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ENTERIC DIARRHOEAL DISEASE IN SHEEP CAUSED BY CAMPYLOBACTER INFECTION IN ALEXANDRIA GOVERNORATE

(With 4 Tables)

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الإسهال المعوى فى الأغنام المتسبب نتيجة العدوى ببكتريا الكامبيلوباكتر فى محافظة الأسكندرية

علا عبد العزيز باشا ، فتح الله على الشابورى

إشتملت الدراسة على فحص عدد ١٠٠ مسحة شرجية من الأغنام المصابة بالإسهال المعوى لدراسة مدى الإصابة بعدوى ميكروب الكامبيلوباكتر. وقد أسفر الفحص البكتريولوجى عن عزل ميكروب الكامبيلوباكتر جوجينى من ١٢ حيوان بينما تم عزل بكتريا الكامبيلوباكتر كولاى من ٩ حيوانات مصابة فقط. ودلت دراسة تأثير المضادات الحيوية على معزولات الكامبيلوباكتر الجوجينى والكولاى أن أفضلها فاعلية كان الكتورامفنيكول يليه النيومايسين والأمبيسيلين والجنتاميسين. بينما كانت معظم المعزولات مقاومة للتتراسيكلين والبنسلين والسلفاميزوكسازول. وتتلخص هذه الدراسة على أن الكامبيلوباكتر تلعب دورا فى الإصابة بالإسهال المعوى فى الأغنام. هذا وقد تم مناقشة الأهمية الصحية العامة لميكروبات الكامبيلوباكتر المعزولة وطرق الوقاية منهما.

SUMMARY

The incidence of Campylobacter jejuni and Campylobacter coli harbouring the intestine of sheep with diarrhea was studied. A total number of 100 rectal swab samples were collected from sheep suffering from diarrhea at Alexandria governorate animal inclosures. The samples were examined bacteriologically. The bacteriological examination revealed the isolation of Campylobacter jejuni from 12 (12 %) out of 100 examined sheep, while Campylobacter coli could be isolated from 9 cases of diarrheic sheep examined. Moreover, Campylobacter isolated in this study were tested for susceptibility of 10 antibiotics. None of the isolates were resistant to Chloramphenicol and high percentages of isolates were resistant to Tetracycline, Penicillin and Sulphamethoxzole.

These results indicate that Campylobacter colonization in the intestine is very common in sheep and the bacteria play a role in aggravation of diarrhea process. The public health importance of Campylobacter jejuni and Campylobacter coli were discussed.

Key words: *Campylobacter, diarrhea, antibiotic sensitivity*

INTRODUCTION

Diarrhoea is the most common symptom of illness in lambs, which can be caused by many organisms, and more than one causative agent can be present in the one animal like Salmonella, E-coli, Viruses such as Rotavirus and Campylobacter which can also cause this problem (Radostits *et al,*. 2000). Campylobacter species are now recognized as one of the most important causes of enteric diarrhoeal disease in domestic animals and humans throughout the world (Atabay and Corry, 1997). hence, Campylobacter enteritis constitutes a zoonoses of major concern in puplic health and indeed, has been shown to be a greater problem than salmonellosis in several countries (Stanley *et al.,* 1998). Campylobacter is one of the most important foodborn microorganisms leading to gastroenteritis in human. These human cases are most likely associated with handling or consumption of undercooked meat products (Oosterom *et al.,* 1984).

The source of infection for humans in developed countries is though to be the massive reservoir of Campylobacter jejuni and to a lesser extent Campylobacter coli in the animal population. It is assumed that man may be infected by direct contact with diseased animals suffering from Campylobacter diarrhoea or by consumption of food or water contaminated by secretion or excretion of diseased animals (Acha and Szyfers. 1991, Shakespeare, 2002 and Harmut *et al.,* 2003). Campylobacter is a Gram- negative cylender, curved, and motile rod. It is a microaerophilic organism, as it requires a reduced levels of oxygen for growth. It is relatively fragile, and sensitive to environmental stresses such as 20% oxygen, drying, heating, disinfectants and acidic conditions. Because of its microaerophilic characteristics, the organism requires 5% oxygen and 10 % carbon dioxide for optimal growth conditions (Betty *et al.,* 1998). It is evident that Campylobacter jejuni and Campylobacter coli are responsible factor of diarrhea in sheep (Diker, 1987; Terzolo, 1988 and Khalil *et al.,* 1993).

Increasing antimicrobial resistance Campylobacter infection cases is a recognized problem, (Saenz *et al.,* 2000 and Jensen and

Aarestrup, 2001). However, Gur *et al.* (1989) stated that quinolone antibiotics were very active against Campylobacter species and showed that there was no any marked difference between the susceptibility patterns of C. jejuni and C.coli.

The present study was designed to investigate the presence of Campylobacter infection in sheep at Alexandria governorate animal inclosures. Moreover, due to the public health hazard of antibiotic resistant for Campylobacter, antibiotic sensitivity patterns of Campylobacter Jejuni and Campylobacter coli were evaluated.

MATERIALS and METHODS

Collection of samples:

Sterile rectal swab samples were collected from 100 diarrheic sheep of different ages and sex suffering from diarrhea, raised in small groups at Alexandria governorate.

Bacteriological examination:

Rectal swab samples were inoculated into sterile tube containing brucella broth (Retting, 1979) to preserve the viability of the organism during transportation to the laboratory. The samples were examined for Campylobacter jejuni and Campylobacter Coli by streaking on Skirrow medium (Skirrow, 1977) containing 5-7 % horse blood and Campylobacter selective supplement (Oxoid). The plates were incubated at 42 °C for 48 hours in an atmosphere of 5 % oxygen, 10 % carbon dioxide and 85 % nitrogen in standard anaerobic jars. The bacterial isolates were purified and identified by using colonial, morphological and biochemical characteristics (Koneman *et al.* 1988 and Quinn *et al.* 2002).

In – vetro antibiotic sensitivity test:

Different types of antibacterial sensitivity discs were used in the sensitivity tests to determine the sensitivity and resistance of different isolated strains of Campylobacter jejuni and Campylobacter coli. The antibiotic discs were obtained from Oxoid. The technique was carried out using disc diffusion method according to Bopp *et al.* (1985). Three colonies of Campylobacter organisms were inoculated into tubes containing 5 ml Muller Hinton broth (Oxoid), then incubated for eight hours under reduced oxygen tension at 37 °C until the turbidity exceeded that of the standard McFarland 0.5 barium sulphate tube (0.5 ml of 1.175 % barium chloride hydrate to 99 ml of 1 % sulphuric acid). The turbidity was adjusted to match that of the McFarland 0.5 barium

sulphate standard tube by adding sterile saline solution. The suspension was then inoculated evenly on 150 mm Muller – Hinton agar plates supplemented with 5 % defebrinated sheep blood. Antibiotic discs 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 hours at 37 °C under the microaerophilic conditions and the inhibitory zone diameters were measured.

RESULTS

Incidence of C. jejuni and C. coli in rectal swab samples collected from diarrheic sheep:

Isolates of Campylobacter jejuni were recovered from 12 (12%) out of 100 rectal swab fecal samples examined while Campylobacter coli was recovered in 9 (9 %) of diarrheic sheep.

Totally C. jejuni and C. coli could be isolated from 21 out of 100 sheep examined Table (1). The biochemical characteristics of Campylobacter isolates are illustrated in Table (2).

In – Vitro antimicrobial sensitivity

As shown in Table (3), C. jejuni isolated were sensitive to Chloramphenicol (100 %) followed by Neomycin and Gentamycin (80 %), while 60 % only for Ampicillin. On the other hand, all isolates of C. jejuni were resistant to Tetracyclin (100 %) and 80 % of isolates were resistant to Erythromycin, Sulphamethoxazole and Pencillin.

As shown in table (4), C. coli isolated from sheep rectal swab samples were sensitive to Ampicillin and Neomycin (100 %) followed by Chloramphenicol (80 %) but they showed high resistance to Penicillin, Tetracyclin and Naladixic acid.

Table 1: Incidence of C. jejuni and C. coli in rectal swab samples collected from diarrheic sheep.

No. of sheep examined	C. jejuni		C. coli	
	No. of Isolates	%	No. of isolates	%
100	12	12	9	9

Table 2: Biochemical reactions of Campylobacter jejuni and Campylobacter coli isolated from sheep suffering from diarrhea.

Test	C. jejuni	C. coli
Catalase test	+ve	+ve
Oxidase test	+ve	+ve
H2S on lead acetate	+ve	+ve
H2S on T S I	-ve	-ve
Nitrate reduction	+ve	+ve
Hippurate hydrolysis	+ve	-ve
Glycin (1%)tolerance test	+ve	+ve
Sodium chloride (3.5%) tolerance test	-ve	+ve
Growth at 25°C	-ve	-ve
Growth at 37 °C	+ve	+ve
Growth at 42°C	+ve	+ve

Table 3: Antimicrobial sensitivity of Campylobacter jejuni isolated from sheep (n = 5)

Antimicrobial agents	Degree of sensitivity					
	Sensitive		Moderate		Resistant	
	No	%	No	%	No	%
Neomycin N3O	4	80	1	20	0	0
Gentamycin GM10	4	80	1	20	0	0
Ampicillin AMP10	3	60	2	40	0	0
Stryptomycin S10	0	0	3	60	2	40
Erythromycin E15	0	0	1	20	4	80
Chloramphenicol C30	5	100	0	0	0	0
Naladixic Acid NA30	1	20	1	20	3	60
Sulphamethoxazole SX T25	0	0	1	20	4	80
Penicillin (10 I U)	0	0	1	20	4	80
Tetracyclin T30	0	0	0	0	5	100

Table 4: Antimicrobial sensitivity of Campylobacter coli isolated from sheep (n = 5)

Antimicrobial agents	Degree of sensitivity					
	Sensitive		Moderate		Resistant	
	No	%	No	%	No	%
Neomycin N3O	5	100	0	0	0	0
Gentamycin GM10	3	60	2	40	0	0
Ampicillin AMP10	5	100	0	0	0	0
Stryptomycin S10	1	20	3	60	1	20
Erythromycin E15	1	20	0	0	4	80
Chloramphenicol C30	4	80	1	20	0	0
Naladixic Acid NA30	1	20	0	0	4	80
Sulphamethoxazole SX T25	3	60	2	40	0	0
Penicillin (10 I U)	0	0	0	0	5	100
Tetracyclin T30	0	0	1	20	4	80

DISCUSSION

Campylobacter species are microaerobic inhabitant of the gastrointestinal tract of various animals including sheep (Betty *et al.* 1998). It have been recognized as a cause of diarrhea in sheep (Radostitis *et al.* 2000). In this study, rectal swab samples were collected from sheep with diarrhea and used for isolation of Campylobacter jejuni and Campylobacter coli. As shown in table 1 Campylobacter jejuni was isolated from examined rectal swab sheep samples at a percentage of 12%. This bacteria was isolated from sheep faeces by many various studies (Turkoson *et al.* 1988 and Adesiyun *et al.* 1992). Nearly similar percentage was recorded by Rosef *et al.* (1983) who isolated 8.1 %. Higher recovery rates were recorded by Manser and Dalziel (1985) and Adesiyun *et al.* (1992) who isolated 22 and 17.9 % respectively.

On the other hand, low rate of 2 % was recorded by Turkoson *et al.* (1988). These differences in isolation rates may be attributed to sample collection in different seasons as high temperature in summer months, while lower recovery rates occurred during winter and spring and this was reported by Hanninen *et al.* (2000). The result illustrated in table 1 showed that Campylobacter coli was isolated from examined rectal swab sheep samples at percentage of 9 %. Nearly similar results

were recorded by Terzolo (1988) and Kakkar and Dogra (1990), and lower result was reported by Rosef *et al.* (1983) but higher result was recorded by Giacoboni *et al.* (1993)

The rate of isolation of Campylobacter jejuni was higher than Campylobacter coli in rectal swab samples of sheep. Table (1). This support the observation of Rosef *et al.*, (1983) and Busato *et al.* (1999) who reported that Campylobacter coli is less frequently involved in animal than Campylobacter jejuni. However, the epidemiological features of both are similar and sometimes not further differentiated in literature (Busato *et al.* 1999).

Campylobacteriosis is acute or chronic infection of humans and animals. Transmission from animals to human has been known for Campylobacter jejuni and Campylobacter coli. The infection in man is manifested generally by acute enteritis, abdominal pain, diarrhea in most cases with 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 and Hartmut *et al.* 2003).

In- vetro antibiotic sensitivity test was done against isolated strains of Campylobacter using a panel of 10 antibiotics (Table, 3 and 4). The most effective antibiotics for all isolates of Campylobacter jejuni and Campylobacter coli were Chloramphenicol. This support the data reported by Diker *et al.* (1990). The isolates of Campylobacter species at this study were resistant to Tetracycline Penicillin and Sulphamethoxazole. These findings agree with that recorded by Erdger and Diker (1995).

In conclusion, presence of Campylobacter in the small intestine of sheep represent a public health hazard. Moreover, because of multidrug resistance among Campylobacters hygienic measures should be undertaken such as cleaning and disinfection of animal houses, avoidance of improper use of antibiotics, hygienic disposal of animal excreta and proper cooking and hygienic handling of meat before consumption.

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