BS. VET. MED. J. 6TH SCI. CONF. VOL. 20, NO.1, P.253-259

Beni-Suef
Veterinary Medical
Journal

Campylobacter infection in broiler breeder flocks in El-Minia governorate

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Existence of Campylobacter species that colonize broiler chickens intestinal tract raised and slaughtered in El-Minia Governorate as a source of animal protein origin for human consumption were studied. Samples were collected from 381 broiler chickens from different private farms distributed in six cities related to El-Minia Governorate and examined for the prevalence of Campylobacter spp. Percentage of *Campylobacter jejuni* isolation was 19.5% , 14.8% , 18.3% , 14.7% , 12.3% and 12.1% from Maggagha, Beni-Mazar, Samalout, Abou-Curcas, Mallawey and Deer-Mouase, respectively. While *Campylobacter coli* was 4.8% , 6.2% , 4.3% , 6.7% , 3.5 % and 0.0% respectively . Higher rate of isolation of *Campylobacter jejuni* 18.0% and *Campylobacter coli* 5.1% were obtained from 216 freshly dead broiler chickens carcasses than 165 diseased and slaughter birds which revealed 13.3% and 4.2% , respectively . Overall , Campylobacter spp. showed a higher tissue affinity for caecum (11.3%) Campylobacter jejuni and (2.6%) Campylobacter coli than for jejunum (4.2%) *C. jejuni* and (1.6%) *C. coli* and liver (1.6%) *C.jejuni* and (1.6%) *C. coli.* Susceptibilities of the recovered *Campylobacter jejuni* and *C.coli,* isolates to fifteen antibiotic discs clarified that gentamycin, neomycin, chloramphenicol and nalidixic acid in addition most of the strain were resistant to penicillin , ampicillin, tetracycline , sulfa methoxazole , trimethoprim, cephalothin and enerofloxacin . Public health hazard of enteropathogenic campylobacter was discussed and suggestive measures for reduction of campylobacter in broiler chicken farms were explained.

The genus campylobacter derived its name from the Greek word for "Curved rod" due to the curved, spiral or S-shaped morphology of these bacteria (Jordan *et al.,* 2001). Campylobacter is Gram negative slender, curved, motile rod and non- haemolytic on blood media. It is a micro-aerophilic organism, which means it has a requirement for reduced level of oxygen. (Requires 5% oxygen, 10% carbon dioxide and 85% nitrogen) for optimal growth. It is relatively fragile and sensitive to environmental stresses e.g 21% O_2, heating, drying, disinfectants and acidic conditions (Baron *et al.,* 1994).

Campylobacteriosis is a contagious disease infected poultry characterized by low mortality and high morbidity with a chronic course (Peckham, 1984). Poultry serve as primary reservoir hosts of thermophilic Campylobacters. Up to 90% of broilers may be infected, while 100% of turkeys and 88% of domestic ducks may harbour the organisms (Calnek *et al.,* 1997). Other than domestic birds, 36% of Japanese quail (Ibrahim *et al.,* 2005) and 78% of young ostriches up to 8 weeks were infected (Stephens *et al.,* 1998). Large numbers of Campylobacter's can be present as commonsal in the avian intestinal tract without any apparent gross lesions where the prevalence of infection in broiler breeder flocks has been found to be as hight as 80% (Evans, 1992).

Campylobacter has been associated with gastro-enteritis in human of all ages (Skirrow, 1977). Campylobacter enteritis is caused by the two closely related species, *Campylobacter jejuni* and *Campylobacter coli* (Skirrow, 1991). *Campylobacter jejuni* is a common and important cause of bacterial diarrhea in humans; equaling or exceeding salmonella and shigella spp.; In prevalence ; While, *Campylobacter coli* is less frequent than *Campylobacter jejuni* as a cause of diarrheic disease in humans (Acha and Szyfers, 1991).

Birds have been implicated as a source for these infections in human beings (Grant *et al.,* 1980; Stern *et al.,* 1985). Infection can be transmitted from chicken carcases and other products either transferred from hands to mouth by inexperienced food handlers. Consumption of raw or under cooked poultry meat or other innocent foods which may become cross contaminated from the raw products by means of hands and utensils, propably considered as the most frequent mode of infection (Kapperud *et al.,* 1992).

The pathogenic mechanisms by which Campylobacter cause diarrhea in humans seem

to be adhesion of the mucous membrane, toxin production and/or invasion of the epithelial cells, since the clinical effect varies between watery diarrhea and bloody diarrhea (Lindblom and Kaijser, 1995). The clinical manifestation of Campylobacter enteritis in human is an acute diarrhoeal illness often with acute abdominal cramping and fever. Other symptoms often present are nausea, headache and muscle pain. The illness usually occurs 2-5 days after ingestion of contaminated food (Blaser, 1997).

Recently the use of antibiotics in feed animal additives particularly in poultry farms has become of great concern for public health, because this practice may promote the emergence of multi- antibiotic resistant mutant of campylobacter species that can be transmitted to humans which are resistant to related human antimicrobial agents leading to difficulties in successfully treatment (Shakespeare, 2002).

The present study was planned to investigate the prevalence of enteropathogenic Campylobacter species in broiler chicken farms in different cities in El-Minia Governorate. In addition, bacteriological identification of isolated campylobacter species. Public health hazards of isolated antibiotic resistant campylobacter species, as well as antibiotic sensitivity patterns were intended to be estimated in vitro.

Materials and methods

Sampling. A total of 381 samples from different broiler chicken private farms located in different cities in El-Minia Governorate were employed during the winter 2008-2009. These were consisted of 216 freshly dead and 165 diseased and slaughtered broiler chickens aged 4-6 weeks. Samples were collected from jejunum, 2 caeci and liver separately from examined birds. Samples were directly transported to the laboratory in ice container within few hours for bacteriological procedures.

Preparation of samples. Small pieces from liver were crushed with one ml of sterile normal saline then thoroughly mixed in a sterile mortar. Also, one gram of faecal and gut fluid or mucous from caecal and intestinal contents were dissolved in one ml sterile normal saline. Centrifugation of the mixture at 1000 r.p.m for 5 min. was applied.

Enrichment procedure. Few drops of the supernatant were aspirated by sterile Pasteur pipette and cultured in semisolid thioglycolate broth in a small screw capped tube and incubated aerobically at 25ºC - 37 ºC and 42ºC for 48 – 72 h.

Isolation technique. Two ml of the positive samples showing characteristic corkscrew motion under phase contrast microscope were aspirated by sterile Pasteur pipette and streaked onto the surface of Blaser's medium (Campy – BAP) containing brucella agar base, 10% defibrinated ovine blood and campylobacter selective supplement (skirrow, SR 69E, Oxoid) contained vancomycin 5mg, polymixin – B 1250 I U and trimethoprim 2.5 mg, was added as one vial for 500 ml prepared medium. All inoculated plates were incubated for 48 – 72 h at 25ºC , 37ºC and 42ºC in gas pack system under microaerophilic conditions using campylobacter gas generating kits (Oxoid , BR38) producing 5% Oxygen , 10% CO2 and 85% nitrogen.

Morphological and biochemical Identification. The suspected colonies were stained by modified Gram stain (with 0.8% solution of basic carbol fuchsin instead of safranine) to detect the morphology, and then examined under the phase contrast microscope to detect the darting motility. Suspected colonies were also subjected to biochemically identified according to (Holt *et al.,* 1994).

Sodium Hippurate hydrolysis test. Colonies from Muller Hinton blood agar culture was inoculated into 0.5 ml of sterile sodium hippurate solution in a small screw capped tube. Tubes were incubated including an un-inoculated control, aerobically at 37ºC for 3 h according to (Lior, 1984). Over layed with 0.2 ml of nine, hydrin solution without mixing. Development of a deep purple colour within 5 min. indicates a positive result while development of a slight bluish colour regarded as negative results.

In-vitro anti- biogram sensitivity testing. The technique was carried out using the disc diffusion method according to Bopp et al., (1985). Campylobacter isolates (15 from each) were tested for resistance using gradient disk diffusion MIC to : naldixic acid (30 µg), cephalothin (30 µg) penicillin (10 IU), ampicillin (10 µg), streptomycin (10 µg), neomycin (30 µg), gentamycin (10 µg) erythromycin (15 µg), chloramphenicol (30 µg), nitrofuranation (300 µg), tetracycline (30 µg), oxytetracycline (30 µg), trimethoprim (1.25 µg) sulfamethoxazole (25 µg), enerofloxacin (10 µg). A swab of campylobacter isolates were inoculated into screw capped tubes containing 5 mL Muller Hinton broth (Dfco) then incubated for overnight at 37ºC. The turbidity was adjusted to match that of the standard Mcfarland 0.5 barium sulfate tube by adding sterile saline solution. From suspension few drops were evenly spread on 15

ml Muller–Hinton agar plates supplemented with 5% defibrinated ovine blood, Inoculated plate was left for approximately half an hour and immediately after plate become dried , antibiotic discs were placed on the surface of agar plate by using discs dispenser (Oxoid). The plates were incubated for 72 hours at 37ºC under the micro-aerophilic conditions and the diameter of the inhibitory zone for each disc was scored as described in the Oxoid manual.

Results

In this study, usually diseased chickens were showing clinical signs of depression, diarrhea and pasty vent plumage. Gross lesions revealed the distension from the jujunal region to the two caeci with accumulation of mucus and watery fluid. Haemorrhages were present in some cases. Incidence of *Campylobacter jejuni* and Campylobacter coli isolated from examined broiler chicken farms at different cities in El-Minia Governorate were illustrated in (Table 1). Detection of *Campylobacter jejuni* and Campylobacter coli from recently dead and diseased and slaughter broiler chickens were recorded in (Table 2). Existence of *Campylobacter jejuni* and Campylobacter coli recovered from different sites of the examined freshly dead and diseased slaughter broiler chickens were showed in (Table 3). Occurrence of Campylobacter species recovered from different sites of the examined broiler chickens were explained in (Table 4). Results of anti-microbial sensitivity tests of campylobacter species isolated from broiler chickens in vitro were represented in (Table 5).

Discussion

Campylobacteriosis is acute or chronic infection of humans caused food borne infection particularly in food of poultry origin. The infection in man manifested by acute enteritis, abdominal pain and diarrhea in most cases stool contain blood, pus or mucous, fever up to 40ºC and sometimes vomiting. Abdominal signs may lead to laparotomy or appendectomy (Acha and Szyfers, 1991; Shakespeare, 2002; Hartmut *et al.,* 2003). However, extra intestinal disease, including meningitis, endocarditis, septic arthritis and proctocolitis, is being increasingly recognized during the past few years, particularly in patients with acquired immuno deficiency syndrome (AIDS).(Koneman *et al.,* 1992; Baron *et al.,* 1994).

Campylobacters are fragile, fastidious and slow growing micro-organisms (Jordan *et al.,* 2001). The recovery of campylobacter microorganisms is greatly influenced by oxygen content of the gaseous atmosphere in contact with solid isolated media. It is sensitive to air, surviving only one to two days on solid enrichment isolation media , two to four days in liquid media, and ten to twenty days in semi-solid media at 25ºC and survival can be enhanced by holding cultures at 4ºC and by reducing oxygen tension (Park *et al.,* 1984). These fact acts as the main failure that encountered with the laboratory technician that working in the identification of the isolated campylobacter microorganisms. Appropriate methods of transport and storage of campylobacters are necessary because these organisms are sensitive to desiccation. An enriched semi-solid brucella medium incorporating 10% ovine blood can be used to maintain viability of cultures for transport at 25ºC for up to 3 weeks (Wang *et al.,* 1980).

It is clear that campylobacter micro-organisms are widely spread in broiler farms as mentioned by both (Jacobs-Reitsma *et al.,* 1994; and Wieliczko, 1995). The chickens can be infected by campylobacter micro-organisms at 4-6 weeks of age. It is obvious from this study that campylobacter micro-organisms are widely infected in broiler farms distributed in different cities in El-Minia Governorate. It is clear from data illustrated in (Table 1) that the high rate of campylobacter species isolation showed, 23.8% , 23.5% and 22.6% from Maggagha, Beni-Mazar and Samalout; respectively. On the other hand, low rate of detection, 21.3 %, 15.8% and 12.1% from Abou-Curcas, Mallawey and Deer-Mouase, respectively. These findings mainly attributed to that El-Minia is a longitudinal Governorate. However, the distance between the east and west deserts become narrow toward the south where the weather is greatly differed from the north to the south.

The detection of *Campylobacter jejuni* and Campylobacter coli from recently dead and diseased slaughter broiler chickens (Table 2). Campylobacter species was mainly isolated from recently dead broiler chickens, 50 isolates with an incidence of 23.1% were recovered from 216

Table (1): Incidence of *Campylobacter jejuni* and *Campylobacter coli* isolated from broiler chicken farms at different cities in El-Minia Governorate.

Name of city	No. of samples	*C. jejuni*		*C. coli*		Total isolates	
		No.	%	No.	%	No.	%
Maggagha	42	8	19.0	2	4.8	10	23.8
Beni- Mazar	81	14	17.3	5	6.2	19	23.5
Samalout	93	17	18.3	4	4.3	21	22.6
Abou- curcas	75	11	14.7	5	6.7	16	21.3
Mallawey	57	7	12.3	2	3.5	9	15.8
Deer- Mouase	33	4	12.1	0	0.0	4	12.1
Total	381	61	16.0	18	4.7	79	20.7

Table (2): Detection of *Campylobacter jejuni* and *Campylobacter coli* from freshly dead and diseased slaughter broiler chickens.

Source of samples	No. of samples	*C. jejuni*		*C. coli*		Total isolates	
		No.	%	No.	%	No.	%
Freshly dead	216	39	18.0	11	5.1	50	23.1
Diseased and slaughter	165	22	13.3	7	4.2	29	17.6
Total	381	61	16.0	18	4.7	79	20.7

Table (3): Existence of Campylobacter jejuni and Campylobacter coli recovered from different sites of the examined freshly dead and diseased slaughter broiler chickens.

Source of samples	No. of samples	Recovery sites	*C. jejuni*		*C. coli*		Total isolates	
			No.	%	No.	%	No.	%
		Jejunum	9	4.2	4	1.9	13	6.0
Dead	216	Cecal contents	28	13.0	6	2.8	32	14.8
		Liver	2	0.9	1	0.5	3	1.4
		Jejunum	7	4.2	2	1.2	9	5.5
Diseased	165	Cecal contents	15	9.1	4	2.4	21	12.7
		Liver	0	0.0	1	0.6	1	0.6
Total	381		61	16.0	18	4.7	79	20.7

Table (4): Occurrence of Campylobacter jejuni and Campylobacter coli recovered from different sites of the examined broiler chickens.

Recovery sites	Total isolates		*C. jejuni*		*C. coli*	
	No.	%	No.	%	No.	%
Jejunum	22	5.8	16	4.2	6	1.6
Cecal contents	53	13.9	22	11.3	10	2.6
Liver	4	1.0	2	0.5	2	0.5
Total	79	20.7	61	16.0	18	4.7

Table (5): Anti-microbial sensitivity tests of *Campylobacter jejuni* and *Campylobacter coli* isolated from broiler chickens in vitro.

Antimicrobial disc	Disc potency	*C. jejuni*	*C. coli*
Naldixic acid	30 µg	S	S
Neomycin	30 µg	S	S
Gentamycin	10 µg	S	S
Chloramphenicol	30 µg	S	S
Enerofloxacin	10 µg	R	R
Oxytetracyclin	30 µg	M	M
Erythromycin	15 µg	M	R
Nitrofuranation	300 µg	M	R
Streptomycin	10 µg	M	R
Sulfamethoxazole	25 µg	R	R
Trimethoprim	1.25 µg	R	R
Penicillin	10 I U	R	R
Ampicillin	10 µg	R	R
Tetracycline	30 µg	R	R
Cephalothin	30 µg	R	R

S=Sensitive (+ + +) M= moderate (+ +) R = Resistant (-)

In vitro drug sensitivity test proved that all tested isolates were sensitive to Naldixic acid , Neomycin , Gentamycin and

chicken carcases. While, the recovery rate from 165 diseased slaughter broiler chickens was 17.6% (29 isolates). Campylobacter infection consider as one of the most important agent that responsible for increase in daily mortality rate in broiler chicken farms. Mortality of 32% in infected chicks by *Campylobacter jejuni* was recorded by (Ruiz-palacios *et al.,* 1981).

The existence of Campylobacter jejuni and Campylobacter coli recovered from different sites of the examined recently dead and diseased slaughter broiler chickens (Table 3). Campylobacter jejuni has been recovered from jejunum, caecal contents and liver with a rate of 4.2%, 13.0% and 0.9%, respectively in recently dead broiler chickens. While, it was recovered from jejunum, caecal contents and liver with a rate of 4.2%, 9.1% and 0.0%, respectively in diseased slaughter broiler chickens.

Campylobacter coli was isolated from recently dead broiler chickens with a rate of 1.9%, 2.8% and 0.5% from jejunum, caecal contents and liver, respectively. While, it was revealed from diseased slaughter broiler chickens with a rate of 1.2%, 2.4% and 0.6% from jejunum , caecal contents and liver, respectively . The high isolation rate of campylobacter species from caecal contents become in a greement with (Sayed and Mohamed, 2004; Fadel and Hamed, 2006). Generally, the principle niche for colonization in the bird is the caecum and caecal contents are the diagnostic sample of choice (Jordan *et al.,* 2001). On the other hand, high isolation rate of campylobacter species from jejunum is recorded by (El-Seedy *et al.,* 2008). The occurrence of *Campylobacter jejuni* and *Campylobacter coli* recovered from different sites of the examined broiler chickens. Table 4 showed that *Campylobacter jejuni* was recovered from broiler carcases with a rate of (16.0%). Several authors comes in agreement with this result, Fernandez and Torres, (2000), (19.7%), Sayed, (2000), (19.2%) and Sayed and Mohamed, (2004), (16.7%)). High prevalence rates of *Campylobacter jejuni* were detected in broilers, (43.0%), (53.7%), (39.6%), (73.2%) and (52.6%) reported by, Atanassova and Ring, (1999); Chou and Tsai, (2001); Wedderkopp *et al.* (2001); Saleha, (2002); El-Seedy *et al.* (2008), respectively. *Campylobacter coli* was isolated also from broiler carcases with a rate of (4.7%).Several authors come in agreement with the present result, (Fernandez and Torres, (2000), (6%) and Wedderkopp *et al* . (2001) , (5%)) . On the other hand, high rates of detection (28.3%), (13%), (26.8%) and (42.3%) were estimated by (Saleha *et al.,* 1996; Atanassova and Ring, 1999; Saleha, 2002; El-Seedy *et al.,* 2008).

The obtained results in Table (5) demonstrated the most effective antibiotics for all isolates (15 from each) in vitro were Gentamycin, Neomycin, chloramphenicol and Naldixic acid. Some similarities with these results as reported by Morris and Patton, (1985); Moffok and Leberes, (1992); Rabie, (1992); Das et al., (1996); Abd EL-Moneim, (1998); Fadel and Hamed, (2006). On the other hand, most isolates of campylobacter species were resistant to penicillin, ampicillin, tetracycline, sulfamethoxazole, trimethoprim, cephalothin and enerofloxacin while oxytetracycline revealed moderate effective for most campylobacter isolates. These results are agreed with those reported by Erdger and Diker, (1995); Sayed, (2000). While disagreed with those, reported by (Salem *et al.,* 1986; Rabie, 1992; Sayed and Mohamed, 2004) as they mentioned that oxytetracycline and tetracycline were highly sensitive.

The high antibiotic resistance rates detected could be due to the wide spread use of antibiotics in broiler chickens, particularly in feed as feed additives as well as due to being use indiscriminately, (Saleh, 2002). In addition, Campylobacter is one of the micro-organisms of moderate intrinsic susceptibility which require only one mutation to become resistant (Wiedemann and Heisig, 1994). Also, resistance to Fluoroquinolones as enerofloxacin appears to develop very rapidly and the incidence of resistant isolates has increased in humans in recent years which have coincided with the approval of these drugs for veterinary use. Consequently the use of these antibiotics in animal production systems may have serious implications for the treatment of human infections (Jordan *et al.,* 2001).

As shown in Table (5) *Campylobacter coli* can be differentiated from Campylobacter jejuni by the hippurate hydrolysis test since, *Campylobacter jejuni* are the only Campylobacters that hydrolyze hippurate (Koneman *et al.,* 1992).

In conclusion, Campylobacter infection in broiler chicken farms as general causing enteritis manifested by diarrhea, hence during missfield diagnosis may be passed as one of common enteric diseases like, Colibacillosis,

Salmonellosis, Coccidiosis and/or clostridial infection and so miss-treatment failure occurs. Also, the high isolation rate of campylobacter spp. in broilers showing chronic respiratory disease (CRD) as a concurrent infection may be attributed to the associated decreased immunity (Fedel and Hamed, 2006). For these reasons, Campylobacteriosis should be correctly diagnosed by laboratory investigation as one of the most important avian pathogens for its public health hazard. Moreover, infected broiler chicken by campylobacter species act as a main reservoir of infection to other domestic farm animals and human beings.

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إصابة قطعان بداري التسمين بالكامبيلوباكتر في محافظة المنيا

أجريت هذه الدراسة لمعرفة مدى تواجد ميكروب الكامبيلوباكتر المعوي في بداري التسمين التي تربى وتذبح للاستهلاك الآدمي في محافظة المنيا . ثم جمع عدد ٣٨١ عينة من كتاكيت بداري التسمين عبارة عن ٢١٦ طائر نافق حديثاً و ١٦٥ طائر مريض ثم ذبحها تتراوح أعمارهم من ٤ إلى ٦ أسابيع من مزارع تربية أهالي منتشرة في ٦ مدن تابعة لمحافظة المنيا وأخضعت العينات للفحص البكتريولوجى بغرض عزل وتضيف ميكروب الكامبيلوباكتر وكانت نسبة عزل ميكروب الكامبيلوباكتر جوجيناى ١٩% ، ١٤,٨% ، ١٨,٣% ، ١٤,٧% ، ١٢,٣% ، ٢١,١% من مركز مغاغة وبني مزار وسمالوط وأبوقرقاص وملوي وديرمواس على التوالي . بينما كانت نسبة عزل ميكروب الكامبيلوباكتر كولاى ٤,٨% ، ٦,٢% ، ٤,٣% ، ٦,٧% ، ٣,٥% ، صفر% على التوالي. وكانت نسبة عزل ميكروب الكامبيلوباكتر جوجيناى والكامبيلوباكتر كولاى هي ١٨% ، ٥,١% على التوالي في البدارى النافقة حديثاً . بينما كانت ١٣,٣% ، ٤,٢% على التوالي في الكتاكيت المريضة والمذبوحة . وتمثل عينات الأعوريين أعلى معدل للعزل ١١,٣% للكامبيلوباكتر جوجيناى ، ٢,٦% الكامبيلوباكتر كولاى بالمقارنة بالجزء الأوسط من الأمعاء (الصائم) ٤,٢% ، ١,٦% وأيضاً الكبد ١,٦ لكل من النوعين من معزولات الكامبيلوباكتر على التوالي . وتم أيضاً دراسة مدى حساسية المعزولات لميكروب الكامبيلوباكتر جوجيناى والكامبيلوباكتر كولاى لعدد ١٥ نوع من المضادات البكتيرية المختلفة المستخدمة في مزارع التربية وتبين أن أعلى حساسية كانت للجنيتاميسين والنيوميسين والكلورامفينكول و حمض النالدكسك في معظم المعزولات وعلى العكس من ذلك كانت معظم المعزولات مقاومة لكل من البنسلين والامبسلين والتتراسيكلين والسلفاميثوكسازول والسلفاترا ميثوبريم والسيفالوسين والانروفلوكساسين . ونوقشت الأهمية الصحية والوبائية لميكروب الكامبيلوباكتر المعزول من بداري التسمين وكذلك الإجراءات الوقائية لحماية صحة الإنسان من الإصابة.