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A SURVEY OF CAMPYIOBACTERS IN HEALTHY AND DIARRHOEIC COWS AND BUFFALOES IN EGYPT

By

Fawzy R. El - Seedy * , Adel M. Khalid ** and Ali S. El - Ged**

*Faculty of Veterinary Medicine , Beni Souif , Cairo University
 , Beni Souif Branch .

** Faculty of Veterinary Medicine , Moshtohor , Zagazig University Benha
 Branch .

SUMMARY

Campylobacters showed a marked increase in their incidence in diarrhoeic cows (21 .2%) than apparently healthy ones (15 .1%) . A great difference could be detected in carriage rates between healthy and diarrhoeal buffaloes . Incidence in diarrhoeic buffaloes was considerably higher than in those with no obvious signs of enteritis (13.1% Vs 4.4%) .

C. jejuni and C. coli were found in higher incidence in bovine with diarrhoea than in those without . Also , C. lariidis was present only in diarrhoeic cows but not in the rectal samples obtained from examined buffaloes

Many campylobacters did not grow onto blood - free selective medium but mostly appeared onto blood containing selective medium . The combination of both media is therefore necessary to ensure the maximum isolation of campylobacters from feces .

It was best to treat diarrhoea in cows or buffaloes due to campylobacter infection by using chloramphenicol , oxytetracycline , neomycin or erythromycin . On the contrary , penicillin - G, streptomycin and sulphonamides should be avoided .

INTRODUCTION

Campylobacters have long been recognized as pathogens in man and animals under this earlier name of vibrio (*Butzler , et al. 1973 and Skirrow 1977*) . Although there is much evidence that campylobacter enteritis is a zoonosis and that farm animals are probably the principal

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sources of infection (*Terzolo 1988 and Meanger and Marshall 1989*) there is little published information on the carriage rates of campylobacters in these animals especially cattle . Moreover , buffaloes need to be examined in this country to establish incidence rates for this organism .

The percentages of positive isolation of campylobacters from the rectal contents of diarrhoeic cows varied from 18% (*Hawari 1979*) , 25.9% (*Blumenshein 1980*) , 19% (*Svedhem and Kauser 1981*) and 22.6% (*Lammerding et al. 1988*) . The number of isolates of campylobacters from cattle has been recorded to increase greatly during the last years (*Bolton et al. 1985*) .

C. jejuni and to a lesser extent *C. coli* are now the most commonly recognized enteric bacterial pathogens (*Hawari 1979 and Svedhem and Jauser 1981*) . Moreover , prescott and *Bruin - Mosch (1981)* found a clear difference in the incidence of *C. jejuni* between the healthy and diarrhoeic cows . . *Waterman et al. (1984)* detected . *C. jejuni* and *coli* in the feces of 13% of the herd during summer and 51% of the same herd during winter and its counts were higher during the winter than the summer . They found that some cows excreted campylobacters during the summer and did not during winter .

Some workers observed that cows with diarrhea had more *C. coli* in their feces than healthy ones (*Aeschbacher and Piffaretti 1989*) .

The purpose of this investigation was to ascertain the incidence of campylobacters in the rectal contents of cows and buffaloes with signs of diarrhoea and those without . Also to provide information about the best media used for the isolation of the organism and to detect the effective antibiotics against the campylobacter isolates to guide therapy .

MATERIAL AND METHODS

Feces were obtained from the rectum of 245 diarrhoeic animals (85 cows and 160 buffaloes) as well as from 123 animals not having signs of enteric illness . All animals were belonging to private and governmental farms at Kaluobia and Beni Souif Governorates .

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On the day of collection, a single 4 mm loopful of feces was inoculated in duplicate onto each of the following media : (a) Oxoid blood agar base No. 2 containing 7% defibrinated horse blood, 1 mg /L rifampicin, 2500 i.u. /L polymyxin - B sulfate and 5 mg/L trimethoprim . (b) Blood - free selective medium after *Bolton et al. (1983)* which consisted of nutrient broth No. 2 (Oxoid) 25 gm /L, New Zealand agar 12 gm /L bacterial charcoal (Oxoid) 4 gm/L, casein hydrolysate (Oxoid) 3 gm /L, ferrous sulphate 0.25 gm /L and sodium pyruvate 0.25 gm/L, autoclaved at 121 C for 15 minutes, cool and selective agents added to final concentrations of polymyxin - B sulphate 150 I.u. /ml, vancomycin 50 ug/ml and nalidixic acid 60 ug /ml .

All inoculated plates were incubated at 42C for 40 hrs in anaerobic jars from which two - thirds of the air were replaced with 5% Co. 95% Nitrogen mixture .

Suspected campylobacters colonies were identified by colonial morphology, catalase and oxidase reactions, typical motility and cell morphology on examination by phase contrast microscopy. Then were subjected to the following tests : growth in 1% glycine, 5% sodium chloride, production of hydrogen sulphate. Campylobacter colonies were finally identified by the biotyping scheme of *Skirrow and Benjamin (1980)* .

Response of isolated campylobacters to various chemotherapeutic agents in vitro :

Forty eight isolates of campylobacter were obtained from apparently healthy and diarrhoeic cows and buffaloes. Stock culture of 7% horse blood agar was used as inocula for sensitivity testing. Their sensitivity to antimicrobials was examined using the disk and agar diffusion method described by *Cruickshank et al. (1975)* .

Plates of 7% horse blood agar were prepared using drug sensitivity test agar (Oxoid) and inoculated to provide a bacterial load. The antimicrobial disks (Difco) were placed on such plates. Tylosin disks were not available and tylosin tartarate of known potency (Elanco) was used to prepare medium containing 2 and 10 ug /ml. All plates were incubated under anaerobic conditions and examined after 48 hrs. The interpretations of the results were carried out according to the manufacture company. The presence or absence of growth on tylosin containing medium was recorded .

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RESULTS AND DISCUSSION

Campylobacters are widely distributed in the animal kingdom. It is believed many human infections result from exposure to animal derived food such as milk, meat and meat products. Increasing attention is being focused upon the health hazards associated with campylobacter infection. Little is known about epidemiology of the infection in Egyptian buffaloes and cows.

Table (1) shows that out of 85 rectal samples obtained from diarrhoeic cows, 18 were positive for campylobacter organisms (21.2%) however, among 33 cows in apparently healthy conditions, were positive (15.1%) isolation rate campylobacters organisms from the Egyptian cows was nearly similar to that found in several other countries. In *England*, *Terzolo (1988)* reported 29.9% and 20.1% campylobacter isolation in diarrhoeic and healthy cows respectively and in Sweden, *Svedhen and Kauser (1981)* found 19%. Also, *Lammerding et al. (1988)* showed that 22.6% of the cows were carriers in their survey in Canada. In the present study, the carriage rate among the Egyptian cows with evidence of enteric disease was considerably higher than in those with no obvious signs of enteritis (21.2% Vs 15.1%). Similar observations were reported by *Prescott and Bruin - Mosch (1981)* who concluded that a great difference could not be detected in the carriage rate between healthy and diarrhoeic cows. In this study, it was noticed that out of 160 buffaloes suffering diarrhoea; 13.1% proved to be positive for campylobacter and the isolation rate reached 4.4% among the apparently healthy buffaloes. Such a clear difference was not observed in cows. Therefore, the presence of campylobacters per gram of feces varied greatly not only from one species of domestic animals to another but also according to the general health condition either with or without obvious signs of enteric diseases. The buffaloes seemed to be of less importance as a source of campylobacters especially in the apparently healthy buffaloes.

Table (2) shows that 18 diarrhoeic cow presented isolated of campylobacters, 9 (50.0%) were *O*-jejuni, 6 (33.3%) were *C. coli* 2 (11.1%) were *C. laridis* and the remaining strain was unidentified. All isolates of campylobacters recovered from the apparently comparison in cows, this biotype was the only one found amongst the isolates from the apparently healthy cows. These findings were in accordance with by *Hawari (1979)*,

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Prescot and Bruin - Mosch (1981) , *Svedhem and Kauser (1981)* and *Bolton et al. (1983)* who showed that *C. jejuni* , then *C. coli* and *C. laridis* were the main cause of cow campylobacter enteritis . About 20 - 50 % of the tested cows excreted campylobacter particularly *C. jejuni* (80 - 90%) .

Table (2) shows the presence of two campylobacter biotypes among the examined buffaloes (*C. jejuni* and *C. coli*) in an incidence of (52.3% and 33.3%) respectively among diarrhoeic buffaloes and (50 .0% and 25.0%) respectively among the healthy ones .

The incidence of *C. laridis* in the cows suffering diarrhoea in our work reached 11.1% and was not isolated from the apparently healthy cows . *C. laridis* was not isolated from buffaloes with or without signs of enteric disease . It is evident that cows form a large carriage rate of campylobacters than buffaloes . *C. jejuni* and *C. coli* presented a higher incidence in animals with diarrhoea than in those without . *C. laridis* was present only in diarrhoeic cows but not in rectal samples obtained from examined buffaloes .

As shows in table (3) , campylobacters were isolated by direct culture onto blood selective medium from 43 (89.6%) of the 48 positive rectal samples . The remaining 5 samples (10.4%) were obtained only by blood free selective medium . However , 32 (66.7%) were obtained only onto blood free selective medium . Many strains did not grow onto blood - free selective medium . This suggests that more isolates could be obtained by blood selective medium . This survey showed that the combination of blood and blood free selective methods are therefore necessary to ensure the maximum isolation of campylobacters from bovine feces . Blood -containing selective media provided a clear and less frequently contaminated culture plates . *Bolton and Coates (1983)* found that no single supplement was as good as horse blood in sustaining the growth of the inoculated campylobacters . However , a combination of charcoal , ferrous sulphate and sodium pyruvate was found to be as effective as blood in isolation of campylobacters .

As shown in table (4) , all the isolates were examined for sensitivity to 14 anti - microbials . All strains of *C. jejuni* were completely sensitive to oxytetracycline , chloramphenicol , neomycin , erythromycin , gentamicin and tylosin (10 ug) with a satisfactory response . On the other hand , ampicillin , tylosin (2 ug) and furazolidone showed variable

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sensitivity to *C. jejuni*. These results agreed with that reported by *Narita et al. (1988)* who showed that none of *C. jejuni* were resistant to chloramphenicol, neomycin, gentamicin and erythromycin but such strains were resistant to penicillin - G, streptomycin and sulphamethoxazole trimethoprim.

A study of the effect of the anti - microbials on *C. coli* showed that ampicillin, oxytetracycline, chloramphenicol, neomycin, erythromycin, gentamicin and tylosin (10 ug) exhibited complete bactericidal action. These findings nearly coincide with the results obtained by *Blumenshein (1980)* and *Lammerding et al. (1988)*.

The growth of *C. laridis* was completely inhibited by ampicillin, oxytetracycline, chloramphenicol, neomycin, erythromycin, gentamicin and tylosin (10 ug). These results agree with the previous studies reported by *Bolton et al. (1983)* and *Narita et al. (1988)*.

These results suggest that wishing to treat diarrhoea in cows or buffaloes in which campylobacter has been shown by laboratory diagnosis to be involved, could use perfectly oxytetracycline, chloramphenicol, erythromycin, meanwhile, penicillin - G, sulphonamides and streptomycin should be avoided.

Table (1): Number and frequency of campylobacters from rectal contents of domestic animals.

Animal species	Diarrhoeic cases			Apparently healthy cases		
	Number tested	Positive for campylobacter		Number tested	Positive for campylobacter	
		No.	%		No.	%
Cows	85	18	21.2	33	5	15.1
Buffaloes	160	21	13.1	90	4	4.4
Total	245	39	15.9	123	9	7.3

Table (2): Display of campylobacter biotypes isolated from examined animals.

Animal species	With symptoms of enteric disease					Those without (Healthy)			
	Campylobacter biotypes					Campylobacter biotypes			
	C. jejuni	C. coli	C. laridis	Unidentified	total	C. jejuni	C. coli	Unidentified	total
Cows	9 (50.0)	6 (33.3)	2 (11.1)	1 (5.6)	18	5 (100.0)	0 (0)	0 (0)	5
Buffaloes	7 (52.3)	0 (33.3)	0 (0.0)	3 (14.3)	21	2 (50.0)	1 (25.0)	1 (25.0)	4
Total	20 (51.3)	13 (33.3)	2 (5.1)	4 (10.3)	39	7 (77.8)	1 (11.1)	1 (11.1)	9

* Number between brackets indicates the incidence percentage.

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Table (3): Comparison of blood-free and blood containing campylobacter media for isolating different biotypes of campylobacter.

Anim. spec-ies	Total NO. of isolates	Cultural method									
		Positive blood selec.media					Positive-free selec. media				
		C.j	C.c	C.l	Unide-ntified	Total	C.j	C.c	C.l	Unide-ntified	Total
Cows	24	14	4	2	1	21	10	3	1	0	14
Buffaloes	25	13	5	-	4	22	12	4	-	2	18
Total	48	27	9	2	5	43	22	7	1	2	32

. C.c = C.jejuni

. C.c = C.coli

. C.l = C.laridis.

Table (4): Results of in-vitro anti-microbial sensitivity tests performed on 48 isolates of campylobacter biotypes.

Anti-microbial used	Disk potency	No. of isolates sensitive			
		C.jejuni (27) [#]	C.coli (14)	C.laridis (2)	Unidentified (5)
Penicillin-G	1.5 unit	0	0	0	0
Ampicillin	10 ug	18	14	2	3
Oxytetracycline	10 ug	27	14	2	5
Streptomycin	10 ug	6	4	0	0
Chloramphenicol	10 ug	27	14	2	5
Neomycin	10 ug	27	14	2	5
Erythromycin	10 ug	27	14	2	5
Cephalothin	30 ug	10	9	1	1
Gentamicin	10 ug	27	13	2	4
Tylosin	2 ug	20	8	1	0
Tylosin	10 ug	27	14	2	2
Furazolidone	50 ug	19	11	0	2
sulfamethoxazole trimethoprim	25 ug	1	0	0	0

[#] Numbers between brackets indicates the tested strains.

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استبيان مدي انتشار ميكروبات الكمبيلوباكتري في البقر والجاموس

السليمه والمصابه بالاسهال في مصر

فوزى الصعيدى - عادل خالد - على الجدى

فى هذه الدراسة أوضحت أن نسبة ميكروبات الكمبيلوباكتري فى الأبقار المصابه بالأسهال (٢١٢٪) عن مثيلاتها فى الأبقار السليمه (١٥١٪) وكذلك فى الجاموس المصابه وجد أن نسبة الإصابة عالية (١٣١٪).

كما وجد أن هناك فى بعض الأنواع من ميكروبات الكمبيلوباكتري توجد بنسب أعلى من الأخرى كما وجد أنواع موجودة فى الحيوانات المصابه بالأسهال ولم توجد على الإطلاق فى الحيوانات السليمه.

كما أثبتت الدراسة أهمية استخدام بعض المضادات الحيويه مثل الكلورامفينيتول والأوكس تتراسيكلين وعلي العكس لا ينصح باستخدام النسلين والسد بنديمين ومركبات السلفا فى العلاج.