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STUDIES ON THE CAMPYLOBACTER ORGANISMS IN CALVES WITH AND WITHOUT DIARROHEA AT KAFR EL-SHEIKH GOVERNORATE EGYPT

Y.R.Azab and * Samy A. El-Midany

Provisional laboratory at Kafr El-sheikh Governorate

Department of Hygiene and Preventive Medicine, Faculty of Veterinary Medicine. Kafr EL Sheikh University

ABSTRACT

The study included data from 543calves with and without diarrhoea. Fecal samples were analyzed for *Campylobacter* sp. The incidence of *Campylobacter* was17.1% among calves with diarrhoea and 14.3% among apparently healthy calves. Of the 86 Campylobacter isolates, 65(12.0%) were identified as *Campylobacter jejuni* and 21(3.9%) isolates were identified as Campylobacter *coli* .It was found that the incidence of *Campylobacter jejuni* was 31(11.1%) and *Campylobacter coli* 10(3.6%) among apparently healthy calves while in calves showing intestinal disorder the incidence of *Campylobacter jejuni* was 31(12.9%) and Campylobacter coli was11 (4.2%). There was no significance variation in the incidence of *Campylobacter* species from apparently healthy and diarrhoeic calves. Five disinfectants were evaluated for their effectiveness. They were phenolic disinfectant (commercial phenol), a chlorine compound (Saniton), an organic acid (Longlife 250 S), a peroxygen compound (Virkon-S) and Glutardialdehyde (TH4). In the houses detergents as soap followed by Polycar were used followed by disinfectants. The results showed that all disinfectants were effective with variation in inactivation time. Screening of selected *Campylobacter* isolates for determining their antimicrobial susceptibility indicated that most of the tested strains were resistance to three or more of antimicrobial examined and exhibit low resistance to gentamicin and chloramphenicol, while no resistance to amikacin.

For the *Campylobacter* virulence properties, an adult mouse model has been used. As regards *Campylobacter jejuni* .The mortality rate reached 80%,60%,and 20% according to the rout of infection I/P,S/C, and orally respectively. On the other hand ,the infection by the *Campylobacter coli,* the mortality rates reached 70%,40%,and 10%by using I/P,S/C and orally respectively. The obtained results showed that, the intestinal tract was the most predominant site for reisolation of *Campylobacter species*, followed by the liver and blood.

INTRODUCTION

Campylobacter jejuni is now recognized as cause of human enteritis throughout the world **(Allos and Blaser, 1995)**. Moreover, it is currently being discussed as the major infectious agent preceding Guillain–Barré syndrome, an inflammatory demyelization peripheral neuropathy of presumptive autoimmune origin **(Rees et al., 1995).** Diarrhoea outbreaks in humans caused by *C*. *jejuni* and *C.coli* are frequently associated with contaminated water and ingestion of unpasteurized milk (**Fahey et al.,1995 and Morgan et al.,1994)** .The consumption of raw chicken, pig and beef meat is also associated with sporadic cases of human diarrhoea **(Butzler, 2004)**. *Campylobacter* infections are predominantly caused by thermophilic Campylobacters, in particular *Campylobacter jejuni* and its close relative *Campylobacter coli* **(Anonymous, 2003 ; Anonymous, 2004 and Butzler, 2004).** The main factors associated with an increased risk of colonization are the lack of hygiene barriers **(Evans and Sayers, 2000** ; **Hald et al., 2000 and Kapperud et al., 1993).** The incidence of *Campylobacter* in animal was variable (**Shane and Montrose, 1985**). Intestinal infection caused by *Campylobacte*r sp. in domestic and wild birds, pigs, cattle, dogs and cats may be considered important reservoirs of *Campylobacter* sp. (**Al-Mashat and Taylor 1980; 1981 and Butzler, 2004)**. In domestic animals they cause diarrhoea, but they are also frequently isolated from asymptomatic animals **(Marks and Kather, 2003 and Modolo et al., 1987**). *Campylobacter* isolated from animal and humans have been shown to have variable resistance to antimicrobial agents depending on their sources (**Bradbury and Munroe, 1983; Butzler et al.1973**).

Little information is available about *Campylobacter* susceptibility to disinfectants. However the survival of *Campylobacter* on surfaces after cleaning and disinfection has been poorly documented. No available studies have been reported on the isolation of *Campylobacter* species from swabs of surfaces in contact with food after cleaning and disinfection procedures **(Cools et al., 2005;Malakauskas et al., 2006 and Miwa et al., 2003)**.It can be routinely detected in floor surface swabs of commercial transport cages after cleaning **(Newell et al., 2001 and Slader et al., 2002).** In addition, for the disinfection of houses, exposure to UV radiation cannot be considered an appropriate method as it is efficacious only when the surfaces are well cleaned and the source of light is positioned very close to the surfaces to be disinfected **(Samberg and Meroz, 1995).**

Most disinfectants have the optimum of efficacy at temperatures above 20ºC **(Meroz and Samberg, 1995**). The most important predictors of protection from campylobacter infection were related to effective hygiene barriers such as housing in buildings in good state of repair and a high standard of cleansing and disinfection **(Evans and Sayers, 2000)**.

The aim of this work was to investigate

1- the Incidence and identification of *Campylobacter* isolated from apparently normal and diarrheic calves

2- the antimicrobial sensitivities of the Campylobacter strains isolated from these animals.

3- the probability of *Campylobacter* isolation from surfaces houses after routine cleaning and disinfection procedures.

MATERIAL AND METHODS

1. Collection of samples

A total of 543 fecal swabs were obtained from the rectum of 280 apparently healthy calves and 263diarrhoeic calves from private farms for isolation of *Campylobacter* sp. The ages of the calves ranged from 2-8weeks. All samples were collected and transported to the laboratory in the transport broth with supplement and examined immediately within 1hr. Also, gas pack jar and *Campylobacter* gas generating kit were used.

2 Disinfectants

1-Aldekol des 03, (Ewabo Chemikalien GmbH Chem-Pharmazeutische Produkla KolpingstraBe 4, Germany), contains Glutardialdehyde 24.8% quaternary ammonium chloride 2.5% and formaldehyde 18.3% The recommended dose is 1L/200L (0.5%).

2-Commercial Phenol, It was used at a concentration of 5%

3- Longlife 250 S, (Antec International Limited, Windham Road, Chilton Industrial Estate, Sudbury, Suffolk Co10 2XD UK), contains an active synergistic blend of organic acids, organic biocides and surfactants. It was used at a concentration of 0.5%.

4- Saniton, (Agropharm), each tablet contains 1670 mg sodium dichloroisocyanurate. It was used at the rate of 1 tablet /4 liter of water.

5-TH4 (Sogeval, Laval-France),each 1L contains Glutardialdehyde (62.50 g)activated by a specific blend of 4 lipophilic biocides (Didecyl dimethyl ammonium chloride 18.75 g, Dioclyl dimethyl ammonium chloride 18.75 g , Oclyl dimethyl ammonium chloride 37. 5 g, Alkyl dimethylbenzyl ammonium chloride 50 g). It was used at a concentration of 0.5%.

6- Virkon S (Antec International Ltd. UK). It is composed of peroxygen compounds, surfactant, organic acids and an inorganic buffer system, proved to be effective because of the acidity (1% solution in water has pH 2.6) combined with other disinfection mechanisms. It was used at a concentration of 1%.

3. Detergents

1-Polycar, (Ewabo, Germany), is a blend of sodium alkyl sulphate 3.4 %, alkyl arylpolyglycol ether sulphate 3.4 %, fatty alcohol ethoxylate 4.4 %, butylglycole 4.5 %, tetrapotassium pyrophosphate 5 %, sodium tripolyphosphate 2.5 % and sodium hydroxide 1 %. It was used at a concentration of 1%.

Neutralizers

The neutralizers were used in recovery broth medium against each disinfectant according to **(Reem-Dosoky et al. 2000).**

1-Lecithin (0.3%) and Tween 80(3%) for phenol and formalin

2-Letheen broth [Letheen broth (2.07%) and Tween 80 (0.05%)] for QAC

3-Sodium sulphite for chlorine neutralization **(Taghi-Kilani et al., 1996).**

Ten percent of disinfectant neutralizer was added to the media taken after cleaning and disinfection swabs.

METHODS

1. Isolation and identification of *Campylobacter* strains

The samples were inoculated onto sterile thioglycollate broth tubes **(Monfort et al., 1990)**. The inoculated tubes were incubated at 42°C for 24-48 h. in microaerophilic condition
(5% O_2, 10% CO_2, and 85% H_2) obtained by using gas pack jar and *Campylobacter* gas generating kit **(Rosef and Kappened, 1983)**. A loopful of the enrichment broth streaked on Preston *Campylobacter* selective medium **(Bolton and Robertson, 1982)**. Then incubated at 42°C for 48h. under microaerophilic condition. The surfaces were sampled before the cleaning and disinfection procedures. The plates were incubated in a modified atmosphere containing 6% CO_2, 6% O_2, and 4% H_2 in N_2 at 42°C for 48 h. *Campylobacter*-like colonies were subcultured one or more times until monocultures were achieved. A single suspected colony was stained with gram stains to demonstrate. Gram negative, slender curved to spiral or comma-shaped rods were sub-cultured onto blood agar plates and pure cultures were subjected to catalase, oxidase tests and added to 1cm thioglycollate broth then examined under the phase contrast microscope using 400X magnification for detection of the characteristic motility and morphology of *Campylobacter.* All positive culture were subjected to biochemical tests according to (**Topely and Wilson (1990) Koneman et al., (1995**)

Isolates were identified as C.jejuni or C.coli as described by **Barrow et al., (1983).**

2. Determination of antibiotic (Bauer et al., 1966).

Antimicrobial susceptibility patterns for 65 C.jejuni strains and 21 C.coli isolated strains were determined according to the agar disk diffusion standard method using Mueller – Hinton agar (Oxoid) supplemented with 5% defibrinated horse blood. The plates were incubated at 42c for 24-48hr in a microaerophilic atmosphere.

The antibiotic tested, namely gentamicin (10ug), streptomycin (10ug), tetracycline (30ug) penicillin (30ug), ampicillin (10ug), chloramphenicol (30ug), kanamycin (30ug), cephalothin, and amikacin

were applied

3. Pathogenicity test for isolated Campylobacter strains.

According to **Stewart -Tull and**

Wardlaw (1984).Pure and well identified isolates from *Campylobacter jejuni* and *Campylobacter coli* were grown onto blood agar media for 3days under microaerophilic condition and single typical colony was subcultured into thioglycollate broth for 24hours and counted by standard McFarland tubes. Thirty adult mice used for each species, 10 for each route of injection (I/P, S/C, orally), each mouse injected with 5X109 of viable organism per ml. All mice were kept under observation during the experimental period (14 days). PM examination was done on mice which died during this period and the bacteriological reisolations of Campylobacter species were done. The surviving mice were killed at the end of the observation time and bacteriological reisolation of *Campylobacter spp.* and were carried out . The last group was kept as a control and injected only with physiological saline

4. Cleaning and disinfection procedures used in houses

The procedure of cleaning was starting with removal of the organic matter with high-pressure water, then application of detergent and disinfectant to surfaces presumed free of organic matter. The houses were disinfected with different disinfectants. A total of 135 environmental swabs were collected from the two houses, 45 before and 45 after the cleaning and 45 disinfection procedures. Sterile gauze swabs (10 cm × 10 cm) soaked in sterile saline was used to collect samples from the surfaces. The swabs were wiped vigorously over the surfaces ($0.5m^2$ ***/*** sample) of wall of 2 calf houses. The swabs were put into jars containing 100 ml of buffered peptone water (BPW) **(Valancony et al., 2001).** All samples were kept at 4 °C until further processing within 48 h.

Result

Table (1): Number of calves positive for one or more species of *Campylobacter*

Farms	No of positive calves/No of examined calves	%	One species	Two species	Total isolates
A	26/143diseased calves 24/158apparently healthy	18.2 15.2	22(84.6) 23(95.8)	4(15.4) 1(4.2)	30 25
B	19/120 diseased calves 16/122 apparently healthy	15.8 13.1	16(84.2) 16(100)	3(15.8) 0(0)	22 16
Total	45/263 total diseased calves 40/280 total apparently healthy	17.1 14.3	38(84.4 39(97.5)	7(15.6) 1(12.5)	52 41

Table (2): Incidence of *Campylobacte*r species isolated from apparently healthy and diarrheic calves

Healthy status	Total Positive calves/total examined calves	*Campylobacter* sp. *C.jejuni*	*C.coli*
Diarrheic	45/263	34 (12.9%)	11(4.2%)
Healthy	41/280	31 (11.1%)	10(3.6%)
Total	86/543	65(12.0%)	21(3.9%)

Table (3): Results of injection of *Campylobacte*r *jejuni* and Campylobacter *coli* in adult mice

			dead		No of dead mice/day						
			No	%	1	2	3	4	5	6	7
C.jejuni	I/P	10	8	80	1	2	3	1	0	1	0
	S/C	10	6	60	0	1	2	1	1	1	0
	Orally	10	2	40	0	0	0	1	1	0	0
C.coli	I/P	10	7	70	1	1	2	2	1	0	0
	S/C	10	4	40	0	1	2	0	1	0	0
	Orally	10	1	10	0	0	0	1	0	1	0

Table (4): Results of reisolation of Campylobacter organism from experimentally dead infected mice

			Sites of re-isolation		
			Blood	Liver	Intestinal content
C.jejuni	I/P	8	6	5	6
	S/C	6	2	3	4
	Orally	2	0	1	2
C.coli	I/p	7	3	2	5
	S/C	4	1	2	4
	Orally	1	0	0	1

Table (5): Number and percentages of Antibiotic resistance Campylobacter strains isolated from apparently healthy and diarrheic calves

Antimicrobial agents	Campylobacter jejuni (65)	Campylobacter coli (21)	Total (86)
Amikacin	0(0%)	2(3.5%)	2(2.3%)
Ampicillin	22((33.8%)	10(47.6%)	32(37.2%)
Kanamycin	24(36.9%)	12(57.1%)	36(41.9%)
Tetracycline	20(30.8%)	9(42.5%)	29(33.7%)
Chloramphenicol	3(4.6%)	2(9.5%)	5(5.8%)
Gentamicin	2(3.1%)	1(4.8%)	2(2.3%)
Nalidixic acid	8(12.3%)	4(19.0%)	12(13.9%)
Streptomycin	5(6.2%)	3(14.3%)	8(9.3%)
Cephalothin	61(93.8)	19(90.5%)	80(93.0%)
Penicillin	57(87.7%)	17(80.9%)	78(90.7%)

Table (6) Efficacy of tested disinfectants on bacteria isolated from calf houses in use condition

TIME	Aldekol des 03	Commercial Phenol	Longlife 250 S	Saniton	TH4	Virkon S
5min.	+	+	+	+	+	-
10min.	-	+	+	+	-	-
15min.	-	+	+	+	-	-
20min.	-	+	-	+	-	-
25min.	-	+	-	+	-	-
30min.	-	-	-	+	-	-
35min.	-	-	-	-	-	-

Discussion

The zoonotic significance of *Campylobacter* infections is well documented in the literature (**Blaser et al., 1980; Doyle, 1981**) and it is apparent that the livestock sampled in this study pose a high zoonotic risk to in contact human, Non-diarrhoeic animals shed *Campylobacter* in their feaces. The finding that 17.1%and 14.3% of diarrhoeic and non diarrhoeic calves respectively tested were positive for *Campylobacter'*s organism is an agreement with published work from other countries by others **Munroe et al.(1983**) isolated *Campylobacter* in an incidence of 17% from diseased calves ,also **Hanninen and Raevuori (1981)** found *Campylobacter* species in a percentage of 16.5% of the examined samples.Moreover,**Toews et al.(1986)** revealed that 13%of calves were infected with *Campylobacter* also **Warner et al. (1986)and Koidis (1991)** who isolated *Campylobacter* organism with percentage 15.5%and14.5% respectively from young calves. In this study, the incidence was considerably significant lower than that recorded by **Kursteiner et al., (1985) and Schiavo et al., (1987)** as they isolated *Campylobacter* at percentage of 65.8% and 33%. Also, **Adesiyun et al., (1992) and Nielsen et al., (1997**) recorded *Campylobacter* at incidence of 32.8%and 42%. Moreover, **Firehammer and Myers (1981**) isolated *Campylobacte*r at incidence of 40% in the diarrhoeic calves and 3(100%) in apparently healthy calves. From the result presented in table (2) it was found that the incidence of *Campylobacter jejuni* were the most predominant isolates at incidence of 13.3% and 11.1% among diseased and apparently healthy calves and followed by *Campylobacter coli* 6.5%and 3.6% in diseased and healthy calves .

Our result are in accordance with those reported by **Gill and Harris(1982**) who showed that *Campylobacter jejun*i was commonly present in the feaces of 2-3 week old unweaned apparently normal calves. Moreover, **Bergmann(1985)** isolated *Campylobacter jejuni* at a percentage of 13% from feaces of 70 calves with diarrhoea and 9.5% of 262 clinically healthy calves .**El-jakee (1985)** showed that *Campylobacter jejuni* was the most predominant isolated from bovine faecal samples at a percentage of 26% . He added that there was no correlation between the isolation of *Campylobacter* and occurrence of diarrhoea .A number of factors may be responsible for its presence as maternal immunity (since a number of the animals were very young). However, such animals may be not having diarrhoea; they may still shed the organism in their feaces. Secondly, amongst older animals, some may have had previous episodes of diarrhoea which may not have been recorded .Thus, they may be still shedding the organism in their feaces .A third possibility is that the *Campylobacter* may colonizing local lesions of enteritis which do not result in diarrhoea. Such isolates may therefore originate from non diarrhoeic calves. Also there was no

difference between *Campylobacter* isolated from diarrhoeic or apparently normal calves. This agree with the result recorded by **Diker et al.,1989 and Giacoboni et al.,1993.**Regarding the pathogenicity of *Campylobacter* species ,table(3)showing the variation in the degree of virulence of organism due to different species as well as the route of infection. *Campylobacter jejuni* injected I/p, S/C and orally the mortality rate reached 80.0%, 60.0%, and 10% respectively. Table (4) showing re-isolation of *Campylobacter* species from dead mice and the most predominant site for reisolation was intestinal tract followed by liver and blood. The results agree with **McCardell et al. (1986)** who reported that experimental infection of mice with *Campylobacter* produce diarrhoea and the organism were reisolated. In the current study, 86 selected *Campylobacter* strains (65strains of *Campylobacter jejuni* and 21 strains of *Campylobacter coli*) were examined for its susceptibility to ten antimicrobial agents(table 5) .The results verified its resistance to cephalothin was the most frequent among the tested antibiotic (93.8%for *C.jejuni* and 90.5%for *C.coli*) followed by resistance to penicillin. Comparable result had been demonstrated that the resistance for tetracycline, ampicilline and kanamycin, which were almost in typical category (33.7%-41.9%). *Campylobacter* strains were resistant to nalixidixic acid, while lower resistance rate (9.3%) was for streptomycin. Only 5 (5.8%) and 2(2.3%) of *Campylobacter* strains were resistant to chloramphenicol and gentamicin respectively .Our result nearly agree with **Altmeyer, et al. (1986)** and **Fox et al. (1984)**.

Antimicrobial resistance was observed for *Campylobacter* isolates and remarkable differences between *C.jejuni* and *C.coli* had been reported .The incidence of resistance to most of the tested antimicrobial agent in this study were higher in case of *C.coli* than in *C.jejuni* (table 5).This finding confirmed by **Avrain et al.(2003**)and **Pezzotti et al.(2003)**. In fact that *C.jejuni* was generally more resistant than was *C.coli* (**Aarestrup and Engberg, 2001).** Most of the risk factors deal with hygienic measures: thorough cleaning, disinfection and hygienic routines for the farm workers have to be implemented. *Campylobacter* species are generally considered susceptible to the disinfectants commonly used. Out of 135 samples (45 before and 45 after the cleaning and 45 after disinfection procedures) collected, 30 *Campylobacter jejuni* strains were recovered from the surfaces before cleaning procedures and 9 *C. jejuni* out of 45 samples collected were found after cleaning. Our findings indicate that *C. jejuni* is able to survive overnight on surfaces after cleaning procedures. Campylobacter was isolated from 67% (30/45) before cleaning and in 20% (9/45) after cleaning (data not shown). All tested disinfectants were effective. The most effective disinfectant was Virkon S, followed by Aldekol des 03 and TH4, then Longlife 250 S, followed by Commercial Phenol and lastly Saniton. This arrangement according to inactivation time that ranged from 5 to 35 minutes (table 6). Inactivation

of *Campylobacter* species by disinfectants was in agreements with the many researchers (Avrain **et al., 2003; Blaser et al., 1986 and Trachoo and Frank, 2002).** C.coli

was found to be more sensitive than *C. jejuni*. Similarly, in other studies **(Peyrat et al., 2008b and Slader et al., 2002).** Although other researchers observed survival of this bacteria in the environment and also its survival after cleaning and disinfection procedures **(Peyrat et al., 2008a&b)**.

Our study confirmed also, that thorough cleaning of some facilities may sufficient to reduce the bacteria provided other sanitary measures, especially mechanical carriers as people, for this reason it is necessary that staff who take part in the decontamination procedures must change clothing, use disposable shoes and overalls before entering the farm and must take a shower when they leave the infected zone. Dry and wet cleaning are very important steps in disinfection procedures. Only when all structures and equipment have been cleaned, disinfection can start in the same order as wet cleaning. Surfaces must be thoroughly wet in order to improve disinfectant activity. During wet cleaning, detergents should be used along with water washing at high pressure. Water sprayed at high pressure may be used to allow the disinfectant to penetrate into cracks or porous surfaces (i.e. wood) **(Meroz and Samberg, 1995)**

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