Microbiological Evaluation of Broiler Carcasses, Wash and Rinse Water from Pluck Shops (Cottage Poultry Processors) in the County Nariva/Mayaro, Trinidad, Trinidad and Tobago, West Indies

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Summary

A study on the prevalence and levels of Campylobacter*,* Salmonella *and* E. coli *on broiler chicken carcasses, wash and rinse water from pluck shops/ cottage poultry processors (CPP) in county Nariva Mayaro Trinidad was done. There are 21 pluck shops/ cottage poultry processors in the county, 14 pluck shops were randomly selected for the study. Samples consisted of 28 broiler carcasses, 14 wash water samples and 14 rinse water samples. Over all the isolation rate of* Campylobacter*, Salmonella and* E. coli *from broiler carcasses wash and rinse water showed significant differences (P< 0.05) between pluck shops. Of the 56 samples examined from the 14 pluck shops sampled, 34 (60.7%) were positive for* Campylobacter*, 34 (60.7%) for* Salmonella *and 40 (71.4%) for* E. coli*. The correlation between the levels of* Campylobacter *found on carcasses and in wash water (r^2= 0.657) and rinse water (r^2= 0.600) was significant (P< 0.05) among pluck shops/CPP. There was also a high correlation (P< 0.05) between wash and rinse water samples (r^2= 0.950) for* Campylobacter*.* Salmonella *levels on carcasses and in wash water were positively (P< 0.05) correlated (r^2= 0.947). Of the 14 pluck shops examined 6 (42.9%) had* Campylobacter *levels that corresponded to infectious dose in humans. The infectious doses for* Salmonella *were isolated from 3 (21.4%) pluck shops and 13 (92.9%) pluck shops evaluated had* E. coli *present at potentially infectious levels. Three pluck shops/CPP (21.4%) had infectious dose for* Campylobacter*,* Salmonella *and* E. coli *where as all others had infectious levels for one or two pathogens. It was concluded that these pathogens are present in pluck shops/CPP in the county, having levels considered to be potentially infectious to humans and as such there should be health concern.*

Résumé

Evaluation microbiologique des carcasses de poulet, de l'eau de lavage et de l'eau de rinçage, des « boutiques pour plumer des volailles » dans le comté de Nariva /Mayaro, Trinidad, Trinidad et Tobago, Les Caraïbes

Une étude a été faite sur la fréquence et les niveaux de Campylobacter*,* Salmonella *et d'*E. coli *dans les carcasses de poulet, dans l'eau de lavage et l'eau de rinçage dans quelques abattoirs/tueries (BPV)/ Transformateurs artisanaux de poulet (TAP) dans le comté de Nariva/Mayaro, Trinidad. Sur les 21 BPV/ TAP situées dans ce comté; 14 ont été sélectionnées pour l'étude. Vingt-huit échantillons de carcasses de poulets, 14 échantillons d'eau de lavage et 14 échantillons d'eau de rinçage ont été prélevés. Globalement, le taux d'isolation de* Campylobacter*, de* Salmonella *et d'*E. coli *des carcasses de poulet, de l'eau de lavage et de l'eau de rinçage ont montré des différences significatives entres les BPV/TAP. Dans les 56 échantillons prélevés sur les 14 TAP testés, 34 (60,7%) ont donné un résultat positif pour* Campylobacter*, 34 (60,7%) un résultat positif pour* Salmonella *et 40 (71,4%) un résultat positif pour* E. coli*. La corrélation entre les niveaux de* Campylobacter *trouvés sur les carcasses, l'eau de lavage (r^2= 0,657) et l'eau de rinçage (r^2= 0,600) était significative entre les BPV/TAP. Il y avait aussi une forte corrélation (P< 0,05) entre les échantillons d'eau de lavage et d'eau de rinçage (r^2= 0,950) pour le* Campylobacter*. Les niveaux de* Salmonella *sur les carcasses et dans l'eau de lavage ont une corrélation positive (r^2= 0,947). Parmi les 14 BPV contrôlées, 6 (42,5%) ont eu des niveaux de* Campylobacter *qui correspondent à une dose infectieuse chez les humains. Des doses infectieuses pour la* Salmonella *ont été isolées de 3 (21,4%) BPV et 13 (92,9%) des BPV évaluées ont montré des taux d'*E. coli *à un niveau potentiellement infectieux. Trois BPV/TAP (21,4%) ont enregistré une dose infectieuse pour les trois agents pathogènes à la fois tandis que*

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tous les autres ont enregistré des niveaux infectieux pour un ou deux des pathogènes. On en conclut que ces pathogènes sont présents dans les BPV/TAP du comté à des doses potentiellement dangereuses pour l'être humain, et donc doivent être considérés comme un problème de santé publique.

1. Introduction

Broiler meat is the most important source of animal protein accounting for 88% of the meat consumed in Trinidad and Tobago (13). Broiler meat consumption per annum in Trinidad and Tobago is among the highest in the world. Per capita consumption averaged 35.5 kg compared to USA 38.3 kg, Canada 25.8 kg and Brazil 24 kg (31). Several foodborne outbreaks of *Campylobacter* (31), *Salmonella* (17, 20) and *E. coli* (16, 27) were attributed to poultry. *Campylobacter*, *Salmonella* and *E. coli* have been isolated from commercial poultry processing plants and broiler production units in Trinidad (2, 3). *Campylobacter jejuni* was recognized as a leading cause of acute bacterial gastroenteritis in humans (33).

In several countries, poultry products destined for human consumption were highly contaminated with *Campylobacter* (4). All serotypes of *Salmonella* are pathogenic for man, although some serotypes are more prevalent in disease conditions than others (18). Contamination of broiler carcasses with *Salmonella* has been a source of concern in the microbiological quality of commercial processing plants (22). *Escherichia coli* are used as an index of faecal contamination of foods and water (3, 7). Adesiyun *et al.* (3) identified evisceration as a significant contributor to the faecal contamination of carcasses during processing of broilers in commercial processing plants in Trinidad. In Trinidad, these large-scale commercial broiler processing plants account for 45% of the broilers processed (chilled and frozen as whole birds or parts), and sold to fast foods outlets, supermarkets and hotels. A major feature of the industry are the pluck shops which account for 55% of the broilers processed and sold to consumers as whole un-chilled birds.

In recognition of the significance of pluck shops (referred to as cottage poultry processors (CPP) in the industry the Caribbean Poultry Association is developing a CPP food safety protocol. This protocol and the regulations therein are consistent with the general principles of food hygiene as contain in the Codex Alimentarius. However, up to date there has

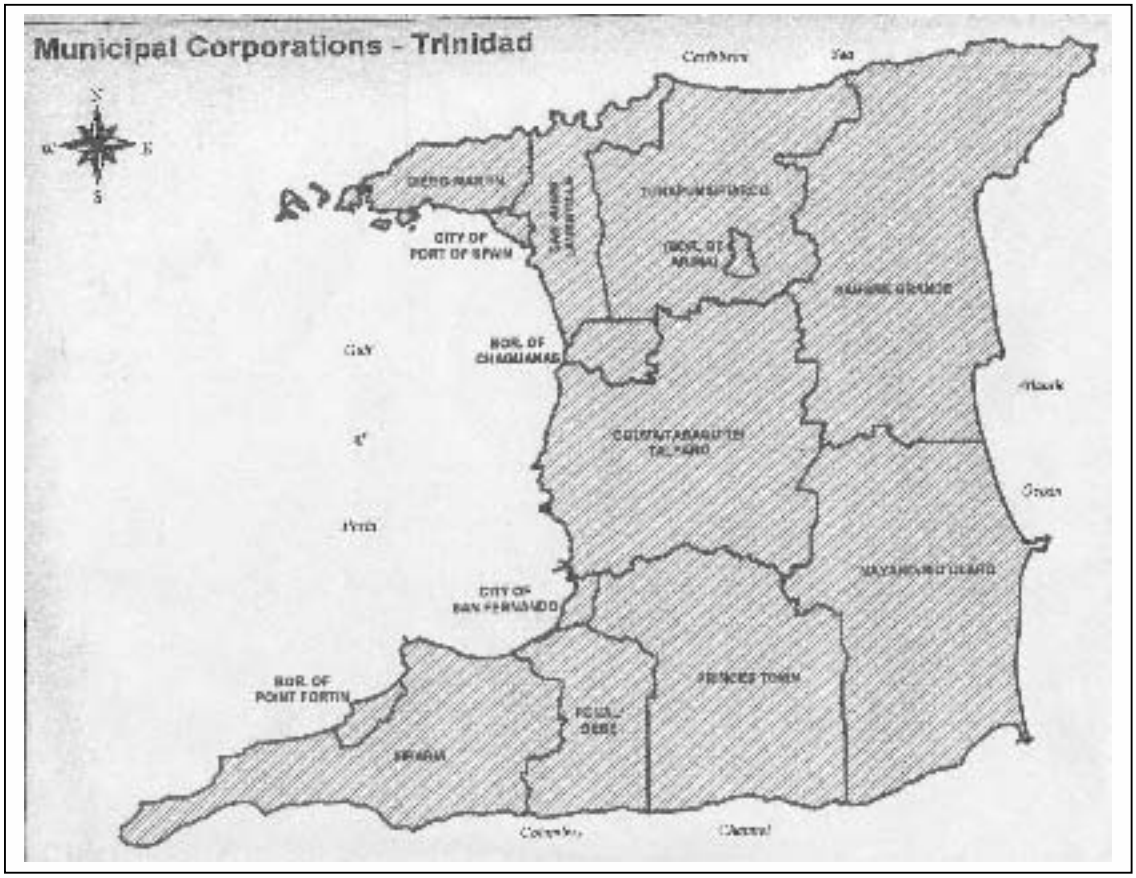

Figure 1:
Map of Trinidad with the location of county Nariva/Mayaro.

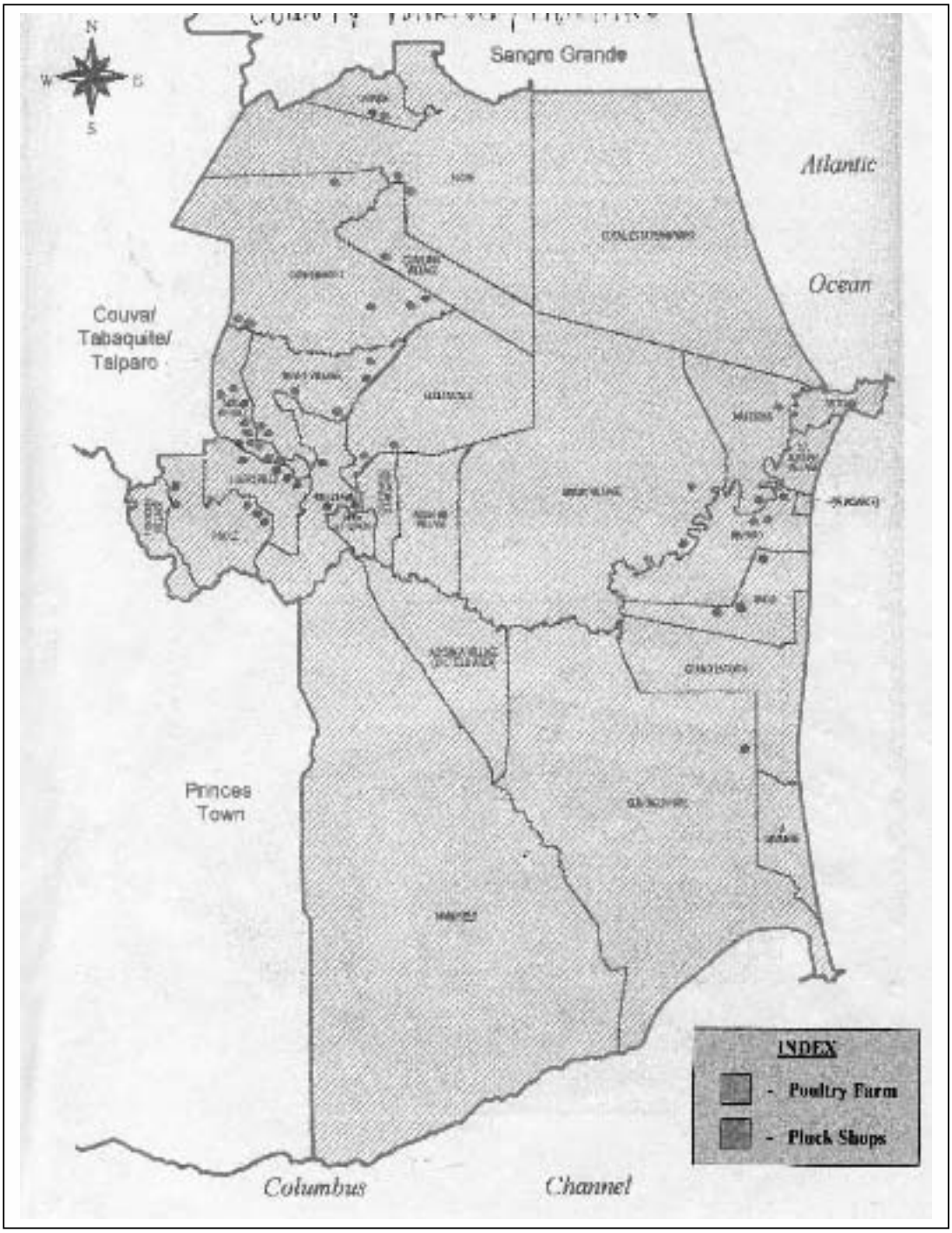

Figure 2: The locations of Pluck Shops/ Cottage Poultry Processors in County Nariva/ Mayaro.

been no published work on the prevalence of microbial organism of public health importance in these pluck shops in Trinidad. Thus, the objective of this study was to determine the prevalence of *Campylobacter*, *Salmonella* and *E. coli* on broiler carcasses, wash and rinse water from pluck shops/CPP in county Nariva/ Mayaro, Trinidad and Tobago.

2. Material and methods

Location and climate

Trinidad is located 10.30 °N latitude and 61.50 °W longitude and has an area of 4828 km^2. Tobago is located 11.15 °N latitude and 60.40 °W longitude and has an area of 300 km^2. There is a wet season from June to December and a dry season from January to May. The maximum temperature ranged from 33.1 to 34.4 °C with a minimum ranging from 20 to 22 °C. Relative humidity ranged from 50.6 to 99.5%. Figure 1 shows the map of Trinidad with the location of the county Nariva/Mayaro

Selection of Pluck Shops/Cottage Poultry Processors

A list of pluck shops/CPP in the county was obtained from the Public Health Inspectorate office Nariva/

Mayaro. The shops were visited and thereby verifying their existence. A total of 21 pluck shops were operating in the county (see Figure 2). 14 pluck shops were selected at random using Minitab (23). The main features of these pluck shops were: a holding area for birds to be slaughtered; a mechanical plucking machine; individual galvanised cone for holding birds during the severing of the jugular; hot water in a 45 gallon galvanised oil drum for scalding birds prior to de-feathering; containers for washing and rinsing carcasses. Washing refers to the first washing of the hot eviscerated carcass where the operator attempts to remove any residual blood, feed material and feather from the inside and outside of the carcass. This was done under running water with a collection pan in a sink. The operator subsequently removes the feet and neck and in a container with potable water the dressed carcass was dipped (rinsing) and then bagged. The number of carcasses rinsed in the same water before it was changed varied among processors. Water used in these facilities came from the municipal supply. Facilities for chilling carcasses were absent in all cases. All birds were slaughtered on demand; hot carcasses were bagged in polyethylene bags and sold to consumers.

Collection of samples

All samples were collected from the 14 randomly selected pluck shops over the period January to March, 2004. Two fresh carcass samples were obtained from each pluck shop. The average weight of each bird ranged from 2.0 to 2.5 kg. Sterile screw cap (20 ml) bottles were used for the collection of wash and rinse water. One sample of wash water and rinse water was taken from the respective container after

Isolation and identification of microorganisms

Each broiler carcass was placed in a sterile polyethylene bag with buffered peptone water (BPW), the volume of BPW was dependent on the weight of the carcasses. The method used was 1 ml BPW per 6 g carcass weight (10). Whole carcass samples were washed for 2 minutes by massaging and shaking according to the surface rinse technique (29, 30). The rinse was then filtered through sterile cheesecloth into sterile bottles. The surface rinse was serially diluted and plated on the various media. The rinse and wash water from the pluck shop was filtered through sterile cheesecloth into sterile bottles to remove any fatty tissue and coarse flesh. The samples were then diluted using BPW, plated and then incubated. All samples were done in duplicates.

Campylobacter

Campylobacter Blood-Free Selective Agar (Modified CCDA-Preston) was used for the isolation and identification of *Campylobacter jejuni.* The medium was prepared as indicated by Bridson (11). Serial dilutions of carcass rinse were poured onto pre-poured selective media in sterile petri dishes. Also, colonies were isolated by the streak plate method, with the use of sterile cotton –tipped swabs. The plates were then incubated at 42 °C for 48 h. *Campylobacter* are strict microaerophile requiring a microaerophilic atmosphere thus, Campy Pak Plus (Difco Microbiology, Voigt Global Distribution, Kansas City USA) was used (30). Two methods of identification were executed the first of which was by colonial morphology. *Campylobacter* is gram negative this was determined via Gram Staining. Instead of using the recommended Safranin as the counterstain as indicated in the standard procedures Ziehl-Neelsen's carbol-fuchsin stain was use (33).

Salmonella

Salmonella cell numbers from carcass surface rinse, rinse and wash water were serially diluted in phosphate buffered solution (14). Dilutions were plated on selective medium Brilliant Green Agar (BGA) and incubated at 37 °C for 24 h. For the isolation of *Salmonella*, fluid samples from carcass surface rinse, wash and rinse water were pre-enriched in buffered peptone water at 37 °C for 24 h. One ml of pre-enrichment culture was inoculated into 9 ml of selective enrichment tetrathionate broth and incubated at 37 °C for 24 hours. A loopful of enrichment culture was streaked on prepared BGA plates and incubated at 37 °C for 24 h. Suspected colonies were compared to reference pure culture of *Salmonella typhimurium* (ATCC14028 C600L, American Type Culture Collection, Rockville, MD, USA) by colour change of selective medium and colonial morphology as described by Bridson (11) and Flowers *et al.* (17). Also, catalase and oxidase tests were performed on isolated *Salmonella* colonies.

Escherichia coli

Like for *Salmonella*, samples were serially diluted and plated onto a selective medium of Eosin Methylene Blue Agar (EMBA) and incubated at 37 °C for 24 h for the isolation and identification of *E. coli.* Isolated colonies were incubated in *E. coli* (EC) broth tubes at 44.5 °C for 24 h and in 4-methylium-billiferyl-beta-D-glucoronic medium (MUG) at 44.5 °C for 24 h. Using reference pure cultures of *E. coli* (ATCC35218 C1971L; American Type Culture Collection, Rockville, MD, USA), the *E. coli* colonies from samples were identified by greenish metallic sheen by reflected light, and dark purple centers by transmitted light on EMBA plates, fermentation in EC broth tubes and by the presence of bluish fluorescence under a long wave (365 nm) ultraviolet ray in MUG broth tubes (11, 21).

Risk analysis

The prevalence of *Campylobacter*, *Salmonella* and *E. coli* at infectious dose levels were determined from

Table 1
Infectious Doses* (cell counts) for *Campylobacter, Salmonella* and *E. coli* (based on the literature)

Pathogen	Infectious doses (counts)	Adopted
[1]*Campylobacter*	500 – 800 cells	Barbut (6) Black *et al., (*9) McClure (24)
Salmonella	10,000 – 1,000,000 cells	Barbut, (6) Conner *et al*., (15) USDA-FSIS (36)
E. coli	100 – 1000 cells	Barbut, (6) Bilgili, (8) USDA-FSIS (36)

[1]Note: There is no international standard set for *Campylobacter* infectious doses for humans
* Total ingested range of cells for typical symptoms of appear in foodborne illness.

the literature. An infectious dose may be defined as the number of counts of a pathogen that must be present to cause an infection in humans. Infectious doses used as reference in this study taken from the literature (6, 8, 9, 16, 24, 36) (Table 1).

Statistical analysis
Microbial colonies formed were recorded as cfu.ml^{-1} (colony forming units per ml of surface rinse, or rinse and wash water). Counts were then transformed to $\log_{10}$ values for subsequent analysis.
One-Way Analysis of variance (ANOVA), Fisher's One-Way Multiple Comparisons, was used to compare counts obtained for *Campylobacter*, *Salmonella* and *E. coli* between pluck shops. The Minitab programme was used for all statistical analyses (23).

3. Results

Prevalence and levels of *Campylobacter, Salmonella* and *E. coli*

The prevalence of *Campylobacter*, *Salmonella* and *E. coli* from broiler carcasses, wash and rinse water sampled from pluck shops in county Nariva/ Mayaro are presented in table 2. Over all the isolation rate of *Campylobacter*, *Salmonella* and *E. coli* from broiler carcasses, wash and rinse water showed significant differences ($P< 0.05$) between pluck shops. Of the 56 samples examined from the 14 pluck shops sampled, 34 (60.7%) were positive for *Campylobacter*, 34 (60.7%) for *Salmonella* and 40 (71.4%) for *E. coli*.

Table 3 summarizes the mean (± SEM) levels of *Campylobacter, Salmonella* and *E. coli* isolated from broiler carcasses, wash and rinse water sampled from the pluck shops in the county of Nariva/Mayaro. *Campylobacter, Salmonella and E. coli* levels from broiler carcasses, wash and rinse water samples, respectively showed significant differences between pluck shops ($P< 0.05$). The correlation between the levels of *Campylobacter* found on carcasses and in wash water ($r^2= 0.657$) and rinse water ($r^2= 0.600$) was significant ($P< 0.05$). There was also a high correlation ($P< 0.05$) between wash and rinse water samples ($r^2= 0.950$) for *Campylobacter*. *Salmonella* levels on carcasses and in wash water samples were positively ($P< 0.05$) correlated ($r^2= 0.947$). However, the levels on the carcasses and in the rinse water samples were not significantly ($P> 0.05$) correlated ($r^2= 0.234$). The correlation between the levels of *E. coli* found on carcasses and in wash ($r^2= 0.199$) and rinse water ($r^2= - 0.195$) was not significant ($P> 0.05$).

Risk analysis
The bacterial count on the product (broiler carcasses) sold to consumers from the pluck shops/CPP was used to assess the risk to the public. Based on table 3 and literature values (Table 1) a risk analysis was done (Figure 3). Of the 14 pluck shops examined 6

Table 2
Prevalence of *Campylobacter, Salmonella* and *E. coli* for carcasses, wash and rinse water sampled from Pluck Shops/Cottage Poultry Processors in County Nariva/Mayaro, Trinidad and Tobago

Samples	Number of Samples	Number/ (%) Positive		
		Campylobacter	*Salmonella*	*E. coli*
Carcasses	28	19 (67.9)	20 (71.4)	25 (89.3)
Wash Water	14	5 (35.7)	8 (57.1)	5 (35.7)
Rinse Water	14	10 (71.4)	6 (42.9)	10 (71.4)
Total	56	34 (60.7)	34 (60.7)	40 (71.4)

Table 3
Mean (± SEM) levels of *Campylobacter*, *Salmonella* and *E. coli* isolated from carcasses, wash and rinse water sampled from Pluck Shops/Cottage Poultry Processors in County Nariva/Mayaro, Trinidad and Tobago

Pathogen	Mean $\log_{10}$ cfu.ml^{-1} (± SEM)		
	Carcass surface rinse	Wash water	Rinse water
Campylobacter	3.33 (±0.26)	1.42 (±0.36)	2.99 (±0.09)
Salmonella	5.08 (±0.26)	2.87 (±0.35)	2.57 (±0.39)
E. coli	5.46 (±0.24)	2.23 (±0.02)	2.04 (±0.45)

(42.9%) had C*ampylobacter* levels that corresponded to potentially infectious dose in humans. The apparent infectious doses for *Salmonella* were isolated from 3 (21.4%) pluck shops and 13 (92.9%) pluck shops evaluated had *E. coli* present at high levels. It was noted that 3 (21.4%) pluck shops had levels for all three organisms that were potentially harmful to human health.

4. Discussion

Food-borne diseases are important public health concerns worldwide and one of the most important food safety hazards is associated with under cooked meat and poultry (19). Broiler meat constituted 88% of all meats consumed in Trinidad and Tobago. A survey of consumers who handled meat at home in Trinidad indicated that 82.2% ($p < 0.01$) considered food safety as very important (5 in press). The Caribbean Epidemiology Centre (CAREC) indicated that reported cases of food-borne illness for member countries had an incidence rate of 12.9 per 1, 000, 000 persons (12). However, in a recent study in Trinidad 52.5% of the respondent consumers had experienced some form of perceived foodborne illness, with 48.8% having symptoms of vomiting and diarrhea, 36.9% blurred vision, nausea and abdominal pain, 20.2% chill and fever, 19% encountered headaches and 1.2% included other e.g. dizziness (34).

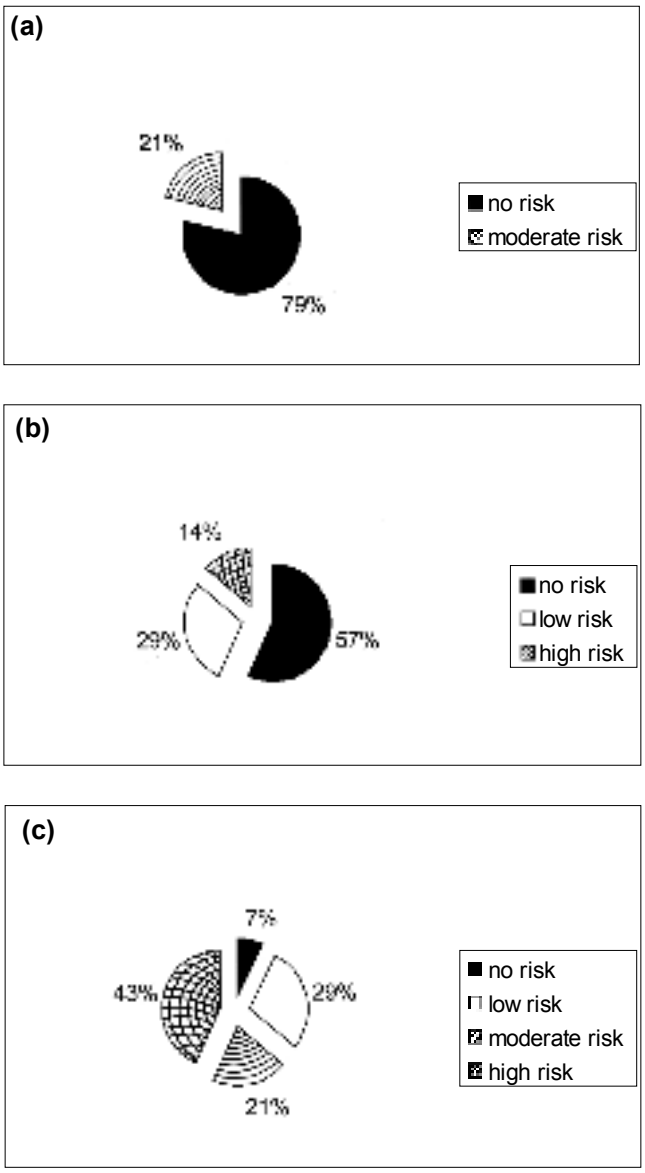

Figure 3: Percentage of pluck shops showing potential risk for (a) *Salmonella,* (b) *Campylobacter,* and *(c) E. coli*.

Pluck shop/CPP owners are faced with a complex challenge of controlling contamination by micro-organisms to carcasses of poultry during slaughter and handling. Further, the processes involved in production and processing of poultry introduce a variety of pathogenic microorganisms from several sources. CPP operating in Trinidad and Tobago does not have a standardized protocol for plant or operational procedures. However, the Caribbean Poultry Association (CPA) is in the process of developing food safety protocol for CPP in the Caribbean Community (CARICOM) Region to be adopted by countries such as Trinidad and Tobago.

Prevalence and levels of *Campylobacter*, *Salmonella* and *E. coli*

It was notable that all pluck shops/CPP evaluated showed presence of *Campylobacter*. *Campylobacter* is a common inhabitant of the gastrointestinal tract of many domestic birds (28). Abu-Ruwaida *et al.* (1) reported 100% recovery rate from chicken carcasses during processing in a modern commercial slaughterhouse in Kuwait. Adesiyun *et al.* (3) reported 56.3% prevalence of *Campylobacter* from commercial broiler processing plants in Trinidad. Shih (30) showed that the isolation rate of *Campylobacter* spp. from conventional retail markets in Taipei was 68% and was not so different to current result. In an earlier study, lower rates (49%) of *Campylobacter* contamination were reported for processed boilers (26). The prevalence of *Salmonella* (60.7%) in pluck shops for county Nariva/Mayaro in Trinidad is lower than the reported 100% for modern commercial plants in Kuwait (1) and lower than that reported on broiler carcasses (73.7%) by Blankenship *et al.* (10). However, the value was higher than that isolated (56.3%) by Adesiyun *et al.* (2) from commercial broiler processing plants in Trinidad. In a recent study Guven *et al.* (19) found the prevalence of *E. coli* was 0% in 1 g of sample for processes raw goose carcasses marketed in Kars (Turkey) compared to 71.4% for *E. coli* in current study.

Under cooked poultry products are perceived to be responsible for significant amount of human illness because of the relatively high frequency of contamination with *Campylobacter*, *Salmonella* and *E. coli* among other pathogens. Higher levels of *Campylobacter, Salmonella* and *E. coli* were found on

carcasses compared to wash and rinse water samples collected from broiler processing at pluck shops/CPP in the current study Table 3. The mean $\log_{10}$ cfu. ml^{-1} (± SEM) levels for *E. coli* on broiler carcasses as reported by Blankenship *et al.* (10) was 1.71 (± 0.76) this was much lower than levels isolated from pluck shops (5.46 ± 0.33) in the current study. *E. coli* presence is generally used as an indication of faecal contamination which increases during slaughtering (1). Microbial contamination may be a direct result of cross contamination which may occur during slaughtering and handling. Microbes may be present on hands, broiler carcasses, equipment e.g. feather picker and in water used. Since wash water was a collection from the washing of several carcasses, it would be consistent with high level of correlation found for *Salmonella* (r^2= 0.947) and *Campylobacter* (r^2= 0.657) on carcasses and in wash water samples. No correlation was however, found between the level of *E. coli* found on carcasses and in wash water (r^2= 0.199) and in rinse water (r^2= -0.195). This may be partially explained by the use of running water to wash carcasses that became soiled by faecal matter. Further, there was a high prevalence of this organism among pluck shops. *E. coli* level on carcasses was 59.2% above that of the wash and 62.6% above the rinse water. These differences observed may be due to the phenomena of surface fixation of bacteria as it impacts on their recovery (22).

Risk levels of pathogens

Over the past decade, *Campylobacter jejuni* has been recognized as an important cause of human enteritis with isolation rates from diarrheic patients often equaling or exceeding the rates of *Salmonella*. Literature shows that significantly more *Campylobacter* – afflicted patients had consumed poultry, further more *Campylobacter* affected patients more often used shorter cooking times for preparing chickens. Figure 3 showed that of the pluck shops/CPP surveyed 6 (42.9%) had apparent infectious levels of *Campylobacter*. Having no set International standard for *Campylobacter* the public health significance of its presence cannot truly be determined. Further more *Campylobacter* has many strains with varying level of virulence and specific strains were not identified. Under cooked poultry products are perceived to be responsible for significant amount of human illness because of high frequency of contamination of poultry with *Salmonella* spp. (20). Only 3 (21.49%) of 14 pluck shops had apparent infectious levels of *Salmonella* (> 10,000 cells). Although the *Salmonella* levels are low it is not consistent with low levels obtained at broiler farms in Trinidad (3). The increased levels noticed may be a direct result of cross contamination. Nisbet *et al.* (25) stated that conditions of slaughter houses tend to allow the spread of *Salmonella* among carcasses with ranges upward from 21%, similar to current results obtained from pluck shops. Thirteen (92.8%) of 14 pluck shops evaluated had high levels for *E. coli*. The presence of such high levels is indicative of poor sanitary conditions during handling (6). High *E. coli* is also indicative of faecal contamination, a major public health concern.

Three pluck shops had levels for *Campylobacter*, *Salmonella* and *E. coli* considered being potentially harmful to human health, where as all others had apparent infectious levels for one or two pathogen. The major differences between the three shops and the others were levels of output and level of infrastructures. The level of pathogen is an indication of the output of the CPP and their ability to avoid/ control the effects of cross contamination.

5. Conclusion

Results obtained may be characterized as an indicator of the occurrence of *Campylobacter, Salmonella* and *E. coli* in Pluck Shops/CPP in county Nariva Mayaro, Trinidad. Since it was outside the scope of this study the strains were not characterized as to their pathogenicity. Thus, making it difficult to ascertain the true risk they pose to consumers. Never the less, it is evident that these pathogens are present in pluck shops/CPP in the county, having levels considered to be potentially infectious to humans and as such there should be health concern. Thus, the competent authority needs to conduct further in-depth studies in this area.

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