Original Article

Polymerase chain reaction in the detection of contamination of chicken meat by Campylobacter jejuni.

T. Arul kumar1* S.Saravanan2 P. Viswanathan3 and M.Sasikala4

1M.V.Sc. graduate, Department of Veterinary Epidemiology and Preventive Medicine Veterinary College and Research Institute, Namakkal, Tamilnadu, India
Phone: 09442276235, E-mail: akumar@altech.com
2Assistant Professor, Department of Veterinary Epidemiology and Preventive Medicine, Veterinary College and Research Institute, Namakkal - 637 002, Tamilnadu, India.
Phone: 94442 19316, E-mail: sarvet_25@yahoo.com
3M.V.Sc. graduate, Department of Veterinary Epidemiology and Preventive Medicine Veterinary College and Research Institute, Namakkal, Tamilnadu, India
Phone: 9344414364
4Department of Veterinary pathology, Veterinary College and Research Institute, Namakkal, Tamilnadu, India
Phone: 9486067886

* Corresponding author: E-mail: akumar@altech.com
Received 25 November 2011; accepted 10 December 2011

Abstract
An investigation was conducted to detect the contamination of chicken meat rendered at various chicken outlets of different locations in Namakkal district of Tamilnadu in India. Polymerase chain reaction was employed in the identification of Campylobacter in meat samples and it could reveal a positivity of 13.33 per cent.

Key words: Chicken meat, Campylobacter jejuni, contamination, polymerase chain reaction, 402 base pairs.

1. INTRODUCTION
Food borne diseases have been documented in recent years as a currently important, communicable and highly infectious disease affecting millions of people in developing countries, as a consequence of poor food hygiene. Poultry and poultry products have repeatedly been implicated as a source of food borne infection in humans (4). Campylobacter jejuni is recognized worldwide as an important cause of food-borne illness and is a major concern to the poultry industry (8).

The rate of Campylobacter infections worldwide has been increasing, with the number of cases often exceeding those of salmonellosis and shigellosis (1). Poultry is recognized as a reservoir for C. jejuni which in man affects both the small and large intestine resulting in diarrhea, nausea, abdominal cramps, muscle pain, headache and fever. Hence, the study aimed at the detection of C. jejuni in chicken samples by employing polymerase chain reaction (PCR), as this molecular technique has been identified as a sensitive and specific technique to detect the pathogen in poultry samples (5).

2. MATERIALS AND METHODS

Two hundred and ten chicken meat samples were collected in separate sterile containers from various retail poultry meat processing plants at five different locations in and around Namakkal of Tamilnadu. To perform PCR for identification of C. jejuni, the bacterial cultures prepared of the samples were diluted with phosphate buffered saline (PBS), centrifuged at 10,000 rpm for 30 min and the pellet was resuspended in 50µl of distilled water. The samples in eppendorf tubes were placed in a boiling water bath for 10
min and immediately kept on ice to destroy any residual enzymatic activity and then used as a template DNA for PCR reaction.

The primers were selected with the following sequence as recommended by Denis et al., 1999 (3) and were custom synthesized (Genei, Bangalore).

Forward: A-5’ – CTA TTT TAT TTT TGA GTG CTT GTG-3’
Reverse: B-5’ – GCT TTA TTT GCC ATI TGT TTT ATT A-3’

The primers were reconstituted in 100 μl of sterile triple distilled water (TDW) and the tubes were kept at room temperature with occasional shaking for one hour. The tubes were spun briefly and the supernatant primer solution was distributed into 10 μl aliquots and stored at -20°C. PCR was performed in a total volume of 50 μl reaction. A master mix was prepared before setting up the PCR reaction by mixing the following reagents in 45 μl volume: PCR reaction buffer -100 mM Tris Hcl, 500 mM Kcl and 15 mM MgCl₂, Primers - 20 pM of each primer, dNTPs - 200 μM of each dNTP and Taq polymerase - 1.5 unit. The mastermix (450μl) consisted of PCR reaction buffer (10x)- 50 μl, Forward primer-10 μl, Reverse primer - 10 μl, dNTP mix - 40 μl, Taq polymerase - 5 μl and TDW - 335 μl. To each PCR tube, 45μl of master mix and 5μl of template DNA were added with a negative control. The tubes were spun briefly and PCR was run in the thermal cycler with the following programme (25 cycles): Initial denaturation -95°C/15 min, denaturation - 94°C / 30 sec, annealing - 48°C / 90 sec, extension - 72°C/ 1 min and final extension - 72°C/10 min.

The PCR products were detected by electrophoresis in a 2 per cent agarose gel in Tris borate ethidium bromide (TBE) buffer (1x) and the results were documented in gel documentation system (Bio-rad laboratories, USA).

3. RESULTS

Campylobacter jejuni DNA in the culture was confirmed by the amplification at 402 bp fragment and no amplification was observed in the negative control kept along with the samples (Figure 1). Out of 210 chicken meat samples, 28 (13.33%) were positive for campylobacter jejuni by PCR.

4. DISCUSSION

Identification of Campylobacters and related bacteria is well known to be problematic, principally because of their complex taxonomies, biochemical inertness, fastidious growth requirements as On, 2001 (7) stated that the phenotypic identification schemes for Campylobacter spp. are often difficult to interpret. However, polymerase chain reaction could be useful in the investigation of C. jejuni contamination in poultry samples (5). In this study, the technique detected the gene of C. jejuni with DNA amplification at 402 bp, which corroborated the finding of King et al., 1997 (6). This study in the above locality revealed a remarkable but rising degree of contamination, however Corry and Atabay, 2001(2) reported a high degree (56.3 per cent) of contamination of chicken meat with Campylobacter.

Food borne pathogens might gain entry into the meat certainly due to faecal contamination, unhygienic slaughter, cleaning with contaminated water, cleanliness of meat handlers and contaminated equipments, and poor environmental hygiene. Hence, the halt of food borne pathogens could be achieved by good rendering plants, optimization of management in rearing houses, hygienic design of all equipments supplemented with good personal hygiene. Further to ensure food safety, poultry meat should be cooked properly before consumption.

5. ACKNOWLEDGEMENTS

I express my gratitude to the Dean and the concerned staff of various departments at Veterinary College and Research Institute, Namakkal of Tamilnadu in India for providing necessary facilities during the study.

6. REFERENCES


Source of support: Nil; Conflict of interest: None declared