

## Approaches for Reduction of Shiga Toxin-Producing *Escherichia coli* and *Salmonella* on Hide of Cattle

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Isolates of Shiga toxin-producing *Escherichia coli* (STEC) were first recognized as human pathogens in 1982 when *E. coli* O157:H7 was identified as the source of two outbreaks of hemorrhagic colitis. Since then other outbreaks of STEC, such as O26, O45, O103, O104, O111, O121, and O145, also have been associated with cases of hemorrhagic colitis [1,7]. However, serotype O157:H7 still is the predominant cause of STEC-associated diseases in the United States and many other countries [2,8].

Epidemiological investigation and animal model studies have revealed that cattle are major reservoir for *E. coli* O157:H7 and other STEC [3-8,10,11]. Recent data indicated that isolation rate of *E. coli* O157:H7 in individual cattle were ranged from 5 to 20%, with enumeration rate in feces at <100 to >10<sup>4</sup> CFU/g [3,8]. STEC can be excreted through feces at cell numbers of 10<sup>6</sup> CFU/g in super-shedding cattle and survive on hides, in drinking troughs, in pens and bedding, on tools, and in the farm environment for several months. Animal hides are an important source of zoonotic pathogens which contaminate carcasses at beef slaughter.

Commercial beef processing plants currently employ several interventions (i.e., trimming, steam vacuuming, steam pasteurization, water washes, and organic acid washes) in combination to achieve large reductions in carcass contamination. Tremendous efforts have been performed for prevention of breaking the gastrointestinal tracts during slaughter processing to make sure that no carcasses leaving the cooler were identified as contaminated with *E. coli* O157:H7 and other STEC.

Epidemiological data have revealed that 76% and 67% of animal hides entering processing plants can be contaminated with *E. coli* O157 and non-O157 STEC; respectively [3]. However, pre-evisceration carcass prevalence of *E. coli* O157:H7 and other STEC serotypes varied greatly, ranging from 0 to 93% for *E. coli* O157:H7 on different days at different plants. Although considerable effort has been applied to reducing *E. coli* O157:H7 and *Salmonella* on and in cattle at pre-harvest, effective hide treatment for pathogen removal is still needed considering the results reported by Bosilevac et al. that the prevalence of *E. coli* O157:H7 and *Salmonella* on pre-evisceration carcasses was 33% and 58%, respectively.

Studies were done by using hides to evaluate their efficacy to kill the inoculated pathogen. Various chemicals (lactic acid 2, 4, and 6%), acetic acid (2, 4, and 6%), chlorine (100, 200, and 400 ppm), alcohol (70, 80, and 90%), peroxyacetic acid (0.05, 0.1, and 0.4%) were evaluated for their effects to kill rifampicin-resistant *Salmonella* Typhimurium inoculated on fresh beef hides. Results indicated that alcohols at all concentrations were effective ( $\geq 5 \log/\text{cm}^2$  reduction)

and acetic and lactic acids at high concentrations (4 and 6%) were effective ( $\geq 3 \log/\text{cm}^2$ ). However chlorine, even at 400 ppm only reduced 1.3log CFU/cm<sup>2</sup>. Cattle washing studies on determination of the impact of various pre-harvest treatments (0.5% lactic acid and 50 ppm chlorine) on microbiological integrity on living animal indicated that the counts of aerobic plate counts, coliforms, *E. coli* had no statistical difference ( $P>0.05$ ) between water wash groups and chemical wash groups.

A method at post-harvest stage was evaluated. Under cooperation between Water Management resources and Cargill, a hide-on-carcass wash machine, as a "car wash for cattle", in which the hides of animals are scrubbed with spinning bristles and a mild bromine solution that kills bacteria at the beginning of the harvesting process was installed at the Fresno beef plant. This process helps better ensure removal of the dirt and debris while washing the animal's exterior, thereby minimizing the potential for contamination from bacteria that potentially pose a health risk to humans. The cost for such a processing is high and it is fine at current high beef price. However it should have an alternative choice in case beef price may go down like oil price.

A food-grade and non-chlorine-based microbicide, containing just two chemicals, levulinic acid and sodium dodecyl sulfate, SDS was developed and thoroughly evaluated in our lab and other labs for effectively killing foodborne pathogens in poultry [12], meat [13], produce [12], and seeds; especially for removal of biofilms in processing plants [6]. This microbicide can also remove dental biofilm in vitro and is 10-fold better than Listerine and when applied in animal mucus for either short term or long term there were no pathological change when compared with water only [9]. Thus its safety for animal application is guaranteed.

Studies were performed to determine the efficacy of this commercial microbicide (Fit-L, HealthPro Inc.) to inactivate STEC and *Salmonella* Typhimurium on cattle hides as a surface spray treatment at different concentrations in vitro and in vivo. A mixture of six isolates of STEC, including serovars O26, O45, O103, O111, O121, and O157 (10<sup>8</sup> CFU/ml) and a mixture of 5 strains of *S. Typhimurium* (10<sup>8</sup> CFU/ml) were sprayed on the surface of 10 × 10 cm sections of cattle hide. The hides were treated by surface spray with this microbicide diluted at different concentrations at 45 psi for 15s. Water only was used as the negative control. For STEC-contaminated hides, 3% levulinic acid plus 0.5% SDS for 5 min reduced STEC populations by 2.3log/cm<sup>2</sup>, compared to the water only treatment. For *S. Typhimurium*-contaminated hides, treatment with 2% levulinic acid plus 0.2% SDS reduced the *Salmonella* population by 3.2log CFU/cm<sup>2</sup>. Scrubbing hides with a brush processing for 30 s followed by the microbicide spray treatment further reduced *Salmonella* contamination by 0.5 log/cm<sup>2</sup>. However, for wet hides, a spray treatment with 4% levulinic

acid plus 2% SDS for 5 min reduced *Salmonella* by only 1.3log CFU/cm<sup>2</sup> when compared with the water-only treatment.

Based on the results obtained from hide studies, commercial “Fit-L” product diluted in tap water at 1:22 (v/v, 2% levulinic acid plus 0.2% SDS) was used for surface wash of live beef cattle. Results revealed the average *E. coli* count before washing (7 cattle with 26 samples) was 6.58log CFU ± 1.0/cm<sup>2</sup>. For tap water only washed cattle (7 cattle with 28 samples) the average *E. coli* count was 6.0log CFU ± 1.10/cm<sup>2</sup> at 5 min and 6.06log CFU ± 1.48/cm<sup>2</sup> at 10 min. Whereas, for “Fit-L”-washed cattle (7 cattle with 28 samples) the average *E. coli* count was 2.6log CFU ± 0.95/cm<sup>2</sup> at 5 min and 2.25log CFU ± 0.89 CFU/cm<sup>2</sup> at 10 min. Following the “Fit-L” washing with a tap water washing resulted in 2.3log CFU *E. coli* ± 0.83/cm<sup>2</sup>. These data revealed that a simple “Fit-L” wash could reduce *E. coli* population by 3.4log and 3.8log on the surface of cattle hide at 5 min and 10 min, respectively when compared with tap-water wash only. A tap water-only wash reduced *E. coli* by 0.5log CFU/cm<sup>2</sup> when compared with samples collected before the wash. Following “Fit-L” washing with one more tap water wash did not further reduce *E. coli* on the surface of cattle hides. These results suggested a simple “Fit-L” wash just before cattle entered the slaughter facility will substantially reduce the population of *E. coli* on cattle hides.

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