Prevalence and Growth of *E. coli* O157:H7 in the Bovine Gall Bladder

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Prevalence and Growth of E. coli O157:H7 in the Bovine Gall Bladder: Project Summary

Background
Since E. coli O157:H7 was first recognized as a human pathogen, bovine have been suspected as a reservoir because ground beef and other foods of bovine origin have been implicated in a majority of foodborne outbreaks. Illness resulting from the ingestion of E. coli O157:H7 ranges from mild diarrhea to hemorrhagic colitis and more serious syndromes, i.e. hemolytic uremic syndrome (HUS). It is estimated that Escherichia coli O157:H7 is responsible for 7,000 to 20,000 cases, 150 to 300 deaths, and $230 to $600 million in medical and productivity costs each year in the United States.

Previous studies indicate that the prevalence of E. coli O157:H7 in cattle is impacted by the following factors:

- Commingling of infected and non-infected cattle
- Age of the cattle
- Sample collection and handling procedures
- The sporadic nature of shedding E. coli O157:H7 (due to diet changes, lack of sensitivity in sampling and detection methods and the low prevalence of E. coli O157:H7 in feces)
- Sporadic intake from the environment; especially in the drinking water.

The findings of Naylor et al. (2003) demonstrated that E. coli O157:H7 localizes at the rectal-anal junction in the bovine intestinal tract. Colonization of the rectal-anal junction (RAJ) is associated with long-term shedders of E. coli O157:H7. Previous inoculation studies have isolated the organism from throughout the digestive tract and sporadically from tonsils and the gall bladder. Collectively, the findings from these studies demonstrate that there is colonization of the RAJ but environmental inputs are likely to contribute to the length and numbers of E. coli O157:H7 shed. The identification of other colonization sites could help explain the sporadic nature of shedding and the long-term shedding by some cattle and ultimately the development of intervention strategies.

The objectives of this study were to determine the prevalence of E. coli O157:H7 in gall bladders from naturally-infected animals and to evaluate the growth and survival of E. coli in bovine bile.

Methodology
A total of 150 gall bladders were collected from a beef processing facility in Wisconsin that receives cattle primarily from three states (Illinois, Iowa, and Wisconsin). Gall bladder samples were collected between May and October of 2005. The sampling period was selected because previous studies have demonstrated a seasonal E coli shedding pattern with the summer months yielding the highest number of positive samples. Samples were collected, transported to the lab on ice, and analyzed within 6 hours of collection. Samples were tested for E. coli O157:H7 using enrichment and immunomagnetic separation procedures.

Separate experiments were conducted to determine if E. coli O157:H7 was capable of growth in bovine bile. Five different O157:H7 strains were tested to ascertain if there were strain-to-strain differences in the ability to grow in bile. In addition, bile from different animals was compared to
determine if there are differences in the antimicrobial properties of bile from different animals. Overnight cultures were used to inoculate filter-sterilized bile at a final concentration of $10^5$ CFU/ml. The inoculated bile was incubated at 39°C and samples removed daily to determine the CFU (numbers)/ml for 7 days.

**Findings**

The results from this study show that serotype O157:H7 strains can grow in bile from cattle and that 3% of gall bladders tested harbored this pathogen. However, a prevalence of approximately 3% does not indicate that the gall bladders of all cattle harboring this pathogen are colonized.

The growth of the *E. coli* O157:H7 strains in 16% bile salts were unexpected and contribute to the ability of this pathogen to compete in the intestinal tract and persist in the environment and throughout the food production chain. The results from this study clearly demonstrate that this pathogen is bile tolerant and can reside and replicate in the gall bladder of infected cattle. What remains unanswered is whether this site is persistently colonized or it transiently occupied. The bile tolerance noted in this study certainly provides a competitive advantage to the organism once it gains access to the bile duct and gall bladder.

**Implications**

It is possible that cattle with *E. coli* O157:H7 in their gall bladder are infected with a strain that is not only bile tolerant but also possesses another characteristic that facilitates colonization. Alternatively, another animal factor or perhaps diet, which can influence bile production, impacts whether the gall bladder becomes colonized. Future studies should ascertain if the presence of *E. coli* O157:H7 in the gall bladder of cattle contributes to the duration (long-term shedders) or the number of the organisms shed in the feces. These findings provide additional information on the location of this organism in cattle that may influence the sporadic nature of pathogen shedding and contribute to the development of animal-treatment strategies.

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