






## Article

# Use of a Mobile Methodology for the Bio-Mapping of Microbial Indicators and RT-PCR-Based Pathogen Quantification in Commercial Broiler Processing Facilities in Honduras

Gabriela K. Betancourt-Barszcz <sup>1</sup>, Sabrina E. Blandon <sup>1</sup>, David A. Vargas <sup>1</sup>, Rosy Bueno López <sup>1</sup>, Daniela R. Chavez-Velado <sup>1</sup>, Angelica Sánchez <sup>1</sup>, Valeria Larios <sup>1</sup>, Nadira J. Espinoza-Rock <sup>1</sup>, Mirian Bueno-Almendarez <sup>2</sup> and Marcos X. Sanchez-Plata <sup>1,\*</sup>

<sup>1</sup> International Center for Food Industry Excellence, Department of Animal and Food Sciences, Texas Tech University, Lubbock, TX 79409, USA; gabbetan@ttu.edu (G.K.B.-B.); sablando@ttu.edu (S.E.B.); andres.vargas@ttu.edu (D.A.V.); rosy.bueno@ttu.edu (R.B.L.); daniela.r.chavez@ttu.edu (D.R.C.-V.); angelica.sanchez@ttu.edu (A.S.); vlarios@ttu.edu (V.L.); nadiespi@ttu.edu (N.J.E.-R.)

<sup>2</sup> Honduran National Service of Agri-Food Safety and Health, SENASA, Tegucigalpa 11101, Honduras; mbueno@senasa.gob.hn

\* Correspondence: marcos.x.sanchez@ttu.edu; Tel.: +1-(975)-595-5208

**Abstract:** Poultry bio-mapping helps identify microbial contamination and process optimization opportunities such as sanitary dressing procedures, equipment adjustments, cross-contamination controls, and chemical intervention applications in commercial poultry processing operations. This study focuses on the development of a preliminary microbiological baseline of microbial indicators, including total viable counts (TVC), Enterobacteriaceae (EB), and the quantification and detection of pathogens such as *Salmonella* and *Campylobacter* spp. in four commercial broiler processing facilities in Honduras. Whole chicken and wing rinses were collected from four poultry processing plants at different locations: live receiving, rehangar, post-evisceration, post-chiller, and wings. The MicroSnap<sup>®</sup> system was used for the enumeration of microbial indicators, the BAX<sup>®</sup>-System-SalQuant<sup>®</sup> was used for the quantification of *Salmonella*, and the BAX<sup>®</sup>-System-CampyQuant<sup>™</sup> was used for *Campylobacter* spp. Negative samples after enumeration were tested with BAX<sup>®</sup>-System *Salmonella* and BAX<sup>®</sup>-System *Campylobacter* for prevalence analysis, respectively. The TVC and EB counts were continuously reduced from the live receiving to the post-chiller location, presenting a statistically significant increase ( $p < 0.01$ ) at the wings location. The *Salmonella* counts were significantly different between stages ( $p < 0.01$ ). The prevalence of *Salmonella* was highest in the live receiving stage, with 92.50%, while that at the post-chiller stage was the lowest, at 15.38%. *Campylobacter* spp. counts were lower than that at the other stages at wings 1.61 Log CFU/sample; however, *Campylobacter* spp.'s prevalence was higher than 62.5% in all stages. Microbial bio-mapping using novel technologies suitable for mobile applications was conducted in this study to establish statistical process control parameters for microorganisms. A nationwide microbial baseline for commercial broiler processing facilities in Honduras was developed. In-country data serve as a benchmark for continuous improvement at each facility evaluated and can assist regulatory officers in the development of risk-based performance standards aimed at reducing the risk of exposure to consumers.

**Keywords:** poultry bio-mapping; microbial baseline; total viable counts; Enterobacteriaceae counts; *Salmonella* enumeration; *Campylobacter* spp. enumeration



**Citation:** Betancourt-Barszcz, G.K.; Blandon, S.E.; Vargas, D.A.; Bueno López, R.; Chavez-Velado, D.R.; Sánchez, A.; Larios, V.; Espinoza-Rock, N.J.; Bueno-Almendarez, M.; Sanchez-Plata, M.X. Use of a Mobile Methodology for the Bio-Mapping of Microbial Indicators and RT-PCR-Based Pathogen Quantification in Commercial Broiler Processing Facilities in Honduras. *Poultry* **2024**, *3*, 330–345. <https://doi.org/10.3390/poultry3040025>

Received: 6 May 2024

Revised: 2 August 2024

Accepted: 23 August 2024

Published: 24 September 2024



**Copyright:** © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

## 1. Introduction

In Central America, poultry production is expected to increase to 259,580 metric tons by 2026. From 1966 to the present, the poultry supply chain has exhibited an average annual growth rate of 4.1% [1]. By the year 2032, poultry meat is expected to provide 41% of the total protein intake derived from meat sources [2]. The consumption of poultry per

capita in Central America is 60 pounds of chicken meat per year [3,4]. Poultry production in Honduras is ~350 million pounds per year, with an annual average growth of ~2.5%, with 85% supplied by national production and 15% by imports [5]. In Honduras, several poultry processing facilities already operate under regulatory schemes similar to the U.S. Department of Agriculture's Food Safety and Inspection Service (USDA-FSIS) standards required to export poultry meat to the United States. The Honduran National Agri-Food Health and Safety Service (SENASA) has been recognized as having an equivalent poultry inspection and certification system to the USDA-FSIS [6].

Chickens' gastrointestinal tracts are often colonized by *Campylobacter* spp. and *Salmonella* [7]. Following the colonization, bacterial fecal shedding may occur, leading to the potential contamination of skin and feathers. Moreover, during the slaughter process, external contamination may occur through the rupture of the gastrointestinal tract and the subsequent transfer of pathogens to uncontaminated carcasses and equipment [8]. Before the chiller stage, the USDA-FSIS enforces a zero-tolerance policy for visible fecal material on carcasses [9]. When carcasses are accidentally contaminated with fecal material, they must undergo reprocessing; this stage is called "salvage" [10]. This process is time-consuming because it involves washing, disinfecting with approved antimicrobials, or trimming the contaminated parts, leading to additional expenses for the facility [11]. The USDA-FSIS tracks the prevalence of *Campylobacter* spp. and *Salmonella* pathogens in chicken carcasses and parts nationwide. Based on the production volume, the estimated nationwide prevalence of *Salmonella* and *Campylobacter* spp. in chicken parts between October 2021 and September 2022 was 7.62% and 16.04%, respectively. Compared to chicken parts, whole carcasses showed a reduced prevalence of *Salmonella* (4.14%), although the percentage of carcasses testing positive for *Campylobacter* spp. was 19.36% [12]. SENASA has established a regulatory national prevalence of 20% of *Salmonella* in whole carcasses, which would result in an expected risk per serving of 1.13 illnesses per 100,000 servings [13,14].

*Campylobacter* spp. and *Salmonella* are considered the leading causes responsible for human gastroenteritis in the U.S. [15]. Among foodborne illnesses, salmonellosis prevails as the most frequent, with an estimated annual incidence of approximately 1.4 million cases, of which 30,000 are culture-confirmed cases [16]. At the time of the study, the USDA-FSIS does not consider *Salmonella* an adulterant in raw chicken since the product must be appropriately handled and adequately cooked before consumption, thus destroying pathogens to lower risk levels [17]. Part of the criteria for the USDA to not recognize *Salmonella* as an adulterant in poultry includes the fact that it is not an added substance but rather occurs naturally within the chicken biome. Nonetheless, in April 2024, the USDA announced a rule to declare *Salmonella* as an adulterant in breaded stuffed raw chicken products when exceeding 1 CFU/g [18]. Breaded stuffed raw chicken products have been implicated in 5% of all chicken-associated outbreaks in the U.S. However, approximately 0.15% of these incidents have been attributed to the domestic chicken supply [18]. Over recent years, these products have been linked to salmonellosis outbreaks in the U.S. due to consumers' difficulty in cooking the product correctly by assuming it to be fully cooked [19]. These products are par-fried and generally sold as frozen, non-ready-to-eat products. Since the product may look cooked when frozen, it has been more difficult for consumers to cook it correctly, reaching the minimum internal temperature needed to destroy *Salmonella* (165 °F) [20]. Also, the product has different ingredients that look cooked at different rates, so it may be more difficult for the consumer to determine an accurate internal temperature [21].

Numerous domesticated and wild bird species have been discovered to have *Campylobacter* spp. In domesticated birds, broiler chickens, breeder flocks, and egg-laying hens frequently have a high prevalence of *Campylobacter* spp. [19]. Therefore, it is acknowledged that chicken serves as the main source of human *Campylobacter* infection [22]. A recent USDA-Economic Research Service (ERS) analysis estimates that USD 1.6 billion of the total USD 15 billion in yearly foodborne disease healthcare expenses in the U.S. are attributable to *Campylobacter* species [23,24]. According to the Centers for Disease Control and Prevention (CDC) data, approximately 1.3 million *Campylobacter* infections occur in the U.S. each

year [25]. Estimates of campylobacteriosis in Honduras were not available at the time of the study.

During processing, the risk of contamination by any pathogen might be present; hence, many interventions in the processing line should be used to reduce the risks. In the poultry industry, chemical, physical, and biological interventions are used to control pathogen loads. Synergistic combinations of interventions are required to ensure that pathogens are controlled throughout the processing chain [26]. Physical interventions like rubber picker fingers and hot washes remove feathers, skin, and visible fecal contamination. Decontamination techniques sometimes involve chemical interventions with chlorine and acidified sodium chlorite, organic acids like lactic and peroxyacetic acid, as well as other interventions like ozone [26,27]. Biological interventions involve the application of phage therapy [28].

The objective of this study was to conduct a bio-mapping study of the main four poultry processing plants that cover 88% of the national chicken production in Honduras. Five sampling locations were included throughout the whole process, including live receiving, rehangar, post-evisceration, post-chiller, and wing locations. This bio-mapping study included indicator microorganisms such as total viable counts and Enterobacteriaceae counts and pathogens such as *Salmonella* and *Campylobacter* spp. loads and prevalence. This information was used to develop a nationwide microbial baseline for commercial broiler processing facilities in Honduras.

## 2. Materials and Methods

### 2.1. Sample Collection

This study was conducted in four different poultry processing facilities based in Honduras, representing 88% of the national chicken production. With the help and consent received from SENASA, a total of 50 samples per processing facility ( $n = 200$ ) were collected, five samples per location, during two different and non-consecutive processing days in June, considered the wet season in Honduras. The first samples were collected during the morning shift, while the second samples were collected during the afternoon shift to account for process variability. During the process, the broiler carcasses pass through chemical interventions, including sodium hypochlorite (Cl) and peroxyacetic acid (PAA), at different locations, such as evisceration, chilling, and deboning. Whole chicken carcass and wings (~2 kg) rinses were collected from five different stages of the poultry processing line: live receiving (LR), during which a rinse from a recently identified death-on-arrival chicken, still warm and intact, was collected; rehangar (R); post-evisceration (PE); post-chiller (PC); and wings rinse composites (W). Chicken carcass/wings were placed in a Whirl-Pak poultry rinse bag (Millipore Sigma, Burlington, MA, USA) with 400 mL of Buffered Peptone Water (BPW; Millipore Sigma, Danvers, MA, USA) and homogenized by hand for 1 min. The rinses collected were placed in a cooler with ice packs to maintain a temperature of  $\leq 7$  °C and transported to the laboratory for microbiological analysis.

### 2.2. Processing Facilities Characteristics and Intervention Parameters

The facilities operated at line speeds of ~150 birds per minute and were inspected by official veterinarians and official online and offline inspectors. The processing capacity of these facilities was 24,294,632.23 birds, 75,954,884.55 birds, 24,179,338.18 birds, and 123,130,774.09 birds annually for plants 1–4, respectively. The four poultry processing facilities employed peroxyacetic acid (PAA) for the pre-chilling stage (30–60 ppm) and the main chilling stage (20–100 ppm). Additionally, one establishment implemented post-chilling interventions, targeting chicken parts such as wings (chlorine 20–50 ppm).

### 2.3. Microbial Indicators Enumeration

The rinses were homogenized by hand for 1 min. Following this, 1 mL was collected from each rinse and diluted in 9 mL tubes of BPW (Millipore Sigma, Danvers, MA, USA). For total viable counts (TVC, aerobic heterotrophic bacteria) and Enterobacteriaceae (EB)

enumeration, the bioluminescence-based MicroSnap<sup>®</sup> system (Hygiena<sup>®</sup>, Camarillo, CA, USA) was used. The MicroSnap<sup>™</sup> system consists of a two-step procedure. The first step is for microbial enrichment, and the second is for bacterial enumeration. The poultry rinse was homogenized by hand for 1 min prior to 1 mL of the rinse being added directly to the MicroSnap Enrichment Device Step 1. The MicroSnap Enrichment Device Step 1 was activated by breaking the MicroSnap-Valve, flushing all the enrichment broth to the bottom of the swab tube, and gently shaking the tube to mix the sample with the enrichment broth. After homogenizing, the MicroSnaps were incubated in portable digital dry block incubators—TVC at  $30 \pm 0.5$  °C for  $7 \text{ h} \pm 10 \text{ min}$  to allow for mesophile and psychrophile growth, while EB MicroSnaps were incubated at  $37 \pm 0.5$  °C for 6 to 8 h following protocol guidelines for the enumeration range. When the incubation was complete, two drops (approximately 100 µL) of the enriched sample were transferred from the MicroSnap Enrichment Device Step 1 to the MicroSnap Detection Device Step 2. The MicroSnap Detection Device Step 2 was activated by breaking the MicroSnap-Valve and flushing all the detection liquid to the bottom of the swab tube. The tube was shaken to mix the enriched sample with the detection liquid. The MicroSnap Detection Device Step 2 tube was immediately inserted into the EnSURE<sup>®</sup> Touch luminometer (Hygiena<sup>®</sup>, Camarillo, CA, USA) to obtain enumerative results, as indicated in the method validation studies conducted by the manufacturer and internally in our laboratory [29,30]. The results reported as relative light units (RLU) results were converted to CFU/mL by the EnSURE<sup>®</sup> Touch and to Log CFU/mL, as described by Vargas et al. [29,30]. (Table S1 and Figure S1).

#### 2.4. *Salmonella* Enumeration and Prevalence

For *Salmonella* enumeration, the protocol AOAC 081201 was followed, as indicated in the USDA-FSIS list of validated test kits [31]. The rinses were homogenized by hand for 1 min prior to 30 mL of the rinse being added to 30 mL of pre-warmed BAX<sup>®</sup> MP Media (Hygiena<sup>®</sup>, Camarillo, CA, USA) containing 1 mL/L of SalQuant solution (Hygiena<sup>®</sup>, Camarillo, CA, USA) at 42 °C and homogenized by hand for 30 s. The samples were immediately incubated at 42 °C for 6 h. After the incubation, the method of BAX<sup>®</sup> System SalQuant<sup>®</sup> (Hygiena<sup>®</sup>, Camarillo, CA, USA) was conducted for the enumeration of *Salmonella* using the Bax System Real Time (RT) PCR Assay for *Salmonella* (Hygiena<sup>®</sup>, Camarillo, CA, USA). The BAX<sup>®</sup> System SalQuant<sup>™</sup> approach uses amplification signal data from the BAX<sup>®</sup> System RT PCR Assay for *Salmonella* and correlates them with statistical models to provide *Salmonella* concentration results for a sample [32]. After the enumeration, an aliquot was removed from the incubator, and the SalQuant test was created; the samples were placed back into the incubator to continue enrichment for 18 h at 42 °C. After the full incubation of 18 h, only the samples that were negative at the enumeration step with BAX<sup>®</sup> System SalQuant<sup>™</sup> were tested for prevalence analysis with the BAX<sup>®</sup> System RT PCR Assay for *Salmonella*. Samples positive on the detection test were considered positive but not quantifiable with the methodology.

#### 2.5. *Campylobacter* spp. Enumeration and Prevalence

For *Campylobacter* spp. enumeration, the protocol AOAC 040702 was followed. The rinses were homogenized by hand for 1 min prior to 30 mL of the rinse being added to 30 mL of pre-warmed (42 °C) 2X blood-free Bolton Selective Enrichment Broth (Thermo Fisher Scientific, Lenexa, KS, USA) plus 2X Bolton Broth Selective Supplement/L (Thermo Fisher Scientific, Lenexa, KS, USA) and homogenized by hand for 30 s. The samples were immediately incubated at 42 °C for 20 h in a BD GasPak EZ CampyPak Container Systems (Becton Dickinson and Company, Franklin Lakes, NJ, USA) anaerobic chamber using BD GasPak EZ Campy Sachets (Becton Dickinson and Company, Franklin Lakes, NJ, USA) to create microaerophilic conditions for *Campylobacter* spp. growth (6–16% O<sub>2</sub> and 2–10% CO<sub>2</sub>). After the incubation, the BAX<sup>®</sup> System CampyQuant<sup>™</sup> (Hygiena<sup>®</sup>, Camarillo, CA, USA) method was conducted for the enumeration of *Campylobacter* spp. After the enumeration, the aliquot was removed from the incubator, the CampyQuant test was created, and the

samples were placed back into the incubator to continue enrichment for 48 h at 42 °C. After 48 h of incubation, only the samples that were negative at the enumeration with BAX<sup>®</sup> System CampyQuant<sup>™</sup> were tested for prevalence analysis with the BAX<sup>®</sup> System RT PCR Assay for *Campylobacter*. Samples positive on the detection test were considered positive but not quantifiable with the methodology.

### 2.6. Statistical Analysis

The data were analyzed using R statistical analysis software (Version 4.2.1). For TVC and EB, the counts were transformed to Log CFU/mL of the chicken rinse. For *Salmonella* and *Campylobacter* spp., the counts were instead transformed to Log CFU/sample of chicken rinse (equivalent to Log CFU/400 mL or 400 CFU/carcass) due to the low concentration of pathogens in the samples for facilitating data visualization.

A one-way ANOVA analysis was performed to compare the counts at each location, followed by a pairwise comparison *t*-test adjusted for Tukey. When parametric assumptions were not met, the Kruskal–Wallis test as a non-parametric alternative for the ANOVA analysis was used, followed by a pairwise comparison of Wilcoxon’s test adjusted by the Benjamini and Hochberg method. To determine significant differences, a *p*-value lower than 0.05 was considered.

As a recommendation from the USDA-FSIS for statistical process control [33], for each microbial indicator and pathogen count, the standard error (SE) was calculated, which helps in the visualization of the dispersion of the sample means around the population mean. The mean plus three standard errors of the mean (Mean + 3SE) shows us the upper control limit. Meanwhile, a statistical process control (SPC) based on Shewhart’s control charts was employed for microbial indicators [34]. The  $\bar{X}$  chart was constructed using Equations (1) and (2), where the overall mean count  $\bar{X}$  for each sampling location was calculated. For the standard factor value ( $A_3$ ), the constant of 1.427 was selected to account for the five samples collected on two different days per facility, while  $\bar{s}$  represents the average standard deviation for each sampled location [34–36].

$$\text{Lower Control Limit (LCL)} = \bar{X} - A_3\bar{s} \tag{1}$$

$$\text{Upper Control Limit (UCL)} = \bar{X} + A_3\bar{s} \tag{2}$$

Tables for the distribution of indicator microorganisms and pathogens’ microorganisms levels were developed [37].

The SalQuant and CampyQuant methodology presents a limit of quantification (LOQ) of 1 CFU/mL according to the manufacturer. The methodology provides a regression equation in order to estimate the bacterial counts; this may be negative and below the LOQ; therefore, a new LOQ, 1% of the real LOQ (0.01 CFU/mL equivalent to 0.6 Log CFU/sample), was established. The samples that presented < 0.6 Log CFU/sample were reported as the new LOQ, equivalent to 0.3 Log CFU/sample. Samples that were not quantifiable during the enumeration but were positive after the prevalence analysis were reported as 0.3 Log CFU/sample, 50% of the estimated LOQ. Samples that were not quantifiable during the enumeration and undetected after the prevalence were reported as 0 Log CFU/sample (Table 1) [38].

**Table 1.** Parameters reported for *Salmonella* and *Campylobacter* spp. quantification and prevalence.

SalQuant/CampyQuant Result (Log CFU/Sample)	Prevalence Result	Reported SalQuant/CampyQuant Result (Log CFU/Sample)	Reported Prevalence Result
No Result	Negative	0	Negative
No Result	Positive	0.3	Positive
Less than 0.6	NA <sup>1</sup>	0.3	Positive
More than or equal to 0.6	NA <sup>1</sup>	SalQuant/CampyQuant result	Positive

<sup>1</sup> Not applicable; prevalence analysis was not conducted in samples quantified by SalQuant and CampyQuant.

### 3. Results

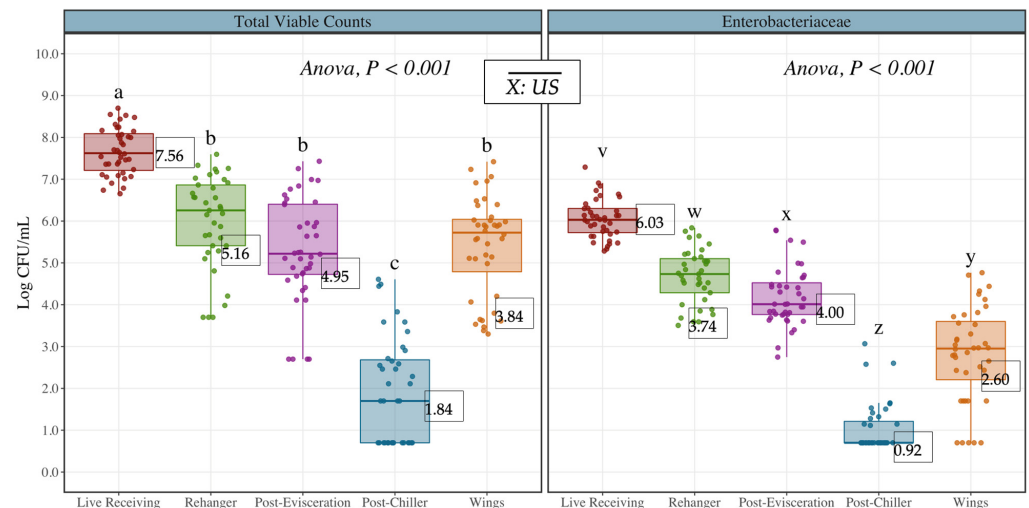
#### 3.1. Microbial Indicators

For TVC counts, the average of the incoming microbial load at the live receiving location was 7.66 Log CFU/mL (Table 2, Figure 1). The broiler carcasses sampled were from animals' recently dead on arrival, so these microbial counts were prior to any treatment. A significant reduction ( $p < 0.001$ ) was observed from the live receiving to the rehanger location with an average of 5.99 Log CFU/mL, showing the effect of scalding in hot water and feather removal (Table 2, Figure 1). The evisceration process includes the removal of the head, feet, and organs with water washes in between the operations for the removal of blood and surface contamination. No statistically significant difference ( $p > 0.05$ ) was found when comparing the rehanger to the post-evisceration (5.30 Log CFU/mL) location (Table 2). After the evisceration, the broiler carcasses passed by the pre-chiller and main chiller. In all plants evaluated, freshwater addition, recirculation, and chemical treatments were used to treat recirculated water. Significant reductions ( $p < 0.001$ ) in the microbial loads were observed to an average of 1.91 Log CFU/mL (Table 2, Figure 1). Finally, after the cutting operations, an increase to 5.42 Log CFU/mL in wings ( $p < 0.001$ ) was observed in the parts' composite rinse (Table 2). The distribution chart for TVC (Table 3) shows that the majority of the samples (20.4%) exhibited a TVC mean from 5.01 to 6 Log CFU/mL.

**Table 2.** Summary chart for total viable counts (Log CFU/mL), including statistical process control parameters on each location sampled (n = 5) at the four poultry processing facilities.

Location	Total Viable Counts (Log CFU/mL)				
	Mean ± SE <sup>1</sup>	Mean + 3SE	$\bar{X} \pm \sigma$	LCL <sup>2</sup>	UCL <sup>3</sup>
Live Receiving	7.66 ± 0.09 <sup>a</sup>	7.93	7.66 ± 0.47 <sup>a</sup>	7.19	8.13
Rehanger	5.99 ± 0.18 <sup>b</sup>	6.53	5.99 ± 0.90 <sup>b</sup>	5.09	6.89
Post-Evisceration	5.30 ± 0.20 <sup>b</sup>	5.9	5.30 ± 0.96 <sup>b</sup>	4.34	6.26
Post-Chiller	1.91 ± 0.20 <sup>c</sup>	2.51	1.91 ± 1.09 <sup>c</sup>	0.82	3.00
Parts (Wings)	5.42 ± 0.19 <sup>b</sup>	5.99	5.42 ± 0.44 <sup>b</sup>	4.98	5.86

<sup>1</sup> Standard error of the mean;  $\bar{X}$  = mean value,  $\sigma$  = average standard deviation of the mean, <sup>2</sup> LCL = lower control limit, <sup>3</sup> UCL = upper control limit. Letters (a-c) mean a statistically significant difference according to ANOVA analysis, followed by a pairwise comparison *t*-test adjusted Tukey at a *p*-value < 0.05.



**Figure 1.** The box plots represent the total viable counts and Enterobacteriaceae counts (Log CFU/mL) on the five locations sampled at the four processing facilities evaluated in this study. For boxplots, the horizontal line represents the median, the upper and lower lines of the box represent the upper and lower quartiles (0.25 and 0.75, respectively), and the vertical upper and bottom lines represent 1.5 times the interquartile range and lower interquartile range, respectively. Dots represent each individual datum collected per indicator. Letters in the boxes (a-c) from the total viable counts

and <sup>(v-z)</sup> from the Enterobacteriaceae counts mean that there is a statistically significant difference according to ANOVA analysis followed by a pairwise comparison *t*-test adjusted for Tukey at a *p*-value < 0.05. The numbers in the white boxes represent the mean of the same stage in a facility in the United States [38], used for comparative purposes.

**Table 3.** Distribution chart of quantified total viable counts samples.

Distribution Log CFU/mL	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<LOD	17	8.7%	17	8.7%
1–2	4	2.0%	21	10.7%
2.01–3	15	7.6%	36	18.3%
3.01–4	16	8.2%	52	26.5%
4.01–5	20	10.2%	72	36.7%
5.01–6	40	20.4%	112	57.1%
6.01–7	36	18.4%	148	75.5%
7.01–8	36	18.4%	184	93.9%
>8.01	12	6.1%	196	100.0%
<b>Total</b>	196	100.0%	-	-

LOD < 10 CFU/mL.

For EB counts, the average of the incoming microbial load at the live receiving location was 6.05 Log CFU/mL (Table 4, Figure 1). A significant reduction (*p* < 0.001) was observed from the live receiving to the rehanger location (4.68 Log CFU/mL). In the post-evisceration location, the average microbial load observed was 4.18 Log CFU/mL, and it was significantly reduced (*p* < 0.001) in the post-chiller (1.03 Log CFU/mL), followed by a significant increase (*p* > 0.05) in wings rinse composites (2.80 Log CFU/mL) (Table 4, Figure 1). The distribution chart for EB (Table 5) reports that the majority of the samples (19.9%) presented EB loads in a range of 4.01 to 5 Log CFU/mL.

**Table 4.** Summary chart for Enterobacteriaceae counts (Log CFU/mL), including statistical process control parameters on each location sampled (n = 5) at the four poultry processing facilities.

Location	Enterobacteriaceae Counts (Log CFU/mL)				
	Mean ± SE <sup>1</sup>	Mean + 3SE	$\bar{X} \pm \sigma$	LCL <sup>2</sup>	UCL <sup>3</sup>
Live Receiving	6.05 ± 0.07 <sup>v</sup>	6.26	6.05 ± 0.64 <sup>v</sup>	5.41	6.69
Rehanger	4.68 ± 0.11 <sup>w</sup>	5.01	4.68 ± 0.74 <sup>w</sup>	3.94	5.42
Post-Evisceration	4.18 ± 0.11 <sup>x</sup>	4.51	4.18 ± 0.59 <sup>x</sup>	3.59	4.77
Post-Chiller	1.03 ± 0.10 <sup>z</sup>	1.33	1.03 ± 0.44 <sup>z</sup>	0.59	1.47
Parts (Wings)	2.80 ± 0.18 <sup>y</sup>	3.34	2.80 ± 0.76 <sup>y</sup>	2.04	3.56

<sup>1</sup> Standard error of the mean;  $\bar{X}$  = mean value,  $\sigma$  = average standard deviation of the mean, <sup>2</sup> LCL = lower control limit, <sup>3</sup> UCL = upper control limit. Letters <sup>(v-z)</sup> mean a statistically significant difference according to ANOVA analysis, followed by a pairwise comparison *t*-test adjusted Tukey at a *p*-value < 0.05.

**Table 5.** Distribution chart of quantified Enterobacteriaceae samples.

Distribution Log CFU/mL	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
<LOD	31	15.8%	31	15.8%
1–2	15	7.7%	46	23.5%
2.01–3	17	8.7%	63	32.2%
3.01–4	37	18.9%	100	51.1%
4.01–5	39	19.9%	139	71.0%
5.01–6	35	17.8%	174	88.8%
6.01–7	21	10.7%	195	99.5%
>7.01	1	0.5%	196	100.0%
<b>Total</b>	196	100.0%	-	-

LOD < 10 CFU/mL.

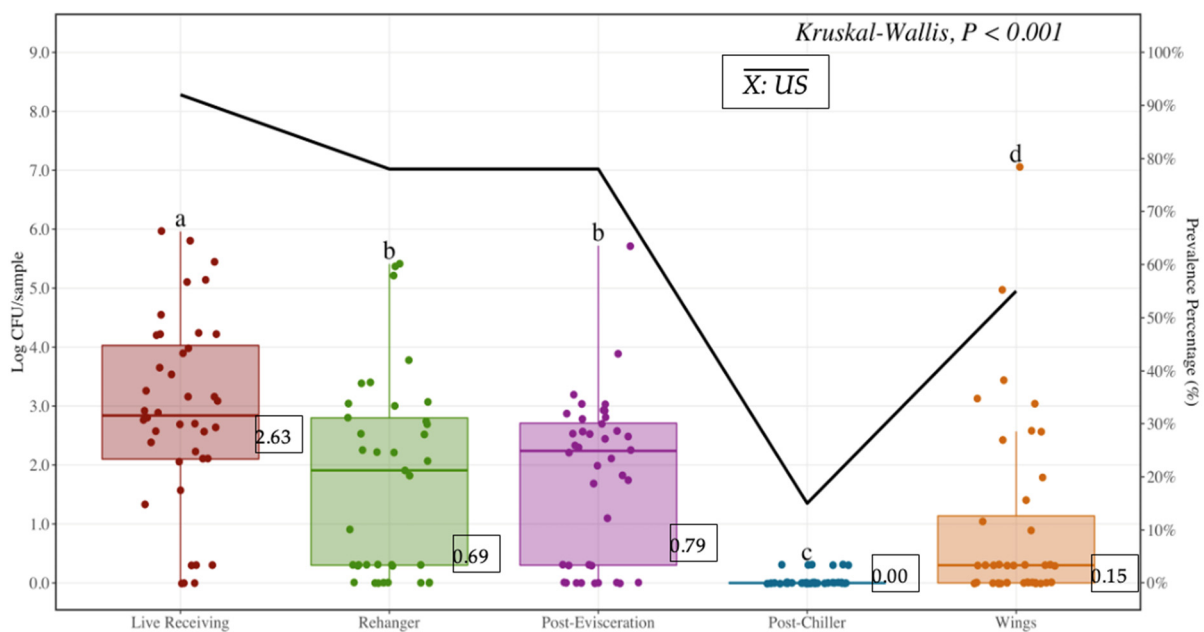
### 3.2. Salmonella Enumeration and Prevalence

The *Salmonella* counts from enumeration were transformed to Log CFU/sample, equivalent to Log CFU/400 mL, which facilitates statistical analysis and the visualization of the data. The average of the incoming microbial load at live receiving prior to any chemical intervention was 2.89 Log CFU/sample (Table 6, Figure 2). After the rehang stage, there was a statistically significant reduction ( $p < 0.01$ ) to 1.75 Log CFU/sample, and there was a similar microbial load at the post-evisceration stage (Table 6). After the post-chiller, there was another statistically significant reduction ( $p < 0.01$ ) to 0.05 Log CFU/sample, followed by a significant increase of an average of 0.93 Log CFU/sample at wings rinse composites (Table 6). Additionally, Table 7 presents the distribution chart of *Salmonella* in the samples.

**Table 6.** *Salmonella* counts (Log CFU/sample) and prevalence (%) on each of the five locations sampled of the poultry processing line.

Location	Salmonella Counts (Log CFU/Sample)		
	Mean $\pm$ SE <sup>1</sup>	Mean + 3SE	Prevalence (%)
Live Receiving	2.89 $\pm$ 0.25 <sup>a</sup>	3.64	92.50%
Rehanger	1.75 $\pm$ 0.27 <sup>b</sup>	2.56	78.38%
Post-Evisceration	1.79 $\pm$ 0.22 <sup>b</sup>	2.45	77.50%
Post-Chiller	0.05 $\pm$ 0.02 <sup>c</sup>	0.11	15.38%
Parts (Wings)	0.93 $\pm$ 0.25 <sup>d</sup>	1.68	55.00%

<sup>1</sup> Standard error of the mean. The different letters (a-d) from *Salmonella* counts mean a statistically significant difference according to Kruskal–Wallis analysis followed by a pairwise comparison of Wilcoxon’s test adjusted for Benjamini and Hochberg at a  $p$ -value  $< 0.05$ .



**Figure 2.** *Salmonella* counts (Log CFU/sample) and prevalence (%) on the five locations sampled of the process. For boxplots, the horizontal line represents the median, the upper and lower lines of the box represent the upper and lower quartiles (0.25 and 0.75, respectively), and the vertical upper and bottom lines represent 1.5 times the interquartile range and lower interquartile range, respectively. Dots represent each individual datum collected per pathogen. Letters in the boxes (a-d) mean that there is a statistically significant difference according to Kruskal–Wallis analysis followed by a pairwise comparison of Wilcoxon’s test adjusted for Benjamini and Hochberg at a  $p$ -value  $< 0.05$ . The numbers in the white boxes represent the mean of the same stage in a facility in the United States [38], used for comparative purposes.



Table 7. Distribution chart of *Salmonella* samples.

Distribution Log CFU/Sample	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
Negative	71	36.2%	71	36.2%
Positive (No Quant)	31	15.8%	102	52.0%
0.4–1	2	1.2%	104	53.2%
1.01–2	12	6.1%	116	59.3%
2.01–3	43	21.9%	159	81.2%
3.01–4	21	10.7%	180	91.9%
4.01–5	6	3.0%	186	94.9%
>5.01	10	5.1%	196	100.0%
<b>Total</b>	<b>196</b>	<b>100.0%</b>	-	-

LOD < 1 CFU/mL.

3.3. *Campylobacter* spp. Enumeration and Prevalence

*Campylobacter* spp. counts from enumeration were transformed to Log CFU/sample, equivalent to Log CFU/400mL. The average incoming load of *Campylobacter* spp. at the live receiving was 4.67 Log CFU/sample (Table 8, Figure 3). There was a statistical difference at the rehanger and post-evisceration locations in this study, with an average of 5.11 Log CFU/sample and 4.71 Log CFU/sample, respectively (Table 8). However, lower counts were observed at the post-chiller and wings rinse composite locations, with an average of 1.92 Log CFU/sample and 1.61 Log CFU/sample, respectively (Table 8).

Table 8. *Campylobacter* spp. counts (Log CFU/sample) and prevalence (%) on each of the five locations sampled of the poultry processing line.

Location	<i>Campylobacter</i> spp. Counts (Log CFU/Sample)		
	Mean ± SE <sup>1</sup>	Mean + 3SE	Prevalence (%)
Live Receiving	4.67 ± 0.18 <sup>a</sup>	5.21	97.50
Rehanger	5.11 ± 0.15 <sup>a</sup>	5.56	100
Post-Evisceration	4.71 ± 0.23 <sup>a</sup>	5.40	97.50
Post-Chiller	1.92 ± 0.30 <sup>b</sup>	2.82	66.67
Parts (Wings)	1.61 ± 0.34 <sup>b</sup>	2.63	62.50

<sup>1</sup> Standard error of the mean. The different letters (a–b) from *Campylobacter* spp. counts mean a statistically significant difference according to Kruskal–Wallis analysis followed by a pairwise comparison of Wilcoxon’s test adjusted for Benjamini and Hochberg at a *p*-value < 0.05.

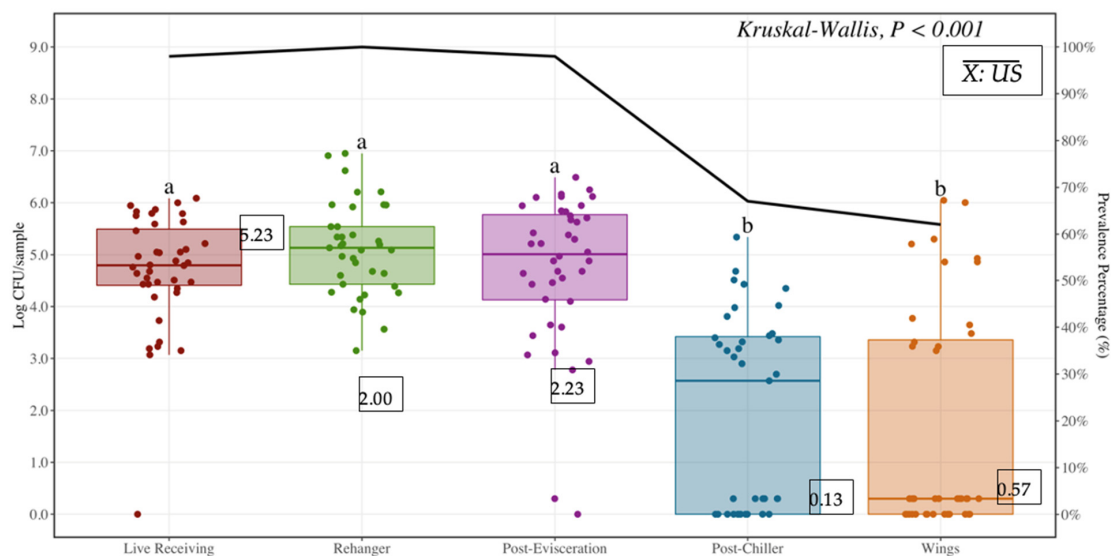


Figure 3. *Campylobacter* spp. counts (Log CFU/sample) and prevalence (%) on the five locations sampled of the process. For boxplots, the horizontal line represents the median, the upper and lower lines of the box represent the upper and lower quartiles (0.25 and 0.75, respectively), and the vertical

upper and bottom lines represent 1.5 times the interquartile range and lower interquartile range, respectively. Dots represent each individual datum collected per pathogen. Letters in the boxes <sup>(a,b)</sup> mean that there is a statistically significant difference according to Kruskal–Wallis analysis followed by a pairwise comparison of Wilcoxon’s test adjusted for Benjamini and Hochberg at a  $p$ -value < 0.05. The numbers in the white boxes represent the mean of the same stage in a facility in the United States (5), used for comparative purposes.

The prevalence values are shown in Table 8. At live receiving, the incoming *Campylobacter* spp. load was 97.5% positive. After the rehangar, the positive samples increased to 100%, and they decreased to 97.50% at the post-evisceration location. The lowest positive samples were observed at the post-chiller and wings locations, with 66.67% and 62.50%, respectively.

All the samples tested for *Campylobacter* spp. were, on average, higher at microbial loads and prevalences than the samples tested for *Salmonella* (Table 6). Additionally, the highest percentage of total positive samples was between 4 and 6 Log CFU/sample, as shown in Table 9.

**Table 9.** Distribution chart of *Campylobacter* spp. samples.

Distribution Log CFU/Sample	Number of Samples	Percent of Total	Cumulative Number	Cumulative Percent
Negative	30	15.3%	30	15.3%
Positive (No Quant)	18	9.2%	48	24.5%
0.4–1	0	0.0%	48	24.5%
1.01–2	0	0.0%	48	24.5%
2.01–3	5	2.6%	53	27.1%
3.01–4	33	16.8%	86	43.9%
4.01–5	48	24.5%	134	68.4%
5.01–6	47	24.0%	181	92.4%
>6.01	15	7.6%	196	100.0%
<b>Total</b>	<b>196</b>	<b>100.0%</b>	<b>-</b>	<b>-</b>

LOD < 1 CFU/mL.

#### 4. Discussion

The goal of this study was to develop a microbiological baseline of microbial indicators and pathogen quantification and detection for four of the largest commercial broiler processing facilities in Honduras. Poultry bio-mapping baselines help identify microbial contamination and process optimization opportunities such as sanitary dressing procedures, equipment adjustments, cross-contamination controls, and chemical intervention applications in commercial poultry processing operations. Bio-mapping baselines, which include the quantification and detection of pathogens, contribute to establishing statistical process control parameters that aid in making decisions for food safety management [38]. When referring to the control, it means that the interventions applied are effective in reducing the microbial levels in the final product, ensuring its safety, and achieving a practical shelf-life [36]. Moreover, poultry processing facilities may utilize bio-mapping to choose the most relevant sampling points that reflect the process’s microbiological effectiveness with the greatest accuracy [38].

The MicroSnap™ technology has proven its utility for estimating microbial indicator loads in chicken rinse samples, and the portability of the system was proven in international settings, particularly when laboratory specialists lack a physical laboratory with all the necessary equipment for analysis [29]. This technology was chosen for its affordability, portability, and ease of use in remote areas like the ones investigated in this research project. Additionally, it allows processing facilities to conduct their own analysis for the quantification of microbial indicators, providing results within the same day without the

need for a complex internal laboratory. The MicroSnap™ technology is a rapid method (6 to 8 h) used for microbial enumeration. It relies on the bioluminescence-based detection of adenosine triphosphate (ATP) in living cells and the ATP's capacity to react with the enzyme complex of the solution present in the swab [39]. Before adopting this methodology, validation methods were checked, and a series of trials were conducted to verify the usefulness and accuracy through internal validation [29,39,40]. The conclusion was that this methodology could be used as a substitute for other standard techniques, offering the benefits of low cost and mobility for the type of project described in this research, as indicated in the manufacturer's AOAC certification [30].

The Hygiena™ BAX® System is a molecular pathogen detection tool that utilizes polymerase chain reaction (PCR) technology to amplify and detect foodborne pathogens. The SalQuant™ and CampyQuant™ methodology features a limit of quantification of 1 CFU/mL for poultry processing rinsates [41]. The BAX® System offers a precise estimation of *Salmonella* and *Campylobacter* spp. pre-enriched Log levels in poultry rinse samples. This rapid estimation provides the poultry industry with a deeper understanding of what is happening during the broiler processing operations and their effects on pathogen reduction, enabling them to assess the effectiveness of interventions and validate new process controls [32].

Indicator organisms help provide information about the hygiene standards maintained during food processing and storage. Inadequate sanitation, re-contamination, and inadequate handling practices can result in elevated levels of these indicator organisms within the product. This study observed a similar trend for both microbial indicators tested. The total viable counts and Enterobacteriaceae counts had a tendency to decrease from the live receiving to the post-chiller location, with a statistically significant increase ( $p < 0.001$ ) at the parts (wings) location when parts composite rinses were used for testing. A previous bio-mapping study conducted in a poultry facility in the United States with reduced chemical interventions showed a similar trend for aerobic counts (AC) and Enterobacteria counts [38]. The average incoming microbial load for TVC was similar in Honduras and the United States, with loads of 7.66 Log CFU/mL and 7.56 Log CFU/mL, respectively. Following the rehangar stage, reductions to 5.16 Log CFU/mL and 4.64 Log CFU/mL were observed for Honduras and the United States, respectively. Subsequently, at the post-evisceration stage, the mean was 5.30 Log CFU/mL for Honduras and 4.95 Log CFU/mL for the U.S. At the post-chiller stage, both countries exhibited similar loads (1.91 Log CFU/mL in Honduras and 1.84 Log CFU/mL in the U.S.). The wing stage was the only stage where significant differences were observed between Honduras and the United States, with loads of 5.42 Log CFU/mL and 3.84 Log CFU/mL, respectively [38]. EB loads were also similar in both countries at the live receiving stage (6.05 Log CFU/mL in Honduras and 6.03 Log CFU/mL in the United States). At the rehangar, reductions to 4.68 Log CFU/mL (Honduras) and 3.74 Log CFU/mL (United States) were noted. At the post-evisceration, post-chiller, and wings stages, similar loads were observed between both countries (4.18 Log CFU/mL, 1.03 Log CFU/mL, and 2.80 Log CFU/mL for Honduras and 4 Log CFU/mL, 0.92 Log CFU/mL, and 2.60 Log CFU/mL in the United States, respectively) [38].

*Salmonella* and *Campylobacter* spp. are pathogens commonly found in poultry products. Both are human pathogens that can exist in high quantities within the gastrointestinal tract of birds, so it is crucial to identify their presence in poultry products, even when they are present at low levels [42]. Pathogen prevalence as the only measure of food safety performance may yield insufficient and incomplete results for proper food safety management. Pathogen quantification can enhance the effectiveness of a risk assessment by allowing interventions to point to particular stages with elevated loads of microbial indicators and pathogens [43]. When comparing *Salmonella* counts to a study that reported the enumeration in three poultry facilities in the United States [44], similar averages were reported in both countries at the live receiving stage, with loads of 2.89 Log CFU/sample in Honduras and means of 2.39 Log CFU/sample, 2.83 Log CFU/sample, and 2.78 Log CFU/sample in

the United States. At the rehanger stage, the Honduran study exhibited pathogen loads of 1.75 Log CFU/sample, compared to 1.85 Log CFU/sample, 0.68 Log CFU/sample, and 0.76 Log CFU/sample in the United States. Similar loads were observed at the post-evisceration stage (1.79 Log CFU/sample in Honduras and 1.59 Log CFU/sample in a facility in the United States); on the contrary, the other two facilities in the United States exhibited lower loads of 0.69 Log CFU/mL and 0.30 Log CFU/mL. Following the post-chiller stage, 0.05 Log CFU/sample was detected in Honduras, compared to loads of 0.25 Log CFU/sample, 0.05 Log CFU/sample, and 0.01 Log CFU/sample in the United States. Microbial loads in wings composite rinses were higher in Honduras (0.93 Log CFU/sample) when compared to the United States (0.29 Log CFU/sample, 0.03 Log CFU/sample, 0.03 Log CFU/sample) [44]. It should be noted that only one of the facilities in this study utilized chemical interventions in the parts processing operations, whereas several interventions with PAA were reported in the U.S. study reported here for comparative purposes.

In this study, the prevalence of *Salmonella* showed a continuous reduction from the live receiving (92.50%) to the post-chiller (15.38%) locations, followed by an increase to 55% at the wings location. In the same study where the enumeration of the three facilities in the United States was reported, *Salmonella* was detected at rates of 86.67%, 98.08%, and 95.65% at the live receiving stage, 88.33%, 61.54%, and 73.91% at the rehanger stage, and 75% at the post-evisceration stage (comparable to Honduras, with 78.38% and 77.50% in the rehanger and post-evisceration stages, respectively). At the post-chiller stage, the prevalence rates were 35%, 7.69%, and 4.38% in the U.S. study, whereas in the Honduran facilities, the average prevalence was 15.38% at post-chilling locations. Finally, at the wings stage, the pathogen prevalence was 58.33%, 9.62%, and 8.70% in the U.S. study [44]. The *Salmonella* prevalence was higher in the post-chiller and wings stages in Honduras compared to the first two facilities in the United States. One potential explanation could be attributed to the lower concentration of chemical treatments during the post-chiller stage featured in the Honduran facilities (20–100 ppm vs. 300–600 ppm in the U.S.). As described above, wings in Honduras do not undergo a final shower/dip with chemicals, such as peroxyacetic acid, while this treatment is generally applied in United States facilities [26,38]. Adopting a multi-hurdle approach to controlling *Salmonella* effectively is recommended in the chicken slaughter process, as diverse reduction stages may help overcome *Salmonella*'s resistance [45].

*Campylobacter* spp.'s prevalence did not exhibit the same tendency as that observed for *Salmonella* in this study. The highest prevalence was found at the rehang location, with 100% positive samples, and from that location, a decreasing prevalence tendency was observed until the parts (wings) stage. Compared to the United States, the most significant difference observed was at the post-chiller stage, with a prevalence of 9.43% compared to 66.67% in Honduras [38]. However, there was an increase to 50% in prevalence in wings samples from a facility in the United States, compared to 62.50% of positive samples in Honduras [38]. The effective implementation of Good Hygiene Practices during the slaughter process plays a crucial role in reducing *Campylobacter* spp. in poultry meat. Moreover, the effectiveness of the process can be increased by combining physical and chemical interventions. However, the efficacy of these interventions is contingent upon the initial concentration of *Campylobacter* spp. in the gastrointestinal tract of the flock since significant reductions may not be sufficient to decrease prevalence in highly loaded samples [46].

In the United States, as well as in Honduras, food safety standards compliance is achieved by the implementation of food safety management systems based on pre-requisite programs and HACCP, as well as the use of physical and chemical interventions during the process. A typical commercial practice for reducing fecal contamination is feed withdrawal prior to slaughter; chemical interventions involving antimicrobial agents such as peracetic acid, cetylpyridinium chloride, acidified sodium chloride, chlorine, and trisodium phosphate are applied to water during the several carcass washes (as immersion and spray)

placed in different locations of the process including before, during, and after chilling in order to reduce the microbial contamination on meat products [27,47]. In the facilities evaluated in this project, the use of chlorine and peracetic acid interventions in the carcass rinse and chilling locations was observed, but at the time of the study, no interventions were evident in the parts processing stages of the process.

This study utilizes novel technologies to not only detect but also enumerate pathogen loads in poultry samples collected during processing. The affordability, portability, and easiness of application were the main criteria for selecting these technologies. To assure the accuracy of the results, the authors not only based their method decisions on validation studies provided by the test kit manufacturers but also conducted internal validations against other validated technologies that may not be as practical in the rural settings described in this study. The comparability of the methodologies was evident, and therefore, despite the potential variability that these rapid methods may present, they provide an important tool for comparing microbial loads in between stages of a processing facility, between facilities when used with the same protocols, and as an overall preliminary baseline for total viable counts, Enterobacteriaceae, and the quantification and detection of pathogens such as *Salmonella* and *Campylobacter* spp. in the four commercial broiler processing facilities that collaborated in this study in Honduras. Additional work is needed to cover other seasons, flocks, and conditions to increase the robustness of the data as a national baseline for decision-making. However, these results provide processors with internal data for process performance and a national reference benchmark for performance comparison that will assist them in the continuous improvement of their operations in support of public health protection in Honduras.

## 5. Conclusions

Applying bio-mapping studies in poultry processing operations can pinpoint locations with higher pathogen contamination and indicator loads, leading to improved process control and facility enhancement. Furthermore, this research incorporates insights from facilities in the United States, serving as a comparative standard for management decisions. This research project has established a microbial baseline for commercial broiler processing facilities in Honduras, evaluating the four largest broiler processing facilities that account for more than 88% of the country's chicken production, thus representing the majority of chicken available for consumption in the country. The findings from this investigation will help regulatory officers consider developing risk-based performance standards based on the average pathogen and indicator loads reported in this study for Honduras. In addition, each processing facility can compare their microbial performance with the data reported in this study for benchmarking their pathogen and indicator control process to consider further food safety management alternatives for continuous improvement aimed at reducing risks of exposure to consumers.

**Supplementary Materials:** The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/poultry3040025/s1>.

**Author Contributions:** Conceptualization, G.K.B.-B. and M.X.S.-P.; methodology, G.K.B.-B., M.X.S.-P., S.E.B., D.A.V., D.R.C.-V., A.S., R.B.L., V.L., N.J.E.-R. and M.B.-A.; software, G.K.B.-B. and D.A.V.; formal analysis, G.K.B.-B.; investigation, G.K.B.-B., M.X.S.-P., S.E.B., D.A.V., D.R.C.-V., A.S., R.B.L., V.L. and N.J.E.-R.; resources, M.X.S.-P.; data curation, G.K.B.-B.; writing—original draft preparation, G.K.B.-B. and S.E.B.; writing—review and editing, G.K.B.-B., M.B.-A. and M.X.S.-P.; visualization, G.K.B.-B.; supervision, M.X.S.-P.; project administration, G.K.B.-B., S.E.B., D.A.V., D.R.C.-V., A.S., R.B.L., V.L., N.J.E.-R., M.B.-A. and M.X.S.-P.; funding acquisition, M.X.S.-P. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was funded by the International Center for Food Industry Excellence (ICFIE) at Texas Tech University and was supported by contributions from Hygiena.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Dataset available on request from the authors. The data are not publicly available due to privacy from the poultry processing partner that allowed the project to be conducted within their poultry processing environment.

**Acknowledgments:** The authors would like to thank all the people from Honduras who helped during their visit to the country.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## References

1. Honduras Poultry Industry Outlook 2022–2026. Available online: <https://www.reportlinker.com/clp/country/1761/726310> (accessed on 11 August 2023).
2. OECD-FAO Agricultural Outlook 2023–2032. Available online: <https://www.oecd.org/publications/oecd-fao-agricultural-outlook-19991142.htm> (accessed on 11 August 2023).
3. Regiones de America: Consumo Percapita de Carne de Pollo 2017–2004 | ILP-ALA. Available online: <https://ilp-ala.org/regiones-de-america-consumo-percapita-de-carne-de-pollo-2017-2004/> (accessed on 27 August 2023).
4. Moreno Servellón, D.A.; Aceituno Alemán, K.J. Pre-Factibilidad de un Food Truck en San Pedro Sula. Master’s Thesis, Facultad de Postgrado Unitec, San Pedro Sula, Honduras, 2020.
5. Rosales Chiessa, I.F. Evaluación Económica de La Producción de Pollos de Engorde Ross® (308) y Cobb CS® (744). Bachelor’s Thesis, Escuela Agrícola Panamericana, Zamorano Honduras, Zamorano, Honduras, 2018.
6. Dos Empresas de Honduras Están Aptas Para Exportar Pollo a EUA-El Sitio Avicola. Available online: <https://www.elsitioavicola.com/poultrynews/32934/dos-empresas-de-honduras-estan-aptas-para-exportar-pollo-a-eua/> (accessed on 27 August 2023).
7. McWhorter, A.R.; Weerasooriya, G.; Kumar, S.; Chousalkar, K.K. Comparison of Peroxyacetic Acid and Acidified Sodium Chlorite at Reducing Natural Microbial Contamination on Chicken Meat Pieces. *Poult. Sci.* **2023**, *102*, 103009. [CrossRef] [PubMed]
8. USDA-FSIS Compliance Guideline for Controlling Salmonella and Campylobacter in Raw Poultry. Available online: <https://www.fsis.usda.gov/guidelines/2021-0005> (accessed on 2 May 2024).
9. USDA-FSIS Compliance Guideline for Training Establishment Carcass Sorters in the New Poultry Inspection System (NPIS). Available online: <http://www.fsis.usda.gov/wps/portal/fsis/topics/regulatory-compliance/compliance-> (accessed on 2 June 2024).
10. USDA-FSIS Meat and Poultry Hazards and Controls Guide. Available online: [https://www.fsis.usda.gov/sites/default/files/import/Meat\\_and\\_Poultry\\_Hazards\\_Controls\\_Guide\\_10042005.pdf](https://www.fsis.usda.gov/sites/default/files/import/Meat_and_Poultry_Hazards_Controls_Guide_10042005.pdf) (accessed on 2 June 2024).
11. Northcutt, J.K. Reference Guide for Solving Poultry Processing Problems. Available online: [https://secure.caes.uga.edu/extension/publications/files/pdf/B%201156\\_1.PDF](https://secure.caes.uga.edu/extension/publications/files/pdf/B%201156_1.PDF) (accessed on 2 June 2024).
12. USDA-FSIS Food Safety and Inspection Service Annual Sampling Summary Report Fiscal Year 2022. Available online: [https://www.fsis.usda.gov/sites/default/files/media\\_file/documents/FY2022-Sampling-Summary-Report.pdf](https://www.fsis.usda.gov/sites/default/files/media_file/documents/FY2022-Sampling-Summary-Report.pdf) (accessed on 2 June 2024).
13. WHO. FAO Risk Assessments of Salmonella in Eggs and Broiler Chickens. Available online: <https://openknowledge.fao.org/server/api/core/bitstreams/84ee367b-fcf3-4b38-8c5f-08123f4dcbe5/content> (accessed on 25 April 2024).
14. SAG Secretaría de Agricultura y Ganadería. Acuerdo No. 552-05. Available online: <https://senasa.gob.hn/wp-content/uploads/2023/03/Acuerdo-No.-552-05.-Reglamento-de-la-inspeccion-sacrificio-e-industrializacion-de-productos-y-subproductos-avicolas.pdf> (accessed on 25 April 2024).
15. Su, C.; de Perio, M.A.; Fagan, K.; Smith, M.L.; Salehi, E.; Levine, S.; Gruszynski, K.; Luckhaupt, S.E. Occupational Distribution of Campylobacteriosis and Salmonellosis Cases—Maryland, Ohio, and Virginia, 2014. Available online: <https://www.cdc.gov/mmwr/volumes/66/wr/mm6632a4.htm> (accessed on 2 May 2024).
16. Koochmaraie, M.; Arthur, T.M.; Bosilevac, J.M.; Guerini, M.; Shackelford, S.D.; Wheeler, T.L. Post-Harvest Interventions to Reduce/Eliminate Pathogens in Beef. *Meat Sci.* **2005**, *71*, 79–91. [CrossRef] [PubMed]
17. Laufer, A.S.; Grass, J.; Holt, K.; Whichard, J.M.; Griffin, P.M.; Gould, L.H. Outbreaks of Salmonella Infections Attributed to Beef—United States, 1973–2011. *Epidemiol. Infect.* **2015**, *143*, 2003. [CrossRef] [PubMed]
18. USDA. USDA Finalizes Policy to Protect Consumers from Salmonella in Raw Breaded Stuffed Chicken Products. Available online: <https://www.usda.gov/media/press-releases/2024/04/26/usda-finalizes-policy-protect-consumers-salmonella-raw-breaded#> (accessed on 2 May 2024).
19. Ford, L.; Buuck, S.; Eisenstein, T.; Cote, A.; McCormic, Z.D.; Kremer-Caldwell, S.; Kissler, B.; Forstner, M.; Sorenson, A.; Wise, M.E.; et al. Salmonella Outbreaks Associated with Not Ready-to-Eat Breaded, Stuffed Chicken Products—United States, 1998–2022. *Morb. Mortal. Wkly. Rep.* **2023**, *72*, 484. [CrossRef] [PubMed]
20. USDA Cooking Meat? Check the New Recommended Temperatures. Available online: <https://www.usda.gov/media/blog/2011/05/25/cooking-meat-check-new-recommended-temperatures> (accessed on 29 September 2023).

21. USDA Federal Register Rule Making. Available online: <https://www.fsis.usda.gov/policy/federal-register-rulemaking/federal-register-notice> (accessed on 29 September 2023).
22. Myintzaw, P.; Jaiswal, A.K.; Jaiswal, S. A Review on Campylobacteriosis Associated with Poultry Meat Consumption. *Food Rev. Int.* **2021**, *39*, 2107–2121. [[CrossRef](#)]
23. Taha-Abdelaziz, K.; Singh, M.; Sharif, S.; Sharma, S.; Kulkarni, R.R.; Alizadeh, M.; Yitbarek, A.; Helmy, Y.A. Intervention Strategies to Control Campylobacter at Different Stages of the Food Chain. *Microorganisms* **2023**, *11*, 113. [[CrossRef](#)]
24. Buzby, J.C.; Allos, B.M.; Roberts, T. The Economic Burden of Campylobacter-Associated Guillain-Barré Syndrome. *J. Infect. Dis.* **1997**, *176*, 192–197. [[CrossRef](#)] [[PubMed](#)]
25. CDC. Campylobacteriosis | CDC Yellow Book 2024. Available online: <https://wwwnc.cdc.gov/travel/yellowbook/2024/infections-diseases/campylobacteriosis> (accessed on 27 August 2023).
26. Chavez-Velado, D. Bio-Mapping Salmonella and Campylobacter Loads in Three Commercial Broiler Processing Facilities in the United States to Identify Intervention Points and Develop Statistical Process Control Parameters for Food Safety Management. Master's Thesis, Texas Tech University, Lubbock, TX, USA, 2022.
27. Ricke, S.C.; Kunderling, M.M.; Miller, D.R.; Keeton, J.T. Alternatives to Antibiotics: Chemical and Physical Antimicrobial Interventions and Foodborne Pathogen Response. *Poult. Sci.* **2005**, *84*, 667–675. [[CrossRef](#)] [[PubMed](#)]
28. Żbikowska, K.; Michalczuk, M.; Dolka, B. The Use of Bacteriophages in the Poultry Industry. *Natl. Libr. Med.* **2020**, *10*, 872. [[CrossRef](#)] [[PubMed](#)]
29. Vargas, D.A.; Betancourt-Barszcz, G.K.; Chávez-Velado, D.R.; Sánchez, A.; Bueno López, R.; Sanchez-Plata, M.X. Bio-Mapping of Microbial Indicators and Pathogen Quantitative Loads in Commercial Broiler Processing Facilities in South America. *Foods* **2023**, *12*, 3600. [[CrossRef](#)] [[PubMed](#)]
30. Meighan, P.; Katz, B.; Nikooei, D. Hygiene MicroSnap Total, AOAC Performance Tested Methods. Available online: <https://www.hygiene.com/documents/62681/microsnap-total-aoac.pdf> (accessed on 23 April 2024).
31. FSIS. Foodborne Pathogen Test Kits Validated by Independent Organizations. Available online: [https://www.fsis.usda.gov/sites/default/files/media\\_file/2020-09/Validated-Test-Kit.pdf](https://www.fsis.usda.gov/sites/default/files/media_file/2020-09/Validated-Test-Kit.pdf) (accessed on 20 July 2024).
32. Applegate, S.F.; Englishbey, A.K.; Stephens, T.P.; Sanchez-Plata, M.X. Development and Verification of a Poultry Management Tool to Quantify Salmonella from Live to Final Product Utilizing RT-PCR. *Foods* **2023**, *12*, 419. [[CrossRef](#)] [[PubMed](#)]
33. FSIS. The Nationwide Microbiological Baseline Data Collection Program: Young Chicken Survey, July 2007–June 2008. Available online: [https://www.fsis.usda.gov/sites/default/files/media\\_file/2020-07/Baseline\\_Data\\_Young\\_Chicken\\_2007-2008.pdf](https://www.fsis.usda.gov/sites/default/files/media_file/2020-07/Baseline_Data_Young_Chicken_2007-2008.pdf) (accessed on 23 April 2024).
34. Jarvis, B. *Statistical Aspects of the Microbiological Examination of Foods*, 3rd ed.; Elsevier: London, UK, 2016.
35. Bueno López, R.; Vargas, D.A.; Jimenez, R.L.; Casas, D.E.; Miller, M.F.; Brashears, M.M.; Sanchez-Plata, M.X. Quantitative Bio-Mapping of Salmonella and Indicator Organisms at Different Stages in a Commercial Pork Processing Facility. *Foods* **2022**, *11*, 2580. [[CrossRef](#)] [[PubMed](#)]
36. Vargas, D.A.; Rodríguez, K.M.; Betancourt-Barszcz, G.K.; Ajcet-Reyes, M.I.; Dogan, O.B.; Randazzo, E.; Sánchez-Plata, M.X.; Brashears, M.M.; Miller, M.F. Bio-Mapping of Microbial Indicators to Establish Statistical Process Control Parameters in a Commercial Beef Processing Facility. *Foods* **2022**, *11*, 1133. [[CrossRef](#)] [[PubMed](#)]
37. FSIS. The Nationwide Microbiological Baseline Data Collection Program: Raw Chicken Parts Survey. Available online: [https://www.fsis.usda.gov/sites/default/files/media\\_file/2020-07/Baseline\\_Data\\_Raw\\_Chicken\\_Parts.pdf](https://www.fsis.usda.gov/sites/default/files/media_file/2020-07/Baseline_Data_Raw_Chicken_Parts.pdf) (accessed on 25 April 2024).
38. De Villena, J.F.; Vargas, D.A.; López, R.B.; Chávez-Velado, D.R.; Casas, D.E.; Jiménez, R.L.; Sanchez-Plata, M.X. Bio-Mapping Indicators and Pathogen Loads in a Commercial Broiler Processing Facility Operating with High and Low Antimicrobial Intervention Levels. *Foods* **2022**, *11*, 775. [[CrossRef](#)]
39. Meighan, P.; Smith, M.; Datta, S.; Katz, B.; Nason, F. The Validation of the MicroSnap Total for Enumeration of Total Viable Count in a Variety of Foods. *J. AOAC Int.* **2016**, *99*, 686–694. [[CrossRef](#)]
40. Meighan, P. Validation of the MicroSnap Coliform and *E. coli* Test System for Enumeration and Detection of Coliforms and *E. coli* in a Variety of Foods. *J. AOAC Int.* **2014**, *97*, 453–478. [[CrossRef](#)] [[PubMed](#)]
41. Wallace, M.F.; Varkey, S.; DeMarco, D.; Tice, G.; Andaloro, B.; Fallon, D.; Rohrbeck, J.; Davis, E.; Tadler, M.; Hoelzer, S.; et al. BAX® System Real-Time PCR Assay for Salmonella. Available online: <http://www.aoac.org> (accessed on 2 June 2024).
42. Rouger, A.; Zagorec, M. Bacterial Contaminants of Poultry Meat: Sources, Species, and Dynamics. *Microorganisms* **2017**, *5*, 50. [[CrossRef](#)] [[PubMed](#)]
43. Vargas, D.A.; De Villena, J.F.; Larios, V.; Bueno López, R.; Chávez-Velado, D.R.; Casas, D.E.; Jiménez, R.L.; Blandon, S.E.; Sanchez-Plata, M.X. Data-Mining Poultry Processing Bio-Mapping Counts of Pathogens and Indicator Organisms for Food Safety Management Decision Making. *Foods* **2023**, *12*, 898. [[CrossRef](#)] [[PubMed](#)]
44. Chavez-Velado, D.R.; Vargas, D.A.; Sanchez-Plata, M.X. Bio-Mapping Salmonella and Campylobacter Loads in Three Commercial Broiler Processing Facilities in the United States to Identify Strategic Intervention Points. *Foods* **2024**, *13*, 180. [[CrossRef](#)] [[PubMed](#)]
45. FAO & WHO. *Measures for the Control of Non-Typhoidal Salmonella spp. in Poultry Meat-Meeting Report*; Microbiological Risk Assessment Series, No. 45; FAO & WHO: Rome, Italy, 2023. [[CrossRef](#)]

- 
46. FAO & WHO. *Measures for the Control of Campylobacter spp. in Chicken Meat-Meeting Report*; Microbiological Risk Assessment Series, No. 46; FAO & WHO: Rome, Italy, 2024. [[CrossRef](#)]
  47. Thames, H.T.; Sukumaran, A.T. A Review of Salmonella and Campylobacter in Broiler Meat: Emerging Challenges and Food Safety Measures. *Foods* **2020**, *9*, 776. [[CrossRef](#)] [[PubMed](#)]

**Disclaimer/Publisher's Note:** The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.