

Mucosal Invasion and Morphological Reaction in Rabbit Ileum Experimentally Infected with Enterotoxigenic *Salmonella bareilly*

S A WANI* and R S GUPTA

Department of Microbiology and Public Health, G B Pant University of Agriculture & Technology, Pantnagar 263145

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The rabbit-ligated-ileal-loops were exposed to whole-cell-culture of *Salmonella bareilly* for different time intervals of post-inoculation. Microscopic examination of sections prepared from the infected loops revealed mild proliferation of mononuclear cells in 3 hrs intervals. After 3 hrs of inoculation different lesions like degeneration, necrosis and desquamation of mucosal epithelium were observed. Heterophilic infiltration with haemorrhages was seen in mucosa, submucosa and lamina propria. These changes were severe in later intervals leading to complete destruction of intestinal villi. These changes together with presence of organisms suggested that strain is also invasive in nature.

Key Words: *Salmonella bareilly*, Whole-cell-culture (WCC), Cell free supernatant (CFS), Rabbit-ligated-ileal-loop (RLIL) model, Histopathology, Degeneration, Necrosis, Invasion

Introduction

Salmonella bareilly has frequently been isolated from both human and non-human sources and has posed public health and animal health problems (Wyllie 1943, Sethi et al. 1979, Kapoor & Chauhan 1980). The serotype has wide host range pathogenicity alongwith world wide distribution. In addition to man and animals, the serotype has been also isolated from free-living fauna including toads, rats, lizards, shrews, squirrels, snakes and cockroachs (Sharma et al. 1980, Sharma et al. 1981). The present work was carried out to study the invasiveness of the organism.

Materials and Methods

Albino rabbits weighing approx. 1 kg procured from IVRI, Izatnagar and *Salmonella bareilly* (JK-I strain)

isolated from faecal contents of a jackal and maintained by the Department were used.

Whole-cell-culture: (WCC) of the *S. bareilly* (JK-I strain) was prepared in Brain-heart infusion broth medium. Cell-free-supernatant (CFS) which contained mainly enterotoxin and other bacterial products was prepared according to the method of Singh (1982). One ml of the WCC and 2 ml of CFS were used for inoculation of ligated-ileal-loop of rabbit (Singh 1982).

Rabbit-ligated ileal-loop: (RLIL) model was prepared by the method of Abdulrashid and Thapliyal (1977). Two adjacent segments were prepared by a 5 cm long portion treated as zero segment wherein no material was inoculated. Ligatures were made ensuring no leakage of fluid and no injury to the intestine. In each rabbit

*Department of Veterinary Microbiology, S K University of Agricultural Sciences & Technology, Nowshehra, Srinagar 190011

after squeezing down the contents four loops were prepared, at a gap of 3 cm each.

For each interval three rabbits were used to with different portions of the ileum exposed, one loop in each rabbit was inoculated with 1 ml of the BHI broth (control).

For enteropathogenicity studies at different post-inoculation timings the inoculated rabbits were sacrificed (by chloroform inhalation) at 15 min, 30 min, 1 hr, 3 hr, 6 hr, 12 hr, 15 hr, and 18 hr post-inoculation.

Histopathology: Specimens from middle portion of the loops exposed to WCC and CFS were fixed in 10% formal saline solution for 72 hr of fixation representative pieces were made from these specimens and were washed overnight under running tap water to remove excess of fixative. They were dehydrated in ascending grades of alcohol. The specimens were cleared in Xylene embedded in paraffin wax of 62°C melting point after infiltration in two stages and the sections were cut. Sections for histopathological examination were stained with haematoxylin and eosin (Lillie & Fullmer 1976) and studied at various magnifications.

To demonstrate the presence of bacteria in gut segments, duplicate sections were stained with modified Gram's method (Culling 1974) and quantitative and qualitative estimates were made. A correlation was made between the bacterial load and severity of histopathological lesions.

Results

At 1 hr post-inoculation with WCC the mucosa of intestine failed to reveal any significant change except the mild proliferation of mononuclear cells in lamina propria (figure 1).

However, the lesions (haemorrhages in the mucosa, degeneration, necrosis and desquamation of mucosal epithelium) increased in severity with the increase in exposure time (figure 2). In submucosa and in serosa the major inflammatory cells were polymorphonuclear leucocytes (figure 3)

In the gut loops tested with CFS for 18 hr interval the submucosa showed edematous changes, mucosal layer showed epithelial necrosis, desquamation and hyperplasia of goblet cells. Marked mononuclear cell

infiltration and haemorrhages were seen in lamina propria. Besides, exudation of serous fluid and haemorrhages were also seen in submucosa. Mucosal degeneration, desquamation resulting in denudation of tips of villi were also seen in the loops exposed for 6, 12 and 15 hrs intervals. Below 6 hr interval, the mucosa and submucosa failed to reveal any significant change.

Sections prepared from the loops receiving WCC of the test strain for 30 min and 1 hr and stained with modified Gram's strain revealed the presence of large number of Gram negative coccobacilli within lamina propria. Similarly the sections of 3, 6, 12, 15 and 18 hrs intervals revealed considerably large number of Gram negative coccobacilli in different layers of gut wall.

Discussion

Different lesions like degeneration, necrosis, desquamation of mucosal epithelium, haemorrhages and infiltrations by mononuclear cells with few polymorphs observed in present work together with presence of gram negative coccobacilli seen in Gram-stained sections of inoculated loops evidenced that the organism is invasive in nature and could not produce any significant change in early phase of infection except mechanical passing through tissues. This coincides with the findings of Carter (1975) who also observed the invasive property of *S. enteritidis* in mice after oral administration. Inflammatory reaction observed in present work from 3 hr onwards in the rabbit ileum by the test strain was in agreement with Giannella et al. (1973) and Giannella (1979). Various lesions as observed in inoculated gut-loops of rabbits in the present work were also observed by Kapoor et al. (1980) in small intestine of the chicken and quails died of *S. bareilly* infection.

Gut loops, tested with CFS of the test strain also showed desquamation of mucosal epithelium, infiltration of mononuclear cells in lamina propria with haemorrhages at few places but the severity was less than that of WCC. Singh (1982) also observed these changes in rabbit ileal loops inoculated with CFS of the two isolates of *S. bareilly*

These results suggest that the organisms possess both invasive and enterotoxigenic activities for rabbit ileal segments.

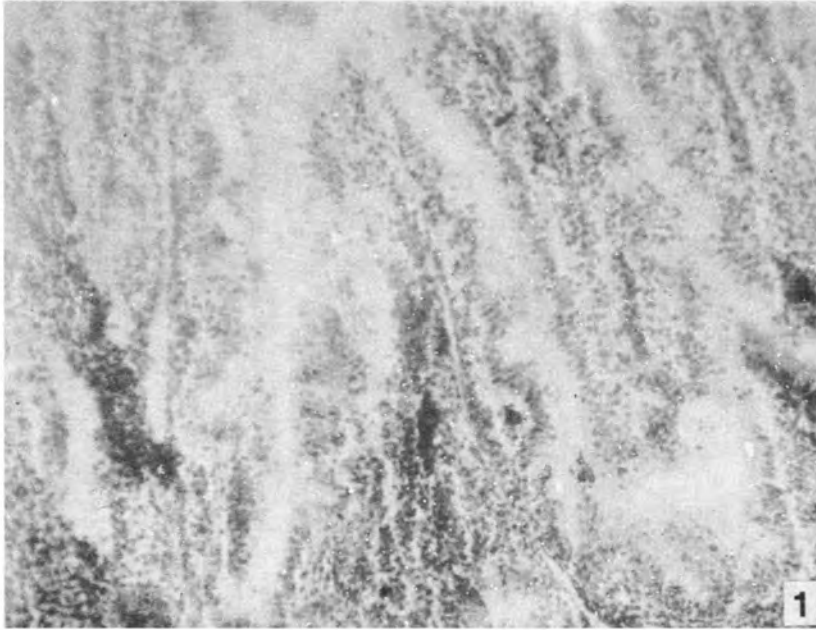


Figure 1 Photomicrograph of ileum of rabbit after 1 hr of inoculation with whole-cell-culture (WCC) of *Salmonella bareilly* showing mild proliferation of mononuclear cells in lamina propria (H & E $\times$ 150)

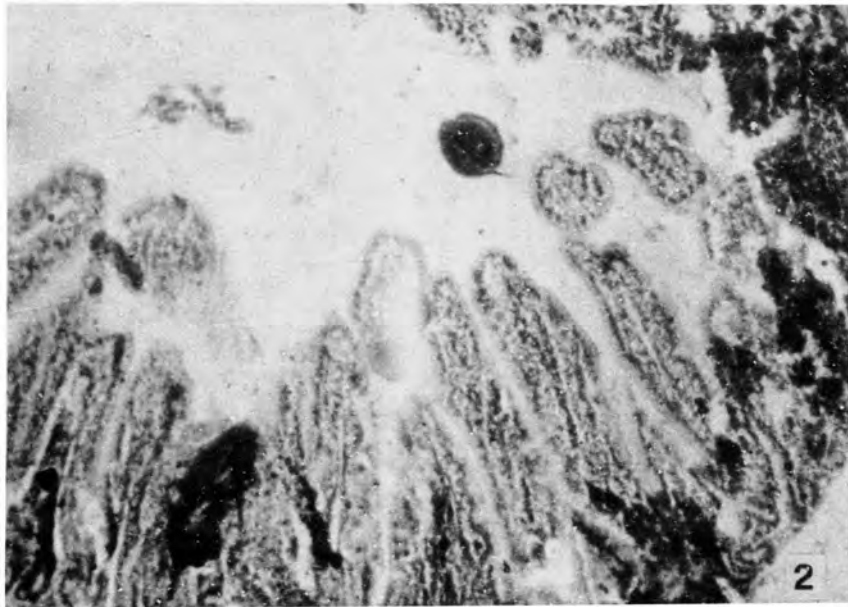


Figure 2 Photomicrograph of ileum of rabbit after 12 hrs of inoculation with whole-cell-culture (WCC) of *Salmonella bareilly* showing marked necrosis and desquamation of mucosal epithelium. Submucosa showing moderate haemorrhages (H & E $\times$ 60)



Figure 3 Photomicrograph of ileum of rabbit after 18 hrs of inoculation with whole cell-culture (WCC) of *Salmonella bareilly* showing vascular congestion and marked haemorrhages in submucosa. Mucosal epithelium showing necrosis and desquamation. There is mononuclear infiltration in lamina propria and submucosa (H & E $\times$ 150)

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