Influence of Colostrum on Transepithelial Movement of
Escherichia coli 055

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ABSTRACT

The effect of colostrum on transepithelial migration of live Escherichia coli 055:B5:H7 in the neonatal calf intestine was determined by microbiological and microscopic investigations. Colostrum-deprived calves (2 to 6 h old) were given E. coli suspended in saline, E. coli suspended in colostrum, or E. coli in saline 1 h after colostrum. Twenty-four hours after exposure, tissues were collected for examination. Escherichia coli were numerous in mesenteric lymph nodes of calves given this organism in saline. Fewer were recovered from nodes of calves that received the bacteria in colostrum. Escherichia coli were not recovered from mesenteric lymph nodes of calves given colostrum before dosage with the organism. Electron microscopic studies of small intestines from calves that received E. coli in saline revealed bacterial attachment sites surrounded by exfoliation of microvilli. Bacteria appeared to enter epithelial cells by invagination of apical plasma membrane or dilation of apical tubules. Intracellular E. coli were enclosed in a surrounding membrane. The organisms were not observed adhering to or penetrating intestinal epithelium of calves that received E. coli in colostrum or 1 h after colostrum.

INTRODUCTION

Intestinal permeability to large proteins occurs in certain neonatal animals. This is the means by which passive immunity is conveyed to the neonatal ungulate via colostrum absorption during the first few hours of life. Absorption of intact proteins is a relatively nonselective process in calves and may be related to immaturity of the intestinal epithelial cell and associated enzyme systems (14). Ultrastructural studies suggest that the intestinal epithelial cell of certain neonates may take in intact proteins and bacteria in a similar fashion (14). The present investigation was to determine the influence of colostrum on transepithelial migration of Escherichia coli in small intestine of the neonatal calf.

EXPERIMENTAL PROCEDURES

Colostrum for this experiment was pooled from cows in the University herd within 24 h after calving. Ten Holstein bull calves obtained at birth before nursing their dams were allotted to treatments differing in intestinal exposure to live Escherichia coli 055:B5:H7. Washed bacteria (1 × 107) suspended in 1 liter of sterile saline were administered via stomach tube to each of four calves 2 to 6 h after birth. Two each were given E. coli in 1 liter of colostrum 2 to 6 h after birth. Two other calves each received 1 liter of colostrum 2 to 6 h after birth 1 h prior to receiving E. coli. Each calf was fed 1 liter of reconstituted whole milk 12 h after dosage with E. coli. Two calves handled in a similar manner but not exposed to E. coli served as negative controls for comparisons of ultrastructural anatomy.

Twenty-four hours after exposure to E. coli, calves were anesthetized with sodium pentobarbital and tissues collected for microbiologic studies. Blood, liver, spleen, and mesenteric lymph nodes draining jejunal and ileal regions of the small intestines were excised, placed in sterile petri dishes, and refrigerated. Immedi-
FIG. 1. Ileal epithelium of calf which had received no colostrum but was exposed to *E. coli* for 24 h. Vacuolization of the epithelium was normal for this area of the intestine. *E. coli* were adherent to the microvilli and envacuolated throughout the epithelium (approximately 1000X).

FIG. 2. Apical ends of several ileal epithelial cells from an *E. coli* exposed calf which had received no colostrum. The microvilli were largely absent at the sites of *E. coli* attachment. *E. coli* were also within the apical cytoplasm (approximately 16,000X).

RESULTS

The ultrastructural anatomy of the small intestine of calves not exposed to *E. coli* was essentially as described (15). Microvilli of the jejunal and ileal cells were well developed and tubules or invaginations in the apical cytoplasm...
FIG. 3. Intracellular cytoplasmic vacuoles normally in the ileal epithelium of neonatal calves, after exposure to E. coli, contained the microorganism apparently in the process of transepithelial migration (approximately 11,000×).

were extensive. Large supranuclear vacuoles were limited to the ileal cells.

In calves given E. coli in saline no cellular changes were observed in duodenal tissue. Intestinal epithelial cells in the mid-region of the villus of both the jejunum and ileum had E. coli adherent to the apical microvillous membranes. Few microorganisms were adherent to the jejunal epithelial cells; however, in the ileal region were large areas of microbial attachment to the absorptive cells and in many instances intracellular penetration (Fig. 1). The microvilli at these attachment sites were exfoliated, and the microorganisms appeared to have been taken into the intestinal epithelial cell by invaginations of the apical plasma membrane (Fig. 2). After the bacteria entered the cell, they always were enclosed in a surrounding membrane. Bacteria occasionally, but not always, entered the large supranuclear vacuoles of the ileal cells (Fig. 3). Subsequently E. coli were in the base of the cells where they were eliminated into the lamina propria.

In calves which received colostrum with or prior to intragastric E. coli, no attachment or intestinal penetration by bacteria could be detected. The apical surface microvilli were intact, and on occasion the apical tubular system was dilated with colostrum (Fig. 4).

Microbiological cultures of mesenteric lymph nodes (MLN) from the jejunal region revealed \(2.3 \times 10^3\) E. coli per g of tissue 24 h after calves received E. coli in saline (Table 1). Ileal MLN of these calves had \(2.9 \times 10^3\) E. coli per g of tissue. No E. coli could be recovered from spleen and liver tissues of these calves, but \(2.6 \times 10^3\) E. coli/ml were recovered from the blood of one calf. Twenty-four hours after exposure to E. coli these calves were severely depressed, nearly moribund, and had profuse watery diarrhea.

Jejunal and ileal MLN cultures from calves that received E. coli in colostrum suspension yielded \(1.6 \times 10^2\) and \(7.8 \times 10^1\) E. coli/g tissue. Liver, spleen, and blood samples from these calves were negative for E. coli, and calves in this treatment group were clinically normal when anesthetized.

No E. coli could be recovered from MLN, liver, spleen, or blood of calves which were given colostrum 1 h prior to administration of E. coli. These calves were apparently in good health when they were anesthetized for sample collection.

DISCUSSION

Exfoliated microvilli, as well as adherence and penetration of E. coli were observed in the ileal mucosa of colostrum-deprived calves that received E. coli. Bacteria apparently gained entrance to the intestinal epithelial cells by invagination of the apical plasma membrane and traversed the cells in surrounding membrane. Similar observations on E. coli penetration in neonatal pigs have been reported (13). Thus, it appears that E. coli 055 may traverse the intestinal epithelium of colostrum deprived pigs and calves in the same manner as macromolecular proteins. This early transepithelial migration of certain E. coli seemingly would predispose the neonate to septicemia and generalized infection.

Others (4) observed no invasion sites in intestines of neonatal pigs exposed to E. coli.
FIG. 4. Ileal epithelial cells from a calf which had received colostrum prior to *E. coli* were unaltered cytologically. Dark aggregations of colostral proteins were in the apical tubular system of the cells (approximately 14,000×).

0138:K81:NM. In addition, they rarely found degeneration of microvilli. Intracellular *E. coli*, which were free within the cytoplasm, were only in intestinal epithelial cells showing regressive changes. Since previous observations of *E. coli* 055 in neonatal pigs were similar to those reported herein (13), the discrepancy presumably is due to dissimilarity in pathogenesis of *E. coli* serotypes.

Transepithelial migration of *E. coli* 055 was limited when the microorganism and colostrum were administered together. However, when colostrum was administered prior to *E. coli*, transepithelial migration did not occur. This is consistent with reports that some *E. coli* become septicemic if given before colostrum but will not if given after colostrum (1, 6). The beneficial effects of colostrum have been recognized, but investigations have not resolved the manner by which these benefits are achieved. The fact that *E. coli* could not be recovered from mesenteric lymph nodes or observed penetrating epithelial cells when given after colostrum suggests the inhibitory effect occurred within the intestinal lumen. Other researchers have found that colostral immunoglobulins have protective action within the intestine against enteric infections (7). Meyers and coworkers (9) found that protective factors transferred to the calf via colostrum did not cause clearance of *E. coli* from the intestinal tract but impaired colonization of the intestinal
TABLE 1. Average numbers of *E. coli* recovered from tissues of calves 24 h after exposure to *E. coli*.

<table>
<thead>
<tr>
<th>Calf treatment</th>
<th>Mesenteric lymph nodes</th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Jejunum</td>
<td>Ileum</td>
<td>Spleen</td>
<td>Liver</td>
<td>Blood</td>
</tr>
<tr>
<td><em>E. coli</em> (4)<em>a</em></td>
<td>$2.3 \times 10^3$</td>
<td>$2.9 \times 10^3$</td>
<td>0</td>
<td>0</td>
<td>2.6 $\times 10^3$/ml (1 calf)</td>
</tr>
<tr>
<td><em>E. coli</em>/colostrum (2)</td>
<td>$1.6 \times 10^2$</td>
<td>$7.8 \times 10^1$</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Colostrum 1 h (2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
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</tr>
</tbody>
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*a*Number of calves in treatment group.  
*E. coli per gram of tissue.

epithelium. Lumenal bactericidal activity was not measured in our experiment.

Previous investigators attributed resistance to colisepticemia to factors in sera of young calves. Gay and coworkers (6) found that hypogammaglobulinemic calves were particularly susceptible to colibacillosis while calves with high serum immunoglobulins were generally resistant. Some researchers have found protection against colisepticemia associated with specific serum factors (2, 3) while others believe nonspecific serum factors render protection (5). Recently, researchers found that intravenous administration of IgM gave neonatal calves protection against *E. coli* antibody could be attributed predominantly to the IgM fraction of colostral whey (8).

Cellular factors may enhance resistance of young calves to colisepticemia. Smith and Halls (12) found *E. coli* in lymph nodes of the alimentary tract of calves following oral inoculation. However, generalized (septicemic) invasion did not occur in calves whose sera contained immunoglobulins. After comparing in vivo and in vitro studies, they concluded that absorbed antibody enhanced phagocytosis of invading microorganisms by the reticuloendothelial system. These studies are in accord with recent reports that when combined with an opsonin, phagocytes from newborn calves were able to kill *E. coli* (11).

In conclusion, these studies further emphasize the need for the young calf to receive colostrum early in life. The benefits of early colostrum in preventing generalized infection may be twofold: a) early exposure to colostrum may prevent transepithelial migration of microorganisms and b) if invasion does occur, the interaction of passively acquired immunoglobulins and phagocytic cells of the neonate result in removal of the invading organism.

REFERENCES


