Using PCR for detection of C*ampylobacter jejuni* in beef minced meat

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Abstract

A survey was conducted in order to determine the incidence of *Campylobacter jejuni* in beef minced meat because *campylobacter spp.*, are characterized by identification of virulence marker genes that are potentially involved in the pathogenesis of food borne diseases. The presence of ceuE, gene cluster among *C.jejuni* isolates was detected by polymerase chain reaction (PCR). Molecular methods are being increasingly applied to detect, quantify and study microbial populations in food or during food processes. Among these methods, PCR-based techniques which reduce the time and labor involved in screening food products for the presence of pathogens 100%. One of its major advantages is to be faster than conventional culture-based methods. It is also highly sensitive, specific and enables simultaneous detection of different microorganisms. In this work the incidence of *campylobacter Jejuni ceuE* reach 30 % using PCR.

Aim of these work

Detected the *Campylobacter jejuni* ceuE directed from minced meat samples without using the traditional methods because PCR test is more rapidly and acquired.

Key words: *Campylobacter jejuni ,* beef minced meat *,* PCR.

Introduction

Campylobacteriosis is one of the most common human bacterial intestinal disorders occurring worldwide **(Tauxe 2001; Butzler 2004; Anonymous and Humphrey 2007)**. Cattle may carry several pathogens in their gastro-intestinal tract. The contamination of beef meat during slaughter and processing of the carcasses is a major risk of subsequent food borne pathogen infection in humans. The most important zoonotic bacteria of them are *Campylobacter* spp., **(Hugas *et al.,* 2009; Westrell *et al*., 2009).** Undercooked meat are the primary sources of a *Campylobacter* infection, beef minced meat can also contribute to illness **(Inglis *et al*., 2004 and Norung and Buncic 2008)**. The infection with *Campylobacter* spp., especially with *C.jejuni*, is one of the leading causes of acute bacterial gastroenteritis in human worldwide **(Wassenaar and Blaser1999; Moore *et al.*, 2005 and On, 2005).**

Thermophilic *Campylobacter* species, mainly *Campylobacter jejuni* has the ability to survive in refrigeration and freezing, which is of obvious relevance to food safety and public health because it responsible for numerous cases of food borne illness and most frequently implicated in clinical diagnosis **(World Health Organization,2000; Newell and Fearnly, 2003; Smulders *et al*., 2008; Hugas *et al*., 2009 and Mor-Mur and Yuste, 2010**). Infected humans exhibit a range of clinical spectra, from mild, watery diarrhea to severe inflammatory diarrhea **(Janssen *et al.,* 2008)**. Besides its role in human enteric illnesses, there are many factors influencing the virulence of *C. jejuni* include motility, chemotaxis, the ability to adhere to and invade intestinal cells, intracellular survival, and toxin production (**Ketley., 1997; Humphrey *et al*., 2007; Young *et al* 2007; Keener *et al*., 2004, and Janssen *et al,* 2008).** Conventional methods for *Campylobacter spp* detection in food involve culturing in selective media at 42°C under micro aerobic conditions and identification of the isolates according to their biochemical and /or immunological characteristics. Although these methods are sensitive and are being continuously improved, they

are relatively complex and time consuming. The identification of a suspected *campylobacter* colony takes 4 to 6 days, and phenotypic identification schemes for *Campylobacter spp.* are often difficult to interpret **(On, 2001).**Furthermore, stressed and/or viable but non-culturable (VBNC) bacteria cannot grow in the selective culture media. Traditionally, detection of pathogens in minced meat has required the use of laborious, time-consuming, pathogen-specific culture methods. Although culture methods are considered to be ''gold standards,'' these methods take several days to perform and are inadequate for making rapid assessments of microbiological safety **(Feng, 2001)**. The molecular methods such as PCR have reduced the time and labor involved in the analysis of food products, the use of molecular tools applied to the study of population dynamics and gene expression in food is only starting **(Falentin *et al*., 2010; Juste *et al*., 2008 and Smith and Osborn, 2009)**. Rapid methods are generally used to screen large numbers of samples and identify those that are negative. As an alternative, molecular amplification techniques can provide highly sensitive and specific methods for the detection, identification and characterization of food-borne organisms,including *Campylobacter spp.* A number of PCR assays that involve DNA amplification have been developed **(Giesendrof and Quint, 1995; Winters and Slavik, 2000; Lilja and Hanninen, 2001; Bolton *et al*., 2002; On and Jordan,2003 and Oliveira *et al*.,2005)**. This technique appears to be the most accurate and reliable for genes or transcripts quantification **(Bustin, 2009).** Molecular techniques such as PCR have proven to be specific and sensitive methods for detecting infectious pathogens (**Koch, *et al.* 1993, Bayardelle, and Zafarullah. 2002** and **Sammarco, *et al.* 2010)**. PCR can detect as few as 100 bacteria per milliliter **(Day *et al.* 1997).**

PCR was highly sensitive method for the detection of all the food borne pathogens tested, several PCR assays designed using single primer sets to identify *Campylobacter jejuni putative virulence gene ceuE encoding a lipoprotein (component of protein - binding - dependent transport system for the siderophore enterochelin it has been identified* ***(Park and Richardson 1995)*** *and characterized* ***(Gonzalez et al 1997).*** *PCR* have been developed **(Linton *et al.*, 1996 and Inglis and Kalischuk, 2003)**. Although PCR assays provide more rapid identification of these species than conventional assays, investigation of unknown samples requires the separate identification of *Campylobacter jejuni* ceuE, extending identification time and increasing reagent costs.

Materials and Methods

Material

Samples collection

One hundred samples of beef minced meat were collected from different supermarket Samples were taken randomly from boxes outside of our region and conveyed respecting the freezing temperature of -18 °C to the Laboratory After suffering a slow thawing at room temperature, the microbiological analysis were performed.

DNA extraction

Genomic DNA was extracted from 50 mg of each minced beef meat sample according to the procedure of (**Rossi *et al.* 2000**) by using a Fermentas kit (K0729) The extracted DNA was performed spectrophotometrically (DU®730 Spectrophotometer, Beckman Coulter, USA). The DNA was stored at −20 °C until use.

PCR reaction

Molecular Testing

Detection of *Campylobacter* ceu E by PCR as described by **(Wieczoek and Osek, 2005)**.

Primers were designed using DNA sequence data (Sigma) for **Table (1)**.

Table (1): Primer sets used in PCR reaction.

C. jejuni	Sequence ″5 →3	Amplicon length (bp)	References
ceuE	Forward CCTGCTACGGTGAAAGTTTTGC Reverse GATCTTTTTGTTTTGTGCTGC	793	**Konkel *et al.* 1999**

Template DNA (5 ml) was added to 20 ml of master mix for a final reaction volume of 25 ml. Reagent concentrations for each pathogen-specific.

PCR reaction and cycling parameters are described in Table (2). Results of amplification reactions are usually obtained by the resolution of products based on size via agarose gel electrophoresis staining with ethidium bromide and visualization by UV-induced fluorescence. The complete PCR amplification and agarose gel analysis generally requires only about 2-4 hours after enrichment. In terms of sensitivity, specificity, and analysis time, selective enrichment followed by PCR is clearly a powerful, rapid, and robust methodology for detecting foodborne bacterial pathogens. A 100-bp standard (#SM0323) Fermentas was used as a size marker **(Linton *et al.*, 1996 and Wang *et al.*, 2002).**

Table (2): Concentrations of PCR reagents and cycling parameters used in this study:

Organism	Mgcl2 (mM)	Primers (mM)	dNTP (mM)	PCR condition
Campylobacter jejuni	2.5	0.5	200	94°C—3 min [94°C—1 min/56°C—1 min/72°C—1 min] X 40 cycle 72°C—5 min

Results

Although the PCR method, in principle, can detect a single bacterial cell with extended cycle regimens (50-60), the detection limit of direct PCR is effectively confined to about 10^4 bacteria per gram of food. This limitation is due to reaction volume constraints (25-100 µl), the increased propensity to amplify nonspecific products at high cycle numbers, and the inhibitory effect of many food components on *Taq* polymerase. Thus, the coupling of enrichment procedures with the PCR has effectively served two purposes: 1) It increases the sensitivity of detection to as few as 0.1 organism per gram of food; 2) it demonstrates by comparison of pre- and post-enrichment inoculate that the food contains viable organisms. The added sensitivity and information regarding cell viability warrants the additional 4-24 hours (depending on the growth characteristics of the organism) required for such a procedure, and overcomes an early criticism that PCR would give false-positive results because it amplifies any DNA, including that of dead or nonviable organisms.

PCR detection of *C. jejuni* putative gene CeuE from the minced beef meat. Single PCR product of CeuE gene with an expected size of 793 bp (Fig.1) were observed in 30 out of 100 minced meat samples.

Rapid technologies facilitate the screening of large numbers of samples in a amount of time. Thus, the PCR assays would be suitable for screening all of the minced meat tested for the presence of*, C. jejuni.*

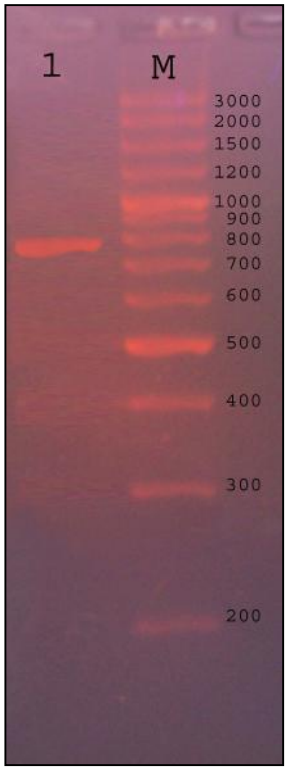

Fig (1) Ethidium bromide stained 2% agarose gel electrophoresis showed the amplified fragment.
M :Marker 100 bp standard (# SM0323) Fermantas .
Line (1) *C. jejuni, ceuE* gene.

Discussion

PCR- method allowed for rapid genetic identification of many *Campylobacter* species direct from the samples. Overall, this method was found to be relatively simple and highly discriminatory and encompassed a broader species range in its application than previously published genotypic methods.

This procedure should add a useful assay to the clinical microbiology laboratory for the differentiation and identification of organisms from these closely related genera (**Eyers *et al*, 1993**, **Hani and Chan 1995, Cardarelli- *et al*, 1996** and **Linton *et al,* 1996**).

The occurrence of food borne pathogens in minced beef was 30% analysed in the present investigations. The finding reinforce the importance of controlling zoonotic pathogens in meat through a complete, continuous farm- to-fork test system(**Kinga *et al*, 2009)**. This present result was slightly lower than which recorded by (**Sherry *et al;* 2009**) as 40 % and by **(Orla *et al* 2011),** who recorde (36%) Campylobacters in beef meat. On the other hand our investigation showed higher than the results which recorded by **Cloak *et al.* (2001)** who recorded incidence 20% in minced beef meat and **Wrong *et al.*(2007)** who recorded the results as 3.5% in beef.

The prevalence of pathogens on the animal from the exsanguinations and the point at which the viscera are removed,the vast majority of the isolates (95.2%) were *C.jejuni* **(Akopayanz *et al*, 1992).**The risk factors of human *Campylobacter sp.* infections include the consumption of contaminated meat, and the handling of the contaminated raw meat, as well as cross contamination with other ready to eat products,also minced meat can also contaminated with different *Campylobacter* **(Humphrey and Jorgensen, 2006** and **Rhoades *et al*, 2009).**The results recommend the importance of adequate cooking of meat and good hygiene to avoid cross- contamination. However, there is no doubt that infection with food borne pathogens in red meat animals represents a substantial risk to human health **(Adak *et al*, 2005; Rhoades *et al*, 2009; Wieczorek and Osek, 2010** and **www.efsa.europe.eu 2010).**

To reduce the number of *campylobacter* in minced meat,it must be stored overnight (4°C), because bacteria died in 17.5% of samples kept at 4 °C, (**Ala'a, and Hamzah 2011).,** and subsequently heated before eat because no bacteria

could be detected after a heating time of 5 min. (**Van Asselt and Zwietering., 2006** and **Aarieke *et al.*, 2012).**The bacterial isolates recovered in the present study were characterized by the determination of several known or putative virulence marker genes using the PCR approach. Also, **Zhao *et al.* (2001)** analyzed 100 beef meat sample for the presence of *Campylobacter sp.* and the results indicated that beef meat,(both at slaughterhouse and retail levels), may be contaminated with more than one food borne bacterial species that are potentially pathogenic for the consumers especially *Campylobacter* **(Bang *et al.*, 2003)** found that a high prevalence (100%) of four different putative virulence and toxin genes (cadF, ceuE, flaA and cdtB) among *C.jejuni* and *C.coli* isolates obtained from cattle determined by PCR.

Abilities of PCR, provides a specific result faster (within 29 h) than traditional culture based methods (72 h). Therefore, in conclusion, this PCR system could prove useful as a routine tool for the swift and reliable detection of *Campylobacter jejuni* in beef minced meat.

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استخدام تفاعل انزيم البلمرة المتسلسل للكشف عن ميكروب الكامبيلوباكتر *المعوية* في اللحوم البقرية المفرومة

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معهد بحوث صحة الحيوان ، الدقي وفرع بنها

الملخص العربى

أجريت هذة الدراسة لتحديد ميكروب الكمبيلوباكتر في اللحم المفرومة بامكانية تحديد الجين الخاص بة باستخدام تفاعل انزيم البلمرة المتسلسل لما لة من احتمالية ان يشارك في الآلية المرضية. من الأمراض التي تنقلها عن طريق الاغذية،و مجموعة الجينات بين الكامبيلوباكتر المعوية فضلا عن جين ceuE تم الكشف عن طريق استخدام اختبارانزيم البلمرة المتسلسل (PCR) التي تقلل من الوقت والعمل تشارك في فحص المنتجات الغذائية لوجود العوامل الممرضة 100%. في هذا العمل تم التوصل إلى الإصابة بالميكروب بنسبة 30%. فى اللحوم المفرومة.