PURCHASED BY THE UNITED STATES
DEPARTMENT OF AGRICULTURE FOR
OFFICIAL USE

Letters in Applied Microbiology 1996, **23**, 61–63

Effectiveness of a steam-vacuum sanitizer for reducing *Escherichia coli* O157 : H7 inoculated to beef carcass surface tissue

W.J. Dorsa, C.N. Cutter and G. R. Siragusa
United States Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, Clay Center, NE, USA.

FS/295: received 12 September 1995 and accepted 19 December 1995

W.J. DORSA, C.N. CUTTER AND G.R. SIRAGUSA. 1996. A steam-vacuum sanitizer reduced aerobic plate counts associated with bovine faecal contamination from 5·5 $\log_{10}$ cfu cm^{-2} to $3{\cdot}0 \pm 0{\cdot}21$ $\log_{10}$ cfu cm^{-2} on beef carcass short plates. The same beef carcass short plates inoculated with $7{\cdot}6 \pm 0{\cdot}09$ $\log_{10}$ cfu cm^{-2} *Escherichia coli* O157 : H7 in faeces, yielded an average residual level of *E. coli* O157 : H7 of $2{\cdot}1 \pm 0{\cdot}21$ $\log_{10}$ cfu cm^{-2}, after steam-vacuum treatments. This study demonstrates the effectiveness of a steam-vacuum sanitizer for removing *E. coli* O157 : H7 from beef carcasses.

INTRODUCTION

A limited number of studies have been conducted to determine the efficacy of using hot water interventions to reduce bacterial populations from beef carcass surfaces (Patterson 1970; Barkate *et al.* 1993). In addition, several workers have determined the effectiveness of hot water interventions for reducing non-specific strains of *Escherichia coli* (Smith and Graham 1978; Davey and Smith 1989; Dorsa *et al.* 1996). These studies have shown hot water washes ranging from 72 to 96°C are effective interventions for decontaminating beef carcasses.

Recent studies by Dorsa *et al.* (1996) determined that hot water (88–94°C) delivered by a steam-vacuum sanitizing system (Vac-San®, Kentmaster Mfg, Monrovia, CA) reduced faecal bacteria and non-specific strains of *E. coli* from carcass surfaces. The United States Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) has recently approved the use of the Vac-San® system in beef processing plants. This is being done in an effort to improve the safety of beef carcasses and to reduce the amount of knife trimming required for the zero tolerance policy. At present this policy requires complete removal of any visible faeces by knife trimming. Consequently, it is important to both the meat industry and FSIS to evaluate this system's ability to remove *E. coli* O157 : H7. This study describes the effectiveness of the Vac-San® system for reducing *E. coli* O157 : H7 contamination from beef carcass short plates.

Correspondence to : Dr Warren J. Dorsa, United States Department of Agriculture, Agricultural Research Service, Roman L. Hruska U.S. Meat Animal Research Center, PO Box 166, Clay Center, NE 68933-0166, USA.
Mention of a trade name, proprietary product or specific equipment is necessary to report factually on available data ; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

© 1996 The Society for Applied Bacteriology

MATERIALS AND METHODS

All treatment applications and sampling were conducted under a biological safety hood (SterilGard Hood, The Baker Co., Inc., Sanford, ME). The Vac-San® system and its operating parameters have been previously described (Dorsa *et al.* 1996). For the present study, water sprayed onto carcass short plates by the system was maintained between 88 and 94°C.

Escherichia coli O157 : H7 (CDC #B6-914 obtained from Dr P.M. Fratamico, USDA-ARS, ERRC) cultures for the study were grown in Tryptic Soy Broth (Difco, Detroit, MI) plus 0·6% yeast extract for 18 h in a shaker incubator (Labline Instruments, Inc., Melrose Park, IL) at 35°C and 100 rev min^{-1}. The culture was harvested by centrifugation at 3500 rev min^{-1} for 10 min at 5°C (Beckman Instruments, Inc., Palo Alto, CA), washed twice in buffered peptone water (BPW; BBL, Cockeysville, MD), and re-suspended to original culture volume in BPW.

The bovine faeces used as the inoculum in the study were collected from three animals the morning of the study and a composite slurry was made by hand mixing 100 g of each faecal sample together with 300 g of sterile deionized water (1 : 1) in a sterile 1 l beaker. The faecal composite was split

into two 180 g samples and placed into separate 500 ml sterile beakers. One of the 180 g faecal samples was autoclaved. The autoclaved faeces was allowed to cool to room temperature, brought back to volume with sterile deionized water, and 20 ml of *E. coli* O157:H7 washed culture was added to the faeces.

Ten pre-rigor beef short plates (13th to the 5th rib) were cut from carcasses immediately after slaughter at a local cow/bull processing facility. Short plates were transported to the laboratory within 1 h and used for the study within 3 h of slaughter.

Five 25 cm^2 (5 cm × 5 cm) areas were marked for sampling on each short plate. The short plates were then u.v. surface sterilized for 20 min under the biological safety hood prior to inoculation with the faecal composite or the *E. coli* O157:H7 spiked faeces. Two of the marked areas on each beef short plate were inoculated with the bovine faecal composite slurry, two other areas were inoculated with sterile faeces spiked with *E. coli* O157:H7, and one area was left uninoculated. The inocolum (bovine faeces or *E. coli* O157:H7 inoculated sterile faeces) was brushed onto the appropriate marked areas with a sterile two-inch paint brush, then allowed to incubate at room temperature for 15 min.

After incubation, one 25 cm^2 area inoculated with both types of inoculum and the uninoculated area were then sampled by excision. The remaining two 25 cm^2 areas were treated using three even passes of the Vac-San® nozzle and then sampled by excision. Excised samples were individually placed in stomacher bags with 25 ml of BPW + 0·1% Tween 20 and pummeled for 2 min (Stomacher 400, Tekmar, Inc., Cincinnati, OH). Appropriate serial dilutions were done in BPW and spiral plated on Trypticase Soy Agar (TSA; faecal samples) or sorbitol MacConkey Agar (SMAC; faecal samples spiked with *E. coli*), using a Model D spiral plater (Spiral Systems Instruments, Bethesda, MD). Plates were incubated aerobically at 35°C for 48 (TSA) and 24 (SMAC) h, then enumerated and counts reported as $\log_{10}$ cfu cm^{-2}.

Analysis of all data was accomplished using the least square means of bacterial populations calculated with the General Linear Model (GLM) procedure of SAS (version 6.06.01, 1989, SAS Institute, Inc., Cary, NC). The probability level was $P < 0{\cdot}05$.

RESULTS AND DISCUSSION

The effectiveness of the Vac-San® system for removing bacterial contamination on beef carcass short plates inoculated with faeces or with *E. coli* O157:H7 in faeces was determined (Table 1). The Vac-San® system reduced aerobic plate counts from inoculation levels of 5·5 $\log_{10}$ cfu cm^{-2} to levels close to that of the bacterial levels normally found on beef carcasses being processed in a commercial plant (2·7 $\log_{10}$ cfu cm^{-2}; Anon. 1994). These residual levels are consistent with previous findings for the system when used on beef carcass short plates contaminated with faeces (Dorsa *et al.* 1996).

Table 1 Effectiveness of the Vac-San® system for removing bacteria of faecal origin and *Escherichia coli* O157:H7 in faeces from beef carcass short plates

Inoculation type	N	$\log_{10}$ cfu cm^{-2} Before Steam-Vac	After Steam-Vac	Log reduction
Faeces	10	5·5 ± 0·09	3·0 ± 0·21	2·5 ± 0·25[a]
E. coli O157:H7	10	7·6 ± 0·09	2·1 ± 0·21	5·5 ± 0·25[b]

Different superscripts denote statistical difference ($P < 0{\cdot}05$).

Results of the Vac-San® system's ability to reduce high levels of *E. coli* O157:H7 in bovine faeces from beef short plates are given in Table 1. High initial inoculation levels of 7·6 $\log_{10}$ cfu cm^{-2} experienced large $\log_{10}$ reductions of *E. coli* O157:H7.

When Cray and Moon (1995) experimentally infected adult cattle with *E. coli* O157:H7 at 10^{10}, 10^7 and 10^4 cfu g^{-1} they observed faecal shedding of the bacterium at levels of $<6{\cdot}9$ $\log_{10}$, $<5{\cdot}0 \times 10^1$ and 0 cfu g^{-1}, respectively. In a different study, Hancock *et al.* (1994) did presumptive *E. coli* O157:H7 colony screenings from sorbitol non-fermenting bacteria of rectal swabs from breeding, fat and dairy cattle, on sorbitol MacConkey agar. They observed average numbers of only 1·6 and 6·6 colonies per faecal sample depending on the season. Apparently *E. coli* O157:H7 is shed in cattle faeces at much lower levels than were used for the inoculations in the present study; however, it was the intent of the present study to observe the Vac-San® system's ability to remove inoculated *E. coli* O157:H7 in a worst-case scenario.

The present study documented the Vac-San® system's ability to reduce high levels of *E. coli* O157:H7 in a faecal menstruum on beef cattle carcass short plates by 5·5 $\log_{10}$ cfu cm^{-2}. Based on these findings, a steam-vacuuming sanitizer system should be very effective for the removal of normal levels of *E. coli* O157:H7 resulting from faecal contamination on beef carcass surfaces in processing facilities.

ACKNOWLEDGEMENTS

The authors wish to thank Mrs Jane Long, Ms Jodi Kuchera and Mrs Carole Smith for their technical support in completing this project. Thanks are also due to Mr James Wray for statistical analyses.

REFERENCES

Anon. (1994) *Nationwide Beef Microbiological Baseline Data Col-*

lection Program : Steers and Heifers. USDA-FSIS Sci. and Tech., Microbiology Division, January 1994. pp. 9–10.

Barkate, M.L., Acuff, G.R., Lucia, L.M. and Hale, D.S. (1993) Hot water decontamination of beef carcasses for reduction of initial bacterial numbers. *Meat Science* **35**, 397–401.

Cray, W.C. and Moon, H.W. (1995) Experimental infection of calves and adult cattle with *Escherichia coli* O157 : H7. *Applied and Environmental Microbiology* **61**, 1586–1590.

Davey, K.R. and Smith, M.G. (1989) A laboratory evaluation of a novel hot water cabinet for the decontamination of sides of beef. *International Journal of Food Science and Technology* **24**, 305–316.

Dorsa, W.J., Cutter, C.N., Siragusa, G.R. and Koohmaraie, M. (1996) Microbial decontamination of beef and sheep carcasses by steam, hot water spray washes, and a steam-vacuum sanitizer. *Journal of Food Protection* **59**, 127–135.

Hancock, D.D., Besser, T.E., Kinsel, M.L., Tarr, P.I., Rice, D.H. and Paros, M.G. (1994) The prevalence of *Escherichia coli* O157 : H7 in dairy and beef cattle in Washington State. *Epidemiology and Infection* **113**, 199–207.

Patterson, J.T. (1970) Hygiene in meat processing plants 4. Hot-water washing of carcasses. *Recent Agricultural Research of the Ministry of Agriculture of NI* **18**, 85–87.

Smith, M.G. and Graham, A. (1978) Destruction of *Escherichia coli* and Salmonellae on mutton carcasses by treatment with hot water. *Meat Science* **2**, 119–128.