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Campylobacter in Food Animals and Humans in Northern Thailand

PAWIN PADUNGTOD[1] AND **JOHN B. KANEENE**[2]*

[1]*Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand; and* [2]*Population Medicine Center, Michigan State University, East Lansing, Michigan 48824-1314, USA*

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ABSTRACT

Cross-sectional, longitudinal, and case-control studies were conducted to describe the epidemiology of *Campylobacter* in chickens, swine, dairy cows, farm workers, nonfarm residents, and children with diarrhea. Samples were collected in Chiang Mai and Lamphung provinces of northern Thailand from 2000 through 2003. A total of 2,360 samples were processed. Results from the cross-sectional study indicated that the prevalences of *Campylobacter* in chickens at the farm, slaughterhouse, and market were 64, 38, and 47%, respectively. In swine, the prevalences at the farm, slaughterhouse, and market were 73, 69, and 23%, respectively. *Campylobacter* prevalence was 14% in dairy cows and 5% in raw milk. The prevalence of *Campylobacter* on farms was lower in environmental samples than in samples collected from live animals. No *Campylobacter* isolates were obtained from healthy nonfarm residents, but isolates were obtained from 5 and 18% of farm workers and children with diarrhea, respectively. The prevalence of *Campylobacter* in pigs in the longitudinal study was 61% at the farm, 46% at the slaughterhouse, and 33% at the market. The majority of *Campylobacter* isolates from chickens (52%), swine (98%), and farm workers (66%) were *Campylobacter coli,* whereas the majority of isolates from dairy cows (63%) and children with diarrhea (62%) were *Campylobacter jejuni.* Most *Campylobacter* isolates from diarrheal children had single-strand conformation polymorphism profiles similar to those of isolates from chickens. None of the risk factors for infection in children with diarrhea were significantly associated with the isolation of *Campylobacter.*

Campylobacter spp. are gram-negative nonsaccharolytic bacteria in the family *Campylobacteriaceae* with microaerobic growth requirements and low G+C content *(34).* There are 14 known species of *Campylobacter,* which can be separated into two groups according to their optimal growth requirements. Most species pathogenic for humans are thermophilic, growing well at 42°C under microaerobic conditions *(24).*

The clinical signs of *Campylobacter* infection in humans include fever, myalgia, athralgia, and gastrointestinal illness *(10, 30). Campylobacter* species have been deemed a major cause of illness in children in developing countries and in young adults in developed countries *(6, 17).* In Thailand, these bacteria are the most common pathogens found in children <12 years old that have dysentery *(2).*

The main source of *Campylobacter* infection is believed to be food of animal origin, such as milk *(12)* and poultry meat *(18).* Human *Campylobacter* infection has been most often attributed to cross-contamination from raw poultry, drinking of nondisinfected water, consumption of poultry bought raw *(13),* and consumption of bird-pecked milk *(32).* In previous studies in Thailand, the prevalence of *Campylobacter* spp. was reported as >60% in broiler chickens *(22)* and 12% in foods of animal origin purchased at the market *(19, 25).*

A few species of *Campylobacter* found in animals result in clinical disease, including *Campylobacter fetus,* which is associated with reproductive disease *(9).* When animals become infected with other species of *Campylobacter,* including those causing enteritis in humans, they do not typically exhibit clinical disease. Consequently, clinically normal animals, especially food-producing animals, can be an important source of human *Campylobacter* infection and disease.

Recent advances in the typing of strains of bacteria have added valuable information to studies exploring the epidemiology of foodborne diseases, and methods for isolation and identification of *Campylobacter* spp. have improved dramatically during the past 20 years. The relationships among *Campylobacter* species isolated from various sources have been determined with selective media, which utilize antimicrobial agents to limit growth of all organisms other than *Campylobacter (7),* with molecular techniques for species identification and molecular profile comparison, and with species identification systems such as API Campy (bioMérieux, Marcy l'Etoile, France). In particular, the single-strand conformation polymorphism (SSCP) assay *(11)* has been used to provide evidence that *Campylobacter* isolates from various sources were genetically and possibly epidemiologically related *(33).*

The purpose of this study was to further understand the epidemiology of *Campylobacter* spp. in Thailand by determining the prevalence of *Campylobacter* spp. (i) in a variety of food-producing animal species by taking samples from live animals on the farm and from foods available for

* Author for correspondence. Tel: 517-353-5941; Fax: 517-432-0976; E-mail: kaneene@cvm.msu.edu.

purchase at the market, (ii) in humans with high and low animal or animal-product contact, and (iii) in farm, slaughterhouse, and market environments associated with these animals. In addition to determining prevalence, the SSCP patterns of *Campylobacter* from food animals and humans were compared to identify any common sources of infection between these groups. Results from these studies should form a basis for risk assessment and future intervention studies intended to reduce the incidence of *Campylobacter* infection in Thailand.

MATERIALS AND METHODS

In this study, *Campylobacter* isolates were collected during three phases of a larger project examining *Campylobacter* in food animals and humans in Thailand: a cross-sectional phase, a longitudinal phase, and a case-control phase. The cross-sectional study was conducted to estimate the prevalence of *Campylobacter* in chickens, swine, dairy cows, farm workers, nonfarm residents, and children hospitalized with diarrhea, the longitudinal study was conducted to identify potential *Campylobacter* contamination points in the pork production system from farm to market, and the case-control study was conducted to identify risk factors associated with *Campylobacter* infection in children hospitalized with diarrhea.

Sample size. The number of individual animal samples collected from each farm was based on a true population prevalence of 50%, a 95% confidence level, and an estimated prevalence within 5% of the true prevalence on the farm *(31)*.

Sample collection. Samples were collected and processed during May through July of 2000 through 2003. All sampling sites were located in the Chiang Mai and Lamphung provinces of northern Thailand within 3 h of the laboratory. Study locations were selected by convenience sampling for logistical reasons. Study locations included farms, slaughterhouses, fresh meat markets, and hospitals. The farms, slaughterhouses, and markets selected were required to keep records so that animals could be tracked from the farm to the slaughterhouse and subsequently from slaughter to the market.

Individual animals were randomly selected for sampling based on their age and stage of production. Pigs <1 month old before slaughter, chickens <2 weeks old before slaughter, and milking cows were included in the on-farm study. The same pigs and chickens were tracked and samples were obtained from them at the slaughterhouse and again at the markets. All workers on the farms and slaughterhouses involved in the study were asked to provide stool samples. The parents of all hospitalized children with diarrhea within the study area were contacted for participation while the child was hospitalized. Because of the low numbers of farm workers, slaughterhouse workers, and children with diarrhea, efforts were made to contact and enroll all possible candidates. Approval for research involving humans and animals was given by the Chiang Mai University Committees on Human Subjects and Animal Research, respectively.

For the cross-sectional study, samples were collected once from each study subject. For the longitudinal study, samples were collected from the same animals at the farm, the slaughterhouse, and market. At the farm, fecal samples were collected from pigs and dairy cows by rectal evacuation and from chickens by cloacal swabbing. Ten-milliliter milk samples were collected from dairy cows.

At the slaughterhouse, samples were collected by swabbing an approximately 50-cm^2 area of the pig carcass with sterile gauze, and cotton swabs were used to swab under the wing and around the cloaca of the chicken carcass. Samples of mesenteric lymph nodes were collected from pigs after butchering. The slaughterhouses participating in our study were small-scale facilities providing meat for local market consumption. The poultry slaughterhouses processed 500 to 800 birds per night. At the slaughterhouse, birds from various farms were kept together in the holding pen while waiting to be slaughtered by hand. After mechanical defeathering, carcasses were chilled in cold water without evisceration, because evisceration is done at the market to provide consumers with viscera for separate purchase. Chicken slaughterhouse samples were collected after defeathering and before chilling. The pig slaughterhouse in this study did not use machinery for the slaughtering process. After slaughter, the pigs were dehaired, eviscerated, and cut into six pieces by hand. Pig carcasses, including visceral organs, were delivered to the market directly after slaughtering. The slaughterhouse samples were collected from the pigs at the end of the butchering process but before shipment to market.

At the market, approximately 100 g of pork from the neck area attached to the head (with the ear tag) and a thigh from each chicken were purchased. All farm, slaughterhouse, and market environmental samples were collected with a sterile gauze swab soaked in sterile skim milk, which was used as the transport medium for these samples. All samples were held in an icebox or refrigerator until further processing, which was to be completed within 48 h of collection.

Farm workers and nonfarming neighbors were asked to submit 10-g stool samples in sterile cups containing Cary-Blair transport medium provided by the investigator. With parental consent, rectal swabs were collected from children with diarrhea at the study hospitals. Swabs were kept in semisolid Cary-Blair transport medium and held as described for fecal and environmental samples.

Pretested questionnaires were administered by the principal investigator at the farm to collect information regarding general farm management and risk factors for *Campylobacter* infection. To ascertain exposure to food animals for the children with diarrhea, pretested questionnaires concerning food consumption and animal contact were administered to the consenting parents by the same nurse who collected the stool specimen from the hospitalized child. Instruction on completion of the questionnaire was provided to the nursing staff by the principal investigator.

Isolation and identification of *Campylobacter* spp. In this study, *Campylobacter* spp. isolation and identification was achieved using selective media and basic biochemical tests.

Approximately 10 g of meat was excised from each purchased sample and minced. All swabs and meat samples were placed in 10 ml of Bolton broth (Oxoid, Basingstoke, UK) supplemented with antimicrobial agents (cefoperazone, trimethoprim, vancomycin, and amphotericin B) as enrichment medium to resuscitate damaged cells and limit growth of other bacteria. The broth was incubated at 42°C in an atmosphere with 5% CO_2 for 48 h. A swab of the broth was inoculated onto Karmali agar (KSA) or Preston agar (Oxoid) supplemented with antimicrobial agents (polymyxin B, rifampin, trimethoprim, and cycloheximide) as the selective medium. Fecal, cloacal swab, and rectal swab samples were inoculated directly onto KSA. All selective media plates were incubated at 42°C in an atmosphere with 5% CO_2 for up to 5 days.

A single colony with typical *Campylobacter* characteristics (gram-negative spiral rod with positive results for oxidase and catalase tests) was selected from each plate for biochemical test-

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TABLE 1. *Primer sets for multiplex PCR (36)*

Primer	Sequence (5′ to 3′)	Target *Campylobacter* gene	Product size (bp)
CJF	ACT TCT TTA TTG CTT GCT GC	*C. jejuni hipO*	323
CJR	GCC ACA ACA AGT AAA GAA GC		
CCF	GTA AAA CCA AAG CTT ATC GTG	*C. coli glyA*	126
CCR	TCC AGC AAT GTG TGC AAT G		
CLF	TAG AGA GAT AGC AAA AGA GA	*C. lari glyA*	251
CLR	TAC ACA TAA TAA TCC CAC CC		
CUF	AAT TGA AAC TCT TGC TAT CC	*C. upsaliensis glyA*	204
CUR	TCA TAC ATT TTA CCC GAG CT		
CFF	GCA AAT ATA AAT GTA AGC GGA GAG	*C. fetus sapB2*	435
CFR	TGC AGC GGC CCC ACC TAT		
23SF	TAT ACC GGT AAG GAG TGC TGG AG	*C. jejuni* 23S rRNA	650
23SR	ATC AAT TAA CCT TCG AGC ACC G		

ing. The colony was then grown on *Brucella* agar supplemented with sheep blood. After 48 h of incubation at 42°C in an atmosphere with 5% CO_2, bacteria were suspended in Mueller-Hinton broth, mixed with an equal volume of 60% glycerol, and stored in a −70°C freezer.

Determination of *Campylobacter* species. For some isolates, API-Campy (bioMérieux) kits were used for species identification following the manufacturer's recommendations. For others, a multiplex PCR (MPCR) assay *(36)* was used to identify the species of *Campylobacter.* A 48-h culture of bacteria was suspended in sterile water, and a phenol-chloroform extraction protocol *(1)* was used to extract the DNA. DNA template (2.5 μl) was added to the MPCR mixture containing 1.25 units of *Taq* DNA polymerase (Pacific Sciences, Bangkok, Thailand) reaction buffer (50 mM Tris-HCl, pH 8.3, 10 mM KCl, and 5 mM $[NH_4]_2SO_4$), 20 mM $MgCl_2$, 200 μM deoxynucleoside triphosphates (dNTPs), and the 12 primers (Table 1). The MPCR assay was carried out in a thermal cycler (Thermo Hybaid, Franklin, Mass.) under the following conditions: initial denaturation at 95°C for 6 min, 30 cycles of denaturation at 95°C for 30 s, annealing at 59°C for 30 s, and extension at 72°C for 30 s, and a final extension at 72°C for 7 min. The resulting product was visualized after electrophoresis through a 1.5% agarose gel, which was then stained with ethidium bromide. The species were identified based on the size of the PCR product compared with that of a known *Campylobacter* control.

Molecular typing of *Campylobacter.* Molecular types for *Campylobacter* isolates were determined using an SSCP assay of the *flaA* gene *(11).* A 2.5-μl phenol-chloroform extraction of bacterial DNA was added to the MPCR mixture containing 3 units of *Taq* DNA polymerase reaction buffer (20 mM Tris-HCl and 50 mM KCl), 2.5 mM $MgCl_2$, 800 μM dNTPs, and 0.4 μM primers CF02 (5′-AAG CCA GAA GTG TCC CAA GTT T-3′) and CF03 (5′-GTC CAA AGT GGT TCT TAT GCA TGG G-3′). The thermocycler conditions were initial denaturation at 94°C for 2 min, 28 cycles of denaturation at 94°C for 1 min, annealing at 56°C for 1 min, and extension at 72°C for 1 min, and final extension at 72°C for 5 min. The resulting product was visualized on a 1.5% agarose gel stained with 0.05% ethidium bromide.

The double-strand MPCR product was separated by incubation in 20 μl of loading buffer with 95% formalin at 95°C for 10 min and incubation at −20°C for 10 min. The single-strand DNA was separated with vertical 10% polyacrylamide gel electrophoresis at 100 V for 2 h at room temperature. The patterns resulting were visualized after silver staining with the following procedure. The gel was fixed in 10% acetic acid for 20 min at room temperature, washed three times for 2 min each time with deionized water, stained with 0.1% silver nitrate solution for 30 min under a dark hood, and then washed with deionized water. Color was developed in developer solution (3% Na_2CO_3, 0.056% HCOH, and 0.0002% $Na_2S_2O_3$) for approximately 5 min or until bands were visible. The gel was then washed again in deionized water three times for 2 min each time. The bands were fixed by immersing the gel in 10% acetic acid for 10 min. The gel was then washed with deionized water for 10 min and air dried or dried in an incubator at 37°C for approximately 1 h.

Pictures of the gel were created with a computer scanner. Comparisons of band patterns were made by visual inspection. Identification numbers were serially assigned and used to approximate molecular type based on various band locations. This information was then evaluated, and a DNA profile number was assigned according to each molecular band pattern. These band patterns were then compared among all of the *Campylobacter* isolates collected.

Statistical analysis. Prevalence was calculated by dividing the number of samples from which a *Campylobacter* isolate was obtained by the total number of samples processed. Differences in *Campylobacter* prevalence between various populations, locations, and sample types were determined with the chi-square test or with Fisher's exact test when numbers did not meet the necessary assumptions for the chi-square test (cell counts < 5). McNemar's test for paired proportions was used to compare prevalences of *Campylobacter* obtained with carcass surface swabs versus other slaughterhouse samples (cloacal swabs for chickens and lymph node samples for pigs). Univariate analysis of risk factors potentially associated with *Campylobacter* isolation was conducted using the chi-square test or Fisher's exact test, and simple odds ratios were computed *(15).*

RESULTS

A total of 2,360 samples were collected and processed (Table 2): 1,852 samples from animal production systems and 508 samples from humans. Animal production system samples were collected from a total of 30 chicken farms, two chicken slaughterhouses, seven pig farms, one pig slaughterhouse, 25 dairy farms, and two markets. Human samples were collected from individuals from 64 farms, 40 nonfarm households, one slaughterhouse, and three hospitals.

Campylobacter was isolated in 22 of the 27 chicken

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TABLE 2. *Number of study sites and number of samples collected, by species and location, in Thailand, 2000 through 2003*

		Study type								
		Cross-sectional and longitudinal				Cross-sectional		Case-control		
		2000		2001		2002		2003		
Species	Location	No. of sites	*n*	No. of sites	*n*	No. of sites	*n*	No. of sites	*n*	Total no. of samples
Chicken	Farm	3	155	3	187	24	192			534
	Slaughterhouse	1	206	1	148					354
	Market			1	72					72
Total		4	361	5	407	24	192	0	0	960
Pig	Farm	3	146	4	285					431
	Slaughterhouse			1	167					167
	Market			1	69					69
Total		3	146	6	521	0	0	0	0	667
Dairy cows	Farm					25	225			225
Total		0	0	0	0	25	225	0	0	225
Human	Farm	7	22	8	41	49	136			199
	Nonfarm community					40	100			100
	Slaughterhouse			1	4					4
	Hospital							3	205	205
Total		7	22	9	45	89	236	3	205	508
Total		14	529	20	973	138	653	3	205	2,360

farms (81.5%), with farm prevalences ranging from 12.5 to 87.5% (mean = 38.8%, median = 42.9%). All pig farms ($n = 7$) yielded *Campylobacter,* with farm prevalences ranging from 24 to 87.5% (mean = 62.5%, median = 68.8%). *Campylobacter* was found on 13 of the 25 dairy farms (52%), with prevalences ranging from 11.1 to 33.3% (mean = 8.9%, median = 11.1%).

The prevalence of *Campylobacter* in individual animals on the farm ranged from 14% in dairy cattle to 64% in chickens and 73% in pigs (Table 3). The prevalence of *Campylobacter* was higher on the farm than at slaughter or at the market for both chickens ($P < 0.01$) and pigs ($P < 0.01$). The prevalence of *Campylobacter* in pigs was higher for carcasses in the slaughterhouse than for meat samples from the market ($P < 0.01$), but prevalence for chickens was lower in the slaughterhouse than at the market ($P < 0.01$). In individual pigs at the farm, at slaughter, and at the market, the prevalence of *Campylobacter* was 61.1% (11 of 18), 46.2% (6 of 13), and 33.3% (5 of 15), respectively.

For chickens at slaughter, the prevalence of *Campylobacter* obtained from cloacal swab samples was not significantly different from that obtained from carcass swab samples ($P = 0.86$). For pigs, the prevalence of *Campylobacter* obtained from lymph node samples was significantly higher ($P < 0.01$) than that obtained from carcass swab samples ($P < 0.01$).

The prevalence of *Campylobacter* on farms was lower in environmental samples than in samples collected from pigs ($P < 0.01$) and chickens ($P < 0.01$) (Table 4). Although the farm prevalence of *Campylobacter* in pigs was higher than in chickens, environmental samples from chicken farms yielded more *Campylobacter* isolates than did environmental samples from pig or dairy farms ($P = 0.03$) (Table 4).

The prevalence of *Campylobacter* in humans was lower than that in farm animals (Table 5). In humans, none of the samples collected from slaughterhouse workers and nonfarming households were positive for *Campylobacter,*

TABLE 3. *Prevalence of* Campylobacter *in animals by location and sample type, in Thailand, 2000 through 2003*

Species	Location	Sample type	No. of samples	No. of positive samples	Prevalence (%)
Chicken	Farm	Cloacal swab	415	265	63.9
	Slaughterhouse	Cloacal swab	73	26	35.6
		Surface swab	73	28	38.4
	Market	Meat	72	34	47.2
Pig	Farm	Feces	361	262	72.6
	Slaughterhouse	Lymph node	70	48	68.6
		Surface swab	75	28	37.3
	Market	Meat	69	16	23.2
Dairy cow	Farm	Feces	125	18	14.4
		Milk	25	1	4.0

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TABLE 4. *Prevalence of* Campylobacter *in the farm environment, by species and sample type, in Thailand, 2000 through 2003*

Species	Sample type	No. of samples	No. of positive samples	Prevalence (%)
Chicken	Feed	6	1	16.67
	Feed tray	24	4	16.67
	Pen floor	29	6	20.69
	Water	26	0	0
	Water tray	23	5	21.74
Pig	Feed	6	0	0
	Feed tray	32	2	6.25
	Pen floor	32	8	25
Dairy cow	Feed	25	0	0
	Feed tray	25	1	4
	Pen floor	25	0	0

and the prevalence of *Campylobacter* in farm workers was relatively low. For children with diarrhea, 17.5% of samples were positive for *Campylobacter*.

Identification of *Campylobacter* to species was completed for 343 isolates. In chickens ($n = 140$), the proportion of *Campylobacter coli* increased as chickens were moved from farm to slaughter and from slaughter to market (Fig. 1). All *Campylobacter* isolates from pigs ($n = 50$) were *C. coli,* regardless of sampling location. In dairy cows ($n = 16$), *Campylobacter jejuni* was found more frequently than *C. coli* (62.5 and 6.3%, respectively). The majority (66%) of *Campylobacter* isolates from healthy farm workers ($n = 5$) were *C. coli,* whereas among isolates from children with diarrhea ($n = 29$), *C. jejuni* ($n = 18$) was more common than *C. coli* ($n = 6$).

A total of 53 different SSCP patterns were identified from 370 *Campylobacter* isolates. Chicken isolates had the highest numbers of SSCP patterns (34) followed by those from pigs (20), dairy cattle (7), and humans (4). When comparing SSCP patterns for animal species at different stages of food production (Table 6), 90% of pig isolates and 83% of chicken isolates at slaughter shared SSCP patterns with the farm isolates, and 93% of isolates from chicken meat at the market shared SSCP patterns with isolates from the farm. When comparing isolates from animals and hospitalized children, none of the *Campylobacter* isolates from children shared SSCP profiles with isolates from pigs, but 71.4% of *Campylobacter* isolates from these children shared SSCP profiles with isolates from chickens (Table 6). All environmental isolates with SSCP profiles (16 chicken, 1 pig, 1 dairy) had fewer different patterns (two patterns in chicken environments and one each in pig and dairy environments) and had patterns matching those found in animals on the farm.

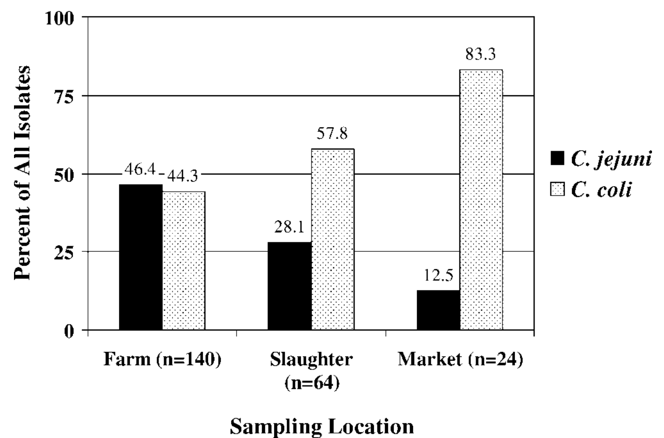

FIGURE 1. *Species of* Campylobacter *isolated from chickens in Thailand, 2000 through 2003.*

Analysis of risk factors for *Campylobacter* infection in children with diarrhea revealed that hospital, gender, age, and consumption of chicken, pork, or milk were not significantly associated with *Campylobacter* isolation (Table 7).

DISCUSSION

***Campylobacter* in animals.** In this study, prevalence of *Campylobacter* (81.5%) in chickens on the farm was relatively high compared with results of other studies (45%) *(21),* whereas the prevalence in pigs on the farm (72.6%) was comparable to that reported previously (63.5 to 79%) *(22, 37).* The *Campylobacter* prevalences observed at the slaughterhouses for chickens (36 to 38%) were relatively low compared with the prevalences reported in studies conducted in similar processing facilities in the UK (83%) and Trinidad (80.2 to 83.9%) *(27),* whereas the slaughterhouse prevalence for pigs (68.6%) was comparable to that in previously reported studies (66%) *(3).* The prevalence of *Campylobacter* on chicken meat at the market in our study was low (47.2%) compared with that in other studies (81.3%) *(22),* whereas the prevalences in pork at the market was higher (23.2%) than that reported in a study in Italy (10.3%) *(22).* The prevalence of *Campylobacter* in food animals in this study was relatively high, suggesting that food animals may be an important source of *Campylobacter* infection (or environmental contamination) in Thailand. Discrepancies between our reported prevalences and those from other studies may be due to differences in farm management and slaughter practices in the various study areas and the use of different selective media and isolation protocols, which may affect the number and species of *Campylobacter* found *(14).*

TABLE 5. *Prevalence of* Campylobacter *in humans, by group and sample type, in Thailand, 2000 through 2003*

Group	Sample	No. of samples	No. of positive samples	Prevalence (%)
Farm workers	Stool	197	9	4.6
Slaughterhouse workers	Stool	4	0	0
Nonfarming community	Stool	100	0	0
Hospitalized diarrhea patients	Rectal swab	205	36	17.6

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TABLE 6. *Comparison of* Campylobacter *SSCP profiles between species and location groups in Thailand, 2000 through 2003*

Group 1			No. of profiles common to both groups	Group 2		
Type	No. of isolates[a]	% similar		Type	No. of isolates[a]	% similar
Chickens on farm	88	51.1	28	Chickens at slaugher	31	90.3
Chickens on farm	88	43.2	13	Chicken meat at market	14	92.9
Chickens at slaughter	31	48.4	4	Chicken meat at market	14	28.6
Pigs on farm	29	41.4	15	Pigs at slaughter	18	83.3
All pigs	47	40.4	21	All chickens	133	39.1
Children with diarrhea	7	0	0	All pigs	0	0
Children with diarrhea	7	71.4	5	All chickens	133	2.3

[a] Number of isolates with SSCP profile determinations.

The relatively high prevalence of *Campylobacter* found in chickens on the farm may be a result of the generally low level of biosecurity in broiler farms in Thailand during the time of study. Our sample farms supplied chickens for local consumption only, and may not have used biosecurity measures as strict as those used on farms raising broilers for export. However, since the study's completion, the Department of Livestock Development has forced all broiler farms to improve their biosecurity measures, particularly after the outbreak of avian influenza in Thailand early in 2004 *(35)*. We found *Campylobacter* in farm environments but at lower prevalences than in live animals. In previous studies conducted outside of Thailand, the environment has been proposed as an important source of infection, which was thought to spread primarily through horizontal transmission *(5)*. Other factors reported to be associated with increased risk of *Campylobacter* infection in chickens at the farm included a chicken house with static air, two or more workers per house, and three or more houses on the farm *(26)*.

TABLE 7. *Risk factors for* Campylobacter *in children hospitalized for diarrhea in Thailand, 2000 through 2003*

Risk factor	No. of samples	No. of positive samples	% positive	Odds ratio	*P*
Hospital					
C	67	14	20.9	1.94	0.579
B	113	19	16.8	1.48	
A	25	3	12.0	Baseline	
Gender					
Female	100	18	18.0	1.06	0.982
Male	105	18	17.1	Baseline	
Age (yr)					
1	119	22	18.5	4.76	0.099
2	50	13	26.0	7.38	
3	22	1	4.6	Baseline	
4	7	0	0.0		
5	5	0	0.0		
Consumed chicken					
Yes	34	7	20.6	1.25	0.817
No	169	29	17.2	Baseline	
Consumed pork					
Yes	57	9	15.8	0.83	0.804
No	146	27	18.5	Baseline	
Consumed milk					
Yes	155	30	19.35	1.68	0.384
No	48	6	12.5	Baseline	

The differences in the prevalence of *Campylobacter* between chickens and pigs at slaughter may be due to several factors. Holding chickens from different locations in a single pen prior to slaughter could result in spread of *Campylobacter* from infected to uninfected birds, and cross-contamination may occur during chilling because all birds were chilled in the same container *(16)*. Because in our study chicken samples were collected prior to chilling, our results may reflect only the preslaughter prevalence of *Campylobacter*. In pigs, slaughterhouse samples were collected from the pigs at the end of the butchering process but before shipment to market. The differences in prevalences between surface swab samples and lymph node samples indicate that the type of sample being collected affects the results. Further studies using standardized sample collection and culturing techniques should be conducted to identify critical *Campylobacter* contamination points during poultry and pork processing.

The highest prevalences of *Campylobacter* in chickens and pigs were observed at the farm, which may indicate that these animals are most likely to acquire *Campylobacter* at the farm. In pigs, the prevalence of *Campylobacter* was lowest at the market, whereas prevalence in chickens slightly increased from slaughter to market. This prevalence increase at the market may be due to the fact that carcass evisceration occurs at the market rather than in the slaughterhouse, which would allow contamination of the carcass with the contents of the gastrointestinal system. Another possibility is that there are other sources of *Campylobacter* contamination at the market that are not present on the farm or at the slaughterhouse.

The identification of *Campylobacter* to species in this study provided insight into the epidemiology of *Campylobacter* in chickens and pigs from farm to market. *C. coli* was the only species of *Campylobacter* found in samples from pigs, similar to findings in France *(20)*, and *C. jejuni* was the most common species found in dairy cattle. However, there was a broad range of relative prevalences of *C. coli* and *C. jejuni* in chickens. The proportion of isolates identified as *C. jejuni* in chickens was slightly higher than

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that for *C. coli* on the farm, but the proportion of *C. coli* increased from farm to slaughter and then from slaughter to market. Given the overall decrease in prevalence and this shift in species proportions, there seem to be important non-farm sources of *Campylobacter* in chickens at market, which has been suggested by other investigators *(16)*. In other studies, *C. jejuni* has been found almost exclusively in chickens at market *(28)*, a finding that may be attributable to differences in slaughter and marketing practices between study areas.

Examinations of SSCP profiles from *Campylobacter* isolates in this study revealed patterns of similarity between different species and different sampling locations (Table 6). More than 90% of *Campylobacter* isolates from chickens at the slaughterhouse and 83% of isolates from pigs at the slaughterhouse shared the same molecular profiles with isolates at the farm, suggesting the possibility of transmission of *Campylobacter* from the farm through the slaughterhouse. This finding in pigs is consistent with the observation made by Borch et al. *(3)*, that the major source of contamination of *Campylobacter* in pigs at slaughter is the pigs themselves. However, in chickens, only 29% of *Campylobacter* isolates from chicken meat at the market shared the same molecular profile with isolates at the slaughterhouse, suggesting that significant contamination in chickens may occur after carcasses arrive at the market.

***Campylobacter* in humans.** There have been several reports concerning *Campylobacter* in humans, particularly in children with diarrhea or dysentery in Thailand. In a case-control study in Thailand, *Campylobacter* was most significantly associated with diarrhea in children <12 months old compared with other organisms, including *Salmonella, Shigella,* and *Escherichia coli (8),* and in a more recent study, *Campylobacter* was the most common pathogen found in children with acute dysentery *(2)*. In a previous study conducted in Chiang Mai *(23), Campylobacter* was found in 6.8% of children with diarrhea, an infection rate lower than the 17.5% found in the present study. This difference suggests the important role of *Campylobacter* in childhood diarrhea in Thailand.

In this study, the majority of *Campylobacter* isolates from children with diarrhea were *C. jejuni,* a finding similar to that reported earlier in Thailand *(2)* and in the United Kingdom, where 93% of *Campylobacter* isolates from patients with diarrhea were *C. jejuni* and only 7% were *C. coli (32)*. Farm workers and slaughterhouse workers harbored a higher proportion of *C. coli* than *C. jejuni* isolates. Given the increasing proportions of *C. coli* in chickens at slaughter and market, this finding in workers suggests that humans and chickens in contact with farm and slaughterhouse workers share a common source of *C. coli.* The source of *C. jejuni* in children with diarrhea is not clear, but the high prevalences of *C. jejuni* in dairy cattle suggest that milk may be a source of *Campylobacter* for children.

When using molecular typing to examine the epidemiology of *Campylobacter* infection in humans, in one study up to 87% of *Campylobacter* isolates from children with diarrhea were the same serotype and biotype as isolates from food of animal origin *(25)*. When molecular profiles were used to compare *Campylobacter* species isolated from chickens, pigs, and diarrheal children in this study, the majority of isolates from children shared similar profiles with isolates from chickens and none shared profiles with isolates from pigs. This finding suggests that there may be a common source of infection for both chickens and children hospitalized with diarrhea. Univariate analysis of chicken consumption as a risk factor for *Campylobacter* in children with diarrhea was not significant, which may be attributable to the fact that children <12 months of age may not be exposed to chicken directly. However, chicken is a very common food in Thailand, and cross-contamination may have occurred in the home where chicken is prepared, as has been documented in other studies *(13)*.

We demonstrated that *Campylobacter* species can be found commonly in various food-producing animals, including chickens, pigs, and dairy cows, in Thailand. Transmission of *Campylobacter* species through the animal processing stages to the market was possible. *Campylobacter* was also isolated in children with diarrhea, and similarities in the molecular profiles of isolates suggest that chickens and children may share an important source of *Campylobacter* infection. Because reduction of *Campylobacter* prevalence at the farm would result in reduction of incidence of *Campylobacter* infection in humans *(29)* and food of animal origin may pose a risk of *Campylobacter* infection for consumers, continued development of methods to reduce the risk of *Campylobacter* infection through reduction of *Campylobacter* in food-producing animals at the farm is critical.

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