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STUDIES ON COMPYLOBACTER INFECTION IN LAYER CHICKENS

(With 5 Tables and 1 Figure)

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دراسات عن الاصابة بالكامبيلوباكتر فى قطعان الدجاج البياض

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تم فحص الأحشاء الداخلية لـ٦٧ عينة من دجاج بياض عمر ٢٥-٣٦ أسبوعا حديث النفوق و٢٥ عينة من قناة البيض و٣٥ مسحة من فتحة المجمع من حالات حية . وقد تم عزل ميكروب الكامبيلوباكتر جو جيناى وكولاى فى ٢١ حالة من ٦٧ بنسبة (٣٤,٣%) و ٣ حالات من ٢٥ بنسبة (١٢%) و١١ حالة من ٣٥ بنسبة (٣١,٤ %) علي التوالي. وقد تم أجراء العدوى الصناعية فى كتاكيت عمر يوم التى أدت إلى ظهور أعراض المرض ونسبة وفيات وصلت إلى ٧٠% ولكن كتاكيت عمر ٤ أسابيع كانت مقاومة لحد ما وبعمل اختبار الحساسية للعترات المعزولة معمليا وجد أن حمض النالاديكس والإريثروميسين والجينتاميسين هى الأدوية الأكثر تأثيرا .

SUMMARY

Sixty seven samples of visceral organs (liver, spleen), blood and bile of freshly dead layers, 25 samples of oviduct and 35 cloacal swabs from living layers 25-36 weeks old collected from farms at Assiut Governorate were examined. Isolation of Campylobacter (C.) revealed 23 positive cases of C. jejuni and C.coli out of 67 (34.3%), 3 out of 25 (12%) and 11 out of 35 (31.4%) respectively. Trials for reproducing the infection in 1 day old Chicks lead to 70% mortality while 4 weeks old chicks were somewhat resistante. In vitro sensitivity test showed that Naladixic acid, Erythromycin and Gentamycin were effective.

Key words: *Compylobacter infection, layer chickens.*

INTRODUCTION

Compylobacteriosis is attributed to infection by thermophilic members of the genus Compylobacter, the three species of clinical significance C.jejun, C.coli and C. laridis are microaerophilic, gram negative, spiral, uniflaglate organisms. (Sebald and Veron, 1963).

Commercial poultry serve as reservoirs of Campylobacter infection with isolation rates in feces reached 72% in chickens. (Simmons and Gibbs, 1979).

Campylobacter jejuni is the most frequently Occurring member of the thermophilic triad (Munroe *et al.*, 1983).

Campylobacter jejuni has been identified as one of the major couses of diarrheal disease in humans throughout the world. (Skirrow 1990).

The organism induced infection of humans is epidemiologically linked to the consumption of improperly prepared poultry products or foods cross-contaminated by poultry products. (Shane, 1992)

Recent evidence suggests that Campylobacter jejuni can colonize the oviduct of laying hens but the source and role of this colonization are unknown. (Camarda *et al.*, 2000)

Modugno *et al.* (2000) found that Campylobacter jejuni biotypes I and II were Common, type III was rare and type IV was absent.

The aim of the present work is:

- Isolation and identifiction of Campylobacter spp. From laying hens.
- Biotyping of Campylobacter jejuni.
- Experimental infection of baby chicks with the isolated organism.
- Testing the isolate against several antibiotic discs to give a suitable treatment.

MATERIALS and METHODS

Samples:

Visceral organs (liver, spleen), bile and blood of freshly dead 67 layers 25 samples of oviduct and 35 cloacal swabs from living layers 25-36 weeks old were used fo isolation.

Media used were:

1. Skirrow selective media for isolation and purification (Skirrow, 1977).
2. Semisolid brucella medium for maintenance. (park *et al.*; 1984 and Mossel, 1985).
3. Triple sugar iron agar (park *et al.*; 1984).

4. Semisolid brucella medium with cysteine for hydrogen sulphide production by lead acetate strips (park *et al.*; 1984).
5. Semisolid brucella medium with sodium selenite for selenite reduction test (Ullmann, 1979).
6. Semisolid brucella medium with potassium nitrate for nitrate reduction test. (Park *et al.*; 1984).

Reagents and Indicators:

1. 3% hydrogen peroxide for catalase test.
2. Sodium hippurate solution (Sigma) and ninhydrin solution for hippurate hydrolysis test.
3. Nitrate solution (A) (0.8% sulphanilic acid in 5N acetic) and nitrate solution (B) 0.5% y-naphty amine in 5N acetic acid) for nitrate reduction test (Diem air, 1957).
4. Tetramethyl paraphenylenediamine – 2Hcl for oxidase test.
5. Lead acetate test paper strips.

Stain used is:

Gram's Stain.

Pathogenicity test:

Twenty, 1day old baby chicks and twenty, 4weeks old chicks were used in our experiment. They were obtained from the Faculty of Agriculture Assiut University poultry farm.

Sensitivity discs used were:

Chloramphenicol (30Mg), Naladixic acid (30Mg), Ampicillin (10Mg), Colistin sulphate (10Mg), Streptomycin (10Mg), Gentamycin (10Mg) Erythromycin (15 Mg), Tetracyclin (30 Mg), and lincomycin (2M).

Methods:

1- Isolation:

Dead laying chickens were subjected to post–mortem examination and swabs were taken from liver, spleen, bile, blood and oviduct. Cloacal swabs from living cases were taken. These swabs were streaked on skirrow selective media and incubated at 37C for 48h. under microaerphilic conditions by use of anaerobic Jar and campy–gas pack – generating packets. Suspected colonies were subcultured in brucella semisolid media and incubated aerobically at 37C for 24h, then maintained in a refrigerator. Further identification and subculture were done weekly.

2- Identification of the organism:

Specimens from suspected colonies were stained by Gram stain to show typical morphology of the organism.

Biochemichal reactions to study: the motility, oxidase test, catalase production, suscedtibility or resistance to naladixic acid, nitrate reduction test, sodium hippurate hydrolysis and hydrogen sulphide production by using lead acetate strips and T S I media.

The differentiation between C. jejuni, C. coli and C. laridis is based on naladixic acid sensitivity and hippurate hydrolysis (Table 1) (Skirrow and Benjamin, 1980, Varnam and Evans, 1991).

Table 1: the differentiation between C. species:

C. species	Naladixic acid sensitvity	Hippurate hydrolysis
C. jejuni	Sensitvie	+
C. coli	Sensitvie	-
C. laridis	Resistant	-

3- Biotyping:

According to lior scheme, (1984). Table 2

Table 2: Shows biotyping scheme for C. jejuni:

Test	C. jejuni			
	I	II	III	IV
Hippurate hydrolysis	+	+	+	+
H_2S production on TSI	-	-	+	+

Pathogenicity test:

Six groups of chicks were divided as follow:

a) 1st group was ten-1day old chicks inoculated orally with 9×10^7 CFU (Ruiz – palacios *et al.*; 1981).
b) 2nd group was five-1day old chicks, left in contact with the 1st group.
c) 3rd group was five-1day old chicks, left as control.
d) 4th group was ten-4week old chicks inoculated orally with 9×10^7 CFU (Ruiz – palacios *et al.*; 1981).
e) 5th group was five-4week old chicks left in contact with 4th group.
f) 6th group was five-4week old chicks and left as control.

Sensitivity test:

Discs were placed on brucella blood agar (without antibiotic supplement) according to Fennel *et al.*. 1984

RESULTS

Some of the naturally infected laying chickens showed diarrhea, while P.M examination of freshly dead cases revealed distension in the intestinal tract extending to the ceca and accumulation of mucus and petechial haemorrhages present in some cases. Other cases showed mottling of the parenchyma of liver and hydropericardium.

Bacteriological examination revealed isolation of small, round, moist, non haemolytic colourless to cream coloured colonies.

By Gram stain showed curved and gull winged forms, the isolates were motile; with a characteristic corkscrew kind of movement.

Results of biochemichal tests are shown in Table 3.

Table 3: Shows Biochemichal tests:

Test	Isolate
1- Catalase	+ ve
2- Oxidase	+ ve
3- Nitrate reduction	+ ve
4- Hippurate hydrolysis	+ ve
5- Sodium selenite reduction	+ve
6- Hydrogen sulphide production:	
a- TSI agar	- ve
b- Lead acetate stirps	+ve

Percentage of C. jejuni and coli in isolated positive cases present in Table 4.

Table 4: Shows percentage of C.jejuni and coli in positive cases:

Visceral Samples (67)				Oviduct samples (25)				Cloacal swabs (35)			
No. of positive	%	C. jejuni	C. coli	No. of positive	%	C. jejuni	C. coli	No. of positive	%	C. jejuni	C. coli
23	34.4	18	5	3	12	3	-	11	31.4	8	3

Biotyping of C. jejuni according to lior scheme(1984) were Biotype I and II

The pathogenicity test:

The 1st group showed signs of diarrhea at the third day post inoculation with mortality reached 70% at the 10th day postinoculation and the gross lesions were enlargement of liver with red and yellow mottling and congestion enlargement of the heart, distention of the intestine extending to the ceca (Fig.1) with prefuse mucus.

The 2nd group-showed mild signs and lesions at the 5th day of inoculation.

The 4th and 5th groups showed mild lesions compared with 1st group at 8th day post inoculation without mortality.

There were no clinical signs in 3rd and 6th groups.

Reisolation of the organism in experimentally infected chicks revealed that the Campylobacter could be reisolated from 1st group.

The sensitivity of C. jejuni against antimicrobial discs illustrated in Table 5.

Table 5: illuestrates the sensitivity of C. Jejuni against antimirobial discs

Types of discs	Sensitivity
Naladixic acid	+++ ve
Erythromycin	++ ve
Gentamycin	++ ve
Chloramphenicol	+ ve
Tetracycline	+ ve
Ampicillin	-ve
Colistin sulphate	- ve
Streptomycin	- ve
Lincomycin	-ve

DISCUSSION

In our study we recovered C. jejuni from reproductive tract beside the internal organs and cloacal swabs this result is in agreement with that reported by Camarda *et al.*, (2000).

The present study revealed isolation of C. jejuni from 31.4% of examined cloacal swabs this percent is less than that recorded by Modugno *et al.*, (2000) who detected C. jejuni from 73% of examined Cloacal swabs in laying hens. The authors also isolated this organism from the oviducts in percentage of (29.4%), this percent is higher than that we recorded in our isolation, where we found C. jejuni in 12% of examined oviducts. The comparatively low percent of isolation that recorded by our result may be due to the explanation of Wegmuller *et al.*, (1993) that the method of culturing with selective enrichment may lose sensitivity because of non optimal growth conditions and the polymerase chain reaction (PCR) has been more sensitive and now the

direct Colony hybridization method is sufficienty sensitive to detect small numbers of C. jejuni in chickens.

C. jejuni biotype I and II were common, this result is similar to that reported by Modugno *et al.* (2000).

The experimentally infected 1day old chicks showed signs of depression and diarrhea, we agreed the result of Ruiz – palacios *et al.* (1981). On the other hand we differ with them in the mortality rate where they recorded low percentage of mortality (32%) compared with 70% in our experiment.

Gross lesions in inoculated 1day old chicks revealed distenstion of intestine to the two cecai with accumlation of the mucus with areas of haemorrhage and hepatic changes were present, this result is in agreement with that observed by Sanyal *et al.* (1983) and Welkos (1984).

The experimental birds kept in contact with inoculated chick revealed signs and lesions similar to that in inoculated chick but in mild picture, this result is somewhate similar to that obtained by Clark and Bueschkens (1988) where they also found beside these lesions focal hepatic necrosis. The reason that contact chicks infected from inoculated one was explained by Lindblom *et al.* (1986) and Pakamunski *et al.* (1986) that the chicks are coprophagic by nature and C. jejuni readily colonizes in the chick, a rapid transmission through an entire flock could be expected. Also Evans (1992) noticed that once C. jejuni is present in a flock, the feed, water, litter and even the air rapidly become contaminated and help to disperse the organism.

The experimentally infected 4 week old chicks showed mild sigs and lesions compared with the group I, We agrred the result of Engvall *et al..*, (1986), Hoop and Ehrsam (1987), where they observed that the flock usually become infected without clinical signs when the chicks are three to five week old, but infection has been observed as early as seven days old.

The sensitivity of the isolates to different antibiotics revealed that Naladixic acid, Erythromycin and Gentamycin were the most effective drugs, this result is similar with the result reported by Das *et al.* (1996). The isolated organism was resistant to Ampicillin as reported by Erdger and Diker (1995).

REFERENCES

Camarda. A, Neweli, D.G., Nasti, R. And Modugno, G.Di (2000): Geno typing Campylobacter jejuni Strains isolated from the gut and oviduct of laying hens. Avian Diseases 44 : 907-912.

Clark, A.G. and Bueschkens, D.H. (1988): Horizontal spread of human and poultry – derived strains of Campylobacter jejuni among broiler Chickens held in incubators and Shipping boxes. Journal of food Protection, 51, 438 – 441.

Das, S.C., Mullick, S.G. and Biswas, G. (1996): Isolation and identification of Campylobacter from poultry: biotyping and in vitro antimicrobial sensitivity. Indian Journal of veterinary Research. 5(1) 29-34.

Diemair, W. (1957): laboratorium subchfur – lebensmittel chemiker., 7 Auflag, verlag von theador steimkoff, leipzig

Engvall, A., Bergqvist, A., sandstedt, K. and Danielsson–Tham, M.L. (1986): Coloniztion of broilers with Campylobacter in convertional broiler – chicken flocks. Acta veterinaria Scandinavica, 27, 540-547.

Erdger, J. and Di Ker, K.S. (1995): Multiple antibiotic resistance in Poultry isolotes of Campylobacter. Veterinar fak Itesi Dergisi Ankara universitesi, Ankara, Turkey.

Evans, S.J. (1992): Introduction and spread of thermophilic Campylobacters in broiler flocks. Veterinary Record. 131,574-576.

Fnnell, C.L., Totten, F.A., Ouinn, T.C., Patton, D.I., Molmes, K.K. and Stamm,W.E.(1984): Characterization of Campylobacter – like organism isolated from homosexualmen J. Inf. Dis. 149: 58-66.

Hoop, R. and Ehrsam, H. (1987): Ein Beitrag zur Epidemiologie von Campylobacter jejuni and Campylobacter Coli in der Huhuermast. Schweizer Archiv fur Tierheilkinde, 129, 193-203.

Lindblom, G.B., Sjogren, E. and kaijser, B. (1986): Natural Campylobacter Colonization in chickens raised under different environmental conditions. Journal of Hygiene, 96,385-391.

Lior, H. (1984): New extended biotyping Scheme for Campylobacter jejuni, Campylobacter coli and Campylobacter laridis. Journal of Clinical Microbiology, 20,636-640.

Modugno, G.D, Camarda, A., Circella, E. and Ficarella, V. (2000): Epidemiological features of Campylobacter jejuni infection amang fowls. Selezione veterinaria 11,978-997.

Mossel, D.A.A. (1985): Media for Campylobacter jejuni and other Campylobacters. International Journal of food Microbiology, 2,119-122.

Munroe, D.L., Prescott, J.F and Penner, J.L. (1983): Campylobacter jejuni and Campylobacter coli Serotypes isolated from Chickens, Cattle and Pigs. Journal of Clinical Microbiology, 18,877-881.

Pakamunski, S., Kass, N., Borochovich, E., Marantz, B. and Rogol, M. (1986): Incidence of Campylobacter Spp. In broiler flocks monitored from hatching to slaughter. Avian Patholgy, 15,83-92.

Park, C.E., Smibert, R.M., Blaser, M.J. Vanderzant, C. and Stern, N.J. (1984): Campylobacter in: " Compendium of methods for the microbiological examination of foods" 2nd Ed. Speck. M. L. (ed) American Public Health Association Washington, D.C.

Ruiz-Palacios, G.M., Escamilla, E. and Torres, N. (1981): Experimental Campylobacter diarrhea in chickens. Infection and Immunity, 34,250-255.

Sanyal, S.C., Neogi, P.K.B., Islam, K.M.N. and Huq, M.I. (1983): Pathogenesis of Campylobacter jejuni diarrhae in an experimental model in: Pearson, A. D., Skirrow, M. B., Rowe, B., Davies, J. R. and Jones, D. M. (eds) Campylobacter II P. 122 (london, Public Health laboratory Service).

Sebald, M., and M. Veron (1963): Teneur en bases de l'dn et Classification des vibrions. Ann Inst Pasteur (Paris) 105:897-910.

Shane, S.M. (1992): The significance of Campylobacter jejuni infection in poultry: a review. Avian pathology. 21: 189-213

Simmons, N.A. and F.J. Gibbs (1979): Campylobacter Spp. in oven-ready Poultry. Journal Infection. 1:159-162.

Skirrow, M.B. (1977): Campylobacter enteritis : a "new" disease. British medical Journal 2,9-11

Skirrow, M.B. (1990): Campylobacter Lancet. 336, 921-923.

Skirrow, M.B. and Benjamin, J. (1980): "1001" Campylobacters : Cultural characteristics of intestinal Campylobacters from man and animals. Journal of Hygiene, 85,427-442.

Ullmann, U. (1979): Methods in compyloacter, In " Methods in Microbiology" 13th Ed. Bergan, T. and Norris, J.R. Academic Press, New York: 435-452.

Varnam, A.H. and Evans, M.G. (1991): Campylobacter in : " food borne Pathogens" Wolfe Publishing Ltd. London England.

Wegmuller, B., Luthy, J. and candrian U. (1993): Applied and Environmental Microbiology. 59:2161-2165.

Welkos, S.L. (1984): Experimental gastroenteritis in newlyhatched chicks infected with Campylobacter jejuni. Journal Medical Microbiology 18:233-248.

Fig. 1:
Distenstion of the intestinal tract in experimentally infected chick by C. jejuni