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J. Heidenreich

C. Aperce

James S. Drouillard

# Increased Concentrations of Bovine Intestinal Mucus Encourage Growth of *Escherichia coli* O157:H7

***J. Heidenreich, C. Aperce, and J. Drouillard***

## Introduction

Cattle have been implicated as carriers of the human pathogen *Escherichia coli* O157:H7. Contamination of the beef supply by *E. coli* O157 can occur during harvest and processing, causing costly recalls or human illness. Many interventions have been applied in attempts to prevent contamination of carcasses in processing plants, such as development of HACCP procedures, carcass washes, and steam pasteurization, but contaminations still occur. Mechanisms that allow *E. coli* O157:H7 to thrive in cattle at sporadic times and in such large numbers are poorly understood. Understanding factors that stimulate *E. coli* O157 growth in cattle will aid in identifying effective interventions that can be applied in feedlots and processing plants to reduce the numbers of this pathogen.

*E. coli* O157 resides in the intestinal tracts of cattle. Mucin is a major component of intestinal mucus and is composed of proteins, lipids, and carbohydrates, which many bacteria can use as a source of food. The amount of mucin available in the intestinal tract depends on the stimulation of intestinal mucus-producing cells (goblet cells), which may be influenced by the animal's diet, stress, and a variety of other factors. Our objective in this experiment was to determine if mucin produced in the small or large intestine could affect growth of *E. coli* O157:H7.

## Experimental Procedures

We isolated mucus from the small and large intestine of freshly harvested cattle. Protein and organic matter concentrations of the mucus were determined.

Five Shiga-toxin-producing *E. coli* O157:H7 strains resistant to the antibiotic nalidixic acid ($Nal^R$) were grown overnight at 98.6°F in a nutrient-rich broth and combined to create a five-strain mixture of *E. coli* O157:H7. To simulate natural conditions of the bovine intestinal tract, a fecal inoculum was prepared. Fresh bovine feces were added to a buffer solution, blended, and then strained through cheesecloth to remove large particulates.

The *E. coli* O157 mixture, McDougall's buffer, and fecal inoculum were added to test tubes containing various concentrations of small intestinal mucus (0, 0.5, 1.0, 2.0, 4.4, 10, or 15 mg organic matter/mL) to determine competiveness of *E. coli* O157:H7 in the presence of background bacteria. The cultures were gassed with oxygen-free $CO_2$, stoppered, and incubated at 104°F. Samples were taken after 0, 6, 8, and 12 hours of incubation and plated onto both Aerobic Petrifilm (3M, St. Paul, MN) for total anaerobic bacteria counts and onto MacConkey sorbitol agar plates containing nalidixic acid (CTN-SMAC) for $Nal^R$ *E. coli* O157:H7 counts. The Aerobic Petrifilm was incubated at 104°F under oxygen-free conditions. The CTN-SMAC plates were incubated at

98.6°F under ambient conditions. The numbers of bacteria were expressed as colony forming units per milliliter of liquid sample (CFU/mL). Additionally, following the same procedures, *E. coli* O157 mixture, McDougall's buffer, and/or fecal inoculum were added to test tubes containing either large or small intestinal mucus at a concentration of 4.4 mg of organic matter/mL.

## Results and Discussion

*E. coli* O157:H7 grew from a concentration of $10^3$ CFU/mL to $10^8$ CFU/mL of culture after 12 hours of incubation (Figure 1). Growth was similar in mucus derived from the small and large intestines ($P>0.10$). *E. coli* O157:H7 grew less in cultures containing feces than in cultures without feces ($P<0.01$). This reduction in growth was probably due to competition for nutrients between *E. coli* O157:H7 and the naturally occurring bacteria present in feces.

The effect of increasing concentrations of small intestinal mucus is shown in Figure 2. The total count of anaerobic bacteria was stable across concentrations from hour 0 to hour 8 ($P>0.10$). Unlike anaerobic bacteria, the number of *E. coli* O157:H7 increased as mucus concentration increased ($P<0.01$). These results suggest that intestinal mucus stimulates growth of *E. coli* O157:H7 and that pathogenic *E. coli* outcompete other intestinal bacteria for utilization of proteins and carbohydrates contained within mucus.

## Implications

These experiments provide valuable information regarding the influence of mucus on growth of *E. coli* O157:H7 in cattle. The study suggests the amount of intestinal mucus available to *E. coli* O157 may influence growth of foodborne pathogens in the intestines of cattle. This provides insight for further investigations toward development of preharvest interventions to limit growth of foodborne pathogens in cattle.

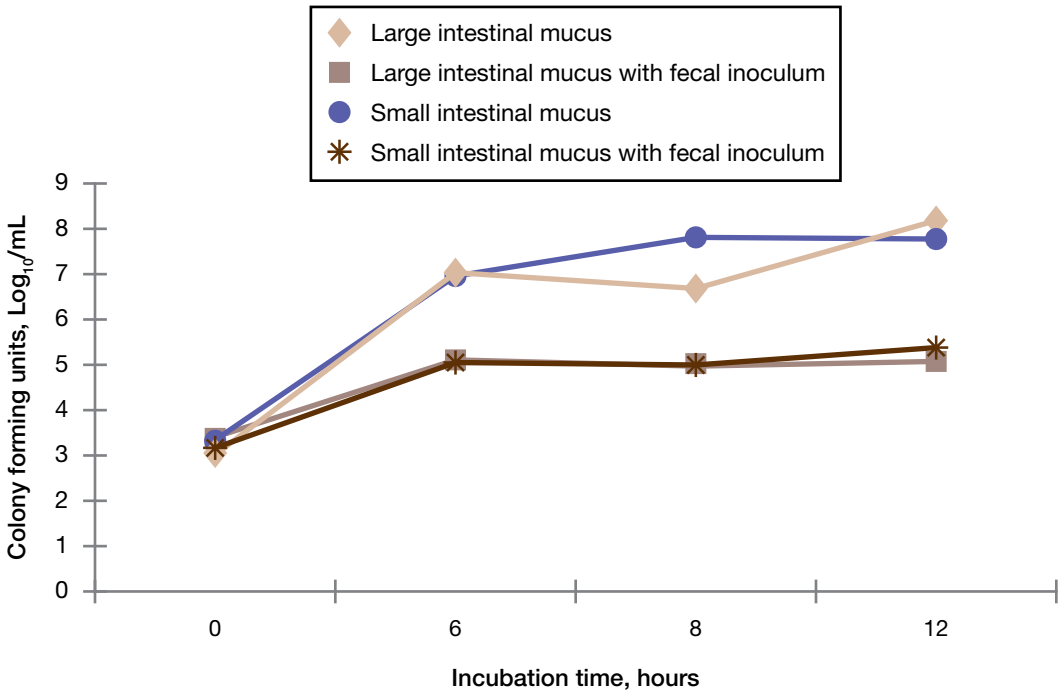


**Figure 1. Growth of *Escherichia coli* O157:H7 in mucus isolated from bovine small intestine or large intestine in the presence or absence of bovine feces.**

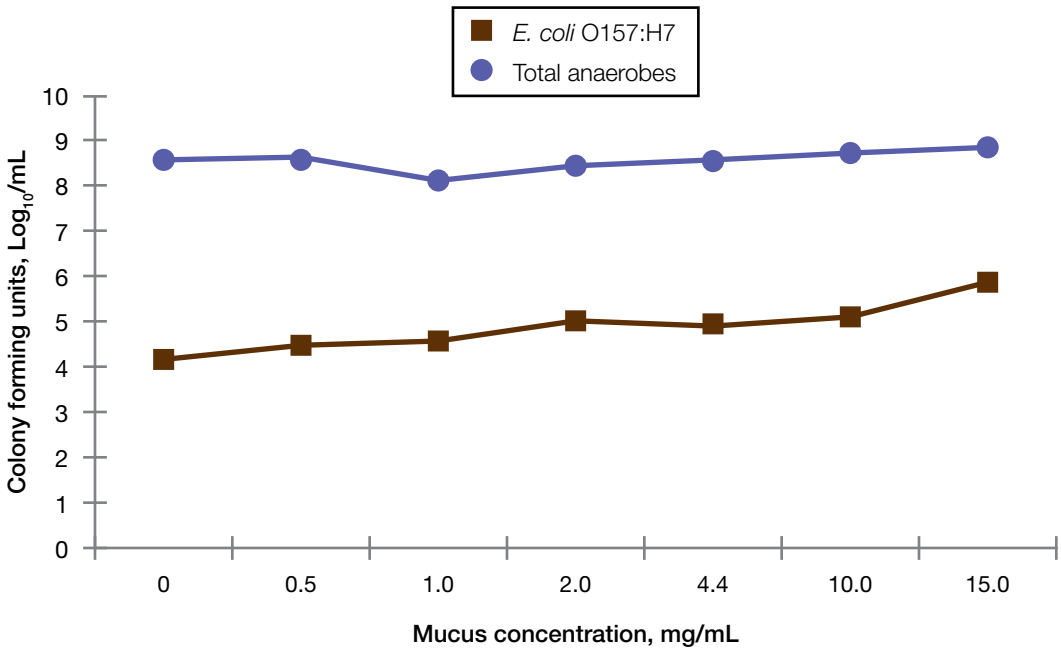


**Figure 2. Growth of total fecal anaerobic bacteria or *Escherichia coli* O157:H7 in response to increasing concentrations of bovine intestinal mucus after 8 hours.**