Animal Health Research Institute,
Assiut Laboratory

SOME STUDIES ON CAMPYLOBACTER INFECTIONS IN PIGEONS IN ASSIUT GOVERNORATE

(With 5 Tables and 3 Figures)

By
FATMA A. MOUSTAFA

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بعض الدراسات عن العدوى بالكامبيلوباكتر فى الحمام بمحافظة أسيوط

فاطمة عبد المجيد مصطفى

أجريت هذه الدراسة على عدد ١٢٠ عينة عشوائية من براز الحمام، عينات الكبد ومسحات الأمعاء بواقع ٤٠ عينة لكل منهم. وقد أسفرت النتائج عن عزل ١٩ عترة من ميكروبات الكامبيلوباكتر بنسبة ١٥,٨% وصنفت العترات إلى كامبيلوباكتر جوجيناى بنسبة ١٤,١% وكامبيلوباكتر كولاى بنسبة ١,٦%. وعند دراسة مدى حساسية الميكروبات المعزولة للمضادات الحيوية بالإضافة إلى دراسة البلازميد بروفيل وجد أن كل من الجنتاميسين والأريثروميسين والنورفلوكساسين كانت أكثرها فاعلية ولذا ينصح باستخدامها وبإجراء أختبار العدوى الصناعية لبيان مدى ضراوة عترة الكامبيلوباكتر جوجيناى فى كتاكيت عمر يوم تبين أنها شديدة الضراوة حيث تم عزلها بنسبة ١٠٠% من الكتاكيت النافقة، أما عند أختبار هذه العترة فى الحمام لوحظ عدم وجود نافق بين الطيور المحقونة ولكن كانت هناك أعراض اكلينيكية واضحة مثل الخمول، وجود اسهال أخضر مائى شديد وانخفاض ملحوظ فى النمو والوزن بالمقارنة بالحمام الغير مصاب. وأهم الافات التشريحية هو احتقان عام فى الأعضاء الداخلية والتهابات دموية شديدة بالأمعاء وتضخم بالكبد والبنكرياس. وقد تم عزل الميكروب بنسبة ٥٠% ، ٧٥% و٨٥% تبعاً لطريقة الحقن.

SUMMARY

One hundred and twenty random samples from cloacal swabs, intestinal content and liver (40 each) were collected from different districts in Assiut Province at the period from November 2003 to May 2004. These samples were examined to determine the incidence of campylobacter species. The obtained results indicated that 19 strains of campylobacter species were isolated with a rate of 15.8%. All isolates were identified as C. jejuni 17 isolates (14.1%) and C. coli 2 isolates (1.6%). Antibiotic

pattern were determined for all isolates as well as the plasmid profile was performed to correlate between antibiotic resistance and plasmid carriage among these isolates. The results obtained revealed that all campy lobacter isolates were sensitive to Gentamycin, Erythromycin and Norofloxacin. The pathogenicity test of C. jejuni was subjected in 45 day-old pigeons and in one day old chicks using different routs of infection. In pigeons the results revealed that there is no mortality among the examined pigeons. The clinical signs in experimentally infected squabs were depression, greenish diarrhae and reduction in body weight gain as compared with control group. The main lesions were congestion of internal organs and sever haemorrhagic enteritis with isolation rates of 50%, 75% and 85% by I/M, S/C and orally inoculation, respectively. C. jejuni were pathogenic to the experimentally infected baby chicks resulting mortality rates of 40, 20 and 10% by the same routs. The isolation rate from dead chicks reached 100%.

Key Words: *Enteropathogenic Campylobacter, pigeons, antibiotic resistance, plasmid profile.*

INTRODUCTION

Campylobacter infections has been implicated as a contagious disease of chickens characterized by low mortality, high morbidity and significant reduction in egg production and body weight. Moreover, campylobactor was considered one of the most important food born microorganisms which cause acute gastroenteritis in poor countries, the number of human cases of campylobacteriosis has increased in the last few years in many countries (Nielsen *et al.*, 2000 and Ring and Atanassova, 2001). These human cases are most likely associated with handling or consumption of undercooked poultry meat products (Doyle, 1990 and Bolder and Mulder, 1991).

Poultry serve as primary reservoir hosts of campylobacter, it causes colonization at the intestinal mucosa in which the flocks became colonized with campylobacter at about 3-4 weeks of age with isolation percentages of 100% and stayed colonized up to slaughter (Jacobs-Reitsma 1997). Also, it cause necrotic liver lesion which observed from day 1 to 7 after the infection (Misawa *et al.*, 1996).

The most important Campylobacters species are Campylobacter jejuni, C. coli, C. lari and C. upsaliensis. Allos and Blaser (1995) reported that C. jejuni and C. coli are responsible for campylobacter

enteritis in industrial countries. The spread of campylobacter in the flock was rapid and usually all samples were positive once colonization has been proven, however, campylobacter did not colonize the intestinal contents in broilers before days 13-14 after hatching. Jacobs-Reitsma (1997) recorded that the flocks become colonized with campylobacter at 3-4 weeks of age with isolation percentage of 100%. Also, Wieliczko (1995) failed to isolate campylobacter from 1-7 days old chicks, the rate of infection was 30.8, 76.5, 72.5 and 66.5% for broilers aged 14, 21, 35 and 47 days, respectively.

In pigeon Campylobacteria were isolated from three pigeons out of 71 pigeons from 129 birds in Norway by Rosef (1981), all the strains isolated had the biochemical characteristics of C. jejuni biotype. The same strain was isolated by Luechtefeld *et al.* (1981) from 8% of 75 wild pigeons trapped on the Denver Zoo during winter months and from 26 of 75 traped during March and April. Megraud (1987) examined 200 pigeon feces samples and could isolate C. jejuni from 106 samples. In Japan, C. jejuni was isolated from 44 of 313 free living birds by Ito *et al.* (1988), the isolation rate was 13% from pigeons (Colombia Livia Domestica).

Both domestic and fecal pigeons may be carriers of hazardous agents for man and animals, feces of pigeons can serve as substrate for the agent of campylobacter infections (Glunder, 1989). He added that an increased risk can be supposed for pigeon breeders and persons feeding feral doves. Casanovas *et al.* (1995) stated that the fecal bacterial flora of pigeon may be the source of infectious diseases in man in the city of Barcelona. Four hundred cloacal specimens were examined, C. jejuni was found in 105 pigeons (26.2%) with a greater incidence in the districts of the city with a high density of pigeons and without seasonal variation. The relationship of farm variables and management practices to fecal shedding of campylobacter on commercial squab (young pigeon) farms by Jeffrey *et al.* (2001). C. jejuni was found in 19/480 (3.96%).

The present work was conducted to investigate the following:

1 - The prevalence of enteropathogenic campylobacter species in pigeons at Assiut Governorate.
2 - Estimation of the antibiotic sensitivity patterns for C. jejuni.
3 - Studying the plasmid profile of the obtained isolates to investigate the relation between antibiotic resistant strains and plasmid carriage.
4 - Determination of the pathogenicity of C. jejuni strains in pigeons and one day old baby chick.

MATERIALS and METHODS

A total of 120 samples from affected pigeons farm including cloacal swabs, liver and intestin (40 each) collected from different localities in Assiut Governorate during November 2003 to May 2004. All examined pigeons age ranged from one month to 1.5 years old pigeons.

Preparation of samples:

All the samples were subjected to bacteriological examination according to Skirrow and Benjamin (1980) and Moller *et al.* (1997) as following: fecal materials were triturated in sterile saline solution (0.9%) and then centrifuged at 3000 r.p.m. for 5 minutes, while liver and intestine samples were cut into small pieces, then crushed and homogenized with normal saline under aseptic condition.

Isolation:

The prepared samples were subjected for campylobacter by incubation in campylobacter enrichment broth, containing 5% lysed horse blood, skirrow campylobacter selective supplement and skirrow campylobacter growth supplement. Then, incubated at 42°C for 48 h in an atmosphere of (5% O_2, 10% Co_2 and 5% N_2) using an anaerobic Jar and Campylobacter gas generating kits (Oxoid, BR 056A). A loopfull from the incubated broth culture was streaked onto campylobacter blood agar base supplemented with skirrow campylobacter selective supplement, 5% lysed horse blood and skirrow campylobacter growth supplement and incubated at 42°C for 48 h in a microaerobic atmosphere (5% O_2, 10% Co_2 and 5% N_2) using Gas-Pak anaerobic Jar and campylobacter gas generating Kits (Oxoid, BR 056A).

Identification of isolates:

Suspected colonies were identified on the basis of typical morphology of the colonies and a microscopic aspect of Gram negative spiral rods. All the strains were identified biochemically according to Baron *et al.* (1994) for oxidase, catalase production, hippurate hydrolysis and sensitivity to Nalidixic acid and Cephalothin.

Isolation of plasmid (DNA):

A single colony of C. jejuni was picked and inoculated in 10 ml Luria-Bertani broth (LB broth) and growth in micro-aerophilic condition at 42°C for 10 hours. Plasmid extraction were done by using the alkaline lysis procedure as described by Woodford *et al.* (1994).

Agarose Gel Electrophoresis:

Electrophoresis was performed in horizontal gel chamber plate (Biorad, Richmond, USA). 10 µl of the extracted plasmid were mixed with 10 µl of loading buffer and the aliquots were loaded onto 0.7% agarose gel stained with ethidium bromide (0.5 µg/ml). Electrophoresis was carried out at 90 v for 2-3 hours and visualized under UV transillumination (Biometra) at 320 nm and photographed (Woodford *et al.*, 1994). The standard marker was E. coli. V 517 of molecular weight ranged from 1.4-35.8 Mdal. The molecular weights of plasmid were calculated by plotting electrophoretic mobility of plasmid and standard marker molecular weights (Log).

Antibiotic sensitivity testing of campylobacter jejuni

Campylobacter enrichment broth inoculated with campylobacter jejuni strains recovered from intestin, liver and cloacal swabs of pigeons by using disc diffusion method (Baron *et al.*, 1994) and followed by incubation at 35°C for 24 h microaerobically. A campylobacter blood agar plates were swabbed with the broth culture and the following antibiotics discs were dropped onto the inoculated agar plates Norfloxacin (10 µg). Tetracycline (30 µg), Gentamycin (10 µg), Kanamycin (30 µg), Erythromycin (15 µg), Chloramphenicol (30 µg), Ampicillin (10 µg).

Pathogenicity of Campylobacter jejuni for squabs and one-day old chicks:

The isolates were tested for pathogenicity according to Baron *et al.* (1994) as follows: A total of 40-one month and half old squabs and one-day old chicks were used to study the pathogenicity of the isolated strains of C. jejuni. Pure culture was suspended in sterile saline solution and matched by standard Macfarland opacity tube No. 3 (Cell density was 10^9). Squabs and chicks were divided into 4 groups (10 squabs and 10 chicks each). First group was administered orally with 0.5 ml of 10^9 cfu. for 2 successive days. Second group was inoculated S/C with 1 ml of 10^9 cfu. Third group was inoculated I/M with 1 ml of 10^9 cfu. Fourth group kept as control and injected with saline. All squabs and chicks were kept under observation for 20 days to observe the general health condition to notice any clinical syndromes of dead and killed birds, post-mortem examination, mortality rate and bacterial reisolation.

RESULTS

Table 1: Incidence of Campylobacter species in the examined pigeons.

Source of samples	No. of samples	Positive samples		Isolated Campylobacter spp.			
				C. jejuni		C. coli	
		No.	%	No.	%	No.	%
Liver	40	2	5.3	2	5	-	-
Intestin	40	7	17.5	7	17.5	-	-
Cloacal swab	40	10	25	8	20	2	5
Total	120	19	15.8	17	14.1	2	1.6

Table 2: Conventional methods for differentiation of the recovered Campylobacter spp.

Species	Sodium hippurate hydrolysis test	Temperature tolerance test		Antibiotic sensitivity	
		25°C	42°C	Nalidixic acid	Cephalothin
C. jejuni	+	+	+	S	R
C. coli	-	+	+	S	R

S: Sensitive.
R: Resist.

Table 3: Antibiotic sensitivity of Campylobacter jejuni isolated from pigeons to antibacterial agents.

Antimicrobial agents	No. of strains sensitive	% of sensitivity	Degree of sensitivity of C. jejuni isolates
Norfloxacin	15/19	79	Sensitive +++
Gentamycin	16/19	84.2	Sensitive +++
Kanamycin	10/19	63.1	Weekly sensitive +
Ampicillin	9/19	47.3	Weekly sensitive +
Chloramphenicol	13/19	68.4	Moderately sensitive ++
Erythromycin	15/19	79	Sensitive +++
Tetracycline	9/19	47.3	Weekly sensitive ++

Table 4: Antibiotic Resistance pattern & plasmid profile of the examined C. jejuni isolates.

Isolate No.	Source of isolates	Antibiotic resistant pattern	No. of plasmids contained	Molecular size of plasmid DNA (Kb)
1	Cloacal swab	Chl. eryth. Tetra-Ampicillin	2	26.8-9
2	Cloacal swab	Amp. Tetra.	-	-
3	Intestine	Amp. ch. Gen, eryth.	2	26.8-9
4	Intestine	Amp. Chl.	-	-
5	Liver	Gent. Chl.	-	-

Table 5: Pathogenicity test in one day old baby chicks (n=10).

Groups	Rout of inoculation	Death	Deaths		Reisolation	
		day	No.	%	No.	%
I	I/M	The 4th, 5th, 8th and 10th day	4	40	4	100
II	S/C	The 9th and 10th day	2	20	2	100
III	orally	The 10th day	1	10	1	100
IV	Control	-	-	-	-	-

DISCUSSION

Campylobacter organisms are wide spreed in broiler farms while few litratures deal with campylobacter infection in pigeons. In Assiut Governorate, 120 samples of pigeon were examined,80 samples were taken from visceral organs (intestine and liver) of dead pigeons and 40 colacal swab were taken from life cases. Campylobacter species was isolated from 19 out of 120 samples (15.8%) with a rate of 5%, 17.5% and 25% respectively. Higher prevalence rates of campylobacter species in pigeons were detected by Megraud, 1987 (53%), Casanovas *et al.*, 1995 (26.2%) and Adesiyun *et al.*, 1998 (17%). In the contrary, lower incidence rates were reported by Rosef, 1981 (9.3%), Ito *et al.*, 1988 (13%) & Jeffrey *et al.*, 2001 (11.1%) (Table 1).

As illustrated in Tables 1 and 2, C. jejuni was recorded from pigeons with the rate of 14.1%. C. jejuni was the most frequently isolated species of campylobacter in different percentages (9.3%, 8%, 53%, 26.2% and 11.1%) by several investigators. Rosef (1981), Luechtefeld *et al.* (1981), Megraud (1987), Casanovas *et al.* (1995) and

Jeffrey *et al.* (2001), respectively. C. coli was isolated in lower incidence (1.6%) where it was recovered from cloacal swab, while, it failed detection from both liver and intestine. These result go parallel with several reports Wieliczko (1995), Uyttendaelf *et al.* (1996) and Chuma *et al.* (1997) they could isolate C. jejuni and C. coli from 19.2% and 2.7%. So it can be concluded that C. jejuni was the most prevalent strains found. The increasing rate of human infections caused by antimicrobial resistant strains of C. jejuni makes clinical management of cases of campylobacteriosis more difficult (Piddock, 1995 and Yan & Taylor, 1996).

Sensitivity test proved that the most effective antibiotics for all isolates were gentamycin, erythromycin and norfloxacin. They were moderately sensitive to kanamycin and chloramphenicol while, these isolates of campylobacter spp. were less sensitive to Ampicillin and Tetracycline. These results go hand to hand with those recorded by Nakai *et al.* (1994) and Das *et al.* (1996) who found that gentamycin, erythromycin and neomycin were highly effective but Erdger and Diker (1995) revealed that the isolates of campylobacter spp. were resistant to Ampicillin, Penicillin and tetracycline (Table 3).

As seen in Table 4 C. jejuni strains harboring plasmids were resistant to the different antimicrobials: Ampicillin, Chloramphenicol, erythromycin, gentamycin and tetracyclin. These results were in agreement with that reported by Lee *et al.*, 1994 and Enberg *et al.*, 2001. The strains which did not posses plasmid and having the antibiotic resistance may be mediated by chromosome and or transposons instead of being plasmid mediated (Saleha, 2002). These findings agreed with that detected by Lee *et al.* 1994 and Saleha, 2002. Figure 1 illustrates that the C. jejuni harboring plasmids were grouped into 2 plasmid profiles. Isolate no. 1 (Lanes : 1) recovered from cloaca samples carried two plasmids of molecular weights (17-5.7 Mda) and isolate no. 3 (Lane: 3) isolated from intestin carried the same plasmid with the same molecular weight (17-5.7 Mda). The similarity in the plasmid profile of C. jejuni strains carring the same plasmid may indicate plasmid relatedness which may reveals the same epidemiological sources (Saleha, 2002).

Regarding the pathogenicity test, no mortality was recorded between the inoculated squabs. The clinical signs appeared as depression, greenish diarrhea and emaciation. Post-mortem findings include congestion of internal organs (liver, kidney, spleen, heart blood

vessels), haemorrhagic enteritis and enlargement of liver and spleen (Figure 2). C. jejuni was isolated in pure culture from cloacal swabs and internal organs of examined living pigeons before and after sacrification. the incidence of bacterial reisolation from squabs infected orally, S/C and I/M was 50%, 75% and 85% respectively. In baby chicks the results revealed that C. jejuni caused mortality of the inoculated chicks which varied according to the rout of infection. The obtained results in (Table 5) declared that the mortality rate was higher among chicks which infected I/M (40%) followed by S/C (20%) and then orally (10%). Deaths occurred between the 4th day up to 10th days post infection. The clinical symptoms included depression, and diarrhea and post-mortem finding lesions showed in (Figure 3) included sever enteritis with enlargement of the two cecae, the liver was highly congested with enlargement of the gall bladder and unabsorbed yolk sac. The bacterial reisolation of the causative agent was successful in chicks infected orally / S/C and I/M with an incidence of 100%. Similar findings were also recorded by Nagwa (1992) who found that the mortality rate ranged from 12.5-37.5% and the pathological lesions of chicks infected with C. jejuni included enlargement of liver and gall bladder.

In conclusion, results obtained in this study revealed that Campylobacters are now recognized as an important enteric pathogen in pigeons. It is universal finding that pigeons for consumption are contaminated with campylobacter organism.

Accordingly, it can be recommended that great attention and efforts should be paid to eliminate. This group of organisms by using the effective antibiotics to avoid the antimicrobial resistance in campylobacter species and C. jejuni.

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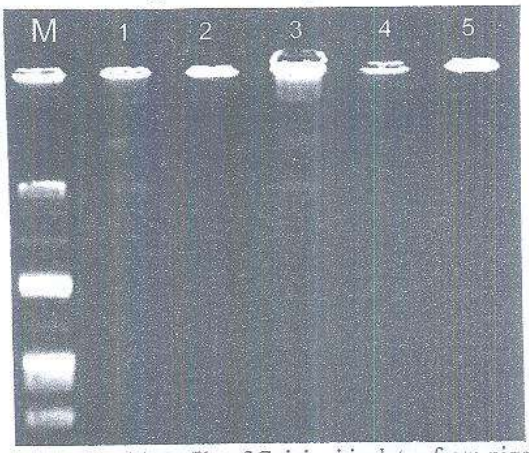

Figure 1: Plasmid profile of C. jejuni isolates from pigeons.
M: E. coli V 517 marker
Lanes: 1, 3: plasmid bearing isolates.
Lanes: 2, 4, 5: plasmid less isolates.
Lanes: 1, 3 (17-5.7 Mda).

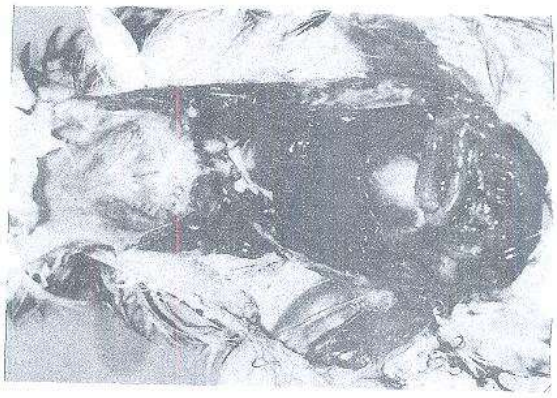

Fig. 2: Experimentally infected squab with C. jejuni showing congestion of internal organs and haemorrhagic enteritis.

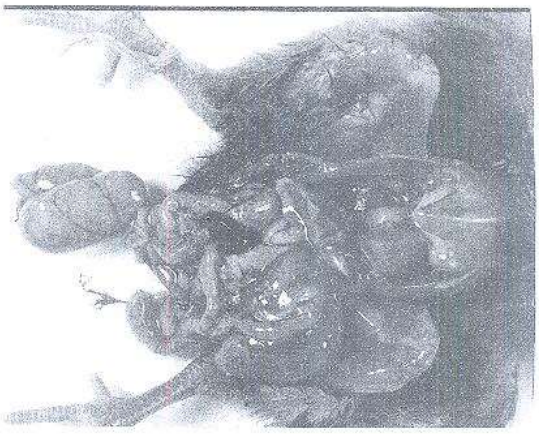

Fig. 3: Experimentally infected baby chick with C. jejuni showing sever enteritis and the liver was congested with enlargement of the gall bladder.