A STUDY OF CALVES SMALL INTESTINE INFECTED WITH *SALMONELLA TYPHIMURIUM* USING TRANSMISSION ELECTRON MICROSCOPIC

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ABSTRACT

The study were designed for*Salmonella typhimurium* infection in calves which carried out on sixteen normal colostrum fed Friesian calves, 3 to 6 weeks old.

Animals were divided into two equal groups. Group 1 inoculated orally with 1.5×10^{11} viablecells of *Salmonella typhimurium* and group 2 served as controls.

The early ultrastructural changes observed in microvilli in infected calves due to *S. typhimurium* were characterized by local derangement with swelling on proximal end of microvilli in addition to presence of many bacteria either associated or adherent to the microvilli.

INTRODUCTION

Salmonellosis has a significant impact on animal health,while cattle may be infected with many different serotypes (1). Bovine salmonellosis is caused mostly by *salmonella enterica*serovarTyphimurium. Salmonella typhimurium is the serotype most frequently isolated from calves (2)(3)(4).

Ultrastructural studies on Salmonellosis were reported in pigs(5), mice(6), rabbits (7) and human(8)..

The present paper is to report the results of our transmission electron microscopic longitudinal study on the small intestine of 3-6 weeks old calves experimentally infected with *Salmonella typhimurium*.

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MATERIALS AND METHODS

Sixteen male Friesian calves, born and reared on dairy farm (Arab Iraq Company for livestock development) have been used. The dairy farm was selected because it had never had a history of any Salmonella isolation. The calves age ranged between 3-6 weeks and had 30-40 kg weight at the time of inoculation.

Challenge Organism:

A highly virulent and multiple drug resistant *S. typhimurium* isolated from feces of a calf at Al-Dahab Al-Abyadh village that died of severe enteritis and septicemia was selected to reproduce the disease experimentally.

Experimental design:
Sixteen calves were randomly divided into two equal groups. Group 1 was served as controls and group 2 was orally inoculated with 1.5x 10^{11} CFU *S. typhimurium* organisms. Control calves were orally inoculated with sterile trypticase soy broth. One calf from each experimental group was killed at each of the following periods: 8, 16, 24, 36, 48, 72, 96 and 120 hours post inoculation (PI).

At the specified time of each experiment, the animal was anesthetized by infiltration of 3% Lidocaine through the flank region. The abdomen was quickly opened and samples were taken which were fixed in 0.75% glutaraldehyde in 0.1 M phosphate buffer, pH 7.3(310milimole) for 2 hours at room temperature.
The blocks were post fixed with 1% osmium tetroxide in 0.1 M phosphate buffer for one hour at 4C0, block stained with 1% uranyl acetate for 2 hours then dehydrated with graded ethanol of 25%, 50%, 65%, 75%, 85%, 95%, 99% and three changes of absolute ethanol. Prophylene oxide was used as an intermediate reagent for embedding with epoxy resin andpolymerized by incubation at 60 c^o in an oven for 48 hrs. Ultrathin sections were made with an ultramicrotome and double-stained with uranyl acetate and lead citrate. The specimens were examined by JEOL, Jem-1200 EX transmission electron microscope (9).

RESULTS AND DISCUSSION

The early ultra-structuralchanges observed in microvilli by TEM in infected calves infected with *S. typhimurium* were characterized by local derangement with little swelling on the proximal end in addition to the presence of many bacteria, either associated or adherent to the microvilli. One of our interesting findings is the ballooning of the microvilli,particularly at the junctional complex between two epithelial cells. The appearance of this ultra-structuralchange in the microvilli might be due to the attachment of *S. typhimurium* to their membrane. In addition to that this lesion indicates the early sign of degeneration of microvilli (10)(11). As the infection becomes more pronounced, the microvilli became short with more effacement from epithelial cells. By 96 hours PI complete disappearance of the microvilli was observed. This observation in the microvilli was different from the experimental study of Willie (12) who found the close association of *S. typhinurium* in experimentally infected pigs with the microvilli was seldomly associated with any morphological alteration(13). While the electron microscopic studies of intestinal tract of infected calves with salmonella found that the only alteration in the microvilli was characterized by irregularly shaped, shortened and thickened microvilli in some sites(14).

The effacement of the brush border of the small intestine by *S.typhimurium* resulted to the loss of brush border enzymes (which hydrolyze small peptides to free amino acids during the process of absorption (15).
The resultant loss of brush border could cause an impaired process of digestion and absorption. Shortening and loss of microvilli is accordance with decreased alkaline phosphatase activity (10). Alkaline phosphatase is located in the plasma membrane of microvilli (16)(17) and is considered as measurement of damage to the digestive absorb surface (18)(19).

The Embage*et al* (17) suggested that the increase in cytoplasmic Ca^{+2}concentration could cause actin filaments of the microvillous core cytoskeleton to be broken into short filaments (20), this would lead to fragmentation and vesiculation of microvilli. We observed that *S. typhimurium* invaded intestinal epithelium as early as 8 hours after challenge exposure through their brush

border(21). In contrast to the present findings Takeuchi (22) observed that penetration to the intestinal epithelium by *S.typhimurium* occurred most often at 12 hours after inoculation in guinea pigs and the usual portal of entry was not only through the brush border, but also occurred via the intercellular junctional complex between epithelial cells(23). Willie etal, (12)(24), detected earlier penetration of intestinal epithelium in pigs by *S. heidelbergand S.typhimurium* at 2 hours after inoculation. Intracellular Salmonella observed in the present study were usually intact and enclosed by a membrane, originating either from the apical or plasma membrane. Those intracellular Salmonella were responsible for the damage to the epithelial cells which started at 16 hours post inoculation and became more sever in epithelial cells of present study were probably due to the presence as the intracellular Salmonella produced cytotoxic effects. In comparison with the present findings Willie etal (12) observed less damage to the epithelial cells due to *S.typhimurium* which was characterized mainly by slight displacement of cytoplasm immediately surrounding the bacterium. The relatively rapid degeneration of mitochondria may affect the ability of cell to generate energy. One of the important energy requiring cell functions is that of active transport, which plays a role in absorption (25). The loss of microvilli reduces the absorptive surface (26). This together with functional deficiency and destruction of many epithelial cells may result in decreased absorption and cause osmotic diarrhea, as suggested for neonatal calf diarrhea (19) and transmissible gastroenteritis (27).
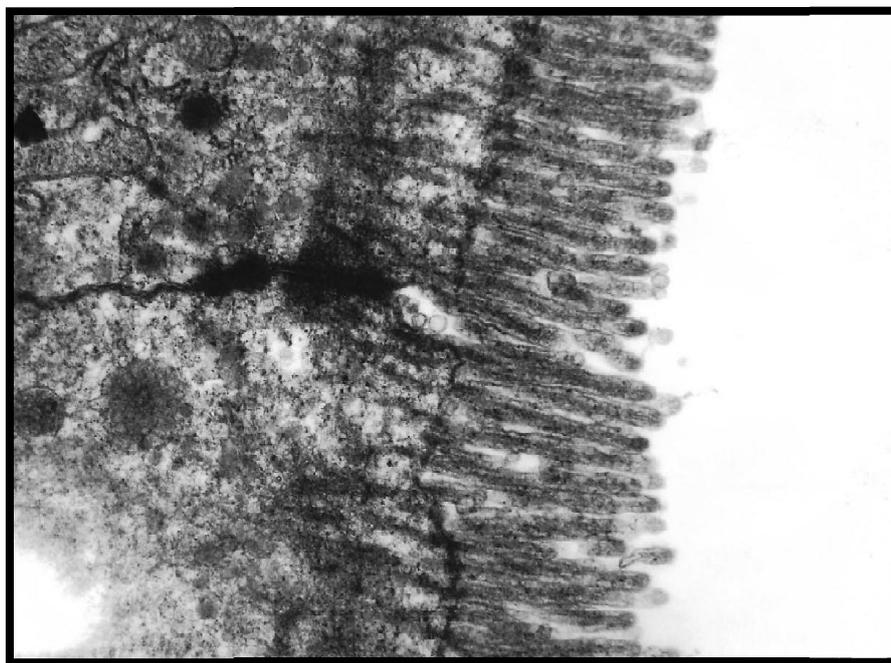

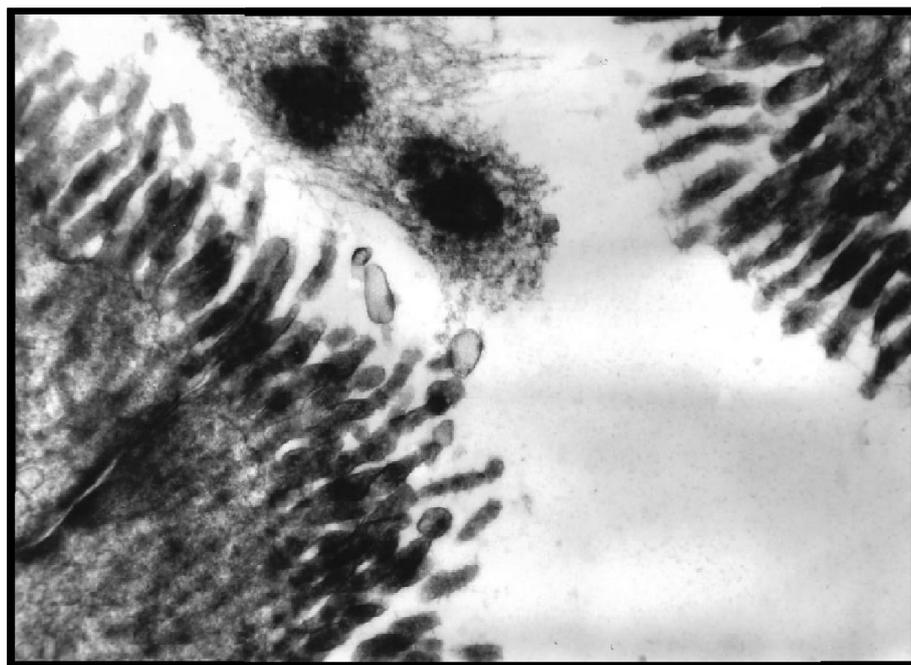Fig.(1) Showing long, closely packed regular microvillus border in the control calf.Scale Bar=0.1μm

Fig.(2) Tow adjacent epithelial cells showing intact tripartite intercellular Junctions, in the ileum of normal calf (TEM).Scale Bar=0.1µm
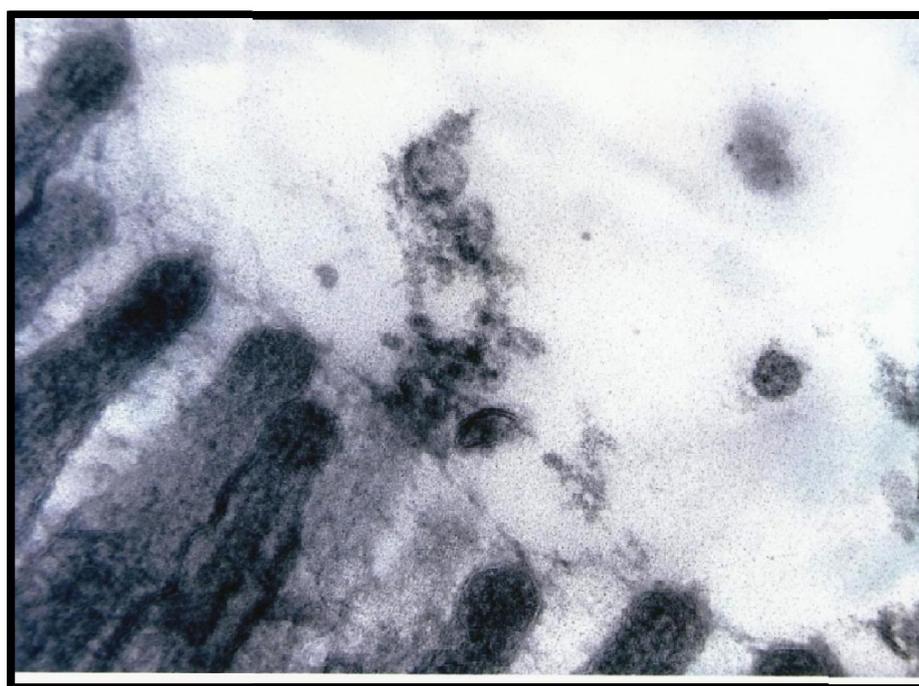]

Fig.(3) Association of bacteria (arrow) with the microvilli at 8 hours (PI). Scale Bar=0.1µm
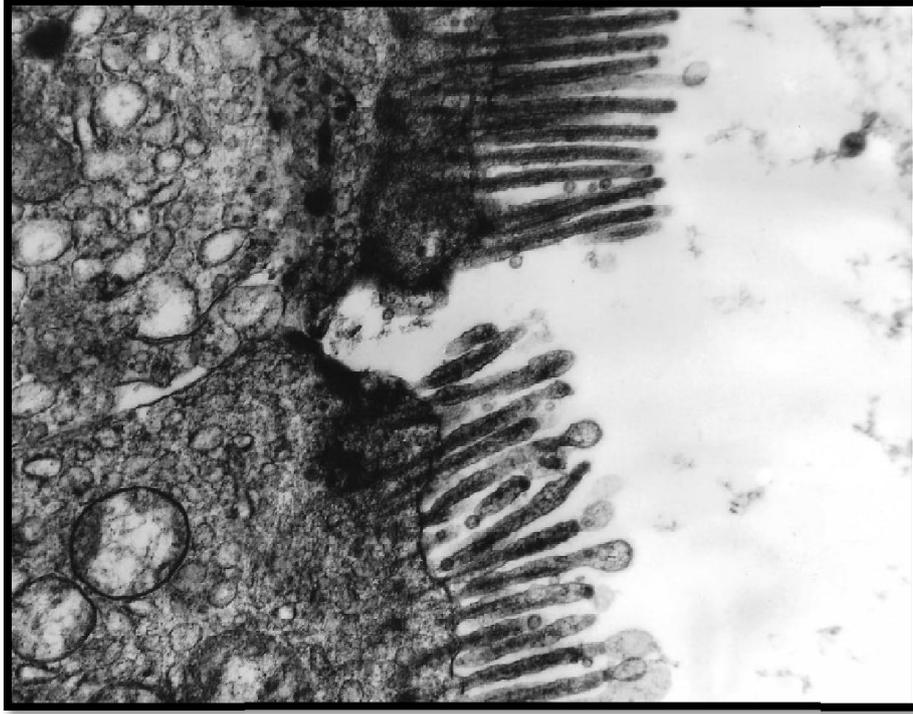
Fig.(4) local derangement with little swelling of the proximal end of the microvilli at 8 hours PI (TEM). Scale Bar=0.1µm

دراسة الأمعاء الدقيقة للعجول المخمجة تجريبيا بجراثيم السالمونيلا تايفيميوريم باستخدام بالمجهر الالكتروني النافذ

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الخلاصة

صممت هذه الدراسة لغرض معرفة جوانب امراض جراثيم السالمونيلا تايفيميوريم في العجول المخمجة تجريبا. اجريت الدراسة على ستة عشر عجلا من نوع الفريزيان، سبق وان تناولت اللبأ وبعمر 3-6 أسابيع. قسمت العجول الى مجموعتين متساويتين ، اعطت المجموعة الاولى جرعة جراثيم السالمونيلا تايفيميوريم مقدارها (١.٥x١٠^{١١}) بينما عوملت المجموعة الثانية كمجموعة سيطره.

تميزت التغيرات الاولية لنتائج الفحص بالمجهر الالكتروني النافذ بتوهان الزغيبات وتورم طفيف في نهاباتها الدانية مع وجود عدد من البكتريا ملتصقة او مجاورة الى نهايتها القاصية.

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