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CAMPYLOBACTER INFECTION IN BROILER CHICKENS IN ASSIUT

(With 4 Tables and 1 Figure)

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اصابات بدارى الطيور بالكامبيلوباكتر في أسيوط

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تم فحص ٤ مزارع للبداري في منطقة أسيوط وذلك لوجود ميكروب الكامبيلوباكتر. ٢٥٤ عينة تم فحصها منها ١٩٠ عينة من الاحشاء الداخلية و ٦٤ عينة من الطيور الحية وذلك من شهر نوفمبر ١٩٩٨ إلى أبريل ١٩٩٩. تم عزل ميكروب الكامبيلوباكتر في ٤٤ عينة بنسبة ٢٣,١ % من الأحشاء الداخلية و ١٢ عينة بنسبة ١٨,٧% من الطيور الحية. وقد كان معدل عزل ميكروب الكامبيلوباكتر جوجوناى في الأحشاء الداخلية ٢٠,٥% وفي العينات الحية ١٥,٦% أما ميكروب الكامبيلوباكتر كولاى فقد تم عزله بنسبة ٢,٦% في الاحشاء الداخلية و ٣,١% من العينات الحية وقد كانت نسبة ميكروب الكامبيلوباكتر جوجوناى في كل العينات هي ١٩,٢% وميكروب الكامبيلوباكتر كولاى هي ٢,٧%. ولقد تم عمل تصنيف بيوكيميائي لميكروب الكامبيلوباكتر جوجوناى وقد بين ذلك التصنيف ان ٢٨ عينة تقع تحت التصنيف I و٢١ عينة تحت التصنيف II . وتم عمل اختبار للمضادات الحيوية ضد ميكروب الكامبيلوباكتر سواء الجوجوناى أو الكولاى وكانت النتيجة كالتالي: حساسية جميع العترات المعزولة الى النالاديكسيك أسيد وجنتاميسين والاريثروميسين وكانت مقاومة للمضادات التالية: التتراسيكلين و الترايمثوبريم والبنسلين والامبيسلين.

SUMMARY

Four broiler farms at Assiut Province were examined for the presence of campylobacter spp. 254 cases (190 visceral samples and 64 cecal swabs) were examined between November 1998 and April 1999. *Campylobacter* spp. were isolated in 44 (23.1%) of the visceral samples and 12 (18.7%) of cecal swabs. The frequency of *C.jejuni* isolation was 20.5 from visceral samples and 15.6% from cecal swabs. Campylobacter coli were

isolated from 2.6% of Visceral samples and 3.1% of cecal swabs. A total percent of C. jejuni in all cases were 19.2% and in C. coli were 2.7%. Biotyping of C.jejuni revealed that 28 (11%) were biotype I and 21 (8.2%) biotype II. All campylobacter isolates (*C.jejuni* and *C. coli*) were sensitive to Naladixic acid, Gentamycin, Erythromycin and resistant to Tetracycline, Trimethoprime, Penicillin and Ampicillin.

Key words: Campylobacter infection in broiler chickens

INTRODUCTION

Campylobacter is one of the most important foodborne microorganisms leading to acute gastroenteritis in humans. These human cases are most likely associated with handling or consumption of under cooked poultry meat products, Oosterom et al. (1984); Harris et al. (1986); Doyle, (1990) and Bolder & Mulder (1991). Horizontal transmission is the primary route of infection in domestic animals particularly poultry, Genigeorgis et al. (1986) and Beard (1993). Vertical transmission is not a natural route of campylobacter infection, Shanker et al., (1986); Rollins, (1991) and Shane (1991). Campylobacter in the broiler chicks could not be demonstrated during the first 2 weeks after hatching. Engvall et al. (1986); Wieliczko (1995) and Jacobs-Reitsma et al. (1995). Poultry serve as primary reservoir hosts of Campylobacter, Genigeorgis (1986) demonstrated that *Campylobacter jejuni* is disseminated from live birds to dressed carcasses and poultry parts during processing. Jacobs-Reitsma et al. (1995) recorded that the flocks became colonized with campylobacter at about 3-4 weeks of age with isolation percentages of 100% and stayed colonized up to slaughter. Wieliczko (1995) failed to isolate campylobacter from 1-7 day-old chicks. The rate of infection was 30.8, 76.5, 72.5 and 66.5 for broilers aged 14, 21, 35, 47 days respectively. The most prevalent strains were *C. jejuni* 1 (51.4), *C.jejuni biotype* 2 (21.3%) *C.coli* (21.9%). Campylobacter did not colonize the intestinal contents in broilers before days 13-14 after hatching. Zaki and Reda (1995) determined the incidence of *Campylobacter jejuni* infection obtained from layer, broiler farms and hatching eggs. The rate of infection in broiler farms reached 2.5%.

Erdger and Diker (1995) isolated 206 thermophilic campylobacter (137 *C. jejuni*, 69 *C. coli*) from the intestine or carcasses. Berndtson et

al., (1996) recorded that all campylobacter isolates belonged to the same sero and biotype *C. jejuni* Panner 2. The spread of campylobacter in the flock was rapid and usually all samples were positive once colonization had been proven. Uyttendaelf et al. (1996) isolated campylobacters from 92 of 162 (57%) samples which were identified as *Campylobacter jejuni* 49.37%, *C. coli* 3.75% , *C. laridis* 3.12% and unclassified 1.25%.

Carvalho et al. (1997) investigated the presence of campylobacter in viscera of chickens with diarrhoea. The frequency of *C. jejuni* isolation was 54.79% from liver samples 35.29% from spleen and 6.9% from bile secretion. Chuma et al (1997) collected samples from broiler flocks. 20% of flocks were positive for *C. jejuni* and 4.7% for *C. coli*.

The aim of the present study was to investigate the prevalence of campylobacter spp. isolates in affected broiler chickens. The strains were biotyped and also tested against several antibiotic discs to give a suitable treatment.

MATERIALS and METHODS

A total of 254 cases from four affected farms at Assiut Governorate were examined between November1998 and April 1999. All examined chickens were of 4-6 weeks of age. The samples were taken from dead birds (190 visceral samples) and live birds (64 cecal swabs).

Media and reagents:

1- Preston's campylobacter broth (Bolton and Robertson 1982)
   a) Brucella broth medium
   b) Preston's campylobacter selective supplement (Oxoid)
   c)Campylobacter growth supplement
   d) Horse blood lysed
2- Preston's campylobacter selective medium (Bolton and Roberston 1982)
3- Semisolid brucella medium (Smibert's medium for maintenance) (Park et al, 1984)
4- Triple sugar iron agar
5- Brucella blood agar medium (Park et al 1984)
6- Brucella FBP agar (Park et al 1984)
7- FBP broth (Skirrow et al 1982)
8- Semisolid brucella medium with potassium nitrate (Park et al 1984)

Reagents and indicators:

1) 3% hydrogen peroxide for catalase test

2) Sodium hipurate sol. and ninhydrin sol. for hippurate hydrolysis test.
3) Solution A and B for nitrate reduction test (Park et al., 1984)
4) Tetramethyl paraphenylenediamine- 2 Hcl for oxidase test.

Stains:

Gram's stain

Antibiotic sensitivity tests: (Fennel et al., 1984)

Drug sensitivity tests were performed on 56 strains of ***Campylobacter jejuni*** and ***C. coli*** with 12 types of antibiotics discs produced by Oxoid. Naladixic acid (30 µg), Gentamycin (10 µg), Erythromycin (15 µg), Nitrofuranation (300 µg), Chloramphenicol (30 µg), Streptomycin (10 µg), Tetracyclin (30 µg), Polymyxin B (300 units), Trimethoprim (1:25 µg), Penicillin (10 units), Enerofloxacin (10 µg) and Ampicillin (10 µg).

Isolation:

The sampling procedure consisted of collecting swabs of cecal content and mucosa, with systemic infection, the organism can also be recovered from liver tissues, bile and blood which were immersed in Preston's media (Boloton and Robertson 1982). Preston enrichment broth comprised brucella broth plus 5% lysed horse blood, Preston campylobacter selective and campylobacter growth supplement.

Inoculated broth was incubated under microaerophilic conditions by the use of anaerobic jars and Campy-pak gas-generating packets and incubated at 42°C for 48 hr. After incubation a loopful was subcultured on Preston agar (brucella agar base supplemented with Preston's selective agents). The plates were incubated under microaerophilic conditions. Colonies thought to be campylobacter were inoculated into semisolid brucella medium containing neutral red indicator (Park et al 1984) followed by aerobic incubation at 42°C for 24 h. The inocualted medium was maintained in refrigerator for futher identification and confirmation. Subcultures were done weekly, or when most of the medium turned yellow.

Identification of isolates:

Suspected colonies were identified on the basis of typical morphology of the colonies, motility under phase-contrast microscopy, oxidase, catalse production, susceptibility or resistance to nalidixic acid or cephalothin, hydrogen sulphide production by using (lead acetate strips, iron containing media, FBP medium) nitrate reduction growth at 25°C or 45°C and hippurate hydrolysis (Varnam and Evans 1991).

Biotyping:

According to the Skirrow biotyping for campylobacter (Skirrow and Benjamin, 1980).

- H_2S production in FBP medium
- Growth at 45.5°C
- Tolerance of TTC cxdsew32 (filter paper strip soaked in triphenyltetrazolium chloride and dried).

Sensitivity tests: (Fennel et al., 1984)

Discs were placed on brucella blood agar (without antibiotic supplement) previously streaked with a suspension of culture. The inoculated plates were incubated at 37°C for 48 hr under microaerophilic conditions.

RESULTS

- In our study usually diseased birds were depressed and diarrheatic.
- The gross lesions revealed that distension in the intestinal tract extending to the ceca, accumulation of mucus and watery fluid and haemorrhages present in some cases. Presence red or yellow motling of the parenchyma of liver, focal hepatic necrosis and subcapsular hemorrhages were present in some cases (Fig.1).
- Incidence of campylobacter species in examined farms were illustrated in Table 1.
- Types and percentages of campylobacter were explained in Table 2.
- Biotyping of ***Campylobacter jejuni*** were recorded in Table 3.
- Results of sensitivity tests for ***Campylobacter jejuni*** and ***coli*** against antimicrobial discs were presented in table 4.

Table 1: Incidence of Campylobacter species in examined farms

Farms	Visceral samples			Cecal swabs		
	No. of samples	+ve cases	%	No. of samples	+ve cases	%
Farm 1	38	9	23.6	9	2	22.2
Farm 2	45	12	26.6	15	3	20
Farm 3	52	10	19.2	23	5	21.7
Farm 4	55	13	23.6	17	2	11.7
Total	190	44	23.1	64	12	18.7

Table 2: Incidence of *Campylobacter jejuni* and *Campylobacter coli* in examined farms

Farms	*Campylobacter spp.*		*Campylobacter jejuni*			*Campylobacter coli*		
	V. Samples	C. Swab	V. Samples	C. Swab	Total number	V. Samples	C. Swab	Total number
Farm 1	9	2	8	2	10	1	-	1
Farm 2	12	3	10	2	12	2	1	3
Farm 3	10	5	10	4	14	-	1	1
Farm 4	13	2	11	2	13	2	-	2
Total	44	12	39	10	49	5	2	7
%	23.1%	18.7%	20.5%	15.6 %	19.2	2.6%	3.1%	2.7%

V = visceral C= Cecal

Table 3: Biotyping of *Campylobacter jejuni*

Farm	Total number	Biotype 1		Biotype 2	
	of *C. jejuni*	V. samples	C.swabs	V. samples	C. swabs
Farm 1	10	5	2	3	-
Farm 2	12	6	1	4	1
Farm 3	14	5	1	5	3
Farm 4	13	6	2	5	-
Total	49	22	6	17	4

Table 4: Sensitivity tests for *Campylobacter jejuni* and *Campylobacter coli.*

Types of discs	*Campylobacter jejuni*	*Campylobacter coli*
Naladixic acid	Sensitive	sensitive
Gentamycin	Sensitive	sensitive
Erythromycin	Sensitive	sensitive
Nitrofuranation	Moderate	moderate
Chloramphenicol	Moderate	resistant
Streptomycin	Moderate	moderate
Tetracyclin	Resistant	resistant
Polymyxin B	Moderate	resistant
Trimethoprim	Resistant	resistant
Penicillin	Resistant	resistant
Enerofloxacin	Sensitive	sensitive
Ampicillin	Resistant	resistant

DISCUSSION

It is clear from this study that campylobacter microorganisms are wide spread in broiler farms. As reported by both wieliczko (1995) and Jacobs-Ristima et al. (1995), the chicken can be infected by campylobacter microorganisms at 4-6 weeks of age.

The rate of infection with campylobacter spp. in visceral organs and cloacal swabs reached 23.1%, 18.7% respectively, nearly the same rate was reported by Brendtson et al. (1996) and Omar et al. (1995) isolated Campylobacter spp. at rate of 19.7% from intestinal swabs.

The types of Campylobacter isolates were differentiated into *Campylobacter jejuni* and *Campylobacter coli.*

The frequency of these isolates reached to 19.2% for *C.jejuni* and 2.7% for *C. coli.* Consistent with several other reports Wieliczko (1995); Uyttendaelf et al. (1996) and Chuma et al. (1997), our results support the conclusion that the most prevalent strains found were *Campylobacter jejuni.*

Campylobacter jejuni biotype I was more prevalent than biotype II, this result is similar as recorded by Wieliczko (1995).

The most effective antibiotics for all isolates were Naladixic acid, Gentamycin and Erythromycin, some similarities with this result as reported by Das et al. (1996) which recorded that all isolates were sensitive to Nalidixic, Gentamycin, Erythromycin and Nitrofurnation. The isolates of Campylobacter spp. were resistant to Tetracycline, Trimethoprim, Penicillin and Ampicillin as reported by Erdger and Diker (1995) called that the isolates of *C.jejuni* and *C.coli* were resistant with variaties to Penicillin, Ampicillin and Tetracycline.

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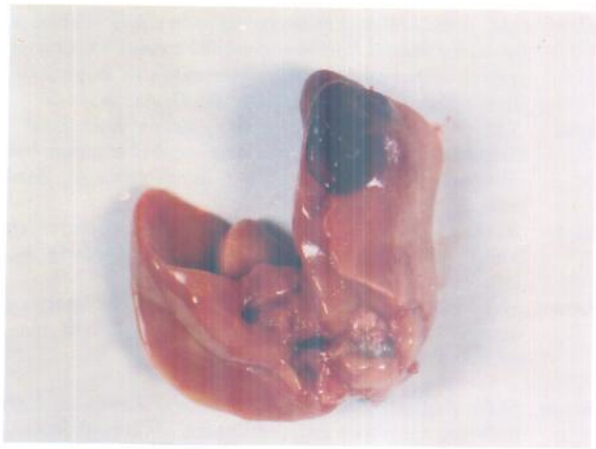

Fig (1)