

**PERCEIVED EFFECTS OF HAZARD ANALYSIS AND CRITICAL CONTROL  
POINT TRAINING ON CALIFORNIA EGG PRODUCERS**

By

**Jay D. Fuller**

Submitted in Partial Fulfillment of the  
Requirements for the Degree

**MASTER OF PREVENTIVE VETERINARY MEDICINE**

School of Veterinary Medicine  
University of California, Davis  
Davis, California

25 May 2000

Reviewed and Approved by

---

Dean O. Cliver, PhD  
Professor of Food Safety

---

Philip H. Kass, DVM, PhD  
Associate Professor of Analytical Epidemiology

20010314 046

## Acknowledgements

I wish to express my sincere appreciation to those who have been instrumental in the completion of this project. Thank you to the following individuals for their contributions:

Dr. David Castellan of the California Department of Food and Agriculture for providing information about the California Egg Quality Assurance Program program, the egg industry, and the CEQAP survey.

Mr. David Goldenberg, CEQAP facilitator, for providing the survey results data.

Professors Dean Cliver and Hans Riemann for their insights into Hazard Analysis Critical Control Point, food safety, and the egg industry, and for their review of the manuscript.

Dr. Phillip Kass for his assistance in statistical analysis and interpretation and review of the manuscript.

Dr. Ralph Ernst, Mr. Doug Kuney, Dr. Castellan, and Mr. Goldenberg for developing the survey questionnaire.

## ABSTRACT

Government regulatory agencies and the food industry are currently incorporating Hazard Analysis and Critical Control Point (HACCP) principles into farm production practices in an attempt to minimize foodborne pathogens. The California Egg Quality Assurance Plan (CEQAP) includes a HACCP training course for egg industry members. The training began in 1995 and questionnaires were mailed to all 162 participants in 1998. Statistical analyses were performed on the 86 surveys returned to assess the effects of the training. Participants belonged to one or more of five industry sectors: egg production, egg packing/processing, pullet production, allied industry, and government/education professional. Five training sessions included introduction and quality assurance plan development, egg processing, flock health, biosecurity and cleaning/disinfection, and pest management/vector control.

Pullet producers rated the egg processing module to be less valuable than pest management training. All other sectors found all training modules to be valuable to their job, with no significant differences in value between modules. The five sectors were equally likely to make management changes after CEQAP training and were most likely to make changes in the area of pest management/vector control. Participants did not increase use of veterinary diagnostic services after training. Live speaker presentations and videotape training sessions were judged to be equally valuable. Overall, the training was perceived to be valuable by all industry sectors and frequently (89%) resulted in management changes.

## **Perceived Effects of Hazard Analysis and Critical Control Point training on California egg producers**

### **Introduction**

Eggs are the most common food vehicle for *Salmonella enteritidis* (SE) in the United States (US) and have been associated with over 50% of SE outbreaks.<sup>1,2,3</sup> Surveillance has indicated that SE is the second most common cause of foodborne illness (FBI) in the US.<sup>1,2,3</sup> During 1997 SE was estimated to have caused 1.3 million cases of FBI, costing over one billion dollars due to lost work productivity and medical costs.<sup>1,2,3</sup> The food industry and government regulatory agencies are working to minimize foodborne pathogen contamination of foods through the use of quality assurance programs, such as the Hazard Analysis and Critical Control Point (HACCP) system.<sup>4,5</sup>

The HACCP system is recognized as the standard for safety assurance in food processing.<sup>4,5,6</sup> The HACCP system requires evaluation of food processing activities and establishment of critical control points in the food process that can be monitored for compliance. The HACCP system is commonly used in food manufacturing, post-harvest processing, and restaurant facilities; however, the United States Department of Agriculture (USDA) and food producers have begun applying HACCP concepts at the farm production level.<sup>7</sup>

The California Egg Quality Assurance Plan (CEQAP) is a voluntary preharvest food safety program targeted at reducing SE in eggs by application of HACCP concepts to egg farm production practices. The CEQAP was developed in 1995 by the California Department of Food and Agriculture (CDFA) in cooperation with the University of California Cooperative Extension, USDA (Animal and Plant Health Inspection

Service/Veterinary Services), US Food and Drug Administration, California Department of Health Services, and the California egg industry. By completing CEQAP training courses, egg industry personnel can become CEQAP-certified in one or more of the following five training module areas: preparing a quality assurance plan, flock health and litter management, cleaning/disinfection and environmental sampling, vertebrate control and biosecurity, and egg processing.<sup>8</sup> Because HACCP training and implementation at the farm level have been initiated only over the past three years, the effects of CEQAP training on egg production practices should be examined. Detailed assessment of CEQAP effects will help determine the effectiveness of individual training modules and allow for further refinement and improvement of the modules.

Essential steps in HACCP include monitoring of critical control points and verification of the monitoring. Environmental SE contamination levels are currently being surveyed on egg farms by the CDFA to determine SE prevalence before and after CEQAP training and possible management changes. Judging CEQAP success strictly by evaluation of microbial reduction in eggs may be difficult, costly, or inaccurate because the prevalence of SE in eggs is already very low.<sup>9</sup> Due to the high level of public concern about food safety, the egg industry will need to promote its reputation and sales through quality assurance.<sup>10,11</sup>

The hypotheses to be tested are as follows:

1. California Egg Quality Assurance Plan participants, representing each of five egg industry sectors (egg production, egg packing/processing, pullet production, allied industry, and government/education professional), found each of the five training courses to be equally valuable to their job. Such analysis will determine whether the training was

perceived to be useful for improving egg quality and which (if any) training modules were perceived to be more or less valuable to each industry sector.

2. Participants from each of the industry sectors were equally likely to incorporate management changes in their operations following CEQAP training. (Government responses were not included because the question was not relevant.)
3. Management changes were made in different operational areas with equal frequency.
4. Participants judged videotape with instructor or home study with videos to be as valuable as live speaker presentations.
5. Participants did not change their use of diagnostic services after CEQAP training.

### **Materials and Methods**

**Study design and subjects**--The CEQAP training began in April of 1995, with live speaker presentations in northern and southern California. Make-up sessions were given throughout the state, using videotapes of the original speakers with a group leader to provide limited instruction and to answer questions. Participants were required to complete a comprehensive exam by home study using CEQAP training manuals and videotapes and to achieve a 90% test score for certification. Over 250 people, responsible for approximately 95% of California egg production, were trained in one or more of the five course topics. Training is ongoing at this time, using video/moderator and home study/video formats.

In December 1998, a three-page questionnaire (appendix), containing 23 questions, was mailed to all 162 participants still actively employed in the egg industry. These included 136 egg producers and/or packing plant personnel, six allied industry

persons (consultants, veterinarians), and 20 government educators or extension representatives.

**Hypothesis testing**-- Survey results were compiled and tabulated into a database using a commercial spreadsheet software package.<sup>a</sup> Codes for responses were No = 0, Yes = 1, Doesn't Apply = 99, did not respond = -2, appropriate to skip question = -1, and the 1-5 range for little to most valuable. All tests were evaluated at a 5% level of significance. Statistical analyses were performed to test each hypothesis as follows:

1. The responses to survey question #1 (respondent activities) were compared with responses to question #3 (usefulness of various training sessions). A Kruskal-Wallis one-way analysis of variance (ANOVA) was performed using a commercial statistical software package<sup>b</sup>, to compare the level of value received for the each of the five training modules on a scale of 1-5 (1 = least, 5 = most valuable) for each of the five activity levels (sectors). A p-value of  $<0.05$  was interpreted to mean that the value received was not equal for all of the modules for that activity level. For rejected global null hypotheses, pairwise contrasts were performed using a multiple comparison adjustment.

For egg producers, the training sessions were not found to be significantly different in value to their job,  $p = 0.10$ .

For egg packagers/processors, the training sessions were not found to be significantly different in value to their job,  $p = 0.37$ .

For pullet (young replacement bird) producers, the pest management session was found to be more valuable than the egg processing session,  $p = 0.0059$ .

For allied industry, the sessions were not found to be significantly different in value to their job,  $p = 0.060$ .

For government and education professionals, the sessions were not found to be significantly different in value to their job,  $p = 0.90$ .

2. The responses to question #5 (management changes made?) were compared with responses to question #1. A  $X^2$  test for homogeneity was performed using a statistical software package<sup>c</sup> comparing the frequency of management changes across four different activities (government excluded). A p-value of  $<0.05$  was interpreted to indicate that management changes were not made in equal frequency by all sectors.

All four sectors (government excluded) were equally likely to make management changes,  $p = 0.16$ . The overall frequency of management changes was 88.6%.

3. The responses to question #6 (areas of management modified) were used to compare management modification frequencies across five areas of management. A  $X^2$  test for homogeneity was used. A p-value of  $<0.05$  was interpreted to mean that management changes were not made in equal frequency in all five areas of management.

Management changes were made with equal frequency in the areas of egg processing, flock health management, cleaning and disinfection, and biosecurity and cleaning/disinfection. Changes were made in pest management/vector control more frequently than for the other areas,  $p < 0.0001$ .

4. The responses to question #8 (value of training format) were used to compare the three different training methods. A Kruskal-Wallis one-way ANOVA was performed comparing the value level on a scale of 1-5 (1 = least, 5 = most valuable) across the three training formats. A p-value of  $<0.05$  was interpreted to mean that participants did not judge all training formats to be equally valuable.

Respondents judged all three training formats to be equally valuable,  $p = 0.063$ .

5. Responses to questions #11 and #12 (frequency of use of veterinary or diagnostic services before/after CEQAP training) were compared. The responses for questions #11 and #12 were assigned numerical values as follows: a) Never = 0, b) 1-2 times/year = 1, c) 3-4 times/year = 2, d)  $\geq 5$  times/year = 3. An exact Wilcoxon Signed-Ranks Test for matched data was then used to determine if any difference existed in use of veterinary or diagnostic services after CEQAP training. A p-value of  $<0.05$  was interpreted to mean that a difference did exist between the use of diagnostics before training versus after training.

Respondents did not significantly change their use of diagnostic or veterinary services after training,  $p = 0.13$ .

## **Results**

**Survey return--** Respondents returned 86 fully or partially completed surveys for an overall return percentage of 53%. The return percentages were 54% (74/136) for egg industry members, 83% (5/6) for allied industry and 35% (7/20) for government or extension persons. The possibility of follow-up bias due to nonrespondents exists; however, no particular biases due to nonrespondents can be hypothesized. Also, most respondents completed training in all five areas, with egg processing (57 completed/86 respondents) being the least attended, and at least 72 of the 86 respondents completing the other four training modules. The respondent industry sector proportions were similar to the overall participant sector proportions. Completing the survey required approximately 15 minutes, and postpaid return envelopes were provided to participants.

**Summary of descriptive data analysis--** The distribution of industry sector association (question #1) is listed in Table 1. The overall median scores for all survey

respondents to question #3 (usefulness of each session to job) were: Introduction and Quality Assurance Plan Development (4), Egg Production (4), Flock Health (4), Biosecurity and Cleaning/ Disinfection (4), and Pest Management/Vector Control (5). The median responses for question #3 are listed by industry sector in Table 2. Of the 85 respondents who replied to question #5, 65 indicated that they did make management changes due to CEQAP training, 10 indicated they made no changes, and 10 indicated “doesn’t apply”. The number of respondents (n) from each production sector and the percentage that made changes were egg production (n= 57, 95%), egg packing/processing (n= 44, 86%), and pullet production (n= 31, 97%). For those who did make management changes, the overall frequencies of changes made in each area of management are depicted in Figure 1. The percentage of changes made in each area is stratified by industry production sector in Table 1. For question #7 (educational training venue participated in), 63 responded that they attended a live presentation (a), 27 attended a make-up videotape with instructor (b), 10 used home study with videos (c), 13 attended (a) and (b), 1 used (a) and (c), and 2 used (a), (b), and (c). The median responses for question #8 (value of training formats) on the 1 (least) to 5 (most) scale were: live presentation (4), make-up video with instructor (4), and home study with videos (5).

The median response for question #11 (frequency of use of veterinary or diagnostics services before training) on the 0 to 3 scale (measuring times per year) was 2 (3-4 times per year). The median response for question #12 (frequency of use of veterinary or diagnostics services after training) was also 2 (3-4 times per year).

## **Discussion**

The results show that CEQAP training made a significant impact on management practices and that all training modules were perceived as valuable to the participants. A high percentage of participants from all sectors made management changes after training and these changes were made in all areas of management with substantial frequency. The high perceived value of pest management training corresponds with the high percentage (>90%) of management changes made in this area. As more scientific knowledge is gained about the epidemiology of foodborne illnesses and potential vectors, it is expected that new management techniques will continue to evolve. The egg industry will need to rely on updated information from CEQAP to ensure that the most effective pest/vector control methods are utilized.

All industry sectors found each of the training modules to be valuable. Some training modules may not have been directly applicable to some sectors, but, for example, even pullet producers found the egg processing module to be at least somewhat valuable. Egg processing is the most automated sector of the egg industry and has had the most established "good manufacturing practices" that might be incorporated into a HACCP program, yet egg processors still found all training modules to be valuable. Various sectors appear to be interested in all aspects of the egg industry. A general understanding of the overall industry can only help make the participants more effective at managing their own facilities.

All three training formats were judged to be valuable. Live speaker presentations may be more entertaining but obviously require more resources to provide. Videotapes should be an acceptable mode of training.

As government and industry are moving to utilize HACCP-type principles on the farm, it is important to assess the actual effects of training on producers. Information on management changes can be coupled with the results of ongoing SE testing on California egg farms. Pathogen reduction levels are the ultimate goal of an on-farm HACCP program; however, a structured effort at quality assurance and the positive image such efforts create are critical to the egg industry in current society.

---

<sup>a</sup> Microsoft Excel 97, Microsoft Corporation

<sup>b</sup> BMDP3S, BMDP Statistical Software, Inc., Los Angeles, Ca.

<sup>c</sup> Epi Info version 6, Centers for Disease Control, Atlanta, Ga.

---

## References

1. Bean NH, Goulding JS, Daniels MT, et al. Surveillance for foodborne disease outbreaks – United States, 1988-1992. *J Food Prot* 1997;60(10):1265-1286.
2. Mead P, Slutsker L, Dietz V, et al. Food related illness and death in the United States. *Emerging Inf Dis* 1999;5(5):<http://www.cdc.gov/ncidod/eid/vol5n05/mead.htm>
3. Baird-Parker A. Foodborne salmonellosis. *Lancet* 1997;16(2):489-495.
4. Bryan F. Hazard analysis and critical point approach to food safety: past, present, and future. *J Environ Health* 1999;61(8):9-15.
5. National Advisory Committee on Microbiological Criteria for Foods. Hazard analysis and critical control point principles and guidelines. *J Food Prot* 1998;61(6):762-775.
6. Mortlock M, Peters A, Griffith C. Food hygiene and hazard analysis critical control point in the United Kingdom food industry: Practices, perceptions, and attitudes. *J Food Prot* 1999;62(7):786-792.
7. United States Department of Agriculture, Food Safety and Inspection Service. Solicitation No. FSIS-13-W-97, 1997:3-4.
8. California Egg Quality Assurance Plan Training Manual, 1995, Sacramento, Ca.

9. Henzler D, Kradel D, Sischo W. Management and environmental risk factors for *Salmonella enteritidis* contamination of eggs. *Am J Vet Res* 1998;59(7):824-829.
10. Zimbelman RG, Wilson LL, Bennett MB, Curtis SE. Public image of animal agriculture in the United States. *Livestock Prod Sci* 1995;43(2):153-159.
11. Fischhoff B, Downs JS. Communicating foodborne disease risk. *Emerg Inf Dis* 1997;3(4):489-495.

Table 1--Frequency of management changes made (%) in various areas of management by specific California egg industry sectors after training\*

Industry sector (n)	Management area				
	Egg Processing	Flock Health	Cleaning/ Disinfection	Biosecurity/ C & D	Pest/Vector Control
Egg Production (49)	35	49	71	63	98
Egg packing/ processing (35)	60	40	49	51	83
Pullet production (30)	37	50	57	70	93

\*Assuming at least one management change was made  
n = Number of respondents  
C & D = Cleaning and disinfection

Table 2--Median responses\* for usefulness of food safety training sessions during 1995-1998 for specific California egg industry sectors

Industry sector (n)	Training session				
	Introduction QAP	Egg Processing	Flock Health	Biosecurity/ C & D	Pest/Vector Control
Egg Production (57)	4	4	4	4	4.5
Egg packing/ processing (44)	4	4	4	4	5
Pullet production (31)	4	3	4	4	5
Allied industry (5)	4	3	5	4	5
Government/ education (7)	3	4.5	4	4	5

\*Responses were made on a scale of 1 (least value) to 5 (most value).  
n = Number of respondents  
QAP = Quality assurance plan development  
C & D = Cleaning and disinfection

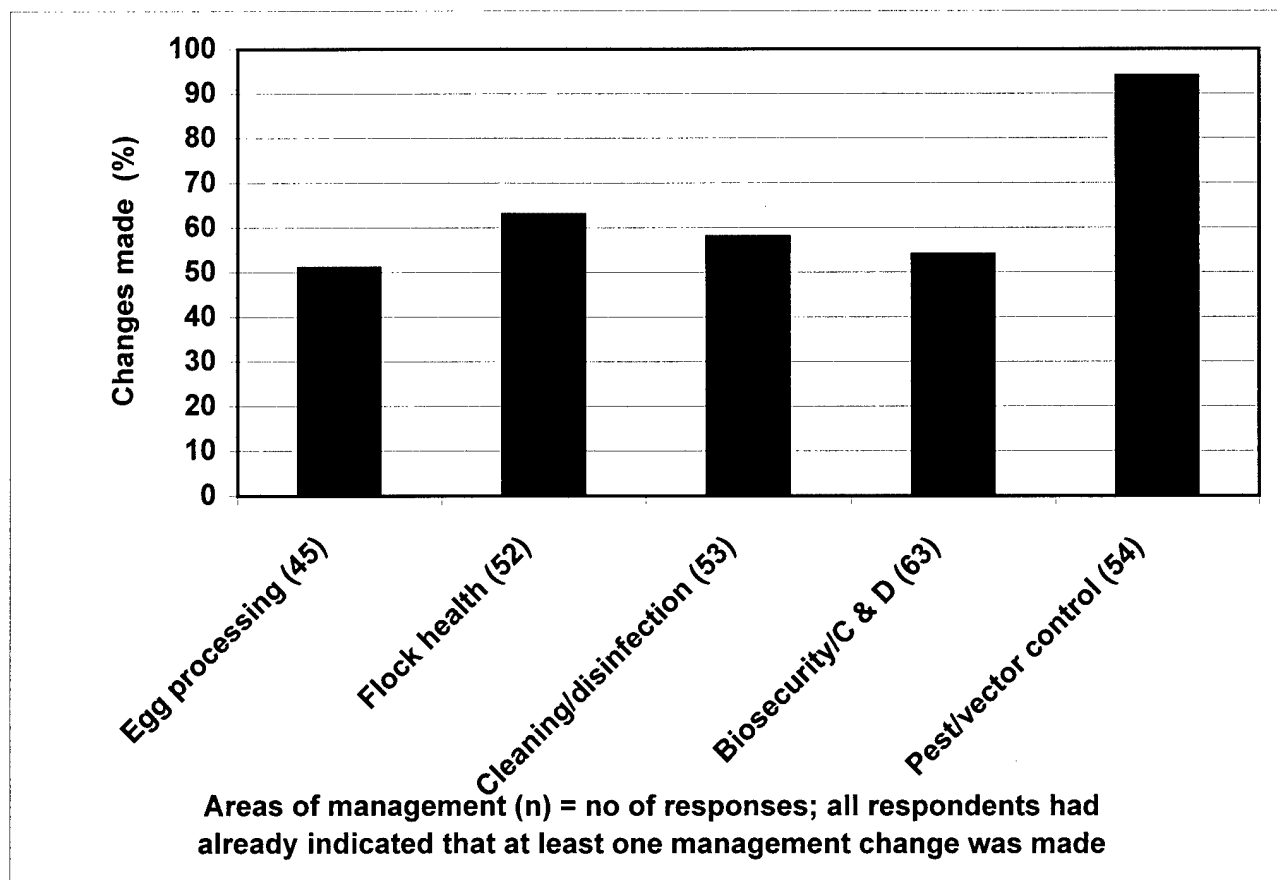


Figure 1. Frequency of management changes made by California egg industry in specific areas after California Egg Quality Assurance Program training in 1995-1998

## **Survival of *Salmonella enteritidis* on orange surfaces using excision method for recovery**

Fresh produce is widely recognized as a potential cause of foodborne illness. Juices made from raw fruit (or vegetables) pose a greater risk to consumers than raw produce because the contamination of one or a few pieces of fruit may be spread into the juice served to many people (11,12). Enteric pathogens such as *Escherichia coli* O157:H7, *Cryptosporidium parvum*, and *Salmonella* spp. have all caused outbreaks associated with the consumption of unpasteurized apple or orange juices. Animal or human fecal contamination may occur in growing orchards, during or after processing, in transport, and at retail establishments. Orange juice has been linked to numerous outbreaks of salmonellosis (1,2,8,10). Fresh squeezed orange juice has become more popular as it has been bottled and chilled for mass marketing and is utilized in frozen smoothie drink preparations. Pasteurized frozen orange juice has also caused foodborne illness outbreaks when contaminated during preparation for consumption in restaurants (1,10).

Although the acidity (pH 2.8-4.0) of orange juice prevents growth of *Salmonella* spp., the organism can survive for at least 15 days in orange juice held at 0 and 4°C (9). The peeled surface of oranges has a pH of 6.0-6.5 and will support growth of *Salmonella* spp. at 24°C. At 4 and 8°C the albedo area (white portion of the orange) allows survival of *Salmonella* spp. for at least 14 days (4,12). The Food and Drug Administration recently provided evidence suggesting that dye and possibly pathogens may enter the albedo or deeper orange tissue areas through the stem scar or peel wounds. *Salmonella* was shown to survive or multiply once inside (12). No information is available regarding survival or growth of *Salmonella* spp. on the outer peel of oranges.

Common processing methods for oranges help reduce microbial counts but do not eliminate all contamination. Pao and Brown (5) demonstrated that standard washing and rinsing procedures using potable water reduced *E. coli* counts by 2.4-log cycles. Addition of the fungicide SOPP (FMC, Lakeland, Fla.) or 200 ppm chlorine did not further reduce *E. coli* populations. Washing and waxing reduced counts by 3.4-log cycles. Further studies demonstrated that during waxing, the synergistic effect of heat ( $\geq 50^{\circ}\text{C}$ ) combined with alkaline pH ( $> 8.0$ ) results in a 4.7-log reduction in *E. coli* on midsection orange surfaces, but only a 1.0 log reduction in stem scar areas (7). Immersion of oranges in various disinfectant solutions resulted in 1.8- to 3.1-log reductions in *E. coli* from midsection surfaces and 1.0-log reductions from stem scar areas. Water immersion alone was almost as effective. Rapid hot water immersion ( $80^{\circ}\text{C}$  for 1 min or  $70^{\circ}\text{C}$  for 2 min) proved more effective, causing a 5-log reduction (6).

Processing methods cannot completely eliminate potential pathogens from oranges. *E. coli* O157:H7 has a very small infective dose and *Salmonella* spp. adapted to acidic conditions such as orange juice might also be expected to have a relatively small infective dose. Post-processing contamination may occur during shipping, handling, and preparation of oranges or fresh juice products. Oranges may be subjected to high ambient temperatures during harvest and to more moderate temperatures (above refrigeration) during handling and retail sales. Baseline data on bacterial survival on oranges must be established to aid in evaluation of sanitation practices for oranges both before and after processing. The object of this study was to determine the survival of *Salmonella enterica* for up to 24 hours on orange peel outer surfaces at  $25^{\circ}\text{C}$  and  $37^{\circ}\text{C}$ .

## MATERIALS AND METHODS

**Inoculum preparation:** Six different naladixic acid resistant serovars of *Salmonella enterica* were cultured from frozen colonies (-78°C). Serovars and sources used were *S. Typhimurium* (bovine feces), *S. Agona* (alfalfa sprouts), *S. Enteritidis* (strain unknown, egg contaminant), *S. Gaminarum* (orange juice), *S. Michigan* (cantalope), and *S. Montivideo* (human, linked to tomatoes). The frozen colonies were initially plated onto Tryptic Soy Agar with 1 g/l of pyruvate and 10 ml/l of 0.5% nalodixic acid (TSAN). After 24 h incubation at 37°C, the colonies were transferred to TSAN slants. The colonies were then transferred two more times after 24 h incubations at 37°C on TSAN slants.

Sterile horse serum (1 ml of 5%) was added to each of the six slant tubes. A sterile loop was used to loosen the colonies as much as possible and the tubes were vortexed slightly to help dislodge colonies into the serum liquid. The mixed-strain inoculum was prepared by mixing 500 µl of fluid from each culture. The inoculum was then plated on bismuth sulfite agar supplemented with 10 ml/l of 0.5% naladixic acid (BSAN).

**Inoculation and testing.** Navel oranges were inoculated with 12.5 µl of the mixed-strain inoculum on a 3 cm demarcated area. The inoculated oranges were stored at 25°C or 37°C for up to 24 h. The inoculated area of the orange skin or similar uninoculated area from control oranges was excised (including a slight amount of albedo) with a sterile scalpel blade and placed into a stomacher bag containing DE neutralizing broth (Difco Laboratories, Detroit, MI). The bag was stomached and the resulting solution was plated on BSAN. Typical black *Salmonella* colonies were counted after 48 h incubation at 37°C. The experiment was repeated four times. At 37°C, the experiment was performed with waxed oranges once and unwaxed oranges three

times. At 25°C, the experiment was performed twice with waxed and twice with unwaxed oranges. Temperature was monitored during the 0-24 h period with Temptale® monitors.

**Statistical analysis.** A paired t-test was used to compare initial inoculum levels with time 0 recovery CFU/orange. Differences were considered to be statistically significant when  $p < 0.05$ . The General Linear Model analysis of variance (SAS Institute Inc., Cary, NC) was used to determine statistical differences between incubation temperatures and between incubation times at 25 and 37°C. Differences were considered to be statistically significant when  $P < 0.05$ .

## RESULTS AND DISCUSSION

No significant difference was found between initial inoculum level and time 0 recovery at 25°C ( $p = 0.344$ ) and 37°C ( $p = 0.789$ ). Initial inoculum and time 0 counts ranged between 7.9- and 9.0-log CFU/orange. Thus, only time 0, 1, and 24 hour data was used for the analysis of variance.

At 25°C the log CFU/orange decreased by 0.2-log cycles from time 0 to 1 h. The log CFU/orange further decreased by 0.4-log cycles from time 1 to 24 h (Table 1). These differences were statistically significant with  $p = 0.0204$  and  $p = 0.0177$ , respectively. The overall difference from time 0 to 24 h was also significant ( $p = 0.0050$ ).

At 37°C the log CFU/orange decreased by 0.8 log-cycles from time 0 to 1 h and by 0.5 log-cycles from time 1 to 24 h (Table 1). The overall difference from time 0 to 24 h was statistically significant ( $p = 0.0328$ ). The differences from time 0 to 1 h ( $p = 0.1001$ ) and from time 1 to 24 h ( $p = 0.2853$ ) were not statistically significant.

An interaction between time and temperature was not found ( $p = 0.2756$ ); thus, the rate of decrease in log-CFU/orange was not shown to be different for 25°C versus 37°C. Survival of

*Salmonella* spp. appears to be similar at both temperatures; therefore, adjustment of temperatures in this range during harvesting or transportation is not likely to improve safety margins.

The decreases in microbial counts (0.6- and 1.3-log CFU/orange) over 24 h are relatively small and of negligible value for providing any kind of safety margin. Such decreases would allow potential pathogens to survive at infective doses. Secondary fecal contamination on the surface of oranges might be a potential source of *Salmonella* spp. infection for at least 24 hours and possibly much longer. These results show the need for effective disinfection methods and sanitary handling of oranges, particularly during post-processing transportation and marketing. Potential contamination from rodents, amphibians, insect vectors, and humans must be minimized wherever possible. Further studies should explore pathogen survival on oranges at longer time periods and at various other temperatures.

TABLE 1. *Salmonella* survival on oranges at 25°C and 37° (log-CFU/orange)

Time (h)	Temperature	
	25°C	37°C
0	8.54 (0.15) <sup>a</sup>	8.35 (0.24)
1	8.30 (0.20)	7.66 (0.20)
24	7.92 (0.24)	7.17 (0.86)

<sup>a</sup>Numbers in parentheses represent standard deviations (n = 4).

## REFERENCES

1. Birkhead, G. S., D. M. Morse, W. C. Levine, J. K. Fudala, S. F. Kondracki, H.-G. Chang, M. Shayegani, L. Novick, and P. A. Blake. 1993. Typhoid fever at a resort hotel in New York: a large outbreak with an unusual vehicle. *J. Infect. Dis.* 167:1228-1232.
2. Duncan, M. D., J. A. Doull, E. R. Miller, and H. Bancroft. 1946. Outbreak of typhoid fever with orange juice as the vehicle, illustrating the value of immunization. *Amer. J. Public Health.* 36:34-36.
3. Eleftheriadou, M., P. Quantick, M. Nolan, and D. Akkelidou. 1998. Factors affecting quality and safety of freshly squeezed orange juice (FSOJ). *Dairy, Food, Environ. Sani.* 18:14-23.
4. Pao, S., E. Brown, and K. R. Schneider. 1998. Challenge studies with selected pathogenic bacteria on freshly peeled hamlin orange. *J. Food Sci.* 63:359-362.
5. Pao, S. and G. E. Brown. 1998. Reduction of microorganisms on citrus fruit surfaces during packinghouse processing. *J. Food Prot.* 61:903-906.
6. Pao, S. and C. L. Davis. 1999. Enhancing microbiological safety of fresh orange juice by fruit immersion in hot water and chemical sanitizers. *J. Food Prot.* 62:756-760.
7. Pao, S., C. L. Davis, D. F. Kelsey, and P. D. Petracek. 1999. Sanitizing effects of fruit waxes at high pH and temperature on orange surfaces inoculated with *Escherichia coli*. *J. Food Sci.* 64:359-362.
8. Parish, M. E. 1998. Coliforms, *Escherichia coli*, and *Salmonella* serovars associated with a citrus-processing facility implicated in a salmonellosis outbreak. *J. Food Prot.* 61:280-284.
9. Parish, M. E., J. A. Narciso, and L. M. Friedrich. 1997. Survival of Salmonellae in orange juice. *J. Food Safety* 17:273-281.
10. Tabershaw, I. R., L. L. Schmelzer, and H. B. Bruhn. 1967. Gastroenteritis from an orange juice preparation. *Arch. Environ. Health.* 15:72-77.
11. Tauxe, R., H. Kruse, C. Heedberg, M. Potter, J. Madden, and K. Wachsmuth. 1997. Microbial hazards and emerging issues associated with produce; a preliminary report to the national advisory committee on microbiologic criteria for foods. *J. Food Prot.* 60:1400-1408.
12. Walderhaug, M. O., S. G. Edelson-Mammel, A. J. DeJesus, B. S. Eblen, A. J. Miller, and R. L. Buchanan. 1999. Preliminary studies on the potential for infiltration, growth, and survival

of *Salmonella enterica* serovar Hartford and *Escherichia coli* O157:H7 within oranges.  
<http://vm.cfsan.fda.gov/~comm/juicstud.html>.

## Effects of hot water on alfalfa seed germination

### Objectives

The study purpose was to determine the effect of hot water treatments on alfalfa seed germination. The primary hypotheses tested were that treatment at 60°C, 65°C, and 70°C for up to 10 min would not adversely affect germination. An initial hypothesis tested (to help determine protocol) was that germination was equal when measured on moist filter paper or plain agar plates.

### MATERIALS AND METHODS

**Measurement of Germination.** Alfalfa seeds sold for commercial sprouting were obtained from Dr. Jeff Farrar, California Department of Health Services, Food and Drug Branch. The seeds were considered to be of high quality for germination capability and were known to be contaminated with low levels of *Salmonella* Mbandaka. The seeds were placed 100 per agar plate (1.5% Difco Granulated Agar, Detroit, MI) or 200 per piece of coarse filter paper (P8, Fisher, Pittsburgh, PA). Seeds were spread out on the agar plates or in envelopes made from the filter paper. The paper envelopes were placed in glass cylinders with a small amount of deionized water to keep the entire paper moist. The glass cylinders were covered with aluminum foil to prevent evaporation. The seeds were incubated at a room temperature (approximately 24°C) for 48 h. A total of 400 seeds was incubated on either agar plates or filter paper in each of two separate experiments. Germination (percentage of seeds germinated per sample) was tabulated for each sample. A seed was considered to have germinated if the seed coat was broken and

a visible sprout extended from the original seed. Swollen seeds or ruptured seeds with sprout tissue inside were not counted as germinated because it was noted that such seeds rarely sprouted completely when incubated for an additional 24 h.

**Heat treatments.** For heat treatment studies, seeds were placed in filter paper envelopes and immersed in a hot water bath (60 to 70°C) for 0 to 10 minutes. At the specified time, the filter paper envelope was immediately immersed in cool water (24°C). Cooled seeds were incubated on filter paper (for 60°C treatment) or plain agar (for 65°C and 70°C treatments) at 24°C for 48 h and germination rates were tabulated. Samples of 200 seeds each were sprouted for each exposure time at 60°C. At least three samples of 100 seeds each were sprouted for each time period at 65°C and 70°C.

**Statistical Analysis.** A two-sample Z-test was used to test for the difference between two populations (filter paper versus agar) for each of the two separate experiments. The level of significance was 0.05. Two-sample Z-tests were used to test for the difference between population proportions (germination percentage) for the control groups (time = 0) versus increasing times of exposure (at each temperature) until a statistically significant ( $\alpha = 0.05$ ) difference was found.

## RESULTS AND DISCUSSION

Germination percentages for the initial two trials with filter paper were found to be 93.8% and 91.5%, while percentages for plain agar were found to be 94.5% and 94.8%. There was no significant difference between the proportions on paper versus agar for either trial ( $p = 0.65$  for trial one and  $p = 0.069$  for trial two). As a result of this

finding, further studies used both paper and agar interchangeably as the media for sprouting.

The germination percentage did not significantly decline ( $p=0.28$ ) when seeds were held for up to 10 min at 60°C (Table 1). When seeds were held at 65°C, germination declined over the entire treatment period but were only significantly reduced ( $p = 0.0026$ ) after a 4-min time period (Table 1). When seeds were held at 70°C, significantly lower germination percentages ( $p=0.0027$ ) were observed after 2, but not after 1 min (Table 1).

Such data, when combined with studies of bacterial destruction by heat treatment of alfalfa seeds may help determine whether hot water treatment is a viable method for minimizing or eliminating potential foodborne pathogens from alfalfa sprouts at the production level.

TABLE 1. *Germination rates (percent) of alfalfa seed heated for various times and temperatures*

Time (min)	Temperature		
	60°C	65°C	70°C
0	97.5	92.0 (4.4) <sup>a</sup>	93.7 (3.3)
1	n.d. <sup>b</sup>	91.3 (0.6)	92.0 (3.0)
2	98.0	89.0 (3.6)	89.2 (2.5)
4	98.5	84.7 (2.1)	73.8 (3.1)
6	96.5	75.3 (4.0)	54.5 (6.3)
8	96.5	58.0 (8.9)	34.3 (4.1)
10	96.5	45.0 (6.9)	25.3 (9.6)

<sup>a</sup>Numbers in parentheses represent standard deviations (n=3-6).

<sup>b</sup>n.d., not determined.

REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0188	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE 18.Jan.01	3. REPORT TYPE AND DATES COVERED MAJOR REPORT		
4. TITLE AND SUBTITLE PERCEIVED EFFECTS OF HAZARD ANALYSIS AND CRITICAL CONTROL POINT TRAINING ON CALIFORNIA EGG PRODUCERS			5. FUNDING NUMBERS	
6. AUTHOR(S) MAJ FULLER JAY D				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) UNIVERSITY OF CALIFORNIA AT DAVIS			8. PERFORMING ORGANIZATION REPORT NUMBER  CI01-7	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) THE DEPARTMENT OF THE AIR FORCE AFIT/CIA, BLDG 125 2950 P STREET WPAFB OH 45433			10. SPONSORING/MONITORING AGENCY REPORT NUMBER	
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION AVAILABILITY STATEMENT Unlimited distribution In Accordance With AFI 35-205/AFIT Sup 1			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words)				
14. SUBJECT TERMS			15. NUMBER OF PAGES 19	
			16. PRICE CODE	
17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT	