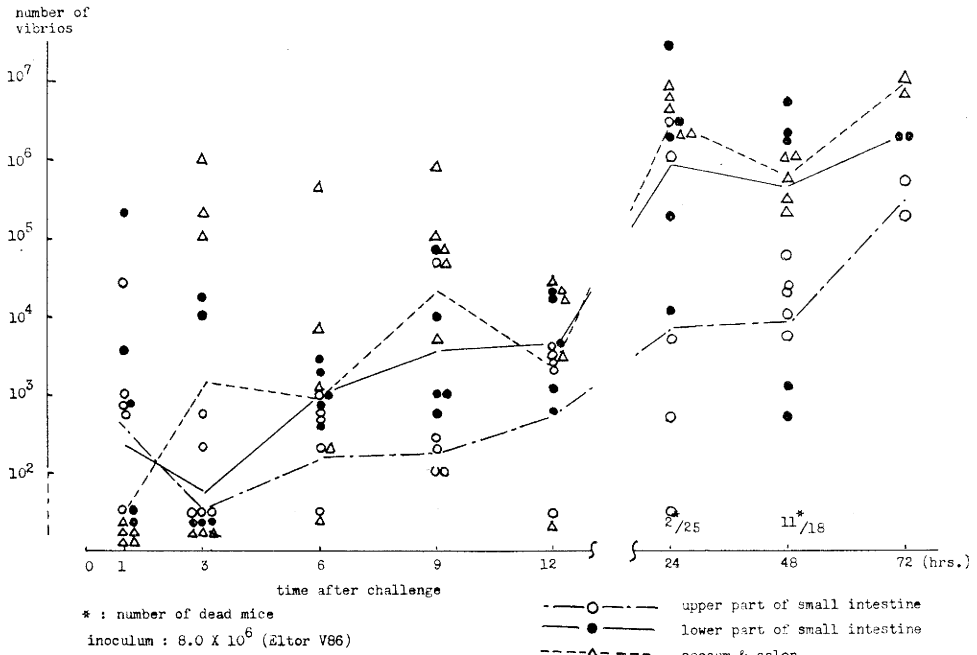


Figure 2 Multiplication of vibrios in the intestine of 8-day-old ICR mouse (counted with TCBS agar)



number of the challenged organisms had decreased from the outset of the infection and reached minimal number at about 3 hours after challenge, and then the vibrios began to multiply and reached near maximal level within 2 days after challenge.

The death of the mice challenged with strain V86, as indicated in Figures 1 and 2, was recognized usually after 1st or 2nd day of the infection.

The accumulation of yellowish or colorless fluid in the small intestine and the cecum, and the diarrhea was macroscopically observed within 1 or 2 days after challenge and the abnormal feature of the intestine was shown in Photograph 3.

The multiplication of vibrios in whole intestine among different aged mice were studied and were indicated in Figures 3 and 4.

In 6- and 8-day-old mice, both groups of the mice were observed to be susceptible to the infection of strain V86 and differences on the multiplication of the vibrios were not recognized between them, however 6-day-old mice were seemed to be more susceptible than 8-day-old mice with respect to the mortality.

The multiplication of the vibrios in 15-day-old mice was not to be distinct and always recognized to be less than above 2 groups of mice.

In 30-day-old mice, none of colonies of the challenged vibrios was isolated with the both agar media from infected samples of intestine - it means by the present method for quantitative cultivation that the number of vibrios in the whole intestine is less than 100 organisms - and the dead mice due to the infection were not recognized in both groups of 15- and 30

Figure 3 Differences on multiplication of vibrios among different mouse-groups (counted with alkaline nutrient agar)

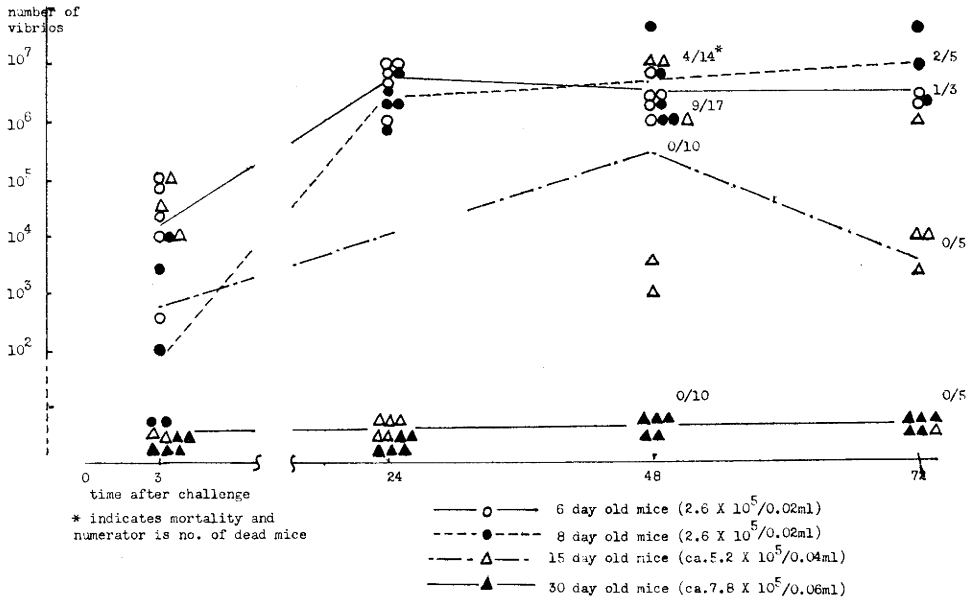
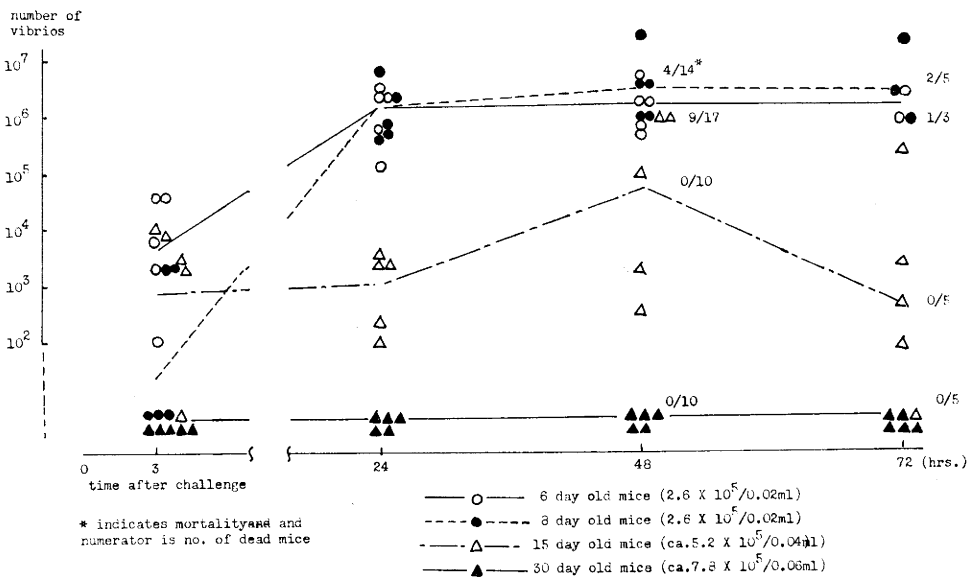


Figure 4 Differences on multiplication of vibrios among different aged mouse-groups (counted with TCBS agar)



day-old mice.

In all experiments, the quantitative cultivation was done using alkaline nutrient agar and TCBS agar, as indicated in the Figures respectively.

The number of the colonies produced

on TCBS agar were always observed to be less than that on alkaline nutrient agar, however the curves of the multiplication were considered to be similar each to each.

Discussion

In succession to Koch's works¹⁾ many trials on the experimental cholera have been carried out with guinea-pig, rabbit, monkey and so on, however very few have been done with mice, and the works using the infant mice seem not to be done up to now.

The past studies on the experimental cholera in adult mice, as mentioned in this introduction, seem not to be of systematic ones and do not afford a definite answer.

Authors tried to induce a cholera in mice and recognized that the accumulation of fluid in the intestine, diarrhea and the multiplication of challenged vibrios, as seen in human, cholera

could be induced by oral challenge only in the infant mice but not in the adult ones.

The present experimental cholera in the infant mice seems to be useful for the studies on infection, immunology and so on, because of following reasons: First, the method of the challenge is very simple - infant mice are able to be infected easily by oral feeding only with one day starvation. Second, small inoculum can induce a cholera-like picture with a high rate.

Third, the infant mice are more convenient than guinea pigs, rabbits, monkeys, etc. with respect to the selecting of experimental conditions.

Summary

The oral infection in mice, strain ICR, was carried out with El Tor vibrio, strain V86 and following results were obtained.

The multiplication of vibrios, accumulation of fluid in the intestine - especially in the cecum -, diarrhea and death were observed only in the suckling mice younger than about 10 days old.

And the multiplication of vibrios in

upper part of the small intestine was observed to be less than that in lower part of the small intestine.

In 15-day-old mice, only slight multiplication of vibrios without death was observed, however in 30-day-old mice, viable organisms of the challenged vibrios could not be isolated from intestinal samples even at 3 hours and within 3 days after challenge.

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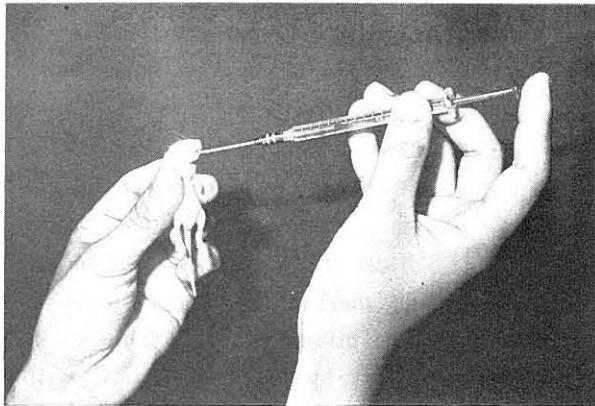


Photo. 1 Challenge by oral route



Photo. 2 Macroscopic appearance of the normal cecum

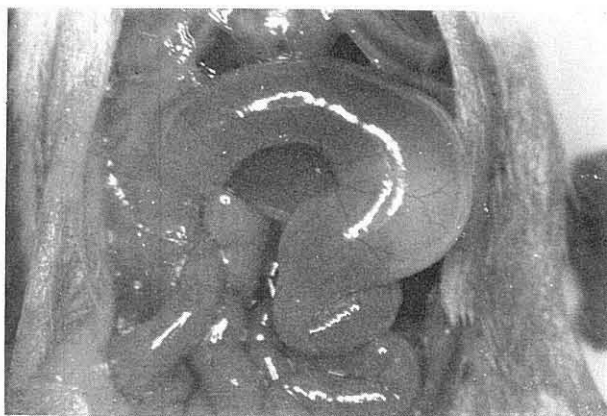


Photo. 3 Greatly distended cecum observed within 24 to 48 hours after oral challenge

マウスの実験コレラ

I 経口感染に関する第1報

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摘 要

マウス（ICR株）を用いてエルトール菌（V86株）による感染実験を行い、次のような結果を得た。

生後10日以下の乳のみマウスにおいてのみ、感染菌の増殖、腸管内特に盲腸に液体の貯溜がみられ、また下痢や死亡がみられた。腸管内における感染菌の増殖は小腸上部と下部では、下部に著明である結果を得た。生後15日マウスでは感染菌の増殖が著明でなく、死亡はみられなかった。生後30日マウスでは、感染菌は感染後3時間ですでにみられず、その後も菌増殖はみとめられなかった。