The Effects of a Three-strain Mixture of Bacteriophage on *E. coli* O157:H7-inoculated Hide Squares over a 10-hour Period*

Mies P.D.[1] and Davis M.J.[2]

[1]Elanco Food Solutions, Overland Park, Kansas, USA
[2]Elanco, Greenfield, Indiana, USA

Abstract — **Bacteriophage is currently being used in United States beef abattoirs under the trade name Finalyse®. Finalyse® is a three-strain mixture of bacteriophages that are effective in reducing the burden of *E. coli* O157:H7 on the hide of cattle. This study was designed to understand the effects of Finalyse against *E. coli* O157:H7 over a 10-hr period. Hide squares (5 squares/time period/treatment) were cut into 2" to 3" sections and incubated on heating pads to mimic body temperature. Hide squares were inoculated with 5.5 logs of recoverable *E. coli* O157:H7, allowing 30 minutes for attachment. Hide squares were treated with Finalyse or a water control. *E. coli* O157:H7 was removed from hide squares by placing the hair side down in a Petri dish with 15 mL sterile water and agitating. Liquid (1 mL) was centrifuged and the supernatant was removed. The pellet was then resuspended in 1 mL of LB broth and plated 10-fold dilutions of BBL™ CHROMager™ O157 plates. Colonies were counted after overnight incubation at 37°C. The maximum reduction of *E. coli* O157:H7 for Finalyse-sprayed hides was 1.3 logs at 1 hour. Finalyse-sprayed hide squares had less recoverable *E. coli* O157:H7 compared to water-sprayed control hide squares throughout the 10-hour study.**

Keywords — **Bacteriophage, E. coli O157:H7, cattle**

I. INTRODUCTION

The prevalence of *E. coli* O157:H7 in fecal matter and on hide samples taken from cattle in feedlots has been shown to be significantly correlated to carcass contamination in abattoirs (Elder et al., 2000). Threshold levels have also been found in fecal pats from feedyards, with prevalence rates of greater than 20% significantly increasing hide and carcass contamination (Woerner et al., 2006). Transportation and lairage also play a key role in *E. coli* O157:H7 prevalence and enumeration on hides and carcasses. Prevalence of *E. coli* O157:H7 increased from 50% to 94% on hides between loading cattle at the feedyard and the time the hide was removed in the abattoir. Enumerative *E. coli* O157:H7 loads also increased from 9 to 70 cattle between the feedyard and the abattoir (Arthur et al., 2007). To decrease the contamination of cattle hides going into the abattoir, a mixture of phage, Finalyse, was developed. This microbial intervention was designed to be applied after transportation and prior to entry into the abattoir. This study was conducted to evaluate a range in dwell time at abattoirs for phage efficacy.

II. MATERIALS AND METHODS

Cattle hide squares were cut into 7.62-cm squares and incubated on heating pads throughout the study. Hide squares were sprayed with avirulent *E. coli* O157:H7 strain ATCC 43888 (American Type Culture Collection, Manassas, VA) and allowed to partially dry for 20 to 40 min. Five (5) hide squares were used per time period per treatment.

0-hour samples were taken prior to treatment with a control sterile water wash or bacteriophage treatment. After treatment with either a control water wash or bacteriophage treatment, samples were taken every hour from 1 to 10 hours.

Finalyse was diluted approximately 1/3 to a concentration of ~6 X 10^{10} pfu/mL in water. Finalyse was placed in a commercially available spray bottle and delivered 3 mL of solution per hide square. For the control samples, sterile water was used instead of the Finalyse product.

Hide squares were placed hair side down in Petri dishes containing 15 mL sterile water and mixed to transfer the hair contents into the water. One (1) mL of the liquid was centrifuged for 1 to 2 min at maximum speed (13,000 x g) and the pellet was resuspended in 1 mL LB. Serial 10-fold dilutions were plated on BBL™ CHROMagar™ O157 (Becton Dickinson and

Company, Sparks, MD). Plates were incubated overnight at 37°C. Results were reported in log CFUs per mL.

III. RESULTS

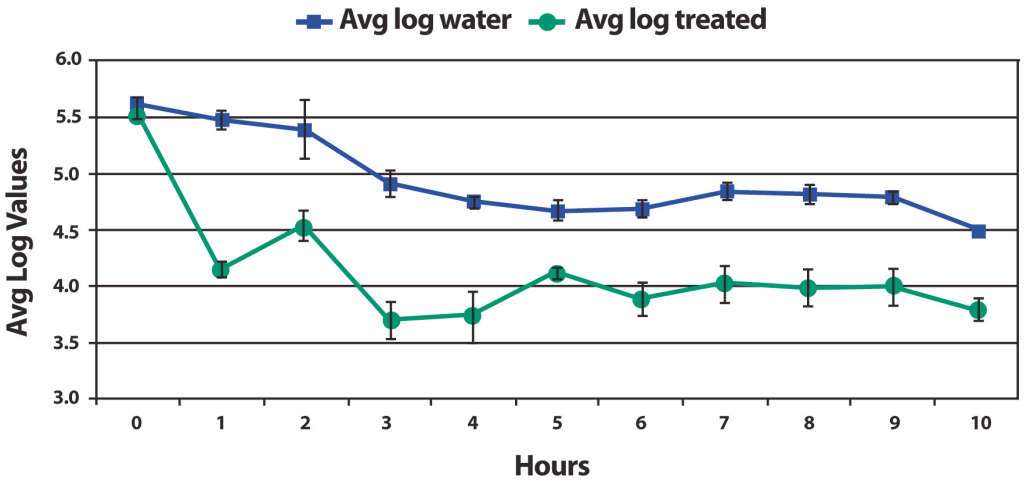
This hide model study used artificially high *E. coli* O157:H7 numbers for the purposes of enumeration. In addition, the Finalyse bacteriophage-to-*E. coli* O157:H7 ratio was very low at 100:1 versus the 10,000:1 ratio that would typically be seen in the field.

Figure 1 displays the results from the study. Similar results were found at time point 0 hr, with the treated hides at 5.55 logs and the water control at 5.61 logs, demonstrating similar inoculation levels for the treatments. At 1 hour, a maximum reduction of 1.27 logs was observed for Finalyse-treated hides compared to the control (water-treated) hides. The greatest advantage in using Finalyse compared to water was seen in the first 4 hours at around a 1-log reduction (1.27-, 0.8-, 1.16- and 0.95-log difference, respectively). Hide squares that were treated with Finalyse maintained a difference of 0.5 logs or more less recoverable *E. coli* O157:H7 compared to the control water wash throughout the 10-hr study.

IV. CONCLUSIONS

The phage mixture, Finalyse, is a viable intervention strategy for *E. coli* O157:H7. Many abattoirs will hold cattle after transportation in lairage pens for 1 to 4 hours and, depending on plant circumstances, up to 10 hours. Finalyse was effective at reducing the burden of *E. coli* O157:H7 prior to entry into the abattoir.

Figure 1.



REFERENCES

1. Elder R, Keen J, Siragusa G, Barkocy-Gallagher G, Koohmarie M, Laegreid W. (2000) Correlation of Enterohemorrhagic *Escherichia coli* O157 Prevalence in Feces, Hides, and Carcasses of Beef Cattle During Processing. *Proc. Natl. Acad. Sci.* 97:2999-3003.
2. Woerner D, Ransom J, Sofos J, Dewell G, Smith G, Salmon M, Belk, K. (2003) Determining the Prevalence of *Escherichia coli* O157 in Cattle and Beef from the Feedlot to the Cooler. *J. Food Prot.*, Vol. 69, No. 12, p. 2824–2827.
3. Arthur T, Bosilevac J, Brichta-Harhay D, Guerini M, Kalchayanand N, Shackelford S, Wheeler T, Koohmaraie M (2007) Transportation and Lairage Environment Effects on Prevalence, Numbers, and Diversity of *Escherichia coli* O157:H7 on Hides and Carcasses of Beef Cattle at Processing. *J. Food Prot.* 70:280-286.

* Elanco Study No. BIOUS090003. 2008. Data on file. Elanco Reference No. 2296.

FIN 21657-2