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PROSPECTIVE STUDIES ON CAMPYLOBACTERIOSIS IN HUMAN AND ANIMALS IN CONTACT

(With 4 Tables)

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دراسات مستقبلية على مرض الكامبيلوباكتريوزس في الإنسان والحيوانات المخالطة له

عـادل حلمـى الجـوهـرى

لقد أجريت هذه الدراسة التحليلية عن وبائية مرض الكامبيلوباكتريوزس على عدد ٣٥٨ حالة اضطرابات معدية معوية منها ٤٧ حالة بنسبة ١, ١٣ ٪ شخصت على انها عدوى بميكروب الكامبيلوباكتر فى الأنسان. وقد تم أيضا جمع عدد ٢١٥ عينة براز من الحيوانات المخالطة له. وقد أثبتت نتائج البحث أن نسبة الإصابة كانت فى الأطفال الذين تتراوح أعمارهم بين ٣-٥ سنوات (٦, ٣ ٪) بينما كانت النسبة فى الأشخاص البالغين (٥, ٩٪). وقد وجد أن من بين عدد ٢١٥ عينة براز تم جمعها من الحيوانات المخالطة عدد ٥١ عينة ايجابية عزل منها الميكروب بنسبة ٧, ٢٣ ٪ منها عدد ١٧ حالة حيوانات منزلية أليفة وعدد ٣٤ حيوانات مزرعة. هذا بالأضافة الى جمع عدد ٩٤ عينة من اللبن وتم عزل الميكروب بنسبة ٦, ٩٪ من تلك العينات. هذا وقد نوقشت الأهمية الصحية المشتركة لميكروبات الكامبيلوباكترز جيجيونى وكولاى ومدى خطورتها على صحة الإنسان .

SUMMARY

This study represents an epidemiological investigation of 47 cases out of 358 cases (13.1%) of gastrointestinal disturbances diagnosed as campylobacter infections in man and 215 domestic farm animals and pets in contact. The incidence of infection was found in young children (3.6%) while in adults was (9.5%). It was found that out of 215 representative animal faecal samples, 51 were positive to campylobacter species (23.7%), 17 cases were pets and 34 were farm animals. In addition, 94 samples of commercial milk were examined and Campylobater jejuni was isolated from

9.6 % of the examined samples. The zoonotic and sanitary importance of the campylobacter organisms and the hazards of its implication in human was discussed.

Key words: ***Campylobacteriosis - Human - Animals***

INTRODUCTION

Campylobacter species are now recognized as one of the most important causes of acute enteric diarrheal disease in humans throughout the world (Walker et al., 1986 and Casini et al. 1997). The thermophilic campylobacter species (Campylobacter jejuni, C. coli and C. laridis) are enteropathogenic for humans (Skirrow and Benjamin 1981 and Skirrow 1982). In addition, Campylobacter hyointestinalis also has been reported as an enteric pathogen for humans (Fennell et al., 1986 and Edmonds et al., 1987). The distribution of disease caused by human campylobacter isolates varies from one geographical area to another. The two predominant species responsible for most common human gastroenteritis are C. jejuni and C. coli (microaerophilic gram-negative bacteria which can now be routinely isolated and identified) (Walker et al., 1986 and Zanetti et al., 1996). There is some evidence that these infections are zoonoses with a world-wide distribution (W.H.O. 1980). They are today, the most frequently isolated enteropathogens found as often in industrialized countries as in the third world.

The zoonotic nature of campylobacteriosis has attracted the attention of many scientists over years. In recent years, C. jejuni and C. coli is the third most common cause of diarrhea in children in developing countries, after enterotoxigenic E.coli and rotavirus (Ruiz-Palacios 1983), the frequency of isolation of campylobacter organisms from stools of human patients has suggested that the organism may be responsible for many cases of severe gastroenteritis (A.P.H.A., 1985). Campylobacter jejui has exploded from obscurity to be recognized as a major human enteric pathogen. This recognition has triggered numerous bacteriological investigations, but the many mysteries and surprises associated with the organism will challenge the creative efforts of scientists for years to come (Walker et al., 1986). Infection has been reported to cause several forms of human disease with a worldwide incidence only within the last 10 years (Blaser et al., 1979), including mild-to-severe diarrhea, non-specific colitis clinically indistinguishable from acute ulcerative colitis (Selkon 1988),

reactive arthritis (Ebright and Ryay 1984) and abortion (Gilbert et al., 1981). The incidence of human abortion induced by this organism is unknown, but disease due to C. jejuni infection is among the most commonly gastrointestinal in nature. However, extra intestinal infection including meningitis (Thomas et al., 1980), cholecystitis (Darling et al. 1979) and urinary tract infection (Davis and Penfold 1979) have been reported. Complications that have been associated with C. jejuni enteritis include Reiter 's syndrome (Jhonsen et al., 1983) and Guillain-Barre syndrome (Kaldor and Speed 1984).

Organisms belong to the C. jejuni and C. coli group have been now firmly established as major enteric pathogens (Karmali et al., 1983). They are widely distributed among animals (Skirrow and Benjamin, 1981) in association with acute diarrheal disease (Winter Scours) in cows and calves. It was also associated with sporadic cases of bovine abortion and avian hepatitis. Epidemiologic evidence indicated the zoonotic significance of the disease. Since many domestic animals frequently excrete campylobacter in their faeces, the potential for its transmission to human-beings is enormous. Infection in man due to contact with sick animals, especially puppies and kittens has been reported (Svedhem and Norkrans 1980). The role of person contact is important when sanitation and personal hygiene are not well practiced (Bokkenheuser et al., 1979).

Campylobacter jejuni is a frequent enteric pathogen that causes diarrheal disease in both developed and developing countries (Merivane and Jean-Claude 1990). In developed countries, C. jejuni has been demonstrated in the stools of 4 to 14% of all patients with diarrheal disease and is estimated to be responsible for as many as two million cases of infection in the U.S.A. each year (Blaser and Reller 1981). Further studies suggest that in developing countries, the rate of infection due to C. jejuni may be even higher than that of developed countries and that the rate of asymptomatic infection may also be high (Walker et al., 1986). The percentage of Campylobacter enteritis due to C. coli varies from 3 to 50% , depending on geographic location (Karmali et al., 1983 and Kalenic et al., 1985). In contrast, C. laridis and C. hyointestinalis are presumed to be present less frequently in human specimens (Walker et al., 1986). Clinically, enteritis due to C. jejuni and C. coli is difficult to distinguish from illness caused by other enteropathogenic bacteria.

Detection of infection with Campylobacter spp. relies chiefly on the initial isolation of the bacteria, using selective media, followed by identification of the organisms isolated by biochemical testing as well as the

determination of antimicrobial susceptibility patterns. However, the fastidious nature of campylobacter spp. can make isolation difficult (Michael et al., 1990).

The pathogenesis of C. jejuni-induced disease is unresolved. Some reports consider adherence or selective adhesion along with invasiveness of the organism to be important virulence factors (Newell et al., 1985; Field et al., 1986; Walker et al., 1988; Sylvester et al., 1996 and Rohner et al., 1997), while others suggest that mucus colonization is a prelude to intestinal infections (Lee et al., 1986 and Linton et al., 1996). Systemic invasion of the blood and extraintestinal organs has been documented in humans (Lastovica and Penner 1983; Luo et al., 1996 and Petersen et al., 1996), though the occurrence is low. C. jejuni enteritis varies from intestinal invasion, characterized by blood and mucus in the stool to watery diarrhea with no sign of invasion (Sangeeta and Frank 1989; Biswas et al., 1996; Notário et al., 1996; and Van Etterijck et al., 1996).

Therefore, on account of the zoonotic importance of campylobacteriosis in man and animals, the object of the present study was to investigate the epidemiology of intestinal thermophilic campylobacter infections in animals and human contact as well as the occurrence of C. jejuni in commercial raw milk in Gharbia Province, in order to try to elucidate the role of various sources of infection in the epidemiology of human campylobacteriosis.

MATERIALS and METHODS

I- Materials:

1- Collection of samples:

a- Stool specimens (Human samples) :

A total number of 358 rectal swabs and fresh stool specimens from freshly voided stools, 283 adults from diarrhetic patients of all ages suffering from severe gastrointestinal symptoms & colitis and 75 ill children from 3-5 years old suffering from diarrhoea were collected. The samples were obtained from patients admitted to El-Mahalla General Hospital. Each sample was collected in sterile container and kept in an insulated box with ice packs (5-10°C) (Shaheen, 1986) and submitted for routine bacteriological examination for isolation of campylobacter species within 24 hours.

b- Faecal samples (Animal samples) :

A total number of 215 fresh faecal samples and rectal swabs / or contents obtained from different animal species which live in contact with

human cases who showed campylobacteriosis after laboratory investigations and originated from many farms representing a wide range of husbandry systems at El-Mahalla Veterinary Investigation Units/or Centers and its suburbs, those were diseased and suffering from enteric illness (enteritis and/or diarrhoea) and submitted to the Public Health Laboratory of El-Mahalla General Hospital - Gharbia Province. Samples were then transfered with minimum of delay to the laboratory for bacteriological examination where usually cultured within 24 hours of the sample being taken for isolation of campylobacters (Atabay et al., 1997).

c- Milk samples :

A total of 94 random milk samples of commercial raw milk collected from the dairy farms, shops, stores and supermarkets survey (packets fresh) in El-Mahalla City and placed in snap-capped vials were screened for the presence of campylobacter species according to the method described by Lovett et al. (1983) . Samples were taken from the bulk cans at each farm and were held at refrigeration temperature until tested. Each milk sample was mixed several times by inversion (Moustafa 1990 and Desmasures et al., 1997).

II- Methods :

2- Isolation of campylobacter species :

For isolation of campylobacter spp., faecal samples or swabs and stool specimens or faecal suspension of 10 µl were directly plated on Preston agar as a selective medium (SKM) containing Vancomycin (10 µg/ml), Polymyxin B (0.25 µ / ml) and Trimethoprim lactate (5 µg/ml) (Bolton & Robertson 1982; Karmali et al., 1986 and Hilton et al., 1997). The plates were then incubated overnight at 42-43°C in a microaerophilic atmosphere (consisting of approximately 5-6 % O_2 , 10 % CO_2 and 84-85 % N_2) in an anaerobic jar with activated Gas Generating Kit by using the GasPak system (BBL). The plates were examined after 48 and 72 hours. Plates showing no growth were re-incubated further and read after 48 hours. Blood agar plates were sometimes used for isolation of campylobacter species after incubation for 24 hours at 42°C in 5 % O_2 .

3- Identification of campylobacter species :

The smears of suspect colonies suggestive of campylobacter were initially identified according to the biotyping scheme described by Skirrow and Benjamin ($1980_{a,b}$ and Smith et al., 1997) , including the microscopical

examination of Gram stained films by phase-contrast microscopy (X 1,000), for typical morphology and motility. Suspect colonies on selective media were examined microscopically on wet mounts and categorized as campylobacter when a typical curved or spiral rod Gram-negative exhibiting darting motility was seen, Catalase production, Cytochrome oxidase, H_2S production and Hippurate hydrolysis tests were used to differentiate between C. jejuni and C. coli.

Catalase production test: catalase activity was tested on microscopic slides by addition of one drop of hydrogen peroxide solution (H_2O_2 3%) directly from a sterile syring to the suspected colony and oxygen was evolved immediately in positive catalase production test (Rogol and Sechter, 1987).

Oxidase test: Oxidase activity was examined on filter paper with 1% aqueous solution of tetramethyl-P-phenylene-diamine dihydrochloride as the reagent. With an inoculating loop a separate well-grown colony was picked up from a fresh culture medium (24 hours) and applied to the reaction zone and spread with the inoculating loop. A positive reaction was indicated by a violet discolouration within 20-60 seconds at the contact point.

H_2S production: By using lead acetate paper strips were suspended over triple suger-iron agar (Difco). Blackening of the strips was graded from trace (slight colour at the tip of the strip) to 4+ (strip completely black). Blckening of the medium itself was also recorded.

Hippurate hydrolysis test: hydrolysis of hippurate is the standard test in most clinical laboratories for distinguishing C. jejuni from C. coli and C. laridis (Skirrow & Benjamin 1980_b and Morris et al., 1985). The rapid method of hippurate hydrolysis test was described by Hwang and Ederer (1975) as modified by Skirrow and Benjamin (1980_b). An aqueous solution (1%) of Sodium-hippurate (Difco) was prepared and dispensed into glass tubes in 0.4 ml amounts , then capped and frozen at -15°C until used. The tubes were thawed before inoculation and a large loopful of the suspected organism was emulsified in the substrate. The tubes were incubated in a heating block or water bath at 37°C for two hours after which 0.2 ml of ninhydrin solution (3.5 gm. of ninhydrin in 100 ml of a 1:1 mixture of acetone & butanol) was added to each tube. The tubes were re-incubated at 37°C for 10 minutes. The production of a deep purple colour was interpreted as an indication of hydrolysis of sodium hippurate with the formation of glycine characteristic for C. jejuni. A colourless to light purple reaction was considered negative (C. coli).

4- Antibiotic sensitivity test:

The sensitivity test was carried out according to D'Amato and Hochstein (1982 and Sicinschi, 1996) using Campylobacter jejuni strains isolated from animal faecal samples, human stool specimens and milk samples.

RESULTS and DISCUSSION

The present study emphasiezed that campylobacteriosis is a true zoonotic disease occurring naturally in many domestic, laboratory, wild animals and birds which may harbour C. jejuni and C. coli serotypes that also occur in humans suffering from Campylobacter enteritis (Fox et al. 1988, Taylor et al., 1989 and Gedlu and Aseffa, 1996).

The summarized results given in Table (1) declared that 358 stool specimens of patients suffering from severe gastroenteritis were examined for their epidemiological characteristics. Campylobacter jejuni/coli was isolated from the stools of 47 patients with an incidence of 13.1%. This is approximatly in accordance with Blazevic (1979).

From Table (1), according to age groups examined, it could be noticed that the incidence of cambylobacter infection among young children (3-5 years old), was 13 cases (3.6%) followed by adults group (patients with diarrhea, 21 cases (5.9%), symptomatic household contacts of positive patients with diarrhea, 9 cases (2.5%) and asymptomatic household contacts of positive patients with diarrhea, 4 cases (1.1%). Such findings could be attributed to many factors, among which the lack of personal hygiene among young children (3-5 years old) and in adults, to consumption of insufficiently cooked food especially those of animal origin. Children are particularly susceptible because they are commonly feed on milk and milk products and in direct contact with pet animals especially puppies and kittens. This data recorded in Table (1), agree with several investigators (Butzler and Skirrow 1979; Blaser et al., 1980 and Karmali et al., 1983). In studies in South Africa and Bangladesh, 40% of children (9-24 months old) excreted C. jejuni (Blaser et al. 1980). In addition, there have been many reported cases of neonatal meningitis caused by campylobacters (Thomas et al., 1980).

It is postulated that the distribution of campylobacter serogroups found in man may be related to their distribution in various animal sources of infection. This investigation showed that campylobacters are commonly present in the faeces of some domestic animals. On the basis of the above mentioned data (Table 2), shows the frequency disribution of

campylobacteriosis and the role of some animals in disseminating the infection. Out of 215 animal faecal samples, 51 samples (23.7%) were positive to campylobacter species (45 C. jejuni 88.2% and 6 C. coli 11.8%). From these positive cases the carriage rates in cattle 6.5%, horses 1.9%, sheep 2.3%, goats 1.9%, dogs 4.6%, cats 3.3%, turkey 1.3% and pigeon 1.9%. It can be concluded that animals especially those sick with diarrhea and in close contact with humans such as pets, may represent an important source of campylobacter infection. Transmission of C. jejuni appears to occur by faecal-oral route through direct contact with faecal materials from infected animals (Svedhem and Norkrans 1980). It was suggested that, dogs and cows with diarrhea may be sources of infection especially infected puppies (Blaser et al., 1978).

Primary epidemiological investigations showed that several food materials especially those of animal origin play a significant role in spreading the infection of campylobacter enteritis. Milk (raw or unpasteurized) represented the main source of infection. The results achieved in the present investigation (Table 3) revealed that the examined milk samples (94) samples studied, C. jejuni was isolated from 9(9.6%) of examined samples. Therefore, it is important to examine milk samples bacteriologically for the presence of campylobacters. Previous studies such as that of Lovett et al. (1983) showed that C. jejuni was present in 1.5% of milk samples taken from bulk tanks. Raw milk has been implicated as vehicle of outbreaks of Campylobacter enteritis (Blaser et al., 1979; Taylor et al., 1979; Robinson & Jones 1981 and Tosh et al., 1981). Robinson (1981) has demonstrated that very few C. jejuni in milk (2-3 cells/ml.) can infect an adult human and produce symptoms of gastroenteritis. Milk-related outbreaks have been reported in Great Britain and also in the United States (Blaser et al., 1979; Robinson 1981 and Tosh et al., 1981). In consequence of these results, the public should be made aware of the hazard of campylobacter and other pathogens in raw milk and should be advised to boil milk before consumption.

The sensitivity test (Table 4) revealed that Erythromycine, Amikacine and Gentamycine were the most effective antibiotics, while Tetracycline, Penicillin and Cephalothine were not effective. Therefore, selected antibiotic to be used in treatment of campylobacteriosis should be based on sensitivity test (D'Amato and Hochstein 1982).

From the above mentioned results and discussion, it can be concluded that control of Campylobacter jejuni/coli infections will ultimately be based

on a better understanding of the reservoirs, epidemiology and pathophysiology of the infection. Since the available evidence shows that households and domestic farm animals constitute a major reservoir for these organisms. Interruption of transmission to human-beings from these sources should have a high priority.

For the prevention of Campylobacteriosis in man, the following instructions and suggested recommended measures must be taken into consideration which include: pasteurization of milk, chlorination of water, thorough cooking of meat and other foods suspected of C. jejuni/coli contamination and careful handling, hand washing after contact with animals or contaminated products. These points and others are particularly very important in view of the results of this research and other scientific investigations reported in this paper.

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Table 1. Frequency distribution of Campylobacter species isolated from human stools.

Human-beings (Cases/or Groups)	Total No of tested stool specimens	No of +ve cases to Campylobacter species	Overall (%)	Hippurate Hydrolysis Test				
				+ve C. jejuni	%	-ve C. coli	%	
1. Adults :								
a- Patients with diarrhea	195	21	5.9	15	31.9	6	12.7	
b- Symptomatic household contacts of positive patients with diarrhea	38	9	2.5	6	12.7	3	6.4	
c- Asymptomatic household contacts of positive patients with diarrhea	50	4	1.1	3	6.4	1	2.1	
2. Children (3-5 years old)	75	13	3.6	10	21.3	3	6.4	
Total	358	47	13.1	34	72.3	13	27.6	

Table 2. Frequency distribution of Campylobacter species isolated from examined animals.

Diarrhoeic animal species	No. of examined samples	No. of +ve for Campylobacter species	Overall (%)	C. jejuni	%	C. coli	%
Cattle	48	14	6. 5	11	21. 6	3	5. 9
Horse	23	4	1. 9	4	7. 8	-	0.0
Sheep	25	5	2. 3	5	9. 8	-	0.0
Goats	20	4	1. 9	4	7. 8	-	0.0
Dogs	29	10	4. 7	8	15. 7	2	3. 9
Cats	25	7	3. 3	6	11. 8	1	2. 0
Turkey	20	3	1. 4	3	5. 9	-	0.0
Pigeons	25	4	1. 9	4	7. 8	-	0.0
Total	215	51	23.7	45	88. 2	6	11. 8

Table 3. Incidence of isolated Campylobacter species in examined milk samples.

Source of samples	No. of samples examined	No. of +ve samples	Overall (%)	C. jejuni	%	C. coli	%
Dairy farms	45	6	6. 4	6	66. 7	-	0.0
Shops	20	2	2. 1	2	22. 2	-	0.0
Stores	15	1	1. 1	1	11. 1	-	0.0
Supermarket survey (packets fresh)	14	-	0.0	-	0.0	-	0.0
Total	94	9	9. 6	9	100.0	-	0.0

Table 4. Antimicrobial sensitivity tests of the Campylobacter jejuni isolated from humans and animals.

Antibiotic used	Human strains No. (34)		Animal strains No. (45)		Milk strains No. (9)	
Erythromycine	S		S[a]		S	
Amikacine	S		S		S	
Gentamycine	S		S		S	
Ampicilline	S (8)	R (26)	S (34)	R (11)	S (5)	R (4)
Sulphonamide	S (16)	R (18)	S (19)	R (16)	S (6)	R (3)
Chloramphenicol	S (26)	R (8)	S (25)	R (20)	S (7)	R (2)
Streptomycin	S (17)	R (17)	S (25)	R (20)	S (4)	R (5)
Tetracycline	R		R[b]		R	
Penicillin G	R		R		R	
Cephalothine	R		R		R	

a = S = Sensitie (+, ++ and +++).
b = R = Resistant.
( ) = Number of strains.