

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 vibronic 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 (oxid) Bolton *et al.* (1992) and gently shaken for 5 min. The mixture was subjected to micro aerophilic with resuscitation 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 sacrificed 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 1×10^8 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 1×10^8 cfu of *C. jejuni*. The third group was infected

orally with 0.5ml of 1×10^8 cfu of *C. coli* for two successive days. The fourth group was inoculated I/M with 0.5 ml of 1×10^8 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	<i>Campylobacter species</i>			
		<i>C. jejuni</i>		<i>C. coli</i>	
		No.	%	No.	%
Cloacal swabs	38	25	65.8	13	34.2
Egg shell	1	1	100	0	0
Egg content	0	0	0	0	0

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

Isolate strains	oxidase	catalase	nitrate reduction	H ₂ S on tsi	growth of	
					25 C°	42 C°
<i>C. jejuni</i>	+	+	+	V	-	+
<i>C. coli</i>	+	+	+	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	<i>C. jejuni</i>	<i>C. coli</i>
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 Messelhauser *et al.*; 2011).

A higher percentage of *Campylobacter species* were found in the cloacal swab and lower incidence in egg and its content perhaps 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 sugar 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.jejuni* 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.jejuni* and *C.coli* isolates revealed that *C.jejuni* 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 molecular biology must be conducted in an attempt to demonstrate viable non. culturable cells of *Campylobacter species* inside eggs.

REFERENCES

- Adesiyun, A.A.; Ojo, M.O.; Mohammed, K. and Garcia, G. (1994): Frequency of isolation of *Campylobacter* and *Salmonella* from live broilers reared by contact farmers in Trinidad. Bulletin of Animal Health and production in Africa. 42(3): 167-172.
- Ahmed, M.M. and Ahmed, F.A. (1994): Occurrence of *Campylobacter species* in broilers and laying hens suffering from diarrhea. Ass. Vet. Med. J. 32 (63): 119-125.
- Bolton, F.J.; Wareing, D.R.A.; Skirrow, M.B. and Hutchinson, D.N. (1992): Identification and biotyping of *Campylobacter*. In Identification Methods in Applied and Environmental Microbiology ed. Board, R.G; Jones, D. and Skinner, F.A. PP. 151-161 Oxford: Black Wdl Scientific Publication.
- Corry, J.E.L. and Atabay, H.I. (2001): Poultry as a source of *Campylobacter* and related organisms. Journal of Applied Microbiology. 90, 96s- 114s
- DeReu, W.; Messens, M.; Heyndrickx, T.B.; Rodenberg, M. and Uyttendaele and Herman, L. (2008): Bacterial contamination of table eggs and the influence of housing systems. Worlds Poultry Science Journal, Vol. 64. 5–19.
- Finegold, M. and Martin, E.J. (1986): Diagnostic Microbiology. 7th Ed. P.P. 186. The C.V. Mosby Company. St. Louis. Toronto. London.
- Gast, R.K.; Guardo Bouldin, J. and Holt, P.S. (2004): Colonization of reproductive organs and internal contamination eggs after experimental infection of laying hens with *Salmonella heidelberg* and *Salmonella enteritidis*. Avian Dis. 48(4): 863-869.
- Ge, B.; White, D.G.; Mc –Dermatt, P.F.; Girard, W.; Zhao, S.; Hubert, S. and Meng, J. (2003): Antimicrobial resistant *Campylobacter species* from retail raw meats. App .Environ-Microbial 69(5): 3005-3007.
- Hangombe, B.M.; Sharma, R.N.; SKjerver, E. and Tuchili, L.M. (1999): Occurrence of *Salmonella enteritidis* in pooled table eggs and market-ready chicken carcasses in Zambia. Avian Dis. 43 (3): 597-599.
- Leuchtefeld, N.A.W. and Wang, W.L. (1981): J. Clin .Microbial 13: 266-268.

- Lin, Y.J. (1988): Survey for *Campylobacter fetus* subspecies. *jejuni* infection in domestic fowls in Fujian province Chinese J. Vet. Sci. and Technol 6: 18-20.
- Messelhauser, U.; Tharigen, D.; Elmer-Englhard, D.; Bauer, H.; Schreiner, H. and Hollar, C. (2011): Occurance of thermotolerant *Campylobacter spp.* On egg shell: a Missing link for food –Borne infection?. Applied and Enviromental Microbiology (2011) p 3896 -97.
- Messens, W.; Grijspeerdt, K.; De Reu, K.; Deketelaere, B.; Mertens, K.; Bamelis, F.; De Baerdemaker, J.; Decuyper, E. and Herman, L. (2007): Egg shell penetration of various types of hens egg by various micro organisms. Journal of Food Protection 70: 623-628.
- Nagla, F. (2005): A study on *Campylobacter species* in chickens In EL-Fayoum Governorate. M.V.SC. Thesis of Microbiology Fac. Vet. Med. Ben-Suef. Univerisity.
- Nor, F.S.; Saleha, A.A.; Jalila, A. and Fauziah, N. (2013): Occurrence of *Campylobacter* and *Salmonella* in ducks and duck eggs in Selangor. Malaysia Tropical Biomedicine 30 (1): 155-158.
- Park, S.F. (2002): The physiology of *Campylobacter* species and its relevance to their role as food borne pathogens. International Journal of Food Microbiology 74, 177-188.
- Peckham, M.C. (1984): Avian vibrio infecions in M.S. Hofstad, H.J. Barnes, B.W. Calvek, W.M. Reid and H.W. Yoder, Jr. (eds) disease of poultry, 8th ED . PP. 221-231. Iow State Univ. Press Ames.
- Ronner, A.C.; Engvall, E.O.; Andersson, L. and Kaijser, B. (2004): Species identification by genotyping and determination of antibiotic resistance in *Campylobacter jejuni* and *Campylobacter coli* from humans and chickens in Sweden. Int. J. Food. Microbiol. 1-96(2) 173-179.
- Ruiz-palacois, G.M.; Escamilia, E. and Torres, N. (1981): Experimental *Campylobacter* diarrhea in chickens. Infect-Immunol. 34 (1): 250-255.
- Sahin, O.; Kobalka, P. and Zhang, Q. (2003): Detection and survival of *Campylobacter* in chicken eggs. Journal of Applied Microbiology 95: 1070-1079.
- Sayed, A.M. (2000): *Campylobacter* infection in boiler chickens in Assiut. J. Assiut. Vet. Medic. 42(84) 213-221.
- Schwaiger, K.; Schmied, FM. and Bauer, J. (2008): Comparative analysis of antibiotic resistance characteristics of Gram-negative bacteria isolated from laying hens and eggs in conventional and organic keeping systems in Bavaria, Germany Zoonoses Public Health 55 (7): 331-341.
- Vashin, I.; Stoyanchev, T. and Roussev, V. (2008): Prevalence of microorganism of the *Campylobacter* genus in Quail (*Coturnix Coturnix*) eggs. Bulgarian Journal of Veterinary Medicine. 11(3): 213-216.
- Wieliczko, A. (1995.a): The role of *Campylobacter* in poultry pathology. Part 1. Epidimiological studies on *Campylobacter* infections in poultry. Medycyna Weterinaryna. 51 (3): 150-152.
- Wilson, I.G. (2003): Antibiotic resistance of *Campylobacter* in raw retail chickens and imported Chicken portions Epidemiol. Infect. 131(3): 1181- 1186.

مدى تواجد ميكروب الكامبيلوباكتري في الدجاج البياض وبيض الماندة بمحافظة سوهاج

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تم جمع عدد ٢٠٠ عينة (١٠٠ من فتحة المجمع و ١٠٠ من بيض الدجاج البياض) في محافظة سوهاج. وفحصت العينات لمعرفة مدى تلوثها ببيكتريا الكامبيلوباكتري المحتمل وجوده على قشر ومحتوى البيض الداخلي وكذلك المسحات. وأسفرت النتائج عن عزل ٣٨ معزولة من فتحة المجمع (٢٥ من الكامبيلوباكتري جوجيناي (٦٥.٨%) و ١٣ من الكامبيلوباكتري كولاى (٣٤.٢%) في حين تم عزل معزولة واحدة من قشر البيض (١%) ولم يتم عزل الكامبيلوباكتري من محتوى البيض الداخلي. بإجراء العدوى الصناعية لميكروب الكامبيلوباكتري جوجيناي وكولاى في الدجاج عمر ١ شهر ثبت أن نسبه النفوق عند الحقن العضلي للجوجيناي 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 الكامبيلوباكتري جوجيناي والكامبيلوباكتري كولاى ووجد أن معظم العترات شديدة الحساسية للأسيبراميسين والأسبكتينومييسين والكلنداميسين والجينتاميسين والكولستين سلفات في حين كانت شديدة المقاومة لكلا من الأموكسيسيلين والبنسلين.