CAMPYLOBACTERIOSIS AMONG LIVESTOCK AND HUMANS

By
M.A.Ibrahim and Abdel-Ghany, A. E.
Hygiene, Management and Zoonoses Dep., Faculty of Vet.Med., Beni-Suef University.

ABSTRACT

In this study 175 stool samples were obtained from humans who were living in rural areas in Beni-Suef Governorate in close contact to animals. Also, 485 fecal samples were obtained from animals (190 dairy cows, 140 calves, 90 adult sheep and 65 lambs) beside 150 milk samples (90 normal milk and 60 mastitic one) were examined for campylobacteriosis. Campylobacter jejuni and Campylobacter coli could be isolated from diarrheic persons at rates of 6.3% and 1.1% respectively whereas in case of persons with normal feces, only C. jejuni was isolated at a rate of 2.5%. This study revealed that campylobacteriosis (mainly caused by C. jejuni) was more prevalent in young age group (2-15 years) than other age groups. The overall prevalence of C.jejuni and C.coli in animals(cattle and sheep) with normal feces was 3.4% and 0.4% respectively. Whereas in case of diarrheic animals, the overall prevalence of C.jejuni and C.coli 6.0% and 0.8% respectively. This study indicated that C.jejuni was more frequent among cattle and humans than C.coli. Also cattle played a significant role as a source and reservoir for Campylobacter to the environment. Finally, C.jejuni could be isolated from normal and mastitic milk at rates of 6.66% and 1.66% respectively whereas C.coli could be isolated from normal milk only at a rate of 1.11%.

INTRODUCTION

Campylobacter infection is a zoonotic disease, observed in most parts of the world as a leading cause of diarrhea and food-borne gastroenteritis (Allos and Blaser 1995). In developed countries, the incidence of *Campylobacter* infection is low and there are a few healthy carriers (Blaser et al., 1983). In developing countries, the incidence of *Campylobacter* infection in diarrheic patients is higher and there are numerous healthy carriers (Blaser et al., 1980).

Campylobacter received serious attention as a causative agent of diarrhea only since1973 with the introduction of a selective medium for isolation from human stool (Butzler et al.,1973). The disease is caused by *Campylobacter jejuni*, or less commonly *Campylobacter coli*. It is estimated to cause 5–14% of diarrhea, worldwide (Coker et al., 2002). *C.jejuni* and *C. coli* are almost identical in behaviour and epidemiology (Butzler and Oosterom

1991) and therefore discussions in this report relating to *C. jejuni,* apply to both organisms.

C. jejuni is predominantly associated with gastrointestinal infections in humans. Most frequently there is a self-limited diarrheal illness with associated abdominal pain that may be severe (Blaser et al., 1979) and (Blaser et al., 1983). Occasional complications of the infection can be manifested as meningitis, pneumonia and a severe form of Guillain-Barre syndrome (a severe paralytic condition) (Blaser et al.,1986) and (Nachamkin et al. 1992). In experimental situations, infection has occurred after as few as 500 organisms were ingested (Robinson, 1981). Moreover the high incidence of clinical disease associated with this organism, its low infective dose in humans and its potentially serious sequelae, confirms its importance as a significant public health hazard (Tauxe, 2002).

Most cases of human campylobacteriosis result from ingesting contaminated foods (such as poultry) (Hopkins et al., 1987), drinking raw milk (Neal
and Slack, 1995), drinking untreated water (Studahl and Andersson, 2000) and (Kapperud et al., 2003) or contact with animal reservoir (Deming et al., 1987). Although the majority of cases are sporadic, outbreaks involving consumption of contaminated raw milk and untreated water occur (Headrick et al.,1994) and
(Bean et al.,1996).

It is well established that *Campylobacter jejuni* is carried in the intestinal tract of a wide variety of wild and domestic animals, especially birds. In most cases, the host is a carrier that does not exhibit symptoms, but in few cases it may have gastroenteritis mainly diarrhea (Berndtson et al., 1996). Furthermore, it is now accepted that foods of animal origin can become contaminated by this pathogen during slaughter and carcass dressing (Madden et al. 2000; Whyte et al. 2003) and campylobacteriosis is predominantly acquired through the consumption of contaminated foods (Anon., 1995).

Reported rates of intestinal *Campylobacter* carriage in food animals have varied widely between studies (Busato et al., 1999). The digestive tract of clinically normal cattle has been demonstrated to be a significant reservoir for a number of *Campylobacter* spp (Atabay and Corry, 1998). *C. jejuni* could be found in the feces of diarrheic and healthy calves, but both *C. jejuni* and *C. coli* can cause a mild self-limiting enteritis and bacteremia when inoculated orally into new born calves (Padungton and Kaneene, 2003). Prevalence of *Campylobacter* in sheep has been shown to be generally lower with approximately 20% of animal's intestinal carriers (Zweifel et al., 2004).

C. jejuni / coli are excreted through feces and animal secretions and dairy cattle get infected through ingestion of water and feeds contaminated with manure. *C. jejuni* can cause mastitis in cows and it can be shed in milk of carrier cows. Direct milk excretion of *C. jejuni / coli* by clinically healthy

cows has been described and implicated in the etiology of human enteritis following consumption of contaminated milk (Orr et al., 1995).

C. jejuni has been isolated from cows with mastitis. However, this organism is isolated infrequently and does not appear to be a significant cause of bovine mastitis (Lander and Gill, 1980). Gudmundson and Chirino-Trejo (1993) indicated that *C. jejuni* mastitis occurred in a Holstein cow 60 days into the first lactation. The infection was characterized by a sudden onset, pyrexia, a painful mammary quarter and pink milk with a few small clots present.

The present study was undertaken to determine the prevalence of *C.jejuni* and *C.coli* in cattle, sheep in Beni-Suef Governorate and to estimate the occurrence of these organisms in humans that live in close contact with cattle and sheep.

MATERIAL AND METHODS

1-Sampling:

A-Human samples:

175 stool samples were taken from the inhabitants residing in close contact with the examined animals (cattle and sheep) in different areas at Beni-Suef Governorate. Data concerning age and clinical signs stressing on diarrhea were registered during taken the samples.

B-Samples from livestock:

a-Fecal samples:

Rectal swabs from 330 cattle (190 dairy cows and 140 calves) were taken beside 155 sheep samples (90 adult sheep and 55 lambs). Both apparently healthy and diarrheic animals were investigated.

b-Milk samples:

A total of 150 milk samples were collected from dairy cows from which the rectal swabs were taken. The udder of cow was examined for mastitis (tenderness and inflammation). Also the milk was examined for sub-clinical and clinical mastitis by California Mastitis test (Romain et al., 2000). The samples (90 normal milk samples and 60 mastitic ones) were collected in sterile screw capped test tubes under aseptic condition directly from the udder.

The different samples collected from animals and humans were transported to the laboratory in an ice box at a temperature of about 4 °C where samples were subjected to bacteriological examination.

2-Preparation of the samples:

a-Human stool:

Human stool samples were inoculated into sterile thioglycollate broth tubes. (Kalpan et al., 1980).

b-Fecal swabs from animals:

The rectal swabs from animals were inoculated also into thioglycollate broth tubes (Monfort et al., 1990).

c-Milk samples:

1-3 ml of each sample was added to about 15 ml thioglycollate broth in sterile test tubes (Waterman et al., 1984).

3-Isolation:

The inoculated thioglycollate broth was incubated at 42 °C for 24-48 h. in a microaerophilic condition (5% oxygen, 10% carbon dioxide and 85% hydrogen) obtained by using gas pack jar and *Campylobacter* gas generating kit (Rosef and Kappened, 1983). A loopful of the enrichment broth was streaked on Preston *Campylobacter* selective medium (Bolton and Robertson, 1982) then incubated at 42 °C for 48 h. under microaerophilic condition.

4-Identification of Campylobacter spp.

Up to three suspect colonies (based on characteristic morphological appearance) were subcultured. Isolates were considered to be *Campylobacter* spp. if they were oxidase positive, motile and Gram stained smears of suspect colonies revealed small tightly coiled spiral organisms. Isolates were identified as *C. jejuni* or *C. coli* as described by Barrow et al. (1993).

RESULTS AND DISCUSSION

Table (1) revealed that *C.jejuni* could be isolated from normal and diarrheic feces of the examined humans at a rate of 2.5% and 6.3% respectively while *C.coli* could only isolated from diarrheic feces at rates of 1.1%. *C. jejuni* and *C. coli* are among the most common causes of bacterial diarrhea in man world-wide (Nachamkin et al. 2000). Among these, *C. jejuni* accounts for the vast majority of infections (Skirrow, 1977; Coles et al., 1985;and Denno et al. 2005). The isolation rate of *C. jejuni* in this study from healthy individuals coincided with those reported by Blaser et al. (1980) who isolated *C. jejuni* from 1 (3%) out of 32 asymptomatic house holds contacts. Also Glass et al. (1983) stated that *Campylobacter* is found commonly both in patients with diarrhea and healthy individuals.

C.jejuni and *C.coli* were isolated at a higher level in the age group from 2-15 years (8.7%) than in the age group 16-30 years (2%) and age group more than 30 years (2.2%). Similar results were reported by many authors as Skirrow, (1977) who mentioned that the peak incidence of *Campylobacter* enteritis appears to be in the 1-5 year old age group and Wright et al. (1983) who reported that during outbreaks of *Campylobacter* enteritis, 45% of the cases were children under the age of 10 years. Also Ahmed et al. (1992) could isolate *C. jejuni* from 4(8.0%) out of 50 infants with acute diarrhea. They recommended that any case presenting with acute diarrhea should be initially screened by fecal leucocyte counting, positive cases should be cultured for *C. jejuni*. Gedlu and Aseffa (1996) demonstrated the important role played by *C. jejuni* in childhood diarrheal illness. They could isolate *C. jejuni* from the stools of 60 children out of 434 (13.8%), the highest isolation rate was in children aged from 6 to 24 months.

The overall prevalence of *C. jejuni* and *C. coli* in cattle and sheep was shown in Table (2). *C. jejuni / C. coli* were isolated from 25 (5.2%) out of 485 fecal specimens taken from cattle and sheep. The highest isolation rate was

recorded among calves (8.6%) followed by cows (5.3%), lambs (3.1%) and adult sheep (1.1%). These results indicated that cattle play a major role as a source of *C. jejuni / C. coli* while the role played by sheep is limited, results that were comparable with those reported by Tesfye et al. (2005). On the other hand Rosef et al. (1983) found that *C. jejuni / C. coli* were isolated at a higher rate in sheep (8.1%) than cows (0.8%). Several studies reported a wide variation of *Campylobacter* carriage rate in domestic food animals. This may reflect the different geographic/climatic conditions, and management practice (Nachamkin et al.1992).

C. jejuni and *C. coli* were isolated from healthy cows (normal feces) at a rate of 4.2% and 0.8% respectively and from diarrheic ones at a rate of 4.3% and 1.4% respectively. On the other hand the results obtained from healthy calves (normal feces) revealed that only *C. jejuni* could be isolated at a rate of 5% whereas in case diarrheic calves *C. jejuni* and *C. coli* could be isolated at a rate of 11.3% and 1.3% respectively. These results indicated that thermotolerant campylobacters (especially *C. jejuni*) might colonize the intestinal tract of healthy cattle.

The prevalence of *C. jejuni* and *C. coli* in dairy cattle ranges from 5 to 53%, depending on methods of isolation (direct plating or enrichment), age of animal (calf or adult), season, and sample analyzed (feces or intestinal contents). (Wesley et al., 2000). *C. jejuni* and *C. coli* could be isolated from calves either healthy or diarrheic by many authors such as Firehammer and Myers (1981), Diker and Istanbulluoglu (1983), Weber et al. (1984), Decastell et al. (1991), Zamora et al. (1992) and Giacoboni et al. (1993). The high frequency of *Campylobacter* isolation in feces of cows suggests that contamination of bulk tank milk occurs primarily via feces of *Campylobacter*-carrier cows during harvesting and/or storage of raw milk (Waterman et al., 1984) beside the healthy cattle can play role in the contamination of the environment and human food chain by Campylobacters (Acik and Cetinkaya, 2005).

Only *C. jejuni* could be isolated from diarrheic sheep and lambs at a rate of 1.8% and 4.4% respectively. Several studies indicated that *C.jejuni* could be isolated from sheep and lambs (Prescott and Bruin-Mosch, 1981, Gill and Harris, 1982, Turkson et al.,1988 and Graham et al., 2003).

Generally the low prevalence rate of *C. coli* in this study either in cattle and sheep or in humans could be explained by the fact that *C. jejuni* predominates among farm animals such as cattle poultry and sheep, whereas *C. coli* is the most commonly found species among pigs (Munrae et al., 1983).

Table (3) indicated the isolation rate of *C. jejuni* and *C. coli* from cow's milk. *C. jejuni* was isolated from normal and mastitic milk at a rate of 6.66% and 1.66% respectively while *C.coli* could be isolated from normal milk only at a rate of 1.11%. *C. jejuni* was isolated from raw cow's milk and bulk-tank milk by many authors such as Lovett et al. (1983) who reported that the isolation rate of *C. jejuni* in milk from bulk-tanks was 1.5%, Hassan,(1987) who could isolate *C. jejuni* from milk of cattle at a rate of 6% and Moustafa (1990) who could isolate *C. jejuni* from 8 out of 92 milk samples obtained from two dairy farms in Assiut City. On the other hand, Decastell et al. (1991) could not

isolate both *C. jejuni* and *C. coli* from milk samples obtained from 160 dairy cows. Christopher et al. (1982) found that *C. jejuni* was not recovered from raw milk (bulk-tank or individual cow's samples). Direct milk excretion of *C. jejuni* by asymptomatic dairy cows may lead to bulk-tank milk contamination and local cases of human enteritis (Orr, et al., 1995).

Results of this study demonstrated that *C. jejuni* can cause mastitis in the cow and suggests that the bovine udder is a potential source of *C. jejuni* in raw milk. This was in accordance with Logan et al. (1982)who isolated *Campylobacter* from milk samples from a dairy herd during an outbreak of mastitis. *C. jejuni* was able to induce mastitis in 22 cows varying from milk in apparent infection to acute severe mastitis. The bacterium multiplied in the udder and was excreted in the milk for 3-73 days in variable numbers (Lander and Baskervilli, 1983). Also Gudmundson and Chirino-Trejo (1993) indicated that *C. jejuni* mastitis occurred in a Holstein cow 60 days into the first lactation. The infection was characterized by a sudden onset, pyrexia, and a painful mammary quarter and pink milk with a few small clots present. On the other hand Waterman et al. (1984) evaluated samples of milk from 1501 cows with mastitis and all were negative for *C. jejuni*.

Consumption of milk contaminated with *C. jejuni* has been described as a cause of human enteritis. Although faecal contamination of milk with the organism has frequently been described, direct milk excretion of *C. jejuni* into milk has rarely been linked with cases of human infection (Orr, et al., 1995). Hutchinson et al. (1985) described a community outbreak of *Campylobacter* enteritis associated with consumption of raw milk, apparently contaminated by two cows with *Campylobacter* mastitis. Raw milk consumption was strongly associated with *C. jejuni* enteritis outbreak among people who had attended a meal where raw milk was served (Peterson, 2003). Also raw cow's milk contaminated with *C. jejuni* was implicated in outbreak of gastroenteritis (mainly diarrhea) of 5 months duration in a farming family. *C.jejuni* of identical genotype was isolated from human and bovine feces, and bulk-tank (Schildt et al., 2006).

Conclusion *The results of this study indicated that C. jejuni was more frequent than C. coli among food animals and humans. Cattle played a significant role as a source and reservoir for Campylobacter in the environment suggesting possible risks of infection to people through the consumption of the contaminated animal products (milk) or through contact with infected animals.*

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Table (1): Distribution of *C.jejuni/C.coli* in the examined humans in relation to age

Age group	No. of samples	No. of isolates	Normal feces			Diarrheic feces		
			No.	C.jejuni	C.coli	No.	C.jejuni	C.coli
2-15 y.	80	7(8.7%)	30	2 (6.7%)	0 (0.0%)	50	4 (8.0%)	1 (2.0%)
16-30 y.	50	1 (2.0%)	30	0 (0.0%)	0 (0.0%)	20	1 (5.0%)	0 (0.0%)
>30 y.	45	1 (2.2%)	20	0 (0.0%)	0 (0.0%)	25	1 (4.0%)	0 (0.0%)
Total	175	9 (5.1%)	80	2 (2.5%)	0 (0.0%)	95	6 (6.3%)	1 (1.1%)

Table (2): Prevalence of *C. jejuni* and *C.coli* in the feces of animals

Animal species	No. of animals	No. of isolates	Normal feces			Diarrheic feces		
			No. of animals	*C. jejuni*	*C. coli*	No. of animals	*C. jejuni*	*C. coli*
Cows	190	10 (5.3%)	120	5 (4.2%)	1 (0.8%)	70	3 (4.28%)	1 (1.4%)
Calves	140	12 (8.6%)	60	3 (5.00%)	0 (0.0%)	80	9 (11.3%)	1 (1.3%)
Sheep	90	1 (1.1%)	35	0 (0.00%)	0 (0.0%)	55	1 (1.8%)	0 (0.0%)
Lambs	65	2 (3.1%)	20	0 (0.00%)	0 (0.0%)	45	2 (4.4%)	0 (0.0%)
Total	485	25 (5.2%)	235	8 (3.4%)	1 (0.4%)	250	15 (6.0%)	2 (0.8%)

Table (3): Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in cow's milk

Specimens	No. of samples	*C. jejuni*	*C.coli*
Normal milk	90	6 (6.66%)	1 (1.11%)
Mastitic milk	60	1 (1.66%)	0 (0.00%)
Total	150	7 (4.66%)	1 (0.66%)

الملخص العربي

مرض الكامبيلوباكتريوزس في حيوانات المزرعة والانسان

في هذه الدراسة تم فحص 175 عينة براز من أشخاص مخالطين لحيوانات المزرعة (الأبقاروالأغنام) في محافظة بني سويف وقد أخذت العينات من أعمار مختلفة ومن أشخاص بعضهم يعاني من اسهال (95) والأخر لايعاني من الاسهال(80). أيضا تم فحص 485 مسحة شرجية من الحيوانات المختلفة (190 أبقار حلابة, 140 عجول رضيعة, 90 أغنام و65 حملان) بالاضافة الي 150 عينة لبن مأخوذة من الأبقار التي تم فحص برازها وكانت عبارة عن 90 عينة لبن مأخوذة من أبقار سليمة و60 عينة مأخوذة من أبقار تعاني من التهاب ضرع.

أظهرت الدراسة ان ميكروب الكامبيلوباكتر جيوجيناي والكامبيلوباكتر كولاي تم عزلهم من الأشخاص الذين يعانون من لاسهال بنسبة 6.3% و1.1% علي التوالي, أما في حالة الأشخاص الذين لا يعانون من اسهال فقد أمكن عزل ميكروب الكامبيلوباكتر جيوجيناي فقط بنسبة 2.5%. كما بينت الدراسة أيضا ان نسبة الاصابة بميكروب الكامبيلوباكتر (وخصوصا الجيوجيناي) كانت أكبر في الأعمار الصغيرة (2-15 سنة) عنها في الأعمار المتوسطة (16-30 سنة) والأعمار الكبيرة (أعلي من 30 سنة).

بالنسبة للحيوانات كانت نسبة عزل ميكروب الكامبيلوباكتر جيوجيناي والكامبيلوباكتر كولاي من الحيوانات السليمة 3.4%, 0.4% علي التوالي بينما كانت نسبة العزل في الحيوانات المصابة باسهال 6%, 0.8% علي التوالي. أوضحت الدراسة أيضا ان ميكروب الكامبيلوباكتر جيوجيناي أمكن عزله من ألبان الحيوانات السليمة بنسبة 6.66% ببينما في الحيوانات المصابة بالتهاب الضرع كانت نسبة الاصابة 1.66%. بالنسبة لميكروب الكامبيلوباكتر كولاي فقد أمكن عزله من الألبان السليمة فقط بنسبة 1.11%.

في النهاية خلصت الدراسة الي أن معدل تواجد ميكروب الكامبيلوباكتر جيوجيناي أعلي من معدل تواجد ميكروب الكامبيلوباكتر كولاي في الحيوانات وكذلك الانسان , كما أن الأبقار تلعب دورا رئيسي كمصدر لميكروب الكامبيلوباكترحيث يمكن أن تلوث المنتجات الحيوانية وخصوصا الألبان من خلال التلوث المحمل بالميكروب مما يشكل خطرا علي الصحة العامة.

في النهاية خلصت الدراسة إلي أن معدل تواجد ميكروب الكامبيلوباكتر جيوجيناي أعلي من معدل تواجد ميكروب الكمبيلوباكتر كولاي في الحيوانات وكذلك الانسان , كما أن الأبقار تلعب دورا رئيسيا كمصدر لميكروب الكامبيلوباكترحيث يمكن أن تلوث المنتجات الحيوانية وخصوصا الألبان من خلال الروث المحمل بالميكروب مما يشكل خطرا علي الصحة العامة.