

Isolation and Identification of Campylobacter organisms From Ducks with Experimental Study in Ducklings and Chicks.

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Abstract

A total of 580 samples (230 from organs of freshly dead ducks, 240 cloacal swabs from apparently healthy ducks and 110 swabs from egg shell surface) were collected aseptically from duck farms of different ages and breeds. In addition to 45 samples were collected from the surrounding environment of ducks (Water, Feeder, and Litter). All these samples were bacteriologically examined for campylobacter infection. The percentage of campylobacter isolates were 22.6% (organs of freshly dead), 12.5% (cloacal swabs) 1.8% egg shell surface, 26.6% water samples, 22.6% feed samples and 63.3% (litter). Isolated campylobacters were identified as *C jejuni* (66%) and *C. coli* (34%). *Campylobacter jejuni* isolates were highly sensitive to norfloxacin, Gentamycin, tetracycline, Flumequin and chloramphenicol, while Erythromycin and Refampicin showed intermediate activity. It was resistant to cefalotin, Nebcin and Unacin.

Pathogenicity for one day old ducklings and chicks with one isolate of *C. jejuni* and/or *S. typhimurium* were done

Introduction

Campylobacter has emerged as a significant infection in a wide range of avian and mammalian species (34; 36). Chickens are considered a source of human infection from which the organism gained its zoonotic importance (12; 28). In chickens campylobacter infection causes a contagious disease characterized by low mortality, high morbidity and chronic course (25).

In the early nineties, Campylobacter species was the most common bacterial enteropathogen isolated from diarrheic stools in Egyptian children. The overall prevalence of campylobacter isolation was 25.9%

from stool of 143 diarrheic children compared to 15.2% of 132 non-diarrheic control children (24).

Campylobacter jejuni biotype 2 was the most prevalent strain in birds (1). Clinical signs observed in infected chickens with *C. jejuni* are listless with pale dry scaly and shrunken combs associated with loss of weight and diarrhea which may affect few birds or the entire flock (10). The most lesions observed in birds infected with *C. jejuni* were enlarged liver and studded with small yellow stellate foci, with haemorrhages beneath the liver capsule while in chronic forms, indurations and atrophy of the livers were frequently accompanied with hydropericardium, ascitis and catarrhal enteritis with petechae (10).

This work was designed to study the incidence of *Campylobacter* species in duck and their surrounding environment in three provinces (El-Garbia, El-Dakahlia and Kafer El-Sheikh) and to detect the sensitivity to various antibacterial agents for the most common isolates. In addition to study the pathogenicity of one isolate to one-day old ducklings and chicks either alone or with *Salmonella typhimurium* organism.

Material and Methods

1- Media:

- For isolation of campylobacter: Thioglycollate broth (Oxoid); Semisolid thioglycollate media; Brucella sheep blood agar plates; Skirrow supplements: for selection of campylobacter (Vancomycin 5 mg; Trimethoprim lactate 2.5 mg and polymyxin B 1250 IU)

- For cultivation and isolation of salmonella typhimurium: Mac-Conkey agar (Oxoid); Selenit F broth (Oxoid)

- Antibiotic sensitivity discs: A total of 10 antibiotic discs (Oxoid, London, England) were used in vitro to test the isolated *Campylobacter jejuni*. The following antibiotics were used: Gentamycin (GM 10 ug), Tetracycline (TE 30 ug), Erythromycin (E 15 ug), flumequine (AR 30 ug), Norofloxacin (Nor 10 ug), Rifampicin (RA 30 ug), Cefalotin (CF 30 ug), Nepcin (TOS 10 ug), Unacin (SEM 10 ug), Chloramphenicol (4; 6).

2- Samples:

- Biological samples: A total of 580 samples were collected aseptically from organs (liver, bile and intestinal contents) of different ages and breeds of duck representing (230 from freshly dead ducks, 240 cloacal swabs from apparently healthy ones and 110 swabs from the surface of egg shells: All samples were examined bacteriologically for the presence of Campylobacter organisms. All dead birds had histories of diarrhea and different mortality rates. Samples of egg shells were collected from 11 breeder farms (8 from El-Gharbia, 2 from El-Dakahlia and one from kafer El-Sheikh).

- Environmental samples: A total of 45 aseptically collected samples from feeders, litter and water (15 samples for each one).

3- Bacteriological examination: Loopfulls from samples were streaked directly onto 3 test tubes of semisolid thioglycollate media. Media was separately incubated at 25°C, 37°C and 42°C for 24 hours then examined under the two phase contrast microscope at 400 X magnification for detection of characteristic motility and morphology of campylobacter organisms (35). Each suspected positive case was filtered by milipore filter of pore size 0.65 um and streaked onto 3 brucella blood agar plates with antibiotics. All cultures were incubated at 25°C, 37°C and 42°C for 42-72 hours in microaerophilic condition of 85% N₂, 10% CO₂ and 5 % O₂.

4- Biochemical identification of pure growth of campylobacter organisms was done (5; 29)

5- Biotyping of *C. jejuni* was carried out (17; 18).

Experimental studies:

- 1- Campylobacter inoculum: Twenty four hours pure culture of one isolated and identified strain of *C. jejuni* biotype 1 was suspended in sterile saline solution (1×10^9 cfu/ml) (3)

- 2- Salmonella typhimurium inoculum: *S. typhimurium* strain was kindly obtained from the Department of Microbiology and Immunity, Animal Health Research Institute, Dokki, Egypt. The organism was inoculated onto nutrient agar and incubated for 24 hours. Colonies were suspended in sterile saline and adjusted to Macferland opacity tube No. 3 (3).

- 3- Experimental Birds: The pathogenicity of *C. jejuni* biotype 1 alone or with *S. typhimurium* were studied by the inoculation of one day old Pekin ducklings and one day old Hubbard chicks. Ten birds from each species were sacrificed and examined bacteriologically to prove its freedom from any pathogens. Table (1) explained the experimental design. The observation period was 45 days. Clinical signs, mortalities and postmortem lesions were recorded daily for 45 days. Trials for reisolation of pathogen(s) from liver, bile and intestinal content from both dead and sacrificed survival birds were done.

Results and Discussion

Bacterial examination of biological samples (230 from freshly died ducks, 240 cloacal samples of apparently healthy ducks and 110 from the surface of egg shells) beside 45 samples from feed, water and litter collected from three provinces resulted in isolation of 100 campylobacter isolates (16%). These isolates include 66 isolates of *C. jejuni* (66%) and 34 isolates of *C. coli* (34%). Tables (2) and (3) summarized these results. *C. jejuni* and *C. coli* were isolated from liver, intestinal content and cloacal swabs from both dead and apparently healthy ducks. These results were in agreement with many authors but the reported incidence may be more (16; 37) or informed by others (31).

The percentage of *C. jejuni* and *coli* isolation from water were (20 and 9%), feed (20 - 9%) and from litter (40 and 13.3%) as seen in Table (3). These results came in agreement with some previous studies (11; 21; 26). They stated that *C. jejuni* was consistently recovered from water and litter in chicken houses. Also, isolation of *C. jejuni* from broiler and layer chickens drinking water, litter and rations were reported in Egypt (27). On the other hand, our results disagreed with other (13) who failed to isolate campylobacter organisms from any hatchery, water, feed or fresh litter samples which were collected from dutch broiler farms. Only two isolates of *C. jejuni* out of 110 egg shell samples (1.82%) were isolated (Table, 3). These results agreed with that of *Doyle* (7) who isolated *C. jejuni* from egg shells in a rate of 7.7%,. Other results (33) recorded that penetration of *C. jejuni* to the table eggs at a rate of 4% with successful isolation from homogenized egg content in a rate of 0.14%. But our results disagreed with results of *Aly* (2) who could not isolate *C. jejuni*, *coli* from duck egg shells.

Biotyping of the isolated *C. jejuni* (66 isolates) showed that biotype I (50 isolates) was more prevalent than biotype II (16 isolates) as seen in tables (4 and 5). These findings came in accordance to that reported previously (31; 39), while *Abrahams et al.* (1) mentioned that biotype II was the most prevalent than I in the examined birds.

In vitro, all tested strains of *C. jejuni* were highly sensitive to norofloxin, gentamycin, nitrofurantoin, tetracycline, flumequinone and chloramphenicol, while erythromycin and rifampicin had a moderate effects and were resistant to cefalotin, nebcin and unacin (Table, 6). These result came in consistent with others (9; 22)).

Experimental infection of one isolate of *C. jejuni* alone or with *Salmonella typhimurium* was done. In one day old ducklings and chicks inoculated with 0.3ml of 1×10^9 cfu of *campylobacter jejuni* resulted in mortality 23.3% and 6.66% respectively (Table, 7). While per os inoculation of one day old duckling and chicks with 0.3ml of 1×10^9 c.f.u of *salmonella typhimurium* resulted in 33.3% mortality in ducks and 40% in chicks as seen in tables (8) and (10). On the other hand dual infection with the two organisms resulted in 50% mortality in both ducks and chickens as seen in table (9)

Inoculated duckling with *C. jejuni* showed depression, loss of appetite and diarrhea with excess amount of mucous or gas, while inoculated chicks showed diarrhea, depression and loss of weight. Similar findings were recorded in some previous investigations (19; 32; 38).

The postmortem lesions of inoculated *C. jejuni* in ducklings and chicks showed emaciated carcasses with congestion of the liver, spleen, kidney, lungs and heart together with ballooned enteritis and small necrotic hepatic foci with sub- capsular hemorrhages in liver. These results came in accordance with some previous reports (10; 15).

Inoculated one day old ducklings and chicks with *Salmonella typhimurium* appeared dejected and stand with dropped wings, the vent was usually pasted with urates and decreased weight. The post mortum examination showed grayish miliary spots in liver and caseous typhilitis. These findings came in agreement with others (14; 20; 23).

Reisolation of inoculated *C. jejuni* and *S. typhimurum* were recorded in tables (10; 11).

The lowering rate of weight gain obtained in all inoculated birds either ducklings or chicks compared to control birds . In this study showed that the relative loss weight of ducks was more than chickens. Average body weight reduction was 31.9% of ducks and 16.5% for chickens as seen in Table (12). The loss of weight due to campylobacter infection in chickens was reported (8; 10; 19; 30; 38).

Based on our study, we can concluded that infected ducks and or chickens with campylobacter organisms may lead to contamination of poultry meat which represents a significant potential for human food borne infection under conditions of defective handling, inadequate refrigeration and improper preparation.

Table (1): Experimental design

Species	Treated groups				Control	
	Inoculate	No	Dose (1×10^8 cfu)	Inoc. Route	Inoculation	No
one day old ducks	<i>C.jejuni</i>	30	0.3	Per os	SC*	10
	<i>S.typhimurium</i>	30	0.3	Per os	SC*	10
	<i>C.jejuni</i> + <i>S.typhimurium</i>	30	0.3	Per os	SC*	10
one day old chicks	<i>C.jejuni</i>	30	0.3	Per os	SC*	10
	<i>S.typhimurium</i>	30	0.3	Per os	SC*	10
	<i>C.jejuni</i> + <i>S.typhimurium</i>	30	0.3	Per os	SC*	10

SC*: Sterilized culture of microorganism

Table (2): Percentage of isolated *campylobacter* from examined ducklings

Farm	Campylobacter jejuni				Campylobacter coli			
	Freshly dead		App. healthy		Freshly dead		App. Healthy	
	No	%	No	%	No	%	No	%
	El-Gharbia Province							
1	3	15.8	2	12.5	2	10.5	1	6.3
2	2	11.11	2	11.11	0	0	0	0
3	3	60	2	40	0	0	0	0
4	3	18.75	1	6.3	3	18.75	1	6.3
5	2	28.6	1	14.3	0	0	0	0
6	3	37.5	2	25	2	25	1	6.3
7	0	0	0	0	2	50	1	25
8	0	0	0	0	2	50	1	12.5
9	3	15	1	5	3	15	1	5
10	2	4.3	2	4.3	1	2.2	1	2.2
11	3	11.11	2	7.4	2	7.4	0	0
S.total	24	13.5	15	8.5	17	9.5	7	3.9

Table (2): continue

El-Dakahlia Province								
1	2	25	2	25	1	12.5	1	12.5
2	2	14.3	1	7.1	1	7.1	1	7.1
3	2	7.7	1	3.8	1	3.8	0	0
S.total	6	12.5	4	66.66	3	6.3	2	33.33
Kafer El-Sheikh Province								
1	2	50	0	0	0	0	1	25
Total	32	22.6	20	8.3	20	9.1	10	4.1

Total number of *Campylobacter jejuni* & *Campylobacter coli* = 52 + 30 = 82

Table (3) : Incidence and percentage of *Campylobacters* isolated from / water, feed, litter and egg shell (layer flocks).

Locality	Water samples						Feed samples			
	Total No	C.jej.		C.coli		Total No	C.jej.		C.coli	
		+ve	%	+ve	%		+ve	%	+ve	%
El-Gharbia province	11	2	18.8	1	9	11	3	27	1	9
El-Dakahlia province	3	1	33.3	0	0	3	0	0	0	0
Kafer El-Sheikh province	1	0	0	0	0	1	0	0	0	0
Total	15	3	20	1	9	15	3	20	1	9
Locality	Litter samples					Egg shell samples				
	Total No	+ve	%	+ve	%	Total No	+ve	%	+ve	%
El-Gharbia province	11	5	45	1	9	80	2	2.5	0	0
El-Dakahlia province	3	1	33.3	1		20	0	0	0	0
Kafer El-Sheikh Province	1	0	0	0	0	10	0	0	0	0
Total	15	6	40	2	13.3	110	2	2.5	0	0

Table (4): Biotyping of *Campylobacter jejuni* isolated from freshly dead ducks and fecal swabs.

Locality	Total No.	Biotype I			Bio type II		
		Dead ¹	Healthy ²	I	Dead ¹	Healthy ²	II
El-Gharbia province	39	19	8	27	9	3	12
El-Dakahlia province	10	6	2	8	1	1	2
Kafer El-Sheikh province	3	2	1	3	0	0	0
Total	52	27	11	38	10	4	14

1- Freshly dead

2- apparently healthy

Table (5): Biotyping of *Campylobacter jejuni* isolated from water, feed, litter and egg shell samples.

Sample	Water		Feed		Litter		egg shell		Total	
	I	II	I	II	I	II	I	II	I	II
El-Biotyping										
El-Gharbia province	2	0	2	1	4	1	2	0	10	2
Dakahlia Province	1	0	0	0	1	0	0	0	2	0
KaferEI-Sheikh province	0	0	0	0	0	0	0	0	0	0
Total	3	0	2	1	5	1	2	0	12	2

Table (6) : Antibiotic sensitivity test

Antibiotics	Concentrations	Susceptibility
1- Norofloxacin	10 ug	S(++++)
2-Gentamycin	10 ug	S(++++)
3-Nitrofurantoin	300 ug	S(++++)
4-Tetracyclin	30 ug	S(++++)
5-Flumequin	30 ug	S(+++)
6-Choloramiphinocle	30 ug	S(+++)
7-Erythomycin	15 ug	MS (++/R)
8-Refampcin	3 ug	MS (++/R)
9-Cefalotine	30ug	R(-)
10-Nebcin	10 ug	R(-)
11-Unacin	10 ug	R(-)

S (++++): highly sensitive ; S (+++): intermediate sensitive

MS (++/R): moderate susceptible with refractory zone

R (-): resistant

Table (7): Mortality pattern in one day old ducks / chicks experimentally inoculated with *Campylobacter jejuni* (0.3 ml of 1×10^9 cfu per os).

Species	group	No	Mortality / days post-inoculation											Survival			
			3	4	5	6	7	8	9	14	16	17	No	%	No	%	
Ducks	treated	30	1	0	1	3	0	1	0	1	0	0	0	7	23.34	23	76.66
	control	10	0	0	0	0	0	0	0	0	0	0	0	0	0	10	100
Chicks	treated	30	0	1	1	0	0	0	0	0	0	0	0	2	6.66	28	93.34
	control	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table (8): Mortality pattern in one day old ducks / chicks experimentally inoculated with *Salmonella typhimurium* (0.3 ml of 1×10^9 cfu per os).

Species	group	No	Mortality / days post-inoculation											Survival		
			3	4	5	6	7	8	9	14	16	17	Total	%	No	%
Ducks	treated	30	1	2	2	1	1	1	1	0	0	1	10	33.33	20	66.67
	control	10	0	0	0	0	0	0	0	0	0	0	0	0	0	100
Chicks	treated	30	1	2	2	1	1	1	1	2	0	1	12	40.00	18	60.00
	control	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table (9): Mortality pattern in one day old ducks / chicks experimentally inoculated with *Campylobacter jejuni* + *Salmonella typhimurium* (0.3 ml of 1×10^9 cfu per os).

Species	group	No	Mortality / days post-inoculation											Survival		
			3	4	5	6	7	8	9	14	16	17	Total	%	No	%
Ducks	Treated	30	2	3	3	2	3	1	0	0	1	0	15	50.00	15	50.00
	Control	10	0	0	0	0	0	0	0	0	0	0	0	0	10	100
Chicks	Treated	30	3	5	4	3	0	0	0	0	0	0	15	50.00	15	50.00
	Control	10	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Tablet (10): Frequency of reisolation of *Campylobacter jejuni* and *Salmonella typhimurium* from different organs of one day old ducks / chicks.

Species	Re-isolate	Intestine		Liver		Gall bladder	
		No	%	No	%	No	%
Ducks	<i>C.jejuni</i>	20/30	66.60	20/30	66.60	22/30	73.30
	Control		0		0		0
	<i>S.typhimurium</i>	22/30	73.30	20/30	66.60	21/30	70.00
	Control		0		0		0
Chicks	<i>C.jejuni</i>	21/30	70.00	19/30	63.30	22/30	73.30
	Control		0		0		0
	<i>S.typhimurium</i>	13/30	43.30	11/30	36.60	50/30	50.00
	Control		0		0		0

Tablet (11): Frequency of reisolation of *Campylobacter jejuni* + *Salmonella typhimurium* from one day old ducklings during and at the end of the experiment.

Species	Re-isolate	Intestine		Liver		Gall bladder	
		No	%	No	%	No	%
Ducks	<i>C.jejuni</i>	18/30	60.00	17/30	56.60	19/30	63.30
	<i>S.typhimurium</i>	16/ 30	53.30	14/30	46.60	18/30	60.00
	Control		0		0		0
Chicks	<i>C.jejuni</i>	11/ 30	36.60	10/30	33.30	13/30	43.30
	<i>S.typhimurium</i>	21/ 30	70.00	19/30	63.30	23/30	76.60
	Control		0		0		0

Table (12): Effect of *C.jenuni* and/or *S.typhymurium* infection (0.3 ml of 1×10^9 cfu, per os) on the body weight of ducks and chicks.

Species	Inoculate	Body weight / week				%*
Ducks	Control <i>S.typhimurium</i>	59.50± 0.55	215± 14.45	610± 44.25	845± 20.34	0
		60.40± 0.42	180± 18.42	495± 10.80	710 ± 2.03	16
	Control <i>C.Jejuni</i>	61.10± 0.56	217 ± 8.24	612± 18.02	870± 42.82	0
		58.30± 0.75	190 ± 0.37	507± 43.01	725± 18.43	16.7
	Control <i>S. Typh</i> + <i>C. Jejuni</i>	60.10± 0.33	217 ± 0.42	618± 72.25	883± 12.32	0
		59.80± 0.63	170± 44.24	480 ± 12.2	697± 84.64	16.9
Chicks	Control <i>S.typhimurium</i>	47.4 ± 14.54	352 ± 14.54	916± 36.48	1815 ± 62.5	0
		48.4 ± 0.51	328 ± 18.42	785.5±62.4	1297 ± 20.34	28.5
	Control <i>C.Jejuni</i>	50.60±0.923	364 ±8.29	812 ± 32.79	1860 ±43.01	0
		48.8 ±0.374	343 ± 18.08	774 ± 48.46	1304 ±78.2	29.9
	Control <i>S. Typh</i> + <i>C. Jejuni</i>	49.8 ±0.374	361 ±27.73	806 ± 41.24	1855 ±108.01	0
		48.4 ±0.748	32 ±8.29	730 ± 32.34	1158 ±72.84	37.5

*% of change from control

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عزل وتصنيف ميكروب الكامبيلوباكتر واحداث عدوي صناعية في البط والكتاكيث عمر يوم

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تم تجميع عدد ٥٨٠ عينة (٢٣٠ من اعضاء بط حديث الرفاة،، ٢٤٠ من مجمع طيور سليمة ظاهريا ، ١١٠ من سطح قشرة البيض) لاعمار وانواع مختلفة من مزارع بط في ثلاث محافظات، كذلك تم تجميع ٤٥ عينة من البيئة المحيطة بالبط (مياة الشرب، العلق والفرشة).

بالفحص البكتيريولوجي لعزل ميكروب الكامبيلوباكتر، كانت نسبة العزل كمايلي:
٢٢,٦% من الطيور النافقة حديثا، ١٢,٥% من مسحات المجمع، ١,٨% من سطح قشرة البيض، ٢٦,٦% من مياة الشرب، ١٢,٦% من العلف، ٦٣% من الفرشة، وتصنيف المعزولات وجد ان ٦٦% من المعزولات تنتمي الي الكامبيلوباكتر جيجيوناى، ٣٤% كامبيلوباكتر كولاي،

وباختبار حساسية المعزولات من كامبيلوباكتر جيجيوناى ن وجد انها شديدة الحساسية لكل من نورفلوكساسين، جنتاميسين، تتراسيكلين، فلوموكين، فلومفينيكول، بينما الارثرومايسين والريفاميسين اظهرت حساسية متوسطة وكانت ائمعزولات مقاومة لكل من السيفالوكين والتبسين والبناسين.

تم احداث العدوي الصناعية بمعزولة من الكامبيلوباكتر جيجيوناى والسالمونيللا تايفيموريوم معا او كلا علي حدة في صغار البط والكتاكيث عمر يوم واحد.