EFFICACY OF LOOP-MEDIATED ISOTHERMAL AMPLIFICATION OF *BACTEROIDALES* ON BEEF CARCASS SURFACES AS AN INDICATOR FOR SANITARY DRESSING PRACTICES

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I. INTRODUCTION

Cattle hides, hair and faeces on live animals has been identified as the major source of Shiga toxin producing *E. coli* (STEC) on the surface of beef carcasses, on manufacturing trimmings and in ground and comminuted beef. However, STECs are inadequate indicators of sanitary dressing due to their low prevalence. Indicator organisms such as *Escherichia coli* biotype I (ECC), *Enterobacteriaceae* (EB) and aerobic plate counts (APC) are commonly used as indicators for carcass surface contamination [1,2]. While these data are valuable, having accurate and reliable information with a rapid time to result would allow more effective corrective measures and mitigation procedures. Use of a rapid loop-mediated isothermal amplification (LAMP) has been developed as a rapid approach for detection of *Bacteroidales* in animal faeces, on leafy greens and from the environment [3]. The objective of this study is to compare the levels (CFU/cm^2) of APC and EB to those of LAMP *Bacteroidales* (BR) in carcass surface samples collected throughout the beef harvest process.

II. MATERIALS AND METHODS

Surface samples were collected using sterile sponge-sticks hydrated with 10 mL of neutralizing buffer from 3 locations throughout the beef harvest process [1]. Samples were collected from the external hide (100 cm^2), prior to evisceration (~8,000 cm^2) and prior to chilling (~4,000 cm^2) with 30 samples being collected at each location over the course of 2 consecutive production days (N = 180). Samples were analyzed for APC and EB following the procedure outlined in EML - O-TC-MET5401 – Aerobic Plate Count – Petrifilm and EML - O-TC-MET6614 - Enterobacteriaceae Count - Petrifilm. Serial dilutions were prepared as necessary, and 1 mL of sample suspension was applied to 3M Aerobic Count Plates and Petrifilm *Enterobacteriaceae* Count Plates and incubated at 35 ± 1° C for 48 ± 2 h and 37 ± 1°C for 24 ± 2 h, respectively. After incubation, colonies within the countable range (10 to 150 colonies) were counted and the data reported as log CFU/cm^2. Fluorometric LAMP reactions were prepared as described by Wang et al. [3] in individual domed PCR tubes (AB0337; Thermo Fisher, USA). A total of 25 µL comprising 12.5 µL WarmStart® _Colorimetric LAMP 2X Master Mix (M1800; New England Biolabs, USA) (final concentration 1X), 2.5 µL 10X LAMP primer mix (16 µM FIP/BIP, 2 µM F3/B3, 4 µM LF/LB) (final concentration 1.6 µM FIP/BIP, 0.2 µM F3/B3, 0.4 µM LF/ LB), 9 µL nuclease-free water, and 1 µL of diluent from sponge-stick samples as previously described. Reactions were run on a BioRad CFX96 at 65° C with a ramp rate of 1° C/s. Microbial plate counts (log CFU/100 cm^2) and LAMP Cq values (time required for the fluorescent intensity to reach/exceed the defined reaction threshold) were analysed using least squares regression models JMP 17.

III. RESULTS AND DISCUSSION

Surface samples from carcasses were found to have quantifiable loads (Table 1) of aerobic bacteria and *Enterobacteriaceae* and as carcasses progressed through the harvest and dressing process, bacterial counts (log CFU/100 cm^2) systematically decreased ($P<0.05$). Sanitary dressing and antimicrobial interventions employed resulted in a 4.6 log CFU/100 cm^2 reduction in APC and 4.6 log

CFU/100 cm^2 reduction in EB counts. Similarly, BR LAMP Cq values increased as cultured bacterial counts decreased through the process. *Bacteroidales* results were identified as "non-detectable" if the Cq values exceed 55 or if fluorescence was not observed; this resulted in 87 out of 180 (48.3%) samples having non-detectable copies of BR. Across sample location the number of non-detectable BR samples increased as carcasses progressed through the sanitary dressing process (Table1).

Table 1. Mean ± SE for Aerobic Plate Counts (APC), *Enterobacteriaceae* (EB) Counts (Log CFU/100 cm^2) and B*acteroidales* Cq (BR Cq) values from swab samples collected from cattle hides (n = 60), carcasses prior to evisceration (n = 60) and after final intervention (n = 60).

Sample Location	APC	EB	BR Cq (n*)
Hide Surface	5.45 ± 0.08^{a}	4.18 ± 0.08^{a}	41.98 ± 1.02^{a} (39)
Pre-Evisceration	2.74 ± 0.08^{b}	1.04 ± 0.08^{b}	44.86 ± 1.07^{ab} (36)
Post-Intervention	0.88 ± 0.08^{c}	-0.39 ± 0.08^{c}	48.11 ± 1.51^{b} (18)
P-value	<0.05	<0.05	<0.05

*Number of samples with detectable Cq values; [a,b,c]Means, within a column, lacking common superscript letters, differ (P<0.05)

Although directionally, Cq values were lower in the presence of higher bacterial counts, the correlation between BR Cq and APC (Figure 1A) and BR Cq and EB counts (Figure 1B) were low.

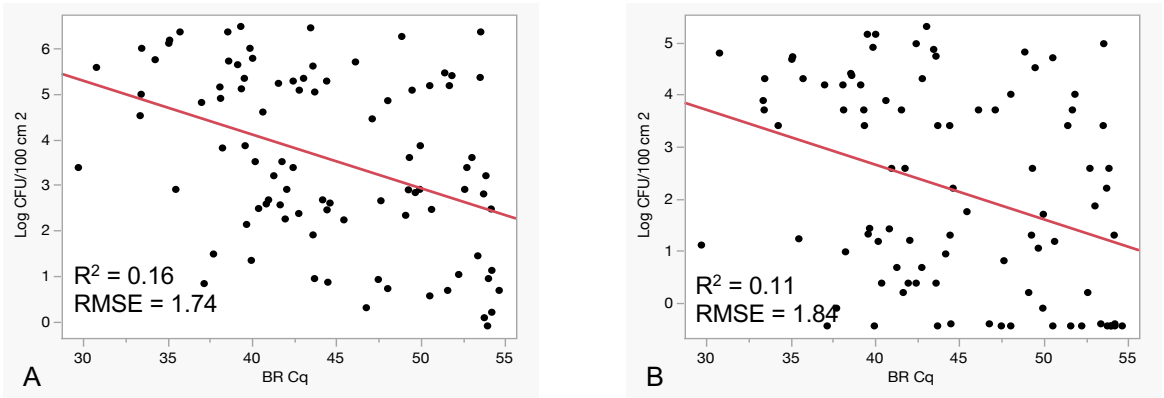

Figure 1. Bivariate fit of APC (A) and EB (B) counts (Log CFU/100 cm^2) with *Bacteroidales* Cq values.

IV. CONCLUSION

Microbiological indicators of contamination provide valuable information to assess the efficacy and performance of sanitary dressing practices and carcass decontamination interventions. Development of faster methodologies would result in more timely corrective actions and product disposition decisions. These results would suggest that populations of BR and EB on cattle hides and subsequently dressed carcasses are not strongly related and that BR populations in cattle may not be as uniformly distributed as EB or aerobic bacteria. Use of the *Bacteroidales* LAMP PCR, while providing more expedient results, requires additional refinement prior to being implemented as a reliable indicator of sanitary dressing practices.

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