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## Original Paper

# Clinicopathological changes associated with *Campylobacter jejuni* infection in broilers Hamada H. El Azzy<sup>1</sup>, El SayedMansour<sup>1</sup>, Nsereen A. Shawky<sup>2</sup>, Mona Salh El Deen<sup>3</sup>

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#### **ABSTRACT**

The present study aimed to investigate the prevalence of Campylobacter jejuniand its clinicopathological changes in broiler chickens in Sharkia province. About 50 diarrheic broilerchicks' cloacal swabs were collected for bacteriological examination. Out of 50 examined swabs; 12 (24%) were positive for Campylobacter [4, C. coli 4 and 8; C. jejuni]. Isolated Campylobacterwassensitive to neomycin and gentamycin. About 45 healthy one-day-old broilerchickens received 5 mg pefloxacin/kg Bw for 5 days to exclude bacterial infections. At 14th day broilers were divided into 3 groups (15/ each). First group; healthy broilers non-treated (control), broilers in 2<sup>nd</sup> and 3<sup>rd</sup> groups were infected with *C. jejuni*. 2<sup>nd</sup> group were infected and nontreated, while 3rd group infected, and treated with 15 mg neomycin/kg Bw. in drinking water for 5 days. At 1st,7th and 14th day post treatment cloacal swabs were collected for re-isolation C. jejuni besideblood samples were collected for hematobiochemical study. Infectedbroilers showed offfood, depression, ruffled feather, diarrhea and mortality rate 40% beside significant decrease in body performance, total protein albumin and non-significant decrease in globulin coupled with non-significant elevation in RBCs, HB, PCV%, significant elevation in WBCs, AST, ALT, ALP, urea and creatinine. Treatment infected broilers by neomycin lead to disappear clinical sing, reduced mortality rate and improved hematobiochemical parameters. It could be concluded that Campylobacter infection induces reversible adverse effect on body performance andhematobiochemical parameters. Neomycin is highly curative against campylobacters

## 1. INTRODUCTION

Poultry has become an important source of meat in developing countries. Enteric disease in broilers is a common and important illness beside a risk for poultry industry in world (Kaakoush, et al. 2015). Campylobacter caused gastroenteritis is caused by two closely related species (Campylobacter jejuni and Campylobacter coli) but Campylobacterjejuni is the more predominant (Leonard, et al. 2020).

Campylobacter can appear in broilers as early as 14-day age at rearing with low percentage and increase to a high percentage at the end of grows out period (Evans, 2012). Most common routes of transmission are feacal-oral ingestion of contaminated food, water and eating of raw meat. Foods implicated in campylobacteriosis (Skarp, et al. 2016). Campylobacter infection is a wide range of avian spp. andrarely transmits vertically from parents to chicks (Huang, et al. 2017). Campylobacter cause diarrhea and health problem contributing substantially to childhood morbidity and mortality (Zhang, et al. 2018). Campylobacters are small and slender gram -ve spiral shaped rods beside its food and water-borne zoonotic diseases (Aneesa and Mohamed, 2019).

Antibiotics are used for bacterial infections (Thomrongsuwannakij, et al. 2018). Campylobacterosis is treated by antibiotics as aminoglycoside which act by irreversible inhibition bacterial ribosomes and impairs protein synthesis of bacteria (Fernandes and Marten, 2017). Neomycin

is a member of aminoglycoside antibiotic against G +ve and G -ve organisms (Gupta and Plazomicin, 2017).

The aim of the present study was isolate, identify *Campylobacter* and its prevalence in broilers in Sharkia province beside its effect on body performance, hematochemical parameters with trail of treatment was studied.

## 2. MATERIAL AND METHODS

## 2.1. Isolation and identification of Campylobacter spp

About 50 diarrhoeic chicks' cloacal swabs were taken from different cities of Sharkia Provence. Swabs were collected aseptically and inoculated into charcoal cefoperazone desoxycholate agar medium (selective medium for isolation of *Campylobacter*). Plates were incubated at 37°C for 72 hrs under special microaerophilic condition (85 % nitrogen 5% oxygen, 10% carbon dioxide) (Murray, et a. 2003). Suspected colonies were identified and Bio-typingby Gram staining, oxidase test, catalase test and standard biochemical methods (Atabay and Corry, 1997).

## 2.2. Antibiotic sensitivity test (In vitro)

Susceptibility of isolated Campylobacter species against different chemotherapeutic agents was tested by disc diffusion method (Quinn, et al. 1994).

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#### 2.3. Antibacterial drugs

2.3.1. Pefloxacin (Peflodad10 %) solution was obtained from Dar Al Dawa Vet and Agri Industrial Co. Itd Jordan. Each ml contains 100 mg of pefloxacin base.

2.3.2. Neomycin sulphate 20% produced from sento care Pharma comp Egypt

#### 2.4. Experimental broilers and experimental design

About 45 apparently healthy one day-old Hubbard broilers nearly equal in live body weight (44.27-46.83gm) and received 5 mg pefloxacin/ kg bw in drinking water for 5 successive days for proving that broilers are free from any bacterial infections. Broilers were fed starter ration from Kahar Company and clean drinking water ad-libtium. At 14 day of age broilers were divided into three equal groups (15/each). Gp (1) healthy chicks (control), Broilers in Gp (2) were orally infected with 0.1ml saline containing(2.5×10<sup>8</sup> CFU) of isolated *C. jejuni*. Gp (2) infected broilers non treated and Gp (3) infected broilers, treated with 15 mg neomycin/kg Bw. in drinking water for 5 consecutive days.

#### 2.5. Body weight:

Chicks were individually weighed at 1<sup>st</sup> day of age and at 1<sup>st</sup> day post treatment for estimation body weight gain and feed conversion rate

## 2.6. Re-isolation of Campylobacter spp.:

At 1<sup>st</sup>, 7<sup>th</sup> and 14<sup>th</sup> day posttreatmentcloacal swabs were collected for Re-isolation *Campylobacter jejuni* 

#### 2.7. Blood samples:

At 1<sup>st</sup>,7<sup>th</sup> and 14<sup>th</sup> day post treatment 2 blood samples were taken.

First sample was taken in a tube contain EDTA for estimation of blood picture Jain (1986).

Second sample was centrifuged to obtain clear serum for estimation of AST and ALT (Reitman and Frankel, 1957) ALP (John, 1982) total protein (Doumas, et al. 1981) albumin (Drupt 1974) globulin (mathematicaly). Uric acid (Artiss 1980) and creatinine (Henry, 1974).

2.8. Statistical analysis was performed by using analysis of variance (ANOVA). Duncan's Multiple Range Duncan, (1955) was used to determine differences among treatments mean at significance level of 0.05. Statistics were run using SPSS program (SPSS, 2004)

#### 3. RESULTS

Examined cloaca swabs revealed 12 (24%) were positive for Campylobacter [4 Campylobacter coli and 8 Campylobacter jejuni].Both Campylobacter coliand Campylobacter Jujuni were negative for gram stain, positive oxidase and positive catalase and grow on 1% glycin, meanwhile campylobacter coli not hydrolysed Hippurate but campylobacter Jujuni hydrolysed Hippurate. (Table 1 and 2). Isolated Campylobacter was sensitive to neomycin and gentamycin (Table, 3). Campylobacter jejuni in broilers induced clinical signs (loss of appetite, depression, ruffled feather; diarrhea and 40% mortality rate at 1st and 7th day post treatment (Table 4). Campylobacterjejuni induced significant decrease (P< 0.5) in body performance, total protein, albumin coupled with nonsignificant changes in globulin beside non-significant elevation in RBCs, HB, PCV% associated with significant increase in WBCs, AST, ALT, ALP, urea and creatinine at 1st and 7th day post treatment. Treatment infected broilers by neomycin showed disappear clinical sing, reduced mortality rate to 20%, not reisolate Campylobacter jejuni and improved hematobiochemical parameters at 14<sup>th</sup> day post treatment (Table 4-8).

Table 1 Prevalence and type of isolated campylobacters

	-ve sample		+ve sample		Type of isolated campylobacters			
Number of cloacal	No	%	No %		Campylobacter jejuni		сатр	ylobacter coli
Swabs					No	%	No	%
50	38	76	12	24	8	66.67	4	33.33

Table 2 biochemical identification of Campylobacter spp in broiler chickens

			C. coli (4)					C. Jujuni (8)	)	
Positive cloacal swabs	Gram stain	Catalase	Oxidase	Growth on 1% glycine	Hippurate hydrolysis	Gram stain	Catalase	Oxidase	Growth on 1% glycine	Hippurate hydrolysis
_	-ve	_	+	+	_	-ve	_	+	_	+

= GS. Catalase= cat. Oxidase=Ox. Growth on 1% glycine = GG. Hippurate hydrolysis= HH

Table3 Antibiotics sensitivity of Campylobacter.isolated from broilers to (n=5).

Antibiotic	Sample	Sensi	Sensitive		Moderate		Resistant	
	number	No	%	No	%	No	%	
Gentamycin	10	8	80	2	20	00	00	
Neomycin	10	6	60	4	40	00	00	
Ciprofloxacin	10	7	70	3	30	00	00	
Erythromycin	10	7	70	3	30	00	00	
Tetracycline	10	4	40	6	60	00	00	
Ampicillin	10	00	00	2	20	8	80	

Table 4 Mortality of healthy and diseased broilers and reisolated campylobacter

	,					
Parameters	Total	Mort ra	ality te	Reisolated of Campylobacter spp post treatment (day)		
Groups	No	No	%	1	7	14
Gp (1)	10	00	00	0.00	0.00	0.00
Gp (2)	10	4	40	10/10	10/10	10/10
Gp (3)	10	2	20	00/10	00/10	00/10

Table 5.Body performance of healthy and diseased broilers (n=5).

Groups	Initial weight(1st day of age)	Final weight (20thday of age)	Weight gain	FC	FCR
Gp (1)	48.33±0.68a	951.16±1.33 <sup>b</sup>	905.71±1.26 <sup>b</sup>	990.45	1.07
Gp (2)	49.67±0.87a	946.32±1.46°	899.86±2.18°	980.85	1.09
Gp (3)	48.80±0.71a	$966.14\pm4.13^{a}$	920.20±5.46a	990.71	1.04

FC=feed consumption, FCR= Feed Conversion rate. \* Significant at P < 0.05. Means with different superscripts of the same column indicate significant difference at P < 0.05.

Table 6 RBCs, Hb, PCV and leukocytic count in healthy and diseased broilers (n=5.)

Groups	RBCs(106/µL)	Hb(g/dl)	PC	V%	WBCs (10 <sup>3</sup> / μL)
1st day	Gp (1)	3.72± 0.51 <sup>a</sup>	13.80±1.93 <sup>a</sup>	39.07±1.73 <sup>a</sup>	12.34±0.76 <sup>b</sup>
	Gp (2)	$4.09\pm0.38^{a}$	$14.69\pm1.60^a$	40.12±1.55a	14.89±0.43a
	Gp (3)	4.21±0.39a	14.89±1.33a	$40.56\pm1.40^{a}$	$13.69\pm0.46^{a}$
7 <sup>th</sup> day	Gp (1)	$3.61\pm0.43^{a}$	13.77±1.82a	39.11±1.57a	12.31±0.79b
	Gp (2)	$4.06\pm0.50^{a}$	14.54±1.54a	40.17±1.43a	14.78±0.36a
	Gp (3)	4.18±0.43 <sup>a</sup>	14.68±1.24 <sup>a</sup>	40.24±1.36a	13.08±0.36 <sup>b</sup>
14 <sup>th</sup> day	Gp (1)	$3.75\pm0.47^{a}$	$13.76\pm1.68^a$	39.12±1.56a	12.31±0.70b
	Gp (2)	4.17±0.33a	$14.60\pm1.69^a$	$40.17\pm1.50^a$	14.49±0.35a
	Gp (3)	4.33±0.34a	14.85±1.28a	40.49±1.37a	13.18±0.46b

Means with different superscripts of the same column indicate significant difference at P < 0.05

Table 7 Protein profile (g/dl) in healthy and diseased broilers (n=5).

Grou	Groups T.Protein Alb		Albumin	Globulin	A/G ratio	
1stday	Gp(1)	5.06±0.13a	2.69±0.19a	2.37±0.11 <sup>a</sup>	1.44±0.21a	
	Gp(2)	$4.62\pm0.12^{b}$	$1.86 \pm 0.21^{b}$	$2.16\pm0.12^{a}$	0.67±0.17 <sup>b</sup>	
	Gp(3)	4.47±0.21 <sup>b</sup>	2.02±0.16b	2.01±0.17 <sup>a</sup>	$0.86\pm0.20^{b}$	
7 <sup>th</sup> day	Gp(1)	$5.90\pm0.50^{a}$	$2.82\pm0.22^{a}$	$2.11\pm0.10^{a}$	1.21±0.23a	
	Gp(2)	$4.60\pm0.68^{b}$	2.38±0.47b	$2.01\pm0.08^{a}$	1.08±0.15a	
	Gp(3)	$6.46{\pm}0.36^a$	$3.50\pm0.28^{a}$	$2.06\pm0.17^{a}$	1.18±0.14 <sup>a</sup>	
14 <sup>th</sup> day	Gp(1)	$5.87{\pm}0.55^a$	2.78±0.24a	$2.07\pm0.14^{a}$	1.25±0.19a	
	Gp(2)	$4.55\pm0.70^{a}$	2.35±0.56a	2.20±0.25a	$1.07\pm0.16^{a}$	
	Gp(3)	6.76±0.41a	$3.60\pm0.44^{a}$	3.06±0.39a	1.21±0.18a	

Means with different superscripts of the same column indicate significant difference at P < 0.05

Table 8 Liver enzymes and kidney functions in healthy and diseased broilers (n=5)

			liver enzymes (U/L)		Kidney fun	ctions (mg/dl)
Groups		AST	ALT	ALP	Uric acid	creatinine
1st day	Gp (1)	89.04±1.46 <sup>b</sup>	56.16±1.02b	40.95±0.90 <sup>b</sup>	4.74±0.29b	1.06±0.09°
	Gp (2)	94.33±1.21a	$59.97{\pm}1.25^a$	44.17±0.78a	$6.14\pm0.28^{a}$	1.72±0.21a
	Gp (3)	92.99±1.01a	$58.52\pm1.04^a$	43.08±0.17a	$5.49\pm0.14a$	1.30±0.10b
$7^{th}day$	Gp (1)	88.99±1.39b	56.13±0.98b	$40.89\pm0.94^{b}$	4.73±0.27b	1.02±0.08b
	Gp (2)	93.97±1.15a	59.91±1.14 <sup>a</sup>	44.21±0.69a	6.11±0.21a	1.70±0.20a
	Gp (3)	91.43±1.32b	57.35±1.35 <sup>b</sup>	$41.56\pm0.89^{b}$	5.40±0.16a	1.34±0.12a
14 <sup>th</sup> day	Gp (1)	89.08±1.42b	$56.15\pm1.04^{b}$	$40.92\pm0.89^{b}$	4.70±0.31b	1.63±0.25 <sup>b</sup>
	Gp (2)	93.68±1.30a	$59.83\pm1.02^a$	44.34±0.71a	6.17±0.25a	$2.64\pm0.2^{a}$
	Gp (3)	90.75±1.19b	57.10±1.85b	41.23±0.88b	5.44±0.21b	1.77±0.19b

Means with different superscripts of the same column indicate significant difference at P < 0.05

#### 4. DISCUSSION

Infected bird with *Campylobacter* carry a very high bacterial concentration in their gastrointestinal tract and the main sites of colonization of *Campylobacter* in poultry are the caeca, colon and cloaca (Facciola, et al. 2017). *Campylobacter* infection is characterized by inflammatory, sometimes bloody diarrhea or dysentery syndrome (cramps, fever, and pain) (Liz, et al. 2020).

In the current study, the prevalence of campylobacter was 24%. Our results are in agreement with Khalifa, et al. (2011) who observed that the prevalence of Campylobacter in broilers in Kaliobia was 26%. Campylobacterprevalence in broilers from Sharkia Provence was 29.3% (Ashraf, et al. 2018). The prevalence of Campylobacterin Assuit Province was 21.5% (Mostafa, et al. 2018) in broilers. Variation in Campylobacter prevalence may be due to difference in sanitation (Leonard, et al. 2020).

In the present study, Campylobacterisolates were identified as *Campylobacter jejuni* 8 (66.67%) and *Campylobacter coli* 4 (33.33%). Same results were reported by Saad (2014) who identified *Campylobacterjejuni* in rate

of 60.9% in Sharkia Province. Comparable percentages of *Campylobacter jejuni*56% were reported by Abd El-Tawab et al. (2015) in Sharkia Provence. Identified *Campylobacter* jejuni in rate of 66% in Egypt (Ashraf, et al. 2018)

Disc diffusion test revealed isolated Campylobacter was sensitive to neomycin and gentamycin. *Campylobacter* isolated from broilers was sensitive to neomycin and gentamycin (Sayed 2000).

Our obtained results revealed that infected broilers with campylobacterjejuni showed clinical signs (ruffled feather, depression, loss of appetite, diarrhea, reduction in body weights and mortality rate was 40%). Diseased broilers treated with neomycin showed disappearance of clinical signs and reduction in mortality rate to 20 % and not reisolate Campylobacter jejuni. Same clinical sigs were observed by Khalil (2002)in broilers infected with Campylobacter jejuni. This result was consistent with Liz, et al. (2020) who stated that broilers infected with Campylobacter jejuni showed loss of appetite, depression, diarrhea, and reduction in body weights. Neomycin is a very effective drug against Campylobacter jejuni as it caused

disappearance of clinical signs and decreased mortality rate in chickens (Krishna, et al. 2018).

Our results revealed that, broilers infected with *Campylobacter jejuni* showed non-significant change in RBCs, Hb, PCV % and significant increase in WBCs. Leukocytosis in infected broiler may be due to inflammatory response in intestinal tract (Radostitis, et.al. 2002). Similar result in blood picture was observed by Thrall (2004) stated that broilers infected with *Campylobacter* showed non-significant elevation in RBCs, Hb, PCV% and significant leukocytosis. *Campylobacter* induce significant elevation in leukocytic count in broilers (Lavini, et al. 2016).

In the present study, *campylobacter* infection induced significant decrease in total proteins, albumin and non-significant decrease in globulin. Reduction in total protein and albumin in broiler infected with campylobacter may be due to liver damage by *campylobacter* toxins in which liver is the sole site of albumin synthesis (Latimer, et al. 2003). Hypoalbuminemia in infected broilers may be due to inappetance and male absorption of nutrients from inflamed intestine (Thrall,2004). *Campylobacter* induce decrease in in total protein and albumin in chickens (Lavini, et al. 2016).

Our results showed that, broilers suffering from campylobacteriosis showed significant increase in AST, ALT, ALP, uric acid and creatinine. Elevationof liver enzyme, uric acid and creatinine comes from Radostitis, et.al. (2002) stated that *campylobacter*toxins induced degenerative changes and necrotic processes in liver and kidneys leading to increase in liver enzymes, uric acid and creatinine. These results were confirmed by result recorded by Lavini, et al. (2016) who stated that with *campylobacterJejuni* showed increase in liver enzymes, uric acid and creatinine in broilers

Our study revealed that, treatment *campylobacters* in broilers using neomycin resulted in disappearance of clinical signs, reduction in mortality rate up to (10%), improved in body weight and not re-isolate *campylobacter* beside improved in hemato-biochemical parameters to normal level at 14<sup>th</sup> day post treatment. Same result were reported previously by Hassanain, (2011) in broilers infected with *campylobacter* and treated withneomycin. Our results were reinforced by Agnes, et al. (2012) who observed an improvement in broilers infected with *campylobacter* and treated with neomycin.

# 5. CONCULOSIONS

It could be concluded that Campylobacter jejuni induce many changes in haemato-biochemical parameters in broilers but neomycin in therapeutic dose was effective in medication of campylobacters infection in broiler chickens.

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