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INCIDENCE OF CAMPYLOBACTER SPECIES IN LAYING HENS AND TABLE EGG IN SOHAG GOVERNORATE

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ABSTRACT

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A Total of 200 samples (100 cloacal swabs and 100 of eggs from laying farms) were collected in Sohag Governorate. The samples were examined for the presence of *Campylobacter*. The bacteriological examination revealed isolation of 38 isolates of *Campylobacter species* from cloacal swabs (25 isolates (65.8%) of *C. jejuni* and 13 isolates (34.2%) of *C. coli*). On the other hand one isolate (1.0%) from egg shell was isolated. While trial of isolation from egg content was unsuccessful. Concerning experimental infection of chickens with *C. Jejuni*, the mortality rate, was (30.0%) among chickens infected intramuscularly, while it was (20.0%) after oral infection .On the other hand the infection with *C. coli* revealed that mortality rate, was (20.0%) among chickens infected I/M and (10.0%) after oral infection. In vitro sensitivity test, most isolates were highly sensitive to spiramycin, spectinomycin, clindamycin, gentamycin and colistin sulphate and were resistant to amoxicillin and penicillin.

Key words: *Campylobacter Jeguni , coli , chicken breeder , eggs.*

INTRODUCTION

Campylobacter species are Gram- negative, spiral and or curved non spore forming, 0.2 – 0.9 µm wide and 0.5- 5.0 µm long. They are oxidase positive with one polar flagellum at one or both ends giving a corkscrew – like motility (Bolton *et al.*; 1992). Although eggs have a high nutritive value, it may be responsible for several outbreaks and acts as a vehicle for transmission of pathogens to consumers (Hangombe *et al.*; 1999 and Gast *et al.*; 2004). Sources of egg contamination are numerous such as eggs may become infected before they are laid at the genital system of birds, when the ovary is infected with bacterial pathogens. After laying, the shell soon become contaminated with a variety of organisms by faecal matter from the bird, contact with dirty surfaces, food stuffs, by washing water, by handling , or perhaps by accumulation of eggs (DeReu *et al.*; 2008). In poultry, *C Jejuni* is responsible for avian vibrionic hepatitis which is a contagious disease of young and mature chickens characterized by low mortality, high morbidity associated with chronic course, poor growth and production (Peckham, 1984). Also, poultry was considered a major reservoir for human Campylobacteriosis, so reduction or elimination of poultry contamination with *C. jejuni* would greatly reduce the risk of *campylobacter* for public health (Park, 2002). Toxic infections caused by microorganisms of the *Campylobacter genus* are food borne diseases. The primary source of which are poultry and poultry products (Corry and Atabay, 2001). The microorganism is microaerophilic and the percentage of oxygen in the atmosphere is toxic to it, so the cultures should be maintained under reduced oxygen tension. A satisfactory mixture is (5.0%) O2, 10.0%) CO2 and (85.0%) N (Leuchtefeld and Wang , 1981). Although critical control measures for the safety of food regarding the health of the consumer had been introduced, serious health hazards outbreaks due to consumption of the eggs still persisted. So the aim of this work is to investigate the incidence of *campylobacter species* which may be found in laying hens and table eggs in Sohag Governorate.

MATERIALS and METHODS

1- Samples:
A total of 200 samples (100 cloacal swab of poultry farm with 16 weeks old and 100 of eggs) were collected in Sohag Governorate. The collected samples were packed in box and aseptically transferred to the laboratory without delay where they were immediately examined bacteriologically.

2- Bacteriological examination:
The cultivation of samples were carried out in Bolton broth supplement with antibiotic (oxoid) Bolton *et al.* (1992) and gently shaken for 5 min. The mixture was subjected to micro aerophilic with resuscitiation 4 hrs at 25-42 °C followed by 40-44 hrs at 25 and 42 °C. The subculture onto Modified Charcoal Cefoperazone Desoxycholate Agar (MCCDA) (Oxoid) and blood agar was carried out. The samples were incubated in

micro aerobic atmosphere 25 °C and 42 °C for 48-72 hrs. Bacterial colonies that exhibited cellular, colonial and biochemical characteristic were observed according to Bolton *et al.* (1992).

3- Experimental infection:

A total of fifty five, one month old chicks obtained from private farms, Sohag Governorate were used to study the pathogenicity of *Campylobacter* species. Before infection, a random sample which included 5.0 chicks were sacrified for postmortem and bacteriological examination to prove that these chicks were healthy. The other chicks were divided into 5.0 groups, each of 10.0 chicks. The first group was infected orally with 0.5 ml of $1x10^8$ colony forming unite adjusted by Macferland density technique of viable identified organism (Finegold and Martin, (1986) of *C. jejuni* for two successive days. The second group was inoculated IIM with 0.5 ml of $1x10^8$ cfu of *C. jejuni.* The third group was infected orally with 0.5ml of $1x10^8$ cfu of *C. coli* for two successive days. The fourth group was inoculated I/M with 0.5 ml of $1x10^8$ cfu of *C.coli.* The fifth group was left as uninfected control. All chicks were kept under observation, symptoms and postmortem finding were recorded.

4 – In vitro – Sensitivity test:

The isolated *C. jejuni* and *C. coli* strains were examined for their susceptibility to the different antibiotics. The paper discs were supplied by Bio-Merieux and Oxoid namely, Spiramycin (10.0mg), Colistin sulphate (10.0 ug), Enrofloxacin (10.0 ug), Gentamycin (10.0 ug), Penicillin (10.0 I.U), Amoxicillin (25.0 ug), Clindamycin (100.0 ug), Spectincmycin (100.0 ug), Neomycin (30.0 ug), Oxytetracyclin (30.0 ug), Ampicillin (10.0 ug) and Streptomycin (10.0 ug). The discs diffusion technique of sensitivity to different chemotherapeutic agents was done according to (Finegold and Martin, 1986).

RESULTS

Table 1: The Incidence of *Campylobacter species* recovered from diseased chickens and eggs.

Examined samples	No. of sample	Positive cases	
		No.	%
Cloacal swabs	100	38	38
Egg shell	100	1	1
Egg content	100	0	0

Table 2: The frequency of *Campylobacter species* recovered from diseased chickens and eggs.

Examined samples	Total number of isolates	*Campylobacter species*			
		C. jejuni		*C. coli*	
		No.	%	No.	%
Cloacal swabs	38	25	65.8	13	34.2
Egg shell	1	1	100	0	0
Egg content	o	o	o	o	o

Table 3: The biochemical characterization of *Campylobacter* isolate strains.

Isolate strains	oxidase	catalase	nitrate reduction	H_2S on tsi	growth of	
					25 C°	42 C°
C. jejuni	+	+	+	V	-	+
C. coli	+	+	+	V	+	+

Table 4: The results of experimental infection of chicks with *Campylobacter species*.

Group no.	No . of infected bird	Route of inoculation	Daily death post infection										Total no. of death	Mortality rate
			1	2	3	4	5	6	7	8	9	10		
1	10	orally	-	-	-	-	-	1	-	1	-	-	2	20%
2	10	I/M	-	-	1	-	1	1	-	-	-	-	3	30%
3	10	orally	-	-	-	-	-	1	-	-	-	-	1	10%
4	10	I/M	-	-	1	-	1	-	-	-	-	-	2	20%
5	10	control	-	-	-	-	-	-	-	-	-	-	-	0 %

Group 1 and 2 = infected orally and injected I / M with *C. Jejuni*
Group 3 and 4 = infected orally and injected I / M with *C coli*

Table 5: Demonstrates the results of in- vitro sensitivity test.

Antimicrobial agent	*C. jejuni*	*C. coli*
Spiramycin	+++	+++
Colistin sulphate	+++	++
Enrofloxacin	++	+
Gentamycin	+++	+++
Penicillin	-	-
Amoxicillin	-	-
Clindamycin	+++	++
Spectincmycin	+++	+++
Neomycin	++	++
Oxytetracyclin	+	+
Ampicillin	-	-
Streptomycin	++	++

+++ = Highly Sensitive
++ = Moderately Sensitive
+ = Weakly Sensitive
- = Resistant

DISCUSSION

Until recently, Little was known regarding bacterial contamination of table eggs. The shell can already be infected when passing through the vent. It is hypothesised that bacterial contamination of the egg content could result from the penetration of the shell by bacteria deposited on the surface of the egg after it has been laid (Messens *et al.*, 2007).

Campylobacter Jejuni and *coli* has become recognized as a common aetiological agent in human diarrhea. These microorganism are wide spread in broiler farms (Wieliczko, 1995.a).

As shown in Table (1) the bacteriological examination of 100 cloacal swabs revealed the prevalence of *Campylobacter species* in a total percentage of (38%). On the other hand *Campylobacter species* was isolated from egg shell with an incidence of (1%) while egg content samples were negative for the isolation of *Campylobacter species*.

Regarding to Table (2), the frequency of *Campylobacter species* recovered from diseased chicken and eggs shown that 25 isolates were *Campylobacter jejuni* (65.8%) and 13 isolates of *Campylobacter coli* with and incidence of (34.2 %).

On the other hand, *Campylobacter jejuni* was isolated by (1%) from egg shell but could not isolate *Campylobacter jejuni* and *coli* from egg content. These results were similar to those recorded by (Lin, 1988; Ahmed and Ahmed, 1994, Adesiyun *et al.;* 1994; Vashin *et al.*; 2008 and Messelhausser *et al.;* 2011).

A higher percentage of *Campylobacter species* were found in the cloacal swab and lower incidence in egg and its contant perhapas vertical transmission of *C.jejuni* and *C.coli* through the egg is probably a rare event and does not play a major role in the transmission of *Campylobacter* on poultry farms.

Biochemical characterization of the isolated *Campylobacter species* was found to be oxidase and catalase positive, reduced nitrates to nitrites, produced no acid in triple suger iron agar and was H2S positive by lead acetate paper strips.

C.jejuni growth occurred at 42° C but not at 25° C while *C.coli* can grow at 25° C and 42° C (Table 3). The experimental infections in chickens with *C.jeuni* and *C.coli* via orally and intramuscularly revealed that, as shown in (Table 4), *C.jejuni* produced 30.0% and 20% mortalities when inoculated I/M and orally respectively. On the other hand *C.coli* produced 20.0% and 10.0% when inoculated I/M and orally respectively. These results agreed with those obtained by (Ruiz –palacois *et al.*, 1981; Sayed, 2000; Nagla, 2005; Sahin *et al.*, 2003) and Nor *et al.*, 2013).

The results of anti microbial sensitivity test for *C.jeguni* and *C.coli* isolates revealed that *C.jeguni* isolates were highly sensitive to spiramycin, colistin sulphate, gentamycin, clindamycin and spectinomycin. Similar results were recorded by Sayed, 2000); Schwaiger *et al.;* 2008); and DeReu *et al.*, 2008) and Nor *et al.*, 2013). On the other hand , *C.coli* isolate were highly sensitive to spiramycin, gentamycin and spectinomycin while resistant to Ampicillin, penicillin and amoxicillin. These are in accordance with the results obtained by (Ge *et al.*, 2003; Wilson, 2003; Ronner *et al.*, 2004; Schwaiger *et al.*, 2008 and Nor *et al.*, 2013).

From this study we concluded that the capability of *Campylobacter* species to interior and /or survive within the egg is quite limited, therefore, it is probable that vertical transmission is an unusual event in breeder hens, and that there are other infection route, moreover future researches using molocular biology must be conducted in an attempt to demonstrate viable non. culturable cells of *Campylobacter species* inside eggs.

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مدى تواجد ميكروب الكامبيلوباكتر في الدجاج البياض وبيض المائدة بمحافظه سوهاج

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تم جمع عدد ٢٠٠عينة (١٠٠من فتحة المجمع و ١٠٠من بيض الدجاج البياض) في محافظه سوهاج. وفحصت العينات لمعرفه مدى تلوثها بيكتريا الكامبيلوباكتر المحتمل وجوده على قشر ومحتوى البيض الداخلي وكذلك المسحات. وأسفرت النتائج عن عزل ٣٨ معزولة من فتحة المجمع (٢٥من الكامبيلوباكتر جوجيناى (٦٥.٨%) و ١٣ من الكامبيلوباكتر كولاى (٣٤.٢%) في حين تم عزل معزولة واحدة من قشر البيض (١%) ولم يتم عزل الكامبيلو باكتر من محتوى البيض الداخلي. بإجراء العدوى الصناعية لميكروب الكامبيلوباكتر جوجيناى وكولاى في الدجاج عمر ١ شهر ثبت أن نسبه النفوق عند الحقن العضلي للجوجيناى هي (٣٠%) وعند العدوى بالفم كانت (٢٠%) في حين كانت (٢٠%) بالحقن العضلي للكولاى و(١٠%) عند العدوى بالفم. تم دراسة تأثير العديد من المضادات الحيوية على الكامبيلوباكتر جوجيناى والكامبيلوباكتر كولاى ووجد أن معظم العترات شديدة الحساسية للأسبيرامسين والأسبكتينوميسين والكلنداميسن والجينتاميسن والكولستين سلفات في حين كانت شديدة المقاومة لكلا من الأموكسيسللين والبنسللين.