Multiple Interventions for Improving Food Safety Practices in 2 Small Beef Abattoirs of Honduras and Associated Impacts on Risk-Mitigation Management

Environmental Health Insights
Volume 14: 1–6
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DOI: 10.1177/1178630220914596

Diego Casas[1,2], Alexandra Calle[1,2], Mariely Bueso[1,2], Nelson Huerta-Leidenz[1,2], Markus F Miller[1,2] and Mindy M Brashears[1,2]

[1]Animal and Food Science Department, Texas Tech University, Lubbock, TX, USA.
[2]International Center for Food Industry Excellence, Texas Tech University, Lubbock, TX, USA.

ABSTRACT: Mitigation of risk for Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* contamination was evaluated after a multiple-intervention approach (comprising food safety education and training, implementation of customized food safety practices and programs, and environmental monitoring programs with audits and corrective actions) in 2 small Honduran beef abattoirs. Previously, neither abattoir had food safety programs in place nor were they subjected to strict food safety regulatory surveillance. Abattoirs A and B were sampled on 4 nonconsecutive months each. Swab samples of abattoir A (n = 160, 40 samples per sampling date) and abattoir B (n = 78, 16-22 samples per sampling date) were taken from direct and indirect food contact surfaces, screened by BAX real-time polymerase chain reaction (PCR) assays and confirmed using immunomagnetic separation, selective media, and latex agglutination. In abattoir A, *Salmonella* presence was negligible, whereas presumptive STECs were present in 10%, 12.5%, 0%, and 5% of the environmental samples respective to each sampling month, indicating a reduction of STEC ($P = .06$) by the third and fourth sampling months. Conversely, presumptive STEC presence was negligible in abattoir B, whereas *Salmonella* presence for each sampling month was of 5.6%, 6.3%, 27.3%, and 0.0%, respectively. Upon the increased pathogen presence detected on the third sampling month, additional actions were taken to reinforce the implementation of food safety practices and programs, which resulted in a *Salmonella* reduction to 0% by the fourth sampling month ($P = .013$). The satisfactory results strongly suggest that a multiple-intervention approach is crucial to improve food safety in this type of premises.

KEYWORDS: *Salmonella*, Shiga toxin-producing *Escherichia coli*, training, food safety

RECEIVED: December 13, 2019. **ACCEPTED:** February 26, 2020.

TYPE: Original Research

FUNDING: The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was funded by the Cooperative Agreement for Research, Technical Assistance and Grant Aid between the Honduran Bank for Production and Housing (BANHPROVI) and Texas Tech University for "Strengthening of Production and Industrialization of Beef, Sheep and Goats".

DECLARATION OF CONFLICTING INTERESTS: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

CORRESPONDING AUTHOR: Nelson Huerta-Leidenz, International Center for Food Industry Excellence, Texas Tech University, Box 42141, Lubbock, TX 79409, USA.
Email: Nelson.Huerta@ttu.edu

Introduction

Protecting public health by assuring the food safety of the meat supply chain must be a top global priority shared by the government and the private sector. Processing of meat products in unsanitary conditions and subsequent sales of these contaminated meat products can have a negative impact on public health.[1,2] However, avoiding the potential meat contamination by foodborne microbial pathogens during cattle harvesting is a more challenging task in developing nations. Greater risks may exist in these settings due to the poor personnel's knowledge in food safety, an educational handicap which is aggravated by cultural gaps and the insufficient outreach of numerous small abattoirs from food safety regulatory authorities. Environmental monitoring plans (EMPs) are very useful to assess the efficacy of food safety programs such as sanitation standard operating procedures (SSOPs), good manufacturing practices (GMPs), and hazard analysis critical control points (HACCPs).[3] Identification of harborage sites by EMP is vital to adequately and timely implement corrective actions (ie, sanitation measures) to mitigate or eliminate pathogen presence.[4] To establish successful EMP, surfaces, equipment, or utensils in contact with microbial contaminants must be sampled to assess the presence of bacterial pathogens and/or indicator microorganisms, as well as to improve the efficacy of applied interventions and sanitation procedures. An EMP is also important to identify the type of microbiota present in a food processing environment and successfully implement cleaning and disinfection procedures.[5]

Food safety practices and microbiological contaminants present in meat plants have been poorly investigated in low-income countries. This type of research is increasingly important in Honduras not only to improve access to safe foods but to ensure that meat is consistently handled in a safe manner. Currently, Honduras holds the status of a country's *equivalence* to the food safety inspection system granted by the USDA Food Safety and Inspection Service (FSIS). This status protects public health and facilitates trade. However, maintaining this status requires to ensure strict compliance with food safety practices.

The Honduran government is designing an aggressive plan for cattle re-population, and the number of beef cattle available for processing is expected to increase.[6] To protect the consumer, meat processing facilities of any size must be ready to receive an increasing amount of cattle and implement effective food safety programs to produce safe products for the domestic and/or international consumers. Therefore, effective in-plant food safety practices and programs such as SSOP, GMP, and HACCP must be urgently implemented because they can lead

to the prevention of foodborne illnesses due to reduced product cross-contamination. We posit that a multiple-intervention approach may improve food safety practices in small beef abattoirs of Honduras with positive, associated impacts on risk-mitigation management. Training can improve the levels of food safety knowledge by abattoir personnel and, in turn, this educational intervention could result in an adequate implementation of SSOP, GMP, and HACCP to potentially prevent the presence of Shiga toxin-producing *Escherichia coli* (STEC) and *Salmonella* in environmental samples. Therefore, the objective of this study was to evaluate the influence of a multiple-intervention approach in 2 small Honduran abattoirs that did not have any food safety program implemented, on the environmental occurrence of STEC and *Salmonella*.

Materials and Methods

Abattoir description

Two small beef abattoirs (A and B) located at different geographical regions of Honduras were chosen for this case study. Abattoirs A and B had different production capacities and were located in the central and eastern regions of the country, respectively. Abattoir A claimed to process 140 head of cattle per week with 45 employees while abattoir B claimed to process 26 head of cattle per week with 8 employees. It is worth highlighting that despite the small size of both abattoirs, their operations could have a big impact on public health. One of these abattoirs satisfies more than half of the beef demand from an agricultural school in Honduras that has had previous food safety complaints and suspected outbreaks on campus. The other sells all its meat products to one of the largest supermarkets in Tegucigalpa.

Before the initiation of the study, employees in both abattoirs declared they were not subjected to strict food safety regulatory surveillance by the local authority and not having received food safety training of any type beforehand. In fact, the Texas Tech University team verified that abattoirs A and B did not have a food safety system in place and were processing 140 and 26 head of cattle per week under those conditions, with 45 and 6 employees, respectively. After a needs assessment visit conducted in their facilities, a monitoring program of pathogen presence in the processing facility was implemented over the course of 4 nonconsecutive months. To monitor the presence of *Salmonella* and presumptive STEC in the food processing environment, environmental swab samples were taken in January, May, June, and July 2017 for abattoir A, and January, March, June, and July 2017 for abattoir B (Supplemental Figure 1).

Training

Food safety training provided to the 2 abattoirs consisted of (a) in-class face to face training with the entire team of both abattoirs (49 attendees); (b) one-on-one training with management to develop HACCP, SSOP, and GMP protocols and implementation of practices; (c) in-plant hands-on training with floor workers on food safety practices; and (d) virtual communication via email to review documented practices.

During May 2017, employees of both abattoirs underwent comprehensive training sessions in HACCP, SSOP, and GMP. Lectures were delivered in Spanish by the Texas Tech University team. Afterward, acquired knowledge about HACCP, GMP, and SSOP was assessed by pre-training and post-training tests consisting of 20 questions (Supplemental Material Training Exam). Training classes were given in a classroom setting in an interactive and dynamic mode and lasted 6 hours with 15-min breaks every 1.5 hours. During the training session, the Texas Tech University team went over each SSOP that was instructed to the abattoirs on the first 2 sampling dates.

In addition to the classroom training, when a surface turned positive to a pathogen, a customized SSOP guidelines' manual, specifically addressing the contaminated equipment, surface, or utensil, was developed and implemented to eventually eliminate pathogen presence on that surface. These SSOP guidelines were provided to the abattoir for implementation after each sampling date. Also, after each sampling date, a report with the developed SSOP was personally delivered and explained to the abattoir managers. This report contained the step-by-step description of how to begin implementing the SSOP and we make sure that it was understood and applied by the abattoir employees.

By May 2017, a customized GMP manual was personally delivered to abattoir B while abattoir A was starting to implement their own. The manual contained GMP for personnel hygiene, buildings and infrastructure, external grounds of the abattoir, pest control, managing and storage of disinfecting chemicals, equipment, process controls, restrooms, and record-keeping of inspection of GMP.

Environmental monitoring program

In both abattoirs, environmental samples were taken in the middle of the production shift with EZ-Reach Sponges hydrated with 25 mL buffered peptone water (BPW, World Bioproducts, Mundelein, IL, USA). A total of 160 samples were taken from abattoir A, 40 samples per each visit. In abattoir B, a total of 78 environmental samples were taken. Abattoir B was considerably smaller, and its equipment was constantly moved around sites or removed from the original site between visits; therefore, the total number of swab samples varied per sampling date. The numbers of samples taken in abattoir B were 18, 16, 22, and 22 respective to each sampling month. Most samples were taken consistently from the same location during each visit to observe potential improvements over time. These samples were taken from tables, saws, equipment, knives, aprons, walls, floors, drains, tubs, baskets, axes, boots, carts, hands, scales, and shelves. The swabbing area consisted of approximately a 6 by 6 inch area when the environmental surface allowed it. Knives, saws, and axes swabbing were made in all the surface areas in direct contact with food. Immediately after collecting each sample, they were placed into insulated bags that were kept cold

with previously frozen ice packs. Insulated bags containing the samples were then sent to the Texas Tech University Food Microbiology Laboratory (Lubbock, TX, USA) by commercial air transportation using USDA-Animal and Plant Health Inspection Service (APHIS) permits (permit 114031) for clearing customs.

Sample preparation

At the Texas Tech University Food Microbiology Laboratory, samples were homogenized in a Stomacher Circulator (Model 400 circulator; Seward, West Sussex, UK) for 30 seconds at 230 r/min. From the homogenized sample, 1 mL was transferred to 9 mL of modified tryptic soy broth (mTSB; Neogen, Lansing, MI, USA) with 8 mg/L novobiocin and acid digest of casein and incubated at 42°C for 24 hours. Before conducting microbial analysis, sample composites were prepared for every 5 samples. To prepare them, 1 mL aliquot from each enriched sample was placed into a sterile test tube and thoroughly vortexed. These composites were used to perform initial BAX real-time polymerase chain reaction (PCR) screening for *Salmonella* and STECs; however, the isolation and confirmation were individually (per-sample basis) accomplished.

Microbiological analysis

Detection and isolation of *Salmonella* were conducted following the methods described by the USDA-FSIS MLG section 4.09.[7] Briefly, overnight enrichments were composited as previously described and underwent BAX system Real-Time PCR assay *Salmonella* detection kits (Dupont, Wilmington, DE, USA). The BAX system Real-Time PCR assay is commonly used in the beef industry when detecting the presence of *Salmonella* and STECs. The *USDA Microbiology Laboratory Guidebook* has used it as a standard detection method until 2020 for isolation protocols of such pathogens. The manufacturer's instructions were followed to screen *Salmonella* spp. and "big 7" STECs. If a composite was found to be positive, the 5 samples corresponding to that composite were further analyzed individually for isolation; 1 mL of each sample was transferred to Rappaport Vassiliadis broth (RV; Oxoid, Hampshire, UK) and Tetrathionate broth (TT; Neogen) with 20% iodine solution, and incubated at 37°C for 24 hours. A loopful of broth from RV and TT enriched tubes was then streaked onto Xylose Lysine Tergitol 4 agar (XLT4; Hardy Diagnostics, Santa Maria, CA, USA) for growth of individual colonies and incubated at 37°C for 40 to 48 hours. Colonies that grew black in color or had a black center and a yellow halo were considered presumptive positive for *Salmonella*. These colonies underwent agglutination with Wellcolex* Colour *Salmonella* agglutination kit (Thermo Fisher Scientific, Lenexa, KS, USA). Positive agglutinating colonies were then subjected to confirmation through real-time PCR (Agilent Technologies, Santa Clara, CA, USA) for *Salmonella*. Real-time PCR was done targeting *ttrC* gene as previously described.[8]

In this study, the presence of presumptive STEC was used as an indication of its presence for control of environmental contamination. Detection and isolation of presumptive STEC were conducted following the *Microbiology Laboratory Guidebook* protocol MLG 5B.05 for non-O157 STEC.[9] Briefly, overnight enrichments of environmental samples were placed in composites and underwent BAX real-time PCR screening assay kits for *stx* and *eae* genes (Dupont) following manufacturer's directions. Composites that were positive had their individual sample enrichments subjected to BAX screening kits for *stx* and *eae* genes. Positive individual samples underwent Dupont BAX System Real-Time PCR assay STEC panel 1 and panel 2 and O157 kits (Dupont) for the detection of big 7 STEC serogroup genes. Positive samples in BAX followed immunomagnetic separation (IMS) for their respective STEC serogroup positive in panel 1, panel 2, or O157 assays. From the resulting cell suspension, 30 µL was streaked onto modified Rainbow Agar (mRBA; Biolog, Hayward, CA, USA) supplemented with 0.150 mg/L potassium tellurite, 0.05 mg/L cefixime trihydrate, and 5 mg/L novobiocin. Presumptive positive colonies in mRBA were confirmed with latex agglutination test kits (Abraxis, Inc, Warminster, PA, USA) corresponding to the presumptive positive serogroup that BAX demonstrated.

Statistical analyses

Statistical analysis of pathogen presence was conducted using the R (v3.4.4) statistical package. Fisher's exact test for equality of proportions was performed for comparison of pathogen presence between sampling months with a significance level of 10%; however, *P* values are reported for any comparison throughout the article. Comparisons were made among the presence of the same pathogen. Mann-Whitney *U* test was conducted to examine the difference between the sample distributions at a .05 significance level for the pre- and post-evaluation test scores of the training session. The Mann-Whitney *U* significance test (a nonparametric equivalent to the 2-sample *t* test) determined whether the samples of indicator bacteria, grouped by the presence or absence of a pathogen, come from the same distribution at the established level of significance.[10] This nonparametric method was chosen over Welch's *t* test because of the lack of normality in the distribution of the test scores.

Results and Discussion

Abattoir A

Presumptive STEC was present in 10%, 12.5%, 0%, and 5% of the environmental samples respective to each sampling month (Table 1). A reduction of presumptive STEC presence ($P = .065$) was observed by the third sampling month and coincided with the completion of GMP, SSOP, and HACCP training of abattoir employees, delivery of customized GMP manuals to the abattoirs, implementation of 2.0% to 2.5% lactic acid spray intervention in final carcasses, and the use of

SSOP targeted to areas, equipment, or utensils that had previously been positive for pathogen presence.

Previous studies have found that food safety training accompanied by improvement and implementation of food safety programs successfully achieved pathogen reduction in abattoirs and food processing environments.[4,11] Abattoir A also added hot water sterilizers to their harvest floor, maintaining water at 180°F (82°C) and monitoring temperature every 1.5 hours. At the harvest floor, it was observed that employees followed the recommendations to use sterilizers for knives, saws, and axes between every carcass.

The absence of pathogens in the targeted areas, equipment, or utensils in subsequent sampling months (Table 2) supported the effectiveness and adequate implementation of recommended SSOP. Recommended disinfectant for the SSOPs for surfaces and equipment was sodium hypochlorite at a concentration of 100 ppm.

Significant reductions of *Escherichia coli* and *Salmonella* have been previously achieved with hypochlorite-based solutions when grown in suspension.[12] Except for one of the sampling months, *Salmonella* was not detected in environmental samples of abattoir A. Thus, this low and inconsistent presence of *Salmonella* did not allow an adequate demonstration of a *Salmonella* reduction in the abattoir A environment.

Table 1. Abattoir A presence (%) of STEC and *Salmonella* throughout the sampling period.

MONTH	STEC (% ± SE_P)	*SALMONELLA* (% ± SE_P)
January	10.0 ± 4.74	0.0
May	12.5 ± 5.23	5.0 ± 3.45
June	0.0	0.0
July	5.0 ± 3.45	0.0

Abbreviations: SE_P, standard error of proportions; STEC, Shiga toxin-producing *Escherichia coli*.

Abattoir B

In abattoir B, the presumptive presence of STEC at the initiation of this assessment was too low to be used as an indicator of the adequate implementation and effectiveness of SSOP and interventions applied. Conversely, *Salmonella* environmental presence was detected for each of the first 3 consecutive sampling months, 5.6%, 6.4%, and 27.3%, respectively (Table 3). Furthermore, *Salmonella* in the stuffer was consistently observed in January and March.

After the second positive sample was found in March, SSOP in-plant training for this particular equipment was conducted, and by June, the stuffer was found free of *Salmonella* (Table 4).

Salmonella enterica has been found to be a foodborne pathogen that can persist in the meat processing environment. In some cases, *Salmonella* can be resistant to disinfectants commonly used in processing plants.[13] Therefore, environmental monitoring programs become critical in identifying the continuous presence of pathogens. Thorough sanitizing of equipment, surfaces, areas,

Table 2. Initial and follow-up *Salmonella* and presumptive STEC contamination in abattoir A.

PROCESSING PLANT AREA	SAMPLE SITE	DATE SAMPLED[a]			
		JANUARY 30, 2017	MAY 15, 2017	JUNE 5, 2017	JULY 5, 2018
Cooking	Drain	–	*Salmonella*	–	–
Cooking	Floor	–	–	–	Presumptive STEC
Fabrication	Grinder	Presumptive STEC	–	–	–
Fabrication	Knife 1	Presumptive STEC	–	–	–
Fabrication	Floor	Presumptive STEC	–	–	–
Harvest	Chiller floor	Presumptive STEC	–	–	–
Harvest	Apron	–	*Salmonella*	–	–
Harvest	Hide	–	–	–	Presumptive STEC
Packaging	Table	–	Presumptive STEC	–	–
Packaging	Floor	–	Presumptive STEC	–	–
Packaging	Drain	–	Presumptive STEC	–	–
Sausage	Table	–	Presumptive STEC	–	–
Sausage	Drain	–	Presumptive STEC	–	–

Abbreviation: STEC, Shiga toxin-producing *Escherichia coli*.
[a]Undetectable presence of pathogens is represented with a dash (–).

or utensils with continuous pathogen presence must be done after identification of areas with possibly persistent pathogens.

The highest *Salmonella* presence was unexpectedly found by the third sampling month, despite personnel training had been provided just recently. For one, the abattoir audit revealed an inadequate implementation of food safety programs. For instance, the employees in charge were not preparing the chlorine solution or did not verify the chlorine concentration as instructed; what they were doing was throwing granulated chlorine on the tables and floors without prior removal of the organic matter (residual processed meat). This unexpected *Salmonella* finding could also occur because the sampling time coincided with the rainy season. It has been found that increased rainfall could favor in-plant *Salmonella* presence.[14] Consequently, additional actions were taken to reinforce the implementation of SSOPs, GMP manual instructions, and verification procedures.

Employees underwent in-plant training of implementation of SSOP, and a matrix for adequate dilution of chlorine solution to 100 and 50 ppm was provided. The use of an adequate concentration of chlorine in disinfection solutions, accompanied by GMP verification procedures, yielded tangible results because by the fourth sampling month, *Salmonella* was not detected

Table 3. Abattoir B presence of STEC and *Salmonella* throughout the sampling period.

MONTH	STEC (% ± SE_P)	*SALMONELLA* (% ± SE_P)
January	11.1 ± 7.4	5.6 ± 5.4
March	0.0	6.3 ± 6.1
June	0.0	27.3 ± 9.5
July	0.0	0.0

Abbreviations: SE_p, standard error of proportions; STEC, Shiga toxin-producing *Escherichia coli*.

(*P* = .0134). Although many other factors can explain this achievement, it can be said that collectively applied, these interventions contributed to the significant pathogen reduction.

Food safety training of employees greatly influenced employee's awareness of adequate sanitary conditions. A deficient food safety knowledge in abattoir employees was observed (Figure 1), and scores for the pre-training exam had an average of 53%. After training, the average score raised to 60%. The 1-tailed Mann-Whitney *U* test detected differences (*P* = .04719) in population distributions of the test scores before and after training. Based on the test results, we can say that the training had a positive, although immeasurable, influence on employee's food safety awareness and perhaps on behavioral changes. In fact, during the last visit, it was noticed that employees were more diligent on cleaning and disinfection procedures; before and after processing, the preoperational sanitation was more thoroughly performed, and the concentration of the disinfectant solution was always checked before sanitizing equipment and surfaces. In addition, evisceration and de-hiding procedures were performed with more caution.

The positive effect of training on food safety behavior has been reported by Pilling et al.[15] The latter authors identified that those establishments in which food safety training was required and implemented exhibited a better score in food safety practices during food preparation.[15] Similarly, Adesokan et al[16] evaluated the improved food safety knowledge and behavior as a result of food safety training; their findings point out that employees under a food safety training program were able to demonstrate significantly higher knowledge and practice when compared with the employees not subjected to the training program. They identified that not only initial training but refreshing concepts provide employees with opportunities to update the learned skills; in their experience, the in-class training was reinforced and flowed up by in-plant refreshers.[16] Briefly, the 4 tactics of Adesokan

Table 4. Initial and follow-up *Salmonella* and presumptive STEC contamination in abattoir B.

PROCESSING PLANT AREA	SAMPLE SITE	DATE SAMPLED[a]			
		JANUARY 16, 2017	MARCH 16, 2017	JUNE 5, 2017	JULY 7, 2017
Cold room	Drain	–	–	*Salmonella*	–
Harvest	Knife 1	–	–	*Salmonella*	–
Processing	Table	–	–	–	–
Processing	Drain A	Presumptive STEC	–	*Salmonella*	–
Processing	Stuffer	*Salmonella*	*Salmonella*	–	–
Processing	Drain B	–	–	*Salmonella*	–
Processing	Faucet	–	–	*Salmonella*	–
Processing	Floor crack	Presumptive STEC	–	*Salmonella*	–

Abbreviation: STEC, Shiga Toxin-producing *Escherichia coli*.
[a]Undetectable presence of pathogens is represented with a dash (–).

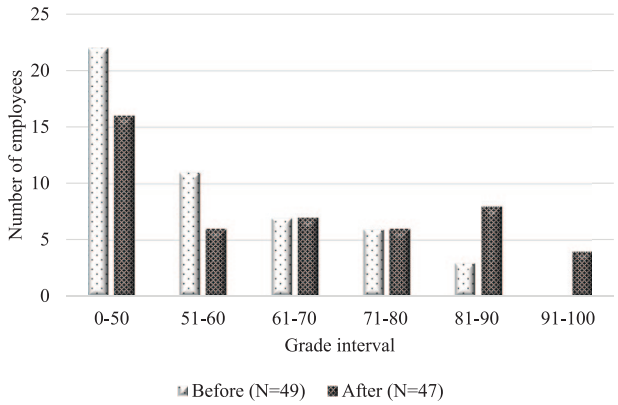

Figure 1. Pre- and post-training exam results from abattoirs A and B employees.

et al[16] were as follows: (a) presenting the employees with the microbial results from their abattoir environmental sampling, (b) modifying their practices while they perform their routine processing operations, (c) modifying written SSOP and GMP to increase practical knowledge, and (d) supporting and justifying the theoretical information provided in the classroom. These tactics were quite similar to those we used in our study.

Conclusions

The multiple-intervention approach is responsible for noticeable improvements in food safety practices and programs which in turn lead to pathogen control in the 2 abattoirs' environments. It is readily evident that, within the undertaken collective actions, education and training in food safety practices and programs play an important role in the achievements. However, better experiment designs and more data collection are needed to demonstrate and measure the importance of training and refreshers of food safety concepts. As expected, an environmental sampling program leads to the identification of sites harboring *Salmonella* or presumptive STEC and the SSOP designed to eliminate those harborage sites effectively assist in controlling pathogen presence and persistence. Despite the promissory results in pathogen reduction, its practical significance in terms of risk mitigation cannot be considered as impactful and much less a long-term achievement in this type of establishment. Therefore, continuous tailored food safety training in conjunction with the EMP must be implemented in these small abattoirs to achieve further and consistent improvements. The experiences and results presented herein must be taken into account by the government agencies responsible for public health and food safety in Honduras to design and implement sanitary inspection policies and regulations to ensure good standards of hygiene and food safety in these small abattoirs and prevent the spread of foodborne disease.

Author Contributions

Conceptualization, D.C., M.MB; Methodology design, D.C., M.M.B and A.C.; Data collection, D.C., M.B., A.C., and N.H-L., Project Administration, M.M.B and M.F.M.; Writing-review and editing, D.C., N.H-L., and A.C. All authors have read and agreed to the published version of the manuscript.

ORCID iD

Diego Casas https://orcid.org/0000-0002-7897-9904

Supplemental Material

Supplemental material for this article is available online.

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