Project Summary

Comparison of Recto-anal Junction Mucosal Swab and Fecal Culture for Detection of *Escherichia coli* O157:H7 in Dairy Heifers

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Background

E. coli O157:H7 can cause severe disease and even death in humans. *E. coli* O157:H7 is transmitted through food, water and direct contact with infected animals. Because domestic ruminants are the most important reservoir for this pathogen, its on-farm ecology has been studied extensively. The niche occupied by *E. coli* O157:H7 is complex, which is in part due to the ability of this pathogen to survive in a variety of environmental conditions. In studies of naturally and experimentally infected animals, a large variation in the duration of colonization between individual animals has been observed. Most animals excrete culture positive feces for less than one week, but there are a few animals that are fecal positive for several weeks, even months. These observations raised the possibility that a minority of "carrier" animals in a herd may play an important role in the maintenance of *E. coli* O157:H7 on the farm. Recent findings that the recto-anal junction mucosa (RAMS) is a major colonization site for *E. coli* O157:H7 in the bovine intestine also suggest that host colonization factors may play an important role. Previous research has demonstrated that RAMS culture is more sensitive than fecal culture as a detection method.

Previous research has also suggested that a correlation exists between positive RAMS culture and duration of infection, because animals that were initially RAMS culture-positive and fecal culture-negative had significantly longer infections than animals with the reverse situation. An inexpensive, rapid and sensitive method for detecting carrier animals could be a valuable management tool for cattle producers to reduce the pathogen load on farms.

To determine the sensitivity of RAMS, researchers compared that method with fecal immunomagnetic separation (IMS). To evaluate the ability of RAMS culture to predict duration of infection, the researchers also conducted a longitudinal study of natural *E. coli* O157:H7 infections among dairy heifers using 1) direct and enriched RAMS culture, 2) direct fecal culture and 3) IMS-enriched fecal culture.

Methodology

Forty dairy heifers from each of two university herds were identified and were sampled once each month for twelve months. Each animal that yielded a positive sample at the monthly visit was resampled one week later. At each visit, two samples were collected from each animal—freshly passed feces and a recto-anal mucosal swab (RAMS).

Microbiological analysis of the RAMS samples was done through direct culture. Swabs that did not yield *E. coli* O157:H7 on direct culture were also analyzed with enriched culture. Direct and enriched cultures were also performed for the fecal samples.

To analyze the genetic relationship between isolates of *E. coli* O157:H7, pulsed-field gel electrophoresis was performed.

Findings

Table 1. Number of heifers longitudinally sampled at each monthly sampling visit.

Sampling Month	Dairy A	Dairy B
April	20[a]	20[a]
May	32	0[b]
June	31	28
July	34	29
August	39	29
September	40	32
October	40	40
November	40	36
December	40	40
January	40	40
February	40	39[b]
March	40	26[c]

[a] Heifers were enrolled at two months of age and sampled repeatedly throughout the study period.
[b] Calves were transported to the heifer raising facility in May and unavailable for sampling
[c] One of the study heifers died of causes unrelated to the study.
[d] Heifers that were close to their calving dates were moved back to the dairy prior to that sampling date and were unavailable for sampling.

RAMS was similar to immuno-magnetic separation in sensitivity as a screening test for *E. coli* O157:H7 in cattle. Overall, RAMS culture (direct and enriched results combined) was as sensitive as IMS fecal enrichment culture. IMS fecal enrichment detected 87 positives from 874 animals (10 percent). RAMS plating including enrichment detected 84 positives out of 790 animals tested (10.6 percent). Direct fecal plating with the IMS enrichment step was the least sensitive method, yielding 28 positive results from 874 samples tested (3.2 percent). Direct RAMS plating alone (without enrichment) detected 62 positives out of 874 samples (7.1 percent). Overall, direct fecal plating was significantly less sensitive than either direct RAMS culture alone or plus enrichment.

RAMS culture-positive status did not predict duration of colonization. To test the hypothesis that positive RAMS culture identifies colonized animals and differentiates them from animals that are not colonized, but are passively shedding *E. coli* O157:H7 in their feces, the researchers compared duration of infection between those heifers that had first positive cultures by fecal IMS culture to those that had first positive culture by RAMS culture. Among 53 infections, seven (13.2 percent) were first detected by all three methods (RAMS culture, direct fecal culture and IMS fecal enrichment). Seventeen (32.1 percent) positives were first detected by RAMS and IMS, 10 (18.9 percent) were first detected by RAMS culture, and 19 (35.8 percent) were first detected by IMS alone. Among 14 infections that had durations of two weeks or longer, five (35.7 percent) were first detected by all three methods, six (42.9 percent) were first detected by both RAMS culture and IMS, and three (21.4 percent) were first detected by IMS. None of the long-duration infections were first detected solely by RAMS culture.

Duration of infection was associated with fecal bacterial counts of *E. coli* O157:H7. The majority of infections were of short duration and a minority of animals was culture positive on consecutive visits over a 60-day interval. The average log fecal counts of *E. coli* O157:H7 associated with short duration infections were likely to be smaller than those associated with infections of longer duration.

Pulsed-field gel electrophoresis (PFGE) results indicated a dominant and persistent strain of *E. coli* O157:H7 in one of the dairies. Among the 54 isolates with PFGE results, 35 (61 percent) were Type A, six (11.1 percent) were Type B, three (5.5 percent) were Type C, three (5.5 percent) were Type D and Types E through K each represented unique patterns. Type A was detected on 13 of 15 sampling dates, and was isolated at least once from 18 different animals in Dairy A.

Implications

This study demonstrated that RAMS culture has a sensitivity similar to that of IMS-enriched fecal culture, and better overall sensitivity than direct fecal plating for *E. coli* O157:H7 screening in cattle. However the researchers did not reproduce results found in previous research that indicated an association between long duration of infection and RAMS culture-positive status and would not necessarily serve as a good way to screen for colonized animals.

There was a positive association between duration of infections and fecal *E. coli* O157:H7 counts. This finding suggested that colonized animals make a significant contribution to pathogen load on farms, and lends support to the concept of “supershedders.” The ability to identify those animals or “supershedders” would potentially allow the prevention of a large contribution of pathogens to farm and ranch environments.

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