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DISSERTATION

MICROBIOLOGICAL PROFILES OF PORK CARCASSES AND PORK VARIETY MEATS AND DECONTAMINATION TECHNOLOGIES FOR PORK VARIETY MEATS

Submitted by

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Department of Animal Sciences

In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

Fall 1999

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COLORADO STATE UNIVERSITY

November 2, 1999

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY HENRY N. ZERBY ENTITLED "MICROBIOLOGICAL PROFILES OF PORK CARCASSES AND PORK VARIETY MEATS AND DECONTAMINATION TECHNOLOGIES FOR PORK VARIETY MEATS" BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

Co-Adviser

Co-Adviser/Department Head

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ABSTRACT OF DISSERTATION

MICROBIOLOGICAL PROFILES OF PORK CARCASSES AND PORK VARIETY MEATS AND DECONTAMINATION TECHNOLOGIES FOR PORK VARIETY MEATS

Experiments were designed and conducted to provide packers, producers, other members of the swine/pork industry and regulatory agencies with information about the microbiological status of pork carcasses and pork variety meats. Collectively, the results offer a snapshot of the occurrence of bacteria on pork carcasses and variety meats collected during only a brief period of time, but provide baseline information for identifying where the industry is and where it should be going in its quest to improve the wholesomeness of products for consumers.

Experiment 1 developed microbiological baseline information for pork carcasses using a 3-site sponge sampling protocol. Experiment 2 compared a 3-site sponge sampling protocol with a 2-site sponge sampling protocol as a means for quantifying and characterizing microbiological populations on pork carcasses. Experiment 3 developed microbiological baseline information for 11 pork variety meats (cheek meat, salivary glands, tongues, livers, hearts, kidneys, stomachs, chitterlings, bungs and front feet). Experiment 4 evaluated the effectiveness of 10 decontamination technologies for their ability to reduce bacterial counts on pork variety meats. Experiment 5 evaluated the effectiveness of 7 decontamination technologies for their ability to reduce incidence of

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Salmonella spp., *Listeria monocytogenes* and *Yersinia enterocolitica* on pork variety meats.

Results from experiment 1 demonstrated that *C. jejuni/coli*, with an incidence of 7.9%, was the most commonly found pathogen (of those tested) on pork carcasses in the cooler, followed by *L. monocytogenes* (5.0%), *Salmonella* spp. (4.6%) and *Y. enterocolitica* (0.9%). Mean (log CFU/cm^2) APC, TCC and ECC were generally lower ($P > 0.05$) during the winter, when compared to the summer season.

The results of experiment 2 indicated that mean (log CFU/cm^2) TCC and ECC did not differ ($P > 0.05$) between the 3-site sponge sampling protocol and the 2-site sponge sampling protocol at pre-wash, final wash or cooler sampling sites. However, the 2-site sponge sampling protocol was not as effective as the 3-site sponge sampling protocol in detecting *Salmonella* spp. In the cooler, the 2-site sponge sampling protocol only detected *Salmonella* spp. at a rate of 2.1% which was less than half of the 4.6% incidence detected using the 3-site sponge sampling protocol.

Results of experiment 3 indicated that general microbiological contamination (APC) was relatively high, for most variety meats sampled, with a mean APC ranging between > 4 log CFU/g to < 7 log CFU/g (hearts were the exception, having a mean APC of 3.4 log CFU/g). Mean TCC and ECC ranged between 1.7 to 4.6 and 1.1 to 4.3 log CFU/g, respectively. There were no positive samples of *Y. enterocolitica* detected on variety meat samples. Less than 1% (4 out of 405) of the samples were positive for *C. jejuni/coli*. For *Salmonella* spp., 15% of the samples were positive and a greater number of positive samples were associated with the head (cheek meat, head meat, salivary gland and tongue) and intestinal tract (chitterlings and bung) than with other types of variety

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meats. *Listeria monocytogenes* occurred on 16% of samples and the highest incidence rates were associated with variety meat products from the head, the stomach and the front feet.

Results from experiment 4 indicated that larger reductions in APC, TCC and ECC on pork variety meats were obtained using trisodium phosphate (12%), acetic acid (2%) and lactic acid (2%) decontamination interventions. Treatments such as hot water (75 to 80°C) or steam resulted in discoloration of red variety meat products due to high temperatures. The hydrogen peroxide (5%) treatment slightly discolored red variety meat products and produced a foam that was undesirable for working/packaging conditions.

Results from experiment 5 indicated that lactic acid (2%) immersion was the most successful and consistent decontamination intervention treatment for reducing *Salmonella*, *L. monocytogenes* and *Y. enterocolitica* counts on variety meats tested. Additionally, a 10 sec application of lactic acid (2%) or acetic acid (2%) was more effective than a 5 sec application in reducing *Salmonella*, *L. monocytogenes* and *Y. enterocolitica*.

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DEDICATION

This dissertation is dedicated to my parents Nevin and Isabelle for their never-ending support and instilling in me a love for animal husbandry and a sense of humor that allows me to work in the field of agriculture. The courage that both have shown over the past years while fighting cancer have allowed me to realize what is truly important in life.

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TABLE OF CONTENTS

Chapter | Page

ABSTRACT ... iii

ACKNOWLEDGEMENTS ... vi

DEDICATION ... viii

TABLE OF CONTENTS ... ix

LIST OF TABLES ... xiii

LIST OF FIGURES ... xv

I. OBJECTIVES OF DISSERTATION ... 1

II. REVIEW OF LITERATURE ... 3
- Introduction ... 3
- Possible Contamination of Pork and Pork Variety Meats During Slaughter ... 4
  - *Salmonella* spp ... 5
  - *Campylobacter jejuni/coli* ... 7
  - *Listeria monocytogenes* ... 7
  - *Yersinia enterocolitica* ... 8
  - Carcass contamination during slaughter ... 9
- Microbiology of Pork Carcasses ... 11
- Microbiology of Pork Variety Meats ... 12
- Microbiological Decontamination Technologies ... 15

III. LEVELS OF MICROBIOLOGICAL CONTAMINATION OF PORK CARCASSES DURING SLAUGHTER ... 18
- Abstract ... 18
- Introduction ... 19
- Materials and Methods ... 20
  - Carcass sampling ... 23
  - Microbiological analysis ... 25
  - Statistical analysis ... 25
- Results and Discussion ... 26
  - Incidence of Pathogens ... 26

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Chapter Page

Salmonella spp. 28
Campylobacter jejuni/coli 30
Listeria monocytogenes 31
Yersinia enterocolitica 32
Microbiological Plate Counts 32
Statistical process control 35
Summary 39

IV. MICROBIAL CONTAMINATION RECOVERED BY 2-SITE VERSUS 3-SITE PORK CARCASS SAMPLING PROTOCOLS 40
Abstract 40
Introduction 41
Materials and Methods 43
Study design 43
Carcass sampling 44
Microbiological analysis 45
Statistical analysis 47
Results and Discussion 47
Incidence of *Salmonella* spp. 47
Microbiological populations 48
Summary 52

V. MICROBIOLOGICAL CONTAMINATION ON PORK VARIETY MEATS 53
Abstract 53
Introduction 54
Materials and Methods 55
Pork variety meat sampling 55
Microbiological analysis 57
Observations concerning in-plant sanitation and handling practices 58
Statistical analysis 58
Results and Discussion 59
Extent of microbiological contamination 59
Incidence of pathogenic organisms 62
Observations concerning in-plant sanitation and handling practices 65
Product transfer chutes 65
Fresh product holding time 66
Personal equipment sanitation 66
Knife sanitizers 67
Cross-contamination 68
Pallet spacing and freezers 68
Weighing 69

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Chapter | Page

Rework.....69
Hand inspection.....70
Handling.....70
Work areas.....71
Clean-up procedures.....72
Summary.....72

VI. APPLICATION OF TECHNOLOGIES TO REDUCE CONTAMINATION OF PORK VARIETY MEATS.....74
Abstract.....74
Introduction.....75
Materials and Methods.....76
Pork variety meat and sample size.....76
Decontamination treatments.....77
Microbiological analysis.....79
Statistical analysis.....79
Results and Discussion.....80
Study I.....80
Study II.....91
Summary.....93

VII. APPLICATION OF DECONTAMINATION TECHNOLOGIES TO REDUCE INOCULATED *SALMONELLA*, *LISTERIA MONOCYTOGENES*, AND *YERSINIA ENTEROCOLITICA* ON PORK VARIETY MEATS.....95
Abstract.....95
Introduction.....96
Materials and Methods.....97
Pork variety meat and sample size.....97
Bacterial cultures.....98
Variety meat inoculation.....99
Decontamination treatments.....99
Microbiological analysis.....100
Statistical analysis.....102
Results and Discussion.....102
Summary.....126

LITERATURE CITED.....128

APPENDIX I.....133

APPENDIX II.....141

APPENDIX III.....147

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Chapter | Page

APPENDIX IV 159

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LIST OF TABLES

Table | Page

Table 2.1 Origin and pH and temperature growth ranges for selected pathogenic bacteria. All values are approximate because the combinations of environmental factors have a substantive influence on the growth and survival of pathogens (modified from Borch *et al.*, 1996) 6

Table 2.2 Possible areas of contamination for hog carcasses during slaughter (modified from Berends *et al.*, 1997) 10

Table 3.1 Initial buffer used to hydrate sponges for sampling and the second buffer added to prepare sponges for shipment to the laboratory for each type of bacteria 22

Table 3.2 Incidence of *Salmonella* spp., *Campylobacter jejuni/coli*, *Yersinia* spp., *Yersinia enterocolitica*, *Listeria* spp. and *Listeria monocytogenes* presented by season and sampling location (across 12 plants; two plants used hot-boning so cooler samples were only collected in 10 plants) 27

Table 3.3 Origin and pH and temperature growth ranges for selected pathogenic bacteria. All values are approximate because the combinations of environmental factors have a substantive influence on the growth and survival of pathogens (modified from Borch *et al.*, 1996) 29

Table 4.1 Ranking of sampling sites on pork carcasses by total plate count (modified from Roberts *et al.*, 1980) 42

Table 4.2 Incidence of *Salmonella* spp. on pork carcasses at pre-wash, final wash and cooler locations. The carcasses were sampled using a 2- or 3-site sampling protocol 49

Table 4.3 Incidence of *Salmonella* spp. across plants[a] for carcasses sampled using a 2- or 3-site sampling protocol 50

Table 5.1 Mean, minimum and maximum aerobic plate counts, total coliform counts and *Escherichia coli* counts for 11 types of pork variety meats 60

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Table | Page

Table 5.2 Incidence of pathogens on pork variety meats at sampling site B (the point at which each variety meat was suitably chilled and deemed ready for domestic distribution or export; a minimum of 20 h in the freezer or chiller after site A) 63

Table 6.1 Reductions of aerobic plate counts on uninoculated pork variety meats resulting from the application of various decontamination treatments 88

Table 6.2 Reductions of total coliform counts on uninoculated pork variety meats resulting from the application of various decontamination treatments 89

Table 6.3 Reductions of *Escherichia coli* counts on uninoculated pork variety meats resulting from the application of various decontamination treatments 90

Table 7.1 Reductions of inoculated rifampicin-resistant *Salmonella* on pork variety meats resulting from the application of various decontamination treatments 112

Table 7.2 Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on pork variety meats resulting from the application of various decontamination treatments 113

Table 7.3 Reductions of inoculated rifampicin-resistant *Yersinia enterocolitica* on pork variety meats resulting from the application of various decontamination treatments 114

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LIST OF FIGURES

Figure		Page
Figure 3.1	3-site sponge sampling protocol for hog carcasses	24
Figure 3.2	Mean (log CFU/cm^2) aerobic plate counts, total coliform counts and E. coli counts obtained from hog carcasses tested at pre-wash, final wash, and cooler locations, presented by season. The season x sampling location interaction was significant for APC ($P < 0.05$); and season and sampling location main effects were significant for TCC and ECC ($P < 0.05$); means bearing a common superscript letter are not different ($P > 0.05$).	33
Figure 3.3	Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler (data from all plants and seasons combined; data collected using the 3-site sponge sampling protocol)	36
Figure 3.4	Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler during the summer season (combined data from all plants; data collected using the 3-site sponge sampling protocol)	37
Figure 3.5	Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler during the winter season (combined data from all plants; data collected using the 3-site sponge sampling protocol)	38
Figure 4.1	Sampling protocols for 2-site and 3-site sponge surface swabbing of hog carcasses	46
Figure 4.2	Mean values (log CFU/cm^2) comparing the 3-site and 2-site sponge sampling protocols using total coliform count and *E. coli* count obtained from hog carcasses at the pre-wash, final wash, and cooler locations, presented by season	51

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Figure	Page

Figure 6.1 Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork cheek meat. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were - CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion). [abcd]Means bearing a common superscript letter within bacteria type are not different ($P > 0.05$). 81

Figure 6.2 Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork heart. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were - CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion). [abcd]Means bearing a common superscript letter within bacteria type are not different ($P > 0.05$). 82

Figure 6.3 Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork liver. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were - CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion). [abcd]Means bearing a common superscript letter within bacteria type are not different ($P > 0.05$). 83

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Figure | Page

Figure 6.4 Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork salivary glands. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were - CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion). [abcd]Means bearing a common superscript letter within bacteria type are not different ($P > 0.05$). 84

Figure 6.5 Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork chitterlings. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were - CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LADP, lactic acid (2%, 48-50°C, 10 sec immersion). [abcd]Means bearing a common superscript letter within bacteria type are not different ($P > 0.05$). 85

Figure 6.6 Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork stomach. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were - CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion); H2O2DP, hydrogen peroxide (5%, 48-50°C,10 sec immersion). [abcd]Means bearing a common superscript letter within bacteria type are not different ($P > 0.05$). 86

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Figure Page

Figure 6.7 Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork tongue. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were - CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion); H2O2DP, hydrogen peroxide (5%, 48-50°C,10 sec immersion). ^{abcd}Means bearing a common superscript letter within bacteria type are not different (P > 0.05). 87

Figure 6.8 Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork tongue. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were - CNTR, control (initial contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); ASCDP, acidified sodium chlorite (1200ppm, 26°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion). ^{abcd}Means bearing a common superscript letter within bacteria type are not different (P > 0.05). 92

Figure 7.1 Reductions of inoculated rifampicin-resistant *Salmonella* on cheek meat resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). ^{abc,yz}Means within an agar type bearing common superscript letters are not different (P > 0.05). 106

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Figure | Page

Figure 7.2 Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on cheek meat resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). [abc, yz]Means within an agar type bearing common superscript letters are not different (P > 0.05)... 107

Figure 7.3 Reductions of inoculated rifampicin-resistant *Yersinia enterocolitica* on cheek meat resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and CIN+RIF (Cefsulodin-Irgasan-Novobiocinagar with 100 mg/ml rifampicin). [abc, yz]Means within an agar type bearing common superscript letters are not different (P > 0.05).. 108

Figure 7.4 Reductions of inoculated rifampicin-resistant *Salmonella* on heart resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). [abcd, xyz]Means within an agar type bearing common superscript letters are not different (P > 0.05).. 109

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Figure | Page

Figure 7.5 Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on heart resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). [abc, wyz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$)........................110

Figure 7.6 Reductions of inoculated rifampicin-resistant *Salmonella* on liver resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). [ab, yz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$)..........111

Figure 7.7 Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on liver resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). [abc, xyz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).........................112

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Figure | Page

Figure 7.8 Reductions of inoculated rifampicin-resistant *Salmonella* on salivary glands resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). $^{abc, yz}$Means within an agar type bearing common superscript letters are not different (P > 0.05).......... 113

Figure 7.9 Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on salivary glands resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). $^{abc, xyz}$Means within an agar type bearing common superscript letters are not different (P > 0.05). 114

Figure 7.10 Reductions of inoculated rifampicin-resistant *Yersinia enterocolitica* on salivary glands resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and CIN+RIF (Cefsulodin-Irgasan-Novobiocinagar with 100 mg/ml rifampicin). $^{abc, yz}$Means within an agar type bearing common superscript letters are not different (P > 0.05)... 115

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Figure | Page

Figure 7.11 Reductions of inoculated rifampicin-resistant *Salmonella* on chitterlings resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). $^{abcd, xyz}$Means within an agar type bearing common superscript letters are not different (P > 0.05)... 116

Figure 7.12 Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on chitterlings resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). $^{abc, wxyz}$Means within an agar type bearing common superscript letters are not different (P > 0.05). 117

Figure 7.13 Reductions of inoculated rifampicin-resistant *Yersinia enterocolitica* on chitterlings resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and CIN+RIF (Cefsulodin-Irgasan-Novobiocinagar with 100 mg/ml rifampicin). $^{ab, yz}$Means within an agar type bearing common superscript letters are not different (P > 0.05)..118

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Figure | Page

Figure 7.14 Reductions of inoculated rifampicin-resistant *Salmonella* on stomach resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); H2ODP, hot water (75-80°C, 10 sec immersion); H2O2DP, hydrogen peroxide (5%, 48-50°C, 5 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). [abcd, yz]Means within an agar type bearing common superscript letters are not different (P > 0.05)... 119

Figure 7.15 Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on stomach resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); H2ODP, hot water (75-80°C, 10 sec immersion); H2O2DP, hydrogen peroxide (5%, 48-50°C, 5 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). [abcd, xyz]Means within an agar type bearing common superscript letters are not different (P > 0.05)... 120

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Figure | Page

Figure 7.16 Reductions of inoculated rifampicin-resistant *Salmonella* on tongue resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). ^{abcd,yz}Means within an agar type bearing common superscript letters are not different (P > 0.05)... 121

Figure 7.17 Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on tongue resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). ^{abcd,vwxyz}Means within an agar type bearing common superscript letters are not different (P > 0.05). 122

Figure 7.18 Reductions of inoculated rifampicin-resistant *Yersinia enterocolitica* on tongue resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and CIN+RIF (Cefsulodin-Irgasan-Novobiocinagar with 100 mg/ml rifampicin). ^{ab,yz}Means within an agar type bearing common superscript letters are not different (P > 0.05)...123

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CHAPTER I

OBJECTIVES OF DISSERTATION

The objectives of the studies reported in this dissertation were as follows:

Overall Objective

To establish baseline contamination levels for aerobic plate counts (APC), total coliform counts (TCC) and *Escherichia coli* counts (ECC) and incidence of *Salmonella* spp., *Listeria monocytogenes, Campylobacter jejuni/coli* and *Yersinia enterocolitica* on pork carcasses and pork variety meats, and to determine the efficacy of various decontamination technologies applied to pork variety meats to reduce bacterial contamination.

Specific Objectives

1. To determine the incidence of selected pathogenic bacteria (*Salmonella* spp., *L. monocytogenes, C. jejuni/coli* and *Y. enterocolitica*) and populations of APC, TCC and ECC on pork carcasses in several swine processing systems currently used in U.S. slaughtering plants.

2. To compare bacterial counts and incidence of *Salmonella* spp. on samples obtained using a 3-site (ham, belly and jowl) sponge swabbing protocol with a 2-site (belly and jowl) sponge swabbing protocol.

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3. To determine the incidence of selected pathogenic bacteria (*Salmonella* spp., *L. monocytogenes, C. jejuni/coli* and *Y. enterocolitica*) and populations of APC, TCC and ECC on 11 pork variety meats (head meat, cheek meat, salivary glands, tongues, livers, hearts, kidneys, stomachs, chitterlings, bungs and front feet).

4. Evaluate decontamination techniques for potential use in pork packing plants to reduce APC, TCC, ECC and to extend the storage life of pork variety meats.

5. Evaluate decontamination techniques for potential use in pork packing plants to reduce pathogen presence and to extend the storage life of pork variety meats.

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CHAPTER II

REVIEW OF LITERATURE

INTRODUCTION

The level of microbiological contamination on meat products has always been a concern. However, it was a concern because fresh meat would spoil before it was consumed due to lack of refrigeration. Now that technology allows us to store fresh meat for days, weeks and even months, microbiological contamination has presented the meat industry with new challenges. Meat spoilage is still a major concern, but, presence of pathogenic bacteria and associated food safety risk has become a growing issue.

Because of major *Escherichia coli* O157:H7 outbreaks that resulted in loss of human life in the U.S. during January of 1993 and in Japan during the summer of 1996, and more recently with *L. monocytogenes* in cooked meat products, significant public attention around the world is now focused on the incidence of foodborne illness. The focus on food safety within all sectors of agricultural food production has intensified. The news media have targeted food safety as an issue that generates substantial consumer interest; thus, a story about potential consumer risk can attract a large audience. More people than ever before are aware of events occurring in the food-safety arena. As a result, U.S. society is conscious of changes that are occurring and that are designed to enhance food safety programs. The implementation of Hazard Analysis and Critical

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Control Points (HACCP) and new developments identified by research in the area of food safety, have been positively portrayed to the public.

Controlling microbiological contamination on pork products enhances their safety and consequently the public perception of pork in the market place. In addition, reducing microbiological contamination can improve shelf-life and retail caselife. Desire to increase fresh pork sales domestically and abroad have increased the need for greater shelf-life and retail caselife. Initial microbial levels and storage conditions (such as temperature and type of packaging) are the most important factors affecting microbial growth which contributes to spoilage and potential health problems (Cannon *et al.*, 1995).

POSSIBLE CONTAMINATION OF PORK AND PORK VARIETY MEATS DURING SLAUGHTER

There are several opportunities for bacteria to contaminate the surface of a pork carcass during slaughter. Berends *et al.* (1998) suggest that, in current industry practices, if contaminated carcasses are entering the fabrication floor to be processed into subprimals and smaller pork products, interim cleaning and disinfection of surfaces and utensils during breaks and at the end of the working day will only prevent about 10% of all cross-contamination that takes place during a working day. Thus, about 90% of the cross-contamination from contaminated carcasses may be unavoidable. Therefore, there is a need to develop and apply Good Manufacturing Practices (GMPs), Standard Operating Procedures (SOPs) and HACCP plans must be designed with the intent of reducing contamination on carcasses before fabrication.

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In order to reduce the potential of carcasses contaminated with pathogenic bacteria from entering the fabrication floor, some information about the processes that take place during slaughter and the source and method of distribution of potential pathogens must be taken into consideration. The major source of *Salmonella* spp., *C. jejuni/coli* and *Y. enterocolitica* contamination on carcasses is the pig, whereas the major source of *L. monocytogenes* contamination on carcasses is from the environment or from within the plant itself (Borch *et al.*, 1996; Wesley and Johnson, 1998). However, the plant may also harbor *Salmonella* spp., *C. jejuni/coli* and *Y. enterocolitica* and conversely the pig may initially introduce *L. monocytogenes* into the plant.

Salmonella spp.

Healthy pigs may be carriers of *Salmonella* spp. (gram negative rods) and can serve as a source of *Salmonella* spp. contamination on pork carcasses (Borch *et al.*, 1996), but, *Salmonella* spp. also can establish themselves in the plant and contaminate carcasses. Berends *et al.* (1997) reported pigs carrying *Salmonella* spp. were 3 to 4 times more likely to have the organism on their carcasses than are *Salmonella*-free pigs. In their study, Berends *et al.* (1997) reported that 5 to 30% of the carcasses were contaminated with *Salmonella* spp., and about 70% of those carcasses that were contaminated with *Salmonella* spp. were contaminated from the animal itself (the remaining 30% of positives were a result of cross-contamination from other positive carcasses). Pork was identified as the source of seven outbreaks of Salmonellosis between 1988 and 1992 (CDC, 1996a). From 1996 through September, 1999 three recalls of pork products were related to *Salmonella* (all three were in 1999; FSIS, 1999c).

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Table 2.1. Origin and pH and temperature growth ranges for selected pathogenic bacteria. All values are approximate because the combinations of environmental factors have a substantive influence on the growth and survival of pathogens (modified from Borch *et al.*, 1996)

Bacteria	Origin[a]	Growth-range	
		pH	Temperature (°C)
Campylobacter spp.	Pig Environment	4.9 to >8.0	30 to 47
Listeria monocytogenes	Pig Environment	4.5 to 9.6	1 to 45
Salmonella spp.	Pig Environment	4.5 to 9.0	5 to 47
Yersinia enterocolitica	Pig Environment	4.2 to 9.0	-2 to 42

[a]Major contamination source is underlined

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Campylobacter jejuni/coli

Campylobacter enteritis is most frequently caused by *C. jejuni* and, to a lesser extent, by *C. coli* (Stern and Kazmi, 1989). *Campylobacter* spp. are usually found in the intestinal tract of pigs in higher numbers than are *Salmonella* spp. or *Yersinia* spp. (Borch *et al*, 1996). *Campylobacter jejuni/coli* are gram negative rods that have microaerophilic characteristics (they require 3 to 5% oxygen and 2 to 10% carbon dioxide for optimal growth conditions; FDA, 1999). *Campylobacter* is reputed to be the most common cause of bacterially-induced diarrheal illness in the U.S., and of the various species, 99% of cases are caused by *C. jejuni* (CDC, 1997).

Borch *et al.* (1996) reported a reduction in the number of positive samples detected in the cooler in comparison to the number of positive samples detected on carcasses before chilling. Borch *et al.* (1996) attributed the reduction in positive samples of *Campylobacter* spp. in the cooler to the fact that *Campylobacter* spp. are microaerophilic organisms, do not grow at temperatures below 30°C, have a low heat resistance, and are sensitive to drying and freezing. The conclusions of Borch *et al.* (1996) support the use of snap chilling as a means of reducing incidence of *C. jejuni/coli* on pork carcasses.

Listeria monocytogenes

Listeria monocytogenes is a gram positive organism. In humans *L. monocytogenes* can cause meningitis, septicemia and abortions and has a high mortality rate -- 20 to 30% of those infected (Mortimore and Wallace, 1994). *Listeria monocytogenes* is normally found in soil and water, but can be carried by animals. Because of its pervasiveness, the organism is constantly re-introduced into the plant

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environment (Tompkin *et al.*, 1999) and re-contamination of meat products with *L. monocytogenes* is common (CDC, 1996b). *Listeria monocytogenes* can survive in processing facilities (e.g., floor drains) for long periods of time and it can form a biofilm on surfaces such as stainless steel, glass and rubber that is resistant to sanitizing agents (Herald and Zottola, 1988; Frank and Koffi, 1990). *Listeria monocytogenes* was associated with 13 recalls of pork products from January, 1999 through September, 1999 (FSIS, 1999c). In a recent study by Duffy *et al.* (1999), *L. monocytogenes* was found on 26.7% of pork retail samples collected from stores located in six U.S. cities.

Yersinia enterocolitica

Yersinia enterocolitica is a gram negative rod and the causative agent of the human foodborne illness yersiniosis. Healthy pigs have been found to be infected with *Y. enterocolitica* and are considered to be the most important source of pathogenic serotypes (Kapperud, 1991; Anderson, 1988; Borch *et al.*, 1996; Wesley and Johnson, 1998). *Yersinia enterocolitica* infections, associated with consumption of pork chitterlings, were ultimately blamed for an outbreak in 1988 that infected several infants in Georgia (CDC, 1996a). In a study conducted by de Boer and Nouws (1991), pathogenic *Y. enterocolitica* strains were isolated from 36 (42%) of 86 porcine tonsils, 8 (20%) of 40 tongues and 17 (17%) of 100 rectal swabs, but was not detected in 20 samples of head meat or in 210 carcasses. The contamination of carcasses, though at a lower rate, seemed to stem from cross-contamination from the feces/intestinal contents or tonsillary region. Funk *et al.* (1998) tested 103 lots of slaughter hogs for *Y. enterocolitica* by swabbing the oral pharyngeal surface on carcasses before scalding. They found that 92.2% of the lots contained at least one hog carcass that was positive for *Y. enterocolitica*. They also

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classified the strains of *Y. enterocolitica* that harbored the *ail* gene as pathogenic strains and reported that 28.2% of the 103 lots had at least one hog that was carrying a pathogenic strain of *Y. enterocolitica.*

Carcass contamination during slaughter

There are several steps during slaughter where pathogens can contaminate carcasses (Table 2.2). The contamination around the stick wound is generally of little importance (Borch *et al.*, 1996). However, if the scald water enters the pharynx and lungs then contamination of the pluck and cross-contamination from the pluck may be of concern. The scalding water is generally 60 to 61.1°C and the carcasses are submersed in the scalding water for approximately 7 minutes. Dehairing machines can increase contamination, especially by spreading fecal matter from carcass to carcass (Borch *et al.*, 1996). Gill and Bryant (1993) isolated *Escherichia coli, Campylobacter* and *Salmonella* spp. from dehairing machines and also then detected the aforementioned bacteria on carcasses that passed through the machines. Singeing has been found to decrease the amount of contamination on a carcass, but does not eliminate microbiological contamination (Gill and Bryant, 1993). Polishing contributes to the spread of bacteria that survive the singeing process and the bacteria may become established on equipment brushes as they are difficult to clean and disinfect (Borch *et al.*, 1996). Nerbrink and Borch (1989) found that levels of microbial contamination sometimes increase on carcasses as a result of the polishing process and Berends *et al.* (1997) reported that 5 to 15% of all carcass contamination with *Salmonella* spp. occurs during polishing.

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Table 2.2. Possible areas of contamination for hog carcasses during slaughter (modified from Berends *et al.*, 1997)

Process step	Source of possible contamination
Transportation	Contamination from other animals and from the trucks
Stunning	
Sticking/shackling	Contamination from knife and equipment
Scalding	Possible contamination of the pluck
Dehairing	Contamination from dehairing equipment
Singeing	
Polishing	Contamination from polishing equipment
Evisceration	Contamination from digestive or intestinal tracts and also from dirty knives or gloves
Splitting	Contamination from splitting/saw
Zero tolerance inspection	Contamination from palpating or handling with dirty gloves/hands
Final wash	Contamination from water or cross-contamination from adjacent dirty carcasses

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During the bunging and evisceration process, there is a risk of contaminating the carcass with ingesta or fecal material if the digestive or intestinal tract is ruptured or cut. Additionally, there is risk of contaminating the pluck from dirty gloves or hands, during the manual process of removing it. The splitting process starts by sawing through the rectal opening and continuing down the spine. During this process, the blade could be contaminated by any contamination present around the rectal area. As the carcass is split, the blade splatters fragments over several areas of the carcass and may potentially cross-contaminate these areas.

Offal or variety meat items also have a high risk of cross contamination. This is partially due to the fact that there is a lot of manual handling of the products to remove them from the carcass and separate them from each other. It is also because variety meats are typically transported through slides or chutes and then stored in lugs or bins where they have an opportunity to cross-contaminate each other. In addition, processing and packaging of these products is often delayed.

MICROBIOLOGY OF PORK CARCASSES

There are numerous studies that indicate some level of contamination or lack of cleanliness of pork carcasses, however most are either from other countries, where the rearing and slaughter practices may be different than are those in the U.S. (slower chain speeds and much lighter carcass weights), or from studies of very small groups of hogs and from only one or two sources. The most conclusive information on the microbiology of hog carcasses was reported by the Food Safety Inspection Service (FSIS, 1996b) in the Nationwide Pork Microbiological Baseline Data Collection Program: Market Hogs (April

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1995 - March 1996). In that study, tissue samples were collected from 2,112 market hogs. The establishments that participated in the testing were responsible for approximately 99.5% of all market hogs slaughtered in the U.S. *Clostridium perfringens*, *Staphylococcus*, *L. monocytogenes*, *C. jejuni/coli* and *Salmonella* spp. were recovered from 10.4%, 16.0%, 7.4%, 31.5% and 8.7% of the tissue samples, respectively. *Escherichia coli* O157:H7 was not recovered from any of the tissue samples in that baseline study. For aerobic plate counts (APC), 91.6% of the samples were below 10^5 colony forming units (CFU) per cm^2, and 84.2% of the samples were less than 10^2 for coliform counts. Results for *Escherichia coli* (Biotype I) counts indicated that 80% of the samples had less than 10 CFU/cm^2 (which corresponds with "m" for *E. coli* performance testing criteria; FSIS, 1996a) and 98.4% had less than 10,000 CFU/cm^2 (which corresponds with "M" for *E. coli* performance testing criteria; FSIS, 1996a). It is important to note that these data were collected from excised tissue samples and that at the present time, most pork plants have adopted sponge swabbing sampling protocols. Data from the FSIS One Year Progress Report on *Salmonella* Testing for Raw Meat and Poultry Products indicated that the incidence of *Salmonella* from January 26, 1998 through January 25, 1999 was 6.5% (FSIS, 1999b); these data were collected using the sponge sampling protocols.

MICROBIOLOGY OF PORK VARIETY MEATS

Unfortunately, pork variety meats are often an afterthought in the overall scope of pork production. Traditionally, variety meats have been considered to have poor microbiological quality and a short shelf-life, particularly when they are distributed as

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chilled products (Gill and Jones, 1992). Contamination and perishability of pork variety meats must be addressed if consumers are to be assured that pork, in general, is safe and of high quality. There is a limited amount of research literature that directly refers to pork variety meat safety or cleanliness, with the possible exception being the pork liver. Gardner (1971) reported that initial APC on fresh livers ranged between 10^4 and 10^5 CFU/cm^2 and, after 7 days of refrigerated storage at 5°C, APC were between 10^8 and 10^9 CFU/cm^2. Caspar *et al.* (1984) reported that pork livers obtained from slaughter houses and retail outlets in the Netherlands had APC of 6.0 to 6.5 log CFU/cm^2 and ECC of 4.0 to 5.0 log CFU/cm^2 at both locations. Oblinger *et al.* (1982) reported that pork livers had initial APC counts between 3 to 3.5 log CFU/cm^2. Hanna *et al.* (1982b) reported that the APC of fresh livers, hearts and kidneys were less than 10^4 CFU/cm^2, and often less than 10^3 CFU/cm^2. After frozen storage for 5 days at -20°C, there were no significant changes in APC for pork livers, hearts and kidneys, but when the products were subjected to temperature abuse for 6 to 12 h at 30°C, there were large significant increases in microbiological counts (Hanna *et al.*, 1982b). Additionally, Hanna *et al.* (1982b) reported that microbiological counts increased during the thawing of frozen products at 25°C for 24 to 48 h. Hanna *et al.* (1982a) reported that pork livers that were stored for up to 14 days at 2°C had lower increases in APC if they were vacuum packaged (APC increased from 2.1 to 5.0 log CFU/cm^2) than pork livers that were wrapped in PVC film (APC increased from 2.5 to 6.4 log CFU/cm^2).

Caspar *et al.* (1984) reported the incidence of *Salmonella* spp. on pork livers at 4.2% and 0.0% for samples collected from slaughter houses during the winter and summer respectively, and 4.4% and 15.4% on pork liver samples collected from retail

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outlets for winter and summer, respectively. Sinell *et al.* (1984) conducted a study in Germany and reported a 72% incidence of *Salmonella* spp. on fresh pork livers from Germany, 29.3% incidence on frozen pork livers from Denmark and Italy, and a 77.8% incidence on pork hearts. Frederick *et al.* (1994) reported that pork cheek meat had a 30% incidence of *Salmonella* spp. and had APC that ranged from 4.1 to 4.6 log CFU/cm^2 and TCC of 2.3 to 2.4 log CFU/cm^2. Laubach *et al.* (1998) reported that pork head meat stored at 3 ±1°C had APC that were consistently in the range of 4 to 5 log CFU/g, TCC of 2 to 3 log CFU/g, ECC of 1.5 to 2.5 CFU/g and a 38% incidence of *Salmonella* spp.

An outbreak of gastroenteritis due to *Y. enterocolitica* serotype O:3 in Atlanta, GA, USA in 1988 was traced to pork chitterlings. During epidemiological investigations of the outbreak, *Y. enterocolitica* was isolated from 3 of 4 unopened containers of chitterlings from one of the case households and from 5 of 11 containers purchased from Atlanta stores that had been the source of chitterlings for some case households (Lee *et al.*, 1990). The eight contaminated containers came from pigs slaughtered in three different regions of the U.S.

Variety meats offered for human consumption often are of poor microbiological quality (Gill and Jones, 1992). High levels of contamination pose a risk to people who consume the product, and increase the potential for cross-contamination from variety meats to other products during processing and preparation in household or food establishments. Caspar *et al.* (1984) stated that microbiological quality of pork livers (presumably this would pertain to all pork variety meats) was dependent on hygienic practices during slaughter, processing as well as storage conditions. The rate of cooling for variety meats is one of the most important factors in preventing high bacterial counts

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(Sheridan and Lynch, 1988; Gill 1988). Laubach *et al.* (1998) explored the idea of altering harvesting practices of pork head meat to reduce microbiological counts and the incidence of *Salmonella*. However, simply changing boning practices was not effective and they concluded that the use of interventions approved for animal carcasses should be investigated to produce variety meat products with lower levels of microbiological contamination.

MICROBIOLOGICAL DECONTAMINATION TECHNOLOGIES

Several decontamination technologies have been evaluated for reducing microbiological levels on the surfaces of carcasses and meat products. These decontamination technologies include: carcass washing with water of various temperatures and pressures, carcass sprays with organic acids (lactic, acetic, citric, propionic, benzoic and sorbic), chlorine solutions, trisodium phosphate solutions, acidified sodium chlorite, steam vacuuming, steam pasteurization, high hydrostatic pressure, sonication pulsed light, pulsed electric fields, chemical dehairing and knife trimming (Ouattara *et al.*, 1997; Sofos and Smith, 1998; Smulders and Greer, 1998; Castillo *et al.*, 1999). Decontamination appears to be most effective when products or carcasses are treated as early as possible in the production system because less bacteria will have established attachment (Caspar *et al.*, 1984; Sofos and Smith, 1998).

Few decontamination technologies have been tested for their ability to reduce microbiological levels on pork variety meats. This is partially due to pork variety meats being of lesser economic value than other parts of the pork carcass, therefore, they may not be harvested with decontamination strategies in mind or they may not even be

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harvested at all. In addition, pork variety meats vary widely in size, shape, color, texture and physical make-up (types of associated muscle, fat and epithelial tissues). This imposes difficulty in devising a system that would economically and effectively reduce the level of bacteria on all, or at least a few, variety meat items. Additionally, many variety meats are harvested by hand and transported in lugs; this makes it difficult to devise an automated system that can be used to apply a decontamination treatment. Following is a review of the decontamination technologies that have been applied to some pork variety meats.

Caspar *et al.* (1984) compared the efficacy of the application of a lactic acid solution (2% v/v, pH 2.8 to 2.9, 5 min) and a hot water (65°C, 15 sec, immersion) to decrease APC on pork livers. After 1 day of storage, the APC for the control samples were 4.4 ±0.4 log CFU/cm^2 while the lactic acid treated samples were 2.2 ±0.3 log CFU/cm^2 and hot water samples were 3.0 ±1.0 log CFU/cm^2. After 5 days of storage, the APC were 5.2 ±0.5, 2.4 ±0.6 and 3.6 ±1.2 log CFU/cm^2 for control samples, lactic acid treated samples and hot water treated samples, respectively. It should be noted that both the lactic acid treated samples and the hot water treated samples were vacuum packaged and the control samples were neither vacuum packaged nor wrapped. Therefore, the vacuum packaging may be responsible, at least in part, for the lower APC levels on the liver samples treated with lactic acid and hot water. Frederick *et al.* (1994) evaluated the ability of water at 20 and 40°C and a 2% acetic acid solution at 20 and 40°C to decrease APC, TCC and *Salmonella* Typhimurium plate counts on pork cheek meat. The 2% acetic acid treatments were generally more effective in reducing APC, TCC and

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Salmonella Typhimurium, however, *Salmonella* Typhimurium was not completely eliminated.

Organic acid treatments are not accepted on product that is being shipped to the European Union (EU). Meat hygiene regulators do not allow any method of product decontamination other than washing with potable water for fear that the decontamination treatment is being implemented to compensate for poor hygienic practices in the slaughter house (Smulders and Greer, 1998).

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CHAPTER III

LEVELS OF MICROBIOLOGICAL CONTAMINATION OF PORK CARCASSES DURING SLAUGHTER

ABSTRACT

The objective of this study was to determine microbiological contamination of pork carcasses processed under a variety of conditions relative to swine processing systems currently used in United States slaughtering plants, as they initiated operation under the new inspection regulations. Data were collected in twelve geographically dispersed pork packing facilities on two separate visits (May-June and November-January). Samples were analyzed for aerobic plate counts (APC), total coliform counts (TCC) and *Escherichia coli* counts (ECC), and for incidence of *Salmonella* spp., *Campylobacter jejuni/coli*, *Listeria monocytogenes* and *Yersinia enterocolitica*. *Campylobacter jejuni/coli* was the most commonly found (7.9%) pathogen in samples taken from chilled carcasses, followed by *L. monocytogenes* (5.0%), *Salmonella* spp. (4.6%) and *Y. enterocolitica* (0.9%). Mean (log CFU/cm^2) APC, TCC and ECC were generally lower during the winter season than during the summer season.

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INRODUCTION

The focus on food safety within all sectors of agricultural food production has intensified. Food safety is an issue that generates substantial consumer interest; thus, a media story about potential consumer risk can attract a large audience. The implementation of HACCP and new developments identified by research in the area of food safety, have been positively portrayed to the public. On the flip side, those in the media seem to focus their attention toward outbreaks of foodborne illness. Several recent occurrences have demonstrated the detrimental impact that a foodborne-illness outbreak can have on companies and major domestic and international markets. This effect of negative publicity is especially devastating for international markets where the perception of product safety is a major criterion for establishing and gaining market share, thus, impacting an entire industry. Fortunately, U.S. pork producers have an excellent reputation for product safety. The International Pork Quality Audit (conducted in 1994-1995) reported that the top reason foreign pork importers purchase U.S. pork is their "confidence in product safety" (Morgan *et al.*, 1995).

Controlling microbiological contamination on pork products enhances their safety and consequently the public perception of pork in the market place. In addition, reducing microbiological contamination can improve shelf-life and retail caselife. Desire to increase fresh pork sales domestically and abroad have increased the need for greater shelf-life and retail caselife. Initial microbial levels and storage conditions (such as temperature and type of packaging) are the most important factors affecting microbial growth which contributes to spoilage and potential health problems (Cannon *et al.*, 1995) and can decrease meat shelf-life by as much as two weeks (Gill *et al.*, 1992). This study

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was conducted to determine the incidence of selected pathogenic bacteria and populations of bacteria on pork carcasses across a variety of swine processing system conditions currently used in U.S. slaughtering plants and to identify practices and procedures used during slaughtering that could be modified to enhance the bacteriological quality of pork carcasses.

It is important to clarify that this study was designed to obtain estimates of the microbiological populations present on pork carcasses and the prevalence of certain pathogens on pork carcasses. This study was not designed to compare types of systems that are used by different plants; therefore, such comparisons are avoided in the following discussion.

MATERIALS AND METHODS

Twelve geographically dispersed U.S. pork packing facilities allowed collection of samples for use in this study. The study included plants in each of the following categories: (1) sow plants that skinned (n = 2), (2) sow plants that scalded (n = 1), (3) market hog plants that skinned (n = 1), and (4) market hog plants that scalded (n = 8). The study also involved plants utilizing the following chilling methods; (1) conventional chilling (-2 to 1°C; with and without spray chilling; n = 4), (2) modified quick chilling (-28°C chill-factor for the initial 45 to 60 min; n = 1) and (3) snap chilling (-62°C chill-factor for the initial 45 to 60 min; n = 5). Two of the plants were "hot-boning" carcasses and therefore did not have carcass coolers. Each plant was visited twice for sample collection; one visit occurred during the "summer season" (May - August) and a second during the "winter season" (November - January).

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The samples were analyzed for aerobic plate counts (APC), total coliform counts (TCC) and *Escherichia coli* counts (ECC), and for the presence or absence of *Salmonella* spp., *Yersinia enterocolitica, Listeria monocytogenes* and *Campylobacter jejuni/coli* at three processing locations within each plant. Processing locations consisted of (1) swabbing carcasses immediately before they entered the final wash cabinet (pre-wash; PW), (2) swabbing hot carcasses immediately after they exited the final wash cabinet (final wash; FW) and (3) swabbing chilled carcasses located in staging coolers after 20 h of chill, just before the carcasses were fabricated (cooler; C). Samples were not collected at the cooler location for the two plants that were hot-boning carcasses.

Samples were collected over a two-day period in each plant for each visit. Twelve samples were collected for each pathogen at each processing location. Six of the samples, for each type of bacteria at each sampling location were collected on the first day and the remaining six were collected on the second day. Sterile sponges in sterile bags (BIOPRO Enviro-sponge Bags, International Bioproducts, Redmond, WA; and Whirl-Pak ®, Nasco, Ft Atkinson, WI) were hydrated with 10 ml of buffered peptone water (BPW) to be used for sample collection of *Salmonella* and *Escherichia coli* (*E. coli* sponges also were used for TCC; Table 3.1) according to the procedures described in the new Meat and Poultry Inspection Regulations (FSIS, 1996a). Sponges used to sample carcasses for analysis of APC, *Y. enterocolitica*, *L. monocytogenes* and *C. jejuni/coli* were hydrated with 10 ml of Butterfield's Phosphate buffer (Table 3.1).

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Table 3.1. Initial buffer used to hydrate sponges for sampling and the second buffer added to prepare sponges for shipment to the laboratory for each type of bacteria

Bacteria Type	Initial Buffer[a] (10 ml)	Second Buffer[b] (15 ml)
ECC and TCC(N = 36)[c]	BPW[d]	BPW[d]
APC (N = 36)[c]	BPD[e]	BPD[e]
Salmonella (N = 36)[c]	BPW[d]	BPW[d]
Yersinia enterocolitica (N = 36)[c]	BPD[e]	BPD[e]
Listeria monocytogenes (N = 36)[c]	BPD[e]	UVM[f]
Campylobacter jejuni/coli (N = 36)[c]	BPD[e]	HEB[g]

[a]Initial 10 ml of buffer used to hydrate the sponge prior to sampling the carcasses.
[b]An additional 15 ml of buffer to hydrate the sponge sample prior to shipment to the lab.
[c]N = 12 at each of three locations (pre-wash, final wash and cooler).
[d]Buffered Peptone Water
[e]Butterfield's Phosphate Diluent
[f]University of Vermont Broth
[g]Hunt's Enrichment Broth

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Carcass Sampling

Sampling of the carcasses with the sponges was conducted using aseptic techniques (sterile sample bags and sponges, and latex gloves and templates that were pre-sterilized, before obtaining each sample, by immersion in water > 82.2°C for 10 sec). The sponging of the carcass was performed according to the procedures described in the new Meat and Poultry Inspection Regulations (FSIS, 1996a). The template was placed on the belly region and the sponge was placed on the surface of the carcass inside the template. The area within the template was swabbed with the sponge by making ten (10) vertical passes and then ten (10) horizontal passes. Then, the template was placed on the ham area using the same template and the same side of the sponge. After completing sponging of the ham, the template was placed on the jowl and the reverse side of the sponge was used to swab the jowl area (Figure 3.1).

Before placing the sponge samples in coolers for shipment, they were hydrated with an additional 15 ml of buffer. The ECC and *Salmonella* samples were hydrated with 15 ml of BPW; APC and *Y. enterocolitica* samples were hydrated with 15 ml of BPD; *L. monocytogenes* samples were hydrated with 15 ml of University of Vermont Broth, and; *C. jejuni/coli* samples were hydrated with 15 ml of Hunt's Enrichment Broth to prepare them for shipment to the laboratory (Table 3.1).

Insulated coolers were placed open under refrigeration conditions to be pre-chilled before their use for shipping samples to the laboratory. Bagged samples were held under refrigeration (1.7 to 4.4°C) and then packaged in pre-chilled, insulated shipping containers. Within 4 h of collection, the samples were shipped via overnight express carrier (in coolers packed with commercial ice substitutes) to a commercial laboratory for

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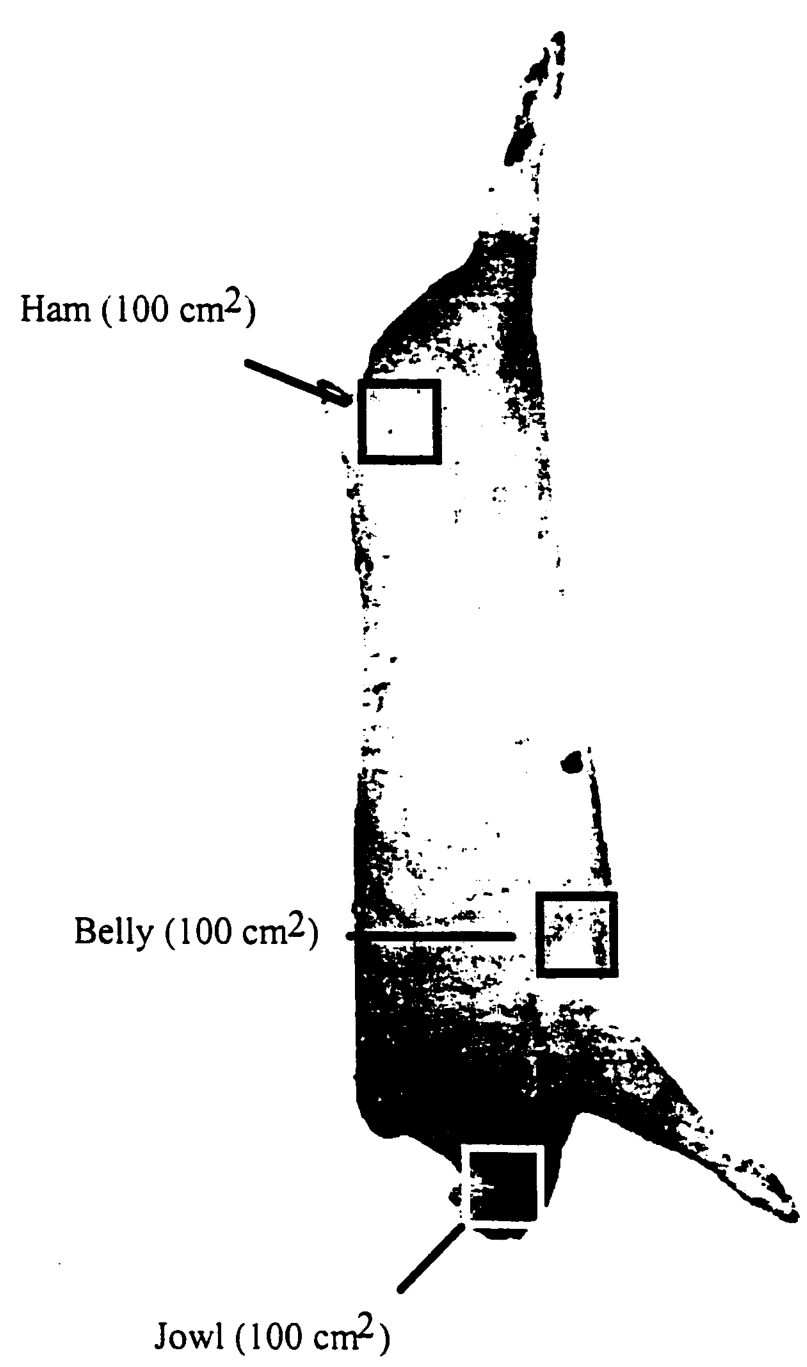

Figure 3.1. 3-site sponge sampling protocol for hog carcasses.

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microbiological analyses. The commercial laboratory monitored the arrival of the sponge samples to ensure that no temperature abuse was encountered (shipping container was < 4.4°C).

Microbiological analysis

To quantifying APC, appropriate decimal dilutions from the samples were plated on Petrifilm™ Aerobic Count Plates (3M™ Microbiology Products, St. Paul, MN). The Petrifilm™ Aerobic Count Plates were incubated for 48 h at 35°C, and colonies were counted manually. To quantify TCC and ECC, appropriate decimal dilutions from the samples were plated on Petrifilm™ *E. coli* Count Plates (3M™ Microbiology Products). Total coliform counts were established by counting all red and blue colonies associated with one or more gas bubbles following 48 h incubation at 35°C and ECC were determined by counting only the dark blue colonies associated with a gas bubble. Presence of *Salmonella* spp., *L. monocytogenes* and *C. jejuni/coli* were determined using procedures recommended in the FSIS-USDA Microbiology Laboratory Guidebook (USDA-FSIS, 1977) and presence of *Y. enterocolitica* was determined using procedures in the Compendium of Methods for the Microbiological Examination of Foods (Speck, 1984). A detailed outline of the procedures that were used to identify *Salmonella* spp., *L. monocytogenes*, *C. jejuni/coli* and *Y. enterocolitica* is presented in Appendix I.

Statistical analysis

Counts below the detectable limit of 1 CFU/cm^2 and 10 CFU/cm^2 were reported as 0.9 CFU/cm^2 and 9 CFU/cm^2, respectively. Bacteriological count data were

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transformed into logarithms before computing means and performing statistical analyses. All bacterial counts were reported as log CFU/cm^2. Data were analyzed by the general linear model (GLM) procedures of SAS® (SAS®, 1995). Plant was not used as a main effect in any of the statistical models in order to protect confidentiality with respect to identity of, and data from, the plants involved in the study. Season and processing location were considered main effects. Data were evaluated using the model $y = a + b_1x_1 + b_2x_2 + b_3x_1x_2$ and least square means were computed for APC, TCC and ECC. When F-test were significant ($P < 0.05$), mean differences were separated by the least significant difference procedure.

RESULTS AND DISCUSSION

Incidence of pathogens

Campylobacter jejuni/coli, with an incidence of 7.9%, was the most common pathogen (of those tested) in cooler samples, followed by *L. monocytogenes* (5.0%), *Salmonella* spp. (4.6%) and *Y. enterocolitica* (0.9%)(Table 3.2). The incidence of the pathogens in samples from the cooler was lower in this study than was the incidence reported by the Food Safety Inspection Service (FSIS, 1996b) in the Nationwide Pork Microbiological Baseline Data Collection Program: Market Hogs (April 1995 - March 1996). In that study, the incidences of *C. jejuni/coli*, *Salmonella* spp. and *L. monocytogenes* were 31.5%, 8.7% and 7.4%, respectively. However, it is important to note that the study conducted by FSIS included more plants, with data collected at several small facilities, and samples were collected via excision sampling rather than sponge sampling over a period of one year. Also presented in Table 3.2 are the incidences of

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Table 3.2. Incidence of *Salmonella* spp., *Campylobacter jejuni/coli*, *Yersinia* spp., *Yersinia enterocolitica*, *Listeria* spp. and *Listeria monocytogenes* presented by season and sampling location (across 12 plants; two plants used hot-boning so cooler samples were only collected in 10 plants)

Location	Season	n	Positive samples					
			Salmonella spp.	*Campylobacter jejuni/coli*	*Yersinia* spp.[a]	*Yersinia enterocolitica*	*Listeria* spp.[a]	*Listeria monocytogenes*
Pre-wash	Summer	144	8	19	10	3	14	0
	Winter	144	8	5	34	0	17	4
Final wash	Summer	144	9	15	6	1	9	1
	Winter	144	9	11	32	0	26	8
Cooler	Summer	120	7	15	5	2	10	2
	Winter	120	4	4	31	1	23	10
Sub-totals			n (%)					
Summer season		408	24 (5.9)	49 (12.0)	21 (5.15)	6 (1.5)	33 (8.1)	3 (0.7)
Winter season		408	21 (5.2)	20 (4.9)	97 (23.8)	1 (0.3)	66 (16.2)	22 (5.4)
Pre-wash		288	16 (5.6)	24 (8.3)	44 (15.3)	3 (1.0)	31 (10.8)	4 (1.4)
Final wash		288	18 (6.3)	26 (9.0)	38 (13.2)	1 (0.4)	35 (12.2)	9 (3.1)
Cooler		240	11 (4.6)	19 (7.9)	36 (15.0)	3 (1.3)	33 (13.8)	12 (5.0)
Total		816	45 (5.5)	69 (8.5)	118 (14.5)	7 (0.9)	99 (12.1)	25 (3.1)
Incidence by plant[b]								
Cooler	Summer	10	4 (40)	4 (40)	4 (40)	2 (20)	4 (40)	1 (10)
	Winter	10	4 (40)	2 (20)	7 (70)	1 (10)	8 (80)	4 (40%)
All locations	Summer	12	10 (83)	6 (50)	8 (67)	3 (25)	8 (67)	3 (25)
	Winter	12	11 (92)	5 (42)	10 (83)	1 (8)	10 (83)	5 (42)
Total in cooler		10	7 (70)	4 (40)	8 (80)	3 (30)	9 (90)	4 (40)
Total all plant locations		12	12 (100)	6 (50)	11 (92)	4 (33)	11 (92)	6 (50)

[a]Includes all positive samples for both pathogenic and nonpathogenic species.
[b]Number of plants that had at least one positive sample.

positive samples for *Listeria* spp. and *Yersinia* spp. bacteria. Information indicating how many plants had at least one positive sample of each pathogen in the cooler is also provided in Table 3.2. At least one positive sample of each of *Salmonella* spp., *C. jejuni/coli*, *L. monocytogenes* or *Y. enterocolitica* was detected in samples from the cooler of 7 (70%), 4 (40%), 5 (50%) and 3 (30%) plants, respectively.

The major source of *Salmonella* spp., *C. jejuni/coli* and *Y. enterocolitica* contamination on carcasses is the pig, whereas the major source of *L. monocytogenes* contamination on carcasses is from the environment or from within the plant itself (Borch *et al.*, 1996; Wesley and Johnson, 1998; Table 3.3). However, the plant may also harbor *Salmonella* spp., *C. jejuni/coli* and *Y. enterocolitica* and conversely the pig may initially introduce *L. monocytogenes* into the plant.

Salmonella spp.

Healthy pigs can be carriers of *Salmonella* spp. (gram negative rods) and can serve as a source of *Salmonella* spp. contamination on pork carcasses (Borch *et al.*, 1996), but, *Salmonella* spp. also can establish themselves in the plant and contaminate carcasses. Berends *et al.* (1997) reported pigs carrying *Salmonella* spp. were 3 to 4 times more likely to have the organism on their carcasses than are *Salmonella*-free pigs. Pork was identified as the source of seven outbreaks of Salmonellosis between 1988 and 1992 (CDC, 1996a). From 1996 through September, 1999 three recalls of pork products were related to *Salmonella* (all three were in 1999; FSIS, 1999c).

In this study, the incidences of *Salmonella* spp. across all locations were 5.5% and in the cooler were 4.6%. The incidence of *Salmonella* spp. in this study corresponds

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Table 3.3. Origin and pH and temperature growth ranges for selected pathogenic bacteria. All values are approximate because the combinations of environmental factors have a substantive influence on the growth and survival of pathogens (modified from Borch *et al.*, 1996).

Bacteria	Origin[a]	Growth-range	
		pH	Temperature (°C)
Campylobacter spp.	Pig Environment	4.9 to >8.0	30 to 47
Listeria monocytogenes	Pig Environment	4.5 to 9.6	1 to 45
Salmonella spp.	Pig Environment	4.5 to 9.0	5 to 47
Yersinia enterocolitica	Pig Environment	4.2 to 9.0	-2 to 42

[a]Major contamination source is underlined

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closely to the 5.5% incidence of *Salmonella* spp. reported by the U.S. Department of Agriculture, Food Safety and Inspection Service from hog carcasses through the first six months of the current *Salmonella* Testing for Raw Meat and Poultry Products (FSIS, 1998).

Campylobacter jejuni/coli

Campylobacter enteritis is most frequently caused by *C. jejuni* and, to a lesser extent, by *C. coli* (Stern and Kazmi, 1989). *Campylobacter* spp. are usually found in the intestinal tract of pigs in higher numbers than are *Salmonella* spp. or *Yersinia* spp. (Borch *et al,* 1996). The number of positive samples for *C. jejuni/coli* was 12.0% during the summer season and 4.9% during the winter season across all locations tested (Table 3.2). The incidence of positive *C. jejuni/coli* was 7.9%, 8.3% and 9.0% in the cooler, at pre-wash and final wash locations, respectively. It should be noted that 15 of the 19 positive samples of *C. jejuni/coli* found in the cooler in this study were from only two of the plants.

These results are consistent with the findings of Borch *et al.* (1996) who reported a reduction in the number of positive samples detected in the cooler in comparison to the number of positive samples detected on carcasses before chilling. Borch *et al.* (1996) attributed the reduction in positive samples of *Campylobacter* spp. in the cooler to the fact that *Campylobacter* spp. are microaerophilic organisms, do not grow at temperatures below 30°C, have a low heat resistance and are sensitive to drying and freezing.

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Listeria monocytogenes

Listeria monocytogenes can survive in processing facilities (e.g., floor drains) for long periods of time and it can form a biofilm on surfaces such as stainless steel, glass and rubber that is resistant to sanitizing agents (Herald and Zottola, 1988; Frank and Koffi, 1990). Because of its pervasiveness, the organism is constantly re-introduced into the plant environment (Tompkin *et al.*, 1999) and re-contamination of meat products with *L. monocytogenes* is common (CDC, 1996b). In humans, *L. monocytogenes* can cause meningitis, septicemia and abortions and has a 20 to 30% mortality rate (Mortimore and Wallace, 1994).

In this study, the incidence of samples from carcasses that were positive for *L. monocytogenes* was 1.4%, 3.1% and 5.0% at pre-wash, final wash and in the cooler, respectively. The incidence of *Listeria* spp. was 16.2% during the winter and 8.1% during the summer and the incidence of *L. monocytogenes* 5.4% during the winter and 0.7% during the summer (Table 3.2). Ten of the 12 positive samples identified in the cooler were from only two plants (one of these plants also had carcasses with a high incidence of positive *C. jejuni/coli* samples). Furthermore, only one plant had positive samples of *L. monocytogenes* at pre-wash, while five plants had positive samples at final wash, and four had positive samples in the cooler. The difference in where there was a higher incidence of carcasses with positive samples of *L. monocytogenes* suggested the source of *L. monocytogenes* contamination may have been from inside the plant, and not from incoming raw materials (e.g. animals), which would agree with the findings of Borch *et al.* (1996). It was interesting to note that the incidence of positive *L. monocytogenes* samples, and of plants with at least one positive sample, was higher in plant locations

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where the carcasses encountered high-moisture environments — the final wash cabinet (3.1%) and the cooler (5.0%).

Yersinia enterocolitica

Healthy pigs have been found to be infected with *Y. enterocolitica* and are considered to be the most important source of pathogenic serotypes (Anderson, 1988; Borch *et al.*, 1996; Wesley and Johnson, 1998). In a study conducted by de Boer and Nouws (1991), pathogenic *Y. enterocolitica* strains were isolated from 36 (42%) of 86 porcine tonsils, 8 (20%) of 40 tongues and 17 (17%) of 100 rectal swabs but was not detected in 20 samples of head meat or on 210 carcasses. The contamination of carcasses, though at a lower rate, seemed to stem from cross-contamination from the feces/intestinal-contents or tonsillary region. The results of this study concur with previous findings in as much as only 3 (0.9%) positive samples of *Y. enterocolitica* were detected on 240 carcasses in the cooler. However, *Yersinia* spp. was detected on 15% of the carcasses in the cooler (Tabel 3.2).

Microbiological plate counts

Mean (log CFU/cm^2) APC, TCC and ECC by season and sampling location are presented in Figure 3.3. For APC, the season × location interaction was significant. The mean (log CFU/cm^2) APC decreased ($P < 0.05$) as carcasses proceeded from pre-wash, to final wash, to the cooler during the summer season, but not during the winter season (APC remained relatively constant from pre-wash through to the cooler). For TCC and ECC, the season × location interactions were not significant ($P > 0.05$) but, season and

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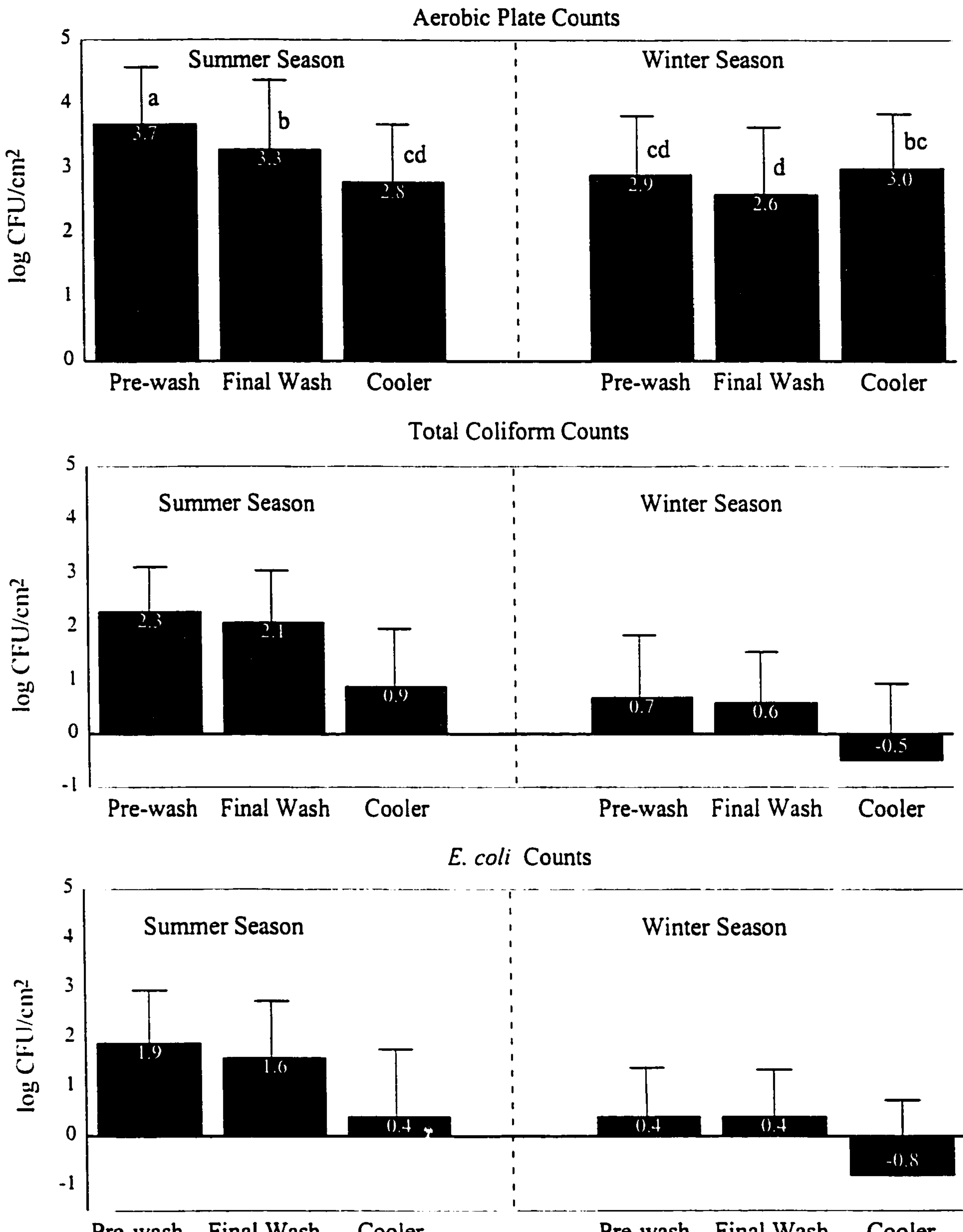

Figure 3.2. Mean (log CFU/cm^2) aerobic plate counts, total coliform counts and *E. coli* counts obtained from hog carcasses tested at pre-wash, final wash, and cooler locations, presented by season. The season x sampling location interaction was significant for APC ($P < 0.05$); and season and sampling location main effects were significant for TCC and ECC ($P < 0.05$); means bearing a common superscript letter are not different ($P > 0.05$).

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location main effects were significant ($P < 0.05$). Mean (log CFU/C TCC and ECC were lower in the winter season than in the summer season ($P < 0.05$) and mean (log CFU/cm^2) for TCC and ECC also were lower ($P < 0.05$; < 1 log CFU/ cm^2) in the cooler than at either the pre-wash or final wash locations. The mean values (log CFU/cm^2) for the ECC and TCC were the same for pre-wash and final wash within both summer and winter seasons.

Differences between seasons in mean (log CFU/cm^2) APC, TCC and ECC may have resulted from a combination of factors. First, in the summer season, ambient (outside) temperatures would usually be higher and so would temperatures inside and outside the plant. This seemed to explain, in part, the seasonal differences as a few of the plants that were sampled had plate counts that were of similar magnitude for both the summer and winter seasons. In most cases, plants with similar plate counts for both summer and winter seasons were from the south-central region of the U.S. The increased ambient temperature and dryness of the summer season also may have had indirect effects that resulted in higher plate counts such as more dust in the air in the plant. Another possibility was that ambient (outside) temperature during transport of the sponge samples to the laboratory may have affected microbial growth in the shipping containers. The temperature of samples was monitored to ensure that the inside of the coolers containing the sponges did not exceed 4.4°C. However, during the winter season, containers may have actually been maintained at less than 4.4°C, thus providing a lower temperature environment during shipping for the samples before they were processed in the laboratory.

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Statistical Process Control

Plants that intend to monitor and evaluate process control will use Statistical Process Control charts based on counts obtained using the sponge sampling method. Figure 3.3 is an example of a Statistical Process Control chart for ECC (log CFU/cm^2) in the cooler and contains data from 10 plants evaluated in this study. In this example, a "critical limit" was established as two standard deviations above the mean. "Critical limit" in this example refers to a verification tolerance level indicating that the process is out of control; it does not refer to a specific critical control point of the HACCP plan. There were a few instances when ECC (log CFU/cm^2) from a sponge sample exceeded the critical limit; in those instances, corrective action and HACCP reassessment could have been initiated, followed by implementation of other constraints or processes identified in the plant's HACCP plan. Statistical Process Control charts by season are presented in Figures 3.4 and 3.5. A Statistical Process Control (SPC) chart for each of the 10 plants in this study can be found in Appendix II. These SPC charts suggested (a) that use of a fixed upper critical limit, rather than use of the mean plus two standard deviations, may be more appropriate for some plants, and (b) in time, when enough samples have been collected an evaluated, the mean plus one standard deviation may be a more useful critical limit than is plus two standard deviations. It is important to remember that the Statistical Process Control method is primarily useful for determining if the system within a given plant is functioning properly—for verification purposes. Use of a Statistical Process Control system is not an effective means for comparing

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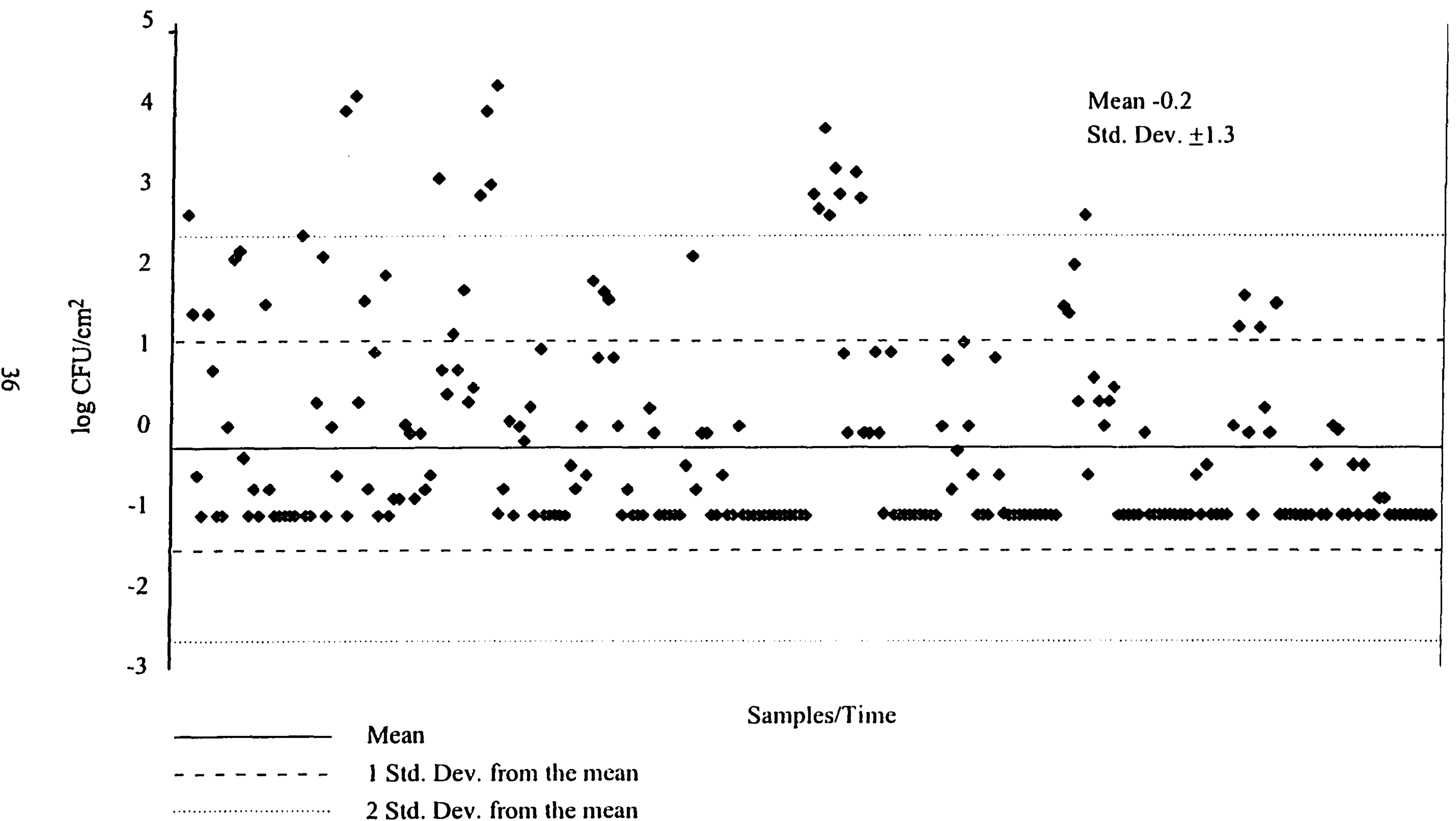

Figure 3.3. Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler (data from all plants and seasons combined).

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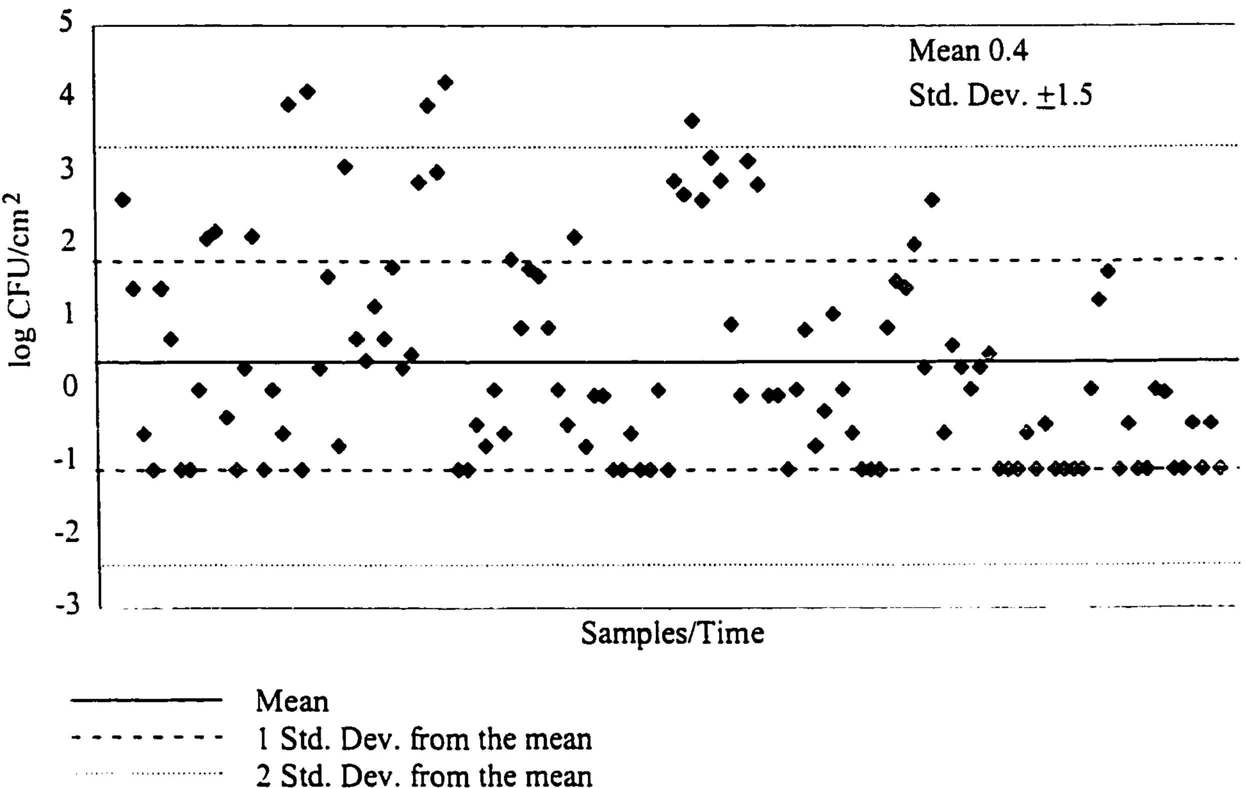

Figure 3.4. Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler during the summer season (combined data from all plants).

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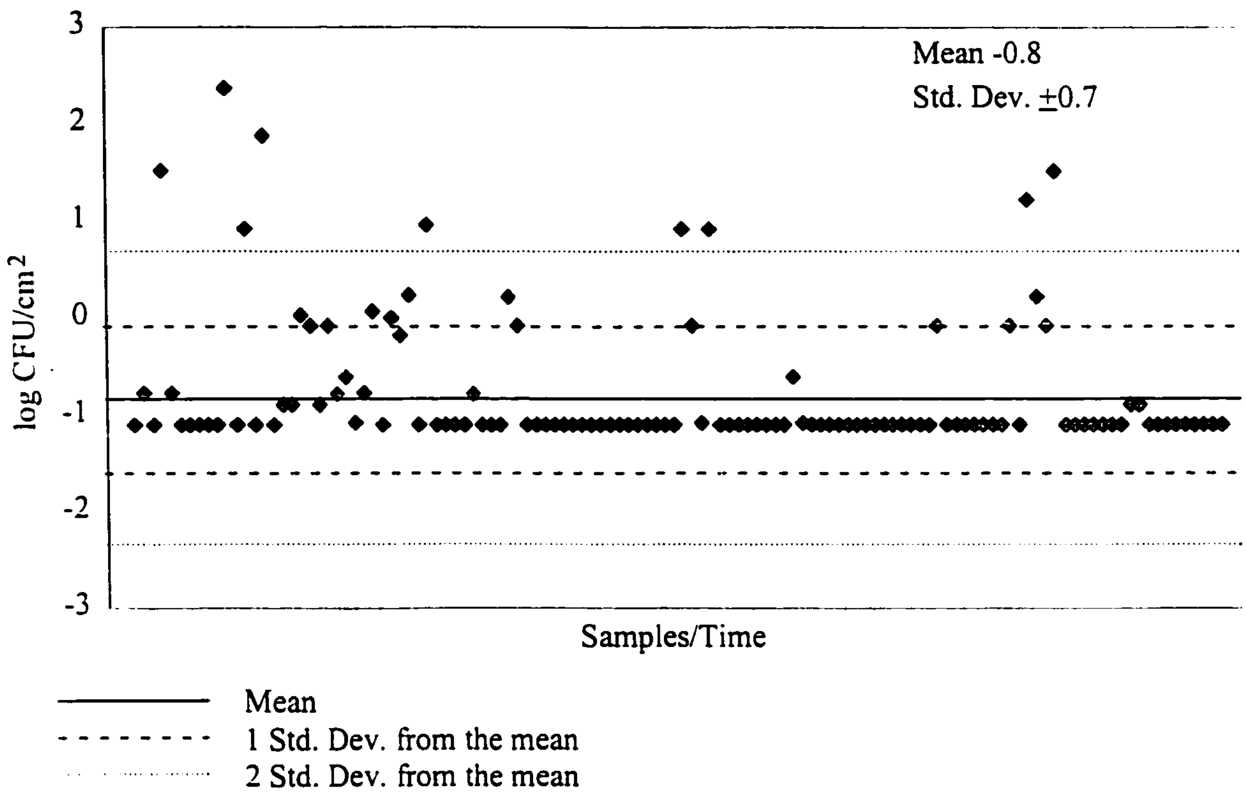

Figure 3.5. Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler during the winter season (combined data from all plants).

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microbiological data from different plants because those plants may use different processing procedures (as was seen in this study).

SUMMARY

Campylobacter jejuni/coli, with an incidence of 7.9%, was the most prevalent pathogen (of those tested) in samples from the cooler, followed by *L. monocytogenes* (5.0%), *Salmonella* spp. (4.6%) and *Y. enterocolitica* (0.9%). The incidence of pathogens detected in this study was lower than reported in a previous USDA baseline study, suggesting substantial improvement, over time, by the pork industry in reducing pathogen incidence on pork carcasses. However, there is continued need for efforts to reduce further the incidence of pathogens on pork. Mean (log CFU/cm^2) APC, TCC and ECC on pork carcasses were generally lower during the winter, than during the summer, season.

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CHAPTER IV

MICROBIAL CONTAMINATION RECOVERED BY 2-SITE VERSUS 3-SITE PORK CARCASS SAMPLING PROTOCOLS

ABSTRACT

The objective of this study was to compare a 3-site (ham, belly and jowl) and a 2-site (belly and jowl) sponge surface swabbing protocol for recovery of microbial contamination from pork carcasses. Samples were collected from 12 geographically dispersed pork packing facilities on two separate visits (May-June and November-January) and were analyzed for total coliform counts (TCC) and *Escherichia coli* counts (ECC), and for incidence of *Salmonella* spp. Mean (log CFU) TCC and ECC expressed on a constant surface area (cm^2) did not differ ($P > 0.05$) when either the 3-site sponge sampling protocol or the 2-site sponge sampling protocol was used to obtain samples before or after the final wash cabinet or in the cooler. However, the 2-site sponge sampling protocol yielded a 2.1% incidence for *Salmonella* spp. in the cooler, which was less than half of the 4.6% incidence detected using the 3-site sponge sampling protocol. The 3-site sponge surface sampling method was a more reliable protocol for identifying carcasses contaminated with *Salmonella* spp. because a larger area was analyzed for presence of the pathogen. Therefore, from a food safety standpoint, it would be in the best interest of the swine industry to continue to use the 3-site sponge sampling protocol.

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INTRODUCTION

On July 25, 1996, the Food Safety and Inspection Service (FSIS) of the United States Department of Agriculture (USDA) established new meat and poultry regulations: the Final Rule on Pathogen Reduction and HACCP Systems (FSIS, 1996a). The requirement of having Sanitation Standard Operating Procedures and *Escherichia coli* testing became effective in all plants on January 27, 1997, and requirements for *Salmonella* spp. performance standards are being phased in and will be present in all plants by January 25, 2000. *Salmonella* spp. was selected because it is one of the most common foodborne pathogens, however, *Salmonella* spp. is not being tested to condemn products, but rather, to verify that HACCP systems are controlling contamination of enteric pathogens (FSIS, 1999a).

In a study by Roberts *et al.* (1980), several sampling sites on pork carcasses were evaluated and ranked according to the extent of bacterial contamination associated with each site (Table 4.1). The distal hind-limb, lateral surface of the abdomen and the cheek (or jowl) were the three areas that consistently had the highest contamination on pork carcasses. A study by Morgan *et al.* (1987) compared contamination levels (total plate count - TPC; *Escherichia coli count* - ECC; and incidence of *Salmonella* spp.) at four different sites on pork carcasses: 1) dorsal to the tail near the anal opening; 2) on the lateral surface of the hind leg, approximately midway on a line between the hip and stifle joints; 3) from the mid dorsal region; and 4) on the lateral surface of the jowl. They concluded that ECC was greatest on the area dorsal to the tail near the anal opening; however, the jowl site was most frequently contaminated with *Salmonella* spp. and had high populations of APC. The high level of contamination on the jowl area was

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Table 4.1. Ranking of sampling sites on pork carcasses by total plate count (modified from Roberts *et al.*, 1980)

Site	Rank[a]
Distal hind limb	12
Lateral surface of the hind limb	7
Lateral surface of the abdomen (belly)	11
Mid-dorsal region (mid-back)	6
Back of neck	9
Throat	5
Cheek or jowl	10
Lateral surface of the thorax	8
Medial surface of the leg	3
Medial surface of the abdomen	4
Medial surface of the thorax	1
Medial surface of the neck	2

[a]The site with the lowest total plate count is ranked "1" and the one with the highest total plate count is ranked "12".

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attributed to the jowl being the drainage point on washed carcasses and therefore bacteria could be expected to accumulate there. Morgan *et al.* (1987) concluded that no single sampling site should be used to assess the microbial status of hog carcasses, as multiple sites may be required to assess the level of contamination by different bacteria.

The protocol for testing pork carcasses for *E. coli* and *Salmonella* spp. outlined in the Final Rule on Pathogen Reduction and HACCP Systems involves sampling the ham, belly and jowl, either by excising and combining the samples, or by swabbing all three sites with one sponge (FSIS, 1996a). However, there was some concern about the safety of employees while sampling the specified ham area and, conversely, there was concern about the efficacy of a sampling protocol that did not include the specified ham area. This study was designed to compare the current FSIS approved 3-site (ham, belly and jowl) sampling protocol with a 2-site (belly and jowl) sampling protocol, by swabbing the surface with sponges, as a means of detecting *E. coli* and incidence of *Salmonella* on pork carcasses in the United States.

MATERIALS AND METHODS

Study design

The study included 12 geographically dispersed pork packing plants. Plants consisted of the following types of categories: (a) sow plants that skinned (n = 2), (b) sow plants that scalded (n = 1), (c) market hog plants that skinned (n = 1), and (d) market hog plants that scalded (n = 8). The study also involved plants utilizing the following chilling methods; (1) conventional chilling (-2 to 1°C; with and without spray chilling; n = 4), (2) modified quick chilling (-28°C chill-factor for the initial 45 to 60 min; n = 1) and (3) snap

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chilling (-62°C chill-factor for the initial 45 to 60 min; n = 5). Two of the plants were "hot-boning" carcasses and therefore did not have carcass coolers. Each plant was visited twice for sample collection; one visit occurred during the "summer season" (May – August, 1998) and a second during the "winter season" (November, 1997 through January, 1998).

Carcass sampling

Sponge samples were collected from 12 carcasses at each of three processing locations within each plant. Sampling locations consisted of: (a) swabbing hot carcasses immediately before they entered the final wash cabinet (pre-wash; PW), (b) swabbing hot carcasses immediately after they exited the final wash cabinet (final wash; FW), and (c) chilled carcasses located in staging coolers just before fabrication (cooler; CH). Samples were not collected at the cooler location for the two plants that were hot-boning carcasses.

Samples were collected over a two-day period in each plant for each visit. Twelve samples were collected for each ECC, TCC and *Salmonella* at each processing location. Six of the samples, for each type of bacteria at each sampling location were collected on the first day and the remaining six were collected on the second day. Sterile sponges in sterile bags (BIOPRO Enviro-sponge Bags, International Bioproducts, Redmond, WA; and Whirl-Pak ®, Nasco, Ft Atkinson, WI) were hydrated with 10 ml of buffered peptone water (BPW) to be used for sample collection according to the procedures described in the new Meat and Poultry Inspection Regulations (FSIS, 1996a).

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For the 3-site surface sampling protocol, the template was placed on the belly region and the sponge was placed on the surface of the carcass inside the template. The area within the template was swabbed with the sponge by making 10 vertical passes and then 10 horizontal passes. Then, the same template was placed on the ham area which was sampled with the same side of the sponge. After completing sponging of the ham, the same template was placed on the jowl and the reverse side of the sponge was used to swab the jowl area (Figure 4.1). For the 2-site sampling protocol, the template was placed on the belly region following the same procedures used in the 3-site sampling method. After the belly region was swabbed, the same template was placed on the jowl and the reverse side of the sponge was used to swab the jowl (Figure 4.1).

Insulated transport containers were placed open under refrigerated conditions and pre-chilled before being used to ship samples to the laboratory. Before placing the sponge samples in coolers for shipment, they were hydrated with an additional 15 ml of BPW buffer. Bagged samples were held under refrigeration (1.7 to 4.4°C) during sampling and then packaged in the pre-chilled, insulated shipping containers. Within 4 h of collection, the samples were shipped via overnight express carrier (in coolers packed with commercial ice substitutes) to the laboratory for microbiological analyses.

Microbiological analyses

Upon arrival, laboratory personnel monitored the samples to ensure that no temperature abuse was encountered (shipping container was < 4.4°C). To quantify TCC and ECC, appropriate decimal dilutions from the samples were plated on Petrifilm™ *E. coli* Count Plates (3M™ Microbiology Products). Total coliform counts were established

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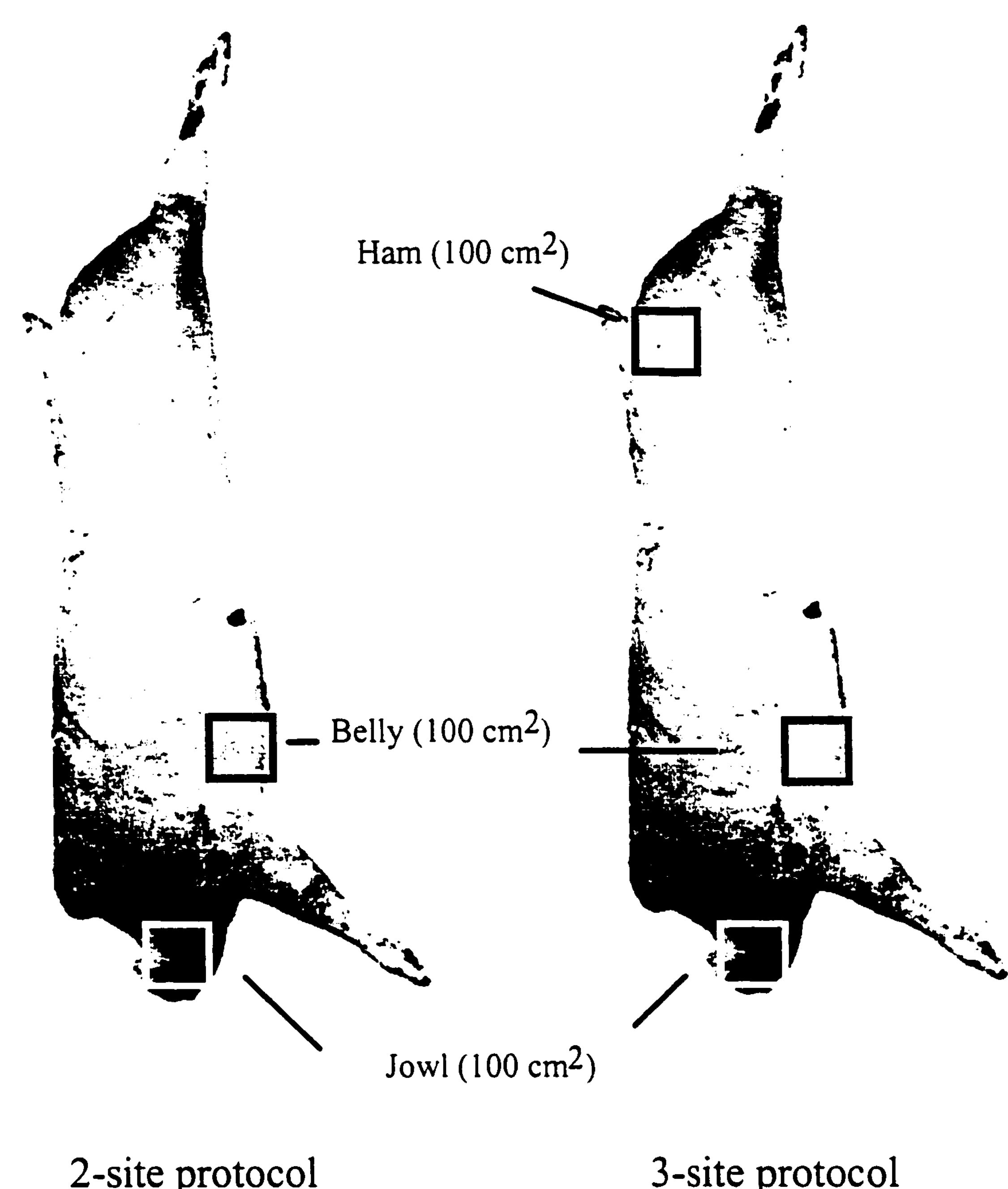

Figure 4.1. Sampling protocols for 2-site and 3-site sponge surface swabbing of hog carcasses.

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by counting all red and blue colonies associated with one or more gas bubbles following 48 h incubation at 35°C and ECC were determined by counting only the dark blue colonies associated with a gas bubble. Presence of *Salmonella* spp. was determined using procedures described in the FSIS-USDA Microbiology Laboratory Guidebook (USDA-FSIS, 1977) as outlined in Appendix I.

Statistical analysis

Counts below the detectable limit of 1 CFU/cm^2 and 10 CFU/cm^2 were reported as 0.9 CFU/cm^2 and 9 CFU/cm^2, respectively. Bacteriological count data were transformed into logarithms before computing means and performing statistical analyses. All bacterial counts were reported as log CFU/cm^2. Data were analyzed by the general linear model (GLM) procedures of SAS® (SAS®, 1995). Plant was not used as a main effect in any of the statistical models in order to protect confidentiality with respect to identity of, and data from, the plants involved in the study. Season, processing location and sampling protocol (2- versus 3-site) were considered main effects. Data were evaluated using the model $y = a + b_1x_1 + b_2x_2 + b_3x_3 + b_4x_1x_2x_3$ and least square means were computed for TCC and ECC. When F-test were significant ($P < 0.05$), mean differences were separated by the least significant difference procedure.

RESULTS AND DISCUSSION

Incidence of Salmonella spp.

The incidence of *Salmonella* spp. for the 3-site protocol was lower in samples collected from the cooler (4.6%) than in samples collected from pork carcasses before

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(5.6%) or after (6.3%) the final carcass wash; the same was true for the 2-site protocol which yielded a 2.1% incidence for samples collected in the cooler and 4.5% before, and 2.8% after, the final carcass wash (Table 4.2). The 2-site sampling protocol (sponging only the belly and the jowl) was not as effective as the 3-site sponge sampling protocol (ham, belly and jowl) in detecting *Salmonella* spp. in the cooler, especially during the summer (Table 4.2). On pork carcasses in the cooler, 2-site samples yielded a *Salmonella* spp. incidence of 2.1% during both seasons which was less than half of the 4.6% detected with the 3-site sampling protocol. The 3-site protocol also yielded positive *Salmonella* spp. carcass samples in the cooler in seven (70%) of the plants, while the 2-site sampling protocol only yielded *Salmonella* positive carcass samples in three (30%) of the plants over both seasons (Table 4.3). Of course, this was not unexpected because the larger surface area sampled for the presence of an organism, the higher the probability of obtaining a positive result.

Microbiological population

Results from the comparison between the 3-site sampling protocol and the 2-site sampling protocol for TCC and ECC, across all 12 plants, are presented in Figure 4.2. No interactions tested significantly different and only the season and sampling location main effects tested different ($P < 0.05$). The sampling protocol (2- versus 3-site) main effect did not test different ($P > 0.05$). Mean TCC and ECC (log CFU/cm^2) were lower ($P < 0.05$) during the winter season than during the summer season, but were the same within both summer and winter seasons for samples collected before and after the final carcass wash. However, mean (log CFU/cm^2) TCC and ECC for samples collected from carcasses in the cooler after chilling were lower (by > 1 log CFU/cm^2) than samples

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Table 4.2. Incidence of *Salmonella* spp. on pork carcasses at pre-wash, final wash and cooler locations for carcasses sampled using a 2- or 3-site sampling protocol

Location	Season	n[a]	Positive *Salmonella* spp. samples	
			3 - Site[b] protocol	2 - Site[c] protocol
Pre-wash	Summer	144	8 (5.6%)	7 (4.9%)
	Winter	144	8 (5.6%)	6 (4.2%)
Final wash	Summer	144	9 (6.3%)	1 (0.7%)
	Winter	144	9 (6.3%)	7 (4.9%)
Cooler	Summer	120	7 (6.8%)	2 (1.7%)
	Winter	120	4 (3.3%)	3 (2.5%)
Sub-totals				
Summer season		408	24 (5.9%)	10 (2.5%)
Winter season		408	21 (5.2%)	16 (3.9%)
Pre-wash		288	16 (5.6%)	13 (4.5%)
Final wash		288	18 (6.3%)	8 (2.8%)
Cooler		240	11 (4.6%)	5 (2.1%)

[a]Two of the 12 plants used hot-boning so cooler data was only collected in 10 plants.
[b]3-site sponge sampling protocol included the ham, belly and jowl.
[c]2-site sponge sampling protocol included the belly and jowl.

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Table 4.3. Incidence of *Salmonella* spp. across plants[a] for carcasses sampled using a 2- or 3-site sampling protocol

Location	Season	n[b]	Positive *Salmonella* spp. samples	
			3 - Site[c] protocol	2 - Site[d] protocol
Cooler	Summer	10	4 (40%)	2 (20%)
	Winter	10	4 (40%)	2 (20%)
All locations	Summer	12	10 (83%)	5 (42%)
	Winter	12	11 (92%)	9 (75%)
Total in cooler		10	7 (70%)	3 (30%)

[a]Number of plants that had at least one positive sample.
[b]Two of the 12 plants used hot-boning so cooler data was only collected in 10 plants.
[c]3-site sponge sampling protocol included the ham, belly and jowl.
[d]2-site sponge sampling protocol included the belly and jowl.

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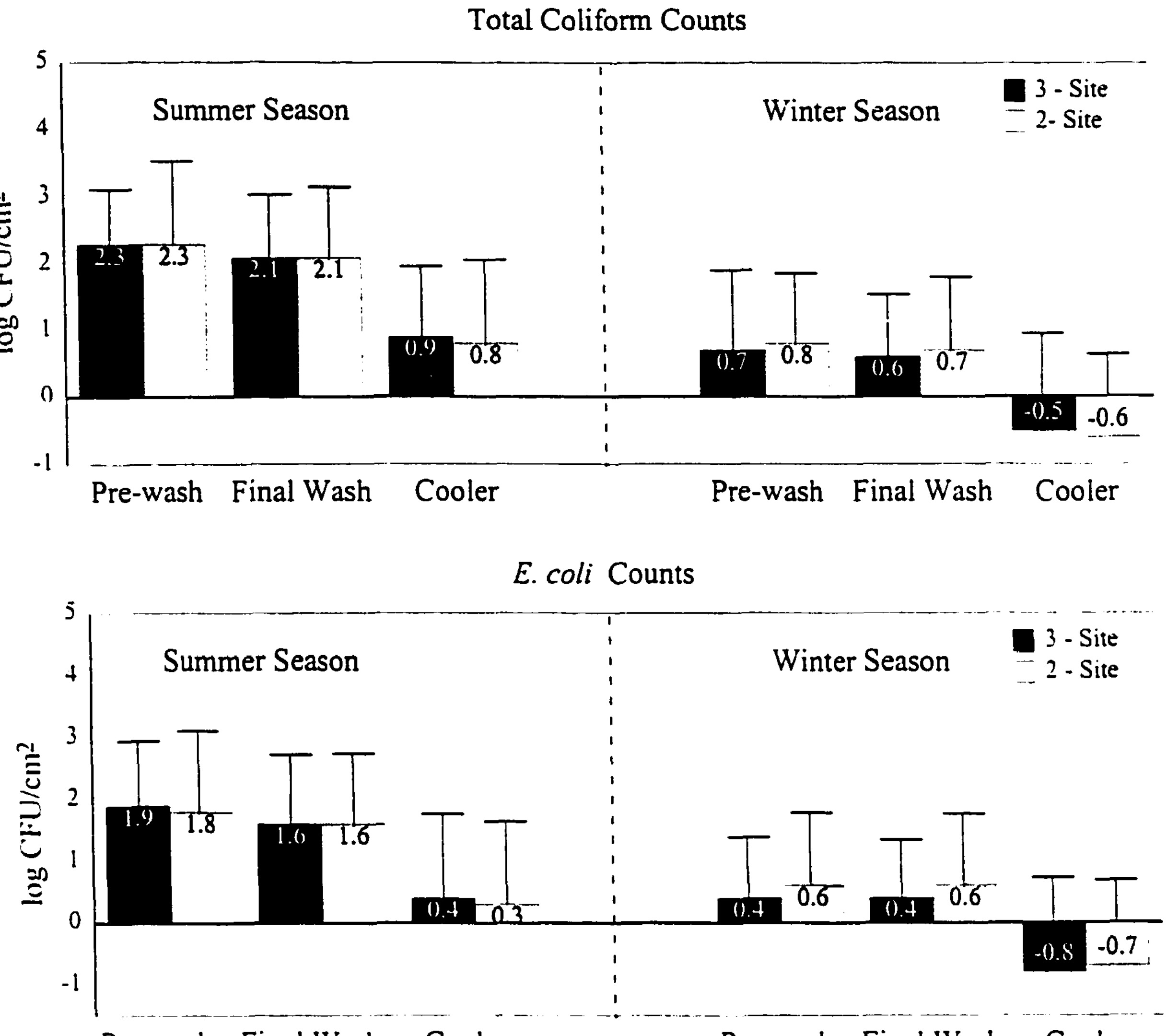

Figure 4.2. Mean values (log CFU/cm^2) comparing the 3-site and 2-site sponge sampling protocols using total coliform counts and *E. coli* counts obtained from hog carcasses at the pre-wash, final wash, and cooler locations, presented by season.

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collected before and after the final carcass wash. Mean ECC (log CFU/cm^2) for samples collected in the cooler were generally low and acceptable across all plants. Mean TCC and ECC (log CFU/cm^2) between the 3-site sampling protocol and the 2-site sampling protocol did not differ ($P > 0.05$) within samples collected before and after the final carcass wash or in the cooler (Figure 4.2). This may have been due to the carcasses having uniform levels of contamination across the swabbing areas on the carcasses. In an future replication of this study or in plants that use different processing systems the results could may find differences between sampling protocols for TCC and ECC.

SUMMARY

Mean TCC and ECC (log CFU/cm^2) for pork carcasses did not differ ($P > 0.05$) when samples were collected using the recommended 3-site sampling protocol versus the 2-site sampling protocol for samples collected before and after the final carcass wash or in the cooler. However, sampling pork carcasses in the cooler with the 2-site protocol resulted in an incidence of *Salmonella* spp. that was less than half of the 4.6% incidence detected with the 3-site protocol. *Salmonella* spp. was selected as the target pathogen for performance standards because it is one of the most common causes of foodborne illness; thus, inability to identify carcasses that have a pathogen on their surface would seem to be a major limitation of using the 2-site versus 3-site pork carcass sampling protocols and, from a food safety standpoint, it would be in the best interest of the swine industry to continue to use the 3-site sponge sampling protocol.

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CHAPTER V

MICROBIOLOGICAL CONTAMINATION ON PORK VARIETY MEATS

ABSTRACT

Bacterial counts and incidence of pathogens were determined from samples of 11 pork variety meats obtained after trimming/washing but before chilling/freezing (Site A) and after chilling/freezing (Site B), during normal production shifts, from 10 packing plants. At Site A, cheek meat, head meat, salivary glands and bung had the highest average aerobic plate counts (5.3 to 6.2 log CFU/g; APC), total coliform counts (4.2 to 4.6 log CFU/g; TCC) and *Escherichia coli* counts (3.6 to 4.3 log CFU/g; ECC). At Site B, APC were highest (5.1 to 5.6 log CFU/g) for cheek meat, salivary glands, tongue, chitterlings and bung, TCC were highest for cheek meat and chitterlings (3.6 and 4.1 log CFU/g, respectively) and ECC also were highest for cheek meat and chitterlings (3.1 and 4.0 log CFU/g, respectively). *Yersinia enterocolitica* was not detected on any pork variety meat samples (n = 405), and *Campylobacter jejuni/coli* was detected in fewer than 1% of variety meat samples. *Salmonella* spp. and *Listeria monocytogenes* were the most frequently detected pathogens on variety meat samples from the 10 plants, with isolation rates on samples of 15% and 16%, respectively. During this study, several opportunities for enhancing good manufacturing practices associated with production of pork variety meats were identified and are discussed.

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INTRODUCTION

Because of recent major *Escherichia coli* O157:H7 and *Listeria monocytogenes* foodborne illness outbreaks that resulted in loss of human life, significant public attention around the world is now focused on the incidence of foodborne illness, especially when it results from contamination that escapes detection by traditional food safety inspection methods. Outbreaks of foodborne illness, in addition to threatening private and public health and endangering lives, dramatically affect market growth and sales of U.S. pork products, particularly in international markets, because of the impact that such attention has on global consumer perceptions of food safety. A food safety dilemma can completely eliminate an opportunity to capitalize on a potential market, as was demonstrated in Taiwan where an outbreak of Foot-and-Mouth Disease resulted in the loss of Taiwanese market share for pork in Japan.

Based on preliminary information obtained for this study, approximately 60-70% of the pork variety meats produced in the U.S. are exported. Markets to which these items are exported currently include Hong Kong, Japan, Korea, China, Russia, Canada, Mexico, the Philippines and Poland. Contamination and perishability of pork variety meats must be addressed if consumers are to be assured that U.S. pork is safe and of high quality. Unfortunately, pork variety meats are often an afterthought in the overall scope of pork production. Traditionally, variety meats have been considered to have poor microbiological quality and a shorter shelf-life, particularly when they are distributed as chilled products (Gill and Jones, 1992). These high levels of contamination not only pose a risk to people who consume the product, but also increase the potential for cross-

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contamination of other food products by contaminated variety meats during processing and preparation in household or food service establishments.

This study was conducted to: (1) determine levels of pathogenic and other microbiological contamination on 11 common U.S. pork variety meats after trimming/washing and before packaging (Site A) and after chilling (Site B), and (2) identify areas of opportunity where practices and procedures used in the handling and processing of pork variety meats could be modified to enhance the bacteriological quality of pork variety meats.

MATERIALS AND METHODS

Pork variety meat sampling

Ten geographically dispersed U.S. pork packing plants were identified for variety meat sample acquisition. Included were plants that operate with various combinations of the following: 1) they utilized either sows/boars, market hogs, or both, 2) they either removed skins at slaughter or scalded and dehaired carcasses, and 3) they chilled carcasses either conventionally (-2 to 1°C; with and without spray chilling), with a moderately-quick chilling system (-28°C chill-factor for the initial 45 to 60 min), or with an extremely-quick chilling system (-62°C chill-factor for the initial 45 to 60 min).

Eleven pork variety meat items were sampled over a two-day period within each plant. Samples (n = 5) of the items produced at each plant (head meat, cheek meat, salivary glands, tongue, liver, heart, kidney, stomach, chitterlings, bung and front feet) were selected randomly (not from the same animals) at each of two locations in the plant: Site A—the point at which each variety meat was boxed or racked after trimming and

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washing, but before chilling or freezing; and, Site B—the point at which each variety meat was suitably chilled and deemed ready for domestic distribution or export (a minimum of 20 h in the freezer or chiller after Site A).

To develop a microbiological profile, samples were evaluated to determine aerobic plate counts (APC), total coliform counts (TCC) and *Esherichia coli* counts (ECC) at both processing sites (A and B) within the plant. Depending on the specific products produced in each plant, a total of 25 to 50 samples of each variety meat was evaluated for APC, TCC and ECC. In addition, all variety meat samples collected at Site B were evaluated for presence of *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni/coli* and *Yersinia enterocolitica*.

Samples were collected using aseptic techniques (sterile sample bags, latex gloves and sampling equipment pre-sterilized or sterilized using 82.2°C water). Due to the variant nature of different types of variety meats (different sizes, irregular surface areas, etc.), a 0.25 to 0.5 kg portion of products or, if appropriate, whole products (for those approximately 0.25 kg in weight) were obtained, cut and placed in a labeled sample bag (Whirl-Pak®, Nasco, Ft. Atkinson, WI). The products were sampled to include as much surface area as possible within the weight range limitations listed. Bagged samples were held under refrigeration (1.7 to 4.4°C) and then packaged in pre-chilled insulated shipping containers. Within 4 h of collection, the samples were shipped via overnight express carrier in coolers packed with commercial ice substitutes to a commercial laboratory for microbiological analyses. The commercial laboratory monitored the arrival of the samples to ensure that no temperature abuse was encountered.

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Microbiological analysis

Upon receiving samples, the microbiological laboratory measured the temperature of the samples in each box (shipments containing samples > 4°C were discarded). On the two separate occasions when samples were in excess of 4°C (due to postal strikes), the shipments were discarded and samples were re-collected during an additional plant visit. Samples were weighed and weights were recorded to allow calculation of $\log_{10}$ bacterial counts/g of product. A surface rinsing procedure was employed to dislodge bacteria from the irregular surface of each variety meat. A measured quantity (100 ml) of a buffer diluent was added to each sample in the bag, which was then sealed. Samples were then rinsed using a rocking motion of 30 shakes (approximately 1 min).

To quantifying APC, appropriate decimal dilutions from the samples were plated on Petrifilm™ Aerobic Count Plates (3M™ Microbiology Products, St. Paul, MN). The Petrifilm™ Aerobic Count Plates were incubated for 48 h at 35°C, and colonies were counted manually. To quantify TCC and ECC, appropriate decimal dilutions from the samples were plated on Petrifilm™ *E. coli* Count Plates (3M™ Microbiology Products). Total coliform counts were established by counting all red and blue colonies associated with one or more gas bubbles following 48 h incubation at 35°C and ECC were determined by counting only the dark blue colonies associated with a gas bubble. Presence of *Salmonella* spp., *L. monocytogenes* and *C. jejuni/coli* were determined using procedures recommended in the FSIS-USDA Microbiology Laboratory Guidebook (USDA-FSIS, 1977) and presence of *Y. enterocolitica* was determined using procedures in the Compendium of Methods for the Microbiological Examination of Foods (Speck, 1984). A detailed outline of the procedures that were

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used to identify *Salmonella* spp., *L. monocytogenes*, *C. jejuni/coli* and *Y. enterocolitica* is presented in Appendix I.

Observations concerning in-plant sanitation and handling practices

During in-plant data collection, records were kept regarding handling, processing and chilling of pork variety meats. These were used along with information and knowledge gained from several hours of in-plant observation, to identify areas of opportunity where practices and procedures used in the handling and processing of pork variety meats could be modified to enhance the bacteriological quality of pork variety meats.

Statistical analyses

Counts below the detectable limit of 1 CFU/cm^2 and 10 CFU/cm^2 were reported as 0.9 CFU/cm^2 and 9 CFU/cm^2, respectively. Bacteriological count data were transformed into logarithms before computing means and performing statistical analyses. All bacterial counts were reported as $\log_{10}$ CFU/cm^2. Data were analyzed using the general linear model (GLM) procedures of SAS® (SAS®, 1995). Plant was not used as a main effect in any of the statistical models in order to protect confidentiality with respect to identity of, and data from, the plants involved in the study. Sampling site was considered a main effect. Data were analyzed using the model $y = a + b_1x_1$ and means were computed for APC, TCC and ECC. When the F-test was significant ($P < 0.05$) means were separated using Tukey's HSD test.

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RESULTS AND DISCUSSION

Extent of microbiological contamination

Means and minimum/maximum values for APC, TCC and ECC obtained from the pork variety meats sampled are presented in Table 5.1. Surface contamination was highly variable from sample to sample, and also appeared to depend on the variety meat product evaluated. Products derived from the head (cheek meat, head meat, salivary glands and tongue) and from the gastrointestinal tract (chitterlings and bung), along with front feet, generally had higher levels of contamination than heart, liver, stomach or kidney. Microbiological profiles for each variety meat are presented in Appendix III and distribution of plate counts for each variety meat are presented in Appendix IV. Minimum and maximum counts for surface contamination levels for most products differed quite markedly. Heart and liver APC were the most variable at site A, with ranges of 6.2 log CFU/g (from 0.7 to 6.9 log CFU/g) and 6.7 (from 0.8 to 7.5 log CFU/g), respectively (Table 5.1).

The lowest average APC were detected on heart (4.0 log CFU/g at site A and 3.4 log CFU/g at site B). The lowest TCC and ECC were detected on heart and front feet (Table 5.1). Of greatest concern were the maximum counts obtained; most suggesting that at least some of each type of product was very close to (if not exceeding) levels of contamination normally thought to be associated with detectable spoilage (APC between 6.0 and 8.0 log CFU/g). Samples derived from salivary glands, chitterlings and bung generally had the highest minimum APC; but more importantly, these variety meats also had the largest minimum TCC and ECC, suggesting that these variety meats should be a

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Table 5.1. Mean, minimum and maximum aerobic plate counts, total coliform counts and *Escherichia coli* counts for 11 types of pork variety meats

			APC $\log_{10}$ CFU/g			TCC $\log_{10}$ CFU/g			ECC $\log_{10}$ CFU/g		
Variety Meat	Site	n	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max
Cheek Meat	A	45	5.3	3.8	7.4	3.9	1.8	6.0	3.6	0.9	6.0
	B	45	5.2	3.6	7.3	3.6	1.0	6.6	3.1	0.9	6.2
Head Meat	A	40	5.7^{a}	4.4	7.5	4.3^{a}	1.4	6.4	3.8^{a}	0.9	6.4
	B	40	4.7^{b}	3.4	5.8	2.8^{b}	1.0	5.3	2.2^{b}	1.0	4.5
Salivary Glands	A	35	6.2^{a}	4.7	7.6	4.6^{a}	2.7	6.2	4.3^{a}	2.2	6.0
	B	35	5.5^{b}	3.9	7.2	3.1^{b}	1.0	6.2	2.8^{b}	1.0	6.2
Tongue	A	45	5.3	3.2	7.2	3.0	0.9	6.0	2.5^{a}	0.8	6.0
	B	45	5.1	3.0	6.7	2.2	0.8	5.6	1.9^{b}	0.7	5.6
Heart	A	44	4.0^{a}	0.7	6.9	2.0	0.6	5.6	1.6	0.6	5.6
	B	45	3.4^{b}	0.7	6.4	1.7	0.7	3.7	1.1	0.6	2.3
Liver	A	35	4.9^{a}	0.8	7.5	3.6^{a}	0.8	6.0	3.2^{a}	0.8	5.8
	B	35	4.0^{b}	1.4	6.6	2.7^{b}	1.0	5.8	2.6^{b}	0.8	5.4
Stomach	A	50	4.8	2.2	7.1	3.4	0.8	6.0	2.9	0.8	6.0
	B	50	4.7	1.6	7.3	3.2	0.8	5.8	2.7	0.8	5.8
Kidney	A	30	5.0^{a}	2.3	6.8	3.2^{a}	0.7	4.9	2.4^{a}	0.7	4.3
	B	30	4.1^{b}	2.4	6.5	2.0^{b}	1.0	4.2	1.4^{b}	1.0	2.4
Chitterlings	A	25	5.5	4.0	7.1	4.2	3.5	5.1	4.1	3.4	5.1
	B	25	5.5	4.9	6.3	4.1	3.5	5.3	4.0	3.2	5.2
Bung	A	30	6.0	3.9	7.3	4.5^{a}	2.2	6.0	4.3^{a}	2.0	6.0
	B	30	5.6	4.6	8.0	2.8^{b}	0.8	4.9	2.6^{b}	0.8	4.9
Front Feet	A	40	5.2^{a}	3.9	6.8	2.0	0.6	4.6	1.3	0.5	4.2
	B	30	4.7^{b}	3.3	6.1	1.8	0.6	4.5	1.5	0.6	3.6

[ab]Means within a variety meat bearing a common superscript letter are not different between sites ($P < 0.05$).

target for use of improved good manufacturing practices and/or decontamination technologies during processing.

Mean APC differed ($P < 0.05$) by sampling site (A vs B) for head meat, salivary glands, heart, liver, kidney and front feet (Table 5.1). Mean TCC differed ($P < 0.05$) by sampling site for head meat, salivary glands, liver, kidney and bung. Mean ECC differed ($P < 0.05$) by sampling site for head meat, salivary glands, tongue, liver, kidney and bung. In all cases (APC, TCC, ECC), counts were lower at site B (the point from which variety meats would be distributed for consumption) than at site A (the point immediately post-harvest at which the products were placed on racks or boxed, before chilling), suggesting that good manufacturing practices are used by the plants during packaging and chilling and that recontamination and growth of bacteria during packaging and handling was minimized. However, neither APC, TCC or ECC for cheek meat, stomach or tongue were reduced between site A and site B, suggesting that it may be important for pork packing plants to consider improving the cooling rates of these products and to consider implementing use of decontamination systems for these pork variety meats.

Caspar *et al.* (1984) stated that microbiological quality of pork liver (presumably this would pertain to all pork variety meats) was dependent on hygienic practices during slaughter, processing and storage. The rate of cooling for variety meats is one of the most important factors in preventing high bacterial counts (Sheridan and Lynch, 1988; Gill 1988). Unfortunately though, most variety meats are collected in bulk containers or lugs, and then packed in boxes before they are adequately chilled (Gill and Jones, 1992). These boxes are then stacked and palletized making it even more difficult to cool the variety meat products. Laubach *et al.* (1998) reported that changing harvesting practices

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was not sufficient, in and of itself, to reduce contamination on pork head meat and they concluded that the use of interventions approved for animal carcasses should be investigated to generate products with lower levels of microbiological contamination.

Incidence of pathogenic organisms

There is strong indirect evidence that pigs and food products of porcine origin are the major sources for human infection with *Yersinia enterocolitica* serogroups O:3 and O:9 (Kapperud, 1991; Funk *et al.*, 1998), which are the predominant types of *Y. enterocolitica* associated with human yersiniosis (de Boer and Nouws, 1991; Funk *et al.*, 1998). Pathogen incidence for pork variety meats sampled in this study are presented in Table 5.2. No *Y. enterocolitica* were detected on any of the variety meats sampled. This may have been because *Yersinia* tends to be found in tonsils and other areas of the throat (Kapperud, 1991; de Boer and Nouws, 1991) and therefore, were not detected in this study. From the positive perspective though, these data implied that the risk of foodborne illness from *Y. enterocolitica* would be relatively low from the pork variety meats evaluated in this study. *Campylobacter jejuni/coli* were only detected on a total of four variety meat samples (an incidence of less than 1%); one positive incident occurred for each of head meat, salivary glands, heart and bung. However, *C. jejuni/coli* was present on samples obtained from three different plants (out of the 10 included in the study), suggesting that, while the overall risk of contamination with *C. jejuni/coli* may be low, its prevalence may be fairly widespread.

Across all variety-meat samples, *Salmonella* spp. and *L. monocytogenes* were the

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Table 5.2. Incidence of pathogens on pork variety meats at sampling site B (the point at which each variety meat was suitably chilled and deemed ready for domestic distribution or export; a minimum of 20 h in the freezer or chiller after site A)

		Campylobacter jejuni/coli		*Salmonella* spp.		*Listeria monocytogenes*		*Yersinia enterocolitica*	
Item	n	Positive[a]	%	Positive[a]	%	Positive[a]	%	Positive[a]	%
Cheek Meat									
Samples	45	0	0	6	13	15	33	0	0
Plants	9	0	0	3	33	5	56	0	0
Head Meat									
Samples	40	1	3	12	30	2	5	0	0
Plants	8	1	13	4	50	2	25	0	0
Salivary Glands									
Samples	35	1	3	5	14	6	17	0	0
Plants	7	1	14	3	43	3	43	0	0
Tongue									
Samples	45	0	0	10	22	5	11	0	0
Plants	9	0	0	3	33	3	33	0	0
Heart									
Samples	45	1	2	2	4	1	2	0	0
Plants	9	1	11	2	22	1	11	0	0
Liver									
Samples	35	0	0	11	31	0	0	0	0
Plants	7	0	0	5	71	0	0	0	0
Stomach									
Samples	50	0	0	0	0	27	54	0	0
Plants	10	0	0	0	0	8	80	0	0
Kidney									
Samples	30	0	0	1	3	0	0	0	0
Plants	6	0	0	1	17	0	0	0	0
Chitterlings									
Samples	25	0	0	8	32	0	0	0	0
Plants	5	0	0	4	80	0	0	0	0
Bung									
Samples	30	1	3	4	13	0	0	0	0
Plants	6	1	17	2	33	0	0	0	0
Front Feet									
Samples	25	0	0	0	0	7	28	0	0
Plants	5	0	0	0	0	2	40	0	0
Total									
Samples	405	4	1	59	15	63	16	0	0
Plants	10	3	30	10	100	8	80	0	0

[a]Number of positive samples from those tested; or number of plants with at least one positive sample.

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most predominant pathogens isolated (present on 15% and 16% of the samples, respectively). In fact, *Salmonella* spp. was detected on all variety meats evaluated except for stomach and front feet, and *L. monocytogenes* was present on all but liver, kidney, chitterlings and bung (Table 5.2). *Salmonella* spp. and *L. monocytogenes* were detected on pork variety meats obtained from 10 and 8 of the 10 plants sampled in this study (Table 5.2), respectively, suggesting that these organisms are prevalent in most pork slaughter facilities and that it is more difficult to control the extent to which pork products are contaminated with these organisms than it is to control contamination from the other pathogens evaluated in this study.

Pork variety meats that were at the greatest risk of being contaminated with *Salmonella* included head meat, tongue, liver and chitterlings (30%, 22%, 31% and 32% of the samples from these variety meats, respectively, tested positive for *Salmonella* spp.; Table 5.2). *Salmonella* spp. was present in 33% to 80% of the plants that produced head meat, tongue, liver and chitterlings (Table 5.2). Products that were at the greatest risk of being contaminated with *L. monocytogenes* included cheek meat, salivary glands, stomach and front feet (33%, 17%, 54% and 28% of the samples from these variety meats, respectively, tested positive for *L. monocytogenes*; Table 5.2). *Listeria monocytogenes* was present in 40% to 80% of the plants that produced cheek meat, salivary glands, stomach and front feet (Table 5.2).

Surprisingly, the list of products that were at risk of being contaminated with *L. monocytogenes* differed completely from those variety meats at risk of being contaminated with *Salmonella* spp. Thus, if packing companies are to improve the safety of pork variety meats, improved good manufacturing practices and/or bacterial

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decontamination techniques should, at a minimum, be utilized to reduce the incidence of *Salmonella* spp. and *L. monocytogenes* on eight of the 11 variety meats (cheek meat, head meat, salivary glands, tongue, liver, stomach, chitterlings and front feet) evaluated in this study. Special attention must also be paid to reducing APC, TCC and ECC on cheek meat, salivary glands, tongue, stomach, chitterlings and bung, especially at site B. Improvements in good manufacturing practices need to be identified at every step of preparation and handling during harvesting, processing and chilling/freezing of pork variety meats, especially (but not exclusively) those that are in direct contact with feed and ingesta.

Observations concerning in-plant sanitation and handling practices

During plant visits to collects samples we also observed handling procedures, application of decontamination procedures and overall plant sanitation that ranged from "very good" to "poor". Overall, a majority of variety meat processing systems were very good, nevertheless, some changes in procedures and practices could substantially improve the microbiological quality of pork variety meat products.

Product transfer chutes

Many plants use stainless steel chutes to transfer variety meats from the slaughter floor to the variety meat processing area. These chutes may become contaminated from "dirty" (microbiologically or physically) variety meats and are, for the most part, in an environment that can be very warm (ambient temperature). These conditions may allow bacteria to proliferate on the surfaces of the chute over the course of a single production

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shift and cross- or re-contaminate many other variety meats that travel down that same chute. Possible solutions to this problem may include redesigning the handling system to eliminate the use of chutes and using a chain system to move product, or utilizing an organic acid drip or spray in the chute that will both facilitate product movement down the chute and help reduce numbers of bacteria that may become attached to the surface of the chute.

Fresh-product holding time

Some products were held for an excessive period of time (> 45 min) on the slaughter floor before being transported to the processing area or until personnel become available to process or move them. This holding time, in a less-than-ideal environment, maximizes the potential for bacteria to multiply during holding. Maximum product holding times on the slaughter and processing floors must be implemented and enforced at all times. Until such time that ambient temperatures in both slaughtering/dressing and variety meat processing areas are reduced from ideal growth temperatures for bacterial proliferation, the time that variety meats are held on the floor must be minimized. This does not mean that products should be processed as fast as possible with no regard to other considerations, but that variety meats cannot be allowed to remain in a plastic lug, box, etc., for a lengthy period of time while waiting to be processed.

Personal equipment sanitation

In many cases, it was observed that no areas were available in which employees could adequately clean their aprons, gloves, knives and other personal equipment. This

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situation resulted in circumstances in which equipment could become encrusted with blood and product residue. The use of rubber or latex gloves is a commonly employed method for reducing the spread of contamination among pork products throughout a meat plant. Gloves can be a useful tool, but often are not washed or changed with sufficient frequency to ensure that their surfaces are always clean and sanitary. Specific areas in the plant must be provided for employees to adequately wash and clean their personal equipment (knives, gloves, etc.). This area must be large enough for all employees, that are moving from workstations to a break room or cafeteria, to have space in which to clean their equipment thoroughly before returning to work.

Knife sanitizers

Hot-water sanitizers (>82.2°C; commonly referred to as sterilizers) were used extensively in meat processing plants as a method for quick decontamination of knives and other hand-held equipment. In many instances, sanitizers were either not present, contained water below 82.2°C, or were not being used continuously/correctly in the variety-meat processing area. Scientists observed sanitizers without adequate water levels, with large amounts of visible contaminants present and in locations that were not close enough in proximity to be used effectively by the employees. Sanitizers must contain adequate water that is of high temperature and allows for complete immersion and sanitizing of the whole knife or other utensil. Most importantly, sanitizers must be located so that employees can reach and readily use them. Employees should be instructed to regularly use sanitizers and be educated in their correct use.

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Cross-contamination

Some variety meats were processed with hot water and chemicals and were probably cleaner after processing than before. However, in some cases, as these products were removed from the scalder, they were cross-contaminated. In several instances, standing water remained on the table surfaces where scalded stomachs were dumped from the scalder before packaging. The high incidence of *L. monocytogenes* on stomachs in this study (Table 5.2) suggested that standing water on tables may be a source for re-contamination with this pathogen just before packaging. Variety meats that have been scalded, cleaned or otherwise decontaminated must not be allowed to come into contact with contaminates (blood, standing water, water splashing from the floor, etc.) or contaminated facilities, equipment, etc. It may be necessary to apply a sanitizing solution or an organic acid rinse to these areas/equipment more frequently to reduce the possibility of re- or cross-contamination.

Pallet spacing and freezers

Many high-velocity blast freezers were well maintained and reduced the core temperature of a pallet of variety meats. But, in some cases, freezers were overstocked with product, and, as a result, airflow around the hot product was severely limited. Many variety meats are small and it may take up to 2 h or more to completely load a pallet with boxed product (depending on the size of the product and the line speed). While waiting to complete a pallet to move it to the freezer, product that was already boxed and stacked on the pallet was often at a temperature that was conducive for bacterial growth. Pallets must be promptly placed in the freezer and in a manner that will allow for maximum

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airflow around the product. Ideally, boxes to be stored frozen should be frozen (via a spiral chilling type system) before palletization. It may be necessary to freeze incomplete pallets and add product to those pallets periodically as more is produced.

Weighing

Certain variety meats were scaled to obtain a "catch weight," while others were scaled to obtain a "fixed net weight" (products packed in pre-labeled containers). For products packed into pre-labeled containers at predetermined net weights, the amount of product in that container was adjusted either by removing or adding pieces of product to arrive at the correct weight. It was common to hold small quantities of product, for use in achieving a specific net weight, in the scaling area. These products sometimes were left in this area for lengthy periods of time and/or without proper refrigeration; a protocol likely to compromise their microbiological integrity. If containers are labeled based upon a preset net weight, then the small pieces of product that are stockpiled in the processing area and used to achieve the acceptable weight should not be stored at, or near, the scaling area for extended periods of time. Using small pieces of product that have been temperature-abused, handled extensively and generally mistreated will succeed in contaminating, cross-contaminating and re-contaminating all of the product in an otherwise clean and sanitary box of product.

Rework

Rework can be derived from many sources, including from damaged boxes in the plant and returned product. Many plants had very specific rework policies that dictated

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that any rework product must be handled before the end of that shift. Immediate disposition of rework must be made a priority. Rework from boxes whose integrity has been compromised and product that has come in contact with pallets should be discarded.

Hand inspection

Several plants used a series of repeated hand inspections (piece by piece) to remove visible defects in products such as cheek meat and lips. In many cases, this type of inspection was conducted with little regard to cross-contamination. In some plants, there was little regard given to the cleaning of gloves as the worker moved from one box to another, and workers handled cardboard boxes and product with the same gloves without cleaning the gloves between handling the product and boxes. Piece-by-piece inspection may be warranted to insure removal of bone chips or physical defects from the product. If every piece of product in a box is going to be physically handled, then specific precautions must be taken. Gloves must be washed and sanitized/decontaminated and, to minimize the spreading of contamination, there should never be an instance in which the outside of a box is touched with gloves and then the product is subsequently handled again without sanitizing those gloves.

Handling

Several plants made use of a variety meat chain (often referred to as a pluck chain) to transport variety meats from the slaughter floor to the processing area. Use of a pluck chain is a good practice because the products are treated as individual units and cross-contamination is minimized. However, in most systems, these advantages were

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quickly lost because the products were removed from the chain (either intentionally or unintentionally) and piled up on a table before further processing. Some plants utilized a pluck chain to carry liver to the processing area where it was then removed from the hook and placed onto an uncleaned table in a pile with other livers that needed trimming. It would be advantageous if variety meats could be treated as separate units throughout the entire processing sequence. The process of trimming livers on an inspection table, transporting them via a pluck chain to the packaging area, bagging them on the chain (using the bag to remove the liver) and then boxing them would unquestionably minimize possibilities for cross-contamination.

Work areas

Proper working-environment temperature and cleanliness of work surfaces were two areas that were observed to be problematic. Processing rooms where ambient temperatures exceed approximately 21-27°C and where working surfaces are covered with a layer of blood residue can negatively impact the bacteriological quality of the products. With improvements that have occurred in refrigeration technology, serious consideration should be given to evaluating use of increased refrigeration (to reduce the temperature) in variety meat processing rooms. In addition, work surfaces must be cleaned at standardized and specific intervals. Regular cleaning of work surfaces will help prevent the growth of bacteria and minimize the cross-contamination of pork variety meats.

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Clean-up procedures

It was customary during breaks in production (including mealtimes) and during the shift change to wash down the production area with water. Product that was in the production area while such washing was performed was usually covered with plastic sheets. During these "clean-up" periods, there were considerable quantities of aerosols created, and water from cleaning splashed onto product. These aerosols and splash-water could have contained bacteria that contaminated the product. Variety meats must be removed from the production area before using water to clean up. As was previously recommended, regular clean-up protocols and procedures are essential to producing clean products. However, every effort must be made to remove all products from the area that is to be cleaned in order to prevent cross- and re-contamination of pork variety meats.

SUMMARY

Variety meats are an important component of pork value; and the bacteriological safety of variety meats can have a major impact on pork consumption, particularly in export markets. In this study, levels of surface bacteria contamination on pork variety meats were variable and somewhat dependent on the specific product evaluated. Aerobic plate counts, total coliform counts and *E. coli* counts were generally higher on the surfaces of products from the head and gastrointestinal tract. Surface bacteria counts for some variety meats (particularly head meat, salivary glands, livers and kidney) that were collected immediately post-harvest, before packaging and chilling, generally were higher than bacteria counts on the same variety meats after chilling and at the point at which they were ready for shipment and distribution. This suggested that pork packing plants

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are using good manufacturing practices during variety meat processing, packaging and chilling. *Yersinia enterocolitica* was not detected on pork variety meats, and *C. jejuni/coli* was detected on less than 1% of variety meat samples. *Salmonella* spp. and *L. monocytogenes* were the most frequently detected pathogens and were found on 15% and 16% of the samples and in 100% and 80% of the plants, respectively. It appears that packing plant efforts to decontaminate at least eight of the 11 products sampled would provide meaningful improvement in the safety of pork variety meats.

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CHAPTER VI

APPLICATION OF TECHNOLOGIES TO REDUCE CONTAMINATION OF PORK VARIETY MEATS

ABSTRACT

Two studies were conducted to determine the effectiveness of decontamination treatments in reducing aerobic plate counts (APC), total coliform counts (TCC) and *Escherichia coli* counts (ECC) on pork variety meats. Study I evaluated immersing pork cheek meat, heart, liver, tongue, stomach, chitterlings and salivary glands in solutions of chlorine (50 ppm, 48-50°C, pH 6.5); hot water (75-80°C); hydrogen peroxide (5%, 48-50°C, pH 6.5); trisodium phosphate (12%, 48-50°C, pH 12.0); acetic acid (2%, 48-50°C, pH 2.8); lactic acid (2%, 48-50°C, pH 2.5) for 10 sec or spraying the variety meats with hot water (75-80°C); acetic acid (2%, 45-50°C, pH 2.8); lactic acid (2%, 45-50°C, pH 2.5) for 10 seconds or applying a steam application (82°C) for 30 sec. Study II evaluated the efficacy of trisodium phosphate, acetic acid, lactic acid or acidified sodium chlorite (1200 ppm, 22°C, 10 sec) immersion solutions for decontaminating pork tongue. In study I, hot water, steam and hydrogen peroxide treatments resulted in discoloration of the red variety meats and immersion in hydrogen peroxide generated a foam that interfered with application and product packaging. The greatest reductions in contamination, without altering the appearance of the products, were obtained with trisodium phosphate, acetic acid and lactic acid. In study II, trisodium phosphate, acetic

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acid, lactic acid and acidified sodium chlorite were all effective in reducing ($P < 0.05$) APC, TCC and ECC. Use of decontamination treatments that are efficacious for reducing microbiological contamination on pork carcasses should reduce the microbiological contamination on pork variety meats.

INTRODUCTION

In contrast to decontamination treatments used for pork carcasses, there is limited information dealing with decontamination techniques for pork variety meats. An extensive amount of the variety meats (offal) offered for human consumption may be of poor microbiological quality (Gill and Jones, 1992). Gardner (1971) reported that initial APC on fresh liver ranged between 4.0 and 5.0 log CFU/cm^2 and, after 7 days of refrigerated storage at 5°C, APC were between 8.0 and 9.0 log CFU/cm^2. Caspar *et al.* (1984) reported that pork liver obtained from slaughter houses and retail outlets for a survey in the Netherlands had APC of 6.0 to 6.5 log CFU/cm^2 and ECC of 4.0 to 5.0 log CFU/cm^2 at both locations. Oblinger *et al.* (1982) reported that pork liver had initial APC between 3 to 3.5 log CFU/cm^2. Hanna *et al.* (1982b), reported the APC of fresh liver, heart and kidney were less than 4.0 log CFU/cm^2, and often less than 3.0 log CFU/cm^2. After frozen storage for 5 days at -20°C, there were no significant changes in APC for pork livers, hearts and kidneys, but when the products were subjected to temperature abuse for 6 to 12 h at 30°C, there were large significant increases in microbiological counts (Hanna *et al.*, 1982b). Frederick *et al.* (1994) reported that pork cheek meat had APC that ranged from 4.1 to 4.6 log CFU/cm^2

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and TCC of 2.3 to 2.4 log CFU/cm^2. Laubach *et al.* (1998) reported that pork head meat stored at 3 ±1°C had APC that were constantly in the range of 4 to 5 log CFU/g, TCC of 2 to 3 log CFU/g and ECC of 1.5 to 2.5 CFU/g.

2. High contamination levels lead to poor keeping qualities and are the result of inappropriate collection and cooling procedures which result in initial contamination and proliferation of spoilage organisms (Gill, 1988). Woolthuis *et al.* (1984) conceded that variety meat products cannot be removed from the carcass without encountering some contamination, and therefore, decontamination treatments are a legitimate means to produce a cleaner, safer product. There is a consensus throughout the literature and among personnel in the industry that decontamination treatments should not be used as a substitute for hygienic practices or SSOPs (Sanitation Standard Operating Procedures) and GMPs (Good Manufacturing Practices) but, rather, as a part of an integrated system to ensure that consumers receive a wholesome, high-quality product. The objectives of this study were to evaluate decontamination techniques for potential use in pork packing plants to reduce APC, TCC, ECC and to extend the storage life of pork variety meats.

MATERIALS AND METHODS

Pork variety meats and sample size

Seven pork variety meat products were selected to test decontamination treatments based on the results of Chapter V - Microbiological Contamination on Pork Variety Meats. These included cheek meat, heart, liver, salivary glands, chitterlings, stomach and tongue. Variety meat samples were collected from two large commercial pork-packing facilities. Within 4 h of collection, the samples were shipped via overnight

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express carrier in insulated containers packed with commercial ice substitutes to Colorado State University, Fort Collins, CO.

Variety meat sample sizes were as follows: cheek meat - whole piece, approximately 150 to 200g; salivary gland - whole piece, approximately 40 to 60g; tongue - whole tongue, approximately 200 to 300g; chitterlings - 13cm portion, approximately 75 to 150g; heart - ½ of a heart, approximately 150 to 300g; liver - a portion of a lobe, approximately 140 to 220g; and stomach - a portion, approximately 100 to 125g.

Decontamination treatments

Treatments evaluated in study I were: control samples (CNTR); chlorine (sodium hypochlorite; 50 ppm; 48-50°C, pH adjusted to 6.5) immersion (i.e., dipping) for 10 sec (CLDP); hot water (75-80°C, 10 sec) immersion for 10 sec (H2ODP); hot water (75-80°C, 10 sec) spray for 10 sec (H2OSP); hydrogen peroxide (5% v/v, 48-50°C, pH 6.5, prepared from 30% hydrogen peroxide, Fisher Scientific, Fair Lawn, NJ) immersion for 10 sec (H2O2DP), trisodium phosphate (12% wt/v, 48-50°C, pH 12.0, prepared from trisodium phosphate hydrate, Rhone-Poulenc, Cranbury, NJ) immersion for 10 sec (TSPDP); acetic acid (2% v/v, 48-50°C, pH 2.8, prepared from glacial acetic acid, Mallinckrodt Baker, Inc., Paris, KY) immersion for 10 sec (AADP); acetic acid (2% v/v, 45-50°C, pH 2.8) spray for 10 sec (AASP); lactic acid (2% v/v, 48-50°C, pH 2.5, prepared from 98% lactic acid, Sigma Chemical Co., St. Louis, MO) immersion for 10 sec (LADP); lactic acid (2% v/v, 45-50°C, pH 2.5) spray for 10 sec (LASP); and steam cabinet (82°C) spray for 30 sec (STSP). The treatments in study II were CNTR, TSPDP,

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AADP, LADP and acidified sodium chlorite (1200 ppm, 26°C, pH 2.5, Sanova; Novus International, Inc., St. Louis, MO) immersion for 10 sec (ASCDP).

For both studies I and II, immersion treatments (CLDP, H2ODP, H2O2DP, TSPDP, AADP, LADP, ASCDP) were prepared (with tap water) by mixing 2 L of the appropriate solution in a stainless steel container and heating the solution (in the container) in a water bath. When the solution reached the proper temperature, the variety meat product was held with sterile forceps and immersed in the liquid for 10 sec. Spraying treatments (H2OSP, AASP and LASP) were prepared (with tap water) by mixing 20 L of the appropriate solution in a plastic container at a temperature slightly higher than the desired temperature. All spraying treatments were applied with a model, custom-made spray-washing/sanitizing unit (M. E. Anderson Engineering, Hallsville, MO). Spraying treatments were applied on each side of the product for 10 sec at pressures of 35 to 40 psi. The steam treatment was conducted utilizing a commercial steam cabinet (Alkar Model 450 Mini Smoker, Alkar, a division of DEC International, Lodi, WI) preheated to 82.2°C. Samples were exposed to steam for 30 sec followed by a 15 sec water (10°C) wash.

Natural levels of contamination on the variety meat products were sufficient to address the objectives of this study and no additional inoculation was necessary. Immediately after application of the appropriate treatment, samples were placed into sterile bags (Whirl-Pak®, Nasco, Ft. Atkinson, WI) and weights were recorded. The bagged samples were then held for 10 min at ambient temperature before microbiological analysis to simulate the time between the application of the treatment and packaging in a commercial facility.

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Microbiological analysis

For both Studies I and II, a surface rinsing procedure was employed to dislodge bacteria from the irregular surface of samples of each variety meat. A 100 ml quantity of Butterfield's phosphate buffer (Difco Laboratories, Detroit, MI) was added to each sample bag. The bags were sealed and the samples were rinsed using a rocking motion for 30 shakes (approximately 1 min). Appropriate decimal dilutions were plated on tryptic soy agar (TSA; Difco Laboratories) using a spiral plating system (Model D, Spiral Biotech, Inc., Bethesda, MD) for determination of aerobic plate counts (APC). Plates were incubated for 48 h at 35°C, and colonies were counted with a laser bacteria colony counter (Model 500 A, Spiral Biotech, Inc.) and a CASBA 4 data processor (Model 800, Spiral Biotech, Inc.). To quantify TCC and ECC, appropriate decimal dilutions from the samples were plated on Petrifilm™ *E. coli* Count Plates (3M™ Microbiology Products). Total coliform counts were established by counting all red and blue colonies associated with one or more gas bubbles following 48 h incubation at 35°C and ECC were determined by counting only the dark blue colonies associated with a gas bubble.

Statistical analysis

Bacteriological count data were transformed into base-ten logarithms before computing means and performing statistical analyses. All bacterial counts were reported as mean value $\log_{10}$ CFU/10g. Counts below the detectable level were reported as 0.2 $\log_{10}$ CFU/10g. Data were analyzed using the general linear model (GLM) procedures of SAS® (SAS®, 1995). Treatment was considered a main effect. Data were analyzed

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using the model $y = a + b_1x_1$ and means were computed for APC, TCC and ECC. When the F-test was significant ($P < 0.05$) means were separated using Tukey's HSD test.

RESULTS AND DISCUSSION

Study I

Comparisons across all decontamination treatments applied to each specific variety meat in study I are presented in Figures 6.1 through 6.7. Effects of each of the 10 decontamination treatments applied to pork variety meat products in reducing ($P < 0.05$) APC, TCC and ECC, are presented in Tables 6.1, 6.2 and 6.3, receptively. The AASP, AADP, LASP and LADP treatments significantly reduced APC on pork cheek meat by 1.3 to 1.7 log_{10} CFU/10g (Table 6.1). For both TCC and ECC, the TSPD, AASP, AADP, LASP and LADP treatments reduced ($P < 0.05$) levels of contamination. Cheek meat treated with LASP and LADP had > 1 log_{10} CFU/10g for both TCC and ECC (Figure 6.1).

All nine decontamination treatments applied to heart reduced ($P < 0.05$) APC by 2.1 to 3.7 log_{10} CFU/10g. The CLDP was not effective ($P > 0.05$) in reducing TCC or ECC on hearts. The TSPDP, AASP, AADP and LADP heart samples had less than 1 log_{10} CFU/10g TCC (Figure 6.2). The control heart samples had relatively low ECC (1.3 log_{10} CFU/10g), however, all treatments except chlorine reduced ECC to 0.3 log_{10} CFU/10g or less.

For liver samples, H2OSP, TSPDP, AASP, AADP, LASP and LADP reduced ($P < 0.05$) APC by 0.7 to 1.2 log_{10} CFU/10g. All treatments except CLDP and STSP significantly reduced TCC on liver samples, with levels of reduction from 1.2 to 2.5 log_{10}

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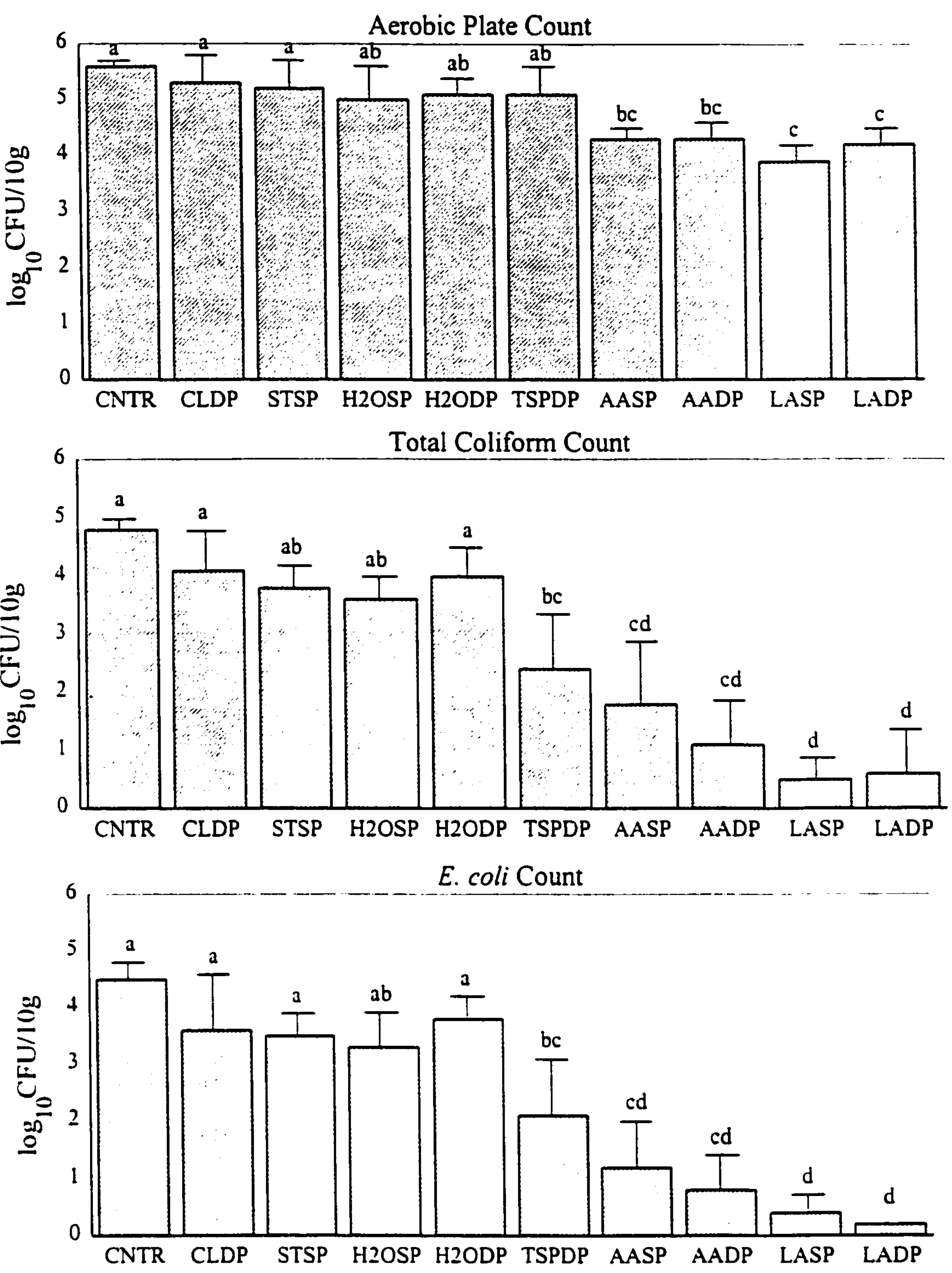

Figure 6.1. Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork cheek meat. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were- CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion). [abcd]Means bearing common superscript letters within bacteria type are not different ($P > 0.05$).

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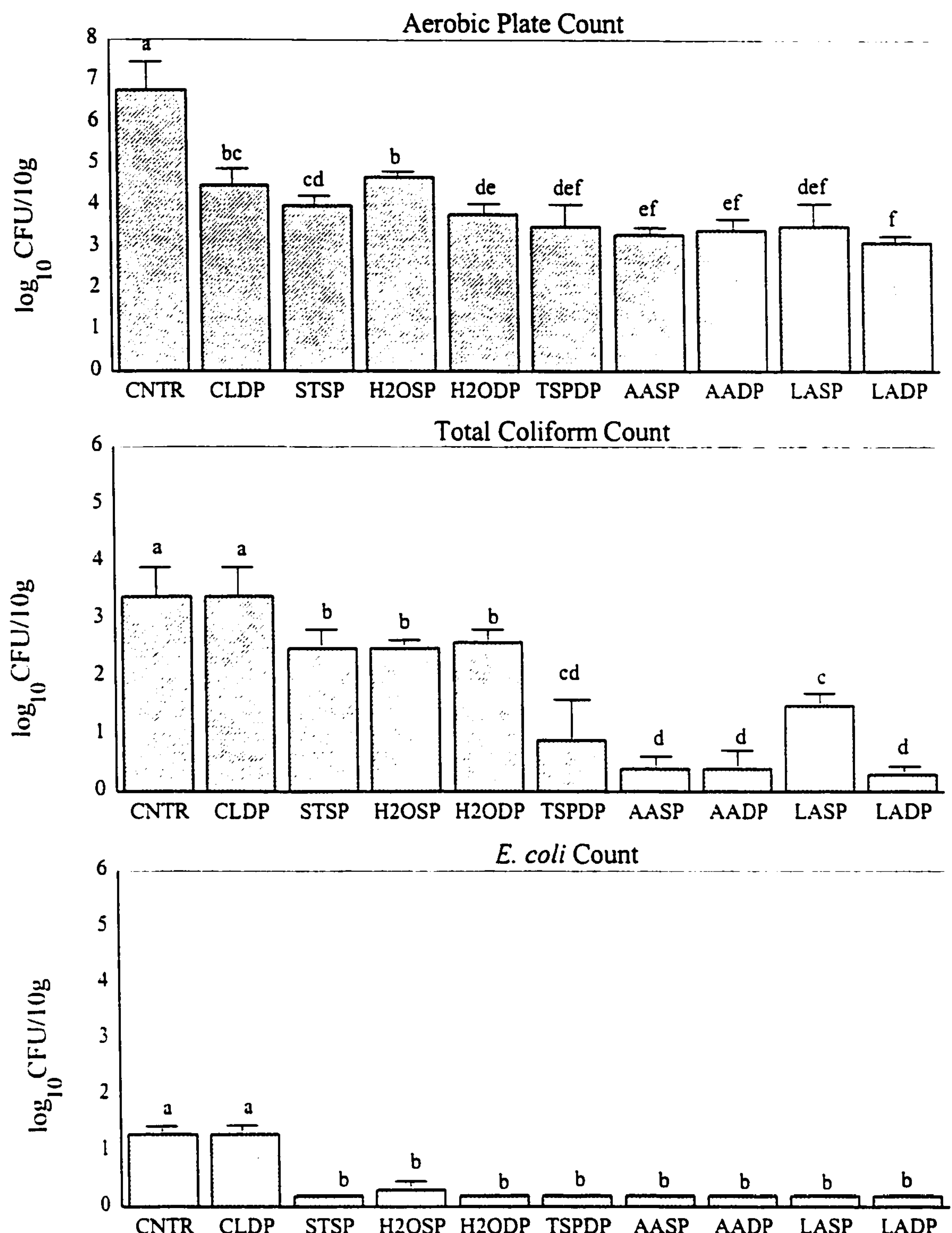

Figure 6.2. Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork heart. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were- CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion). [abcd]Means bearing common superscript letters within bacteria type are not different ($P > 0.05$).

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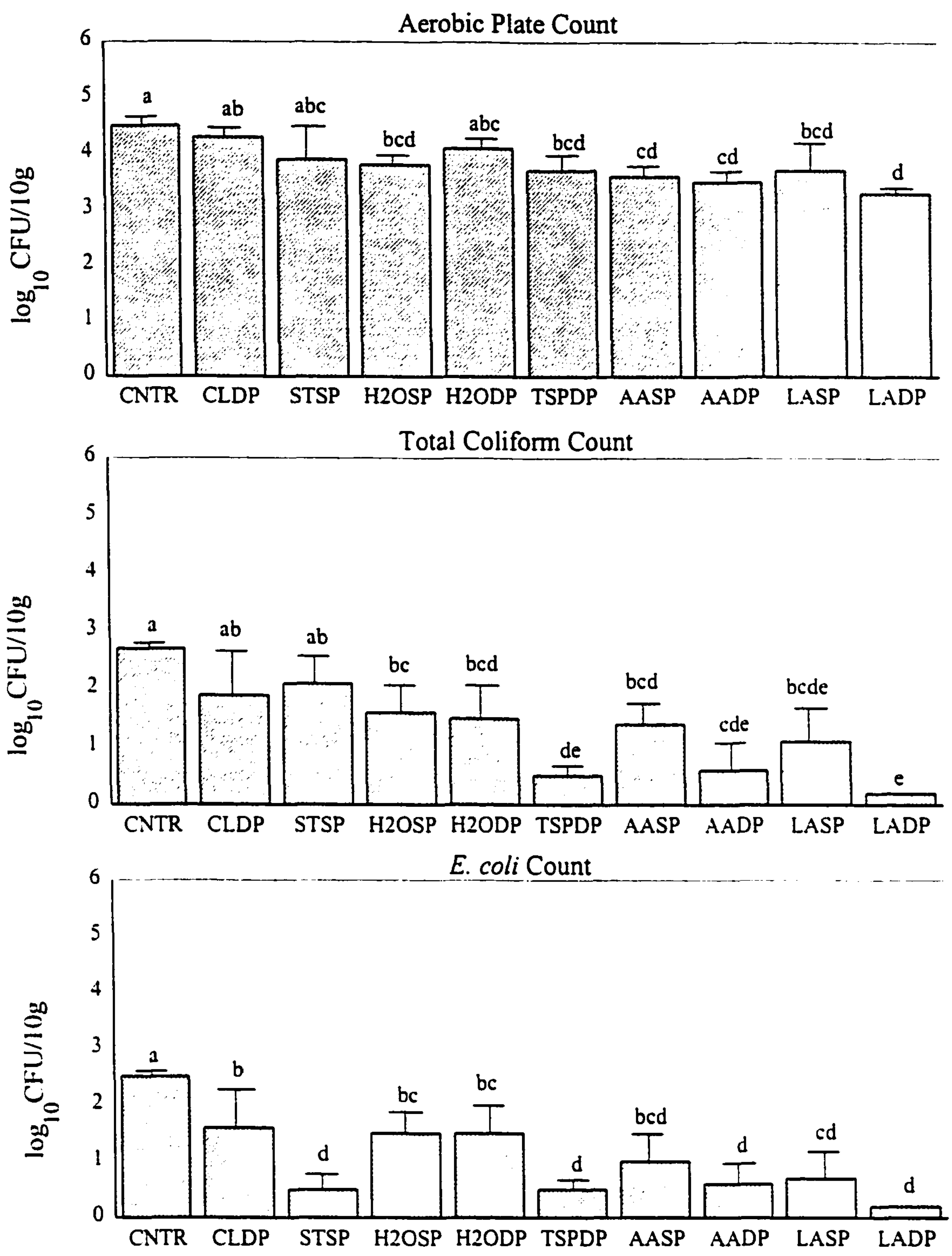

Figure 6.3. Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork liver. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were- CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion). [abcd]Means bearing common superscript letters within bacteria type are not different ($P > 0.05$).

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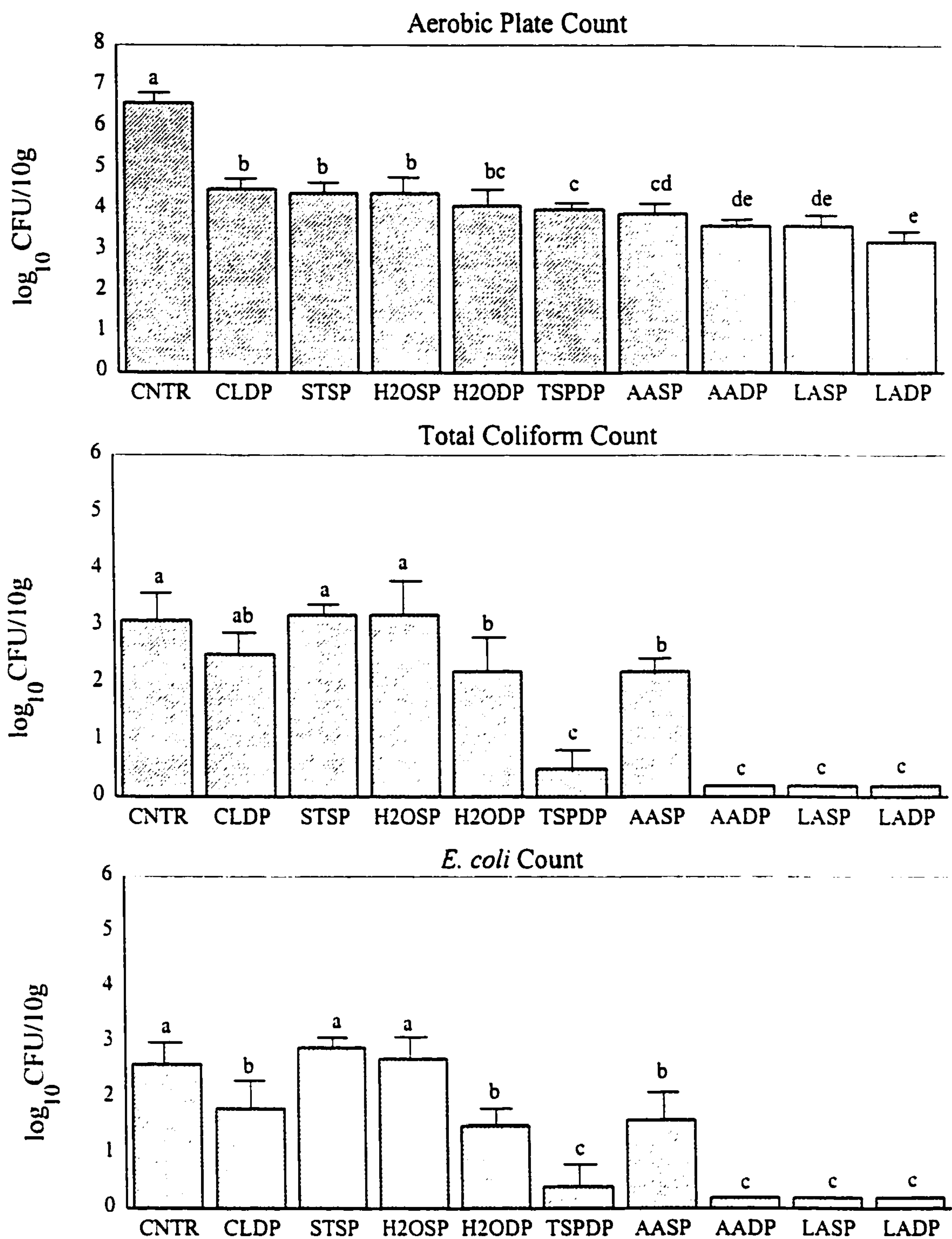

Figure 6.4. Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork salivary glands. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were- CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion). [abcd]Means bearing common superscript letters within bacteria type are not different ($P > 0.05$).

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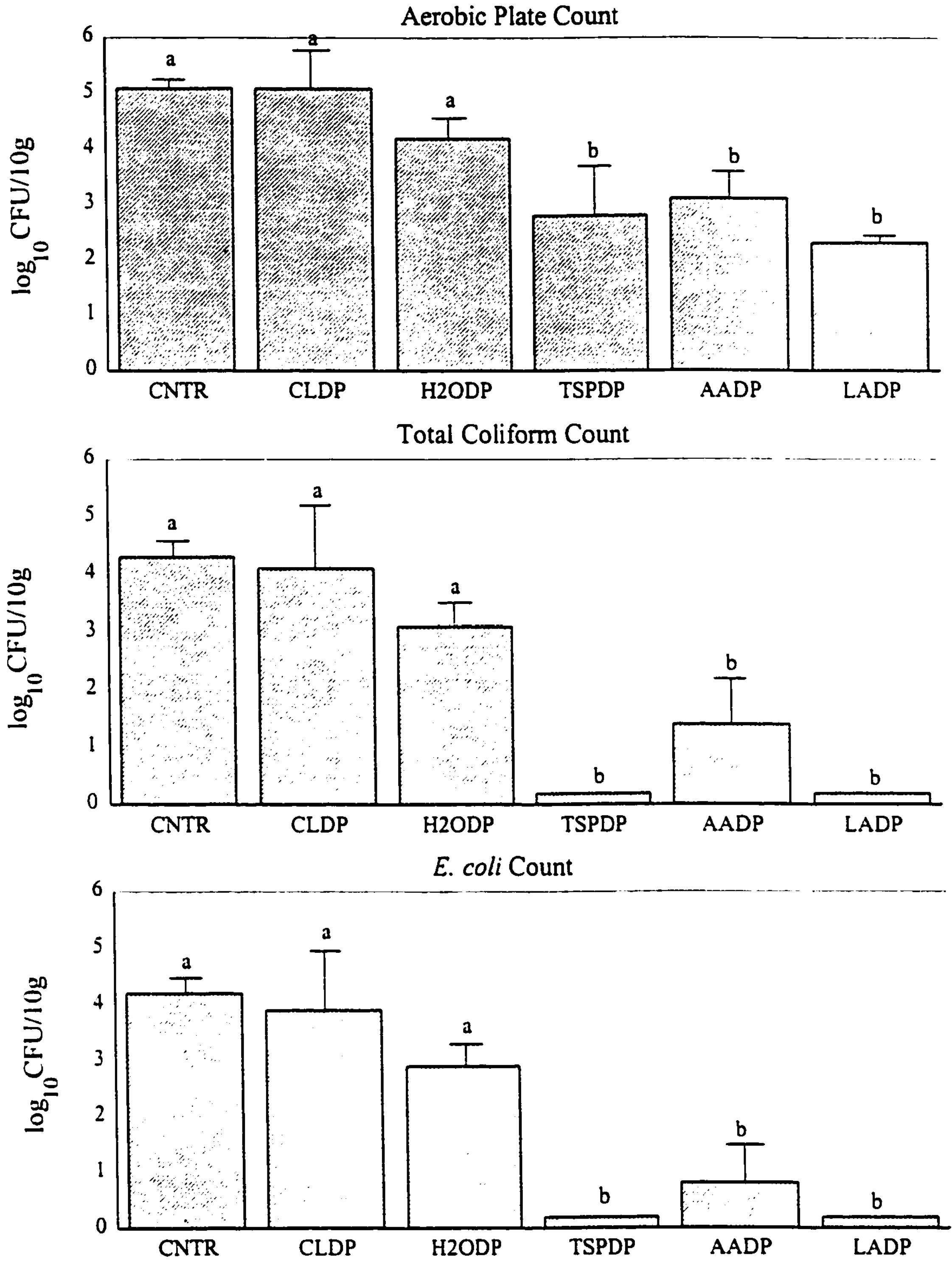

Figure 6.5. Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork chitterlings. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were- CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LADP, lactic acid (2%, 48-50°C, 10 sec immersion). [abcd]Means bearing common superscript letters within bacteria type are not different ($P > 0.05$).

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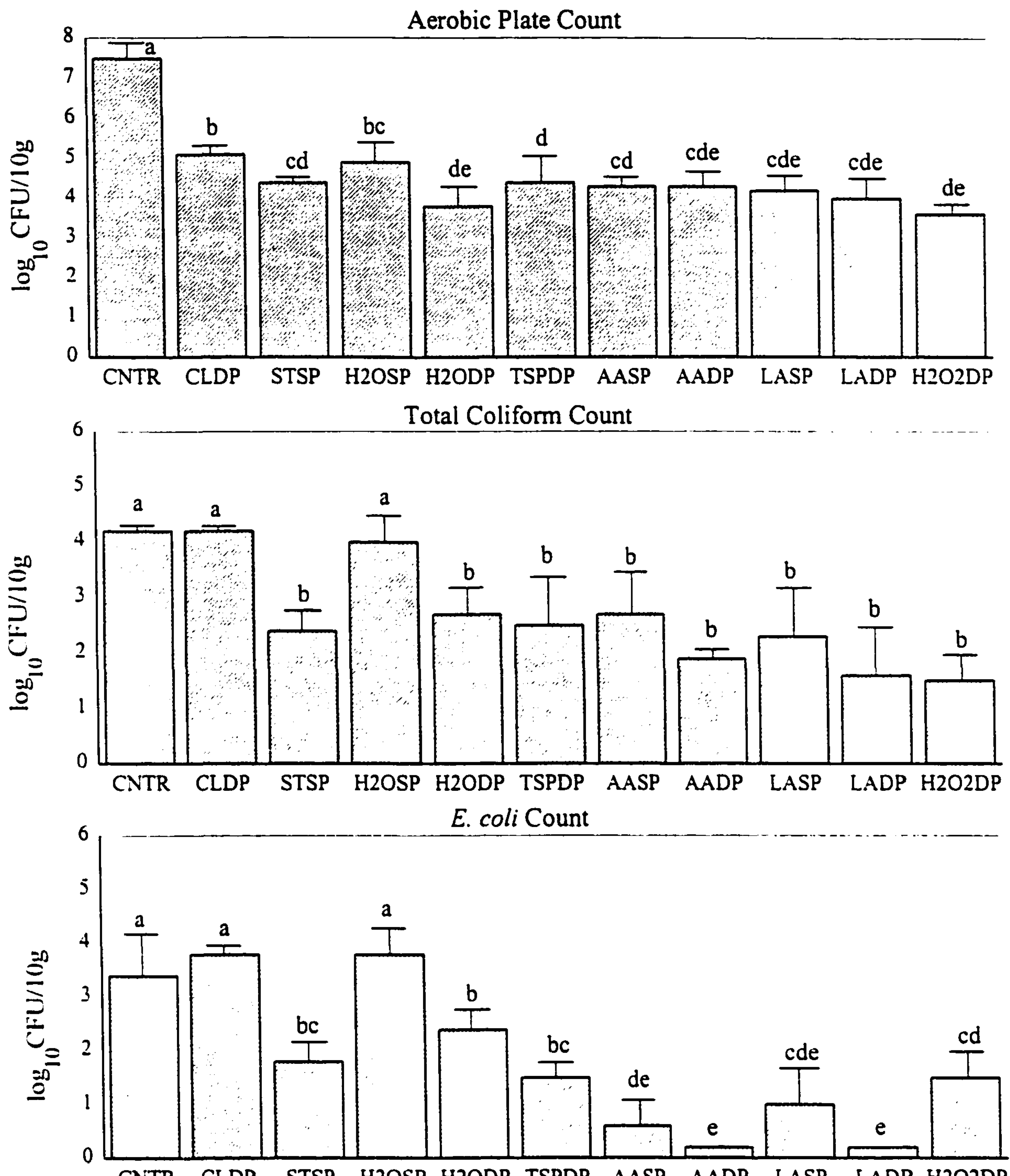

Figure 6.6. Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork stomach. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were- CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion); H2O2DP, hydrogen peroxide (5%, 48-50°C,10 sec immersion). [abcd]Means bearing common superscript letters within bacteria type are not different ($P > 0.05$).

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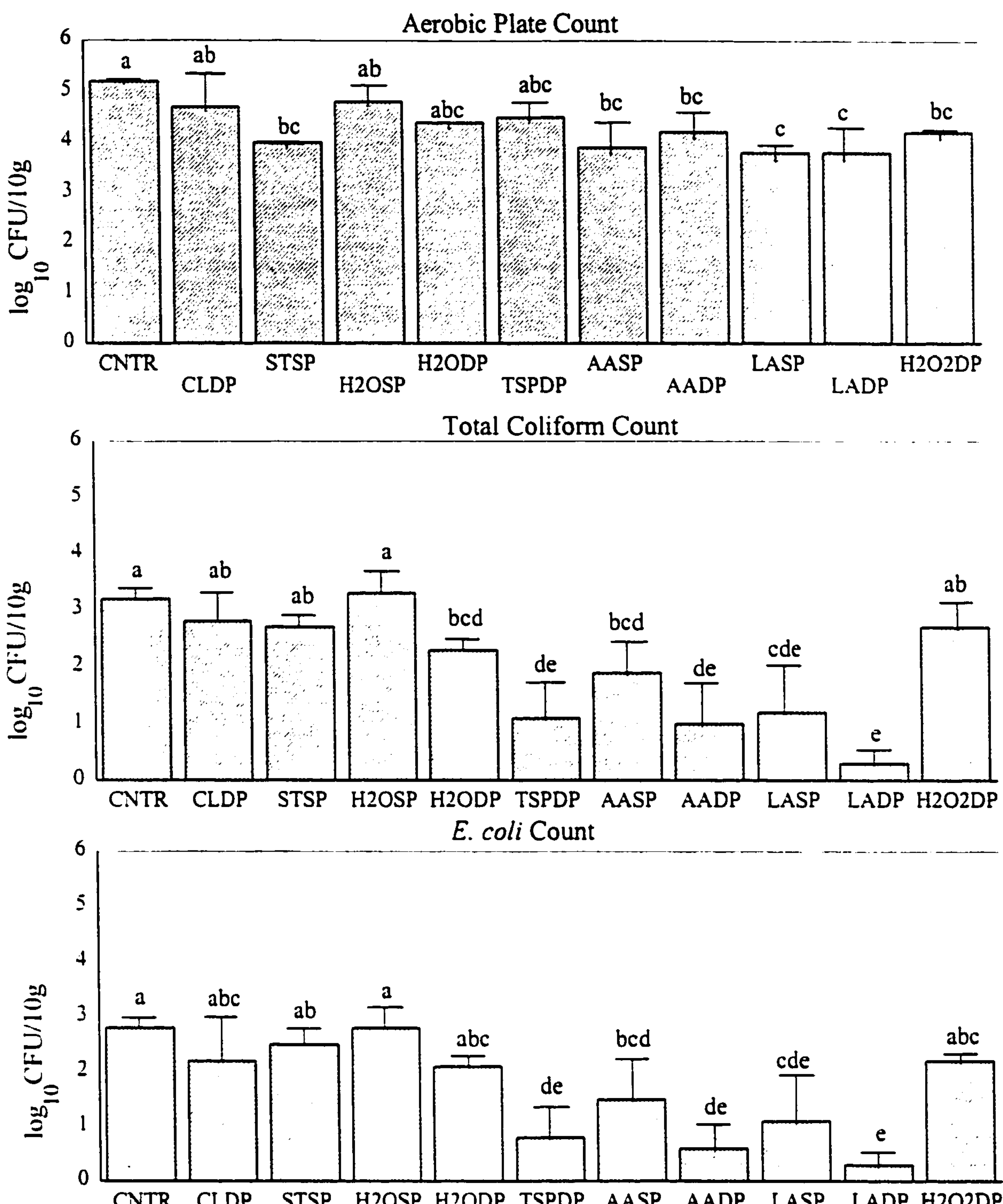

Figure 6.7. Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork tongue. Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were- CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion); H2O2DP, hydrogen peroxide (5%, 48-50°C,10 sec immersion). [abcd]Means bearing common superscript letters within bacteria type are not different ($P > 0.05$).

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Table 6.1. Reductions of aerobic plate counts on uninoculated pork variety meats resulting from the application of various decontamination treatments

		Log reduction (CFU/10g) after treatment application									
Product	CNTR log CFU/10g	CLDP	STSP	H2OSP	H2ODP	H2O2DP	TSPDP	AASP	AADP	LASP	LADP
Cheek meat	5.6	0.3	0.4	0.6	0.5	---	0.5	1.3*	1.3*	1.7*	1.4*
Heart	6.8	2.3*	2.8*	2.1*	3.0*	---	3.3*	3.5*	3.4*	3.3*	3.7*
Liver	4.5	0.2	0.6	0.7*	0.4	---	0.8*	0.9*	1.0*	0.8*	1.2*
Salivary glands	6.6	2.1*	2.2*	2.2*	2.5*	---	2.6*	2.7*	3.0*	3.0*	3.4*
Chitterlings	5.1	0.0	---	---	0.9	---	2.3*	---	2.0*	---	2.8*
Stomach	7.5	2.4*	3.1*	2.6*	3.7*	3.9*	3.1*	3.2*	3.2*	3.3*	3.5*
Tongue	5.2	0.5	1.2*	0.4	0.8	1.0	0.7	1.3*	1.0*	1.4*	1.4*

Decontamination treatments: CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec dwell time); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); H2O2DP, hydrogen peroxide (5%, 48-50°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50 °C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50 °C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion).

*Indicates that the decontamination treatment resulted in a significant reduction ($P < 0.05$) in comparison to the control (CNTR).

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Table 6.2. Reductions of total coliform counts on uninoculated pork variety meats resulting from the application of various decontamination treatments

Product	CNTR log CFU/10g	Log reduction (CFU/10g) after treatment application									
		CLDP	STSP	H2OSP	H2ODP	H2O2DP	TSPDP	AASP	AADP	LASP	LADP
Cheek meat	4.8	0.7	1.0	1.2	0.8	---	2.4*	3.0*	3.7*	4.3*	4.2*
Heart	3.4	0.0	0.9*	0.9*	0.8*	---	2.5*	3.0*	3.0*	1.9*	3.1*
Liver	2.7	0.8	0.6	1.1*	1.2*	---	2.2*	1.3*	2.1*	1.6*	2.5*
Salivary glands	3.1	0.6	+0.1	+0.1	0.9*	---	2.6*	0.9*	2.9*	2.9*	2.9*
Chitterlings	4.3	0.2	---	---	1.2	---	4.1*	---	2.9*	---	4.1*
Stomach	4.2	0.0	1.8*	0.2	1.5*	2.7*	1.7*	1.5*	2.3*	1.9*	2.6*
Tongue	3.2	0.4	0.5	+0.1	0.9*	0.5	2.1*	1.3*	2.2*	2.0*	2.9*

Decontamination treatments: CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82 °C, 30 sec immersion); H2OSP, hot water (75-80 °C, 10 sec spray); H2ODP, hot water (75-80°C, 10 sec immersion); H2O2DP, hydrogen peroxide (5%, 48-50°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50 °C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion).
*Indicates that the decontamination treatment resulted in a significant reduction ($P < 0.05$) in comparison to the control (CNTR).

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Table 6.3. Reductions of *Escherichia coli* counts on uninoculated pork variety meats resulting from the application of various decontamination treatments

		Log reduction (CFU/10g) after treatment application									
Product	CNTR log CFU/10g	CLDP	STSP	H2OSP	H2ODP	H2O2DP	TSPDP	AASP	AADP	LASP	LADP
Cheek meat	4.5	0.9	1.0	1.2	0.7	---	2.4*	3.3*	3.7*	4.1*	4.3*
Heart	1.3	0.0	1.1*	1.0*	1.1*	---	1.1*	1.1*	1.1*	1.1*	1.1*
Liver	2.5	0.9*	2.0*	1.0*	1.0*	---	2.0*	1.5*	1.9*	1.8*	2.3*
Salivary glands	2.6	0.8*	+0.3	+0.1	0.9*	---	2.2*	1.0*	2.4*	2.4*	2.4*
Chitterlings	4.2	0.3	---	---	1.3	---	4.0*	---	3.4*	---	4.0*
Stomach	3.4	+0.4	1.6*	+0.4	1.0*	1.9*	1.9*	2.8*	3.2*	2.4*	3.2*
Tongue	2.8	0.6	0.3	0.0	0.7	0.6	2.0*	1.3*	2.2*	1.7*	2.5*

Decontamination treatments: CNTR, control (initial contamination); CLDP, chlorine (50 ppm, 48-50°C, 10 sec immersion); STSP, steam spray (82°C, 30 sec immersion); H2OSP, hot water (75-80°C, 10 sec spray); H2ODP, hot water (75-80 °C, 10 sec immersion); H2O2DP, hydrogen peroxide (5%, 48-50°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AASP, acetic acid (2%, 48-50°C, 10 sec spray); AADP, acetic acid (2%, 48-50°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion).
*Indicates that the decontamination treatment resulted in a significant reduction ($P < 0.05$) in comparison to the control (CNTR).

CFU/10g. All decontamination treatments were effective ($P < 0.05$) in reducing ECC on livers, with reductions of 0.9 to 2.3 $\log_{10}$ CFU/10g.

All treatments reduced ($P < 0.05$) APC on salivary glands and the TSPDP, AADP, LASP and LADP treatments produced the largest ($P < 0.05$) reductions for TCC and ECC resulting in counts less than 1 $\log_{10}$ CFU/10g (Figure 6.4). Only the immersion treatments were applied to chitterlings; the CLDP and H2ODP treatments did not significantly reduce APC, TCC or ECC. However, TSPDP, AADP and LADP reduced ($P < 0.05$) APC, TCC and ECC on chitterlings. Both TSPD and LADP reduced TCC and ECC to below the detectable levels (> 0.2 $\log_{10}$ CFU/10g; Figure 6.5).

All treatments (including H2O2DP) reduced ($P < 0.05$) APC on stomach by as much as 2.4 to 3.9 $\log_{10}$ CFU/10g. All treatments except CLDP and H2OSP significantly reduced TCC and ECC on stomach samples. The AADP and LADP reduced ECC below the detectable limit (Figure 6.6). For pork tongue, STSP, AASP, AADP, LASP, and LADP reduced ($P < 0.05$) APC by as much as 1.2 to 1.4 $\log_{10}$ CFU/10g. The H2ODP, TSPDP, AASP, AADP, LASP and LADP significantly reduced TCC, and TSPDP, AASP, AADP, LASP and LADP significantly reduced ECC on tongue.

Study II

The results from study II (which included acidified sodium chlorite as a decontamination treatment) are presented in Figure 6.8. All treatments in study II (TSPDP, ASCDP, LASP and LADP) reduced APC, TCC and ECC on pork tongue to < 1 $\log_{10}$ CFU/10g.

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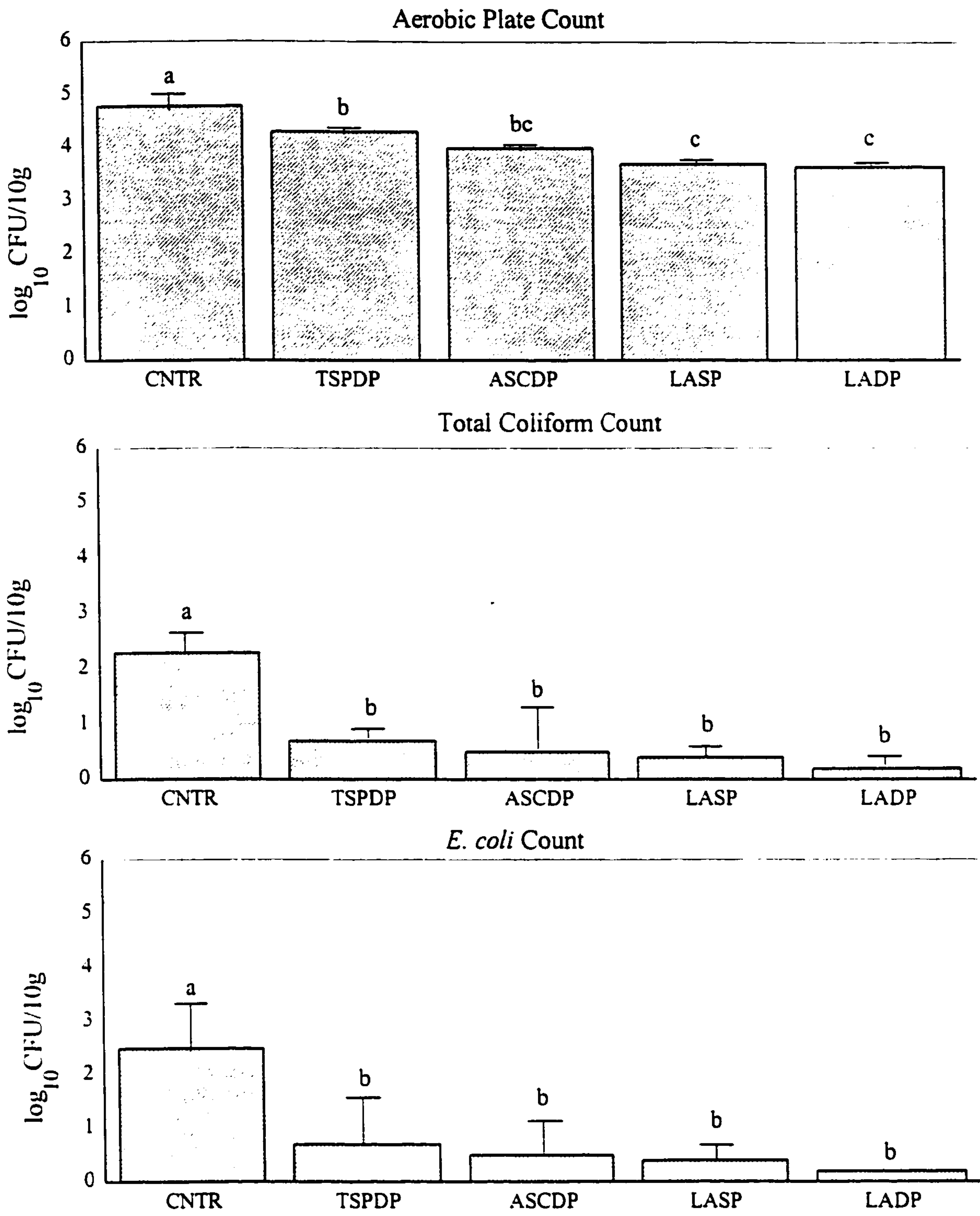

Figure 6.8. Effects of using decontamination treatments for reducing bacterial contamination on uninoculated pork tongue Five samples were evaluated for each treatment and control; CFU/10g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were- CNTR, control (initial contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); ASCDP, acidified sodium chlorite (1200pmm, 26°C, 10 sec immersion); LASP, lactic acid (2%, 48-50°C, 10 sec spray); LADP, lactic acid (2%, 48-50°C, 10 sec immersion); [abcd]Means bearing common superscript letters within bacteria type are not different ($P > 0.05$).

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Treatments involving trisodium phosphate, lactic acid and acetic acid were the most consistent in achieving significant log CFU/10g reductions in APC, TCC and ECC compared to the control. All ECC were less than 1 log after the acetic acid or lactic acid immersion treatments. Chlorine, steam and hot water spray treatments were the most inconsistent in obtaining significant log reductions compared to the control. The chamber used in this study was large and the steam was not applied directly onto the surface of the product; this may explain the lower reductions in microbiological counts obtained with steam compared to significant reductions reported in carcass studies. The hot water, steam and hydrogen peroxide treatments discolored the red variety meat products (liver, cheek meat, heart, salivary gland and tongue). In most cases, application of a decontamination solution by immersion produced a greater log reduction (numerically) than applying the solution via spraying. Although use of a hydrogen peroxide rinse reduced APC, TCC and ECC on stomachs, it produced a foam that interfered with application and may be a problem during packaging.

SUMMARY

Microbiological profiles of pork variety meats clearly indicate that decontamination protocols should be considered as a means for improving microbiological quality of variety meat products. The ability of solutions of lactic acid, acetic acid and trisodium phosphate to effectively reduce bacterial contamination in this study concurs with results of several carcass decontamination studies in the current literature. It is important to reiterate that decontamination treatments can be very useful if incorporated into plant decontamination systems that include appropriate GMPs and

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SSOPs and/or if they are used as a component of multiple hurdles microbiological intervention systems. Decontamination treatments should not be relied on to substitute for proper management or to correct for negligent handling or storage of these or any other type of food products.

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CHAPTER VII

APPLICATION OF DECONTAMINATION TECHNOLOGIES TO REDUCE INOCULATED *SALMONELLA*, *LISTERIA MONOCYTOGENES*, AND *YERSINIA ENTEROCOLITICA* ON PORK VARIETY MEATS

ABSTRACT

The objective of this study was to determine the effectiveness of decontamination treatments in reducing inoculated *Salmonella*, *Listeria monocytogenes*, and *Yersinia enterocolitica* counts on pork variety meats (cheek meat, heart, liver, tongue, stomach, chitterlings and salivary glands). Decontamination treatments evaluated included hot water (75-80°C, 10 sec); hydrogen peroxide (5%, 48-50°C, pH 6.5, 10 sec); trisodium phosphate (12%, 48-50°C, pH 12.0, 10 sec); acetic acid (2%, 48-50°C, pH 2.8, 10 and 5 sec) and lactic acid (2%, 48-50°C, pH 2.5, 10 and 5 sec) immersions. Lactic acid immersion was the most effective and consistent (> 2 log CFU/g reductions) for reducing *Salmonella*, *L. monocytogenes* and *Y. enterocolitica*. *Listeria monocytogenes* was more resistant to decontamination treatments than *Salmonella*, while *Yersinia enterocolitica* was the least resistant. Use of decontamination treatments that are efficacious for reducing microbiological contamination on carcasses will reduce microbiological contamination on pork variety meats.

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INTRODUCTION

In contrast to availability of research data supporting use of decontamination treatments for pork carcasses, there is limited information dealing with decontamination techniques for pork variety meats. An extensive amount of the variety meats (offal) offered for human consumption may be of poor microbiological quality (Gill and Jones, 1992).

Sinell *et al.* (1984) conducted a study in Germany and reported a 72% incidence of *Salmonella* spp. on fresh livers from Germany, 29.3% incidence on frozen livers from Denmark and Italy and a 77.8% incidence on pork hearts. Stewart and Martin (1984) tested pork chitterlings, liver puddings and stomachs in five processing plants and recovered *Salmonella* spp. on chitterlings at two plants, on liver puddings at two plants and on stomachs at one plant (three of the five plants had a product with *Salmonella* spp.). Frederick *et al.* (1994) reported that pork cheek meat had a 30% incidence of *Salmonella* spp. and Laubach *et al.* (1998) reported that pork head meat had a 38% incidence of *Salmonella* spp.

Doyle *et al.* (1981) sampled 31 tongues from apparently healthy slaughter hogs and reported that 16 of the tongues had *Yersinia enterocolitica* of various serotypes. An outbreak of gastroenteritis due to *Y. enterocolitica* serotype O:3 was detected in Atlanta, GA, USA in 1998 and was traced to pork chitterlings. During epidemiological investigations of the outbreak, *Y. enterocolitica* was isolated from 3 of 4 unopened containers of chitterlings from one of the case households and from 5 of 11 containers purchased from Atlanta stores that had been the source of chitterlings for some of the

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case households (Lee *et al.*, 1990). The eight contaminated containers came from pigs slaughtered in three different regions of the U.S.

Woolthuis *et al.* (1984) conceded that variety meat products cannot be removed from the carcass without encountering some contamination, and therefore, decontamination treatments are a legitimate means to produce a cleaner, safer product. There is a consensus throughout the literature and among personnel in the industry that decontamination treatments should not be used as a substitute for hygienic practices or SSOPs (Sanitation Standard Operating Procedures) and GMPs (Good Manufacturing Practices), but rather as a part of an integrated system to ensure that the consumer receives a wholesome high quality product. The objective of this study was to evaluate decontamination techniques for potential use in pork packing plants to reduce pathogen presence.

MATERIALS AND METHODS

Pork variety meats and sample size

Seven pork variety meat products were selected to test decontamination treatments based on the results of Chapter V - Microbiological Contamination on Pork Variety Meats. These included cheek meat, heart, liver, salivary glands, chitterlings, stomach and tongue. Variety meat samples were collected from two large commercial pork-packing facilities. Within 4 h of collection, the samples were shipped via overnight express carrier in coolers packed with commercial ice substitutes to Colorado State University, Fort Collins, CO.

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Variety meat sample sizes were as follows: cheek meat - whole piece, approximately 150 to 200g; salivary gland - whole piece, approximately 40 to 60g; tongue - whole tongue, approximately 200 to 300g; chitterlings - 13cm portion, approximately 75 to 150g cut along the longitudinal axis; heart - ½ of a heart, approximately 150 to 300g; liver - a portion of a lobe, approximately 140 to 220g; and stomach - a portion, approximately 100 to 125g.

Bacterial cultures

The pathogens used for this study were isolated from positive samples collected from the studies presented in Chapter III - Levels of Microbiological Contamination Of Pork Carcasses During Slaughter and Chapter V - Microbiological Contamination Of Pork Variety Meats. Initial testing was conducted with variety meat samples from the participating processing plants to determine the level of antibiotics needed to inhibit the growth of naturally occurring microflora that were resistant to streptomycin and rifampicin. It was determined that 800 µl/ml streptomycin sulfate (Sigma Chemical Company, St. Louis, MO) and 100 µl/ml rifampicin (Sigma) were needed to eliminate background microflora during the initial testing and plating.

A streptomycin-resistant strain (PVM 4R2, Colorado State University, Fort Collins, CO) of *L. monocytogenes* from a culture isolated from a pork carcass in a commercial processing plant was developed following growth (48 h at 35°C) on tryptic soy agar (TSA; Difco Laboratories, Detroit , MI) with 0.6% yeast extract (Difco) and 800 µl/ml streptomycin sulfate (Sigma) following procedures described by Kallander *et al.* (1991). Rifampicin resistant strains of each, *Salmonella* (PVM 6R2, Colorado State

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University) and *Y. enterocolitica* (PVM 19, Colorado State University) were developed from cultures isolated from pork carcasses in commercial pork processing plants following growth (48 h at 35°C) on TSA (Difco) with 100 µl/ml rifampicin (Sigma) following procedures outlined by Hardin *et al.* (1995). Growth curves were obtained during preliminary testing to ensure that the resistant strains grew equally as well as did the parent strains, and cultures of the resistant strains were sent to a commercial laboratory to verify that they were indeed pure cultures of the parent strain. Resistant *L. monocytogenes* was maintained in tryptic soy broth (TSB, Difco) and on TSA (Difco) with 0.6% yeast extract (Difco) and 800 µl/ml streptomycin sulfate (Sigma). Resistant strains of *Salmonella* and *Y. enterocolitica* were maintained in tryptic soy broth (TSB, Difco) and on TSA (Difco) with 100 µl/ml rifampicin (Sigma).

Variety meat inoculation

Variety meat samples were inoculated with 0.5 ml of an inoculum containing 6 to 8 log CFU/ml of the resistant strains. The inoculum was spread over the surface using a sterile bent glass rod and the samples were held at ambient temperature for 30 min to allow for bacterial attachment.

Decontamination treatments

Decontamination treatments evaluated for their effectiveness in reducing *Salmonella*, *L. monocytogenes* and *Y. enterocolitica* were selected based on the results of Chapter VI - Application of Decontamination Technologies to Reduce Contamination on Pork Variety Meats. The immersion treatments from study I that were effective in

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reducing plate counts for each particular variety meat were selected also to determine their efficacy on the aforementioned pathogens (not all treatments were applied to each variety meat). The treatments included were: control samples (CNTR); hot water (75-80°C) immersion for 10 sec (H2ODP); hydrogen peroxide (5% v/v, 48-50°C, pH 6.5 prepared from 30% hydrogen peroxide, Fisher Scientific, Fair Lawn, NJ) immersion for 10 sec (H2O2DP), trisodium phosphate (12% wt/v, 48-50°C, pH 12.0, prepared from trisodium phosphate hydrate, Rhone-Poulenc, Cranbury, NJ) immersion for 10 sec (TSPDP); acetic acid (2% v/v, 48-50°C, pH 2.8, prepared from glacial acetic acid, Mallinckrodt Baker, Inc., Paris KY) immersion for 10 sec (AADP 10) and immersion for 5 sec (AADP 5); and lactic acid (2% v/v, 48-50°C, pH 2.5, prepared from 98% lactic acid, Sigma Chemical Co., St. Louis, MO) immersion for 10 sec (LADP 10) and immersion for 5 sec (LADP 5).

Treatments were prepared (with tap water) by mixing 2 L of the appropriate solution in a stainless steel container and heating the solution (in the container) in a water bath. When the solution reached the proper temperature, the variety meat product was held with sterile forceps and immersed in the liquid for the appropriate amount of time.

Microbiological analysis

Immediately after application of the appropriate treatment, samples were placed into sterile bags (Whirl-Pak®, Nasco, Ft. Atkinson, WI) and weights were recorded. The bagged samples were then held for 10 min at ambient temperature before microbiological analysis to simulate the time between the application of the treatment and packaging in a commercial facility. A surface rinsing procedure was employed to dislodge bacteria from

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the irregular surface of each variety meat. A 100 ml quantity of Butterfield's phosphate buffer (Difco Laboratories, Detroit, MI) was added to each sample bag. The bags were sealed and the samples were rinsed using a rocking motion for 30 shakes (approximately 1 min).

Dilutions from the samples inoculated with *L. monocytogenes* were plated on TSA (Difco) agar with 0.6% yeast extract and 800 µl/ml streptomycin sulfate (TSA+STREP) and lithium chloride-phenylethanol-moxalactam (LPM; Difco) agar (selective for *L. monocytogenes*) with 0.6% yeast extract and 800 µl/ml streptomycin sulfate (LPM+STREP). Dilutions from samples inoculated with *Salmonella* were plated on TSA (Difco) with 100 µl/ml rifampicin (TSA+RIF) and xylose lysine desoxycholate (XLD; Difco) agar (selective for *Salmonella*) with 100 µl/ml rifampicin (XLD+RIF). Dilutions from the samples inoculated with *Y. enterocolitica* were plated on TSA+RIF and cesulodin-irgasan-novobiocin (CIN; Difco) agar (selective for *Y. enterocolitica*) with 100 µl/ml rifampicin (CIN+RIF). Plating was accomplished using a spiral plating system (Model D, Spiral Biotech, Inc., Bethesda, MD)

Plates were incubated for 48 h at 35°C, and colonies were counted with a laser bacteria colony counter (Model 500 A, Spiral Biotech, Inc.) and a CASBA 4 data processor (Model 800, Spiral Biotech, Inc.). All colonies that grew on the different types of agar with the aforementioned levels of streptomycin and rifampicin were assumed to be the resistant strains of bacteria that were inoculated on the variety meat products.

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Statistical analysis

Bacteriological count data were transformed into base-ten logarithms before computing means and performing statistical analyses. All bacterial counts were reported as mean value $\log_{10}$ CFU/g. Counts below the detectable level were reported as 0.2 $\log_{10}$ CFU/g. Data were analyzed using the general linear model (GLM) procedures of SAS® (SAS®, 1995). Treatment was considered a main effect. Data were analyzed using the model $y = a + b_1x_1$ and means were computed and analyzed separately for each agar within each respective pathogen. When the F-test was significant ($P < 0.05$) means were separated using Tukey's HSD test.

RESULTS AND DISCUSSION

The reduction of *Salmonella*, *L. monocytogenes* and *Y. enterocolitica* contamination levels after the application of decontamination treatments is presented in Table 7.1, 7.2 and 7.3, respectively. The comparisons among decontamination treatments used on each variety meat are presented in Figures 7.1 through 7.18.

The AADP 5, AADP 10, LADP 5 and LADP 10 treatments significantly reduced *Salmonella*, *L. monocytogenes* and *Y. enterocolitica* plate counts on cheek meat (Figures 7.1, 7.2 and 7.3). The TSPDP treatment reduced ($P < 0.05$) plate counts of *Salmonella*, but not of *L. monocytogenes* on cheek meat (TSPDP was not tested on *Y. enterocolitica*). The AADP 10 and LADP 10 treatments reduced levels of *Salmonella* and *Y. enterocolitica* on cheek meat below the detectable limit (< 0.2 log CFU/g) on the XLD+RIF and CIN+RIF, respectively.

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Table 7.1. Reductions of inoculated rifampicin-resistant *Salmonella* on pork variety meats resulting from the application of various decontamination treatments

Product	Agar	CNTR log CFU/g	Log reduction (CFU/g) after treatment application[a]						
			H2ODP	H2O2DP	TSPDP	AADP5	AADP10	LADP5	LADP10
Cheek meat	TSA	3.1	---	---	1.2*	1.9*	1.8*	2.1*	2.7*
	XLD	2.5	---	---	2.2*	2.3*	2.3*	2.3*	2.3*
Heart	TSA	3.8	---	---	---	1.1*	1.7*	1.9*	2.4*
	XLD	3.6	---	---	---	3.0*	3.4*	3.4*	3.4*
Liver	TSA	3.5	---	---	---	1.7*	2.4*	1.9*	2.0*
	XLD	3.3	---	---	---	2.4*	3.0*	2.6*	3.1*
Salivary glands	TSA	2.8	---	---	---	2.0*	2.1*	2.3*	2.6*
	XLD	2.2	---	---	---	2.0*	2.0*	2.0*	2.0*
Chitterlings	TSA	5.7	---	---	---	1.1	2.1*	3.4*	4.4*
	XLD	5.7	---	---	---	1.6	2.1	2.6*	3.9*
Stomach	TSA	2.8	1.6*	2.4*	2.4*	1.7*	2.6*	2.2*	2.6*
	XLD	2.4	1.5*	2.4*	2.0*	2.0*	2.2*	2.2*	2.2*
Tongue	TSA	3.4	---	---	---	1.0*	1.5*	1.4*	2.3*
	XLD	3.1	---	---	---	2.7*	2.7*	2.8*	2.8*

[a]Decontamination treatments - CNTR, control (initial contamination); H2ODP, hot water (75-80°C, 10 sec immersion); H2O2DP, hydrogen peroxide (5%, 48-50°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion).
*Indicates that the decontamination treatment resulted in a significant reduction ($P < 0.05$) in comparison to control (CNTR).

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Table 7.2. Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on pork variety meats resulting from the application of various decontamination treatments

			Log reduction (CFU/g) after treatment application[a]						
Product	Agar	CNTR log CFU/g	H2ODP	H2O2DP	TSPDP	AADP5	AADP10	LADP5	LADP10
Cheek Meat	TSA	4.3	---	---	0.8	1.6*	1.6*	2.5*	2.5*
	LPM	3.3	---	---	0.7	1.4*	1.2*	1.9*	2.3*
Heart	TSA	4.8	---	---	---	1.3*	1.6*	1.8*	2.1*
	LPM	4.9	---	---	---	1.3*	2.1*	2.5*	3.6*
Liver	TSA	3.3	---	---	---	0.8	1.6*	1.3*	2.4*
	LPM	3.5	---	---	---	1.2*	2.2*	1.7*	2.3*
Salivary Glands	TSA	3.6	---	---	---	1.1*	1.5*	3.1*	3.1*
	LPM	3.6	---	---	---	1.5*	2.1*	3.4*	3.4*
Chitterlings	TSA	5.4	---	---	2.5*	2.0*	2.3*	4.5*	5.1*
	LPM	4.8	---	---	3.6*	1.6*	2.3*	3.0*	4.0*
Stomach	TSA	4.9	1.9*	1.7*	2.2*	1.0*	1.6*	2.1*	2.7*
	LPM	4.4	1.5*	2.5*	1.8*	0.8	1.8*	1.9*	2.7*
Tongue	TSA	3.3	---	---	0.6*	1.3*	1.8*	2.5*	2.9*
	LPM	3.4	---	---	0.6*	1.5*	2.1*	2.9*	3.2*

[a]Decontamination treatments - CNTR, control (initial contamination); H2OD hot water (75-80°C, 10 sec immersion); H2O2, hydrogen peroxide (5%, 48-50°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion).

*Indicates that the decontamination treatment resulted in a significant reduction ($P < 0.05$) in comparison to control (CNTR).

Table 7.3. Reductions of inoculated rifampicin-resistant *Yersinia enterocolitica* on pork variety meats resulting from the application of various decontamination treatments

Product	Agar	CNTR log CFU/g	Log reduction (CFU/g) after treatment application[a]				
			TSPDP	AADP5	AADP10	LADP5	LADP10
Cheek Meat	TSA	5.5	---	4.2*	4.4*	4.7*	5.1*
	CIN	4.8	---	4.5*	4.6*	4.5*	4.6*
Salivary Glands	TSA	5.0	---	4.4*	4.7*	4.1*	4.8*
	CIN	3.9	---	3.3*	3.5*	3.3*	3.7*
Chitterlings	TSA	6.2	5.6*	6.0*	6.0*	6.0*	6.0*
	CIN	5.3	5.1*	5.1*	5.1*	5.1*	5.1*
Tongue	TSA	4.8	---	4.6*	4.6*	4.6*	4.6*
	CIN	4.7	---	4.5*	4.5*	4.5*	4.5*

[a]Decontamination treatments - CNTR, control (initial contamination); H2OD hot water (75-80°C, 10 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 10 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion).
*Indicates that the decontamination treatment resulted in a significant reduction ($P < 0.05$) in comparison to control (CNTR).

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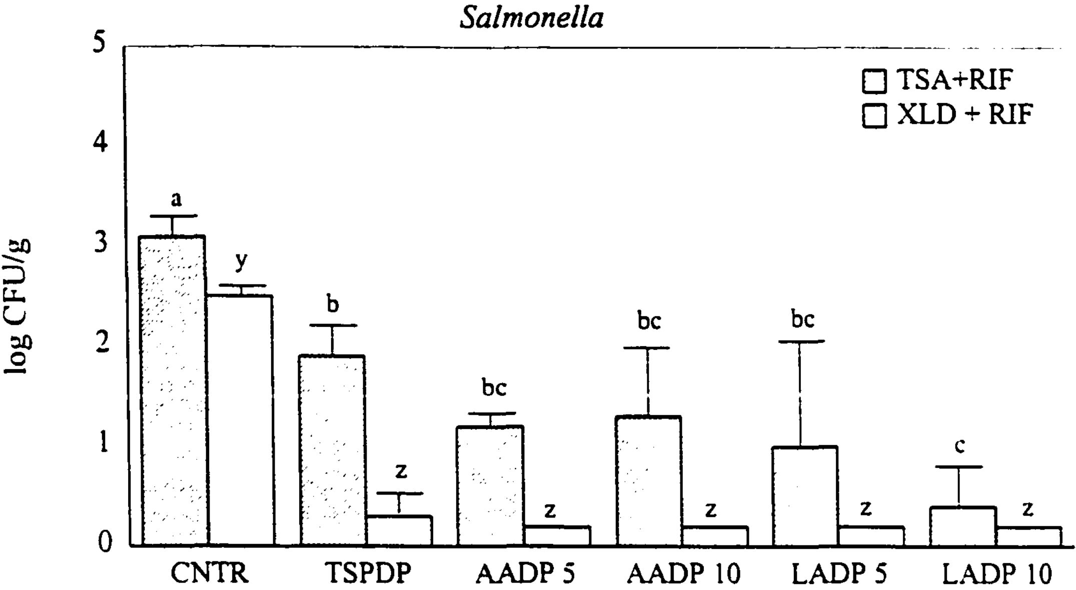

Figure 7.1. Reductions of inoculated rifampicin-resistant *Salmonella* on cheek meat resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). [abc, yz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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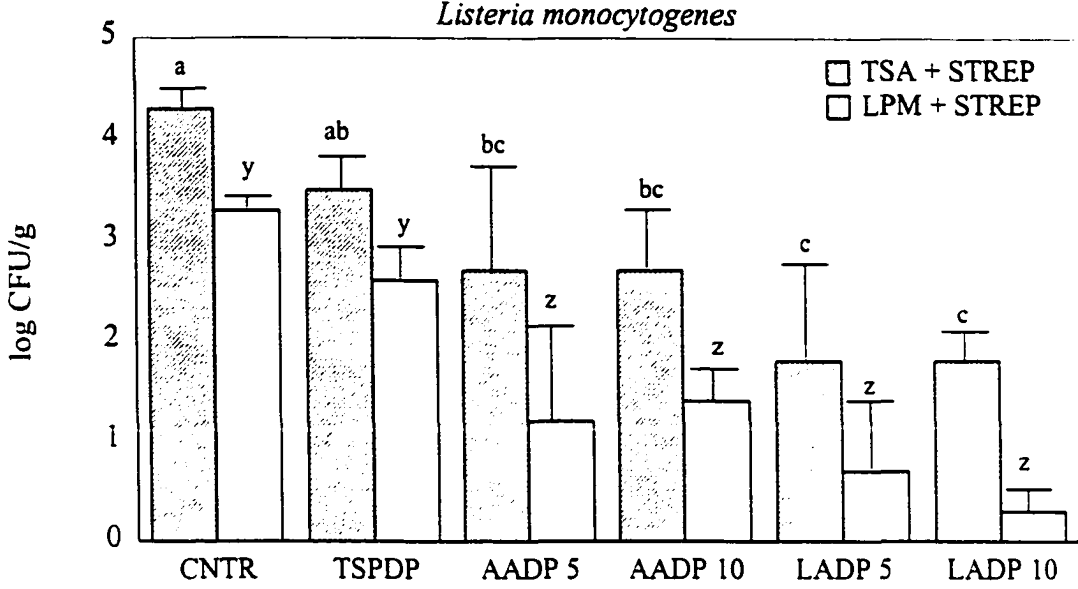

Figure 7.2. Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on cheek meat resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). [abc, yz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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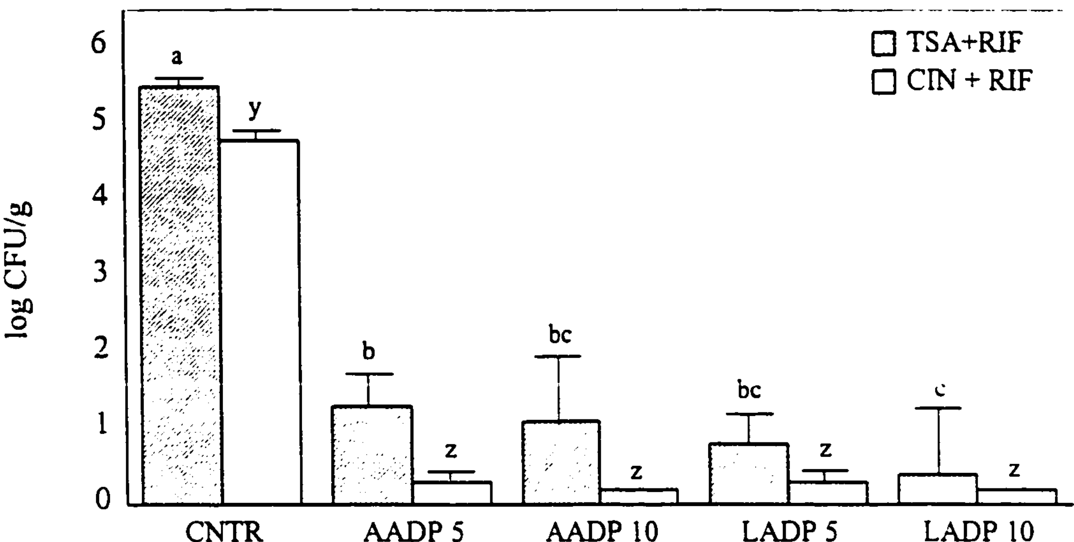

Figure 7.3. Reductions of inoculated rifampicin-resistant *Yersinia enterocolitica* on cheek meat resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and CIN+RIF (Cefsulodin-Irgasan-Novobiocinagar with 100 mg/ml rifampicin). [abc, yz]Means within an agar type bearing common superscript letters are not different (P > 0.05).

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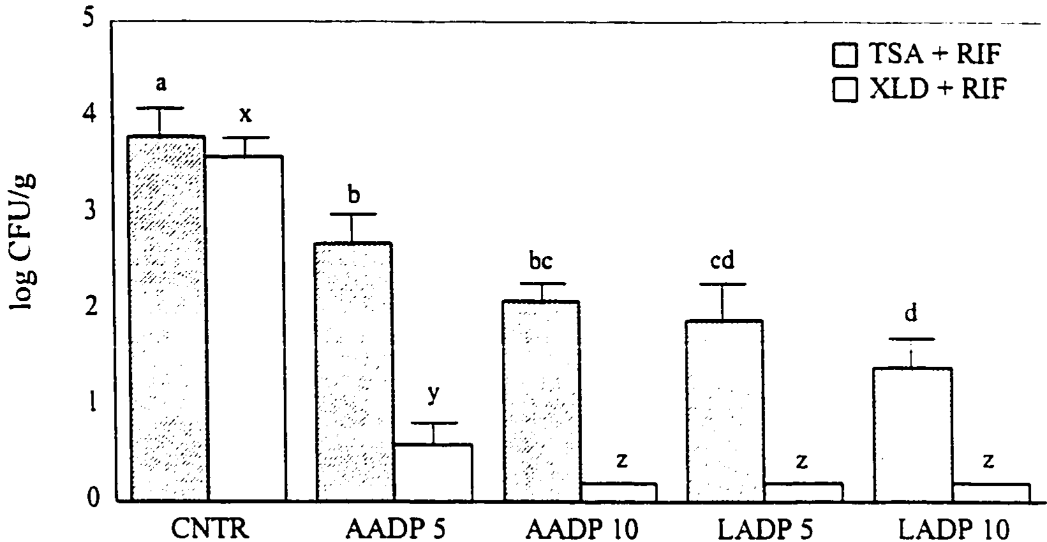

Figure 7.4. Reductions of inoculated rifampicin-resistant *Salmonella* on heart resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). [abcd, xyz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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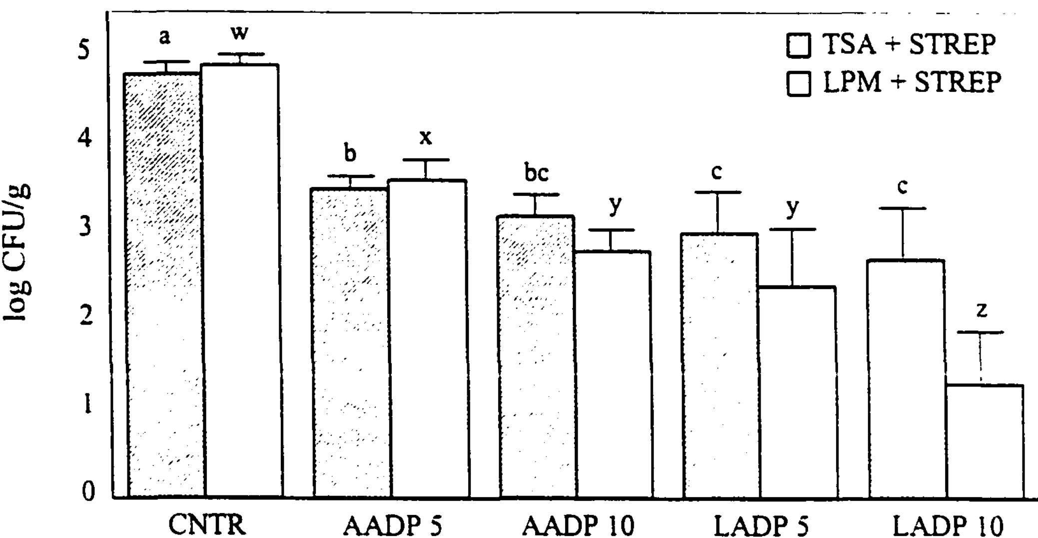

Figure 7.5. Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on heart resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). [abc, wyz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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Salmonella

TSA + RIF
XLD + RIF
log CFU/g
CNTR
AADP 5
AADP 10
LADP 5
LADP 10

Figure 7.6. Reductions of inoculated rifampicin-resistant *Salmonella* on liver resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). [ab, yz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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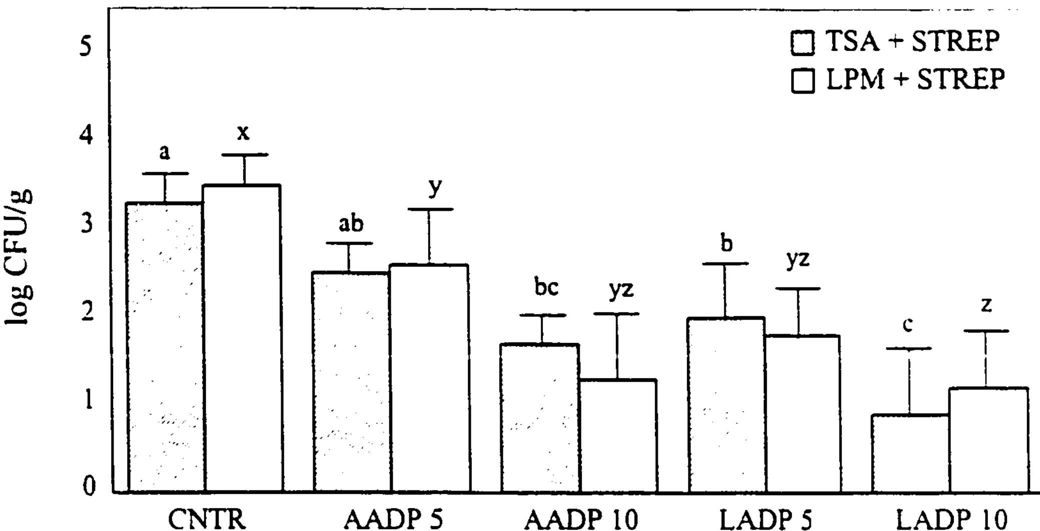

Figure 7.7. Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on liver resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). [abc, xyz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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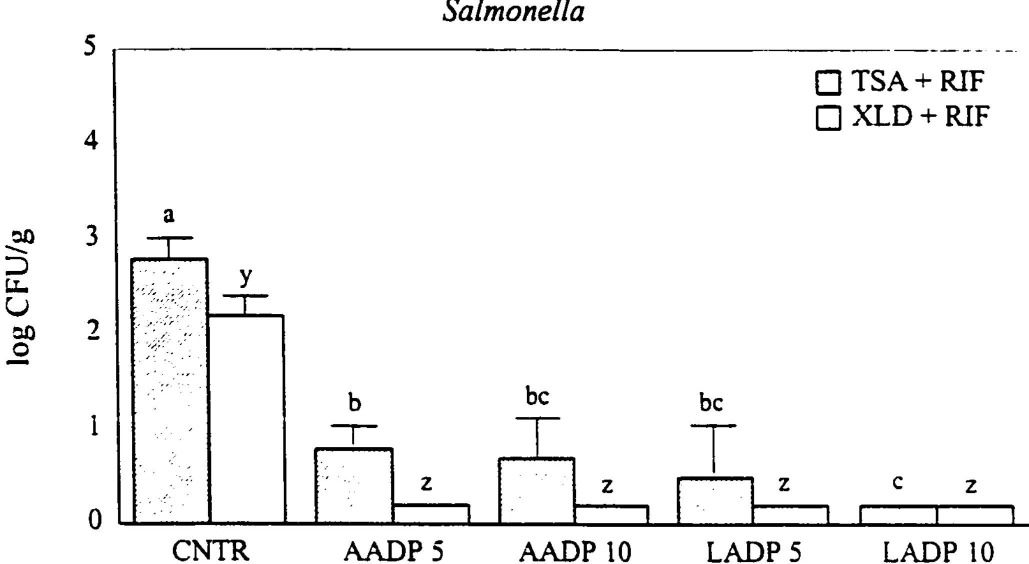

Figure 7.8. Reductions of inoculated rifampicin-resistant *Salmonella* on salivary glands resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). [abc, yz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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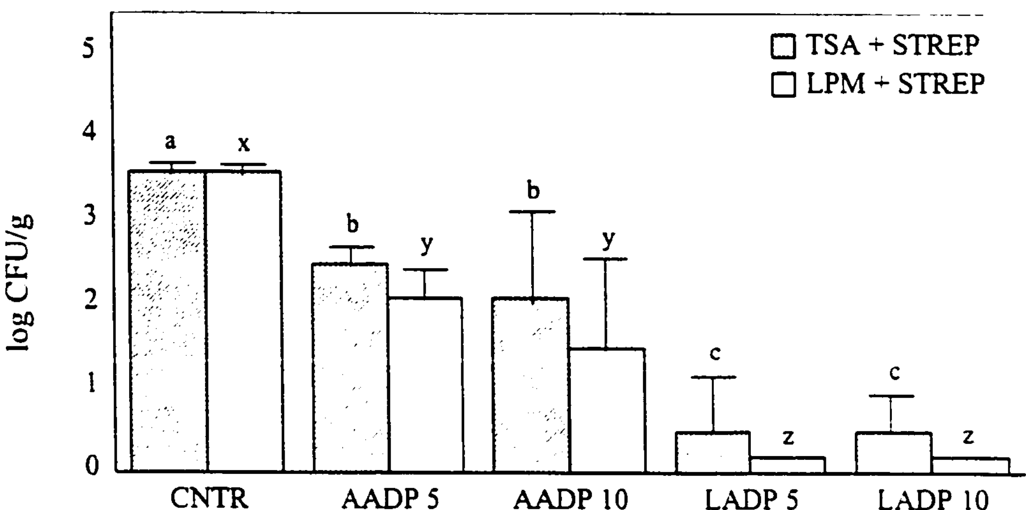

Figure 7.9. Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on salivary glands resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). [abc, xyz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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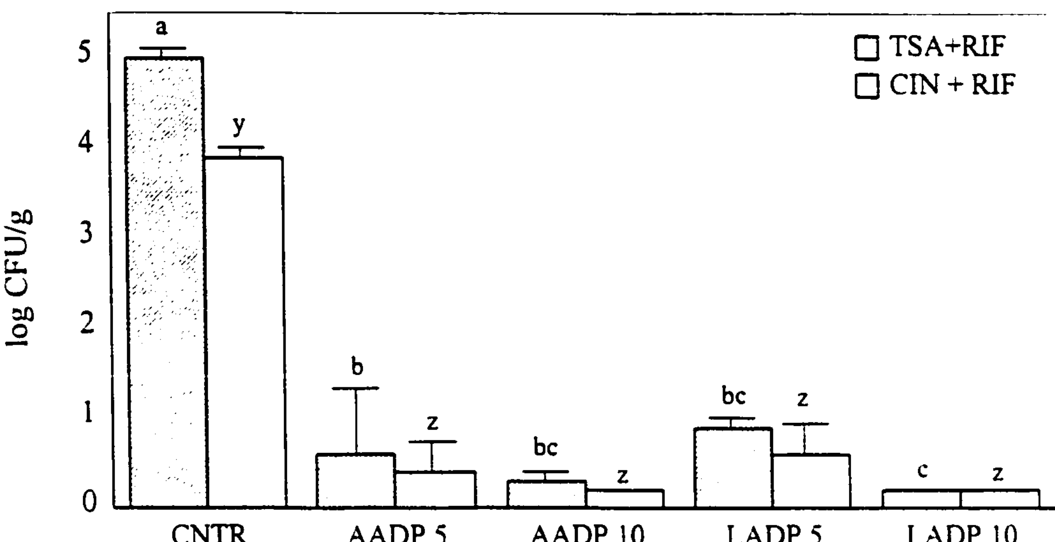

Figure 7.10. Reductions of inoculated rifampicin-resistant *Yersinia enterocolitica* on salivary glands resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and CIN+RIF (Cefsulodin-Irgasan-Novobiocinagar with 100 mg/ml rifampicin). [abc, yz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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Salmonella

Figure 7.11. Reductions of inoculated rifampicin-resistant *Salmonella* on chitterlings resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). $^{abcd, xyz}$Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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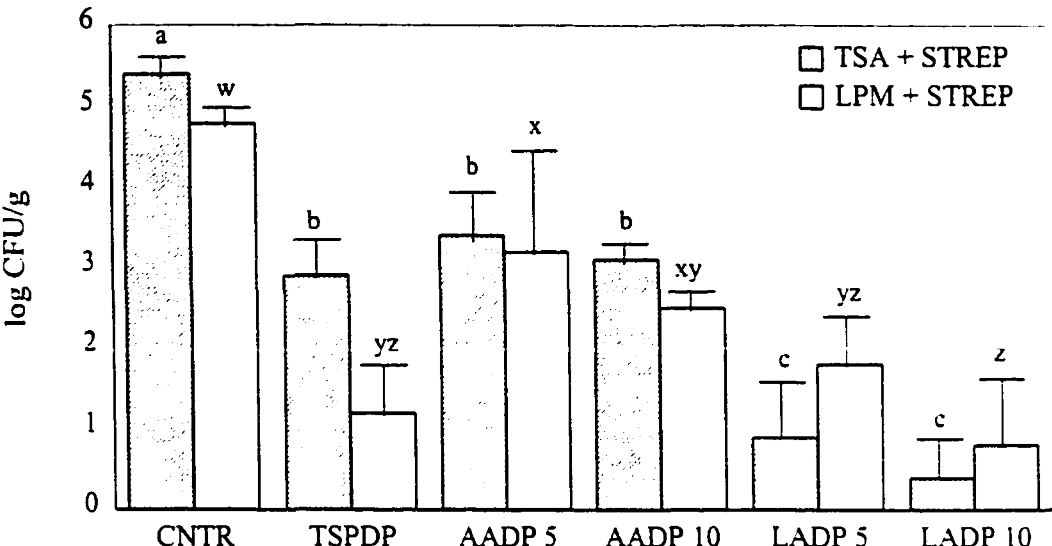

Figure 7.12. Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on chitterlings resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). $^{abc, wxyz}$Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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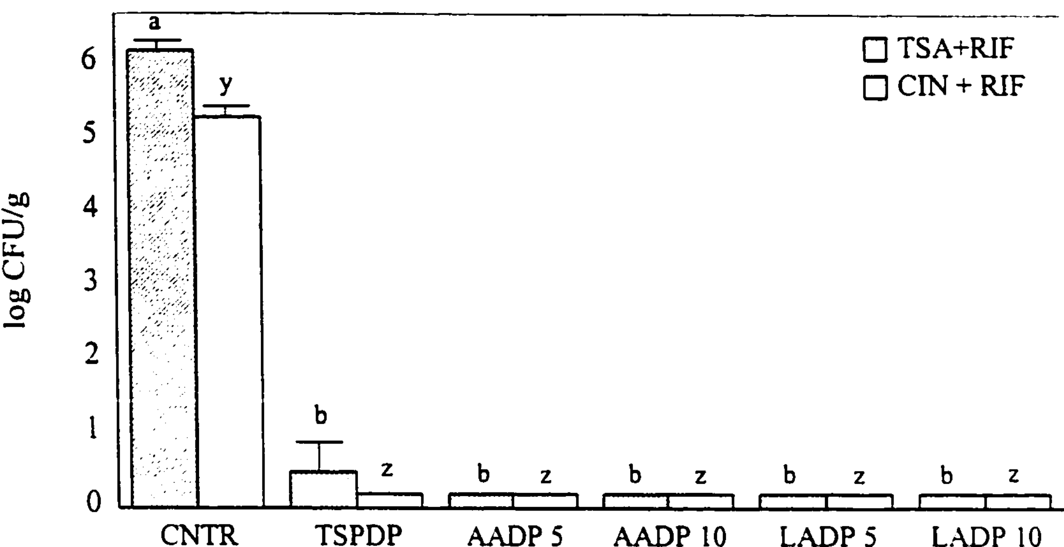

Figure 7.13. Reductions of inoculated rifampicin-resistant *Yersinia enterocolitica* on chitterlings resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and CIN+RIF (Cefsulodin-Irgasan-Novobiocinagar with 100 mg/ml rifampicin). [ab, yz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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Figure 7.14. Reductions of inoculated rifampicin-resistant *Salmonella* on stomach resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); H2ODP, hot water (75-80°C, 10 sec immersion); H2O2DP, hydrogen peroxide (5%, 48-50°C, 5 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). [abcd, yz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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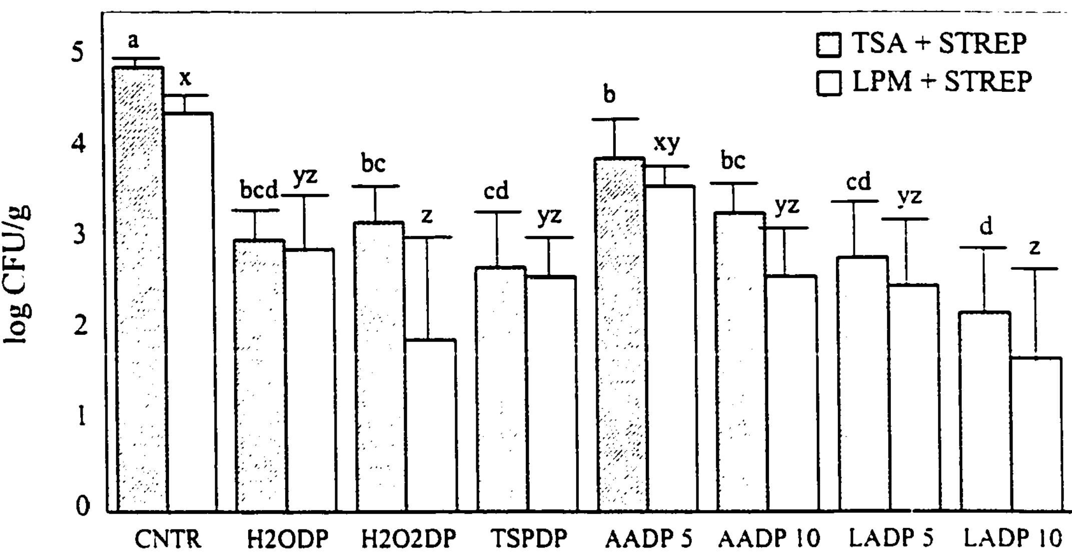

Figure 7.15. Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on stomach resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); H2ODP, hot water (75-80°C, 10 sec immersion); H2O2DP, hydrogen peroxide (5%, 48-50°C, 5 sec immersion); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). [abcd, xyz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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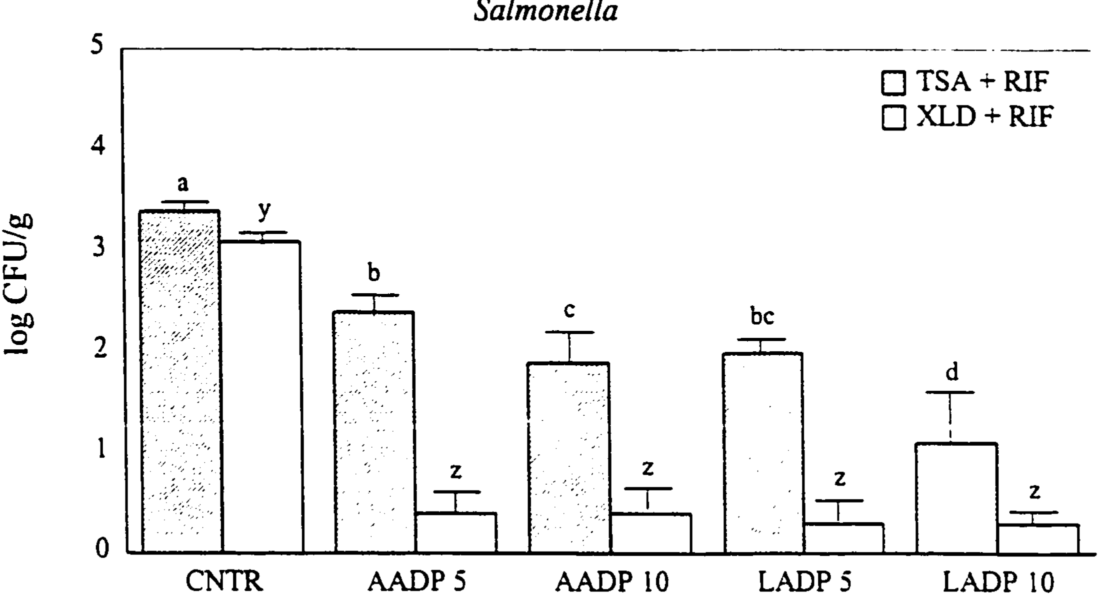

Figure 7.16. Reductions of inoculated rifampicin-resistant *Salmonella* on tongue resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and XLD+RIF (xylose lysine desoxycholate agar with 100 mg/ml rifampicin). [abcd, yz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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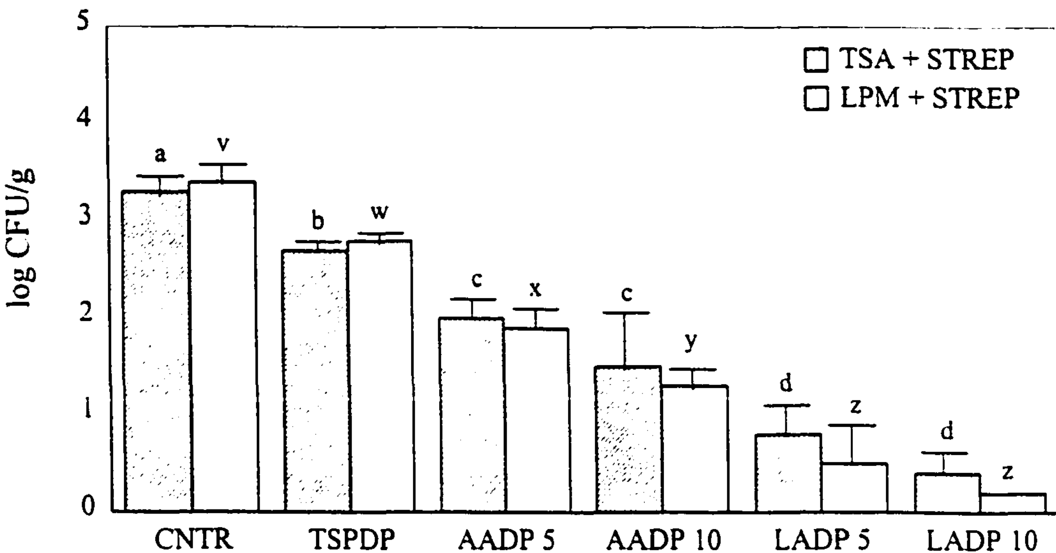

Figure 7.17. Reductions of inoculated streptomycin-resistant *Listeria monocytogenes* on tongue resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); TSPDP, trisodium phosphate (12%, 48-50°C, 10 sec immersion); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+STREP (tryptic soy agar with 800 mg/ml streptomycin sulfate) and LPM+STREP (lythium chloride-phenylethanol-moxalactin agar with 800 mg/ml streptomycin sulfate). [abcd, vwxyz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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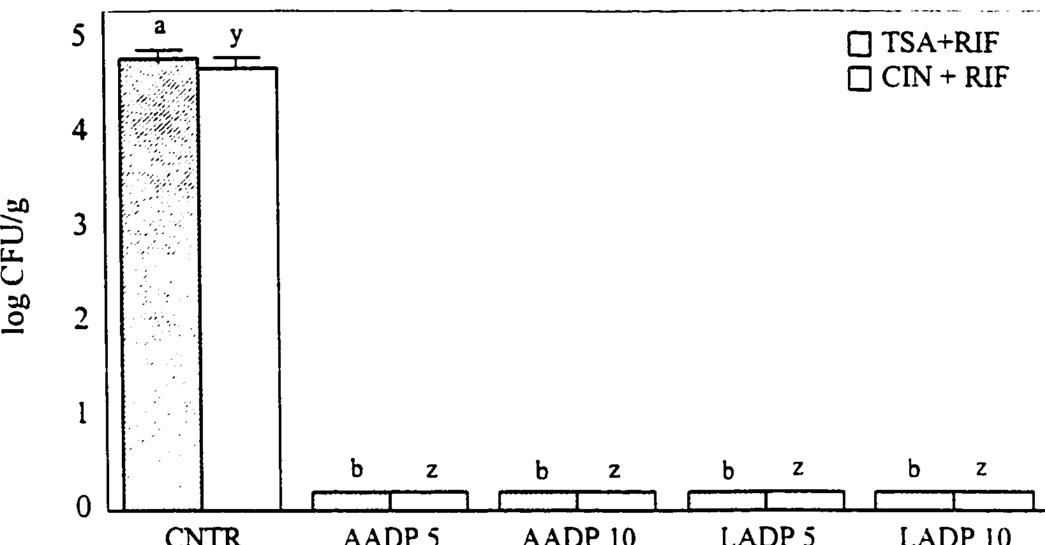

Figure 7.18. Reductions of inoculated rifampicin-resistant *Yersinia enterocolitica* on tongue resulting from the application of various decontamination treatments. Five samples were evaluated for each treatment and control and CFU/g = 0.2 log indicates recovery was below the detectable level. Decontamination treatments were: CNTR, control (inoculated contamination); AADP 5, acetic acid (2%, 48-50°C, 5 sec immersion); AADP 10, acetic acid (2%, 48-50°C, 10 sec immersion); LADP 5, lactic acid (2%, 48-50°C, 5 sec immersion); LADP 10, lactic acid (2%, 48-50°C, 10 sec immersion). Agar types were TSA+RIF (tryptic soy agar with 100 mg/ml rifampicin) and CIN+RIF (Cefsulodin-Irgasan-Novobiocinagar with 100 mg/ml rifampicin). [ab, yz]Means within an agar type bearing common superscript letters are not different ($P > 0.05$).

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Salmonella and *L. monocytogenes* levels on inoculated heart were consistently reduced ($P < 0.05$) by use of AADP 5, AADP 10, LADP 5 and LADP 10 (Figure 7.4 and 7.5). The plate counts for *Salmonella* were below the detectable limit on heart for samples treated with AADP 10, LADP 5 and LADP 10 (Figure 7.4). Levels of *Salmonella* and *L. monocytogenes* were reduced ($P < 0.05$) on liver following decontamination treatments of AADP 10, LADP 5 and LADP 10 (Figure 7.6 and 7.7). While use of AADP 5 reduced *Salmonella*, it did not significantly reduce *L. monocytogenes* on inoculated liver.

The *Salmonella* plate counts on salivary glands were significantly lower than the control counts following treatments of AADP 5, AADP 10, LADP 5 and LADP 10 treatments. The levels were below the detectable limits on XLD+RIF for all four treatments, and below detectable limits on TSA+RIF for LADP 10. Both LADP 5 and LADP 10 were more effective ($P < 0.05$) in reducing *L. monocytogenes* levels on salivary glands than were AADP 5 and AADP 10, which were intermediate to use of the lactic acid treatments and the control (Figure 7.9). *Yersinia enterocolitica* was significantly reduced by AADP 5, AADP 10, LADP 5 and LADP 10 (Figure 7.10).

Only LADP 5 and LADP 10 consistently reduced ($P < 0.05$) *Salmonella* levels on pork chitterlings on both TSA+RIF and XLD+RIF (Figure 7.11), while treatment with TSPDP, AADP 5, AADP 10, LADP 5 and LADP 10 all reduced ($P < 0.05$) plate counts for *L. monocytogenes. Yersinia enterocolitica* levels also were significantly reduced with the use of TSPDP, AADP 5, AADP 10, LADP 5 and LADP 10 (Figure 7.13), and plate counts recovered from CIN+RIF were below the detectable limit for all treatments, and below the detectable limit on TSA+RIF for AADP 5, AADP 10, LADP 5 and LADP 10.

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Several decontamination treatments (H2ODP, H2O2DP, TSPDP, AADP 5, AADP 10, LADP 5 and LADP 10) were applied to stomach, and all significantly reduced *Salmonella* levels (Figure 7.14). All treatments except AADP 5 also reduced ($P < 0.05$) the levels of *L. monocytogenes* on stomach; but resulting plate counts were > 1.5 log CFU/g (Figure 7.15).

Salmonella plate counts were significantly reduced on tongue by treatments of AADP 5, AADP 10, LADP 5 and LADP 10 (Figure 7.16). The lactic acid treatments were more effective ($P < 0.05$) in reducing *L. monocytogenes* levels on tongue than were TSPDP, AADP 5 and AADP 10, which were intermediate to the use of lactic acid treatments and the control (Figure 7.17). The levels of *Y. enterocolitica* recovered from tongue after application of AADP 5, AADP 10, LADP 5 and LADP 10 were all below the detectable limit (Figure 7.18).

Lactic acid treatments generally resulted in a larger numeric log reduction than did use of the other decontamination treatments. Inoculated samples of variety meats were immersed in lactic acid solutions and in acetic acid solutions for two different time periods; 10 sec. and 5 sec. The 10 sec immersion consistently produced a greater log reduction than did the 5 sec immersion for both lactic acid and acetic acid solutions except when reductions resulted in counts below the detectable limit. Although hydrogen peroxide reduced *Salmonella* and *L. monocytogenes* on stomach, it produced a foam that interfered with application and may be a problem during packaging.

As was previously indicated, two different agar media (TSA+STREP and LPM+STREP for *L. monocytogenes*, TSA+RIF and XLD+RIF for *Salmonella*, and TSA+RIF and CIN+RIF for *Y. enterocolitica*) were used for each bacterium to evaluate

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their comparative abilities to enumerate viable cells present on variety meat samples. Figures 7.1 through 7.18 show mean counts for *Salmonella*, *L. monocytogenes* and *Y. enterocolitica* grown on the nonselective agar (TSA+RIF and TSA+STREP) and grown on the selective agars (XLD+RIF, LPM+STREP and CIN+RIF). The difference between the means from the non-selective agar and the selective agar for each treatment reflected, in part, differences in viable versus non-viable bacteria injured, but not killed, by heat and/or chemicals.

Salmonella spp. counts for inoculated cheek meat, heart and tongue grown on the XLD+RIF were near or below the detectable level, while, except for cheek meat immersed in LADP 10, *Salmonella* spp. counts on the TSA+RIF were between 1 and 2 log CFU/g. The TSA+STREP and LPM+STREP counts for *L. monocytogenes* were closer to the same than were those of *Salmonella* grown on two different agars, indicating that *L. monocytogenes* was more resistant to these decontamination treatments than were *Salmonella* This concurs with the results of studies by van Netten *et al.* (1995; as summarized by Smulders and Greer, 1998) in which use of a 2% lactic acid solution at 37°C for 60 to 90 sec eliminated *Salmonella* but not *Listeria*. Decontamination treatments were very effective in reducing *Y. enterocolitica* counts, thus *Y. enterocolitica* contamination was easily controlled on the surface of pork variety meats (Table 7.3 and Figures 7.3, 7.10, 7.13 and 7.18).

SUMMARY

Several researchers have documented that decontamination treatments can be used to decrease bacterial populations on pork carcasses. In support of those results, this

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study demonstrated that various decontamination treatments can effectively reduce the levels of *Salmonella*, *L. monocytogenes* and *Y. enterocolitica* on pork variety meats. It is important to reiterate that decontamination treatments can be very useful if incorporated into plant decontamination systems that include GMPs and SSOPs and/or if they are used as a component of multiple hurdles microbiological intervention systems. Decontamination treatments should not be relied on to substitute for proper management or to correct for negligent handling or storage of these, or any other type of food products. More research is needed in actual plant situations to validate those decontamination treatments that were successful in the laboratory, to determine their effectiveness and practicality for use by the industry.

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APPENDIX I

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SALMONELLA METHOD - USDA/FSIS METHOD, MEAT PRODUCTS

This section outlines the procedure used to culture *Salmonella* spp. from raw meat.

1. Stomach for 2 min

2. Incubate enrichment bag for 24 $\pm$ 2 h at 35°C.

3. After incubation, add 1 ml from enrichment bag to one 9 ml Tetrathionate and Selenite Cysteine broth. Vortex.

4. Incubate 24 $\pm$ 2 h at 35°C.

5. From each Tetrathionate and Selenite Cysteine broth tube, streak to one plate each of Hektoin enteric agar (HE), XLD agar, and bismuth sulfite agar (BS).

6. Incubate plates 24 to 48 h at 35°C.

7. If plates have few typical colonies when first examined, return to incubator and re-examine the next day.

8. Typical *Salmonella* colonies are as follows:
   XLD - select pink colonies with or without black centers. Many cultures produce colonies with large, glossy black centers or appear as almost completely black centers.
   HE - select blue-green to blue colonies with or without black centers. Many cultures may produce colonies with large, glossy black centers or may appear as almost completely black centers.
   BS - select colonies that are black and are surrounded by a blackened medium that exhibits a metallic sheen by reflected light. Occasionally, colonies may be green.

9. Pick three colonies from a plate. Avoid touching the agar. Inoculate LIA and TSIA with each colony. Incubate at 35°C for up to 24 h.

10. LIA Cultures:
    (+) will have a purple butt
    (-) will have a yellow (acid) butt

11. TSIA Cultures:
    (+) will have a red (alkaline) slant and a yellow (acid) butt with or without production of H_2S (blackening)

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12. Take any presumptive + cultures and place into one drop each of *Salmonella* O Antiserum (poly A-1 and Vi) and *Salmonella* H Antiserum (poly A-Z). Test for agglutination.

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LISTERIA MONOCYTOGENES USDA/FSIS METHOD

This section outlines the procedures used to culture *Listeria monocytogenes* from raw meat.

1. Stomach for 2 min and incubate enrichment bag for 20 to 24 h at 30°C.

2. After incubation, add 0.1 ml Fraser supplement to Fraser broth tube. Transfer 0.1 ml of broth from enrichment bag into Fraser broth. Vortex.

3. Incubate Fraser broth at 35°C for 26 ± 2 h and 48 ± 2 h.

4. Streak black or darkening tubes to Modified Oxford Agar (MOX). Incubate at 35°C for 24 to 48 h.

5. Typical presumptive *Listeria monocytogenes* colonies should be small colonies with black zones around the colonies.

6. Pick 5 presumptive *Listeria* colonies and streak for isolation onto horse blood overlay agar plate. Incubate overnight at 35°C.

7. Examine the blood agar plates with a fluorescent lamp. Select translucent colonies, about 1 to 2 mm in diameter, with a narrow zone of beta hemolysis surrounding the colony and complete clearing of the blood underneath.

8. Pick isolated colonies and transfer to BHI broth and Bacto Motility medium by stabbing. Incubate overnight at 30 to 25°C.

9. Inoculate Bile Esculin Agar, MR-VP medium, 0/F medium, Nitrate broth and Rhamnose, Xylose, and Mannitol fermentation broths from the BHI broth. Also test for oxidase and catalase reactions.

10. Inoculate the Motility medium for 2 d. Observe for an umbrella type of growth. Discard cultures that do not have this type of growth.

12. Perform Modified CAMP test and confirmatory tests. Refer to directions in the USDA Microbiology Laboratory Guidebook (MLG).

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ISOLATION OF CAMPYLOBACTER JEJUNI/COLI; USDA/FSIS METHODS

A. Equipment, Reagents, and Media

1. Equipment
    a. Agitating incubator(s) for 37° and 42° C
    b. Balance
    c. Stomacher and sterile bags
    d. Anaerobic jars
    e. Campy-Pak Plus (BBL 71045) - Do not use an additional catalyst with this.
    f. Filter paper (for glycerol humectant and oxidase test)
    g. Petri-dishes (100 x 15 mm disposable)
    h. Sterile plastic inoculating loops and needles
    i. Glass microscope slides and cover slips
    j. "Hockey sticks"
    k. Pipettes

2. Reagents
    a. Glycerol
    b. 3% Hydrogen Peroxide solution
    c. Oxidase reagent
    d. Nalidixic acid antibiotic susceptibility discs (30μg)
    e. Cephalothin antibiotic susceptibility discs (30μg)

3. Media
    a. Hunt Enrichment Broth
    b. 0.1% peptone water
    c. Modified Campylobacter Charcoal Differential Agar (MCCDA)
    d. Semisolid Brucella Glucose Medium
    e. Brucella-FBP (BFBP) Broth
    f. Brucella-FBP (BFBO) Agar
    g. Enriched Semisolid Brucella Medium

B. Media and Reagent Preparation

1. <u>Hunt Enrichment Broth</u>

<u>Basal Broth</u>

Nutrient broth #2 (Oxoid CM67)	25.0 g
Yeast Extract (Oxoid L21 of Difco 0886-17)	6.0 g
Distilled Water	950 ml

Dissolve the nutrient broth #2 and yeast extract in distilled water. Autoclave at 121°C for 15 min. Cool media and add the following per liter of basal broth:

Sterile, lysed horse blood (Oxoid)	50.0 ml
Vancomycin (Dissolve 0.25 g vancomycin hydrochloride in DI water, mix well, and filter sterilize.	4.0 ml

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Trimethoprim Lactate (Sigma T-0067) - Dissolve 0.3125 g in DI water, mix, and filter sterilize.	4.0 ml
Sodium Cefoperazone (Dissolve 0.375 g in DI water, mix, and filter sterilize.	4.0 ml
Sodium Cyclohexmide (Sigma) Prepare as a 10% solution in 50% ethanol. Dissolve 5 g sodium cycloheximide in 50 ml 50% ethanol. Mix, and filter sterilize.	1.0 ml
FBP Supplement (Oxoid SR84, *Campylobacter* Growth Supplement)	2.0 ml/ 500 ml

C. Isolation Procedure

1. Place sponge samples in 100 ml Hunt Enrichment Broth (HEB).

2. Incubate samples in anaerobic jar with Campy-Pak at 37°C, shaking at 100 RPM for 4 h.

3. After 4 h incubation, add additional sterile cefoperaone solution. Remove the Campy-Pak and add a new Campy-Pak. Increase temperature to 42°C. Continue the incubation for 20 h shaking at 100 RPM.

4. Streak enrichments directly and at a 1:100 dilution onto MCCDA plates. Swirl a swab in the enrichment bag and add to a 9.9 ml tube of 0.1% peptone water and vortex. Inoculate the plates by placing a swab into the enrichment or dilution and removing excess liquid. Swab approximately 40% of the plate, then streak from the swabbed area to yield isolated colonies.

5. Incubate the plates at 42°C for 24 h in an anaerobic jar with a Campy-Pak. Add 4 drops of glycerol to a filter paper and place it in the jar to diminish typical confluent and swarming growth of *Campylobacter*. If no growth is achieved at 24 h, reincubate the plates for an additional 24-48 h to attempt recovery.

D. Identification of *Campylobacter*

1. *Campylobacter* colonies on MCCDA are smooth, shiny, and convex with a defined edge, of flat, transparent or translucent, and spreading with an irregular edge; colorless to grayish or light cream, and usually 1 to 2 mm in diameter buy may be pinpoint to several mm in diameter.

2. Transfer suspect colonies for each sample from the MCCDA plates and transfer each to 10 ml of Brucella-FBP (BFBP) broth. Loosen the tube caps to allow atmosphere entry. Do not vortex culture tubes, as this will introduce oxygen into the media. Incubate tubes for 24-48 h at 42°C with the addition of a Campy-Pak.

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3. Perform the following identification tests on each BFBP broth culture:
   a. Inoculate a tub of semisolid brucella glucose medium with several drops of the above BFBP broth culture (using a Pasteur pipette) into the top 10 mm layer of the medium. Incubate tubes with Campy-Pak for 1 to 3 d.
   b. Glucose fermentation test - *Campylobacter* are nonfermentative, so the color of the medium will remain red-orange. A positive reaction shows a yellow color (acid with phenol red indicator) in the semisolid brucella glucose medium.
   c. Catalase test. After reading the results of the glucose fermentation test, add 1 ml of 3% hydrogen peroxide to the semisolid brucella glucose medium culture, then gently invert the tube several times to distribute the reagent. Examine after 1 to 10 min for formation of bubbles, indicating a positive reaction. *Campylobacter jejuni* and *Campylobacter coli* are catalase positive.

4. Add about six drops of the BFBP broth culture to a BFBP agar plate, and spread over the surface with a sterile hockey stick. Place a disc of nalidixic acid (30 ug) and a disc of cephalothin (30 ug) on each plate. Press each disc on the agar in a sterile manner to assure attachment. Incubate the plates at 42°C for 1 to 3 d with a Campy-Pak.
   a. Observe the growth patterns surrounding the antibiotic impregnated discs. C. *jejuni* and C. *coli* are sensitive to nalidixic acid, and a clear zone of inhibition will exist around the disc. A zone of any size indicates sensitivity. The organisms are both resistant to cephalothin, so growth will be present right up to the disc.
   b. Oxidase test - Heavily smear cells from the above BFBP agar plate onto a filter paper and drop oxidase reagent onto the culture. The test is positive if the cell mass turns dark purple within 30 sec.

5. Retain positive cultures for future use.

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YERSINIA ENTEROCOLITICA

This method was taken from the Compendium of Methods for the Microbiolical Examination of Foods, Chapter 27.

1. Stomach for 2 min.

2. Incubate enrichment bag for 24 ± 2 h at 35°C.

3. Incubate Bile-Oxalate-Sorbose (BOS) selective enrichment broth with 0.1 ml from enrichment bag to 10 ml of BOS. Incubate at 22 to 25°C for a total of 5 d.

4. Streak onto *Yersinia* Agar (DIFCO) with supplement at day 3 and day 5 of BOS. Incubate at 30°C for 18 to 24 h.

5. Presumptive colonies on agar have a red "bullseye" with a transparent border.

6. Transfer presumptive colonies to KIA slants. Incubate at 35°C for 18 to 24 h. Presumptive *Yersinia* will give an Alkaline/Acid reaction without H_2S and little or no gas. Inoculate Christensen's Urea Agar Slant and read after 2 to 3 h. Hold negative slants overnight. *Yersinia* urease positive will produce a "hot pink" color on slant.

7. Take positive presumptive isolates to the following biochemicals:
    a) Simmons Citrate - *Yersinia enterocolitica* is negative (no color change).
    b) Purple Broth Base with 0.3 ml of Sucrose - *Yersinia enterocolitica* is positive (broth turns yellow).
    c) Purple Broth Base with 0.3 ml of L-Rhamnose - *Yersinia enterocolitica* is negative (no color change). Incubate at 35°C for 24 h.

8. Take presumptive isolates to Micro-ID strip. Incubate at 35°C for 4 h.

Positive *Yersinia enterocolitica* isolates should be saved, and then biotyped to determine whether the isolate is a pathogenic strain of *Yersinia enterocolitica.*

Isolates will be sent out for "O" serotyping and other indirect markers of pathogenicity.

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APPENDIX II

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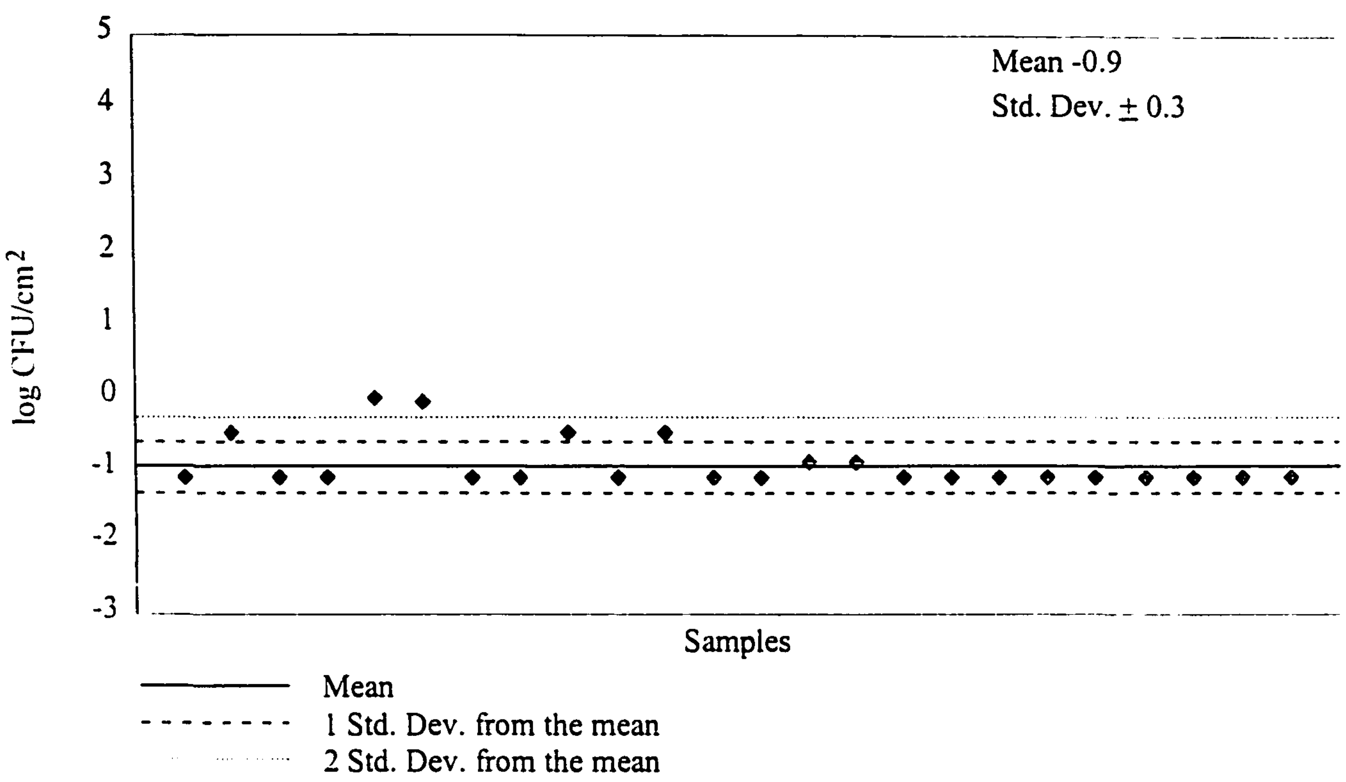
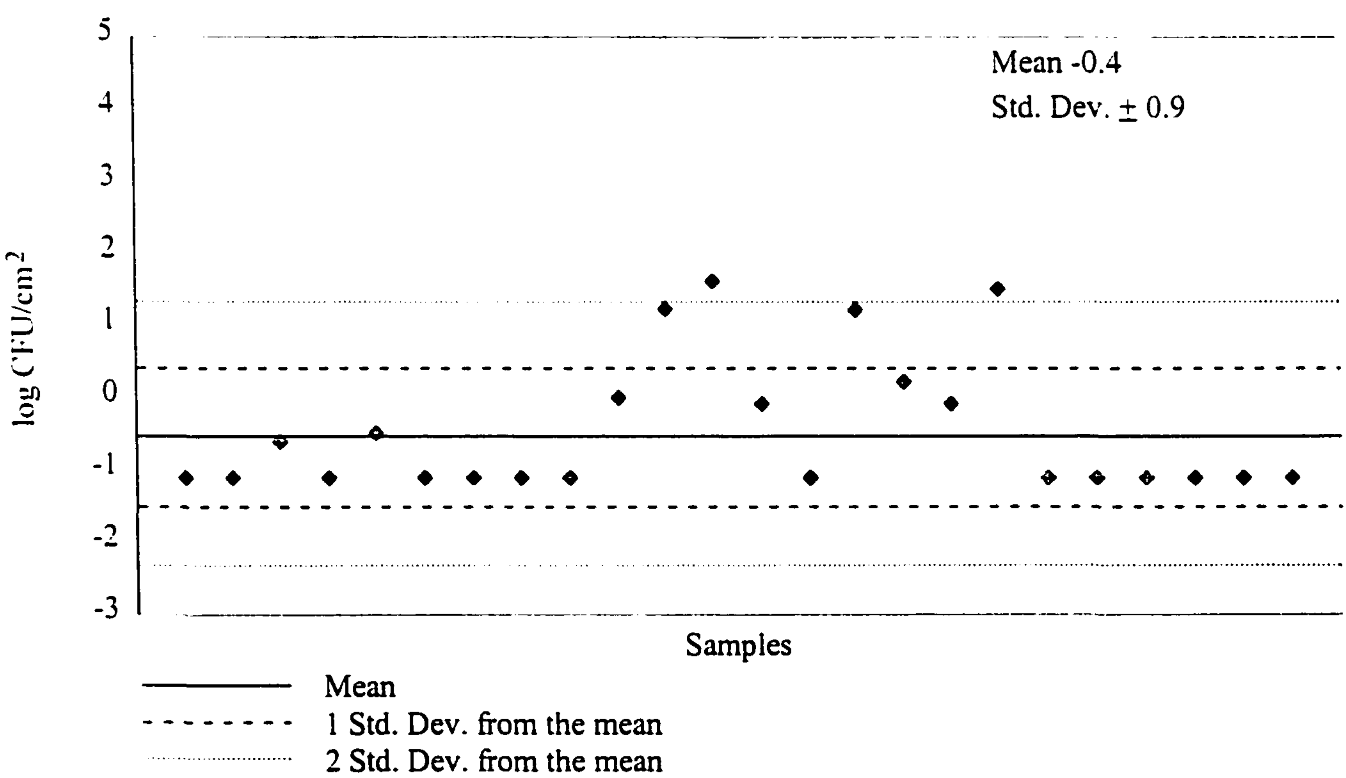


Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler of plant A across both seasons (data collected using the 3-site sponge sampling protocol).



Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler of plant B across both seasons (data collected using the 3-site sponge sampling protocol).

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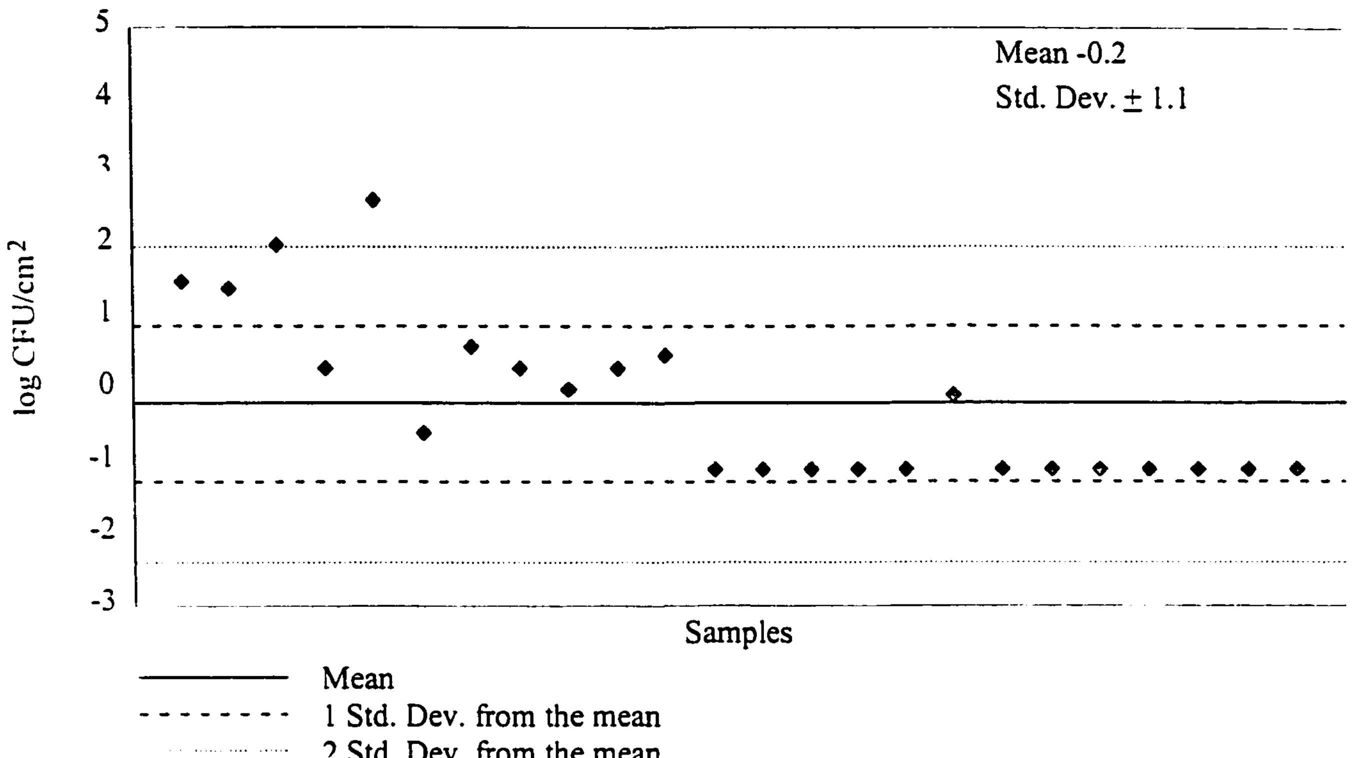
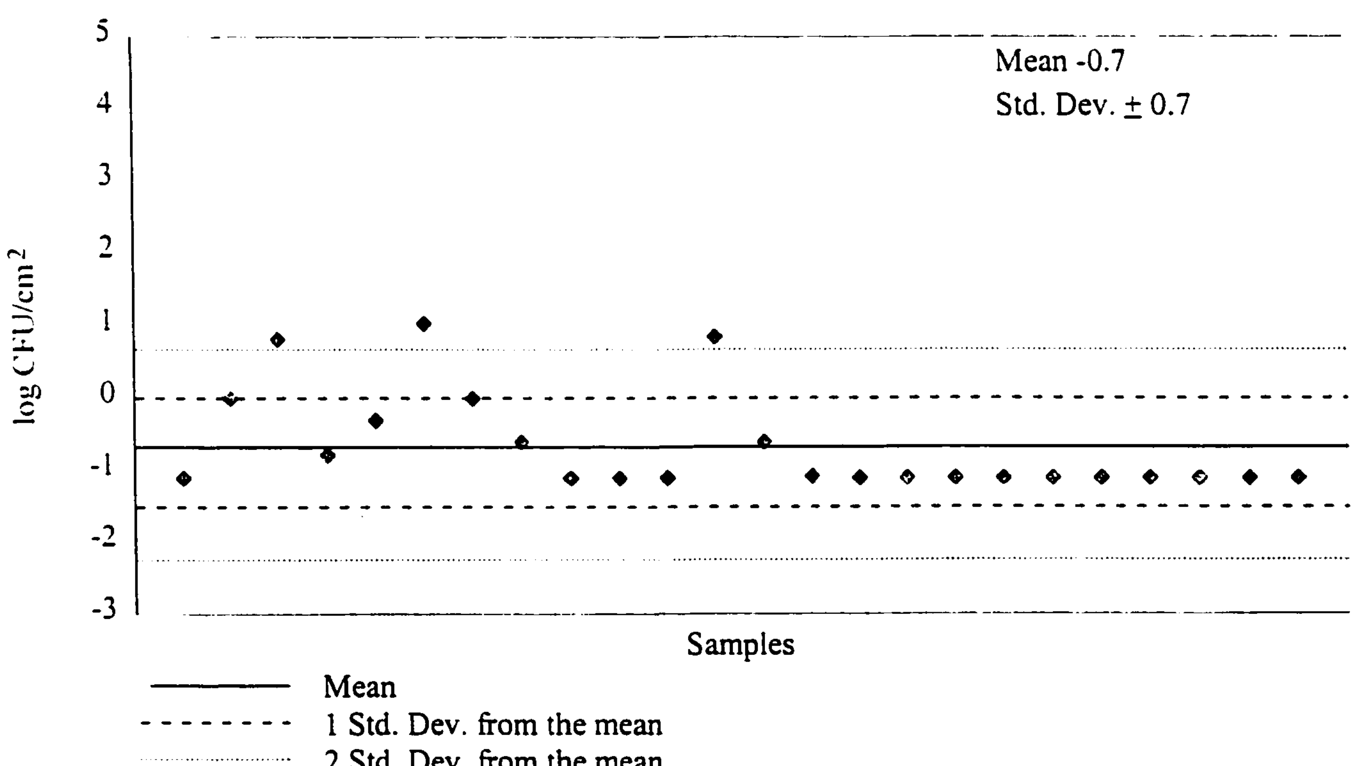


Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler of plant C across both seasons (data collected using the 3-site sponge sampling protocol).



Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler of plant D across both seasons (data collected using the 3-site sponge sampling protocol).

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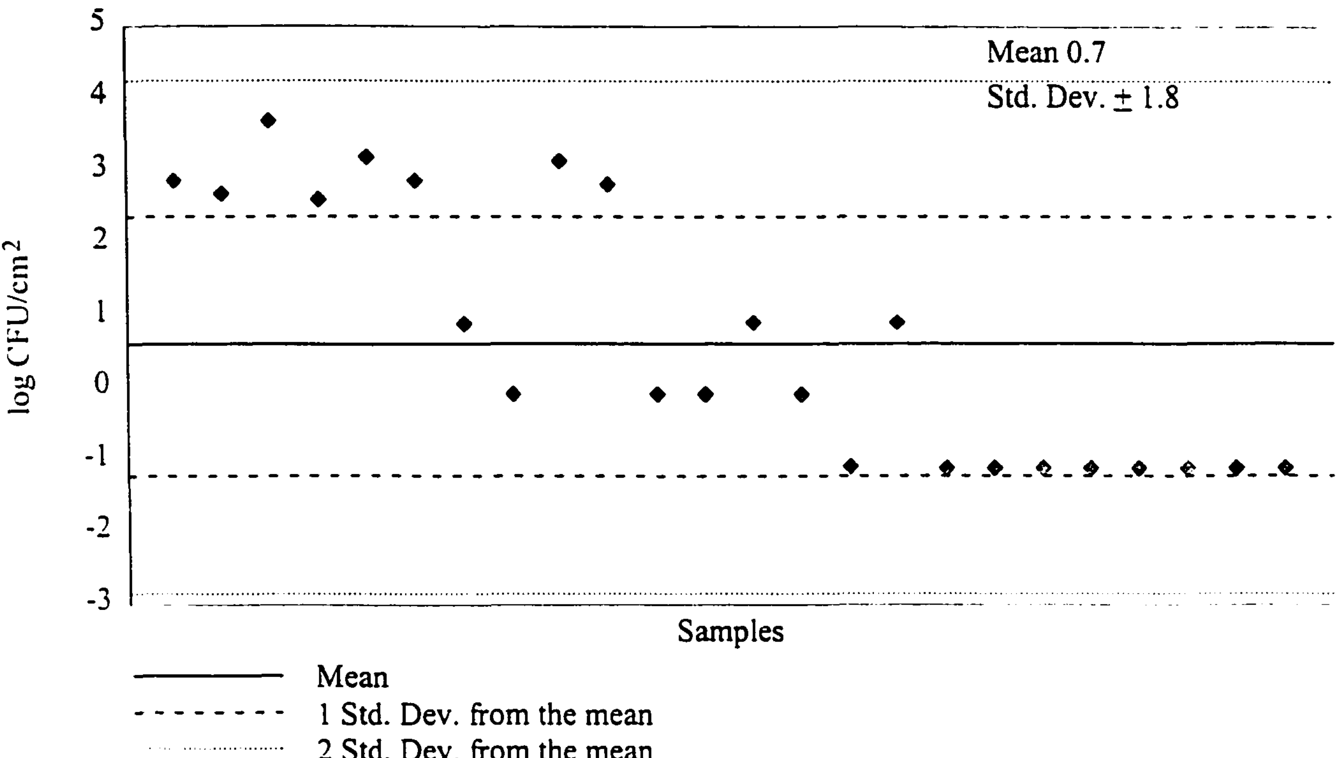
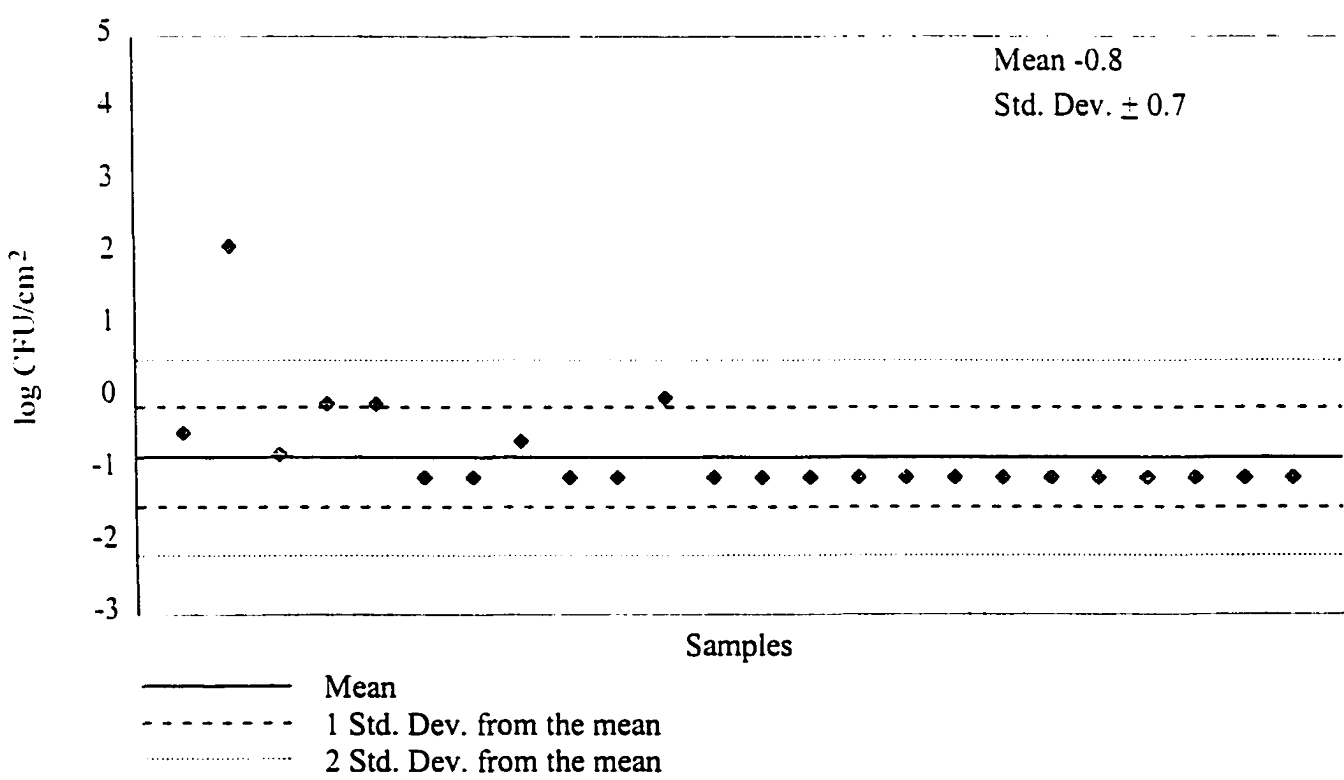


Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler of plant E across both seasons (data collected using the 3-site sponge sampling protocol).



Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler of plant F across both seasons (data collected using the 3-site sponge sampling protocol).

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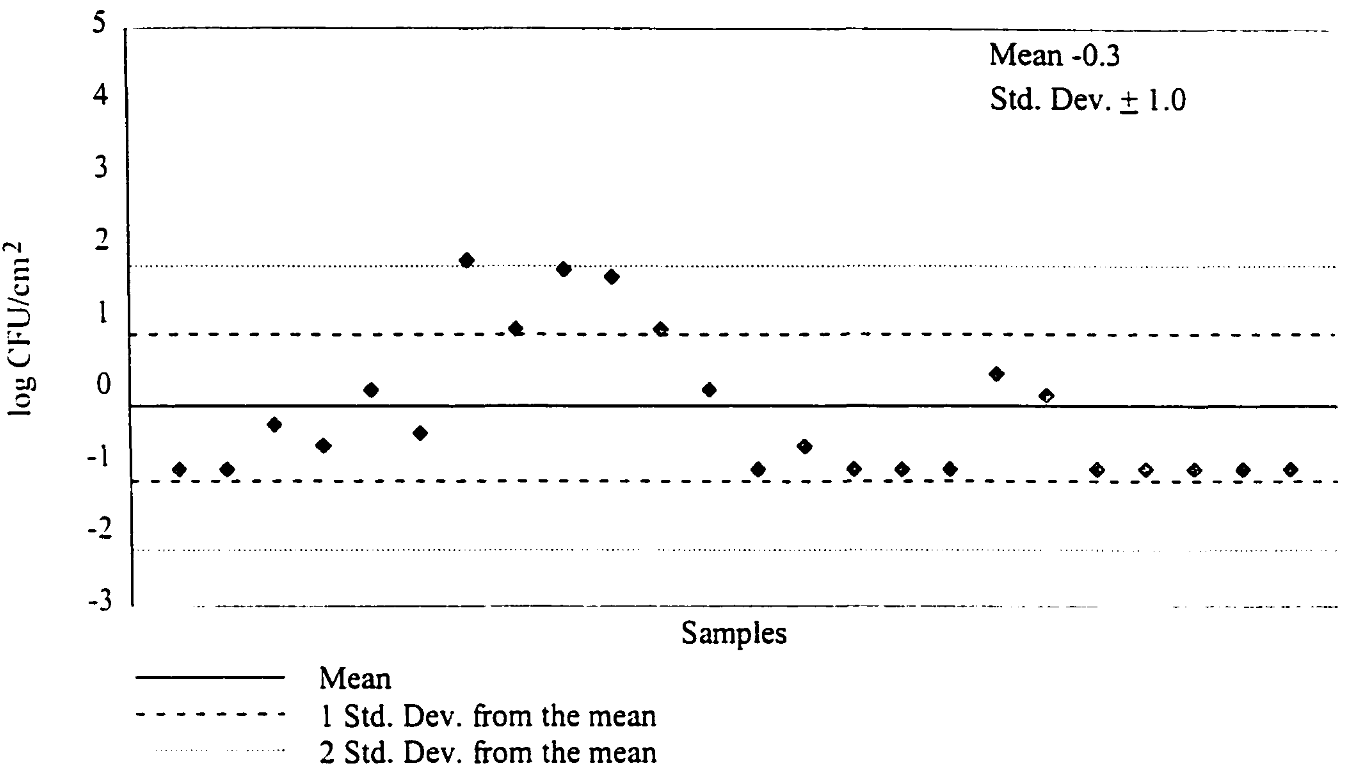
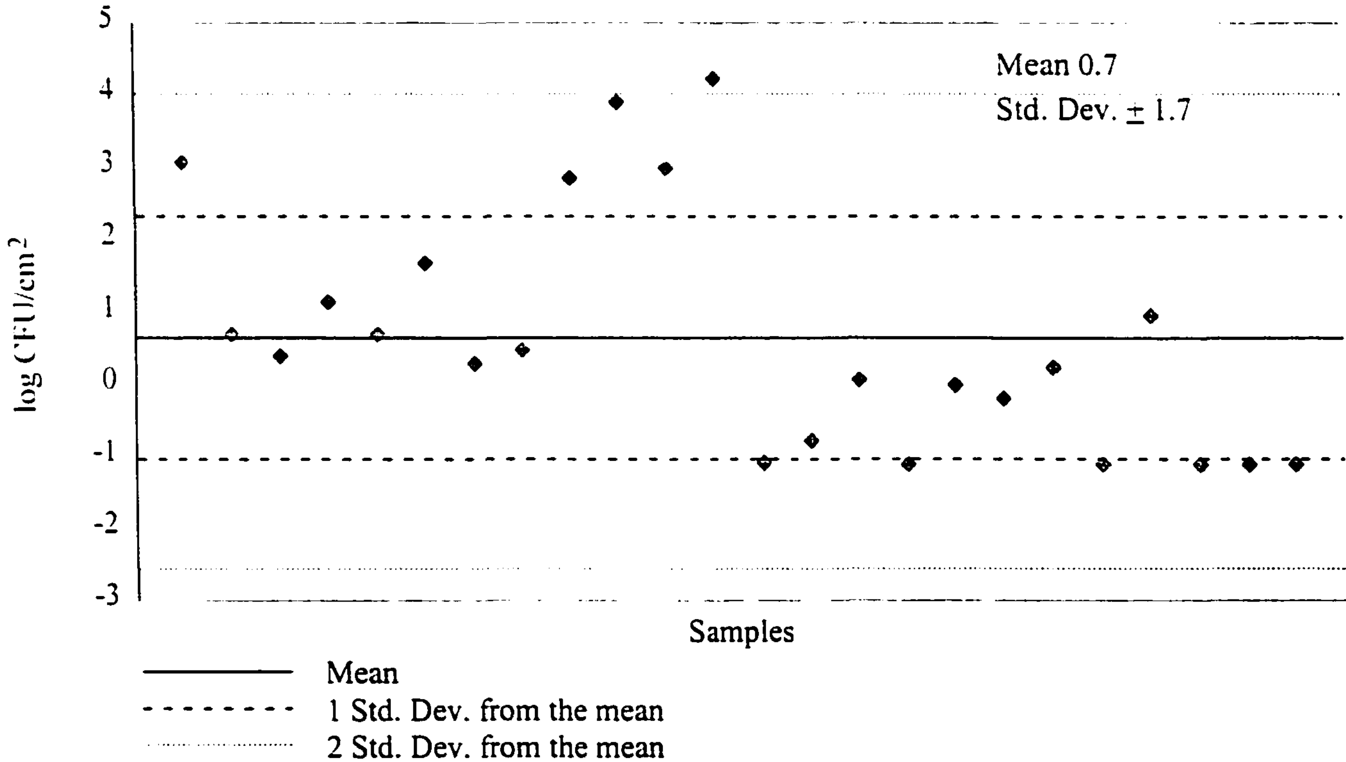


Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler of plant G across both seasons (data collected using the 3-site sponge sampling protocol).



Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler of plant H across both seasons (data collected using the 3-site sponge sampling protocol).

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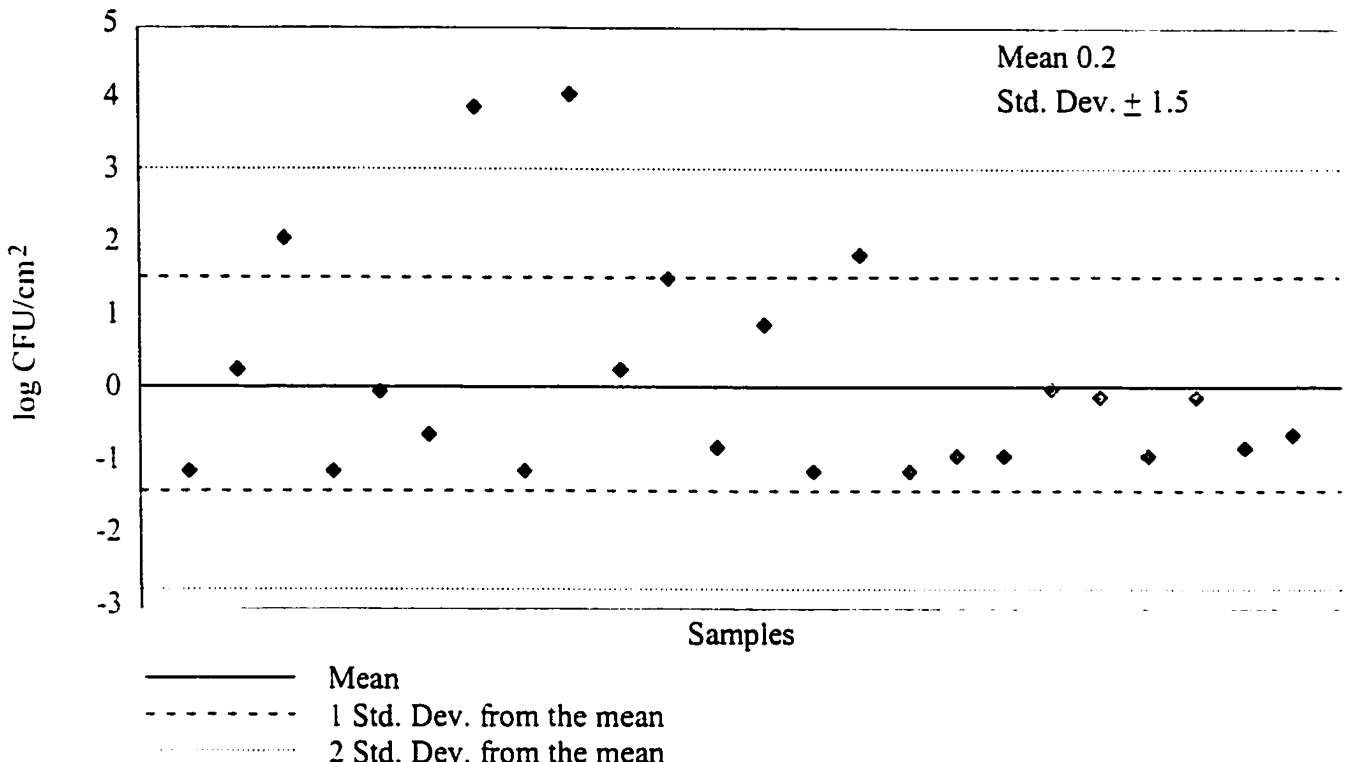
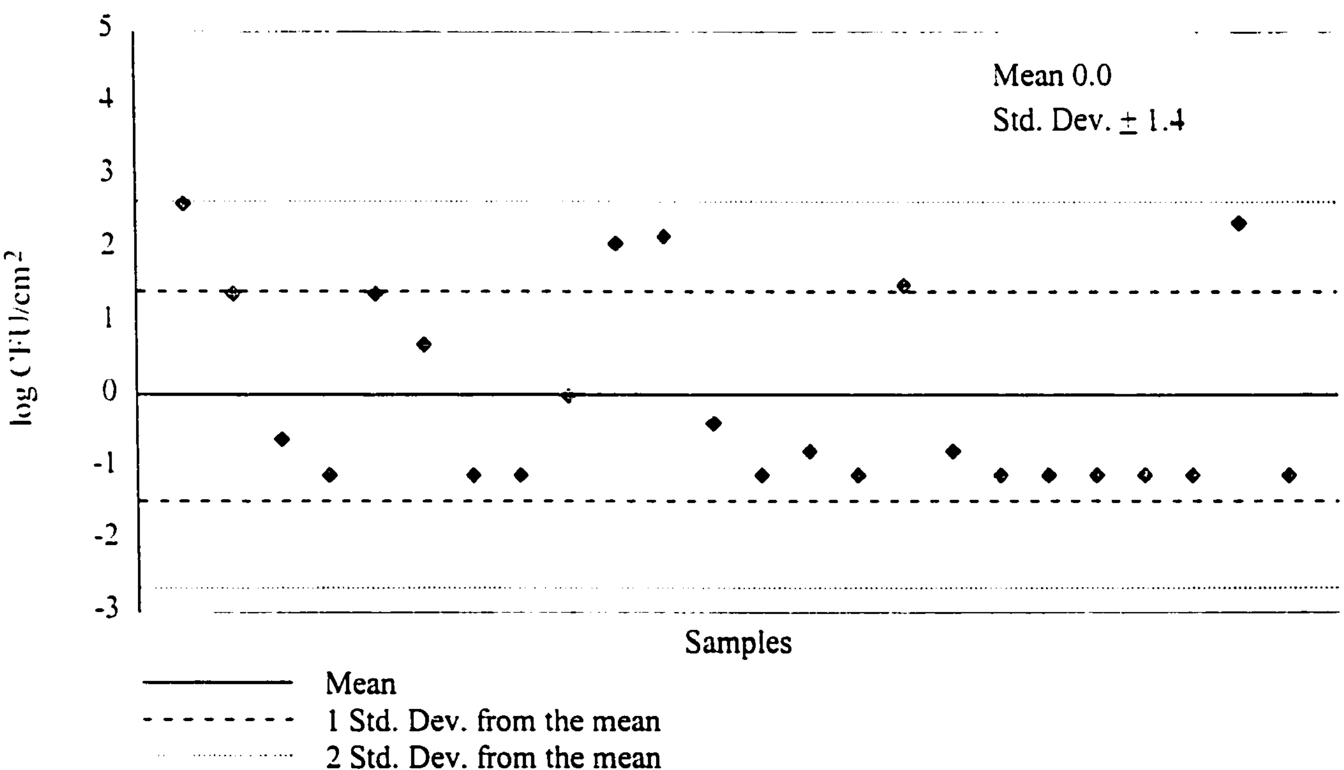


Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler of plant I across both seasons (data collected using the 3-site sponge sampling protocol).



Statistical Process Control chart of *E. coli* counts obtained from hog carcasses in the cooler of plant J across both seasons (data collected using the 3-site sponge sampling protocol).

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APPENDIX III

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Pork Variety Meats - Bung

Bacteria Counts

			CFU/g			Log CFU/g		
Bacteria	Site	n	Min	Max	Mean	Min	Max	Mean
APC	A	30	8,300	21,000,000	3,415,910	3.9	7.3	6.0^{a}
	B	30	36,000	97,000,000	6,122,500	4.6	8.0	5.6^{a}
TCC	A	30	160	1,000,000	235,322	2.2	6.0	4.5^{a}
	B	30	6	70,000	8,271	0.8	4.9	2.8^{b}
ECC	A	30	110	1,000,000	204,588	2.0	6.0	4.3^{a}
	B	30	6	70,000	7,983	0.8	4.9	2.6^{b}

Site A - samples were collected after trimming and handling and just prior to packaging and chilling.
Site B - point at which variety meat was suitably chilled and deemed ready for distribution (~24 hours in the freezer or chiller after Site A).
[ab] Means in a column, within bacteria and between sites, bearing different superscript letters, differ ($P < 0.05$).

Incidence of Pathogens

		Campylobacter jejuni/coli		*Salmonella* species		*Listeria monocytogenes*		*Yersinia enterocolitica*		% Total
	n	Positive[†]	%	Positive[†]	%	Positive[†]	%	Positive[†]	%	(n = 120)
Samples	30	1	3	4	13	0	0	0	0	4
Plants	6	1	17	2	33	0	0	0	0	

[†]Number of positive samples from those tested; or number of plants with at least one positive sample.

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Pork Variety Meats - Cheek Meat

Bacteria Counts

			CFU/g			Log CFU/g		
Bacteria	Site	n	Min	Max	Mean	Min	Max	Mean
APC	A	45	6,100	24,000,000	1,934,069	3.8	7.4	5.3
	B	45	4,000	20,000,000	2,511,518	3.6	7.3	5.2
TCC	A	45	60	1,100,000	189,460	1.8	6.0	3.9
	B	45	11	3,500,000	268,983	1.0	6.6	3.6
ECC	A	45	8	1,100,000	187,275	0.9	6.0	3.6
	B	45	7	1,600,000	143,314	0.9	6.2	3.1

Site A - samples were collected after trimming and handling and just prior to packaging and chilling.
Site B - point at which variety meat was suitably chilled and deemed ready for distribution (~24 hours in the freezer or chiller after Site A).

Incidence of Pathogens

		Campylobacter jejuni/coli		*Salmonella* species		*Listeria monocytogenes*		*Yersinia enterocolitica*		% Total
	n	Positive[†]	%	Positive[†]	%	Positive[†]	%	Positive[†]	%	(n = 180)
Samples	45	0	0	6	13	15	33	0	0	18
Plants	9	0	0	3	33	5	56	0	0	

[†]Number of positive samples from those tested; or number of plants with at least one positive sample.

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Pork Variety Meats - Front Feet

Bacteria Counts

Bacteria	Site	n	CFU/g Min	CFU/g Max	CFU/g Mean	Log CFU/g Min	Log CFU/g Max	Log CFU/g Mean
APC	A	40	8,100	6,500,000	483,153	3.9	6.8	5.2^{a}
	B	30	2,100	1,300,000	185,517	3.3	6.1	4.7^{b}
TCC	A	40	4	40,000	3,295	0.6	4.6	2.0^{a}
	B	30	4	33,000	3,550	0.6	4.5	1.8^{a}
ECC	A	40	3	14,000	996	0.5	4.2	1.3^{a}
	B	30	4	3,900	813	0.6	3.6	1.5^{a}

Site A - samples were collected after trimming and handling and just prior to packaging and chilling.
Site B - point at which variety meat was suitably chilled and deemed ready for distribution (~24 hours in the freezer or chiller after Site A).
[ab] Means in a column, within bacteria and between sites, bearing different superscript letters, differ ($P < 0.05$).

Incidence of Pathogens

	n	*Campylobacter jejuni/coli* Positive†	*Campylobacter jejuni/coli* %	*Salmonella* species Positive†	*Salmonella* species %	*Listeria monocytogenes* Positive†	*Listeria monocytogenes* %	*Yersinia enterocolitica* Positive†	*Yersinia enterocolitica* %	% Total (n = 100)
Samples	25	0	0	0	0	7	28	0	0	6
Plants	5	0	0	0	0	2	40	0	0	

†Number of positive samples from those tested; or number of plants with at least one positive sample.

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Pork Variety Meats - Head Meat

Bacteria Counts

Bacteria	Site	n	CFU/g Min	CFU/g Max	CFU/g Mean	Log CFU/g Min	Log CFU/g Max	Log CFU/g Mean
APC	A	40	24,000	31,000,000	2,615,700	4.4	7.5	5.7[a]
	B	40	2,600	570,000	106,380	3.4	5.8	4.7[b]
TCC	A	40	23	2,400,000	271,800	1.4	6.4	4.3[a]
	B	40	9	210,000	7,359	1.0	5.3	2.8[b]
ECC	A	40	8	2,400,000	235,918	0.9	6.4	3.8[a]
	B	40	9	28,000	1,565	1.0	4.5	2.2[b]

Site A - samples were collected after trimming and handling and just prior to packaging and chilling.
Site B - point at which variety meat was suitably chilled and deemed ready for distribution (~24 hours in the freezer or chiller after Site A).
[ab] Means in a column, within bacteria and between sites, bearing different superscript letters, differ ($P < 0.05$).

Incidence of Pathogens

	n	*Campylobacter jejuni/coli* Positive[†]	*Campylobacter jejuni/coli* %	*Salmonella* species Positive[†]	*Salmonella* species %	*Listeria monocytogenes* Positive[†]	*Listeria monocytogenes* %	*Yersinia enterocolitica* Positive[†]	*Yersinia enterocolitica* %	% Total (n = 160)
Samples	40	1	3	12	30	2	5	0	0	13
Plants	8	1	13	4	50	2	25	0	0	

[†]Number of positive samples from those tested; or number of plants with at least one positive sample.

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Pork Variety Meats - Heart

Bacteria Counts

Bacteria	Site	n	CFU/g Min	CFU/g Max	CFU/g Mean	Log CFU/g Min	Log CFU/g Max	Log CFU/g Mean
APC	A	44	5	7,500,000	586,755	0.7	6.9	4.0^{a}
	B	45	5	2,300,000	197,852	0.7	6.4	3.4^{b}
TCC	A	44	4	360,000	13,284	0.6	5.6	2.0^{a}
	B	45	5	5,400	503	0.7	3.7	1.7^{a}
ECC	A	44	4	360,000	9,487	0.6	5.6	1.6^{a}
	B	45	4	200	26	0.6	2.3	1.1^{a}

Site A - samples were collected after trimming and handling and just prior to packaging and chilling.
Site B - point at which variety meat was suitably chilled and deemed ready for distribution (~24 hours in the freezer or chiller after Site A).
[ab] Means in a column, within bacteria and between sites, bearing different superscript letters, differ ($P < 0.05$).

Incidence of Pathogens

	n	*Campylobacter jejuni/coli* Positive[†]	*Campylobacter jejuni/coli* %	*Salmonella* species Positive[†]	*Salmonella* species %	*Listeria monocytogenes* Positive[†]	*Listeria monocytogenes* %	*Yersinia enterocolitica* Positive[†]	*Yersinia enterocolitica* %	% Total (n = 180)
Samples	45	1	2	2	4	1	2	0	0	3
Plants	9	1	11	2	22	1	11	0	0	

[†]Number of positive samples from those tested; or number of plants with at least one positive sample.

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Pork Variety Meats - Kidney

Bacteria Counts

Bacteria	Site	n	CFU/g Min	CFU/g Max	CFU/g Mean	Log CFU/g Min	Log CFU/g Max	Log CFU/g Mean
APC	A	30	210	6,000,000	692,023	2.3	6.8	5.0[a]
	B	30	260	3,300,000	252,695	2.4	6.5	4.1[b]
TCC	A	30	5	76,000	14,186	0.7	4.9	3.2[a]
	B	30	11	15,000	986	1.0	4.2	2.0[b]
ECC	A	30	5	21,000	3,272	0.7	4.3	2.4[a]
	B	30	10	240	44	1.0	2.4	1.4[b]

Site A - samples were collected after trimming and handling and just prior to packaging and chilling.
Site B - point at which variety meat was suitably chilled and deemed ready for distribution (~24 hours in the freezer or chiller after Site A).
[ab] Means in a column, within bacteria and between sites, bearing different superscript letters, differ ($P < 0.05$).

Incidence of Pathogens

	n	*Campylobacter jejuni/coli* Positive[†]	*Campylobacter jejuni/coli* %	*Salmonella* species Positive[†]	*Salmonella* species %	*Listeria monocytogenes* Positive[†]	*Listeria monocytogenes* %	*Yersinia enterocolitica* Positive[†]	*Yersinia enterocolitica* %	% Total (n = 120)
Samples	30	0	0	1	3	0	0	0	0	1
Plants	6	0	0	1	17	0	0	0	0	

[†]Number of positive samples from those tested; or number of plants with at least one positive sample.

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Pork Variety Meats - Liver

Bacteria Counts

			CFU/g			Log CFU/g		
Bacteria	Site	n	Min	Max	Mean	Min	Max	Mean
APC	A	35	6	35,000,000	1,902,489	0.8	7.5	4.9^{a}
	B	35	26	3,800,000	323,337	1.4	6.6	4.0^{b}
TCC	A	35	6	980,000	130,256	0.8	6.0	3.6^{a}
	B	35	9	660,000	55,225	1.0	5.8	2.7^{b}
ECC	A	35	6	680,000	99,232	0.8	5.8	3.2^{a}
	B	35	6	240,000	23,220	0.8	5.4	2.6^{b}

Site A - samples were collected after trimming and handling and just prior to packaging and chilling.
Site B - point at which variety meat was suitably chilled and deemed ready for distribution (~24 hours in the freezer or chiller after Site A).
^{ab} Means in a column, within bacteria and between sites, bearing different superscript letters, differ ($P < 0.05$).

Incidence of Pathogens

		Campylobacter jejuni/coli		*Salmonella* species		*Listeria monocytogenes*		*Yersinia enterocolitica*		
	n	Positive[†]	%	Positive[†]	%	Positive[†]	%	Positive[†]	%	% Total (n = 140)
Samples	35	0	0	11	31	0	0	0	0	9
Plants	7	0	0	5	71	0	0	0	0	

[†]Number of positive samples from those tested; or number of plants with at least one positive sample.

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Pork Variety Meats - Salivary Gland

Bacteria Counts

			CFU/g			Log CFU/g		
Bacteria	Site	n	Min	Max	Mean	Min	Max	Mean
APC	A	35	52,000	40,000,000	5,827,486	4.7	7.6	6.2[a]
	B	35	8,100	16,000,000	1,751,670	3.9	7.2	5.5[b]
TCC	A	35	540	1,700,000	358,346	2.7	6.2	4.6[a]
	B	35	11	1,500,000	101,427	1.0	6.2	3.1[b]
ECC	A	35	160	1,100,000	250,874	2.2	6.0	4.3[a]
	B	35	10	1,500,000	96,216	1.0	6.2	2.8[b]

Site A - samples were collected after trimming and handling and just prior to packaging and chilling.
Site B - point at which variety meat was suitably chilled and deemed ready for distribution (~24 hours in the freezer or chiller after Site A).
[ab] Means in a column, within bacteria and between sites, bearing different superscript letters, differ ($P < 0.05$).

Incidence of Pathogens

		Campylobacter jejuni/coli		*Salmonella* species		*Listeria monocytogenes*		*Yersinia enterocolitica*		
	n	Positive[†]	%	Positive[†]	%	Positive[†]	%	Positive[†]	%	% Total (n = 140)
Samples	35	1	3	5	14	6	17	0	0	10
Plants	7	1	14	3	43	3	43	0	0	

[†]Number of positive samples from those tested; or number of plants with at least one positive sample.

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Pork Variety Meats - Small Intestine

Bacteria Counts

Bacteria	Site	n	CFU/g Min	CFU/g Max	CFU/g Mean	Log CFU/g Min	Log CFU/g Max	Log CFU/g Mean
APC	A	25	10,000	12,000,000	1,324,760	4.0	7.1	5.5
	B	25	74,000	1,900,000	396,280	4.9	6.3	5.5
TCC	A	25	2,900	130,000	31,368	3.5	5.1	4.2
	B	25	2,900	180,000	26,380	3.5	5.3	4.1
ECC	A	25	2,600	130,000	28,784	3.4	5.1	4.1
	B	25	1,500	160,000	20,052	3.2	5.2	4.0

Site A - samples were collected after trimming and handling and just prior to packaging and chilling.
Site B - point at which variety meat was suitably chilled and deemed ready for distribution (~24 hours in the fieezer or chiller after Site A).

Incidence of Pathogens

	n	*Campylobacter jejuni/coli* Positive[†]	*Campylobacter jejuni/coli* %	*Salmonella* species Positive[†]	*Salmonella* species %	*Listeria monocytogenes* Positive[†]	*Listeria monocytogenes* %	*Yersinia enterocolitica* Positive[†]	*Yersinia enterocolitica* %	% Total (n = 120)
Samples	30	0	0	8	32	0	0	0	0	7
Plants	6	0	0	4	80	0	0	0	0	

[†]Number of positive samples from those tested; or number of plants with at least one positive sample.

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Pork Variety Meats - Stomach

Bacteria Counts

Bacteria	Site	n	CFU/g Min	CFU/g Max	CFU/g Mean	Log CFU/g Min	Log CFU/g Max	Log CFU/g Mean
APC	A	50	150	13,000,000	843,460	2.2	7.1	4.8
	B	50	44	18,000,000	1,435,110	1.6	7.3	4.7
TCC	A	50	6	1,100,000	88,671	0.8	6.0	3.4
	B	50	6	630,000	50,873	0.8	5.8	3.2
ECC	A	50	6	1,100,000	64,180	0.8	6.0	2.9
	B	50	6	630,000	36,283	0.8	5.8	2.7

Site A - samples were collected after trimming and handling and just prior to packaging and chilling.
Site B - point at which variety meat was suitably chilled and deemed ready for distribution (~24 hours in the freezer or chiller after Site A).

Incidence of Pathogens

	n	*Campylobacter jejuni/coli* Positive[†]	*Campylobacter jejuni/coli* %	*Salmonella* species Positive[†]	*Salmonella* species %	*Listeria monocytogenes* Positive[†]	*Listeria monocytogenes* %	*Yersinia enterocolitica* Positive[†]	*Yersinia enterocolitica* %	% Total (n = 200)
Samples	50	0	0	0	0	27	54	0	0	23
Plants	10	0	0	0	0	8	80	0	0	

[†]Number of positive samples from those tested; or number of plants with at least one positive sample.

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Pork Variety Meats - Tongue

Bacteria Counts

			CFU/g			Log CFU/g		
Bacteria	Site	n	Min	Max	Mean	Min	Max	Mean
APC	A	45	1,500	16,000,000	1,905,222	3.2	7.2	5.3[a]
	B	45	900	5,500,000	666,367	3.0	6.7	5.1[a]
TCC	A	45	8	1,100,000	101,889	0.9	6.0	3.0[a]
	B	45	6	420,000	16,277	0.8	5.6	2.2[a]
ECC	A	45	6	1,100,000	95,179	0.8	6.0	2.5[a]
	B	45	5	420,000	13,252	0.7	5.6	1.9[b]

Site A - samples were collected after trimming and handling and just prior to packaging and chilling.
Site B - point at which variety meat was suitably chilled and deemed ready for distribution (~24 hours in the freezer or chiller after Site A).
[ab] Means in a column, within bacteria and between sites, bearing different superscript letters, differ ($P < 0.05$).

Incidence of Pathogens

		Campylobacter jejuni/coli		*Salmonella* species		*Listeria monocytogenes*		*Yersinia enterocolitica*		Total
	n	Positive[†]	%	Positive[†]	%	Positive[†]	%	Positive[†]	%	(n = 180)
Samples	45	0	0	10	22	5	11	0	0	13
Plants	9	0	0	3	33	3	33	0	0	

[†]Number of positive samples from those tested; or number of plants with at least one positive sample.

APPENDIX IV

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Pork Variety Meats - Bung

Bacteria counts comparing site A (point of packaging) and site B
(after 24 h of frozen storage) from the six plants sampled.

Distribution of Aerobic Plate Counts

Percent of Total Samples

0 20 40 60 80 100

■ Site A
Site B

CFU/g

0 < 1
>1 ≤ 10
>10 ≤ 100
>100 ≤ 1,000
>1,000 ≤ 10,000
>10,000 ≤ 100,000
>100,000 ≤ 1,000,000
>1,000,000 ≤ 10,000,000
> 10,000,000

Distribution of Total Coliform Counts

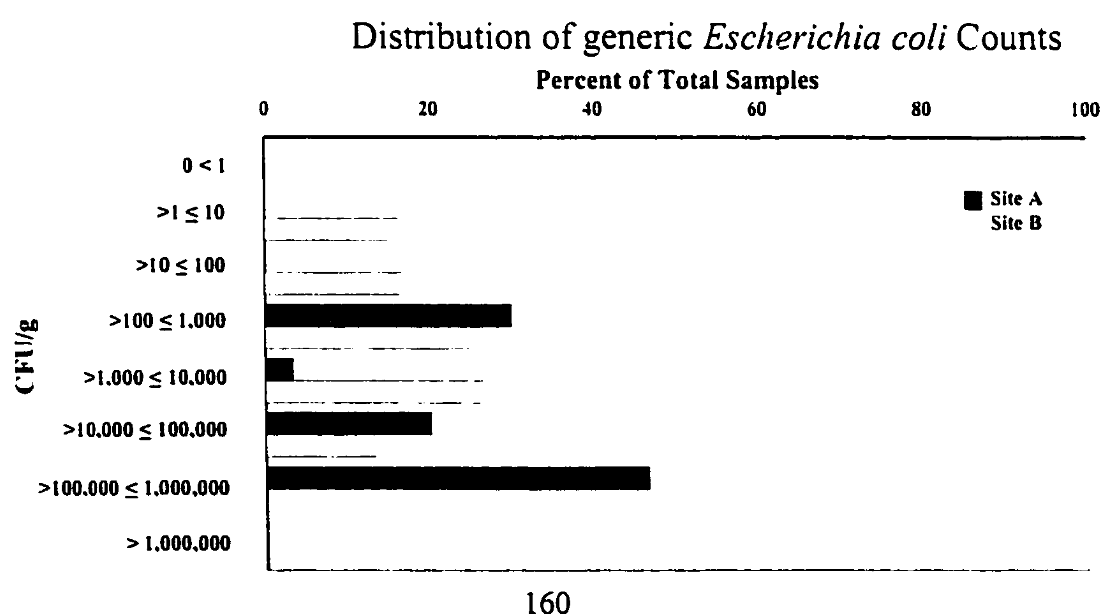
Percent of Total Samples

0 20 40 60 80 100

■ Site A
Site B

CFU/g

0 < 1
>1 ≤ 10
>10 ≤ 100
>100 ≤ 1,000
>1,000 ≤ 10,000
>10,000 ≤ 100,000
>100,000 ≤ 1,000,000
> 1,000,000



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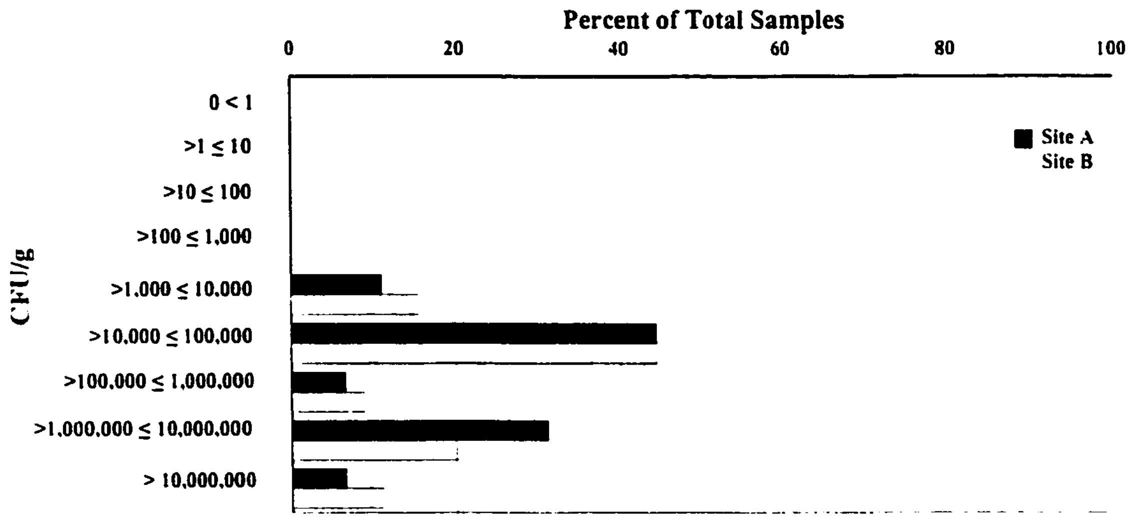
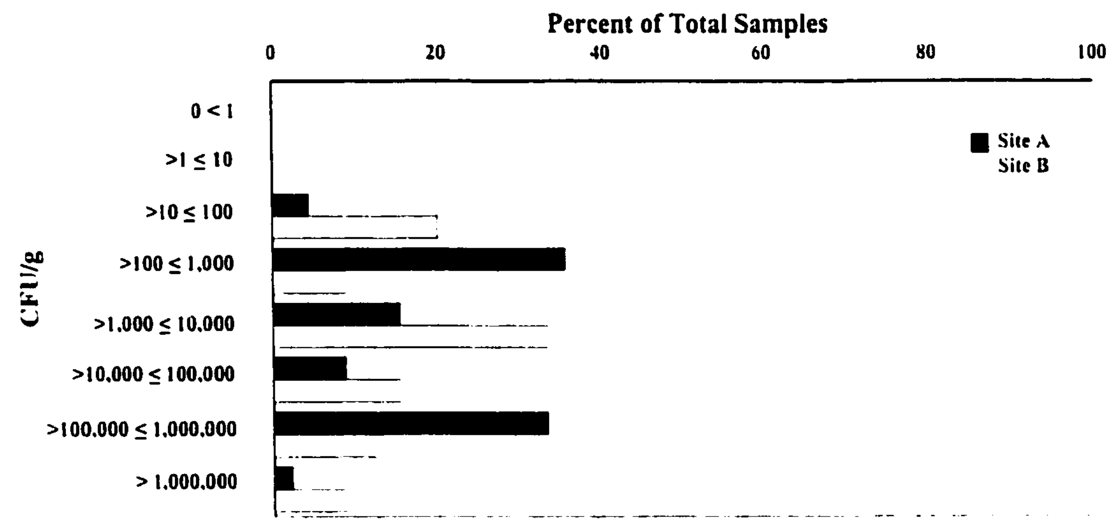
Pork Variety Meats - Cheek Meat

Bacteria counts comparing site A (point of packaging) and site B (after 24 h of frozen storage) from the nine plants sampled.

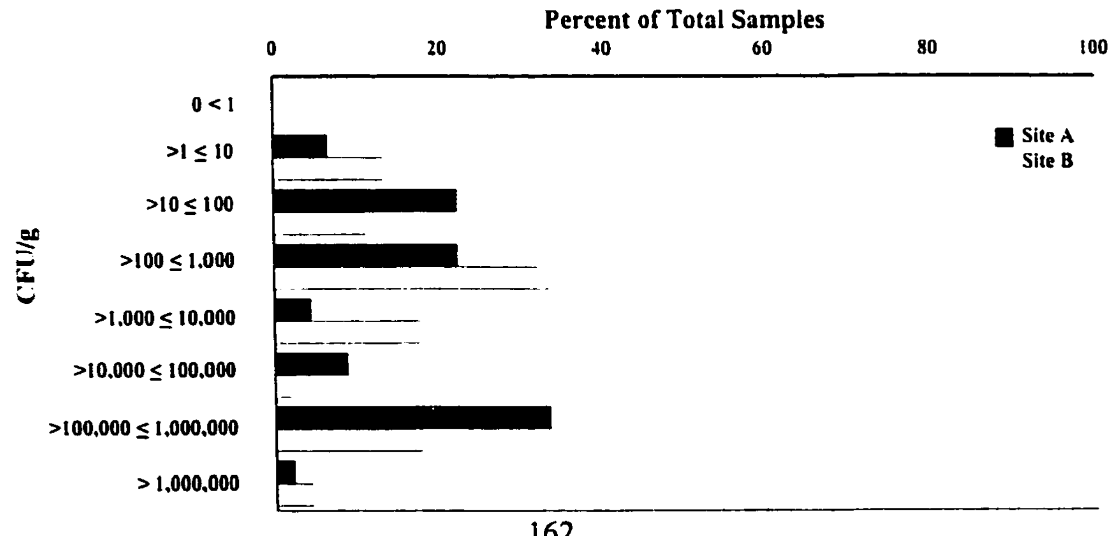
Distribution of Aerobic Plate Counts



Distribution of Total Coliform Counts



Distribution of generic *Escherichia coli* Counts



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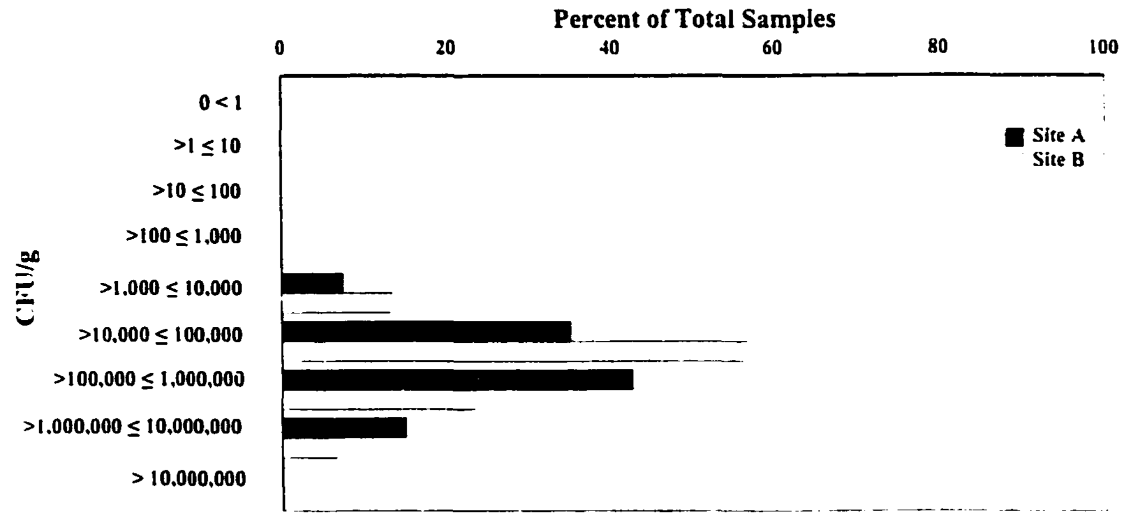
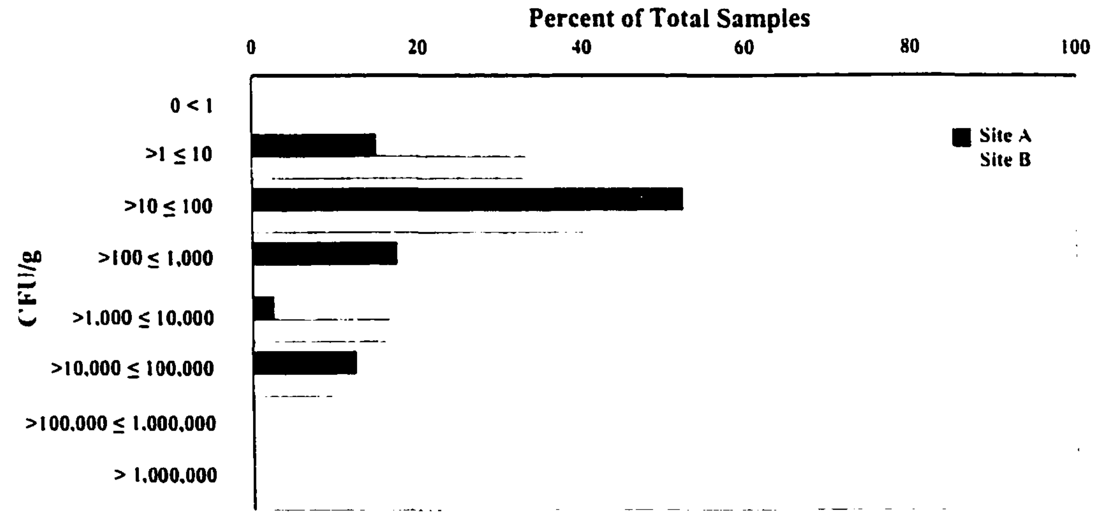
Pork Variety Meats - Front Feet

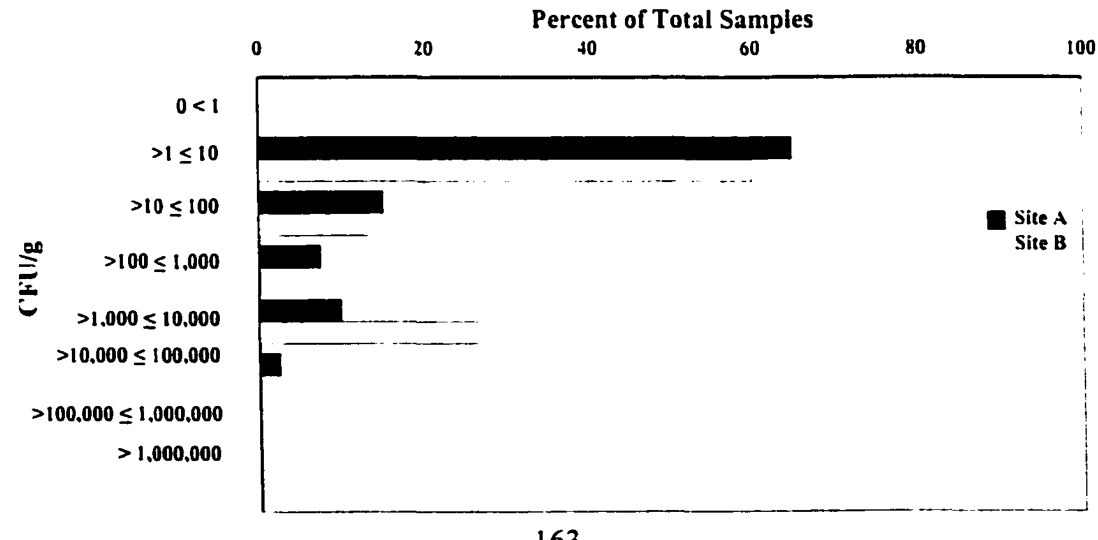
Bacteria counts comparing site A (point of packaging) and site B (after 24 h of frozen storage) from the six plants sampled.



Distribution of Total Coliform Counts



Distribution of generic *Escherichia coli* Counts



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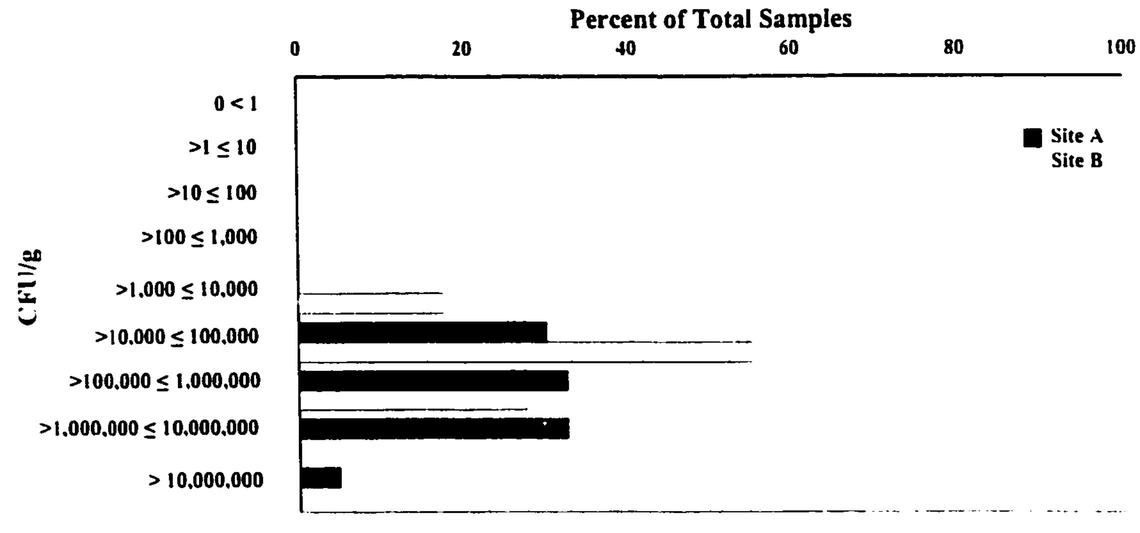
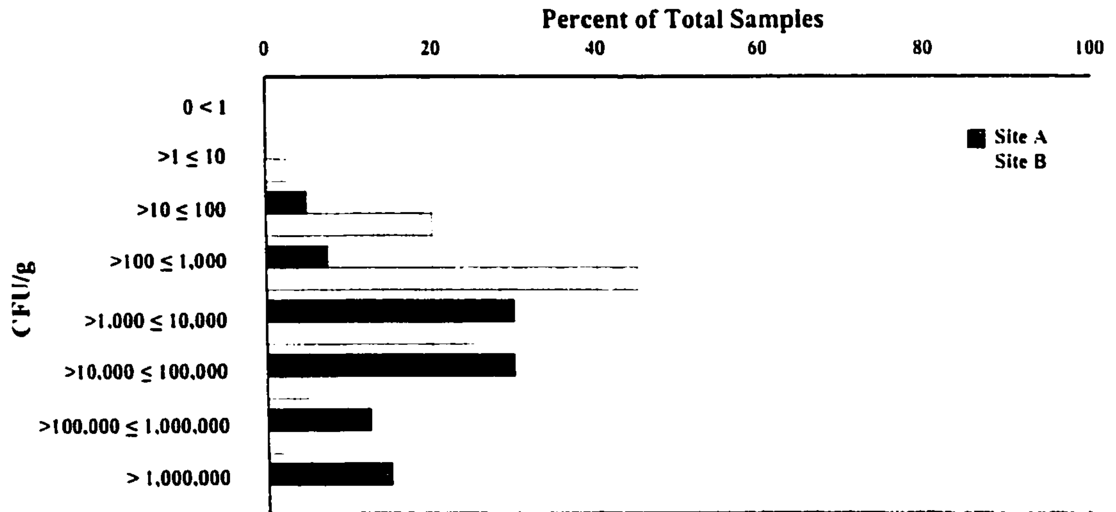
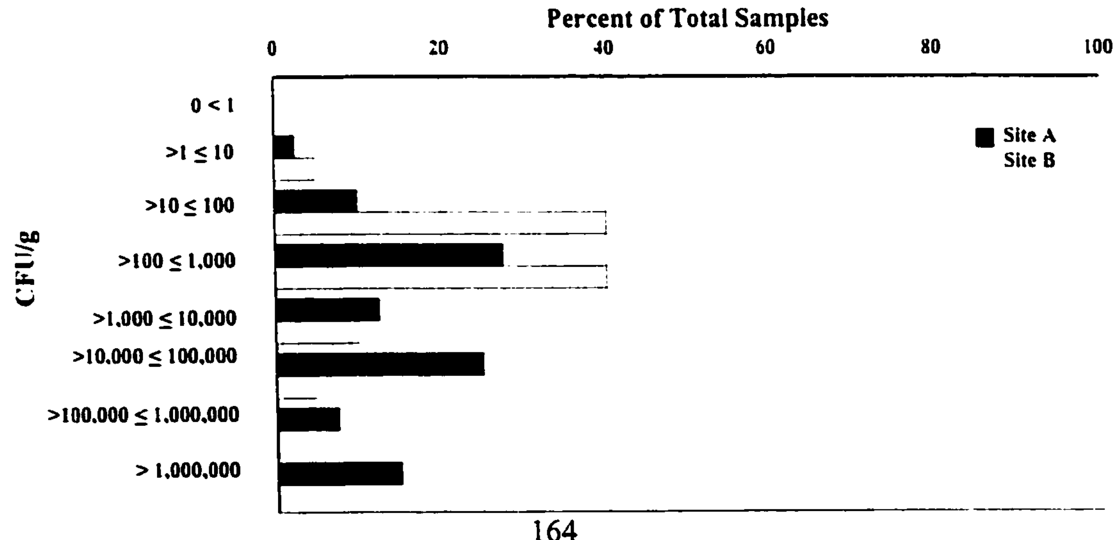
Pork Variety Meats - Head Meat

Bacteria counts comparing site A (point of packaging) and site B (after 24 h of frozen storage) from the eight plants sampled.







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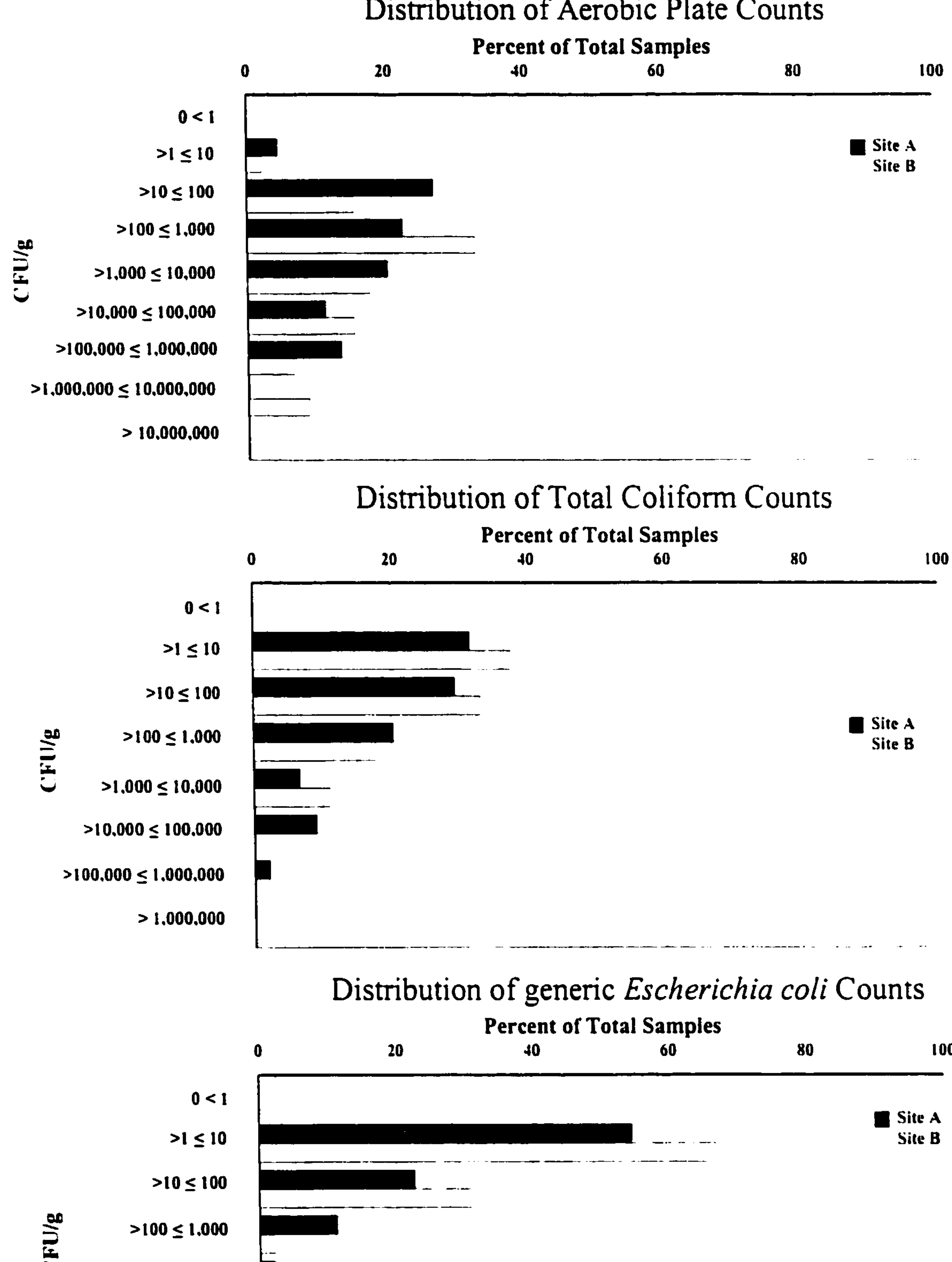
Pork Variety Meats - Heart

Bacteria counts comparing site A (point of packaging) and site B (after 24 h of frozen storage) from the nine plants sampled.



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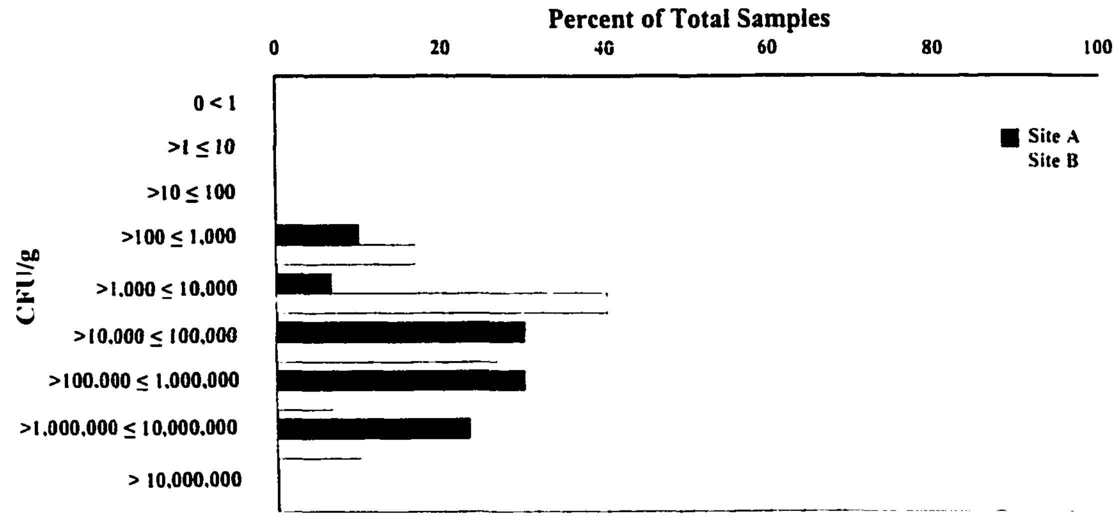
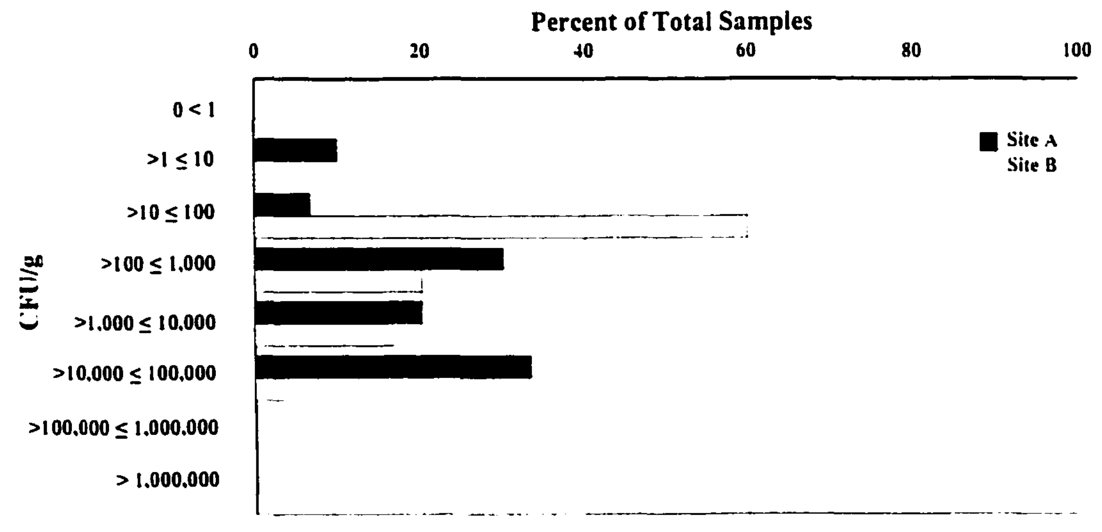
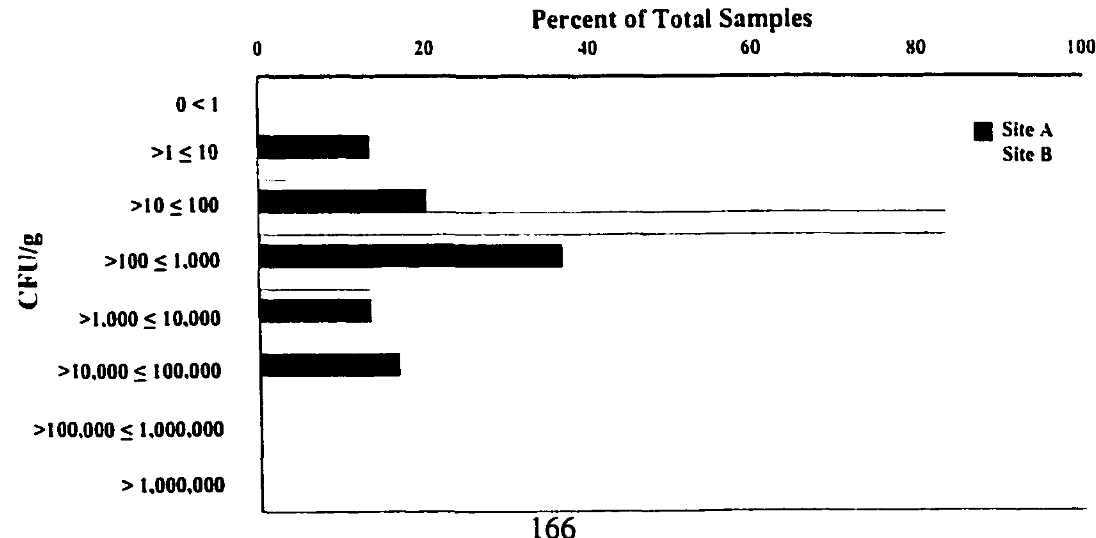
Pork Variety Meats - Kidney

Bacteria counts comparing site A (point of packaging) and site B (after 24 h of frozen storage) from the six plants sampled.







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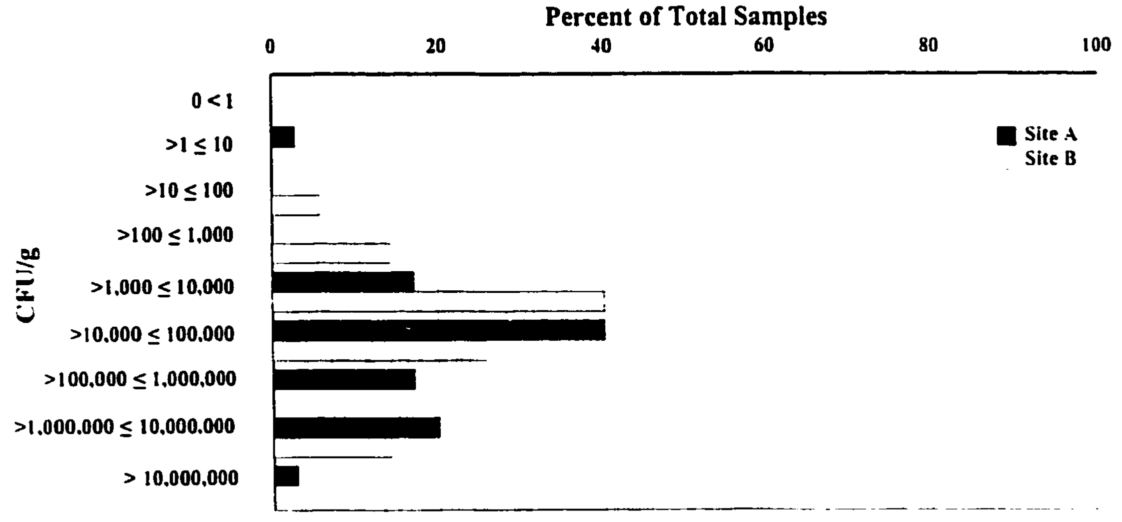
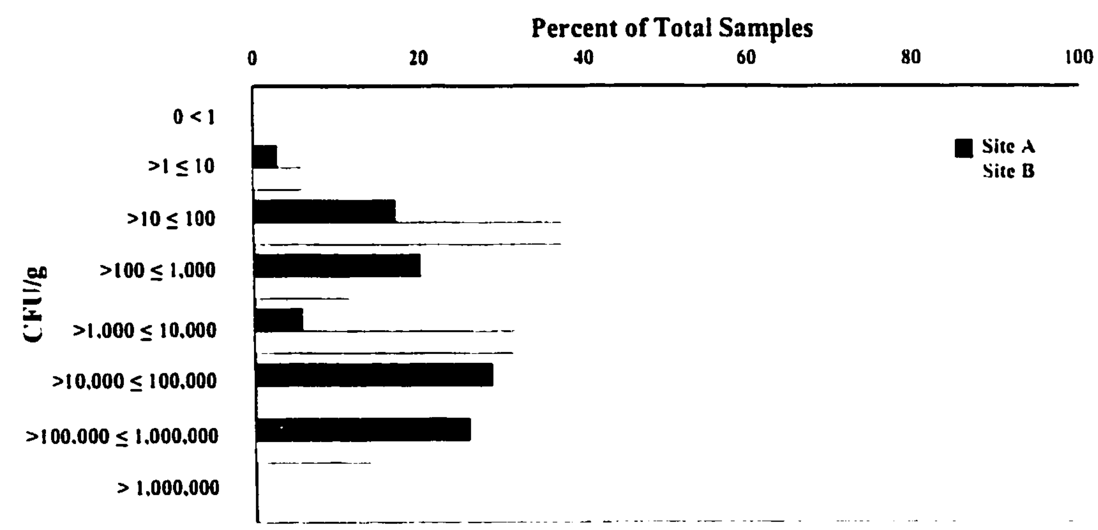
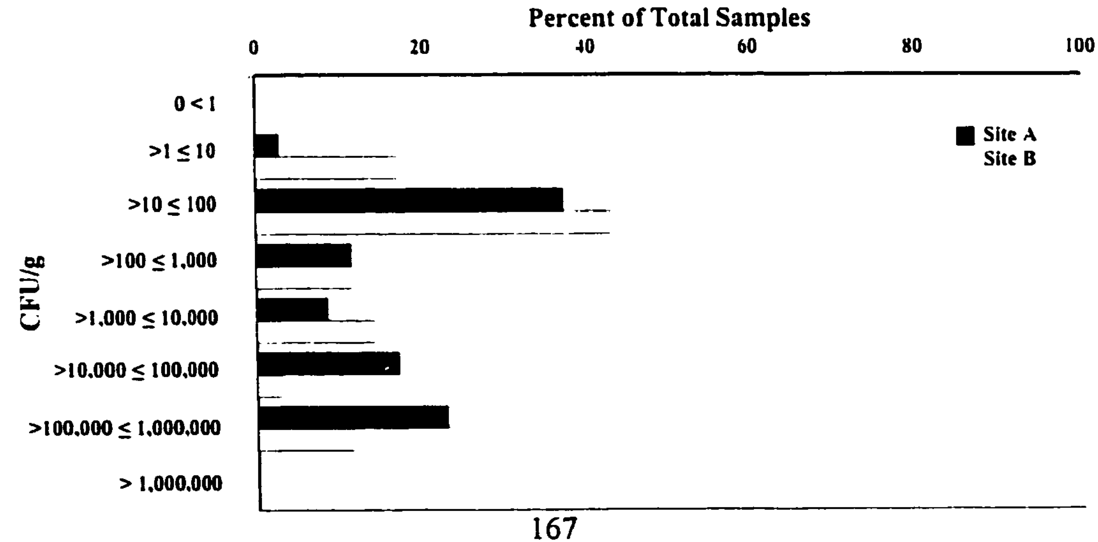
Pork Variety Meats - Liver

Bacteria counts comparing site A (point of packaging) and site B (after 24 h of frozen storage) from the seven plants sampled.







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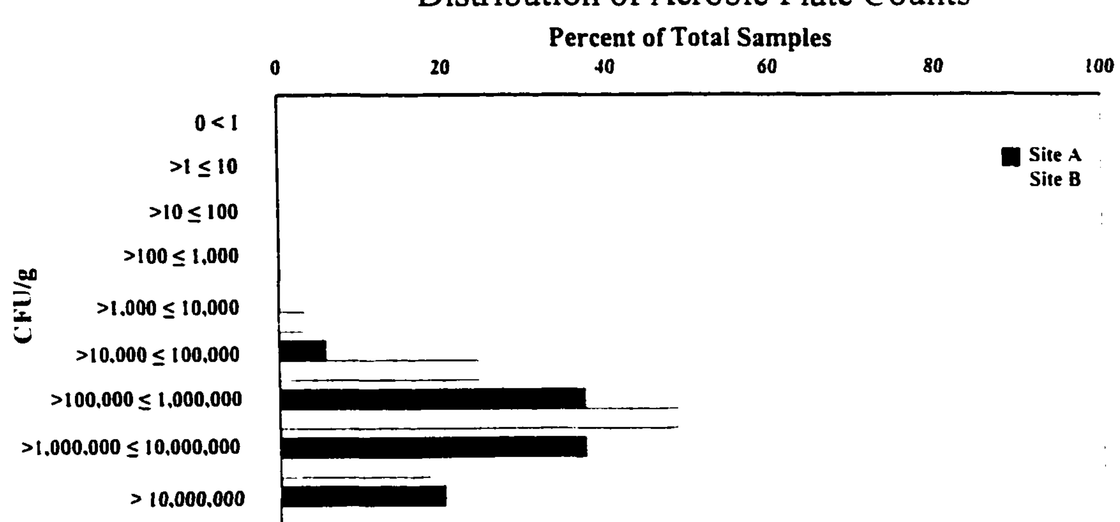
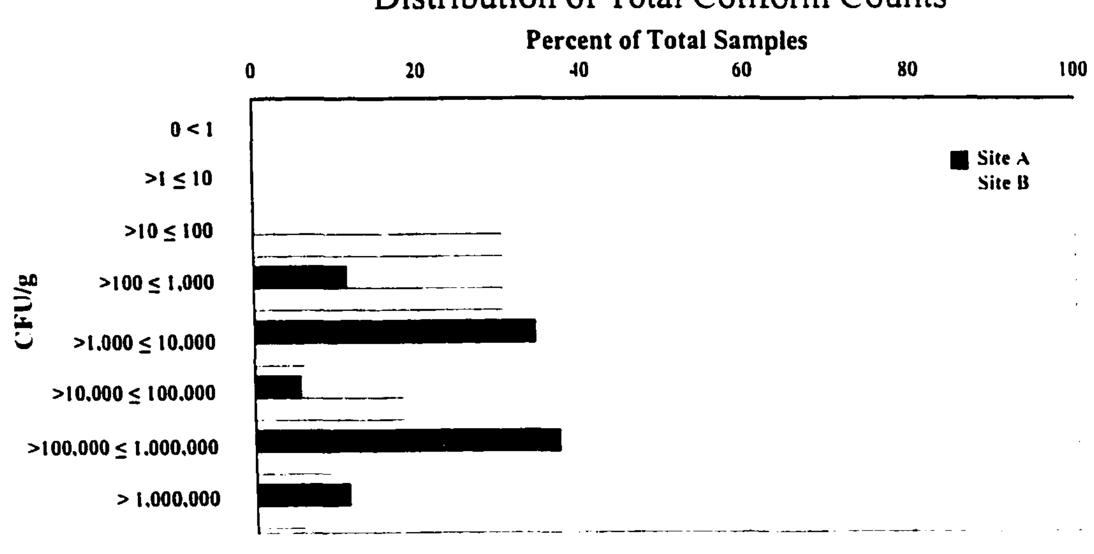
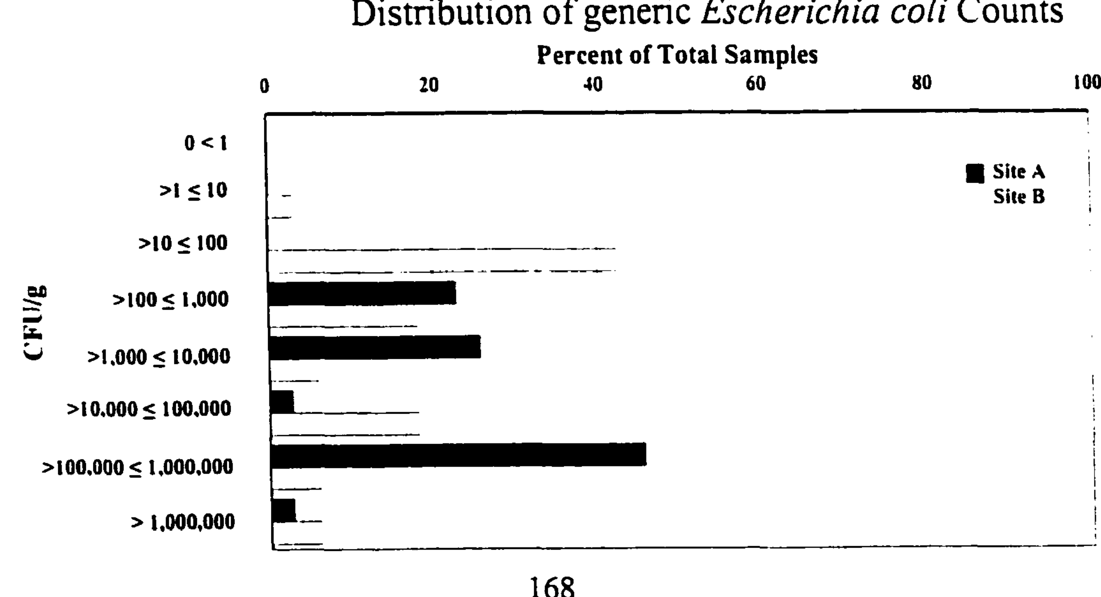
Pork Variety Meats - Salivary Gland

Bacteria counts comparing site A (point of packaging) and site B (after 24 h of frozen storage) from the seven plants sampled.







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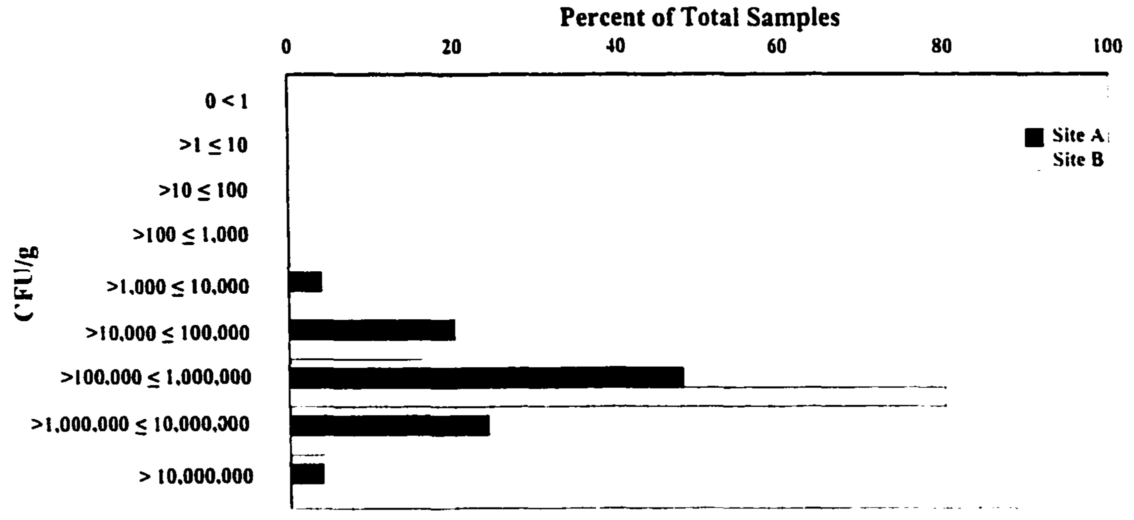
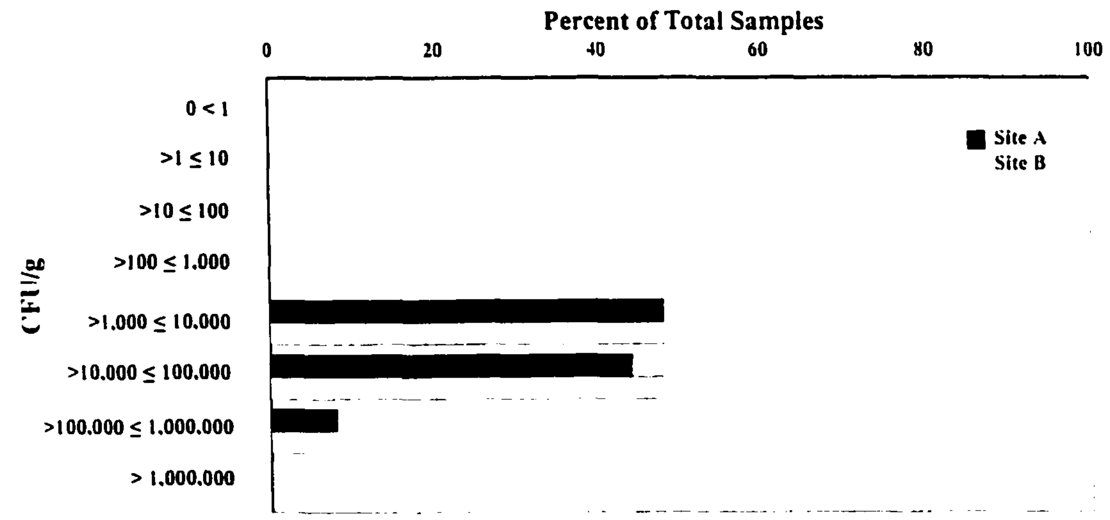
Pork Variety Meats - Small Intestine

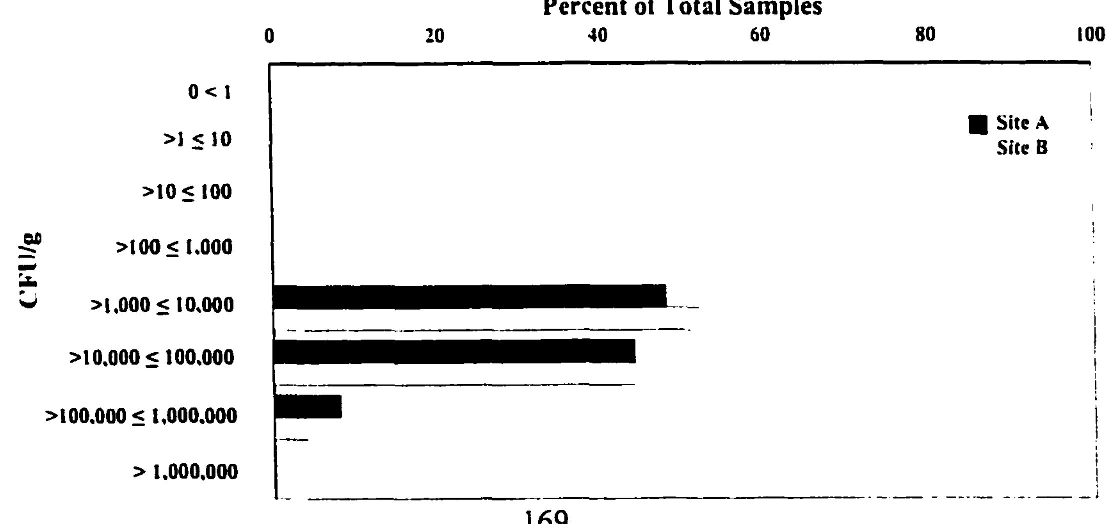
Bacteria counts comparing site A (point of packaging) and site B (after 24 h of frozen storage) from the five plants sampled.



Distribution of Total Coliform Counts



Distribution of generic *Escherichia coli* Counts



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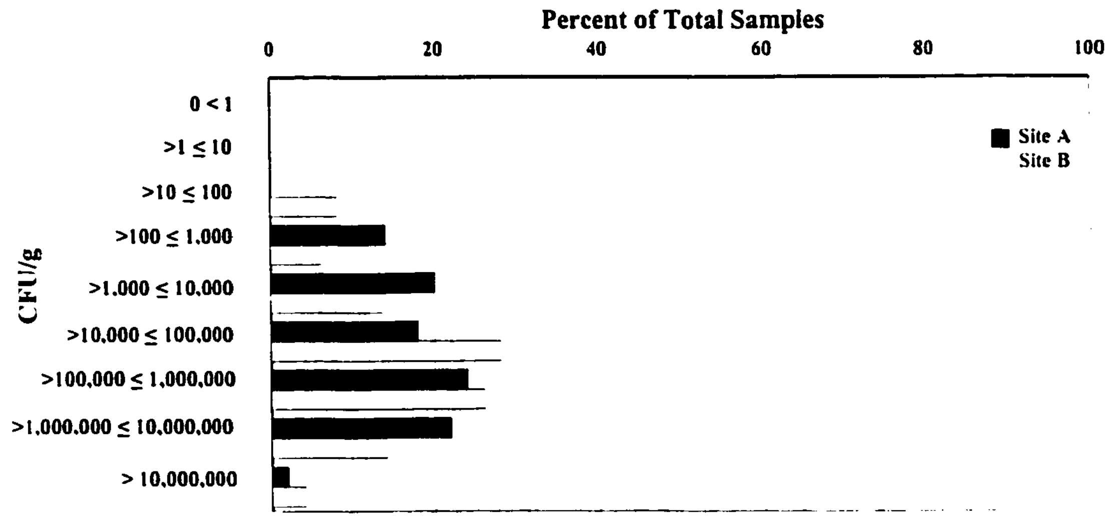
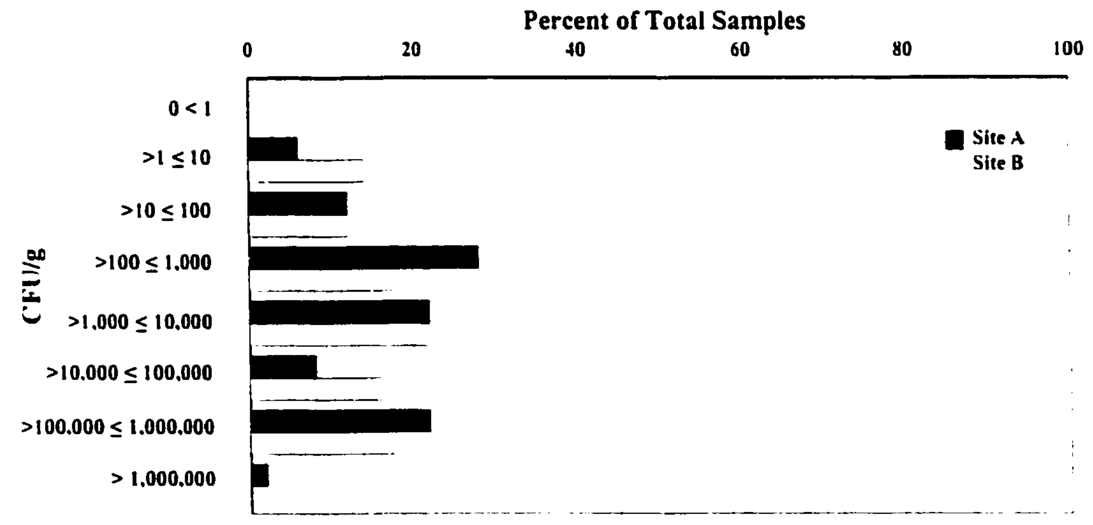
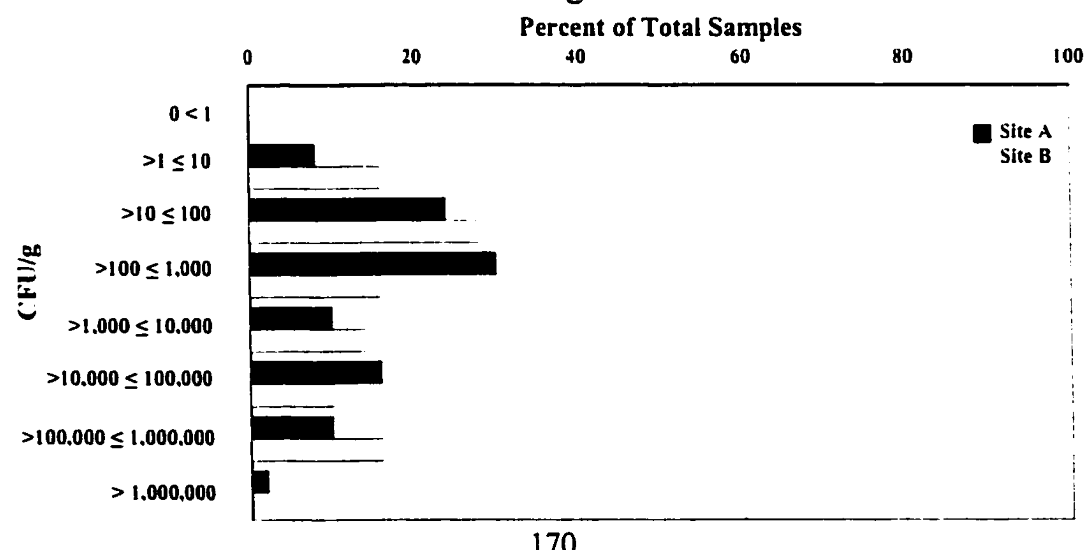
Pork Variety Meats - Stomach

Bacteria counts comparing site A (point of packaging) and site B (after 24 h of frozen storage) from the ten plants sampled.







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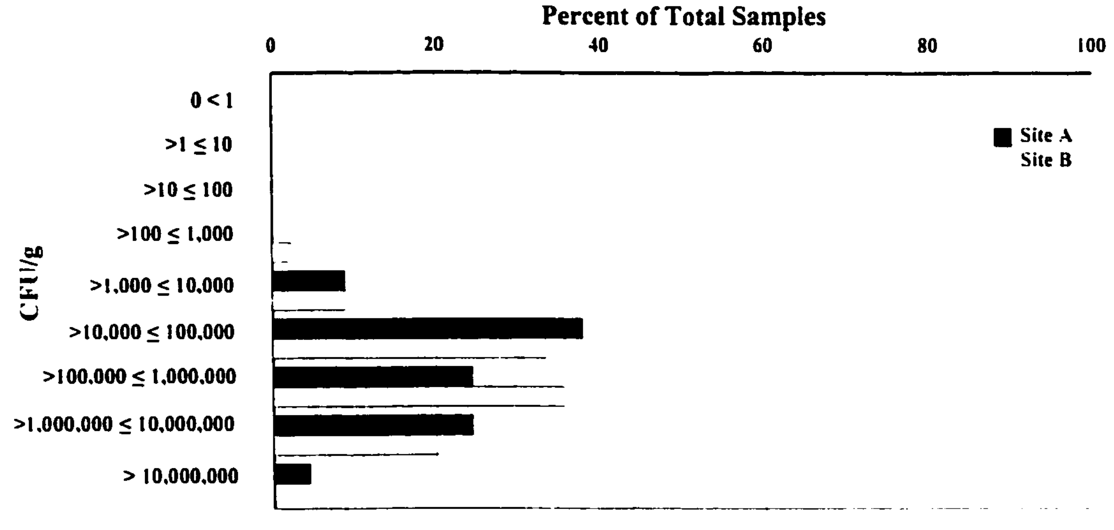
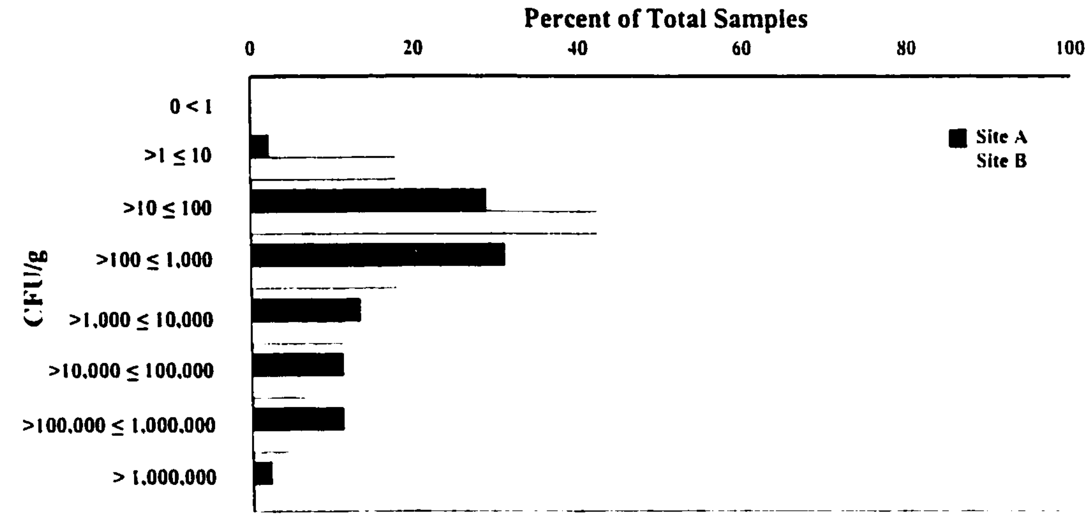
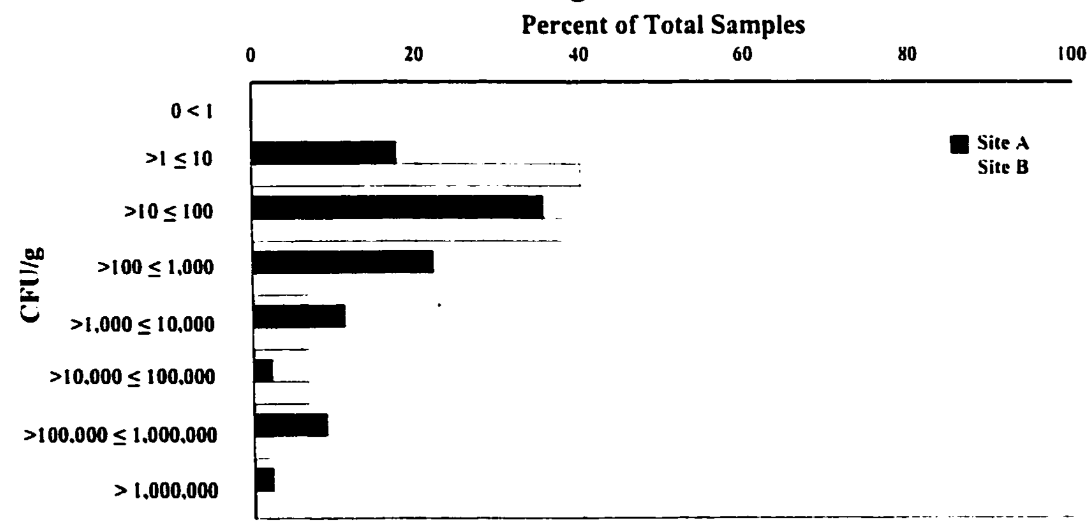
Pork Variety Meats - Tongue

Bacteria counts comparing site A (point of packaging) and site B (after 24 h of frozen storage) from the nine plants sampled.







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