RESEARCH NOTE

DETECTION OF *CAMPYLOBACTER* IN DUCK USING STANDARD CULTURE METHOD AND MULTIPLEX POLYMERASE CHAIN REACTION

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Abstract. In 2005, total of 140 samples of duck meat and intestine from slaughterhouses in Nakhon Pathom Province, Thailand, were analyzed for *Campylobacter* spp. Twenty-eight samples (20%) were positive for *Campylobacter* spp using the standard culture method (SCM) with 21 samples of *C. jejuni* and the other 7 *C. coli*. Forty-four samples (31%) were positive using multiplex polymerase chain reaction, with 34 samples of *C. jejuni* and 10 of *C. coli*. This is the first report of *Campylobacter* contamination in duck in Thailand.

INTRODUCTION

Campylobacter are gram-negative bacteria in the family Campylobacteriaceae with microaerobic growth requirement. *Campylobacter* spp is recognized as one of the most prevalent causes of human foodborne diarrheal illness in children and young adults in developing countries (Rasrinual *et al*, 1988; Oberhelman and Taylor, 2000). Human *Campylobacter* infection is widely held to be the result of handing and consuming of raw poultry and cross contamination of uncooked products (Tauxe, 1992). The prevalence of *Campylobacter* in animals has been reported from many countries (Pezzotti *et al*, 2003; Boonmar *et al*, 2005; Padungtod and Kaneene, 2005, Tsai and Hsiang, 2005), but there is no information about prevalence of

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Campylobacter in duck in Thailand.

Differentiation of *C. jejuni* and *C. coli* has traditionally relied on the hippurate hydrolysis test; *C. jejuni* hydrolyses hippurate whereas *C. coli* does not. Polymerase chain reaction (PCR) has become a reliable alternative to the traditional biochemical method of detection. PCR can identify bacteria at the gene level and this assay has been used for the detection of *Campylobacter* in poultry (Giesendorf *et al*,1992; Hazeleger *et al*, 1994), chicken litter (Itoh *et al*, 1995), raw milk and dairy products (Wegmuller *et al*, 1993). Harmon *et al* (1997) and Wesley *et al* (1997) have succeeded in differentiating *C. jejuni* and *C. coli* using PCR. The purpose of this study was to determine the presence of *Campylobacter* spp in duck using standard culture method (SCM) in comparison with PCR.

MATERIALS AND METHODS

Duck samples

A total 140 samples of duck meat and duck intestine were collected from slaughterhouses in Nakhon Pathom Province, Thailand,

between December 2004 and November 2005. All samples were kept on ice and sent to the laboratory within 24 hours of collection.

Campylobacter strains

Campylobacter coli NCTC 11353 and C. jejuni ATCC 33291 reference strains were obtained from The National Institute of Health, Ministry of Health,Thailand.

Isolation and identification of Campylobacter spp

Campylobacter spp isolation and identification were conducted according to Boonmar et al (2005). In brief, 1 g of meat or intestine sample was added to 9 ml of Preston Campylobacter selectve enrichment broth (Brucella broth supplement with Preston Campylobacter selective supplement SR 117, Campylobacter growth supplement SR 84 (Oxoid, Hamshire, UK) and 5% lysed horse blood), and incubated under microaerobic conditions (5% O_2, 10%CO_2, 85%N_2) at 42ºC for 24 hours. The enrichment broth culture was then streaked onto modified CCDA-Preston medium plates, Campylobacter blood-free selective agar base (Oxoid) supplemented with Campylobacter CCDA selective supplement SR 155E). Agar plates were incubated under microaerobic conditions at 42ºC for 3-5 days using a gas pack jar system (Mitsubishi Chemicals, Tokyo, Japan). A typical Campylobacter colony was selected for further identification by Gram staining and by biochemical tests (catalase, oxidase and hydrolyse hippurate test).

PCR assay

Campylobacter spp identification was conducted using a modification of the PCR method of Harmon et al (1997). A sample from Preston broth culture was used for DNA extraction (Wesley and Bryner,1989). PCR primers (Table 1) were commercially synthesized. Amplification was performed in a volume of 50 μl containing 5.0 ng DNA, 40 pmol each of C-1, C-4, pg 3 and pg 50, 1 U of Taq DNA polymerase (Invitrogen Corperation, USA), 200 mM each dATP, dCTP, dTTP, and dGTP, 10 mM Tris-HCl and 5.5 mM $MgCl_2$. Samples were subjected to an initial denaturation step at 94ºC for 4 minutes, followed by 30 amplification cycles of 1 minute at 94ºC, 1 minute at 45ºC, and 1 minute at 72ºC, with a final step of 72ºC for 7 minutes. PCR reaction products were separated in a 1% agarose gel following electrophoresis at 100 V for 60 minutes. Gels were stained with ethidium bromide (0.2-0.25 mg/ml) and visualized under uv-lighter.

RESULTS

Amplification of C .jejuni DNA ATCC 33291 yielded two bands of approximately 460 bp and 160 bp (Fig 1), whereas a single band of approximately 460 bp was generated from amplification of C .coli DNA NCTC 11353. Typical results observed after PCR analysis of the duck samples are shown in Fig 1. Amplicons of Preston enrichment broth clearly differentiated C. jejuni from C. coli ; lanes 3, 4, 5 were C. jejuni and lane 6 was C. coli. Of
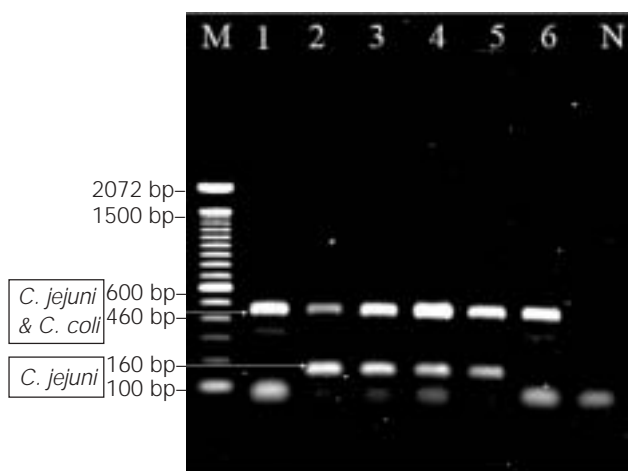

Fig 1–Agarose gel-electrophoresis of PCR reaction products from duck isolates. Lane M, 100 bp ladder (Boehringer Mannheim); lane 1, C. coli NCTC 11353; lane 2, C. jejuni ATCC 33291; lanes 3-6, amplicons from Preston enrichment broth; lane N, a negative control (no template DNA).

Table 1
PCR primers employed for *C. jejuni* and *C. coli* detection.

Species	Product size (bp)	Primer	Nucleotide sequence (5´ to 3´)
C. coli	460	pg3	GAA-CTT-GAA-CCG-ATT-TG
C. jejuni	480	pg50	ATG-GGA-TTT-CGT-ATT-AAC
C. jejuni	160	C-1	CAA-ATA-AAG-TTA-GAG-GTA-GAA-TGT
		C-4	GGA-TAA-GCA-CTA-GCT-AGC-TGA-T

Table 2
A comparison of *Campylobacter* detection using SCM and PCR.

Test		SCM	
		+	-
PCR	+	28	16
	-	0	96
Total		28	112

the 140 duck samples examined, 34 were identified as *C. jejuni* and 10 as *C .coli.*

A comparison of detection of *Campylobacter* spp from 140 duck samples using SCM and PCR is shown in Table 2. SCM produced 28 (20%) positive samples whereas 31% were positive by PCR. Comparing PCR with SCM, it was found that sensitivity of PCR was 100%, specificity 85.71% and efficacy 88.57%.

DISCUSSION

Isolation and identification of *Campylobacter* spp have traditionally involved the use of selective culture media combined with biochemical tests. This method is expensive, laborious and time consuming whereas PCR is cheaper and nearly 4 times faster than SCM. In recent years, PCR has increasingly been applied in detection and identification of *Campylobacter* spp. Several reports using PCR method have shown great improvement in accuracy and sensitivity, associated with fast sample processing (Englen and Fedorka-Cray, 2002; Wang *et al*, 2002). Our study showed

prevalence of *Campylobacter* spp in duck samples was about 31% by PCR but only 20% by SCM (p<0.01). Stoyanchev (2004) found prevalences of 38.3% and 40.8% of *Campylobacter* spp isolated from poultry samples in Bulgaria using SCM and PCR, respectively. Tsai and Hsiang (2005) also found 43.5% of *Campylobacter* isolated from cloacal swabs of ducks using SCM in Taiwan. In the Philippines, Magritrado *et al* (2001) found only 6% prevalence of *Campylobacter* isolated from a total 135 duck and chicken samples using both SCM and PCR. Prevalence of *Campylobacter* isolated from duck in Thailand is higher than that in the Philippines. Padungtod and Kaneene (2005) found 36% of *Campylobacter* from slaughterhouse chickens by SCM in northern Thailand, a relatively low prevalence compared to that in the UK (83%) and Trinidad (80-83%) (Rodrigo *et al*, 2005).

To know the real presence of *Campylobacter* in duck, it is necessary to collect fresh samples and culture immediately after collection. In our study, there was a time delay in bringing samples from slaughterhouse to the laboratory, even though all samples were kept in an icebox. The *Campylobacter* spp positive rate by SCM in this study may be an underestimation. Antimicrobial sensitivity of the duck isolates should also be examined in order to determine the importance of duck as a reservoir of *Campylobacter* infection in Thailand.

from INTERVET company, Thailand.

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