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Beef Science Cluster

Understanding non-O157 STEC associated with cattle and beef carcasses

Project Title:	
Prevalence, persistence and control of non-O157 shiga toxin producing Escherichia coli	

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Background

There are between 50,000 and 100,000 different serotypes (strains) of *E. coli*. Most are harmless, some may be beneficial, but some produce a very dangerous Shiga toxin. Shiga toxigenic *E. coli* (STEC) can cause vomiting, diarrhea and abdominal pain in people. *E. coli* O157:H7 is the most well-known STEC, but it is not the only one.

All STEC's carry at least one *stx* gene coding for the Shiga toxin, an *eae* gene coding for a protein that helps *E. coli* attach to the intestinal surface, and a *wzx* gene that codes for an "O" antigen. All three of those genes must be present in the *E. coli* cell for it to be a STEC.

Non-O157 STEC infections are rare, but in 2011 the United States Department of Agriculture's Food Safety and Inspection Service gave the "Top 6" most common non-O157 STECs (O26, O45, O103, O111, O121, and O145) the same status as*E. coli* O157:H7. The US is Canada's main beef export market, so our beef processing industry needs to understand how we may be impacted.

Objectives

The objective of this study is to fill many of the knowledge gaps that exist with respect to non- O157 STEC associated with beef.

What they did

Two beef packing plants in southwestern Ontario were visited four times over a five-month period. Swabs were collected from the neck, flank and round of cattle, their carcasses and the plant environment (holding pens and kill floor). In all, 300 swabs were collected. The swabs contained many kinds of bacteria and contaminants, so each one was enriched to encourage the *E. coli* to grow. DNA was extracted from each sample, and two different PCR tests were used to detect whether the *eae*, *stx* and *wzx* genes were present. Samples that tested “presumptive positive” were subjected to more specific tests to confirm whether STEC were present.

What they learned

The two initial PCR tests agreed closely, and found “presumptive positive” results for more than 92% of the hide, 72% of the carcass and 84% of the environmental samples. The O103, O121 and O45 serotypes were more common than O125, O111 and O26. All presumptive positive samples contained *eae*, *stx* and *wrx* genes from at least three of the Top 6 non-O157 STECs. Some were positive for all six Top 6 non-O157 STECs

But these initial presumptive positive PCR results only mean that the *eae*, *stx* and *wrx* genes were present in the sample, and each sample contained many different *E. coli* bacteria. The presumptive positive results don’t necessarily mean that all three genes were in the same *E. coli* bacterium. Some *E. coli* may have only had one or two of the three required STEC genes. If an individual *E. coli* doesn’t carry all three genes at the same time, it’s not an STEC. 120 of the presumptive positive samples were cultured, 10 distinct *E. coli* colonies were isolated from each, and each colony was individually PCR tested. This was done twice, so 2400 individual *E. coli* isolates were examined overall.

These confirmation tests produced very different results than the presumptive tests. In fact, no culture-positive Top 6 non-O157 STEC were recovered at all. They did find *E. coli* with O103, O121, O45, O125, O111 or O26 antigens coded by the *wzx* gene. But they didn’t find any *E. coli* that contained those antigens as well as both the *eae* and *stx* genes. In other words, the O103, O121, O45, O125, O111 and O26 *E. coli* found in these samples weren’t STEC’s.

What it means

In *E. coli* O157:H7, the presence of the O157:H7 antigen is a very good indicator that the *eae* and *stx* genes will be present as well. In other *E. coli*, the *stx* gene is quite mobile because it is part of a prophage. A prophage is the DNA that codes for a phage, and has been incorporated into the bacteria’s own DNA. A phage is a virus that infects bacteria. When the *stx* gene is turned on in *E. coli*, the shiga toxin gets produced, and new copies of the phage get manufactured. When the *E. coli* gets too full of phage particles, the bacteria bursts and releases both the phage and the shiga toxin. When the new phages infect other susceptible *E. coli*, they also introduce the *stx* gene. This means that some *E. coli* may have an O103, O121, O45, O125, O111 or O26 antigen, but if they haven’t been infected with the prophage they may not be a STEC.

Non-O157 STEC’s were not found in this study, but rare doesn’t mean non-existent. Improved diagnostic tests that combine the speed, ease, and price of PCR tests with the accuracy of slow, difficult and costly culture tests would benefit both processors and regulators, particularly when the detection of non-O157 STEC causes a recall.

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