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FREQUENCY OF THERMOPHILIC *Campylobacter* IN COMMERCIAL BROILER FARMS IN SOUTHERN BRAZIL USING DIFFERENT CULTURING TECHNIQUES AND SELECTIVE MEDIA

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ABSTRACT

Broiler is a potential reservoir for thermophilic *Campylobacter* species, whose laboratory culturing is difficult because of environmental stress effect and presence of competitor cells. *Campylobacter* frequency was analyzed in broiler feces, cloacal swabs, drag swabs and litter taken from 22 commercial broiler flocks with 3 to 5 weeks of age in Southern Brazil from 2010 up to 2011. Samples were direct plated in Preston Agar (PA), modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) and Campy-Line Agar (CLA) or enriched in Bolton Broth (BB) for 24h and 48h following plating in each selective media. All samples were incubated in microaerobic atmosphere at 41.5 °C. Samples enriched in BB for 24h or 48h had lower *Campylobacter* isolation frequency than directed-plated samples. In addition, enriched samples showed an abundant growth of non-*Campylobacter* cells in mCCDA and CLA. Analyzing the directed-plated samples in each selective medium, the highest *Campylobacter* frequencies were detected in litter samples inoculated in PA (63.9%); drag swabs streaked either in CLA or mCCDA (69.4%); feces plated in PA (88.9%) and cloacal swabs streaked in mCCDA (72.2%), respectively. PA was the best selective media to isolate *Campylobacter* from litter samples ($P<0.05$), while there was not significant difference between PA, mCCDA or CLA to detect *Campylobacter* in directed-plated drag swabs, feces or cloacal swabs. *Campylobacter* isolated were biochemically identified as *C. jejuni* or *C. coli.* This study showed high frequency of *Campylobacter* in Brazilian broiler flocks sampled, suggesting that the performance of the culturing technique should be considered to *Campylobacter* analysis in broiler samples.

KEYWORDS: Broilers, *Campylobacter* jejuni, *Campylobacter* coli, microbiology culture.

INTRODUCTION

Thermophilic *Campylobacter* (C.) species are often found in high levels in the intestinal tract of broilers, which are considered potential reservoirs of the bacteria. Despite the absence of clinical disease in broilers, thermophilic *Campylobacter* are a leading cause of human bacterial gastroenteritis mostly associated to handling or consumption of contaminated raw or undercooked broiler meat worldwide (Lee & Newell, 2006).

On the other hand, detection of *Campylobacter* is crucial to evaluate the contamination of commercial broiler flocks. Currently, a number of culture protocols have been used for *Campylobacter* detection in a variety of samples, such as broiler meat. However, limited information is available on the performance of enriched or direct culture for *Campylobacter* detection in other broiler samples (Musgrove *et al*., 2001; Rodgers *et al*., 2010). *Campylobacter* is extremely susceptible to a variety of environmental stresses, such as variations in temperature, humidity, osmolarity, presence of sunlight, atmospheric oxygen, freezing and the presence of competitor cells (Lee & Newell, 2006); hence the difficulty to establish cultures of the bacteria in the laboratory. For these reasons, the aim of this study was to analyze enriched or direct culture to detect *Campylobacter* in samples collected from broiler farms.

MATERIALS AND METHODS

Broiler feces (36), cloacal swabs (36), drag swabs (36) and broiler litter samples (36) were taken

from 22 commercial broiler flocks with 3 to 5 weeks of age in Southern Brazil from 2010 up to 2011. Samples were transported to the laboratory in insulated boxes with ice packs and processed within 4 hours of sampling. Further, samples were direct plated in Preston Agar (PA), modified Charcoal Cefoperazone Deoxycholate Agar (mCCDA) and Campy-Line Agar (CLA) or enriched in Bolton Broth (BB) for 24h and 48h following plating in each selective media. All samples were incubated in a microaerobic atmosphere (5% O_2, 10% CO_2 with the balance N_2) at 41.5 °C.

Suspect bacterial colonies were subcultured in Blood Agar no 2 (BA2) plates for confirmation, which were incubated in the microaerobic atmosphere at 41.5 °C for 24 h to 48 h. Gram negative colonies exhibiting curved or spiral rods were presumptively identified as *Campylobacter* and were tested for catalase, oxidase, hippurate hydrolysis and hydrolysis of indoxyl acetate. *C. jejuni* subsp. *jejuni* (ATCC 33560) was used as positive control. The effect of enrichment culture and direct plating to detect *Campylobacter* in samples analyzed was determined by Fisher's Exact test.

RESULTS AND DISCUSSION

Samples enriched in BB for 24h or 48h had lower *Campylobacter* isolation frequency than directed-plated samples (Table 1). In addition, enriched samples showed an abundant growth of non-*Campylobacter* cells in mCCDA and CLA. As previously described, the enrichment culture of fecal samples from broilers can be severely compromised by the many competing non-target bacteria present in the sample (Musgrove *et al*., 2001; Rodgers *et al*., 2010).

Table 1 - Detection of thermophilic *Campylobacter* in broiler samples according to direct plating or enrichment culturing.

Selective media	Culturing procedure		
	Direct culture	Enriched culture[1] (24 h)	Enriched culture (48 h)
Broiler litter			
PA[2]	63.89%5 (23/36) aA[6]	30.56% (11/36) aB[7]	2.78% (1/36) C
CLA[3]	25.00% (9/36) bA	5.56% (2/36) bB	0.00% (0/36) B
mCCDA[4]	22.22% (8/36) bA	8.33% (3/36) bAB	0.00% (0/36) B
Cloacal swabs			
PA	61.11% (22/36) A	69.44% (25/36) aA	11.11% (4/36) B
CLA	66.67% (24/36) A	25.00% (9/36) bB	5.56% (2/36) C
mCCDA	72.22% (26/36) A	38.89% (14/36) bB	22.22% (8/36) B
Broiler feces			
PA	88.89% (32/36) A	30.56% (11/36) aB	2.78% (1/36) C
CLA	77.78% (28/36) A	8.33% (3/36) bB	0.00% (0/36) B
mCCDA	83.33% (30/36) A	16.67% (6/36) abB	0.00% (0/36) C
Drag swabs			
PA	61.11% (22/36) A	47.22% (17/36) aA	0.00% (0/36) B
CLA	69.44% (25/36) A	13.89% (5/36) bB	2.78% (1/36) B
mCCDA	69.44% (25/36) A	11.11% (4/36) bB	2.78% (1/36) B

1 - Bolton Broth enriched culture. 2 - Preston Agar. 3 - Campy-Line Agar. 4 - modified Charcoal Cefoperazone Deoxycholate Agar. 5 - Percentage of samples found positive to thermophilic *Campylobacter.* 6 - Percentages followed by different lower case letters in columns differ significantly ($P<0.05$). 7 - Percentages followed by different capital letters in rows differ significantly ($P<0.05$).

Analyzing the directed-plated samples in each selective medium, the highest *Campylobacter* frequencies were detected in litter samples inoculated in PA (63.9%); drag swabs streaked either in CLA or

mCCDA (69.4%); feces plated in PA (88.9%) and cloacal swabs streaked in mCCDA (72.2%), respectively. PA was the best selective media to isolate *Campylobacter* from litter samples (P<0.05), while there was not significant difference between PA, mCCDA or CLA to detect *Campylobacter* in directed-plated drag swabs, feces or cloacal swabs. Direct plating of broilers caecal contents in mCCDA has already been most sensitive than direct culture in PA (Rodgers *et al*., 2010), while directed-plated broilers caecal contents in mCCDA had a higher *Campylobacter* detection rate than CLA (Potturi-Venkata *et al*., 2007). According to Musgrove *et al*. (2001), the large number of viable *Campylobacter* cells in the intestinal content of broilers allows detecting the bacteria by direct plating onto selective media. Nevertheless, the choice of selective media might influence the efficiency of isolating *Campylobacter* from broiler samples (Potturi-Venkata *et al*., 2007).

On the other hand, *Campylobacter* strains isolated in this study were biochemically identified as *C. jejuni* or *C. coli* (Table 2). C. jejuni, the most prevalent specie found, has been responsible for the majority of human bacterial gastroenteritis associated to handling and consumption of contaminated broiler meat worldwide (Lee & Newell, 2006).

Table 2 - *Campylobacter* species (%) detected in broiler samples according to direct culture in Preston Agar.

Specie	Broiler litter	Cloacal swabs	Broiler feces	Drag swab
C. coli	0.00% (0/36)	2.78% (1/36)	2.78% (1/36)	0.00% (0/36)
C. jejuni	63.89% (23/36)	58.33% (21/36)	86.11% (31/36)	61.11% (22/36)

CONCLUSION

Directed culture allowed detecting higher levels of *Campylobacter* in broiler feces, cloacal swabs, drag swabs and broiler litter samples than enriched culture. Moreover, the present study showed high frequency of *Campylobacter* in Brazilian broiler flocks sampled, pointing to the need for additional studies to identify interventions strategies to reduce *Campylobacter* contamination in broiler flocks.

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