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Campylobacter spp. isolation from infected poultry livers with and without necrotic lesions

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ABSTRACT

This study was developed in order to understand the possible intervention of *Campylobacter* spp. as etiological agent of necrotic lesions in poultry livers.

This way, *Campylobacter* spp. was isolated from poultry livers with and without necrotic lesions. Additionally, virulence factors (*cad*F and *cdt*B) and antimicrobial resistance profile of the isolated strains were analyzed. From a total of 39 liver samples analyzed, 21 presented lesions and 18 were clean. *Campylobacter* spp. was isolated from 80.9% of liver samples with necrotic lesions (17/21) and from 38.9% of liver samples without lesions (7/18). These results indicate poultry liver as a potential source of human *Campylobacter* infection, since this bacteria may remain viable in the internal liver tissue in undercooked conditions.

A high resistance to nalidixic acid (100%), norfloxacin (100%), ciprofloxacin (95.8%), ampicillin (91.6%) and tetracycline (75%) was observed among *Campylobacter* spp. isolates. Also, PCR detection of *cdt*B and *cad*F virulence and toxin genes, revealed 75% and 68.8% of positive samples, respectively. Strains isolated from livers with and without lesions presented similar results with respect to virulence factors and to antimicrobial resistance profiles, evidencing that these putative pathogenic determinants are widespread among the isolates from poultry livers.

Phi coefficient calculated in order to measure the degree of association, revealed a highly significant association (Phi = 0.472; p-value < 0.01) between the presence of livers with hepatic necrosis lesions and *Campylobacter* isolation. This result indicates the possibility of using these macroscopic lesions as visible and reliable indicator of *Campylobacter* spp. presence in poultry flock, and, thus, becoming an important tool to support the implementation of corrective measures at poultry farms level. This methodology could contribute for an accurate time-efficient monitoring and the development of effective prevention and intervention measures for *Campylobacter* spp. infection with reduced cost.

1. Introduction

Since 2005 until 2012, Campylobacteriosis continued to be the most frequently reported zoonotic disease in humans in the European Union. In 2012, 214,268 confirmed cases of human campylobacteriosis were reportedin the EU and 31 deaths were reported by 14 Member States (EFSA, 2014).

Foods of animal origin, in particular broiler meat is considered to be the main food-borne source of *Campylobacter* human infection (EFSA, 2014; Nadeau, Messier, & Quessy, 2003; Nielsen, Fussing, Engberg, Nielsen, & Neimann, 2006; Silva et al., 2011). Several studies had already emphasized the importance of poultry as a reservoir and source of *Campylobacter jejuni* as a result of its infection and contamination at the farm and slaughterhouse levels (Herman et al., 2003; Sasaki et al., 2013). For instance, in Japan, *C. jejuni* was isolated from 71.2% of retail poultry samples (Saito et al., 2005) and, in Denmark, *Campylobacter* spp. was found in 36% of poultry at the slaughterhouse level (Nielson et al., 1997). Later, Son, Englen, Berrang, Fedorka-Cray, and Harrison (2007) reported a higher (78.5%) occurrence of *Campylobacter* spp. from poultry carcasses in slaughterhouses after cooling.

Poultry livers are also a potential risk to the health of consumers, as *Campylobacter* spp. is often isolated from this organ, as

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demonstrated by studies developed by Barot, Mosenthal, and Bokkenheuser (1983); Fernández and Pisón (1996),White et al. (2006),Kenar, Akkaya, and Birdane (2009) and Sasaki et al., 2013. Also, as it was previously referred by Barot et al., (1983), ingestion of undercooked chicken livers infected with *Campylobacter* has been reported to be a cause of intestinal Campylobacteriosis in humans. The proportion of foodborne outbreaks of *Campylobacter* related to poultry liver parfait or pâté reported to the Health Protection Agency (HPA) has been increasing: 1992–2006, 9.5% (9/95); 2007–2009, 75% (15/20). In England, in 2009, from the 11 outbreaks reported, 9 (82%) outbreaks at catering premises were linked to poultry liver parfait or pâté consumption (8 and 1 prepared from poultry and duck livers, respectively) (HPA, 2009). *Campylobacter* outbreaks linked to pâté consumption have also been reported in Scotland (Forbes et al., 2009). In 2010, Inns, Foster, and Gorton (2010) reports an outbreak of 13 *Campylobacter* infection confirmed cases from 24 cases of gastroenteritis among guests at one wedding reception. The cohort study using an univariate analysis revealed a strong association with consumption of chicken liver parfait.

Poultry liver foods represent a potential source of human *Campylobacter* infection as this bacteria can be present both in the external and internal liver tissue, and may remain viable after an insufficient cooking (Whyte, Hudson, & Graham, 2006). Measures required to prevent transmission of *Campylobacter* infection to humans depend upon the location of the organisms in the organ. If the infection is located in the hepatic tissue, and is probably secondary to bacteremia the burden of providing a clean product falls on the farmer. If, on the other hand, *Campylobacter* occurs in a surface contamination due to unhygienic handling of offal, the clean product has the responsibility of the slaughterhouse or the retailer or both (Barot et al., 1983).

All the previous studies underline the importance of healthy poultry liver as a source of *Campylobacter* spp. to human infection. Nevertheless, in some cases, this agent can be responsible for the development of clinical infection disease in poultry. In these cases, the principal changes are located at the intestinal tract (Shane, 1997). However it is also possible to observe necrotic lesions in the liver that can be identified during *post mortem* inspection at slaughterhouse level (Boukraa, Messier, & Robinson, 1991).

In the last two years, hepatic necrotic lesions have been frequently observed during poultry *post mortem* inspection in Portuguese abattoirs. This was the glint for the present study which main aim is to understand the possible intervention of *Campylobacter* spp. as etiological agent of the hepatic necrotic lesions. The confirmation of a relationship between the presence of poultry livers with necrotic lesions and the *Campylobacter* spp. infection, allow us to consider these macroscopic lesions as visible and reliable indicators of the presence of *Campylobacter* spp. in the poultry flock, thus becoming an important tool to support the implementation of corrective measures at poultry farms level.

Additionally it was evaluated the presence of some virulence factors that may suggests their potential role as important biological and pathogenic factor involved in *Campylobacter* spp. infection (Ripabelli, Tamburro, Minelli, Leone, & Sammarco, 2010), as well as the profile of resistance to antibiotics since it is recognized as an emerging public health problem (Engberg, Aarestrup, Taylor, Gerner-Smidt, & Nachamkin, 2001).

2. Material and methods

2.1. Sampling procedure

During the period from September 2010 to June 2011 a total of 8 livers lots from poultry slaughtered for consumption were analyzed. Each lot consisted of six poultry livers (3 livers with necrotic lesions and 3 without lesions) from animals that belonged to the same poultry flock. As it was referred in the introduction chapter, *Campylobacter* spp. can be isolated from apparently healthy livers. For this reason, in this study, they were also analyzed in each lot, livers without lesions, in order to compare the level of *Campylobacter* spp. isolation between these samples and those with hepatic necrosis. This way, it was possible to figure out the involvement of this agent in the occurrence of the hepatic lesions.

All the samples were properly identified (slaughter day, poultry flock), individually packed in a sterile named recipient, and transported to the laboratory under refrigerated conditions.

Table 1
Primers and PCR conditions used for identified the gender *Campylobacter* spp., *Campylobacter jejuni* and *Campylobacter coli* isolates (Linton et al., 1997).

Target genes	PCR reaction	Primers names: sequences (5′–3′) (amplicon sizes)
rrs	94 °C 1 min; 58 °C 1 min; 72 °C 1 min (25cycles)	CCCJ609F: AAT CTA ATG GCT TAA CCA TTA; CCCJ1442R: GTA ACT AGT TTA GTA TTC CGG; (854 bp)
hip	94 °C 1 min; 66 °C 1 min; 72 °C 1 min (25 cycles)	HIP400F: GAA GAG GGT TTG GGT GGT G; HIP1134R: AGC TAG CTT CGC ATA ATA ACT TG; (735 bp)
CCCH	94 °C 1 min; 60 °C 1 min; 72 °C 1 min (25 cycles)	CC18F: GGT ATG ATT TCT ACA AAG CGA G; CC519R: ATA AAA GAC TAT CGT CGC GTG; (500 bp)

- *rrs*, 16S rRNA gene-based PCR assay; -*hip*, hippuricase gene-based PCR assay; -*CCCH*, *C. coli*-specific PCR assay.

2.2. Laboratory procedure

2.2.1. Sample preparation

At the laboratory (4 h after samples collection), all the samples, were submerged in absolute alcohol for 10 s for surface decontamination. This procedure was applied in order to guarantee that a positive sample correspond to an infected organ (internal *Campylobacter* spp.) and not to a superficial contaminated organ (external *Campylobacter* spp.). This means that a positive result allows identification of an animal infection status.

From every lot, a fragment from each liver with necrotic lesion was removed for further histopathological analysis.

2.2.2. Campylobacter isolation

Campylobacter spp. isolation was performed according to the guidelines of the ISO 10272:2006 Norm- Horizontal method for detection and enumeration of *Campylobacter* spp. (ISO, 2006).

In a first stage, 10 g of each liver was suspended in 90 ml Preston enrichment broth (Nutrient Broth No. 2, CM0067B, Oxoid; added with 5% ovine blood, *Campylobacter* Growth Supplement, SR0232E, Oxoid and Modified Preston *Campylobacter* selective supplement, SR0204, Oxoid) and homogenized in a Stomacher (90 s). The diluted samples were incubated under microaerophilic atmosphere (5% O_2, 10% CO_2, and 85% N_2 – microaerophilic generator (Oxoid)) at 42 ± 2 °C for 48 h.

In a second stage, one loop of the selective enrichment broth was streaked onto the surface of two selective solid media: Modified Charcoal Cefoperazone Deoxycholate Agar (Lab 112, Lab M with selective supplement, Lab X 112, Lab M) and Preston Agar (Columbia Agar, CM0331B-Oxoid plus 5% ovine blood, *Campylobacter* Growth Supplement, SR0232E, Oxoid, Modified Preston *Campylobacter* Selective Supplement, SR0204, Oxoid). The plates