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Short Communication

Detection of thermophilic *Campylobacter* sp. in raw chicken sausages by methods ISO 10272: 2006 in Curitiba – Parana State – Brazil

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Abstract

The aim of this study was the detection of *Campylobacter* sp. in raw chicken sausages using the methods ISO 10272-1 and ISO 10272-2. The overall prevalence of *Campylobacter* sp. in the samples tested was 16.67%, representing a serious risk to the health of consumers, particularly if measures guaranteeing proper cooking of foods and prevention of cross-contamination are not adopted. Furthermore, the majority of campylobacteriosis cases in humans are caused by consumption or improper handling of contaminated raw or undercooked poultry meat, which constitute the main vehicle of this infection.

Key words: *Campylobacter* sp., poultry meat, chicken sausages.

Campylobacter jejuni and *Campylobacter coli* are the leading bacterial cause of gastroenteritis in humans worldwide. Infected humans can manifest a series of clinical symptoms ranging from mild watery diarrhea to severe inflammation (Humphrey *et al.*, 2007). Moreover, *C. jejuni* has been identified as a major infectious agent in Guillain-Barre syndrome, a disease affecting the peripheral nervous system that causes neuron demyelination (Godschalk *et al.*, 2004). The majority of campylobacteriosis cases in humans is sporadic and caused by consumption or improper handling of contaminated raw or undercooked poultry meat, which constitute the main vehicle of infection (EFSA, 2009).

Brazil is a world leader in the production and processing of poultry meat, production in 2012 was 12,645,000 tons. The state of Paraná is considered the biggest producer of broilers in Brazil, contributing with 28.0% of the production (IBGE, 2013). The prevailing Brazilian legislation (Brazil, 2001) does not regulate for *Campylobacter* contamination in foodstuffs. Although Brazil is a major exporter of poultry meat, scant information is available on the contamination of these products and subproducts by *Campylobacter*.

Refrigerated sausages are regarded as a food with high exposure to contamination given the intense manipulation inherent to their manufacturing process, where the presence of these micro-organisms suggests contamination during meat processing (Barros *et al.*, 2007).

The aim of the present study was the detection of *Campylobacter* sp. in raw chicken sausages using the methods ISO 10272-2: 2006 (colony-count technique) and ISO 10272-1: 2006 (detection method). The sausages assessed were commercially available from markets in Curitiba city, Paraná State, Brazil. A further analysis was carried out comparing the two methods for the detection of *Campylobacter* sp.

A total of 30 samples of raw chicken sausages were purchased from different commercial outlets in the municipality of Curitiba – Paraná State, both in pre-packed and loose-weighed form, over the period spanning from November to December 2011. The minimum amount of 200 g was acquired for each sample to ensure representativity as recommended by RDC (Board of Commerce Resolution) No. 12 of 02/01/2001 of ANVISA/MS (National Health Surveillance Agency/Health Ministry) (Brazil, 2001). After collection, samples were sent to the laboratory under re-

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frigerated storage. For dilution, portions of 5 g from each sample were taken for analysis and placed into adapted flasks (7) containing 45 mL of Bolton broth supplemented with cefoperazone (10.0 mg), vancomycin (10.0 mg), trimethoprim (10.0 mg) and amphotericin B (5.0 mg) (SR 208E) (volume corresponding to 500 mL of medium) (Oxoid, England).

For the colony-count technique (ISO10272-2: 2006), aliquots of 0.1 mL of the mixture (sample + Bolton broth) were streaked onto plates containing modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) (CM 739) (Oxoid, England) supplemented with cefoperazone (16.0 mg) and amphotericin B (5.0 mg) (SR 155E) (volume corresponding to 500 mL of medium) (Oxoid, England) and Modified Bolton Agar (MBA) in duplicate. The MBA was prepared from the Bolton broth (CM 983) (Oxoid, England) with agar-agar (1.5%), ferrous sulphate (0.5 g/L) (Carlo Erba) and 2,3,5-triphenyltetrazolium chloride solution (TTC) (200 ppm) (Merck). Thereafter, a selective supplement with cefoperazone (10 mg), trimethoprim (10 mg), vancomycin (10 mg) and cycloheximide (25 mg) was added (SR 183E) (volume corresponding to 500 mL of medium) (Oxoid, England). Plates were incubated (41.5 ± 0.5) °C for 48 h in a microaerophilic atmosphere developed by an injection system (5% O_2, 10% CO_2, 85% N_2) (Air Liquide) for 20 s. MBA and the microaerophilic injection system were prepared as described by Franchin *et al.* (2005).

Five typical colonies from each plate were observed under a microscope in freshly prepared samples stained with 1% methylene blue solution (Laborclin, Brasil), and gram stain (Laborclin, Brasil). The typical colonies exhibiting characteristic morphology were reisolated onto Tryptic Soy Agar (TSA) supplemented with 5% defibrinated sheep blood (Newprov, Brasil), and incubated (36 ± 1) °C for 24 and 48 h in a microaerophilic atmosphere. Identification was performed by biochemical tests: catalase, oxidase, hippurate hydrolysis, indoxyl acetate; and evaluated the sensibility to nalidixic acid and cephalotihn. In addition to the biochemical tests was performed identification employing the API CAM® system (Biomerieux, France) according to manufacturer's directions.

For the detection method (ISO 10272-2: 2006), the prepared mixture containing 5 g of sample and 45 mL of Bolton broth was incubated (37) °C for 4 h and subsequently incubated (41.5 ± 0.5) °C for 48 h, under microaerophilic injection (Air Liquide, Brazil) for 10 s. The enriched mixture was plated on mCCDA and MBA, and then incubated (41 5 ± 0.5) °C for 48 h under a microaerophilic atmosphere. Typical *Campylobacter* colonies were identified using the procedure outlined in method ISO 10272-2 (2006).

The prevalence of *Campylobacter* sp., and analysis of the detection methods were compared with the use of global percentage of agreement accuracy and the proportion of true results (Hong Kong University on line statistical package) (Hong Kong University, 2013) and Z-test for two proportion of independent samples (Epi Tools on line statistical package) (Epi Tools, 2013). Significance was set at $p < 0.05$ for the null hypothesis.

Based on the results of the two analysis methodologies, the prevalence of *Campylobacter* sp. in the samples was 16.67%, of which 13.33% represented *Campylobacter jejuni* subsp *doylei*, and 3.34% for *Campylobacter lari*.

This study yielded 03 (10.00%) positive samples by the colony-count technique, and 02 (6.67%) by the detection method. Positive results were found by one or another method, with none of the samples testing positive on both methods (±). It was figured 90.0% of global concordance for colony count technique and 100% for detection method. Detection method did not show any discordance either positive or negative (Table 2).

Three samples (samples 12, 15 and 22) identified by direct plating but not isolated after enrichment, suggest this event was due to the presence of a microbiota contaminant not inhibited by the mix of antibiotics in the Bolton broth. This microbiota contaminant thus inhibited the growth of the viable *Campylobacter* population.

However, two samples (samples 17 and 23) identified only upon enrichment may be explained by them having a low concentration of *Campylobacter* or a non-viable population, thus precluding detection at the direct plating stage yet allowing recovery after enrichment.

Taken together, the results found for both methodologies tested highlight the importance of concomitant use of the two methods given the micro-organism is both fastidious and difficult to culture from food samples.

The results in Table 1 indicate 4 positive values (13.34%) for MBA, and only 3 (10.0%) for mCCDA. Thus, MBA shows a superior performance, although without statistical significance (Z test, $p = 0.687$). The ISO standard stipulates the use of mCCDA and recommends a complementary selective medium at the discretion of the analyst.

Ferreira *et al.* (2007), in a study adopting the method ISO 10272-1, failed to detect *Campylobacter* sp. in samples of *alheiras*, traditional sausages from Northern Portugal. In

Table 1 - Positive results identified by colony-count technique and detection method.

	Colony-count technique		Detection method	
Sample	mCCDA	MBA	mCCDA	MBA
12	-	+	-	-
15	-	+	-	-
17	-	-	+	+
22	+	-	-	-
23	-	-	+	+

Table 2 - Concordance analyses of selective media for the two methods used for *Campylobacter* detection.

	Colony count technique	Detection method
Positive concordance	0	2
Positive discordance	1	0
Negative discordance	2	0
Negative concordance	27	28
Global percentage of concordance	90.00%	100.00%

Selective media used were mCCDA and MBA.

addition, the authors attributed the absence of pathogens to the smoking process used to cure the sausages.

Similarly, Cortez *et al.* (2004) detected no *Campylobacter* in their analysis of 106 sausage samples. The authors hold that *Campylobacter* is sensitive to factors intrinsic to sausage production, such as the addition of sodium chloride and spices.

Therefore, it is clear that the detection level of *Campylobacter* sp. in 16.67% of chicken sausage sample assessed in this study is significant comparing to the no pathogen isolation by Cortez *et al.* (2004); although this study have used a different protocol for the isolation.

The results of this study showed that factors intrinsic to the production of sausages for human consumption were unable to inhibit *Campylobacte*r growth in the samples tested.

These findings suggest that refrigeration during storage and sale of the product did not hamper recovery of *Campylobacter*. These results corroborate the observations of Maziero and Oliveira (2010), who reported no significant difference in the prevalence of *C. jejuni* between fresh samples and those stored at low temperatures, indicating that the organism is able to survive under frozen storage conditions.

Nevertheless, the source of potential contamination of our samples could not be established. Since the samples were purchased in loose-weighed and pre-packed form, cross-contamination may have taken place at the commercial outlets from other poultry subproducts through contaminated equipment and work surfaces.

Concerned with this scenario, Burgess *et al.* (2005) assessed the external packaging of raw poultry meat and confirmed the presence of *Campylobacter* on 1.1% of items, pointing to the need for rigorous care in the handling of foodstuffs and their contact with surfaces, as well as the cleaning of utensils, equipment and exposed surfaces.

Overall, the isolation of *Campylobacter* in the raw chicken sausage samples tested indicates that good hygiene practices are not being adhered to during the processing, storage and distribution stages, representing basic measures for the prevention of contaminants, whose absence poses a safety hazard for human consumption of the product.

To conclude, considering that we dealt with raw product, the presence of *Campylobacter* sp. in 16.67% samples, poses a serious threat to public health, since it suggests that measures for guarantee of proper cooking of foods and prevention of cross-contamination were not. Since data was obtained under a pilot experiment further studies must be conducted to understand the behavior of *Campylobacter* in raw chicken sausages, and thus to reduce the contamination possibility.

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