Original article

The prevalence of thermotolerant *Campylobacter* species in food animals in Jimma Zone, southwest Ethiopia

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Abstract

Background: Thermotolerant *Campylobacter* spp. is known to occur in the intestinal systems of a wide variety of domestic and wild animals. Although *Campylobacter jejuni* and *Campylobacter coli* cause acute diarrhoeal diseases in humans worldwide, they mostly manifest themselves in an apparently healthy carrier state in other mammalian species. However, little is known about the presence of campylobacter bacteria in various food animals as possible sources of infection to humans in Ethiopia.

Objective: The aim of this study is to determine the prevalence of thermotolerant *Campylobacter* spp. in various food animals in Jimma zone, southwest Ethiopia.

Methods: A cross-sectional study was conducted in urban and rural farm animal settings in Jimma, southwest Ethiopia in the period between January 2004 to April 2004. Fecal specimens were collected from 485 various food animals (cattle, n=205; poultry, n=191; pigs, n=18; sheep n=71) and cultured using standard methods.

Results: *Campylobacter* spp. were isolated from 192 (39.6%) out of 485 fecal specimens taken from various urban and rural farm animals. The highest isolation rate was recorded among chickens (68.1%), followed by pigs (50.0%), sheep (38.0%) and cattle (12.7%). Among the 192 thermophilic campylobacters isolated, 135 (70.3%) were identified to be *C. jejuni*, 51 (26.6%) were *C. coli* and 6 (3.1%) were *C. lari*. *C. jejuni* was the most prevalent species in chickens (80.8%), followed by sheep (59.3%) and cattle (53.8%). All isolates found in pigs were identified to be *C. coli* (100%).

Conclusion: The results of this study indicate that thermophilic campylobacters are very frequent among various food animals in Ethiopia, suggesting possible risks of infection to people through the consumption of contaminated animal products or through contact with infected animals. [*Ethiop.J.Health Dev.* 2005;19(3):225-229]

Introduction

The thermophilic campylobacters- *C. jejuni* and *C. coli* - account for the majority of gastrointestinal diseases attributed to the genus *Campylobacter*. *Campylobacter* is now considered to be one of the causes of diarrhoeal diseases in humans, resulting mainly from the contamination of poultry and other animal products (1, 2). However, the pathogenesis of *Campylobacter* gastro-enteritis is not fully characterized (3). A serious conse-quence of diarrhoeal diseases is the Guillain-Barrè syndrome (GBS) characterized by polyneuritis of the peripheral nerves, which may lead to either short-term or lengthy paralysis (4). *Campylobacter* received serious attention as a causative agent of diarrhoea only since 1973 with the introduction of a selective medium for isolation from human stool (5). A total of 15 species and six subspecies are now categorized in the genus *Campylobacter* and 12 of these species are associated with human diseases (6, 7). Only the five significant human pathogens are considered in detail,-the enteropathogens *C. jejuni, C. coli, C. lari, C. upsalinesis*, and the systemic pathogen *C. fetus* (3). Campylobacters are small gram-negative, non-spore-forming, helical bacteria with a distinctive `darting' motility, and are catalase and oxidase positive. *Campylobacter* spp. can be found in the reproductive organs, intestinal tracts, and oral cavity of animals and humans (7, 8). These organisms are widely distributed in nature and are mainly recognized as zoonotic infections in a multitude of animal reservoirs, particularly avian species, with shedding into the environment. Although *C. jejuni* and *C. coli* cause acute diarrhoeal diseases in people worldwide (9), they are mostly present in an apparently healthy carrier state in other mammalian species (10). Humans acting as accidental hosts typically acquire these potential pathogens through the ingestion of contaminated food, particularly poultry, unpasteurized milk, improperly treated water, or animal contact with sporadic infections far outnumbering those occurring in point source epidemics (11, 12). The few reported studies of *Campylobacter* spp. as human enteric pathogens in Ethiopia showed isolation rates ranging from 13.6% to 13.8% (13, 14). Several countries have reported the epidemiology of *Campylobacter* bacteria in different wild and domestic animals (10, 15-17). However, little is known about the presence of *Campylobacter* spp. in various food animals as possible sources of infection for humans in Ethiopia. Therefore, the present study was undertaken to determine the prevalence of thermotolerant *Campylobacter* species in cattle, sheep, pigs and chickens in Jimma zone, southwest Ethiopia.

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Methods
Study area
A cross-sectional study was conducted in urban and rural farm animal settings in Jimma, southwest Ethiopia during the period from January 2004 to April 2004. The urban farm animal settings were located at Jimma University College of Agriculture (JUCA) and the Jimma Dairy Development Enterprise (JDDE), while the rural animal farm settings were located at the Merrewa Peasant Association located about 10 km north of Jimma town.

Collection of samples
As outlined in Table 1, a total of 485 fresh faecal samples/rectal swabs were collected from apparently healthy rural and urban farm animals. The 112 cattle, 94 chickens and 71 sheep from the Merewa rural setting (n=277) and the 93 cattle, 97 chickens and 18 pigs from the urban setting (n=208) were included in the study. Approximately 1-5 grams of fecal samples were obtained using direct rectal retrieval and rectal swab systems. The samples were taken with a sterile cotton wool swab moistened in nutrient broth and transported in Carry-Blair Transport medium (Oxoid Ltd, Basingstoke, Hampshire, England). The sample size was calculated based on the reported mean carrier rate in different food animals to be 50% or higher (18).

Culture and identification of thermotolerant Campylobacter spp
All fecal samples/ rectal swabs obtained from different food animals were inoculated directly onto Modified Charcoal Cefoperazone Deoxycholate Agar (Blood free selective medium) (Oxoid Ltd, Basingstoke, Hampshire, England), which is appropriate for the isolation of thermophilic campylobacters (19). The cultures were incubated in a microaerophilic atmosphere, achieved in anaerobic jars (Oxoid, UK) without catalyst and by using CampyGen® gas generating kits (5% O_2 and 10% CO_2) (Oxoid, UK) at 42°C for 48 hours. The growth of thermophilic campylobacters was detected by their characteristic appearance on culture media i.e. the presence of flat grayish colonies resembling droplets of water sprayed on the medium. Preliminary identification was performed based on the characteristic Gram-staining reactions and positive tests for oxidase and catalase agents. The type strains *C. jejuni* (NCTC 11351), *C. coli* (LMG 6440) and *C. lari* (NCTC 11352) were included as positive controls.

All the isolated *campylobacter* strains were kept frozen at -70°C as stab culture in 1% nutrient gar (Oxoid, UK) until species differentiation was done.

Species differentiation
All isolated strains identified as thermophilic campylobacters were tested for hippurate hydrolysis, H_2S production and susceptibility to nalidixic acid and cephalothin. These parameters formed the basis for the identification of *C. jejuni*, *C. coli* or *C. lari*, as proposed by On *et al*. (7). For the hippurate hydrolysis test, a 48-hour pure culture taken from blood agar plates was duly emulsified in 1% sodium hippurate and incubated in a heating block at 37°C. After 2 hours of incubation, 0.2 ml of the ninhydrin reagent was slowly added on the sides of the tubes. Without mixing, the tubes were returned to the heating block for 20 minutes and examined immediately, without shaking for color development. A deep purple crystal violet like color, which indicates the presence of glycine that results from the hydrolysis of hippurate, was considered a positive test. Those organisms yielding a positive test were considered *C. jejuni*, while those organisms that showed a negative reaction (no color development) were considered *C. coli* and *C. lari*.

For hydrogen sulfide (H_2S) production test, pure culture was inoculated on a triple sugar iron agar (Oxoid, UK) and incubated at 37°C. Hydrogen sulfide production turned parts of the agar black indicating a positive test. All the tested campylobacter strains were found to be negative for H_2S production.

Susceptibility tests to nalidixic acid (30 µg) (Oxoid, UK) and cephalothin (30 µg) (Oxoid UK) were performed for all isolates of *Campylobacter* spp. in accordance with the criteria set by the National Committee for Clinical Laboratory Standards (NCCLs) using the disk diffusion method (20). The isolates were classified as sensitive and/or resistant according to the standardized tables supplied by the NCCLs (20). *Campylobacter* strains that were sensitive to nalidxic acid but which are resistant to cephalothin were considered *C. jejuni* and *C. coli*, while strains that were resistant to both drugs were considered *C. lari*.

Statistical analysis
The Epi info version 2000 (approved by CDC, Atlanta, Georgia, USA) was used for statistical analysis. A Chi-Square test was applied to examine whether the differences between the values were significant.

Results
Isolation of Campylobacter spp
The numbers and percentages of the theromophilic campylobacters isolated from 485 different food animals are presented in Table 1. *Campylobacter* spp. were isolated from 192 (39.6%) of the 485 fecal samples investigated from various urban and rural farm animals. The highest isolation rate was recorded among chickens (68.1%), followed by pigs (50.0%), sheep (38.0%) and cattle (12.7%). The rate of *Campylobacter* spp. isolated in all animals was found to be higher in animals from urban areas than from rural settings (56.7% vs. 26.7%, p<0.05).

Phenotype distribution
Among the 192 thermophilic *Campylobacter* strains

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isolated from various food animals, 135 (70.3%) were found to be *C. jejuni*, 51 (26.6%) were *C. coli* and 6 (3.1%) were found to be *C. lari* (Table 2). The number of *C. jejuni, C. coli* and *C. lari* isolated per food animal species were: cattle, 14/10/2; chickens, 105/21/4; pigs, 0/9/0 and sheep, 16/11/0. respectively. *C. jejuni* was found to be the most prevalent species in chickens (80.8%), followed by sheep (59.3%) and cattle (53.8%). All isolates found in pigs were *C. coli* (100%).

Discussion

Many different animal species maintain *Campylobacter* spp. without showing clinical signs (18). The most important species of *Campylobacter* in veterinary medicine are *C. fetus* subsp. *fetus* and *venerealis* (21). Of the *Campylobacter* spp. that are pathogenic in food animals, *C. fetus* could cause reproductive disorders such as abortion and infertility in cattle and sheep (21), and *C. hyointestinalis* and *C. mucosalis* have been associated with enteritis in pigs and cattle (21). But, the role of *C. jejuni* as a primary pathogen in farm animals is uncertain (18). *C. jejuni* could be found in the faeces of diarrheic and healthy calves and piglets, but both *C. jejuni* and *C. coli* can cause a mild self-limiting enteritis and bacteremia when inoculated orally in to new born calves (18). Isolation of *C. jejuni*, *C. coli* and *C. lari* from the intestinal contents of domestic livestock revealed considerable intra-and interspecies variation (10).

Overall, this study has found a high frequency of *Campylobacter* spp. in chickens (68.1%) (Table 1). The reported prevalence of *Campylobacter* bacteria in broiler flocks ranged between 35-57% in Europe (22) and 64-100% in Africa (17, 23, 24). In this study, among the thermophilic campylobacters isolated from chickens, *C. jejuni* accounted for 80.8%, *C. coli* for 16.2% and *C. lari* for 3.0% (Table 2). Similar results have also been reported in other countries indicating a higher prevalence of *C. jejuni* than *C. coli* in poultry farms (18). Poultry appeared to be significant sources of *Campylobacter bacteria,* and chickens were found to be healthy carriers of campylobacters, mostly *C. jejuni* (18). Chicken products were found to be important sources of *Campylobacter* infection in humans in both Asia (25) and Africa (24), and chickens were also found to be potential sources of *Campylobacter* bacteria in farm workers (24). Although a higher prevalence of *Campylobacter* on poultry products has been reported in developing countries, local customs like eating well-cooked poultry are believed to reduce the risk of infection, compared to developed countries in Europe, where the consumption of undercooked poultry is more common (18). The sources of *Campylobacter* infections in chickens were more likely to be horizontal contamination from the environment or the sewerage system, rather than from direct flock to flock transmission (18). The present study also showed that there is a high prevalence of *Campylobacter* bacteria in chickens in urban settings that have extensive indoor systems (90.7%) than the rural settings where animals are reared under free-range conditions (44.7%) (p< 0.05). Other studies showed that the prevalence of *Campylobacter* colonization was higher in organic farms (100%) compared to extensive indoor systems (49.2%) or conventional farms (36.7%) (26). The highest levels of recovery of *Campylobacter* bacteria in chickens usually occurred during the warmer months of the year (June-October) in developed countries, and more *C. jejuni* (43%-86%) than *C. coli* (11%-39%) were recovered (18). The seasonality of *Campylobacter* bacteria found in poultry products in the markets coincides with the peak incidence of *Campylobacter* in humans, which demonstrates the importance of chicken as a source of campylobacter infection for humans in developed countries (18).

Table 1: **The prevalence of thermophilic campylobacters from feces/rectal swabs of 485 different food animals in Jimma, southwest, Ethiopia**

Food animals	No. of samples examined and no. of cases positive for campylobacter (%)							
	Urban setting				Rural setting			
	JUCA[a]		JDDE[b]		Merrewa			Total
Cattle	42	10 (23.8)	51	11 (21.6)	112	5 (4.5)	205	26 (12.7)
Chicken	97	88 (90.7)	-	-	94	42 (44.7)	191	130 (68.1)
Pigs	18	9 (50.0)	-	-	-	-	81	9 (50.0)
Sheep	-	-	-	-	71	27 (38.0)	71	27 (38.0)
Total	**157**	**107 (68.1)**	**51**	**11 (21.6)**	**277**	**74 (26.7)**	**485**	**192 (39.6)**

[a]Jimma University College of Agriculture; [b]Jimma Dairy Development Enterprise

In this study, only *C. coli* was isolated from pigs (100%) (Table 2). Most workers have reported a higher carriage rate of *C. coli* than *C. jejuni* among healthy pigs, providing evidence that *C. coli* is a normal flora of these animals (10, 15, 16, 27). *C. jejuni* is recognized as the most important causal agent of human *Campylobacter* enteritits, whereas *C. coli* is less commonly involved in human infections (28). *C. coli* accounted for 17.6% of the

cases of human infection in Ethiopia (13). The contamination of pig carcasses during slaughter processes also represents a potential source of human infection.

Table 2: **The phenotype distribution of thermophilic *Campylobacter* spp. from various food animals in Jimma, southwest, Ethiopia**

Food animals	C. jejuni No. (%)	C. coli No. (%)	C. lari No. (%)
Cattle (n=26)	14 (53.8)	10 (38.5)	2 (7.7)
Chickens (n=130)	105 (80.8)	21 (16.2)	4 (3.0)
Pigs (n=9)	- -	9 (100.0)	- -
Sheep (n=27)	16 (59.3)	11 (40.7)	- -
Total (192)	**135 (70.3)**	**51 (26.6)**	**6 (3.1)**

The frequency of *Campylobacter* spp. isolation (38.0%) in sheep in this study was found to be higher than that reported in studies conducted in Norway (8.1%) (10), Portugal (15%) (15), and Brazil (20%) (16). The main species of *Campylobacter* isolated from the faeces of sheep in this study is *C. jejuni* (59.3%), followed by *C. coli* (40.7%). The same pattern is observed with campylobacters isolated from the intestines of sheep at slaughter houses in Preston, Lancashire, the United Kingdom (29). Although it is likely that intestinal infection is close to 100%, the shedding of campylobacters in the faeces varies considerably with the time of year (29). In some occasions, 100% of sheep were shedding camylobacters and not shedding on other occasions. This may show seasonal patterns. The shedding of campylobacters by sheep has the potential to contaminate pastures and surface waters. Contamination of surface and sub-surface waters may transmit *Campylobacter* within herds and between farms and other livestock groups (30).

In this study, the prevalence of thermophilic *Campylobacter* spp. in cattle was found to be 12.7%. Previous research in cattle has been limited, but it has been increasing as outbreaks of human campylobacteriosis were traced to foods of cattle origin (18). The prevalence of *Camplobacter* was found to be 15% in beef from calves, 37.7% in dairy herds and 89.4% on beef cattle at slaughter (18). The prevalence in beef cattle was high in the summer, which coincides with the seasonal peak of human *Campylobacter* infections (18). However there are reports from different parts of the world, which show that in domestic livestock, different carriages exist ranging from 0 to 100% (31, 32), with isolation most frequently seen in confined herds of dairy cattle (10). Variables, such as herd size and type, season, age of animal, sample site, sample frequency and isolation method, geography, diet and husbandry practices, have been suggested to account for the differences (33). Raw and improperly pasteurized milk from cows has been incriminated as a means of transmission of the infection in several outbreaks of milk borne campylobacteriosis (10, 34). Since cows might be intestinal carriers of

Campylobacter spp, the faecal contamination of milk represents a potential route leading to human infection. But on rare occasions outbreaks have been traced to asymptomatic *Campylobacter* mastitis in cows, which causes a high number of organisms to be directly excreted into the milk (33).

In conclusion, the results of this study indicate that thermophilic campylobacters, *C. jejuni* and *C. coli/C. lari* are very frequent among food animals in Ethiopia, suggesting possible risks of infection to people through consumption of contaminated animal products or by direct contact with infected animals. Since the available evidence shows that food animals constitute a major reservoir for these organisms, interruption of transmission to human beings from these sources should be given a high priority. Awareness of the necessity for hand washing after contact with animals and their products and the importance of proper cooking and handling of foods of animal origin are probably as important in preventing *C. jejuni/coli* infections as they are for preventing salmonella infections. Pasteurization of milk, chlorination of water supplies and proper cooking of foods readily kill these organisms.

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