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Evaluation of intestinal damage caused by *V. cholerae* O139, an *in vivo* study

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ABSTRACT

Cholera, a severe form of gastroenteritis, is one of the most widespread diseases in developing countries. The mechanism of intestinal infection caused by *V. cholerae* O139 remains unclear. In order to explore some morphological aspects of its infection in the intestine including Peyer's patches, we investigated the *V. cholerae* O139 infection at intestinal site of the rabbit gut-loop model. The electron microscopic analysis revealed denuded mucosal surface with loss of microvilli and integrity of the surface epithelium. Infection of the intestine with *V. cholerae* O139 induces destruction of villi, microvilli and lining epithelium with exposure of crypts of Lieberkuhn.

1. Introduction

Cholera, produced by *V. cholerae*, is one of the most frequent endemic diseases in developing countries [1]. It is an extremely virulent disease; patient can develop acute watery diarrhoea with severe dehydration that can lead to death if untreated [2]. At least 1 million people are at risk of exposure to *V. cholerae* infection. The World Health Organization estimates that 91,000 people (uncertainty range: 28,000 to 142,000) die of cholera in endemic countries and 2500 people die of the disease in non-endemic countries annually [3].

In late 1992, *V. cholerae* serogroup O139 caused a massive cholera epidemic in southern India and then spread rapidly to different cholera endemic areas of the country [4]. This newly identified strain might have emerged from O1 El Tor biotype [5]. The strains differ in its surface antigens of somatic 'O' type and are encapsulated [6]. Histopathological study carried out in adult rabbit of infected intestinal tissue revealed that *V. cholerae* O139 infected intestinal tissue is more pathogenic than O1 El Tor [7].

Interaction between *V. cholerae* and intestine is fundamental for producing infection. Lack of ultrastructural study conducted on intestinal tissue infected by *V. cholerae* has prevented definitive statements from being made concerning its pathogenesis. The purpose of this study was to investigate the effect of *V. cholerae* O139 at distinctive intestinal site of the rabbit gut-loop model, towards advancing our knowledge on feasible pathogenesis of this bacterium. The significant of study is that any intestinal damage in animal to be caused by this pathogen can be observed in the laboratory, where we rely on animal models to understand the pathogenesis of *V. cholerae* O139 in human.

2. Material and methods

2.1. Bacteria preparation

V. cholerae O139 Bengal wild type (WT) was obtained from Department of Microbiology and Parasitology, Universiti Sains Malaysia, Kubang Kerian, 16150, Kelantan, Malaysia. Luria-Bertani (LB) was used for both liquid and agar media; polymyxin B (Poly),

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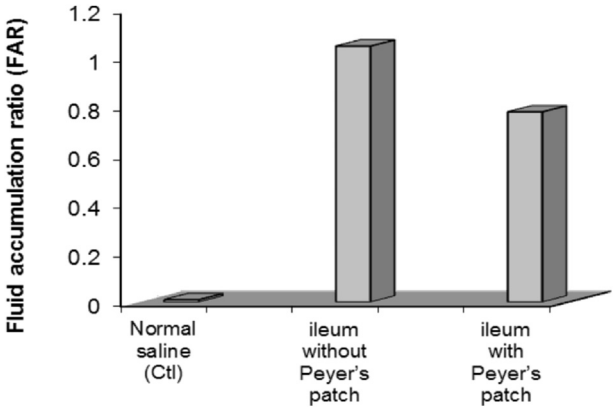

Fig. 1. Fluid accumulation ratio in ileum loops of rabbit when 10^6 cfu/ml doses of O139 *V. cholerae* were inoculated.

0.75 μg/ml, was added in the medium for selective growth of *V. cholerae.*

2.2. Animals

New Zealand White adult rabbits were bred in the Animal House, Universiti Sains Malaysia (USM) Health Campus. New Zealand White adult rabbits were used only if they were healthy and showed no evidence of diarrhoeal disease during the time period. For the experiment, 2.0–2.5 kg rabbits were starved for 24 h before the surgery. All procedures involving the use of animals had the approval of the Animal Ethics Committee (AEC), USM. The institution's law on the care and use of laboratory animals was followed.

2.3. Rabbit infection

Anesthesia for surgery was induced by intramuscular injection of Ketamine (50 mg/kg), Xylazine (8 mg/kg) and Acepromazine

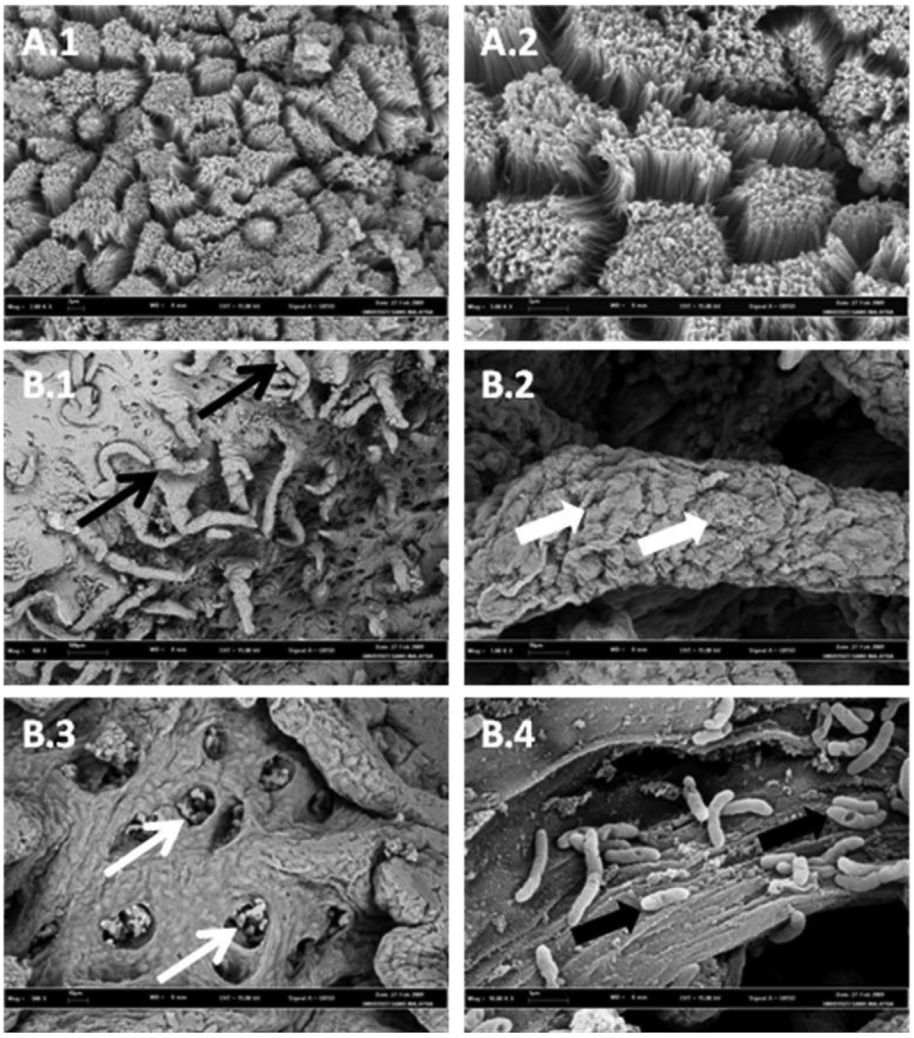

Fig. 2. A & B: Scanning electron micrograph of the intestine of rabbit at 18 h after inoculation of normal saline (NS) and 1×10^6 CFU of Wild Type (WT) respectively.
A.1-A.2: Control sections of ileum injected with NS. Scale bar, A.1 = 2 μm; A.2 = 2 μm.
Ultrastructural morphology of ileum injected with NS showing normal villi and microvilli.
B.1-B.4: Sections of ileum injected with WT. Scale bar, B.1 = 100 μm; B.2 = 10 μm; B.3 = 10 μm; B.4 = 1 μm.
SEM of WT injected ileum shows extensive damage to the villi (thick white arrow) and villi (black arrow) with pore formation (white arrow), also damaged ileum colonized by *V. cholerae* O139 (thick black arrow).