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PREVALENCE OF *CAMPYLOBACTER JEJUNI* AND *CAMPYLOBACTER COLI* IN CALVES AND LAMBS WITH AND WITHOUT DIARRHEA AND THEIR PUBLIC HEALTH IMPORTANCE

(With 3 Tables)

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نسبة انتشار الكامبيلوباكتر جوجيني والكامبيلوباكتر كولاي في العجول والحملان المصابة أو غير المصابة بالإسهال وأهميتهما على الصحة العامة

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أجريت هذه الدراسة على عدد ٣٠٩ مسحة شرجية من العجول (١٦٧) والحملان (١٤٢) المصابة بالإسهال و السليمة ظاهريا لدراسة نسبة انتشار الكامبيلوباكتر جوجيني والكامبيلوباكتر كولاي. وقد أسفر الفحص البكتريولوجى عن عزل الكامبيلوباكتر جوجيني من ٢٤ (١٤,٣٧%) من العجول وكانت نسبة انتشار الكامبيلوباكتر جوجيني أعلى في العجول المصابة بالإسهال (٢٣,٤%) من العجول السليمة ظاهريا (١٠,٨٣%). بينما في الأغنام تم عزل الكامبيلوباكتر جوجيني من ١٣ (٩,١٥%) من الحملان وكانت النسبة في الحملان المصابة بالإسهال ١٢,٢٤% بينما كانت النسبة في الحملان السليمة ظاهريا ٧,٥٢%. وقد تم عزل الكامبيلوباكتر كولاي من ١٥ (٨,٩٨%) من العجول وكانت نسبة انتشار الكامبيلوباكتر كولاي أعلى في العجول المصابة بالإسهال (١٧,٨٢%) من العجول السليمة ظاهريا (٦,٦٧%). بينما في الأغنام تم عزل الكامبيلوباكتر كولاي من ١٠ (٧,٠٤%) من الحملان وكانت النسبة في الحملان المصابة بالإسهال ٨,١٦% بينما كانت النسبة في الحملان السليمة ظاهريا ٦,٤٥%. دلت دراسة تأثير المضادات الحيوية علي معزولات الكامبيلوباكتر جوجيني و الكامبيلوباكتر كولاي أن أفضلها فاعلية كان الكلورامفينيكول ويليه كل من الجنتاميسين والنيوميسين. وكانت معظم المعزولات مقاومة للسلفاميسوكسازول و الأمبيسيلين والتتراسيكلين وكانت نسبة ٧٠-٨٠%من المعزولات مقاومة للإريثرومايسين. وكانت نسبة المقاومة للمضادات الحيوية أعلى فى الكامبيلوباكتر كولاي عنها في الكامبيلوباكتر جوجيني. وتلخص هذه الدراسة على أن الكامبيلوباكتر جوجيني والكامبيلوباكتر كولاي تلعب دورا في الإسهال في العجول وأن نسبة انتشار الكامبيلوباكتر جوجيني في العجول والحملان أعلى من نسبة انتشار الكامبيلوباكتر كولاي

وإن كانت الكامبيلوباكتر كولاي أكثر مقاومة للمضادات الحيوية. هذا وقد تم مناقشة الأهمية الصحية العامة لكل من الكامبيلوباكتر جوجيني والكامبيلوباكتر كولاي وطرق الوقاية منهما.

SUMMARY

The prevalence of *Campylobacter jejuni* and *Campylobacter coli* harboring in the intestine of calves and lambs with or without diarrhea was studied. A total of 309 rectal swab samples were collected from calves (167) and lambs (142) with and without diarrhea, the samples were examined bacteriologically. The bacteriological examination revealed the isolation of *C. jejuni* from 24 (14.37%) of 167 examined calves. The prevalence was significantly higher in the diarrheic calves (23.4%) than from the apparently healthy calves (10.83%). In addition, *C. jejuni* was isolated from 13 (9.15%) of 142 sheep examined, where higher (but non significant) percentage (12.24%) was reported in diarrheic lambs than (7.52%) reported in apparently healthy lambs. Also, lower prevalence of *C. coli* was observed in examined samples of calves (8.98%) and lambs (7.04%). The prevalence of *C. coli* was significantly higher in diarrheic calves (17.82%) than apparently healthy calves (6.67%), and higher in diarrheic lambs (8.16%) than apparently healthy lambs (6.45%) (Non significant). Moreover, Campylobacter isolated in this study were tested for susceptibility to 9 antibiotics. None of the isolates were resistant to Chloramphenicol and high percentages of isolates were resistant to Tetracycline, Erythromycin, Ampicillin, Penicillin, Naldixic acid and Sulfamethoxazole, while drug resistance was more frequent in *C. coli* than *C. jejuni.* These results indicate that Campylobacter colonization in the intestine is very common in young calves and lambs and the bacteria play a role, at least in the aggravation of the diarrhea process. Additional studies are needed to assess the role of campylobacter in spontaneous enteric disease of calves and lambs. The results of this study demonstrate that *C. jejuni* is widely distributed, while *C. coli* is more narrowly distributed but significantly more resistant to antimicrobial. The public health importance of *C. jejuni* and *C. coli* were discussed.

Key words: *Campylobacter, C. jejuni, C. coli, calves, lambs, diarrhea.*

INTRODUCTION

Diarrhea is the most common symptom of illness in young calves and lambs. Scours can be caused by many organisms, and more than one

causative agent can be present in the one animal. Salmonella, *E coli* and viruses such as Rotavirus are the most common cause of scours in young calves, but protozoa such as Cryptosporidia and Coccidia, and bacteria such as Campylobacter can also cause this problem (Radostits *et al.*, 2000).

Campylobacter is a Gram-negative slender, curved, and motile rod. It is a microaerophilic organism, which means it has a requirement for reduced levels of oxygen. It is relatively fragile, and sensitive to environmental stresses (e.g., 21% oxygen, drying, heating, disinfectants, and acidic conditions). Because of its microaerophilic characteristics the organism requires 3 to 5% oxygen and 2 to 10% carbon dioxide for optimal growth conditions (Betty *et al.*, 1998).

It is evident that *C. jejuni* and *C. coli* are responsible factor of diarrhea in several animal species and human (Sato *et al.*, 2004). However, the role of Campylobacter in calf diarrhea is not entirely assured. Experimental data (Al-Mashat and Taylor, 1983) indicate that infection with Campylobacter can induce diarrhea in calves. In other studies, no differences in prevalence of *C. jejuni* between healthy and diseased calves were observed (Busato *et al.*, 1998).

C. jejuni is a common and important cause of bacterial diarrhea in humans, equaling or exceeding Salmonella and Shigella spp. in prevalence. *C. coli* is less frequent than *C. jejuni* as a cause of diarrheic disease in humans. The source of infection for humans in developed countries is thought to be the massive reservoir of *C. jejuni* and to a lesser extent *C. coli* in the animal population. It is assumed that man may be infected by direct contact with diseased animals suffering from Campylobacter diarrhea or by consumption of food or water contaminated by secretion or excretion of diseased animals (Acha and Szyfers, 1991; Shakespeare, 2002 and Hartmut *et al.*, 2003).

Increasing antimicrobial resistance in Campylobacter is a recognized problem, (Sáenz *et al.*, 2000 and Jensen and Aarestrup, 2001). The increasing uses of antibiotics in treatment of animal diseases especially enteric diseases may create more resistant strains of Campylobacter in human and animal.

This work was planned to:

- Study the prevalence of *C. jejuni* and *C. coli* in the feces of calves and lambs with and without diarrhea, and its possible role in enteric diseases in animals.
- Study the role of calves and lambs as a source of infection to human with *C. jejuni* and *C. coli.*

- Study the susceptibility of Campylobacter isolated from calves and lambs to a panel of 9 antibiotics.

MATERIALS and METHODS

1- Collection of samples:

Rectal swab samples were collected from 120 apparently healthy calves and 47 calves suffering from diarrhea (the calves were 15 days to 6 months age). Also, 93 rectal swab samples were collected from apparently healthy lambs (one week to 3 months age) and 49 from lambs suffering from diarrhea. The samples were collected from Behera and Alexandria Provences. The animals were raised individually or in small groups.

2- Bacteriological examination of samples:

Rectal swab samples were inoculated into sterile tube containing brucella broth to preserve the viability of the organism during transportation to the laboratory. The samples were examined for *C. jejuni* or *C. coli* by streaking on Skirrow medium contained, 5-7% horse blood and campylobacter selective supplements (Oxoid). Plates were incubated at 42°C for 48 h in an atmosphere of 5% oxygen, 10% carbon dioxide, and 85% nitrogen in standard anaerobe jars. Then the bacterial isolates were purified and identified by using colonial, morphological and biochemical characteristics (Koneman *et al.*, 1988 and Quinn *et al.*, 2002).

3- In-vetro antibiotic sensitivity testing:

The technique was carried out using the disc diffusion method according to Bopp *et al.* (1985). Bacterial isolates (10 from each) were tested for resistance using gradient disk diffusion MIC to Neomycin (30 μg), Gentamycin (10 μg), Ampicillin (10 μg), Streptomycin (10 μg), Naldixic acid (30 μg), Erythromycin (15 μg), Chloramphenicol (30 μg) and Sulfamethoxazole (25 μg), Tetracycline (30 μg) by Oxoid. Three colonies of Campylobacter organisms were inoculated into tubes containing 5 ml Muller Hiton broth (Oxoid), then incubated for eight hours under reduced oxygen tension at 37°C. The turbidity was adjusted to match that of the standard McFarland 0.5 barium sulfate tube (0.5 ml of 1.175% barium chloride hydrate at 99 ml of 1% sulfuric acid) by adding sterile saline solution. The suspension was then inoculated evenly on 150-mm Mueller-Hinton agar plates supplemented with 5% defibrinated sheep blood Antibiotic disc were placed on the surface of agar plate in a radial pattern with the lowest concentration toward the

center. The plates were incubated for 72 h. at 37°C under the microaerophilic conditions, and the inhibitory zone diameters were measured.

RESULTS

The prevalence of *C. jejuni* and *C. coli* in rectal swab samples collected from calves and lambs (Table 1).

Isolates of *C. jejuni* were recovered from 11 (23.4%) of the 47 diarrheic calves which was significantly higher than from 13 (10.83%) of the 120 apparently healthy calves. Total number of positive samples for *C. jejuni* was 24 (14.37%) of 167 rectal swab samples from calves. *C. coli* was recovered from 7 (17.82%) of the 47 diarrheic calves which was significantly higher than apparently healthy calves (6.67%). Totally, *C. coli* was isolated from 15 (8.98%) of examined samples of calves.

Isolates of *C. jejuni* were recovered from 6 (12.24%) of the 49 diarrheic lambs and from 7 (7.52%) of 93 apparently healthy sheep. Totally, *C. jejuni* was isolated from 13 (9.15%) of 142 lamb's fecal samples. *C. coli* was recovered from 4 (8.16%) of the diarrheic lambs and from 6 (6.45%) of apparently healthy sheep. Totally, *C. coli* was isolated from 10 (7.04%) of samples collected from lambs.

Table 1: Prevalence of *Campylobacter jejuni* and *Campylobacter coli* in rectal swab samples collected from calves and lambs.

Animal		*C. jejuni*			*C. coli*		
		No of samples	No of +ve	% of +ve	No of samples	No of +ve	% of +ve
Cattle	Diarrhea	47	11	23.4	47	7	17.82
	Healthy	120	13	10.83	120	8	6.67
	Total	167	24	14.37	167	15	8.98
	X	4.34			2.08		
	P	0.037 (P<0.05)*			0.094 (P>0.05)**		
Sheep	Diarrhea	49	6	12.24	49	4	8.16
	Healthy	93	7	7.52	93	6	6.45
	Total	142	13	9.15	142	10	7.04
	X	0.86			0.14		
	P	0.354 (P>0.05)***			0.705 (P>0.05)***		

χ is chi square P is probability * Significant difference
** Approach significance *** Non significant

In-vetro antimicrobial sensitivity

As shown in Table (2), *C. jejuni* isolated from cattle and sheep were sensitive to Chloramphenicol (100%) followed by Gentamycin and Neomycin. All *C. jejuni* isolated from cattle and sheep were resistant to Sulfamethoxazole, Penicillin, Ampicillin, Erythromycin and Tetracycline.

As shown in Table (3), all *C. coli* isolated from cattle were sensitive to Chloramphenicol followed by Gentamycin and Neomycin but show high resistance to Erythromycin, Tetracycline, Penicillin, Sulfamethoxazole, Ampicillin and Naldixic acid. The percentages of resistant strains were higher in *C. coli* than *C. jejuni*

Table 2: Antimicrobial sensitivity of *Campylobacter jejuni* isolated from calves (n =10) and lambs (n =10):

Antimicrobial	Calves						Lambs					
	Sensitive		Moderate		Resistant		Sensitive		Moderate		Resistant	
	No	%	No	%	No	%	No	%	No	%	No	%
Neomycin	6	60	4	40	0	0	5	50	5	50	0	0
Gentamycin	7	70	3	30	0	0	7	70	3	30	0	0
Ampicillin	0	0	2	20	8	80	0	0	1	10	9	90
Erythromycin	0	0	3	30	7	70	0	0	2	20	8	80
Chloramphenicol	9	90	1	10	0	0	9	90	1	10	0	0
Nalidixic acid	1	10	3	30	6	60	0	0	4	40	6	60
Sulfamethoxazole	0	0	2	20	8	80	0	0	2	20	8	80
Tetracycline	0	0	1	10	9	90	0	0	2	20	8	80
penicillin	0	0	0	0	10	100	0	0	0	0	10	100

n= number of examined isolates.

Table 3: Antimicrobial sensitivity of *Campylobacter coli* isolated from calves (n=10) and lambs (n =10):

Antimicrobial	Calves						Lambs					
	Sensitive		Moderate		Resistant		Sensitive		Moderate		Resistant	
	No	%	No	%	No	%	No	%	No	%	No	%
Neomycin	6	60	3	30	1	10	5	50	3	30	2	20
Gentamycin	7	70	2	20	1	10	6	60	4	40	0	0
Ampicillin	0	0	2	20	8	80	0	0	1	10	9	90
Erythromycin	0	0	1	10	9	90	0	0	1	10	9	90
Chloramphenicol	7	70	3	30	0	0	7	70	3	30	0	0
Nalidixic acid	0	0	2	20	8	80	0	0	1	10	9	90
Sulfamethoxazole	0	0	1	10	9	90	0	0	2	20	8	80
Tetracycline	0	0	1	10	9	90	0	0	1	10	9	90
penicillin	0	0	0	0	10	100	0	0	0	0	10	100

n= number of examined isolates.

DISCUSSION

Campylobacter spp. have been recognized as a cause of diarrhea in cattle and sheep (Radoststits *et al.*, 2000). In this study, rectal swab samples were collected from calves and lambs with and without diarrhea and used for isolation of *C. jejuni* and *C. coli.*

As shown in Table (1) *C. jejuni* was isolated from 24 out of 167 examined samples of calves (14.37%). Nearly similar isolation rates were reported by Hoar *et al.* (1999) and Beach *et al.* (2002). However, higher isolation rates were reported by Adesiyun *et al.* (1992), Giacoboni *et al.* (1993) and Pezzato *et al.* (2003) who isolated *C. jejuni* at rates from 11 to 54%. On the contrary lower rates of isolation were reported by Das *et al.* (1993) and Rosef *et al.* (1983). The prevalence of Campylobacter species in cattle varies from very low to 100% (Rosef *et al.*, 1983; Warner *et al.*, 1986 and Busato *et al.*, 1999). The difference in prevalence may be due to epidemiological situation, season, the age of animals, and the method of diagnosis (Busato *et al.*, 1999 and Sato *et al.*, 2004).

The data presented in Table (1) revealed that *C. jejuni* was isolated from examined sheep samples at a percentage of 9.15%. This bacteria was isolated from sheep feces by many previous studies like Turkson *et al.* (1988) and Adesiyun *et al.* (1992).

The results illustrated in Table (1) showed that *C. coli* was isolated from examined samples of calves and lambs at a percentage of 8.98 and 7.04%, respectively. Nearly similar result was recorded by Terzolo (1988) and Kakkar and Dogra (1990), and lower result was reported by Rosef *et al.* (1983). On the other hand higher result was recorded by Terzolo (1988) and Giacoboni *et al.* (1993).

The prevalence of *C. jejuni* was higher than *C. coli* in calves and lambs (Table 1). This support the observation of Rosef *et al.* (1983) and Busato *et al.* (1999) who reported that *C. coli* is less frequently involved in animal than *C. jejuni*. However, the epidemiological features of both are similar and some times not further differentiated in the literature (Busato *et al.*, 1999).

As shown in Table (1), the prevalence of *C. jejuni* and *C. coli* was significantly higher in calves and lambs suffering from diarrhea. The study provided evidence of significant associations between diarrhea and infection with *C. jejuni* and *C coli*. This support the observations of previous studies where; Diker *et al.* (1990); Busato *et al.* (1998), Acha *et al.* (2004) and Dodson and Lejeune (2005) who isolated

Campylobacter from enteric diseases in calves. Further more, in experimental study, oral inoculation of campylobacter was able to produce enteritis in calves (Al-Mashat and Taylor, 1983). Kerr (2004) mentioned that *C. jejuni* can cause mild to moderate diarrhea in calves that is often thick and contain mucous and or blood. In the other hand, Busato *et al.* (1998) could not find differences in prevalence of *C. jejuni* between healthy and diseased calves. This result (no significant differences between diarrheic and healthy) was observed in sheep in this study (Table, 1).

Campylobacteriosis is acute or chronic infection of humans and animals, transmission from animals to human has been known for *C. jejuni* and *C. coli.* The infection in man manifested by acute enteritis, abdominal pain, diarrhea in most cases stool contain blood, pus or mucous, fever up to 40 °C and in few cases vomiting. Abdominal symptoms may lead to laparotomy or appendectomy (Acha and Szyfers, 1991, Shakespeare, 2002 and Hartmut *et al.,* 2003).

An in-vetro antibiotic sensitivity test was done against isolated strains of Campylobacter using a panel of 9 antibiotics (Table, 2 and 3). *C. jejuni* and *C. coli* were sensitive to Chloramphenicol. This support the data reported by Diker *et al.,* (1990) who treated cases of bovine diarrhea due to Campylobacter successfully with Chloramphenicol. In addition, high percentage of isolates of *C. jejuni* and *C. coli* were resistant to Ampicillin, Penicillin, Tetracycline, Erythromycin, Naldixic acid and Sulfamethoxazole but drug resistance was more frequent in *C. coli* than *C. jejuni*. These findings are agree with that recorded by Thwaites and Frost (1999), Chuma *et al.* (2001) and Bae *et al.* (2005) who reported hat there are multiple-antibiotic resistance in *C. coli*, but disagree with Leatherbarrow (2004) who demonstrated that there was a very low prevalence of resistance among the *C. coli* isolates. It is known that *C. jejuni* and *C. coli* have different susceptibility profiles, but resistance traits are known to be readily transferred among species of Campylobacter (Bodeis *et al.,* 2002 and Sato *et al.,* 2004).

However, because of the susceptibility tests for Campylobacter species are not standardized and the use of antibiotics in the animals may promote the emergence of multi-antibiotic resistant mutant of campylobacter species (Betty *et al.,* 1998 and Shakespeare, 2002). In addition, Campylobacter is one of the microorganisms of moderate intrinsic susceptibility which require only one mutation to become resistant (Wiedemann and Heisig, 1994).

In conclusion:

Due to the presence of Campylobacter in the small intestine of diarrhea calves, a contribution of these bacteria within the pathogenesis of calf diarrhea is possible. Final evaluation of their pathogenic importance is only positive by means of virulence tests. The results of this study demonstrate that *C. jejuni* is widely distributed among cattle farms, while *C. coli* is narrowly distributed but significantly more resistant to antimicrobial. In addition, calves and lambs especially those suffering from diarrhea are a potential source of *C. jejuni* and *C. coli* for human, which can be transmitted to man in-contact. So, the following hygienic measures should be under-taken, periodical cleaning and disinfection of animal houses, hand washing and immersion in a mild antiseptic after handling of infected animals, hygienic disposal of animal excreta and attention should be paid to food and water given to animals.

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