Prevalence of *Campylobacter jejuni* in broilers and the adhesion and invasion abilities

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Abstract

Campylobacter jejuni **is the major cause of food borne pathogen in human which the major reservoir of this pathogen is poultry. The *C. jejuni* in broilers had been investigated from the ceca of broilers received from the slaughter house. Twenty broilers/flocks of 7 flocks had been performed. The prevalence of *C. jejuni* in each flock was following 80%, 70%, 55%, 75%, 55%, 65% and 55%. The average prevalence of *C. jejuni* was 65% from the broiler flocks. Adhesion and invasion of 44 strains of *C. jejuni* on INT 407 had been studied. The adhesion and invasion abilities of 44 *Campylobacter* isolates from caecal contents were analyzed with Human embryonic intestine (INT-407) cells using a gentamicin resistance assay. The 44 *Campylobacter* isolates adhered and invade to INT-407 cells at 0.022 to 0.0896% and 0.000035 to 0.00142% of the starting viable inoculum. No correlation between adhesion and invasion abilities of *C. jejuni* to INT-407 had been found. Our findings indicated that *C. jejuni* present in the ceca of broilers were diverse in their abilities to adhere and invade human intestinal epithelial cells among the *Campylobacter* isolates.**

Keywords— *Campylobacter jejuni*, Broilers, prevalence, adhesion, invasion

Introduction

Campylobacter **is one of the most leading causes of acute bacterial diarrhea worldwide (Mead et al., 1999). Infection with *C. jejuni* or *C. coli* is characterized by the sudden onset of fever, abdominal cramps and diarrhea with blood and leukocytes (Blaser et al, 1979; Blaser et al., 1983). There are many possible sources of infection with *C. jejuni* and *C. coli*, as they are part of the normal intestinal flora in a wide range of birds and mammals. Large-scale outbreaks of human campylobacteriosis are rare and are usually linked to the consumption of polluted water or raw milk. Sporadic cases of campylobacteriosis are more common and are associated with the consumption of undercooked chicken. In the United States, case-control studies have attributed 48-70% of the sporadic infections to the consumption of *Campylobacter*-contaminated chickens (Deming et al., 1987; Harris et al., 1986). The percentage of *Campylobacter*-contaminated chicken carcasses varies, often between 50 and 90%, depending on the time of year and the number of carcasses tested. One study found that as many as 98% of chicken carcasses may be contaminated with *C. jejuni* by the time of sale (Stern and Pretanik, 2006). The ability of *C. jejuni* to adhere and invade the epithelial cells of the gastrointestinal tract is important for the development of *Campylobacter*-mediated enteritis (Pei et al., 1998; Russell et al., 1994). The adherence to and invasion of *C. jejuni* into host cells has been studies in a variety of cell lines (de Melo, et al., 1989; Hu and Kopecko, 1999; Konkel et al., 1992; Oelschlaeger et al., 1993). Human embryonic intestine (INT-407) had been widely used to assess the ability of enteric bacteria to adhere and invade epithelium. The objective of this study was to study the prevalence of *C. jejuni* in broiler intestine and the abilities of their adherence and invasion.**

Materials and Methods

BACTERIAL STRAINS AND CULTURE CONDITIONS

Campylobacter isolation was previously described by membrane filtration techniques (Kulkarni et al., 2002). Briefly, caecal contents of 7 broiler flocks were collected for 20 samples of each using a sterile swab. The swabs were incubated in Preston broth (Nutrient broth no.2) (Oxoid, Hampshire, England) containing 5% lysed horse blood, *Campylobacter* growth supplement and modified Preston selective supplement (2500 IU of polymyxin B, and 5 mg each of rifampicin, trimethoprim and amphotericin B) (Oxoid, Hamshire, England) for 24 h or overnight, 37ºC. Eight drops of each broth culture were spotted on a cellulose acetate membrane with 0.45 µm pores, diameter 47 mm (Sartorius, Goettingen, Germany). The membrane was placed on the surface of a blood agar base no. 2 (Oxoid, Hamshire, England) containing 5% sheep whole blood and *Campylobacter* growth supplement (Oxoid, Hamshire, England). The membrane was left on the agar surface for 30 min to let all the fluid passed through. The pores allowed relatively slender and naturally spiraling 'cork screw' motile to pass through whereas other bacteria harbored in intestine were excluded on the 0.45 µm cellulose membrane. The culture plates were incubated for 48 h, 37ºC in an atmosphere of 5% O_2, 10% CO_2 and 85% N_2 using an anaerobic jar with CampyGen (Oxoid, Hampshire, England). Colonies of *Campylobacter* were identified to the genus level by typical morphology on Gram stain (slender, curved, 'seagull wing' shaped or spiral, Gram negative rods). The species differentiation was performed on the basis of nalidixic acid sensitivity and hippurate hydrolysis (Lior, 1984; Smibert, 1963). The *C. jejuni* strains were suspended in Brain Heart Infusion containing 15% glycerol and stored at -80ºC until use.

CELL CULTURE

Human embryonic intestine (INT-407) cells (ATCC CCL-6) were maintained in DMEM (Gibco, Auckland, New Zealand) with 5% FBS (fetal bovine serum), penicillin and streptomycin (Gibco, Auckland, New Zealand) in 5% CO_2-humidified incubator. Confluent stock cultures were trypsinized and new stock cultures were seeded with 10^5 cells/ml onto 24-well tissue culture plates (Corning, USA) for the adherence and invasion assay and incubated at 37ºC in a humidified 5% CO_2 incubator for 48 h, and a semi-confluent monolayer was obtained. Prior to the experiment, the monolayer was washed and incubated with DMEM without antibiotic and FBS.

ADHESION AND INVASION ASSAY

The adherence and invasion assays were performed by the method of Konkel et al. (1989) with some modification. Briefly, *C. jejuni* strains were grown microaerobically on Blood agar No. 2 with supplement for 48 h, 37ºC. Bacteria were harvested from plates with PBS and adjusted spectrophotometrically to approximately $1x10^7$ bacteria/ml. Containing CFU (colony forming unit) approximately 100 times higher than cell number was inoculated into duplicated wells of a 24-well tissue culture plate containing semiconfluent monolayers of INT-407 cells. The actual numbers of bacteria in the inoculums added to monolayers were determined retrospectively by serial dilution and plate counting. Infected monolayers were incubated for 3 h at 37ºC under a 5% CO_2 humidified atmosphere to allow bacterial adherence and internalization. For determination of adherence, the cells were washed 3 times with PBS (phosphate buffered saline) and the cell monolayer was lysed with 0.5% deoxycholate (W/V) (Sigma-Aldrich, Auckland, New Zealand) total bacteria associated with the cells (intracellular and extracellular bacteria) were enumerated by plating serial dilutions of the lysates on MH (Müller Hinton) agar (Oxoid, Hamshire, England) with 5% sheep blood and counting the resultant colonies. In order to measure bacterial invasion, the infected cells were washed 2 times with PBS and incubated in fresh PBS containing 1% fetal bovine serum (FBS) and 150 µg/ml gentamicin for 2 h to kill remaining viable extracellular bacteria. In preliminary experiments 150 µg/ml of gentamicin killed all bacterial strains 3 h after exposure. Quantification of viable intracellular bacteria was performed by washing the infected eukaryotic cells with PBS twice and subsequent lysing with 0.5% deoxycholate (W/V). Following serial dilution in PBS, released intracellular bacteria were enumerated as described for the adherence assay.

Adherence bacteria were expressed as the percentage of bacteria counted without antibiotic treatment referred to the infection dose. Because the bacterial invasion rate is very low it can be neglected without marked influence on the result. Invasion ability was expressed as the percentage of the bacterial inoculum surviving for the gentamicin treatment.

Results and Discussion

PREVALENCE OF *C. JEJUNI* IN BROILERS

The *C. jejuni* in broilers had been investigated from the caeca of broilers received from the slaughter house. Twenty broilers/flocks of 7 flocks had been performed. The prevalence of *C. jejuni* in each flock was following 80%, 70%, 55%, 75%, 55%, 65% and 55%. The average prevalence of *C. jejuni* was 65% from the broiler flocks. This data accords to the previous report that the prevalence of *Campylobacter spp.* in Thai broiler is 60% (Padungtod et al., 2002)

ADHESION AND INVASION BY *C. JEJUNI*

To test the pathogenic properties of *Campylobacter* isolates, the adhesion and invasion abilities of 44 *Campylobacter* isolates from caecal contents were analyzed with Human embryonic intestine (INT-407) cells using a gentamicin resistance assay. After 3 h incubation, the 44 *Campylobacter* isolates adhered to INT-407 cells at 0.022 to 0.0896% of the starting viable inoculum. The invasion abilities of the 44 isolates to INT-407 were 0.000035 to 0.00142% of the starting viable inoculum. No correlation between adhesion and invasion abilities of *C. jejuni* to INT-407 cells had been found. The percentage of recovery in this method showed lower adherence and invasiveness than a report by Biswas et al. (2000) that 0.7416-2.1714% and 0.0012-0.4226% of the range of adherence and invasion, repectively. Our findings indicated that *C. jejuni* present in the caeca of broilers were diverse in their abilities to adhere and invade human intestinal epithelial cells among the *Campylobacter* isolates.

Table 1 The adhesion and invasion abilities of 44 strains of Campylobacter jejuni

strains	Adhered	Adhered (%)	Invaded	Invaded (%)
81116	$(4.04\pm0.71)\times10^3$	0.0404	12.5	0.000125
11168	$(2.48\pm0.71)\times10^3$	0.0248	28.5	0.000285
C57	$(5.22\pm1.41)\times10^3$	0.0522	11.0	0.00011
C67	$(2.89\pm2.83)\times10^3$	0.0289	17.0	0.00017
BK11	$(5.19\pm4.24)\times10^3$	0.0519	4.5	0.000045
BK12/5	$(2.96\pm2.83)\times10^3$	0.0296	25.0	0.00025
BK15	$(2.88\pm2.12)\times10^3$	0.0288	7.5	0.000075
BK21/23	$(4.04\pm1.41)\times10^3$	0.0404	12.5	0.000125
BK22/16	$(8.95\pm2.83)\times10^3$	0.0895	13.5	0.000135
BK22/17	$(2.84\pm3.54)\times10^3$	0.0284	3.5	0.00035
BK22/19	$(4.04\pm2.12)\times10^3$	0.0404	13.0	0.00013
BK23/23	$(7.20\pm0.71)\times10^3$	0.0720	67.5	0.000675
BK24/7	$(5.60\pm2.83)\times10^3$	0.0560	15.5	0.000155
S1H6	$(4.39\pm3.54)\times10^3$	0.0439	9.0	0.00009
S1H7	$(2.19\pm2.83)\times10^3$	0.0219	14.0	0.00014
S1H8	$(6.72\pm0.71)\times10^3$	0.0672	28.5	0.000285
S1H9	$(3.65\pm0.71)\times10^3$	0.0365	16.5	0.000165
S1H12	$(8.96\pm2.12)\times10^3$	0.0896	59.5	0.000595
S3H1	$(4.59\pm0.71)\times10^3$	0.0459	42.0	0.00042
S3H5	$(1.65\pm2.83)\times10^3$	0.0165	98.5	0.000985
S3H8	$(3.01\pm1.41)\times10^3$	0.0301	9.0	0.00009
S4H3	$(2.20\pm2.83)\times10^3$	0.022	3.5	0.000035
S4H5	$(3.62\pm2.12)\times10^3$	0.0362	8.5	0.000085
S5H5	$(2.53\pm2.12)\times10^3$	0.0253	34.0	0.00034
S5H6	$(4.62\pm2.83)\times10^3$	0.0462	43.0	0.00043
S5H10	$(5.65\pm0.71)\times10^3$	0.0565	39.5	0.000395
S5H11	$(3.20\pm3.54)\times10^3$	0.032	24.5	0.000245
S5H15	$(8.65\pm1.41)\times10^3$	0.0865	26.5	0.000265
S6H6	$(5.89\pm0.71)\times10^3$	0.0589	10.0	0.0001
S6H16	$(3.90\pm2.12)\times10^3$	0.0390	14.0	0.00014
S7-9	$(4.18\pm2.83)\times10^3$	0.0418	12.5	0.000125
S11/1	$(5.66\pm1.41)\times10^3$	0.0566	27.0	0.00027
H16	$(2.81\pm3.54)\times10^3$	0.0281	11.5	0.000115
FFW4-5	$(5.0\pm2.83)\times10^3$	0.05	13.0	0.00013
GH17	$(3.90\pm1.41)\times10^3$	0.039	13.5	0.000135
G2H7	$(4.12\pm3.54)\times10^3$	0.0412	6.0	0.00006
G3-16	$(3.26\pm2.83)\times10^3$	0.0326	11.5	0.000115
G6H9	$(4.65\pm0.71)\times10^3$	0.0465	12.0	0.00012
G7H10	$(7.01\pm2.12)\times10^3$	0.0701	30.5	0.000305
G7H13	$(6.90\pm0.71)\times10^3$	0.069	41.5	0.000415
G7H16	$(4.89\pm3.54)\times10^3$	0.0489	52.0	0.00052
GLB3-5	$(6.79\pm2.12)\times10^3$	0.0679	141.5	0.00142
GLB3-9	$(4.15\pm2.83)\times10^3$	0.0415	20.0	0.0002
GLB3-18	$(4.57\pm0.71)\times10^3$	0.0457	11.0	0.00011

ACKNOWLEDGEMENTS

Our works were supported by The Thailand research fund.

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