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Destructive and Nondestructive Procedures to Obtain Chicken Carcass Samples for *Escherichia coli* and *Salmonella* spp. Detection

Marcus Vinícius Coutinho Cossi, Michelle Vieira de Almeida, Mariane Rezende Dias,
Paulo Sérgio de Arruda Pinto, and Luís Augusto Nero

Abstract

Destructive and nondestructive sampling procedures were compared for *Escherichia coli* and *Salmonella* spp. detection in 60 fresh chicken carcasses, which were submitted to the following sampling procedures: rinsing, skin swabbing, tissue excision, and skin excision; the proximity or not to the cloacae region was also considered. The obtained results were compared to identify significant differences ($p < 0.05$). Forty eight chicken carcasses were positive for *E. coli*, and five were positive for *Salmonella* spp. For *E. coli*, nonsignificant differences were observed between rinsing and tissue excision, rinsing and skin excision, and skin excision and tissue excision ($p > 0.05$), thus indicating equivalencies between these techniques. Skin swabbing produced a statistically significant lower frequency of positive results ($p < 0.05$) than all other techniques for *E. coli*, thus indicating its inadequacy for detection of this microorganism. For *Salmonella* spp., no significant differences were observed between the sampling techniques ($p > 0.05$), possibly due to the low overall frequency of positive carcasses. No significant differences in the number of positive samples (*E. coli* or *Salmonella* spp.) were observed between samples collected near or far from the cloacae region ($p > 0.05$), regardless of the sampling technique. The obtained results demonstrate that the tested sampling techniques were equivalent for *Salmonella* spp. detection in chicken carcasses, as observed for *E. coli* with the exception of skin swabbing.

Introduction

Spoilage and pathogenic microorganism contamination of chicken carcasses and avian products are constant concerns for the food industry and public health agencies in several countries (Álvarez-Astorga *et al.*, 2002). *Salmonella* spp. is the main foodborne pathogen associated with these products, and is recognized as responsible for several food poisoning outbreaks, due to the consumption of contaminated avian products (Rasschaert *et al.*, 2008; Vandeplas *et al.*, 2010). When present in birds' gastrointestinal systems, *Salmonella* spp. can easily contaminate carcasses during slaughter, usually by some processing failures, such as bowel rupture (Reiter *et al.*, 2007; Rasschaert *et al.*, 2008).

The presence of enteric microorganisms in foods is used as a possible indicative of *Salmonella* spp. contamination; and in addition, it suggests poor hygienic conditions during production and processing (Álvarez-Astorga *et al.*, 2002; Ghafir *et al.*, 2008). *Escherichia coli* is considered a good indicator of both poor hygiene in industrial slaughter and production, and the possible presence of foodborne pathogens (Ghafir *et al.*, 2008). In addition, several *E. coli* strains are also pathogenic, justifying testing for this microorganism in foods, including avian products (Tsola *et al.*, 2008).

Sampling procedures are fundamental to the reliability of tests for the presence of specific microbial groups of foodborne pathogens in food. Several sampling procedures are used for animal carcasses, and are typically classified as destructive or nondestructive (Snijders *et al.*, 1984; Capita *et al.*, 2004). Considering their specific advantages and disadvantages (Palumbo *et al.*, 1999; Capita *et al.*, 2004), the best sampling procedure can be chosen by specific food industries or regulatory agencies to obtain reliable data for microbial monitoring (Gill and Jones, 2000). The objective of the current study was to compare destructive and nondestructive chicken carcass sampling techniques for the detection of *E. coli* and *Salmonella* spp., and to evaluate their limitations and possible equivalencies.

Materials and Methods

Chicken carcasses

A total of 60 fresh chicken carcasses were obtained from commercial establishments in Viçosa city and the surrounding region, in the state of Minas Gerais, Brazil. Each carcass

Departamento de Veterinária, Universidade Federal de Viçosa, Viçosa, Brazil.

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was collected in its commercial package, and kept under refrigeration in isothermal containers until analysis.

Sampling techniques and dilution

Under aseptic conditions, each carcass was divided into two halves along the longitudinal section of its spine by using a sterile knife (Fig. 1). One half of the carcass was used to obtain a rinsing (nondestructive) sample, using a procedure modified from the USDA/FSIS (2008). The other half of the carcass was submitted to sampling by using two destructive (tissue excision and skin excision) and one nondestructive (skin swabbing) procedure, according to Gill *et al.* (2006). The breast and back regions of this half-carcass were divided into six areas of 25 cm^2 (5×5 cm) by using sterile templates for reference, to obtain samples according to cited procedures (see Fig. 1). For each procedure (with the exception of rinsing), two areas were randomly selected from the breast and back regions of the carcass and from areas near or far from the cloacae region.

For rinsing (nondestructive), the half carcass was placed in a sterile bag and weighed, and an equal amount (in mL) of buffered 0.1% peptone water (Oxoid Ltd., Basingstoke, England) was added. Then, the contents of the bag were manually homogenized for 5 min, and the final homogenate was collected in a sterile flask. The final sample concentration was defined as 1 mL = 1 g of the carcass.

Skin swabbing (nondestructive) samples were obtained by swabbing selected areas of the carcass with moistened (5 mL of buffered 0.1% peptone water; Oxoid) sterile sponges. Sponges were collected in sterile bags with 45 mL of buffered 0.1% peptone water and automatically homogenized for 5 min (Stomacher® 400 Circulator; Seward Ltd., Sussex, England). The final sample concentration was defined as 1 mL = 1 cm^2 of the carcass.

Tissue excision (destructive) samples were obtained by excision of skin and tissue fragments from selected areas of the carcass by using sterile scalpels and pincers. Tissue excision samples were collected in sterile bags until a weight of 25 g was obtained. Then, 225 mL of buffered 0.1% peptone water (Oxoid) was added, and the mixture was automatically homogenized for 5 min (Stomacher 400 Circulator). The final sample concentration was defined as 1 mL = 0.1 g of the carcass (1:10).

Skin excision (destructive) samples were obtained by excision of skin from selected areas of the carcass by using sterile scalpels and pincers. Skin excision samples were collected in sterile bags containing 50 mL of buffered 0.1% peptone water (Oxoid) and automatically homogenized for 5 min (Stomacher 400 Circulator). The final sample concentration was defined as 1 mL = 1 cm^2 of the carcass.

All the obtained homogenates were then ten-fold diluted by using buffered 0.1% peptone water (Oxoid).

E. coli *detection*

For each chicken carcass and sampling technique, a 1:100 sample dilution was plated on Petrifilm™ *Escherichia coli* (3M Microbiology, St. Paul, MN) for *E. coli* detection, followed by incubation at 35°C for 48 h. The presence of counts of 100 colony forming units per g or cm^2 (cfu/g or cfu/cm^2) or higher was considered a positive result for *E. coli* (a typical colony is blue in color, associated with gas formation). This concentration was considered a reference for the presence of *E. coli*, once it is used as a quality and safety parameter for foods in the United States and other countries (Álvarez-Astorga *et al.*, 2002; USDA, 2003).

Salmonella *spp. detection*

Samples from each sampling technique were submitted for *Salmonella* spp. detection according to a protocol modified from ISO 6579 (ISO, 2002). Aliquots containing 25 g (tissue excision samples) or 25 mL (rinsing, skin swabbing, and skin excision samples) of the final homogenates were added to 225 mL of buffered 1% peptone water (Oxoid), and incubated at 37°C for 18 h (pre-enrichment step). Then, 1 mL of the resulting culture was inoculated in 10 mL of selenite cysteine broth (Oxoid; incubated at 37°C for 24 h). About 0.1 mL of this culture was then inoculated in 10 mL of Rappaport-Vassiliadis enrichment broth (Oxoid; incubated at 42.5°C for 24 h) (selective enrichment step). After incubation, culture aliquots were streaked on brilliant green phenol red lactose sucrose

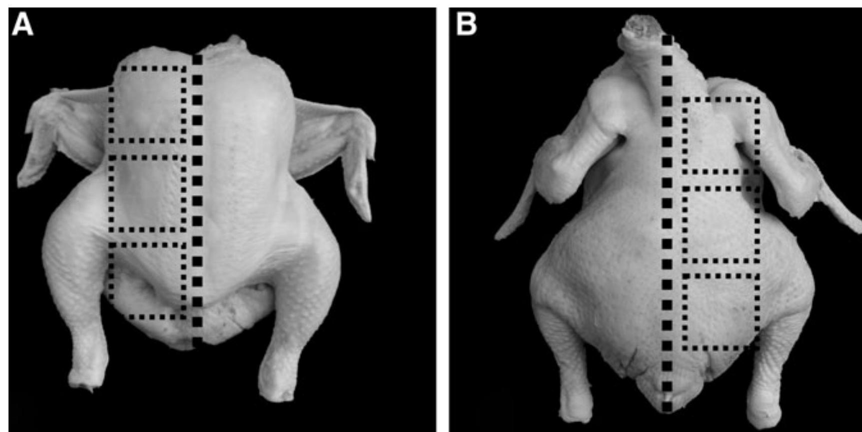

FIG. 1. Front **(A)** and dorsal **(B)** view of a typical chicken carcass, demonstrating division of the carcass into two halves (a dotted line marks the middle of the carcass). One half of the carcass was reserved for sampling by rinsing. Six areas (dotted line squares) were defined on the other half of the carcass to obtain samples by tissue excision, skin excision, and skin swabbing.

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Table 1. Overall Frequency of Positive Results for *Escherichia coli* (≥100 cfu/g or cm^2) and *Salmonella* spp. Obtained by Distinct Sampling Procedures of Fresh Chicken Carcasses Collected from Retail Stores Located at Viçosa, Minas Gerais, Brazil

Sampling procedure	Escherichia coli (≥100 cfu/cm^2 or g)	Salmonella *spp.*
Rinsing carcass	40	2
Tissue excision	40	2
Skin excision	34	2
Skin swabbing	24	0
Total	48	5

agar (PBLS; Oxoid) and xylose lysine deoxycholate agar (XLD; Oxoid), and incubated at 37°C for 24 h (selective plating). Typical or suspected *Salmonella* colonies (PBLS: small, transparent, colorless or pink or opaque white, usually surrounded by a pink or red halo; XLD: pink with or without black centers) were transferred to triple sugar iron (TSI, Oxoid) and lysine iron (LIA; Oxoid) slants, and incubated at 37°C for 24 h. When a typical reaction was observed in at least one of the slants (TSI: red slant and yellow butt, with or without H_2S formation; LIA: purple butt, with or without H_2S formation), cultures were subjected to serological testing with somatic (O) and flagelar (H) polyvalent antisera (Probac do Brasil SA, São Paulo, SP, Brazil), followed by molecular confirmation by using the polymerase chain reaction to detect the presence of *inv*A gene (Galan *et al.*, 1992). Taking into consideration all the confirmation steps just mentioned, final results were expressed as positive or negative for *Salmonella* spp. in 25 g or 25 cm^2 of each sample and sampling procedure.

Data analysis

Results from *E. coli* and *Salmonella* spp. detection tests for each chicken carcass and each of the four sampling procedures were compared by McNemar test to verify the statistical significance of differences between sampling techniques ($p < 0.05$). Frequencies of positive results were compared by considering the sampling site (near or far from cloacae) and sampling procedure, using the Chi-square test ($p < 0.05$) to verify statistically significant differences. All statistical analyses were conducted by using Statistica 7.0 (StatSoft Inc., Tulsa, OK) and XLSTAT 2009.1.02 (Addinsoft USA, New York, NY).

Results

The frequencies of positive *E. coli* (≥100 cfu/g or cm^2) and *Salmonella* spp. samples are presented in Table 1. Comparisons of the results obtained from each sampling procedure are presented in Tables 2 and 3. For the detection of *E. coli* positive carcasses, only nonsignificant differences were observed between samples obtained by rinsing and tissue excision, rinsing and skin excision, and skin excision and tissue excision ($p > 0.05$). However, skin swabbing presented significant differences when compared with the other sampling procedures for *E. coli* detection ($p < 0,05$). For *Salmonella* spp., no significant differences were observed among all tested sampling protocols ($p > 0.05$).

The results of frequencies of *E. coli* and *Salmonella* spp. isolation considering the tested sampling procedures (except rinsing) as well as while considering the proximity of the cloacae region are shown in Table 4, and no statistically significant differences were observed ($p > 0.05$).

Discussion

Considering the obtained results, rinsing and tissue excision sampling procedures resulted in positive *E. coli* detection with a higher frequency than skin swabbing. In addition, only skin swabbing was unable to detect *Salmonella* spp. from chicken carcasses, whereas all other tested procedures resulted in two positive samples each (Table 1). In a similar study, Gill *et al.* (2005) were able to identify *E. coli* in 100% of chicken carcasses analyzed by using skin excision samples of different sizes (1, 10, and 100 cm^2). Gill and Jones (2000) compared tissue excision with swab samples obtained by using three distinct swab materials, and found similar frequencies of positive results for *E. coli*. In the same work (Gill and Jones, 2000), the influence of the size of the sample area was investigated, and higher frequencies of positive *E. coli* detection were found to correlate with larger carcass sampling areas. Further, rinsing of chicken carcasses was associated with higher frequencies of positive results for *E. coli* detection than both tissue excision and skin swabbing (Table 1), and is considered the most adequate sampling procedure for the recovery of microorganisms present at low levels, such as *E. coli* (Gill *et al.*, 2005).

Rinsing and tissue excision protocols yield final results in the same units (≥100 cfu/g), thus enabling direct comparison. Moreover, the observed equivalence between rinsing and

Table 2. Comparison of Distinct Sampling Procedures for the Detection of *Escherichia coli* (≥100 cfu/g or cm^2) in Fresh Chicken Carcasses Collected from Retail Stores Located at Viçosa, Minas Gerais, Brazil

	Coincident		*Divergent*			
Paired comparison of sampling procedures	*Positive*	*Negative*	*Positive: Negative*	*Negative: Positive*	Q^a	p
Skin swabbing: Skin excision	20	22	4	14	5.56	0.031
Rinsing: Tissue excision	32	12	8	8	0.00	0.804
Skin swabbing: Rinsing	21	17	3	19	11.64	0.001
Skin swabbing: Tissue Excision	21	17	3	19	11.64	0.001
Skin excision: Rinsing	29	15	5	11	2.25	0.210
Skin excision: Tissue Excision	29	15	5	11	2.25	0.210

[a]McNemar test. *p*-Values lower than 0.05 indicate significant differences between the paired compared sampling methods.

Table 3. Comparison of Distinct Sampling Procedures for the Detection of *Salmonella* spp. in Fresh Chicken Carcasses Collected from Retail Stores Located at Viçosa, Minas Gerais, Brazil

Paired comparison of sampling procedures	*Coincident*		*Divergent*			
	Positive	*Negative*	*Positive: Negative*	*Negative: Positive*	*Q*[a]	P
Skin swabbing: Skin excision	0	58	0	2	2.00	0.500
Rinsing: Tissue excision	0	56	2	2	0.00	0.625
Skin swabbing: Rinsing	0	58	0	2	2.00	0.500
Skin swabbing: Tissue Excision	0	58	0	2	2.00	0.500
Skin excision: Rinsing	0	56	2	2	0.00	0.625
Skin excision: Tissue Excision	1	57	1	1	0.00	0.500

[a]McNemar test. *p*-values lower than 0.05 indicate significant differences between the paired compared sampling methods.

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tissue excision (Table 2) is economically valuable, once rinsing is a nondestructive sampling technique. In agreement with the current study, Cox *et al.* (2010) compared positive results for *E. coli* from chicken carcasses obtained by rinsing and tissue excision, and also failed to observe significant differences between the sampling protocols. Considering skin swabbing and skin excision, procedures that yield final results in the same units (≥ 100 cfu/cm^2), skin excision was able to recover higher frequencies of positive results for *E. coli* (Table 1) and presented significant differences when compared with skin swabbing (Table 2), thus being considered a better option despite being destructive.

The absence of differences between the sampling procedures for *Salmonella* spp. (Table 3) was probably caused by the overall low frequency of positive results identified in the current study. However, in a similar study, Cox *et al.* (2010) compared the frequencies of *Salmonella* spp. detection in chicken carcasses sampled by rinsing and tissue excision, and did not find significant differences between the tested procedures. *Salmonella* spp. is usually present at low levels in chicken carcasses, limiting the use of sampling procedures with low sensitivity, which can underestimate its presence (Cox *et al.*, 2010). Considering this, King *et al.* (2008) obtained higher frequencies of positive results for *Salmonella* spp. in chicken carcasses when larger sample volumes were used for microbiological analysis, a procedure that improved the sensitivity of the isolation.

Table 4. Comparison of Distinct Sampling Procedures Versus Carcass Sampling Sites (Near and Far from the Cloacae Region) for the Detection of *Escherichia coli* (≥ 100 cfu/g or cm^2) and *Salmonella* spp. in Fresh Chicken Carcasses Collected from Retail Stores Located at Viçosa, Minas Gerais, Brazil

Sampling procedure	*Sampling sites*	n	Escherichia coli	Salmonella
Tissue excision	Near cloacae	37	26	1
	Far away cloacae	23	14	1
Skin excision	Near cloacae	36	20	2
	Far away cloacae	24	14	0
Skin swabbing	Near cloacae	36	14	0
	Far away cloacae	24	10	0
	χ^2		9.375	4.268
	p		0.095	0.512

Since *E. coli* and *Salmonella* spp. are enteric microorganisms, the sampling site on the carcasses might influence the final results, once regions near the cloacae may be potentially contaminated with Enterobacteriaceae (Palumbo *et al.*, 1999; Cason and Berrang, 2002). However, the absence of significant differences between the results considering the sampling sites and procedures frequencies of *E. coli* and *Salmonella* spp. positive results in chicken carcasses submitted to distinct sampling procedures considering the sampling sites (Table 4) may be explained by the characteristics of chicken slaughtering. The avian evisceration step tends to contaminate the carcass in a similar way, and the several subsequent washing steps result in a uniform distribution of microbial contamination throughout the carcass (Mead *et al.*, 1994; Gill *et al.*, 2005). The sampling site of the carcass tends to be more relevant for microbiological analysis of other animal carcasses, such as swine (Palumbo *et al.*, 1999) and cattle (Grau, 1986).

Considering the obtained results, relevant information is presented for food industries and official regulatory organs in choosing the sampling technique that is most convenient and appropriate to obtain the desired microbiological control during production and inspection. These findings are economically valuable, because rinsing preserves the integrity of the carcass for commercial use by the food industry, and provides similar results when compared with tissue and skin excision for detection of both *E. coli* and *Salmonella* spp. So, this procedure could be adequately employed in quality control programs and in monitoring the presence of these microorganisms in chicken carcasses.

The current study provided specific information about distinct sampling procedures, allowing both the food industry and official regulatory agencies to apply the most appropriate technique during chicken production, after taking into consideration safety and quality goals. The tested sampling techniques were equivalent for *Salmonella* spp. detection, as observed for *E. coli* with the exception of skin swabbing.

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Disclosure Statement

No competing financial interests exist.

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Address correspondence to:
Luís Augusto Nero, Ph.D.
Departamento de Veterinária
Universidade Federal de Viçosa
Campus Universitário
36570-000 Viçosa
Brazil

E-mail: nero@ufv.br

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