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 handling 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 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 selective 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% O₂, 10%CO₂, 85%N₂) at 42°C 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 42°C 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 Corporation, USA), 200 mM each dATP, dCTP, dTTP, and dGTP, 10 mM Tris-HCl and 5.5 mM MgCl₂. Samples were subjected to an initial denaturation step at 94°C for 4 minutes, followed by 30 amplification cycles of 1 minute at 94°C, 1 minute at 45°C, and 1 minute at 72°C, with a final step of 72°C 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.
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.

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Detection of Campylobacter in Duck, Thailand

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REFERENCES


