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SURVEILLANCE OF ENTEROPATHOGENIC CAMPYLOBACTER IN RAW POULTRY MEAT AND SOME POULTRY PRODUCTS IN ASSIUT CITY

(With 5 Tables and 1 Figure)

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مدى تواجد ميكروبات الكامبيلوباكتر الممرضة فى لحوم الدواجن وبعض منتجاتها فى مدينة أسيوط

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يعتبر ميكروب الكامبيلوباكتر من أهم الميكروبات التى تسبب حالات النزلات المعوية فى الانسان، ولقد اثبتت الدراسات الوبائية وجود ارتباط وثيق بين هذه الحالات وتناول لحوم الدواجن ومنتجاتها، ولذلك فقد اجريت هذه الدراسة لتحديد دور الدواجن ومنتجاتها لنقل العدوى للانسان. لذلك فقد تم جمع عدد ١٨٠ عينة عشوائية من لحوم ،اكباد، قوانص وامعاء بدارى التسمين بعد الذبح مباشرة فى الفترة من فبراير الى ابريل ٢٠٠٤ وكذلك بعض منتجات الدواجن المعروضة للبيع فى مدينة اسيوط مثل اللحم المفروم واللانشون بواقع ٣٠ عينة لكل منهم، وقد اسفرت النتائج عن تواجد ميكروب الكامبيلوباكتر بنسبة ٣٩,١٧% ، ١٠% ، فى العينات المأخوذة من بدارى التسمين ومنتجات الدواجن على التوالى. وقد تم عزل ميكروب الكامبيلوباكتر من لحوم بدارى التسمين، عينات الكبد، ومسحات الأمعاء بنسبة ٥٣,٣٣% ، ٢٠% ، ٨٣,٣٣% على التوالى. وقد تم عزل ميكروب الكامبيلوباكتر جيوجيناى من لحوم بدارى التسمين (١٦,٧%)، الكبد (٣,٣٣%) مسحات الأمعاء (٤٦,٧%) ، هذا بالأضافة إلى عزل ميكروب الكامبيلوباكتر كولاي من لحوم الدواجن (٣٦,٧%)، عينات الكبد (١٦,٧%) ومسحات الأمعاء (٣٦,٧%). كما أظهرت النتائج تواجد ميكروب الكامبيلوباكتر جيوجيناى فى لحوم الدواجن المفرومه بنسبة ٢٠% . وقد تمت دراسة مدى حساسية عترات الكامبيلوباكتر جيوجيناى المعزولة من عينات اللحوم ، عينات الكبد ولحوم الدواجن المفرومة للمضادات الحيوية حيث أظهرت النتائج ان ٧٥% من العزلات قاومت الأمبسيلين ،٥٠% قاومت الكلورامفينيكول و٢٥% قاومت كل من اللأريثروميسين ،الجينتاميسين والتتراسيكلين. هذا بالأضافة إلى دراسة البلازميد بروفيل وعلاقته بالحساسية للمضادات الحيوية ، وقد تمت مناقشة نتائج كل منهما. وقد تمت مناقشة الأهمية الصحية والوبائية لميكروب الكامبيلوباكتر ومصادر التلوث المختلفة، هذا بالإضافة إلى مناقشة التوصيات لكيفية الحد من تواجد الكامبيلوباكتر فى لحوم بدارى التسمين ومنتجات الدواجن.

SUMMARY

Campylobacter is considered among the most important pathogens reported as a cause of bacterial enteritis in human. Epidemiological evidence has linked *Campylobacter* infection in human with poultry and poultry products. One hundred and eighty random samples from broiler carcasses and some poultry products including cecal contents, muscles, liver, gizzard, chicken minced meat and chicken luncheon samples (30 each) were aseptically collected from local poultry slaughter shops and supermarkets at different districts in Assiut province at the period from February to April 2004.These samples were examined for the occurrence of *Campylobacter* species. The obtained results indicated that *Campylobacter* species were isolated from 39.17% and 10% of the examined broiler carcasses and poultry products, respectively. *Campylobacter* species were recovered from muscles, liver and cecal contents with a rate of 53.33% , 20% and 83.33%, respectively. *C. jejuni* was detected in muscles (16.7%), liver (3.33%), cecal contents (46.7%) and chicken minced meat (20%), meanwhile *C. coli* was determined in muscles (36.7%), liver (16.7%) and cecal contents (36.7%). Antibiotic resistance patterns were determined for *C. jejuni* isolates obtained from muscles, liver and chicken minced meat. In addition plasmid profile were performed to correlate between antibiotic resistance and plasmid carriage among these isolates. It was found that 75% of *C. jejuni* isolates obtained from muscles, liver and chicken minced meat showed resistance to ampicillin, followed by 50% of the strains were resistant to chloramphenicol in addition 25% of the strains were resistant to erythromycin, gentamycin and tetracycline. Correlation between plasmid profile analysis and antibiotic resistance of the examined strains were discussed. Public health hazard of multiple antibiotic resistant enteropathogenic *Campylobacter* was discussed and suggestive measures for reduction of *Campylobacter* in broilers and poultry products were explained.

Keywords: *Enteropathogenic Campylobacter, Broiler carcasses, Poultry products, Antibiotic resistance, Plasmid profile.*

INTRODUCTION

Campylobacter species are among the most frequently reported causes of bacterial enteritis in the developed countries (Nielsen *et al.*, 2000),where the number of human cases of campylobacteriosis has been

steadily increased recently in many countries (Ring and Atanassova, 2001). Recently the use of antibiotics in food animal production, particularly in poultry 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).

Most *Campylobacter* species colonize the intestinal mucosa of warm blooded hosts, including all food producing animals and humans (Newell, 2001), however the favored environment appears to be the intestine of all avian species. (Stephens *et al.*, 1998; Hald and Brandsted 2000 & Waldenstrom *et al.*, 2001). Epidemiological evidence has linked *Campylobacter* with poultry and poultry products (Humphrey, 1995; Jacobs-Reitsma, 1997 and Kramer *et al.*, 2000) as it has been reported that there is a linear relationship between prevalence in broiler flocks and the probability of human campylobacteriosis (WHO, 2002).

Clinically the most important campylobacters are enteropathogenic species, members of the thermotolerant groups: *Campylobacter jejuni*, *C. coli*, *C. lari* and *C. upsaliensis*. It has been reported that *C. jejuni* and *C. coli* are responsible for the majority of *Campylobacter* enteritis in industrial countries (Allos and Blaser, 1995).

A wide range of zoonotic and environmental risk factors have been identified (Petersen *et al.*, 2001). Environmental sources are believed to be important reservoirs for *Campylobacter* infections in broiler chicken flocks which is considered the most frequently identified risk factors in *Campylobacter* foodborne outbreaks around the world (Eberhart-Phillips *et al.*, 1997 & Ring and Atanassova, 2001). Large numbers of campylobacters can be present in the avian intestinal tract without any apparent gross pathology where the prevalence of infection in broiler breeder flocks has been found to be as high as 80% (Evans, 2001) and consequently *Campylobacter* spp inevitably find its way onto chicken meat and organs when carcasses are contaminated with intestinal contents during slaughtering, defeathering and evisceration (Kapperud *et al.*, 1992).

Infection can be transmitted from chicken carcasses and other products either transferred from fingers to mouth unthinkingly by inexperienced food handlers, consumption of raw or undercooked poultry meat or products or other (innocent) foods which may become cross contaminated from the raw products by means of hands and

utensils and this is probably considered the most frequent mode of infection (Kapperud *et al.*, 1992).

The present study was designed to investigate the prevalence of enteropathogenic *Campylobacter* species in broiler chicken carcasses at slaughter and some poultry products as chicken minced meat and chicken luncheon marketed at Assiut province. Moreover, due to the public health hazard of antibiotic resistant *C. jejuni*, antibiotic sensitivity patterns of *C. jejuni* (isolated from poultry meat, organs and poultry products) were estimated. Furthermore, plasmid profile of these isolates were investigated to study the relation between antibiotic resistance strains and plasmid carriage as well as to spotlight on possible contamination sources and suggestive measures for control of *Campylobacter* at each link in the food chain.

MATERIALS and METHODS

Collection of samples:

One hundred and eighty random samples from broiler carcasses including cecal contents, muscles, liver, gizzard and poultry products constituting chicken minced meat and chicken luncheon (30 each) were collected from local poultry slaughter shops and supermarkets at different districts in Assiut province at the period from February to April 2004. All samples were kept in an insulated ice box and transported to the laboratory with a minimum of delay.

Preparation of samples:

Small pieces were cut from pectoral and thigh muscles, liver as well as gizzard from broiler carcasses at slaughter, then crushed and homogenized separately with normal saline under complete aseptic condition. Swabs were prepared from cecal contents. Small pieces were prepared from luncheon samples, while frozen chicken minced meat samples were thawed by overnight refrigeration. Each sample was aseptically and carefully freed from its casing then mixed thoroughly in a sterile mortar before being examined.

Enrichment procedure: (Varnam and Evans, 1991):

The prepared samples were selectively enriched for *Campylobacter* by incubation in *Campylobacter* enrichment broth containing (*Campylobacter* skirrow's supplement and growth supplement at 42^0C for 48h. in a microaerobic atmosphere (5% O_2, 10% CO_2 and 85% N_2) using Gas-Pak anaerobic jar and *Campylobacter* gas generating kits (Oxoid, BR 056A).

Isolation technique:

A loopfull from the incubated broth culture was streaked onto Brucella agar base supplemented with blood, *Campylobacter* skirrow's supplement & growth supplement and incubated at 42°C for 48h. in a microaerobic atmosphere (5% O_2, 10% CO_2 and 85% N_2) using Gas-Pak anaerobic jar and *Campylobacter* gas generating kits (Oxoid, BR 056A). Suspected colonies were identified biochemically according to Baron *et al.*, (1994). Biochemically identified colonies were maintained in semisolid brucella medium containing neutral red indicator and incubated at 42°C for 24 h.

Antibiotic susceptibility test:

The antibiotic sensitivity patterns were determined for 12 srtrains of *C. jejuni* recovered from muscles, liver and chicken minced meat by using the disc diffusion method (Bopp *et al.*, 1985). Brucella agar base was supplemented with 5% sheep blood and incubated at 37°C for 48h. in a microaerophilic condition. The following antibiotic discs were used: ampicillin 10µg, chloramphenicol (30µg), erythromycin (15µg), gentamycin (10µg) and tetracycline (30µg) according to Bopp *et al.*, (1985).

Extraction of plasmid DNA:

The *C. jejuni* isolates obtained from liver, muscles and chicken minced meat were purified on Brucella agar base containing growth supplement and incubated in microaerophilic condition at 42°C for 24h. Single colony was picked and inoculated in 10ml of Luria Bertani broth (LB) and grown in microaerophilic condition at 42°C for 10h. Plasmid extraction were done by using the alkaline lysis procedure as described by Woodford *et al.*, (1994).

Agarose gel electrophoresis:

10µl of the extracted plasmid were mixed with 10µl of loading buffer and the aliquots were loaded onto 0.7% agarose gel stained with ethidium bromide (0.5µg/ml). Electrophoresis was carried out at 90 v for 2-3h and visualized under UV transillumination (Biometra) at 320 nm and photographed (Woodford *et al.*, 1994). *E.coli* (V517) containing plasmids of molecular weight ranged from 1.4-35.8 Mda was used as molecular weight standard marker. The molecular weight of plasmids were calculated by blotting electrophortic mobility of plasmid and the standard molecular weight marker.

RESULTS

Table 1: Occurrence of *Campylobacter* species in broiler carcasses and some poultry products.

Broiler carcasses				Poultry products			
Source of samples	No. of samples	*Campylobacter* spp. No.	%	Type of product	No. of samples	*Campylobacter* spp. No.	%
Muscles	30	16	53.33	Minced meat	30	6	20
Liver	30	6	20				
Gizzard	30	-	-	Luchcon	30	-	-
Cecal contents	30	25	83.33				
Total	120	47	39.17	Total	60	6	10

Table 2: Detection of *Campylobacter jejuni* and *Campylobacter coli* in broiler carcasses.

Source of samples	No. of samples	*Campylobacter* species			
		C. jejuni		*C. coli*	
		No.	%	No.	%
Muscles	30	5	16.7	11	36.7
Liver	30	1	3.33	5	16.7
Gizzard	30	-	-	-	-
Cecal contents	30	14	46.7	11	36.7
Total	120	20	16.7	27	22.5

Table 3: Existence of *Campylobacter jejuni* and *Campylobacter coli* in some poultry products.

Type of product	No. of samples	*Campylobacter* species			
		C. jejuni		*C. coli*	
		No.	%	No.	%
*Minced meat	30	6	20	-	-
Luncheon	30	-	-	-	-

*Chicken minced meat.

Table 4: Correlation between antibiotic resistance pattern and plasmid carriage of the examined *C. jejuni* isolates.

Antimicrobial agents	Sensitive		Resistance to antimicrobials					
			Resistant with plasmids		Resistant without plasmid		Total of resistant isolates	
	No./12	%	No./12	%	No./12	%	No./12	%
Ampicillin	3	25	7	58.33	2	16.67	9	75
Chloramphenicol	6	50	4	33.33	2	16.67	6	50
Erythromycin	8	66.67	3	25	1	8.33	4	33.33
Gentamycin	9	75	3	25	-	-	3	25
Tetracycline	8	66.67	3	25	1	8.33	3	25

Table 5: Antibiotic resistance pattern and plasmid profile of the examined *C. jejuni* isolates.

Isolate No.	Source of isolates	Antibiotic resistant pattern	No.of plasmids	Plasmid size (Mda)
1	Liver	Chl.Gen.	2	4.3 - 3.4
2	Muscles	Amp.	2	4.3 - 3.4
3	Muscles	Amp. Chl.	1	35
4	Muscles	Amp.	1	22
5	Muscles	Chl. Eryth. Gen.	1	22
6	Muscles	Amp. Chl. Eryth. Gen.Tetra.	1	22
7	Minced meat	Amp. Chl.	-	-
8	Minced meat	Amp.	1	22
9	Minced meat	Amp. Eryth. Tetra.	3	22 –4.3 - 3.4
10	Minced meat	Eryth. Tetra.	-	-
11	Minced meat	Amp.	3	22 – 8 - 1.6
12	Minced meat	Amp.Chl.	-	-

Amp.=Ampicillin,Chl.=Chloramphenicol,Eryth.=Erythromycin,Gen.Gentamycin, Tetra.=Tetracycline

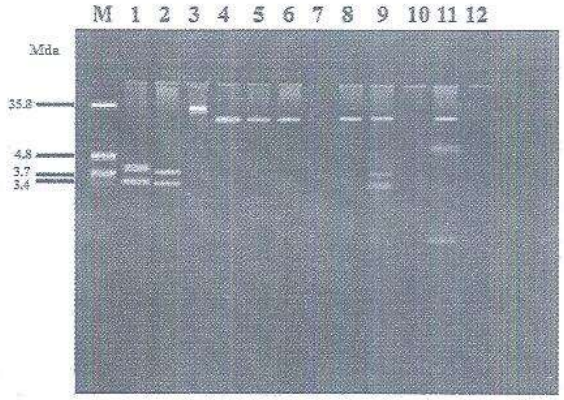

Figure1: Plasmid profiles of *C.jejuni* isolates.
M: E. coli V517 marker.
Lanes: 1, 2, 3, 4, 5, 6, 8, 9, 11 : plasmid bearing isolates.
Lanes: 7, 10,12: plasmidless isolates.
Lanes: 1, 2 (4.3-3.4Mda), lane: 3 (35 Mda), Lanes :4, 5, 6 ,8 (22 Mda).
lane: 9 (22-4.3-3.4 Mda).
Lane: 11 (22 -8 -1.6Mda).

DISCUSSION

Campylobacter species are commonly found in broiler chickens at slaughter where the ceca and intestine of infected birds have been shown to contain very large numbers of the organism. The pathogen appears to survive the processing operation and cross-contamination during procedures such as scalding, plucking and evisceration which may allow the prevalence of carcass contamination to exceed that of infection in the live birds, where several retail surveys have shown that typically more than 50% of chicken carcasses were contaminated with *Campylobacter* species (Evans, 2001).

It is evident from data presented in Table 1 that *Campylobacter* species were isolated from 47 out of 120 samples of broiler chicken carcasses at slaughter with a percent of 39.17%. *Campylobacter* species

were recovered from muscles, liver and cecal contents with a rate of 53.33%, 20% and 83.33%, respectively. However the organism couldnot be isolated from gizzard samples. Higher prevalence rates of *Campylobacter* species in broilers were reported by Zeenathul, 1994 (97.1%); Atanassova and Ring, 1999 (41.1%); Refregier-Petton *et al.*, 2001 (42.7%); Stern *et al.*, 2001 (87.5%); Wedderkopp *et al.*, 2001 (42.5%) and Saleha, 2002 (72.6%). In the contrary, lower prevalence rates were detected by Kappeured *et al.*, 1993 (18%) and Berndtson *et al.*, 1996 (27%).These variation in the recovery rates may be attributed to differences in geographic distribution of *Campylobacter* species among broilers and variation of isolation methods (ICMSF, 1996). Although we could able to isolate *Campylobacter* species from 6 out of 30 samples of chicken minced meat with a rate of 20%, we could not recover it in chicken luncheon samples.

C. jejuni was recovered from broiler carcasses with a rate of 16.7% (Table 2). It has been isolated from muscles, liver and cecal contents with a rate of 16.7%, 3.33% and 46.7%, respectively. *C. jejuni* was the more frequently isolated species of *Campylobacter* by several investigators from broilers with a rate of 43%, 19.7%, 53.7%,39.6% and 73.2% detected by Atanassova & Ring,(1999); Fernandez & Torres, (2000); Chou&Tsai, (2001); Wedderkopp *et al.*, (2001) and Saleha, (2002), respectively.

C. coli was isolated with a rate of 22.5% from broilers (Table2) where it was recovered from muscles, liver and cecal contents with a rate of 36.7%, 16.7% and 36.7%, respectively. Moreover, both of *C. jejuni* and *C. coli* were not recovered from gizzard samples. Higher prevalence rates of *C.coli* were detected in broilers 28.3% and 26.8% reported by Saleha (1996) and Saleha (2002), respectively. On the other hand, lower rates of detection 13%, 6%, and 5% were estimated by Atanassova & Ring, (1999); Fernandez & Torres, (2000) and Wedderkopp *et al.*, (2001), respectively.

It is clear from data illustrated in Table 3 that *C. jejuni* was isolated from 6 (20%) out of 30 samples of chicken minced meat, meanwhile we could not isolate it from chicken luncheon samples. Moreover, we could not isolate *C. coli* from both minced meat and chicken luncheon samples. It was reported in literature that *Campylobacter* species can be isolated with a high percentage from poultry products (Nielsen & Nielsen, 1999; Uyttendaele *et al.*, 1999 and Berrang *et al.*, 2001) in which it was revealed that microbial contamination of poultry meat is largely a surface phenomenon

contaminating the skin either directly from intestinal contents and the dropping or indirectly via contaminated knives or cutting boards and other equipments (Saleh *et al.*, 2003) and consequently the nature of poultry processing systems makes cross contamination from *Campylobacter* infected to *Campylobacter* free carcasses unavoidable (ICMSF, 1998), hence the possibility of recovery from poultry products is high however, the inability to isolate *Campylobacter* species from luncheon samples may be due to the heat treatment of the product which may had destruct the pathogen during manufacturing.

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). *C. jejuni* can harbor a large array of resistance determinants including several genes that are plasmid or chromosome mediated or both (Saleha, 2002). Evaluation of both antibiotic resistance and plasmid profile of the isolated strains is useful as epidemiological markers to understand the epidemiology of the disease and the nature of antibiotic resistance (Ali *et al.*, 1996).Hence we study the antibiotic resistance and plasmid profile of the 12 *C. jejuni* isolates recovered from muscles, liver and chicken minced meat to reveal the hazard that may constitute a health risk.

The obtained results in Table 4 declared that 75% of the examined strains were resistant to ampicillin followed by 50% of the strains were resistant to chloramphenicol and in addition 25% of the examined strains were resistant to erythromycin, gentamycin and tetracycline. The high antibiotic resistance rates detected could be due to the wide spread use of antibiotics in chickens, particularly in feed, as well as due to being use indiscriminately (Saleha, 2002). It was found that *C. jejuni* strains harboring plasmids were resistant to the different antimicrobials: ampicillin, chloramphenicol, erythromycin, gentamycin and tetracycline with a rate of 58.33%, 33.33%, 25%, 25% and 25%, respectively. These results are in agreement with that reported by Lee *et al.*, 1994 and Engberg *et al.*, 2001). However the data in Table 4 illustrated that 16.67% of the examined *C. jejuni* strains were resistant to ampicillin and chloramphenicol, moreover, 8.33% of the isolates were resistant to erythromycin and tetracycline without carrying any plasmid. The antibiotic resistance in those strains which did not possess plasmid 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).

Table 5 and Figure 1 illustrates that the *C. jejuni* harboring plasmids were grouped into 5 plasmid profiles. Isolate no.1 and 2 (lanes: 1 & 2) recovered from liver and muscles, respectively carried two plasmids of molecular weights (4.3 - 3.4 Mda). In addition isolates no. 4, 5, 6 and 8 (lanes:4, 5, 6 & 8) isolated from muscles and chicken minced meat carried the same plasmid with molecular weight of 22 Mda, however isolates no. 3, 9 and 11 (lanes: 3, 9&11) recovered from muscles and chicken minced meat carried plasmids of different molecular weights (35 Mda, 22 - 4.3 - 3.4 Mda & 22 - 8 - 1.6 Mda), respectively. The similarity in the plasmid profile of *C.jejuni* strains carrying the same plasmid may indicate plasmid relatedness which may reveals the same source of contamination (Saleha, 2002), however the different size plasmid in the remaining strains may indicates different epidemiological sources.

In conclusion, results obtained in this study revealed that locally produced broilers are a potential source of multiple –antibiotic resistant enteropathogenic *Campylobacter* species which pose a public health hazard to consumers. Reinforcing hygienic practices at each link in the food chain from producer to consumer is critical to diminish *Campylobacter* contamination rates in retail broilers. These practices including reduce pathogen carriage in broilers at farm level, increased hygiene at slaughter and continued implementation of hazard analysis critical control point (HACCP) systems as well as increased consumer education to reduce cross contamination during handling and preparation.

REFERENCES

Ali, A.A.; Al-Bakkri, G.H. and Saliih, D.S. (1996): Role of plasmids in antibiotic resistance of *Campylobacter* species isolated from children. J. of Islamic Academy of Sciences 9 (4): 1-6 .

Allos, B.M., and Blaser, M.J. (1995): Campylobacter jejuni and the expanding spectrum of related infections. Clin. Infect. Dis. 20: 1029 -1099.

Atanassova, V. and Ring ,C. (1999): Prevalence of *Campylobacter* spp. in poultry and poultry meat in Germany. Int. J. Food Microbiol. 51: 187-190.

Baron, E.J.; Peterson, L.R. and Finegold, B.M. (1994): Bailey and Scott's Diagnostic Microbiology.9th Ed.; Mosby St. Louis, Baltimore.

Berndtson, E.; Danielsson- Tham, M.L. and Engvall, A. (1996): Campylobacter incidence on a chicken farm and the spread of Campylobacter during the slaughter process. Int. J. Food Microbiol.32: 35-47.

Berrang, M.E.; Buhr, R.J.; Cason, J.A. and Dickens, J.A. (2001): Broiler carcass contamination with campylobacter from feces during defeathering. J. Food Prot. 64 (12): 2063-2066.

Bopp, C.A.; Birkness, K.A.; Wachsmuth, I.K. and Barrett, T.J. (1985): In vitro antimicrobial susceptibility plasmid analysis, and serotyping of epidemic-associated *Campylobacter jejuni*. J. Clinic. Microbiol. 21(1):4-7.

Chou, J. and Tsai, H. (2001): Prevalence and antimicrobial susceptibility of Salmonella and Campylobacter from meat-type chickens in Taiwan. J. Chin. Soc. Vet. Sci. 27: 27-38.

Eberhart-Phillips, J.N.; Walker, N.; Garrett, D.; Bell, D.; Sinclair, W.R. and Bates, M (1997): Campylobacteriosis in New Zealand: results of a case -control study. J. Epidemiol. Community Health 51: 686-691.

Enberg, J.; Aarestrup, F.M.; Taylor, D.E.; Gerner-Smidt, P. and Nachamkin, I. (2001): Quinolone and macrolide resistance in *Campylobacter jejuni* and *C.coli*. Resistance mechanisms and trends in human isolates. Emerg. Infect. Dis. 7 (1): 24-34.

Evans, Sarah.(2001): Campylobacter. In: Jordan, F.; Pattison, M.; Alexander, D. and Faragher, T. Poultry diseases. W.B. Saunders. pp.170-177.

Fernandez, H. and Torres, N. (2000): Occurrence of *Campylobacter jejuni* and *C.coli* in three groups of hens of different geographic origin in Southern Chile. Arch. Med. Vet. 32: 241-244.

Hald, T. and Brandsted, T. (2000): Annual report on Zoonoses in Denmark 2000. Ministry of Food, Agriculture and Fisheries, Copenhagens, Denmark. Cited in Newell et al., 2003.

Humphrey, T.J. (1995): Human campylobacter infections: epidemiology and control. Sci. Prog. 78:135-146 cited in: Berrang et al., 2001.

ICMSF (1996): Microorganisms in Foods, Microbiological specifications of food pathogen. Co. Pub. by James & James Ltd, London.

ICMSF (1998): Microorganisms in foods. Microbial Ecology of Food.Commodities-London: Blackie. pp. 72-129.

Jacobs-Reitsma, W.F. (1997): Aspects of epidemiology of Campylobacter in poultry. Vet. Q. 19: 113-117.

Kapperud, G.; Skjerve, E.; Bean, N.; Osroff, S.M. and Lassen, J. (1992): Risk factors for sporadic campylobacter infections: results of case control study in Southern Norway. J. Clinic. Microbiol.30:3117-3121.

Kapperud, G.; Skjerve, E.; Vik, L.; Hauge, K.; Lysaker, A.; Aalmen, I.; Ostroff, S. and Potter, M. (1993): Epidemiological investigation of risk factors for campylobacter colonization in Norwegian broiler flocks. Epidemiol. Infect. 111: 245-255.

Kramer, J.M.; Frost, J.A.; Bolton, F.J. and Waring, D.R.A. (2000): Campylobacter contamination of raw meat and poultry at retail sale. Identification of multiple types and comparison with isolates from human infection. J. Food Prot. 63: 1654-1659.

Lee, CH-Y.; Tai, CH -L.; Lin, SH-CH. and Chen, Ya-T. (1994): Occurrence of plasmids and tetracycline resistance among *Campylobacter coli* isolated from whole market chickens and clinical samples. Int. J. Food. Microbiol. 24: 161-170.

Newell, D.G. (2001): Animal models of *Campylobacter jejuni* colonization and disease and the lessons to be learned from similar *Helicobacter pylori* models. Symp. Ser. Soc. Appl. Microbiol. 30:575-675.

Nielsen, E.M. and Nielsen, N.L. (1999): Serotypes and typability of *Campylobacter jejuni* and *Campylobacter coli* isolated from poultry products. Int . J. Food Microbiol. 46 (3):199-205.

Nielsen, E.V.A.; Engberg, J.; Fussing, V.; Petersen, L.; Brogren, C-H. and Stephen, I.W.O. (2000): Evaluation of phenotypic and genotypic methods for subtyping *Campylobacter jejuni* isolates from humans, poultry and cattle. J. Clinic. Microbiol. 38 (10): 3800-3810.

Petersen, L.; Nielsen, E.M.; Enberg, J.; On, S.L.W. and Dietz, H.H. (2001): Comparison of genotypes and srotypes of *Campylobacter jejuni* isolated from Danish wild mammals and birds and from broiler flocks and humans. Appl. Environ. Microbiol. 67 (7): 3115-3121.

Piddock, L.J.V. (1995): Quinolone resistance and *Campylobacter* spp. Antimicrobiol. Agents Chemotherapy. 36: 891-898.

Refrgier-Petton, J.; Rose, N.; Denis, M. and Salvat, G. (2001): Risk factors for *Campylobacter* spp. Contamination in French broiler chicken flocks at the end of the rearing period. Prev. Vet. Med. 50: 89-100.

Ring, CH. And Atanassova, V. (2001): Surveillance of poultry flocks kept in three different housing systems for the prevalence of *Campylobacter* and *Salmonella* spp.1st Cong. of Food Hygiene and Human Health,6-8 Feb. Dept. of Food Hygiene, Fac. Vet. Med. Assiut, Egypt pp. 91-97.

Saleha, A.A. (2002): Isolation and characterization of *Campylobacter jejuni* from broiler chickens in Malaysia. Int. J. of Poultry Science.1 (4): 94-97.

Saleha, A.A.; Ibrahim, A. and Kamarzaman, A.(1996): Campylobacter in village chickens: Prevalence and biotypes. J. Vet. Malaysia 8:25-27.

Saleh, E.A.; Abdel-Dayem, R.H. and Corry, J.E.L. (2003): Campylobacter contamination of poultry ,beef , sheep and goat carcass during processing.2nd ICOFHHH,21-23 Oct. Assiut Egypt. pp. 127-130.

Shakespeare, M. (2002): Zoonoses. Pharmaceutical Press. New York pp.248-250.

Stephens, C.P.; On, S.L. and Gibson, J.A. (1998): An outbreak of infectious hepatitis in commercially reared ostriches associated with *Campylobacter coli* and *Campylobacter jejuni* Vet. Microbiol. 61:183-190.

Stern, N.J.; Fedorka-Cray, P.; Bailey, J.S.; Cox, N.A.; Craven, S.E.; Hiett, K.L.; Musgrove M.T.; Ladely, S.; Cosby, D.; and Mead, G.C. (2001): Distribution of *Campylobacter* spp.in selected US poultry production and processing operations. J. Food Prot. 64:1705-1710.

Uyttendaele, M.; De Troy, P. and Debevere, J. (1999): Incidence of *Samonella, Campylobacter jejuni, Campylobacter coli* and *Listeria monocytogenes* in poultry carcasses and different types of poultry products for sale on the Belgian retail market. J. Food Prot.62 (7): 735-740.

Varnam, A.H. and Evans, M.G. (1991): Foodborne pathogens. An illustrated Textbook. Wolfe Publishing Ltd., New York pp.209-234.

Waldenstrom, J.; Broman, T.; Carlsson, I.; Hasselquist, D. and Olsen, B. (2001): Prevalence of *Campylobacter* spp. in migrating wild birds in Sweden-ecological considerations of carriership. Int. J. Med. Microbiol. 291:37-38.

Wedderkopp, A.; Gradel, K.; Jorgensen, J. and Madsen, M. (2001): Preharvest surveillance of *Campylobacter* and *Salmonella* in Danish broiler flocks: a 2 year study. Int. J. Food Microbiol. 68:53-59.

WHO (2002): The increasing incidence of human Campylobacteriosis . Report and proceedings of WHO consultation of experts, Copenhagen, Denmark, 21-25. Nov. 2000 WHO. /CDS/ CSR/ APH publication Geneva, Switzerland.

Woodford, N.; Johnson, A.P. and Threfall, E.J. (1994): Extraction and fingerprinting of bacterial plasmids. In Methods in Practical Laboratory bacteriology Chart, H (ed.) CRC. Boca Raton, pp.93-105.

Yan, W. and Taylor, D.E. (1996): Characterization of erythromycin resistance in *Campylobacter jejuni* and *Campylobacter coli*. Antimicrobiol. Agents Chemotherapy. 35.

Zeenathul, N.A. (1994): Prevalence of *Campylobacter jejuni* in chickens. Cited in Saleh. (2002).