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PREVALENCE AND PATHOGENISITY OF *CAMPYLOBACTER SPECIES* IN CHICKENS IN EL FAYOUM GOVERNORATE

(With 4 Tables)

By

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مدى تواجد وخطورة ميكروبات الكامبيلوباكتر فى الدجاج فى محافظة الفيوم

فوزى رياض الصعيدى ، أسماعيل عبد الحفيظ رضوان ، مرفت ميلاد عبدالله، نجلاء فتحى قرنى

تم تجميع عدد ٣٠٠ عينة من السلالات المحلية للدجاج عمر ٤ – ١٦ أسبوع من المزارع الحكومية والخاصة بمحافظة الفيوم وبنى سويف (٢٥٠ من المريض – ٥٠ من السليم ظاهريا). الفحص البكتريولوجى أظهر أن ٩٧ (٣٢,٣%) أيجابيا للكامبيلوباكتر. تم عزل ٨٨ عترة كامبيلوباكتر من الدجاج المريض (٤٦,٤%) C.coli , C.jejuni, (٥,١%) C.lardi. (٣٩,٢%) ٩عترة من الدجاج السليم ظاهريا. وكانت أهم العترات المعزولة من الدجاج المريض كامبيلوباكتر جنجواى بالعدوى الصناعية بميكروب كامبيلوباكتر جنجواى وملاحظة الوفيات بعد ٦ أيام من العدوى الصناعية عن طريق الفم و٤ أيام للحقن العضلى كانت نسبة الوفيات للمجموعة المعدية عن طريق الفم (٢٠ %) – المحقونة عضليا (٣٠%) وتم أعادة العزل للميكروبات المحقونة من الكبد والأمعاء من الدجاج وملاحظة التغيرات الباثولوجية على هذه الأعضاء.

SUMMARY

A total of 300 (250 clinically diseased and 50 apparent healthy) local breeds chickens (4 to 16 weeks old) were collected from different governmental and private farms at El-Fayoum and Beni Suef Governorates were employed. Bacteriological examination showed that 97 (32.3%) were positive for *Campylobacter species*. Those cases consisted of 88 isolates were recovered from 250 clinically diseased chickens and 9 isolates from 50 apparent healthy ones. Out of the examined 88 isolates of *Campylobacter* recovered from the diseased chickens, 45 were *C. jejuni*, 38 were C. coli and 5 were *C. lardi* with an

incidence of 46.4 %, 39.2 % and 5.1% respectively. From apparent healthy chickens the 9 recovered isolates consisted of 6 *C. jejuni* and 3 *C. coli*. The recovery rate from different sites of all the examined chickens was almost equal from the Jejunum (44.3%) and caecum (43.3%), while it was in a descending rate from the liver (9.3%), gall bladder (2.1%) and heart blood (1.0%) as the number of *Campylobacter species* that were isolated from the jejunum, caecum, liver, gall bladder and heart blood was 43, 42, 9, 2, and one respectively. From diseased chickens, *Campylobacter jejuni* was mainly isolated from jejunum (29 isolates), followed by caecum (12 isolates), liver (3 isolates) and once from gall bladder contents. *Campylobacter coli* was mainly recovered from caecum (26 isolates), followed by jejunum (8 isolates), liver tissues (2 times), gall bladder and heart blood (1 isolate each). *Campylobacter lari* was recovered from jejunum (2 isolates) and liver tissues (3 isolates). In apparent healthy chickens, *Campylobacter jejuni* was isolated from jejunum (3 isolates), followed by caecum (2 isolates) and liver tissues (1 isolate), while *Campylobacter coli* was recovered from caecum (2 isolates) followed by jejunum (1 isolate). Experimental infection with *Campylobacter jejuni* showed that the first mortalities was recorded 6 days post infection in orally infected group and 4 days in intramuscularly infected group of chickens. Mortality rate was 20.0% (4/20) in orally inoculated group and 30% (6/20) in I.M. infected group. Re-isolation of the inoculated microorganism was recorded from the liver, caecum and jejunum from 3 (out of the 4 dead) orally infected chickens, and from. 5 (out of the 6 dead) I.M. inoculated ones. Rectal swabs that were collected from living chickens at the end of the experiment showed that *C. jejuni* was recovered from 3 living chickens of orally infected group and 2 from I.M. injected group. Experimental infection with *C. coli* produced 25.0% and 20.0% mortalities when inoculated I.M and orally respectively. The principal changes were in the form of distension of the intestinal tract. Intramuscular inoculation of *C. jejuni* and *C. coli* in a mixed form produced 35.0 % mortality rate with severely detectable pathological changes in the liver, accumulation of mucus and watery fluid in duodenal loop and hemorrhages in the intestinal tract. The mortality rate lowered to be 25.0% accompanied with severe dehydration and blood tinged duodenal mucosa when the two organisms were inoculated orally.

Key words: *Chickens, Campylobacter species, C.jejuni, C.coli.*

INTRODUCTION

Campylobacteriosis, caused by *Campylobacter species*, is an important food–borne disease of specific concern to consumers of undercooked poultry meat (Simon, 1992). The contamination of poultry meat usually occurs after slaughtering when the organisms spread from the intestinal contents of the affected birds to the carcasses during the process of evisceration (Simmons and Gibbs., 1979). In chicken farms, the main transmission route of this microorganism is horizontal, as the vertical route continues to be the object of inconclusive researches (Fonseca *et al.*, 2006).

Campylobacter microorganisms are Gram-negative, spiral and/or curved non-spore forming, 0.2 – 0.9 µm wide and 0.5-5 µm long. They are oxidase positive with at least one polar flagellum at one or both end, giving a corkscrew or darting motility (Bolton *et al.*, 1992).

In poultry, *Campylobacter jejuni* is responsible for avian vibrionic hepatitis, which is a contagious disease of young and mature chickens characterized by low mortality, high morbidity as associated with chronic course, poor growth and productions (Peckham, 1984).

It is reasonable to assume that *Campylobacter* is the cause of a significant percentage of bacterial food-borne illness in humans and that poultry may be a vehicle responsible for a substantial portion of these illnesses (Sayed, 2000). It is imperative that sensitive methods for detection, enumeration, isolation and identification be used to assess the presence of *C. jejuni* and *C. coli* in poultry (Steinhauserova, 2001).

The present study was planned to investigate the prevalence of *Campylobacter species* in clinically diseased and apparent healthy, local bread chickens, to characterize biochemically the isolated *Campylobacter* strains and to clarify the frequency of recovery from different internal sites of studied chickens. Also to asses the pathogenisity, of one or more of the recovered organisms, by experimental chickens infection.

MATERIALS and METHODS

Collection of samples:

A total of 300 local breeds chickens (4 to 16 weeks old) which were collected from "El-Azzab project" at El-Fayoum Governorate, "Sedse project" at Beni Suef Governorate as well as from different private farms at El-Fayoum Governorate were employed. These were

consisted of 250 clinically diseased sacrificed (emergency slaughtered) chickens and 50 apparent healthy cases.

Samples were collected from jejunum, caecum and gall bladder contents, liver tissues and heart blood. Samples from each case were separately collected using sterile mono-use disposable plastic glove, which was inverted after sampling and on which, sample information was written. All samples were transported to the laboratory in cold chamber container, within few hours for bacteriological procedures.

Bacteriological examination:

In the laboratory, a loop full from the homogenized suspension of liver tissues and the contents of gall bladder were directly streaked in duplicate onto plates of Bacto – Campylobacter agar, and Camp BAP medium (Blaser *et al.*, 1984) and incubated anaerobically at 37 °C and 42 °C for 48 – 72 hours for direct isolation.

The pre-enrichment method was used for recovery of *Campylobacter species* from caecal and intestinal contents by dissolving one gram of fecal and gut fluid or mucous in one ml of sterile physiological saline. Centrifugation of the mixture at 1000 rpm for 5 minutes was applied and few drops of the supernatant were inoculated into thioglycolate and tryptone - soy – broth and incubated anaerobically at 25 °C, 37 °C and 42 °C for 48 hours. A loop full of each broth was streaked onto the surface of sheep blood agar with antibiotics and growth supplement. Also other few drops of supernatant were streaked directly on to the surface of Camp BAP medium and Bacto Campylobacter agar. All solid media were incubated microairophilically at 25, 37 and 42 °C for 48–72 hours for direct plating from intestinal samples.

The isolated colonies were identified morphologically and culturally and all Gram negative bacterial isolates showing the typical shape of *Campylobacter* (Hanninen, 1982) were carefully selected and the biochemical characterization tests were used according to Cruickshank *et al.* (1975); Collee *et al.* (1992); Koneman *et al.* (1996) and Tolba (2005).

Experimental infection:

One hundred and forty apparently healthy thirty days old local breed chickens received all vaccines against Newcastle, BD and IB viruses were divided into 7 equal groups (20 each). These chickens were reared on clean litters separately in a good ventilated conditions and fed on ration free from any antimicrobial feed additives.

Chickens of the first group were infected orally with 0.5 ml saline suspension of $1X10^9$ cfu/ 1 ml saline of *C. jejuni* for 2 successive days (Barson *et al.*, 1994). The second group was I/M inoculated with 0.5 ml of $1X10^9$ cfu/ 1 ml saline of *C. jejuni*. The third and fourth groups were also treated like group 1 and 2 respectively, but using *C. coli*:

The fifth and sixth groups were similarly treated like group 1 and 2 respectively, but were simultaneously infected with each of *C. jejuni* and *C. coli*. The seventh group was kept as a control non infected control group.

Clinical signs, morbidity, mortality and post mortem examination of freshly dead experimental chickens were recorded as well as re-isolation of *Campylobacter species* from both dead and survived experimental chickens was employed.

RESULTS

The incidence of *Campylobacter species* recovered from diseased sacrificed (emergency slaughtered) and apparently healthy chicken samples are shown in Table (1). Out of the examined 88 isolated of campylobacter recovered from the diseased chickens, 45 were *C. jejuni*, 38 were *C. coli* and 5 were *C. lardi* with an incidence of 46.4 %, 39.2 % and 5.1% respectively (Table 1). From apparent healthy chickens the 9 recovered isolates consisted of 6 *C. jejuni* and 3 *C. coli*.

The total number of *Campylobacter* microorganism recovered from different sites of examined chickens is given in Table (2). The recovery rate was almost equal from the Jejunum (44.3%) and caecum (43.3%), while it was in a descending rate from the liver (9.3%), gall bladder (2.1%) and heart blood (1.0%) as the number of *Campylobacter species* that were isolated from the jejunum, caecum, liver, gall bladder and heart blood was 43, 42, 9, 2, and one respectively. Eighty-five out of 97 Campylobacter isolates were recovered from the intestinal samples collected from all the examined chickens.

The frequency of *Campylobacter species* recovered from different sites of the examined diseased chickens is shown in Table (3). *Campylobacter jejuni* was mainly isolated from jejunum (29 isolates), followed by caecum (12 isolates), liver (3 isolates) and once from gall bladder contents. *Campylobacter coli* was mainly recovered from caecum (26 isolates), followed by jejunum (8 isolates), liver tissues (2 times), gall bladder and heart blood (1 isolate each). *Campylobacter*

lari was recovered from jejunum (2 isolates) and liver tissues (3 isolates).

In apparent healthy chickens, *Campylobacter jejuni* was isolated from jejunum (3 isolates), followed by caecum (2 isolates) and liver tissues (1 isolate), while *Campylobacter coli* was recovered from caecum (2 isolates) followed by jejunum (1 isolate).

Results of experimental infection with *Campylobacter jejuni* showed that first mortalities was recorded 6 days post infection in orally infected group and 4 days post infection in group of chickens inoculated intramuscularly. Mortality rate was 20% in orally inoculated group of chickens and 30% in intramuscularly infected group (Table 4). Re isolation of the inoculated microorganism was recorded from the liver, caecum and jejunum from 3 (out of the 4 dead) orally infected chickens and from.5 (out of the 6 dead) intramuscularly inoculated ones. Rectal swabs that were collected from living chickens at the end of the experiment showed that *C. jejuni* was recovered from 3 living chickens of orally infected group and 2 from I.M. injected group.

Results of experimental infection with *Campylobacter coli* showed that first mortalities was recorded 5 days post infection in orally infected group and 3 days post infection in group of chickens inoculated intramuscularly. Mortality of 20.0 % and 25.0 % was recorded in oral route and intramuscular respectively (Table 4). Affected chickens showed, within 3-5 days, depression, fecal saturation of the vent plumage, and watery dropping, which persisted for 8-10 days. The principal change associated with *C. coli* infection comprises distention of the intestinal tract extending from duodenal loop to the bifurcation of the caeca. Re-isolation of the microorganism was recorded from the clotted heart blood of from the caeca of two out of the four dead orally infected group and from 3 out of 5 intramuscularly freshly dead chickens.

Results of concurrent experimental infection with *Campylobacter jejuni* and *Campylobacter coli* by oral infection, showed that 5 chickens (25.0%) were died. Those consisted of 2 chickens died 5 days post infection, another 2 died 3 days later and 1 at the eleventh day post oral infection. The recorded lesions were mainly intestinal with clear or blood tinged mucus in duodenum, the caeca were distended and severe dehydration was noted in two dead chickens. Re-isolation of the two inoculated organisms was achieved from the intestinal contents of two dead chickens. Rectal swabs from living chickens gave positive results for *C. jejuni* only in two cases.

In case of I.M. route of infection, death started two days post infection for 2 chickens and for other 3 chickens one day later, the last recorded mortality was in two chickens after 12 days from the beginning of experimental infection. Mortality rate amounted 35 %. The severity of clinically detectable changes was confines to depression and diarrhea. The presence of yellow/red mottling of the liver parenchyma was noted. Accumulation of mucus and watery fluid in duodenal loop was also noted. Haemorrhages in the intestinal tract were recorded in 3 chickens. Re-isolation of *C. jejuni* in combination with *C. coli* in a mixed from was achieved twice from heart blood and liver, while, *C. jejuni* was re-isolated alone from other two chickens from the liver.

Table 1: Incidence of *Campylobacter species* recovered from the examined chickens.

Health condition	Total No. of isolates	Isolated Campylobacter microorganisms					
		C.jejuni		*C.coli*		*C.lari*	
		No.	%	No.	%	No.	%
Diseased:	88	45	46.4%	38	39.2%	5	5.1%
Apparent healthy	9	6	6.2%	3	3.1%	0	0.0%
Total	97	51	52.6%	41	42.3%	5	5.1%

Table 2: Total number of *Campylobacter species* recovered from different sites of the examined chickens.

Health condition	Recovery site				
	Jejunum	Cecum	Liver	Gall bladder	Heart blood
Diseased	39	38	8	2	1
Apparent healthy	4	4	1	-	-
Total (%)	43 (44.3%)	42 (43.3%)	9 (9.3%)	2 (2.1%)	1 (1.0%)

Table 3: Frequency of *Campylobacter species* recovered from different sites of the examined diseased chickens.

Recovery site	Campylobacter species					
	C.jejuni		*C.coli*		*C.lari*	
	No.	%	No.	%	No.	%
Jejunum	29	33.0%	8	9.1%	2	2.3%
Cecum	12	13.6%	26	29.6%	-	0.0%
Liver	3	3.4%	2	2.3%	3	3.4%
Gall bladder	1	1.1%	1	1.1%	-	0.0%
Heart blood	-	0.0%	1	1.1%	-	0.0%
Total	45	51.1%	38	43.2%	5	5.7%

Table 4: Mortalities of experimentally infected chickens with the isolated Campylobacter strains.

Microbe used / infection route / Chickens Mortalities	Campylobacter species used					
	C.jejuni		*C.coli*		*C.jejuni* and *C.coli*	
	Oral	I.M.	Oral	I.M.	Oral	I.M.
	Number of experimental chickens used					
	20	20	20	20	20	20
Deaths:						
48 hours						2
3 days				3		3
4 days		1				
5 days		2	2		2	
6 days	1		1	1		
7 days				1		
8 days	1	2			2	
9 days		1				
10 days			1			
11 days	2				1	
12 days						2
Total deaths	4	6	4	5	5	7
Death rate	4/20	6/20	4/20	5/20	5/20	7/20
Percentage	20%	30%	20%	25%	25%	35%

DISCUSSION

Campylobacter species is an important agent that causes foodborne infection, particularly in food of poultry origin. *Campylobacter jejuni* and C. *coli* has become recognized as a common etiological agent in human diarrheas. This fact had led to announce (on December, 2007) that "This has been the year of eating dangerously" (Lundy, 2007). He declared that "Consumer Reports" got things rolling in the Cincinnati Enquirer reported that 83 percent of all raw chickens harbor *Campylobacter or Salmonella*, leading causes of food- borne disease.

Campylobacter microorganisms are widely spread in boiler farms as reported by both wieliczko (1995A) and Jacobs-Reitsma *et al.* (1994) and the chickens can be infected by *Campylobacter* microorganisms at 4-6 weeks of age. Therefore, the efficient control of the transmission routes in chicken farms is of outmost importance to prevent it from spreading (Fonseca *et al.*, 2006).

It is well known that the recovery of *Campylobacters* is greatly influenced by oxygen content of the gaseous atmosphere in contact with

enrichment and isolation media. *Campylobacter jejuni* and *C. coli* are sensitive to air, surviving only 1 to 2 days in solid media, 2 to 4 days in liquid media, and 10 to 20 days in semisolid media at room temperature and survival can be enhanced by holding cultures at 4°C and by reducing oxygen tension. (Park *et al.*, 1984). These fact acts the main obstacles that encountered in the laboratories that working in the isolation and identification of *Campylobacter* microorganisms.

In this study, 97 cases were found to be positive for campylobacter with an incidence of 32.3%. Those cases consisted of 88 isolates were recovered from 250 clinically diseased sacrificed (emergency slaughtered) chickens and 9 isolates from 50 apparent healthy ones.

Out of the examined 88 isolated of *Campylobacter* recovered from the diseased chickens, 45 were *C. jejuni*, 38 were *C. coli* and 5 were *C. lardi* with an incidence of 46.4 %, 39.2 % and 5.1% respectively (Table, 1). From apparent healthy chickens the 9 recovered isolates consisted of 6 *C. jejuni* and 3 *C. coli*.

The total number of *Campylobacter* microorganisms recovered from different sites of the examined chickens is given in Table (2). The recovery rate was almost equal from the Jejunum (44.3%) and caecum (43.3%), while it was in a descending rate from the liver (9.3%), gall bladder (2.1%) and heart blood (1.0%) as the number of *Campylobacter species* that were isolated from the jejunum, caecum, liver, gall bladder and heart blood was 43, 42, 9, 2, and one respectively.

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In apparent healthy chickens, *Campylobacter jejuni* was isolated from jejunum (3 isolates), followed by caecum (2 isolates) and liver tissues (1 isolate), while *Campylobacter coli* was recovered from caecum (2 isolates) followed by jejunum (1 isolate).

Our results agreed with that of different authors, as the higher incidence of *C. jejuni was* explained as because chicks are coprophagic by nature, and *C. jejuni* readily colonizes in the chick, a rapid

transmission through an entire flock could be expected (Pokamunski *et al.*, 1986). Jacobs-Reitsma *et al.* (1994) recorded that the flocks became colonized with *Campylobacter* at about 3-4 weeks of age with isolation percentage of 100% and stayed colonized up to slaughter. Wieliczko (1995B) failed to isolate *Campylobacter* from 1-7 day-old chicks and the rate of isolation was 30.8 , 76.5 ,72.5 and 66.5% for broilers aged 14, 21, 21, 35 and 47 days respectively. The most prevalent strains were *C. jejuni* (51.4%), *C. coli* (21.9%) and *Campylobacter* did not colonized the intestinal contents in broilers before days 13-14 after hatching

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In apparent healthy chickens, *Campylobacter jejuni* was isolated from jejunum (3 isolates), followed by caecum (2 isolates) and liver tissues (1 isolate), while *Campylobacter coli* was recovered from caecum (2 isolates) followed by jejunum (1 isolate).

The presented results showed that it was found that jejunal and caecal contents followed by liver tissue, bile and finally heart blood were the sites of recovery in a descending manner. Also, *C. lari* was recovered from liver and jejunum only. Eighty-five out of 97 *Campylobacters* isolates were recovered from the intestinal samples collected from the examined chickens. This result almost agreed with Vaema *et al.* (2000) who succeeded to isolate *C. jejuni* from 25 cloacal samples of live poultry and from 15 intestinal swabs from dead birds. They added that 48% of cloacal samples and 33.4% of intestinal swabs were confirmed to contain *C. jejuni* .Recently,Burgess *et al.* (2005) concluded that, *C. jejuni* and *C. coli* accounted for 59% and 24% of the raw chickens contaminating microorganisms. Mean while, Stern and Robach (1995) stated that caecal droppings were the most suitable non destructive samples for assessing *Campylobacter species*, while on the other hand, Oyarzabal *et al.* (1995) succeeded to isolate *Campylobacter species* only from 18 out of 91 avian intestinal swabs. Generally, the mechanisms by which *C. jejuni* is introduced into the poultry house

remains unclear, although a variety of potential sources have been implicated, including water (Pearson *et al.* (1993), insects (Rosef and Kapperud, 1983), farm personal (Kazwala *et al.*, 1990) and rodents (Stern, 1992).

In this work an experimental design was planned to study the pathogenisity of *C. jejuni* and *C. coli*, in a single or mixed form, in the production of disease condition in chickens. Inoculation of *C. jejuni* in single form intramuscularly produced 30.0% mortalities compared with 20.0% mortalities when given orally. The mostly affected organs were the liver, caeca and jejunum with reisolation of the inoculated organism from the liver, caeca and jejunum.

On the other hand, *C. coli* produced 25.0% and 20.0% mortalities when inoculated intramuscularly and orally respectively. The principal changes were associated with *C.coli* infection was in the form of distension of the intestinal tract.

Intramuscular inoculation of *C. jejuni* and *C. coli* in a mixed form produced 35.0% mortality rate with severely detectable pathological changes in the liver, accumulation of mucus and watery fluid in duodenal loop and haemorrhages in the intestinal tract. The mortality rate lowered to be 25.0% accompanied with severe dehydration and blood tinged duodenal mucosa when the two organisms were inoculated orally.

Our results agreed with different reports, Ruiz-palacois *et al.* (1981) who performed experimental infection with $9x10^7$ CFU of *C. jejuni* orally and the recorded mortality was 32%. Clarck and Bueschens (1988) mentioned that the pathological lesion of chicks infected with *C. jejuni* included distended intestinal tract and abnormal gross liver pathology. Berndtson *et al.* (1996) concluded that the colonization of poultry by *C. jejuni* induces a specific secretory IgA response which appears to play an important role in colonization reduction. On the other hand, Young *et al.* (1999) concluded that different *C. jejuni* isolates vary in both their ability to colonize the caeca and their ability to invade the liver.

For conclusion, this study provided that *Campylobacter species* are widely spread in local breed broiler farms with a mean incidence of 32.3 % which ranged from 35.2 % in diseased and 18 % in apparent healthy chickens. These microorganisms mainly colonize the jejunum (mainly *C. jejuni*) and caecum (mainly *C. coli*) and also, can colonize the liver tissues. Regarding the biochemical identification of *Campylobacter species*, there were some differences within the same

species in its biochemical activities. Experimental infection with more than one type of *Campylobacter species* increased the mortality rates and severity of post mortem findings than those obtained when each organism was inoculated singly

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