EFFICACY OF INTERVENTIONS DURING PRIMARY PROCESSING ON CONTAMINATION OF BEEF CARCASSES WITH *ESCHERICHIA COLI*: A SYSTEMATIC REVIEW-META-ANALYSIS

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Abstract – Results of intervention strategies for *Escherichia coli* reduction on beef carcasses during primary processing are inconsistent or contradictory. Our objective was to identify, critically evaluate and synthesize published intervention research reporting treatment efficacy at the abattoir on *E. coli* contamination of beef carcasses using systematic review (SR)-meta-analysis (MA) methodology. Four electronic bibliographic databases were searched. Separate random-effects MAs were conducted for unique intervention datasets. SR-MAs included 36 citations reporting 202 trials. Although 44 interventions were identified, MA was precluded for most due to small study numbers with high risk of bias and large heterogeneity. MA of final carcass washing (OR 0.56, CI: 0.41-0.77), pasteurization (OR 0.09, CI: 0.06-0.14) and 24 hour dry chilling (OR 0.17, CI: 0.11-0.24) data showed a reduced odds of *E. coli* carcass contamination. The combined effects of potable water wash, steam or hot water pasteurization and a 24 hour dry chill, assuming no additional contamination and all other variables constant, resulted in the reduced generic *E. coli* prevalence of 1.22% (CI 0.17, 3.57).

Key Words – Abattoir, pasteurization, water wash

I. INTRODUCTION

Verotoxigenic *E. coli* (VTEC) cause haemorrhagic colitis, haemolytic ureamic syndrome and significant morbidity and mortality. Transmission to humans occurs through contaminated food, water, environment, or person-to-person contact. The Canadian annual average of 1,453 VTEC cases reported 1996 to 2004 [1] is an underestimate since for every case there are 10-47 not reported [2]. Beef contaminated with pathogenic strains of *E. coli* is thought to be the source of 37% of human infections in Canada [3].

As part of Hazard Analysis and Critical Control Points (HACCP) plans, processing plants implement interventions during primary beef processing to prevent or reduce *E. coli* contamination. A SR of HACCP implementation studies indicated that HACCP validation is more consistently associated with reduction of carcass contamination with indicator rather than pathogenic bacteria [4].

Research knowledge synthesis methods such as SR-MAs can formally evaluate intervention research [5]. Transparent and replicable methods identify, evaluate, critical appraise and summarize or synthesize data from several studies evaluating the same interventions under similar conditions. Resulting pooled effect estimates are more informative, and in the absence of considerable biological and statistical heterogeneity across studies, have more power than estimates from single studies [6]. Beef carcass processing intervention research has evaluated the efficacy of bacterial decontamination measures; however, results are inconsistent or contradictory. A SR-MA evaluating intervention research at the abattoir level is necessary to complement this information and to generate transparent, evidence-based inputs for risk assessment and management.

The study objective was to evaluate the efficacy of interventions applied in beef plants during processing (until completion of chilling), for the prevention or reduction of beef carcass contamination with generic and/or pathogenic strains of *E. coli*, using SR-MA methodology.

II. MATERIALS AND METHODS

Agricola, CAB International, PubMed, and Food Science and Technology Abstracts® were searched on June 20, 2008 and updated on September 22, 2009. Relevance screening of abstracts identified primary research in English investigating the efficacy of any intervention, applied to beef carcasses during primary processing, to prevent or reduce *E. coli* contamination. The full paper was screened for relevance, to characterize intervention type, outcome and point in chain. Laboratory and *'in vitro'* research is not reflective of commercial processing and was excluded, as were studies with intervention or laboratory protocols not described sufficiently to allow reproduction, and/or raw or adjusted data not reported to allow *post-hoc* calculation for MA. Raw data from prevalence studies formed 2x2 tables and odds ratios (OR) and standard errors were computed.

Separate MAs were conducted at the trial level on data subsets investigating washing, pasteurization, and chilling, stratified by study design and outcome. Given an *a priori* assumption of significant heterogeneity, random-effects MAs were conducted [7]. MA was conducted in Stata/IC 10.1 (StataCorp. 2007. *Stata Statistical Software: Release 10*. College Station, TX: StataCorp LP). Publication bias was assessed if a MA did not result in statistically significant heterogeneity ($P< 0.1$) and the dataset included ≥10 studies [7, 8]. Heterogeneity was assessed via a χ^2 test using the Q statistic and quantified on a relative scale using the I^2 value [8].

III. RESULTS AND DISCUSSION

Thirty-six unique citations, reporting 24, 10 and 3 before-and-after, control and challenge studies were included in the SR. Many reported multiple study designs and unique trials per citation with almost 90% conducted in North America. Sample size per trial ranged from 31-99 and ≥ 100 in 20 and 18 studies, respectively; a study often reported multiple trials with varying sample sizes. In 75.6% of studies, carcass swabs were tested for generic *E. coli* (30/37). Intervention protocols were heterogeneous. Studies of pre-evisceration interventions were fewer than post-evisceration. Final wash interventions included potable room temperature water, hot water (74-87.8^{o}C), and high and low pressures, occasionally combined with an organic acid, ozonated water or hydrogen peroxide. Pasteurization methods included steam and hot water for various lengths of time, sometimes combined with an organic acid spray. Chilling interventions included air or water spray chilling for various amounts of time (16h – 7 days), sometimes preceded by a chlorine or organic acid spray. Generic *E. coli* was investigated in 30 and pathogenic strains in 10 studies. Prevalence and concentration outcomes were reported in 26 studies and 29 studies, respectively.

Twenty-seven relevant studies were excluded because intervention or laboratory protocols were not adequately described to allow replication, and/or raw or adjusted data were not reported.

A reduced odds of generic *E. coli* carcass contamination was shown in MAs of 20 unique data subsets; results for final carcass washing (OR 0.56, CI: 0.41-0.77), pasteurization (OR 0.09, CI: 0.06-0.14) and dry chilling at 24 hours (OR 0.17, CI: 0.11-0.24). Assuming a baseline risk (ACR) of 50% of beef carcasses contaminated with *E. coli* prior to each intervention, 14, 42 and 35 carcasses per 100 will become *E. coli* negative upon final wash, carcass pasteurization and 24hr dry chill, respectively, similar to trends for concentration. Sub-grouping by specific protocol decreased heterogeneity but also MA robustness. The MA results pertain only to generic *E. coli* but experts indicate that similar results are likely for pathogenic strains [9]. Our results indicate that final wash using potable water, pasteurization with steam or hot water with or without an acid treatment, and dry chilling are effective interventions for reducing generic *E. coli* contamination of finished beef carcasses. The apparent efficacy of final wash may be influenced by initial levels of bacterial contamination.

Carcass pasteurization consistently reduced the odds of an *E. coli* contaminated carcass across all types of pasteurization. Steam pasteurization was almost as effective (RD of 38 (32-43) vs. 42 (37-45) per 100 carcasses with 50% ACR) as hot water pasteurization. Applying lactic acid further

decreased the prevalence of *E. coli* contamination vs. pasteurization alone.

Prevalence of generic *E. coli* should be low before chilling due to previous interventions. Chilling maintains rather than reduces bacterial contamination on finished carcasses [10]; however, our chilling MA indicated that the odds of detecting a positive carcass and the concentration of generic *E. coli* on contaminated carcasses were reduced except after a spray chill. Our MAs suggest that an acid rinse prior to a dry chill is more effective compared to dry chill alone; however, this should be interpreted with caution due to moderate heterogeneity (I^2 = 32.9%). Several studies reported the effect of processing interventions used in sequence reducing bacterial contamination by 3- to 4-log from pre-evisceration to completion of chilling. This hurdle effect causes interventions applied later in the chain to appear less effective, as the remaining contamination decreases. This could have affected the apparent lack of significance for the effectiveness of some interventions.

Significant heterogeneity (P <0.1) observed in 10 of 20 MA data subsets was likely due to variable intervention protocols and other study design factors (e.g. sampling methods and size of sampled area).

Publication bias was not examined in 14/20 data subsets as the tests do not perform well with < 10 trials [8]. The summary estimate was adjusted using the trim-and-fill method [11] for detecting publication bias in the final wash prevalence MA and it suggested that if five un-published studies were imputed, the pooled effect estimate would be more significant (from OR 0.56 [(0.41-0.77] to OR 0.45 [0.32-0.64]). We cannot know the extent of research that has been conducted but never published. In this case the impact of this bias was a slight alteration of the summary outcome, not changing the final conclusions.

IV. CONCLUSION

Final carcass wash, pasteurization and chilling effectively decrease the odds and concentration of generic *E. coli* contamination on beef carcasses according to SR-MA. Other interventions such as pre-chill acid rinse may only be efficacious if chilled *E. coli* prevalence is consistently higher than expected and other changes to the HACCP program have been ineffective. Pasteurization had the largest potential impact on decreasing contamination. Lack of intervention research measuring pathogenic strains of *E. coli* was identified.

Many intervention strategies are available to beef processors but they must, under government regulations, validate methods under their particular production conditions. Validation of each operation should determine the selection of intervention protocol parameters.

A lack of large controlled trials and well reported relevant intervention research was identified. Inadequate reporting of intervention and sampling protocols, measurement units and results were common, resulting in exclusion of studies. The pharmaceutical and processing industries might possess intervention efficacy data not publicly available for proprietary reasons. Data sharing could result in better global intervention knowledge and more robust MAs.

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