Pathological And Bacteriological Studies On Campylobacter In Pigeon At Sharkia Governorate

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ABSRACT

One hundred pigeons, 2 - 40 weeks old, were collected from different farms at sharkia province representing 34 flocks, suffering from depression, watery or bloody diarrhea,and loss of body weight. Macroscopically, the intestine ,liver and spleen were congested .Necrotic foci of about 1-2 mm in diameter were seen on the hepatic surface. Bacterial isolation revealed ten bacterial isolates of *Campylobacter jejuni*. The isolates were identified morphology and biochemical with an incidence of 10% . The isolates were biotyped as biotype 1 (7) and biotype 1a (3) isolates.

Experimental infection with bacterial isolates on 20 -30 days old pigeons was carried out. The morbidity and mortality were 90% and 30% respectively . Microscopically,the epithelial lining of intestine was hyperplastic and transformed into undifferentiated cells with vacuolated cytoplasm and large vesicular nuclei (intestinal adenomatosis). The mucosa and submucosa were highly infiltrated with infilammatory cells with decreased number of intestinal crypts . The hepatic parenchyma revealed severe congested blood vessels and hemorrhages.Necrotic foci were focally replaced the hepatic tissue , infiltrated with fibroblasts and roud cells in addition to thickened capsule.Other necrotic foci are highly infiltrated with round cells mainly heterophils.Hyperplastic epithelial lining of the bile ducts was seen. The spleen showed severe depletion of the white pulp.Subcapsular and red pulp infiltiration with round cells was detected. All *Campytobacter jejuni* isolates were found highly sensitive to gentamycin, cholestine and trimethobrim

INTRODUCTION

Pigeons had been domesticated thousands of years ago. Nearly all houses of Egyptian villages raise pigeons where it is considered a source of protein supply for human because of it's palatability and cheap price.

The genus campytobacter species and specifically *Campylobacter jejuni* is the predominant species causing a disease in both breeding and commercial flocks of chicken, turkey, pigeons, ducks ***(1)*** and mammalian species ***(2, 3)***

Campylobacter is the cause of contagious diseases of chicken and ducks characterized by low mortality, high morbidity and chronic course. The disease is associated with significant reduction in egg production ***(4, 5)***.The post mortem recorded in chickens and quails are mainly in the liver , where it appeared congested,friable beside supcapsular haemorrhges ***(6, 7)***. Focal hepatic necrosis and moderate mononuclear cells infiltiration could be observed in illium and cecum of chicken and quails ***(8,9)***.

The purpose of this study was to record the pathological changes related to infection by *Campylobacter jejuni* in pigeons at Sharkia governorate with an attemps to detect the drug of choice against the isolates.

MATERIAL AND METHODS

One hundred diseased pigeons suffering from diarrhea at different ages (2-40 weeks) were collected from 34 flocks at different localities, Table (1). Bacteriological examination and postmortem changes were carried out and recorded .

Table 1. Localities and number of examined pigeons .

Locality	Age / weeks	Number of examined pigeons
Kanayat	2-40	15
Dearb negem	2-30	25
Abokaber	2-35	12
Zagazig	2-38	8
Belbis	2-25	18
Abo-Hamad	2-40	22

Experimental birds

For study the pathogenicity of campylobacter in pigeons,40 balady pigeons, 20-30 days old were purchased from the markets .They were apparently healthy and tested bacteriologically for campylobacter and found to be free from campylobacteriasis infection.

Media

-Thioghycolate broth ***(10)*** brucella sheep blood agar ***(11)***

-Campylobacter was isolated ***(12)*** and identified ***(13,14)*** as previousiy described.

The Pathogenicity

Pigeons were classified into 2 equal subgroups. Each bird of the first group was inoculated orally with 1 ml 10^9 live cells of *Campylobacter jejuni* isotates. The 2nd group kept as control .All birds were kept under observation 14 days. The mortality and morbidity rates, symptoms and postmortem lesions were recorded daily. Intestinal and cecal content from dead pigeons were cultured and the disc diffusion techniques was applied to detect the drug of choice against the isolates.

Histopathological examination

Specimens from intestine ,liver and spleen were collected from the natural and experimental infected pigeons and fixed in 10% buffered neutral formalin solution. Five-micron thick paraffin section were prepared,stained with H&E and examined microscopically ***(15).***

RESULTS

The clinical sings

The infected pigeons suffered from depression, watery or bloody diarrhea,and loss of body weight.

Bacterial isolation

Ten isolates could be isolated from 100 pigeons out of 34 flocks at sharkia province .

Campylobacter identification

Ten isolates could be identified morphologically, motility test. and culture basis as campylobacter colonies were small, moist, transperant gram negative and curved rods (Table 2).

Biochemical assay carried showed that the ten isolates identifiend biochemically were *Campylobacter jejuni* (Table3) .

Biotyping of *Campylobacter jejuni* revealed that the isolates belonged to 2 biotypes (7 biotype 1 and 3 isolates to biotype 1 a (Table 4)

The pathogenicity had a short incubation period 18-72 hrs with 30% mortality rate and90% morbidity rate (Table 5).

The isolate *Campylobacter jejuni* was sensetive to gentamycin, cholestine and trimethobrim (Table 6) .

Pathological findings

Macroscopically, the intestine was congested with thickened wall and the lumina filled with watery or bloody fluid. In some cases erosions and ulcers were seen in mucosa. The liver was congested, enlarged with necrotic foci of about 1-2 mm in diameter beside subcapsular hemorrahages. The spleen was slightly enlarged.

Microscopically,the intestinal mucosa was thickened with hypertrophied of tunica muscularies (Fig.1). The epithelial lining was hyperplastic and transformed into undifferentiated cells with vacuolated cytoplasm and large vesicular nuclei(intestinal adenomatosis) (Fig. 2). The mucosa and submucosa were highly infiltrated with infilamatory cells with decreased number of intestinal crypts (Fig.3). The intestinal crypts were atrophied and infiltrated with round cells beside hemorrhages (Fig.4). The number of goblet cells was decreased with depletion of lymphoid follicles (Fig.5) .In some casses the tunica muscularies and serosa were infiltrated with round cells and hemorrhages (Fig.6). Other casses showed severe desquamation of the intestinal villi which filled the lumina (Fig.7). The hepatic parenchyma revealed severe congested blood vessels and hemorrhages (Fig.8). Necrotic foci were focally replaced the hepatic tissue, infiltrated with fibroblasts and round cells, in addition to

thickened capsule (Figs.9,10). Other necrotic areas were highly infiltrated with round cells mainly heterophils (Fig.11). Hyperplastia of the epithelial lining of the bile ducts was seen (Fig.12). The spleen showed severe depletion of the white pulp (Figs.13,14). Subcapsular infiltration of round cells with cavitations of white pulp were detected (Fig15). The red pulp was infiltrated with round cells, (Fig.16).

Table 2 . Culture characteristics identification of suspected campylobacter isolates.

isolates	Growth temp			aerobic growth	Anaerobic growth	Growth in 5% Co2	Motility
	25c	37c	42c				
1	-	+	+	-	-	+	+
2	-	+	+	-	-	+	+
3	-	+	+	-	-	+	+
4	-	+	+	-	-	+	+
5	-	+	+	-	-	+	+
6	-	+	+	-	-	+	+
7	-	+	+	-	-	+	+
8	-	+	+	-	-	+	+
9	-	+	+	-	-	+	+
10	-	+	+	-	-	+	+

Table 3. Biochemical identification of suspected campylobacter isolates

Isolates No	Catalase test	Oxidase test	Glycine tolerance	H2S production lead acetate	Hippurate hydrolysis
1	-	+	+	+	+
2	-	+	+	-	-
3	-	+	+	-	+
4	-	+	+	+	+
5	-	+	+	+	+
6	-	+	+	-	+
7	-	+	+	+	-
8	-	+	+	+	+
9	-	+	+	+	+
10	-	+	+	+	+

Table 4. Biotyping C-jejuni isolates.

Isolates	Hipurate hydrolysis	Rapid H2S	DNA Hydrolysis	Biotype
1	+	-	-	1
2	+	-	-	1
3	+	-	+	1a
4	+	-	+	1a
5	+	-	-	1
6	+	-	-	1
7	+	-	-	1
8	+	-	+	1a
9	+	-	-	1
10	+	-	-	1

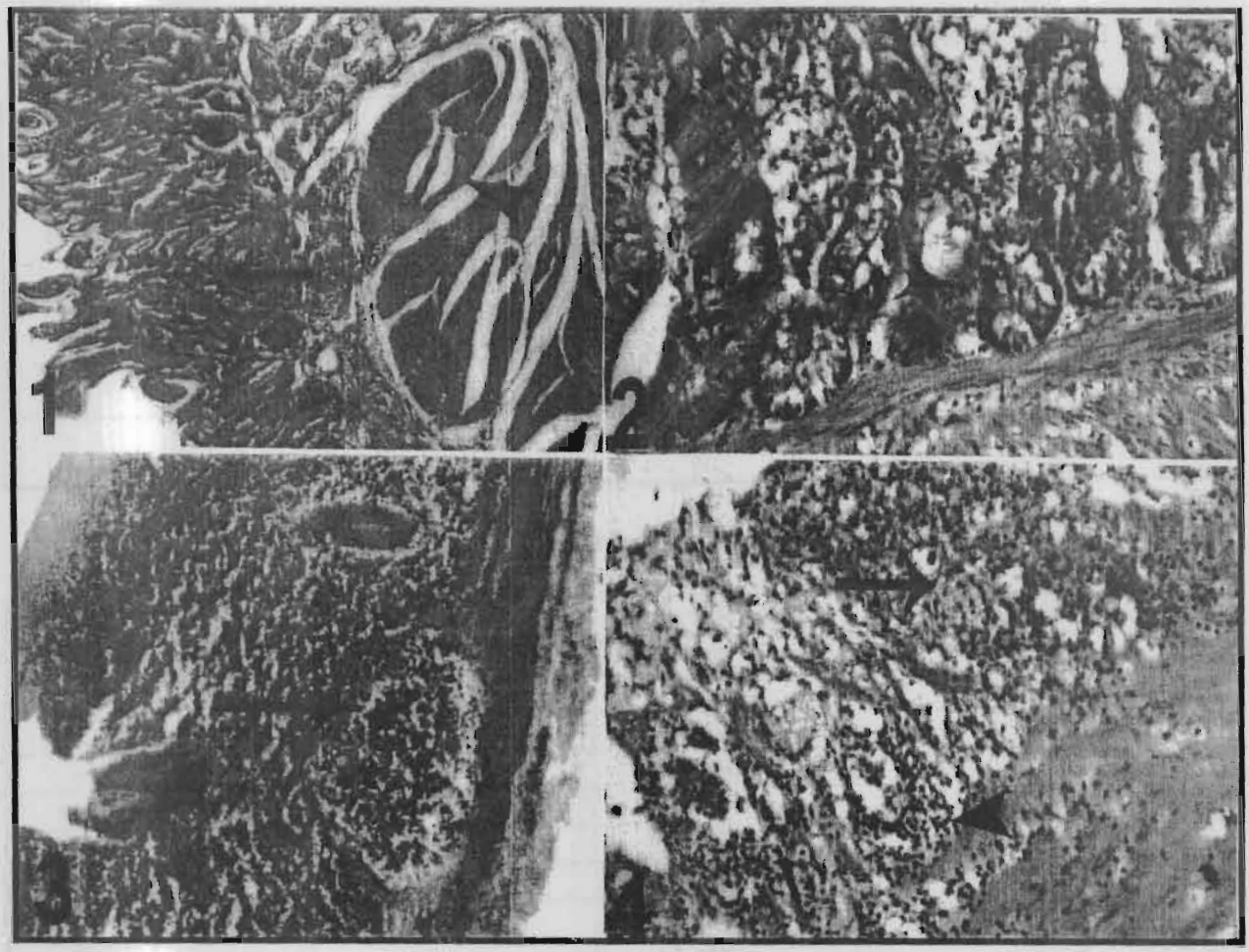
Table 5 Results of oral infection with C-jejuni in pigeons.

Group No	Number Of Birds	Dose / Birds	Rout	Morbidity	Mortality	P.M	Reisolation
1	20	10^{9cfu}	orally	18/20	6/20	Small necrotic foci on the surface of liver, enteritis and enlarged spleen	+
control	20	-	-	-	-	-	-

Table 6. Result of *in vitro* sensitivity testing of isolation C.jejuni strain.

Antimicrobial agent	Disk potency	Standerd sensitivity zone	
Gentamycin	10	>15>19	+++
Naldixic acid	3	>16>19	++
Flumequien	30	>13>18	+
Neomycin	30	>15>19	+
Cholistine	30	>13>18	+++
Oxytetracycline	30	>14>18	++
Trimethoprime	1.25+2375	>11>15	+++
Kanamycine	30		R
Ampicillin	30		R
Novoblocin	30		R

+++= high sensitive ++ intermediate R=resistance



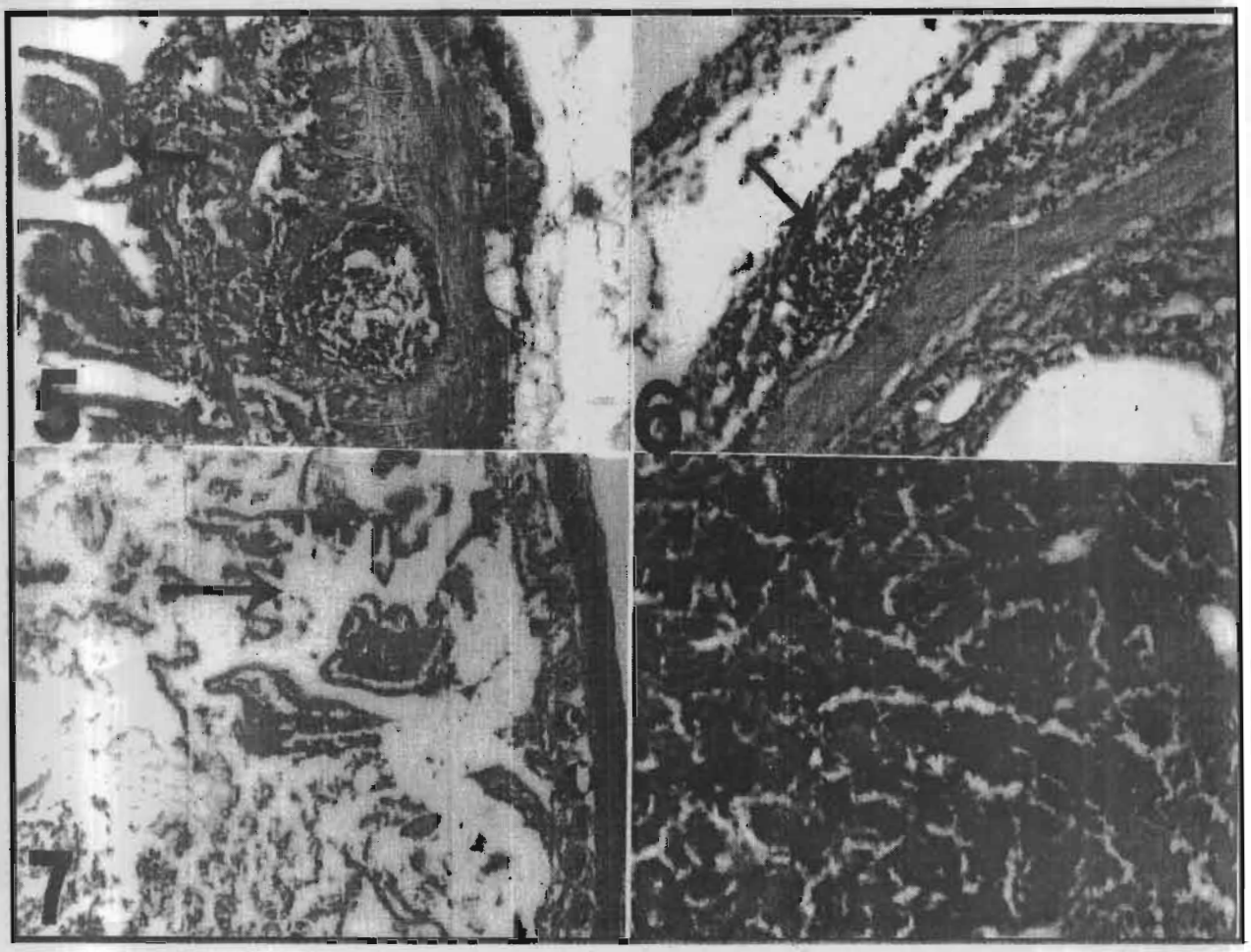
Figs.1-4. Intestine showing

Fig.1. Thickening mucosa with hypertrophied of tunica muscularies. H & E x 150

Fig.2. Hyperplasia of the epithelial lining which transformed into undiffeientiated cells with vacuolate cytoplasm and large vesicular nuclei (intestinal adenomatosis). H & E x 1200

Fig.3. The mucosa and submucosa, infiltrated with infilamatory cells with decreased number of intestinal crypts. H & E x 300.

Fig.4. The intestinal crypts were atrophied and infiltrated with round cells beside hemorrhages. H& E X 600.



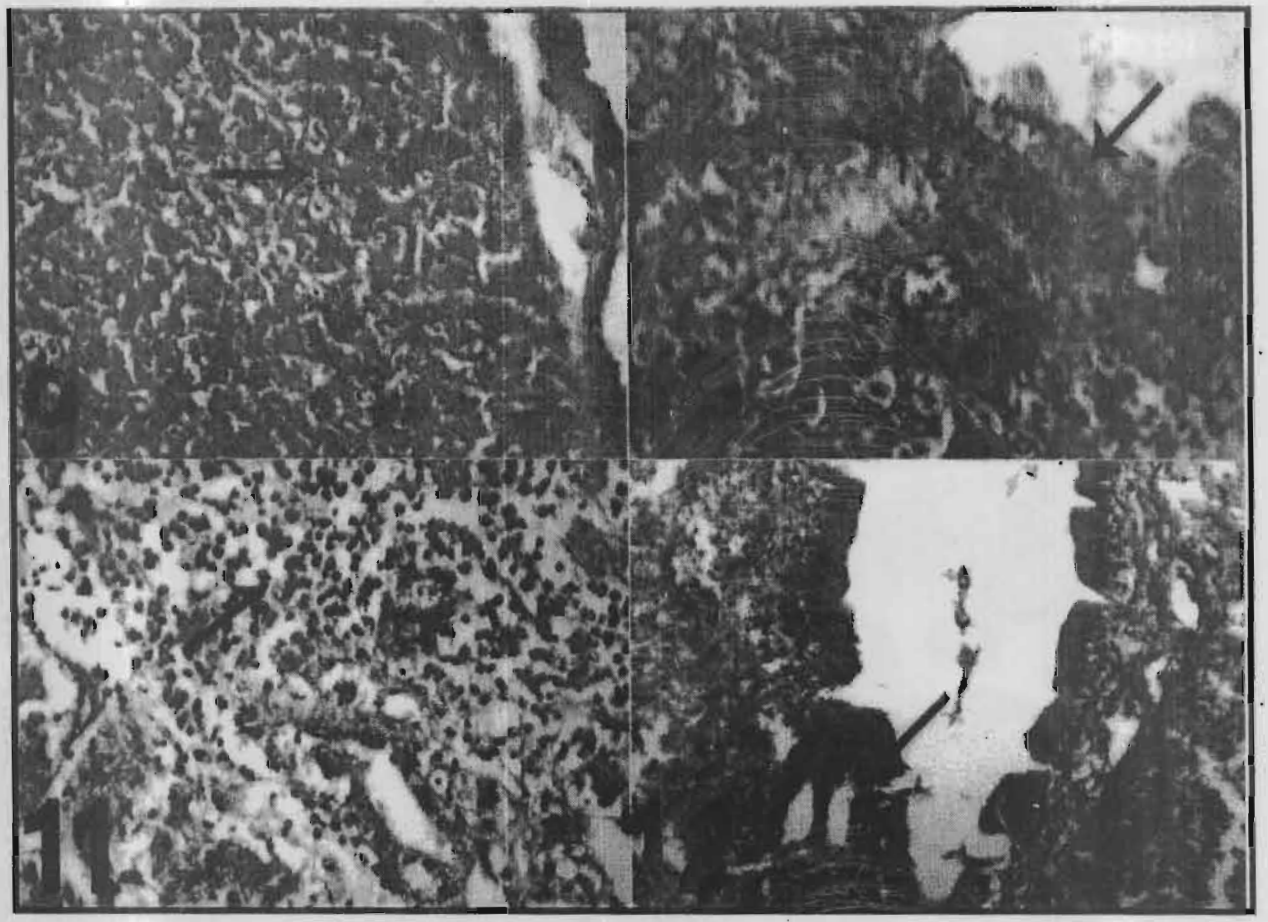
Figs. 5-7. Intestine showing

Fig.5. Decreased number of goblet cells of mucosa with depletion of lymphoid follicles. H & E x 300.

Fig.6. The infiltration of tunica muscularies and serrosa with round cells beside hemorrhages. H & E x 600.

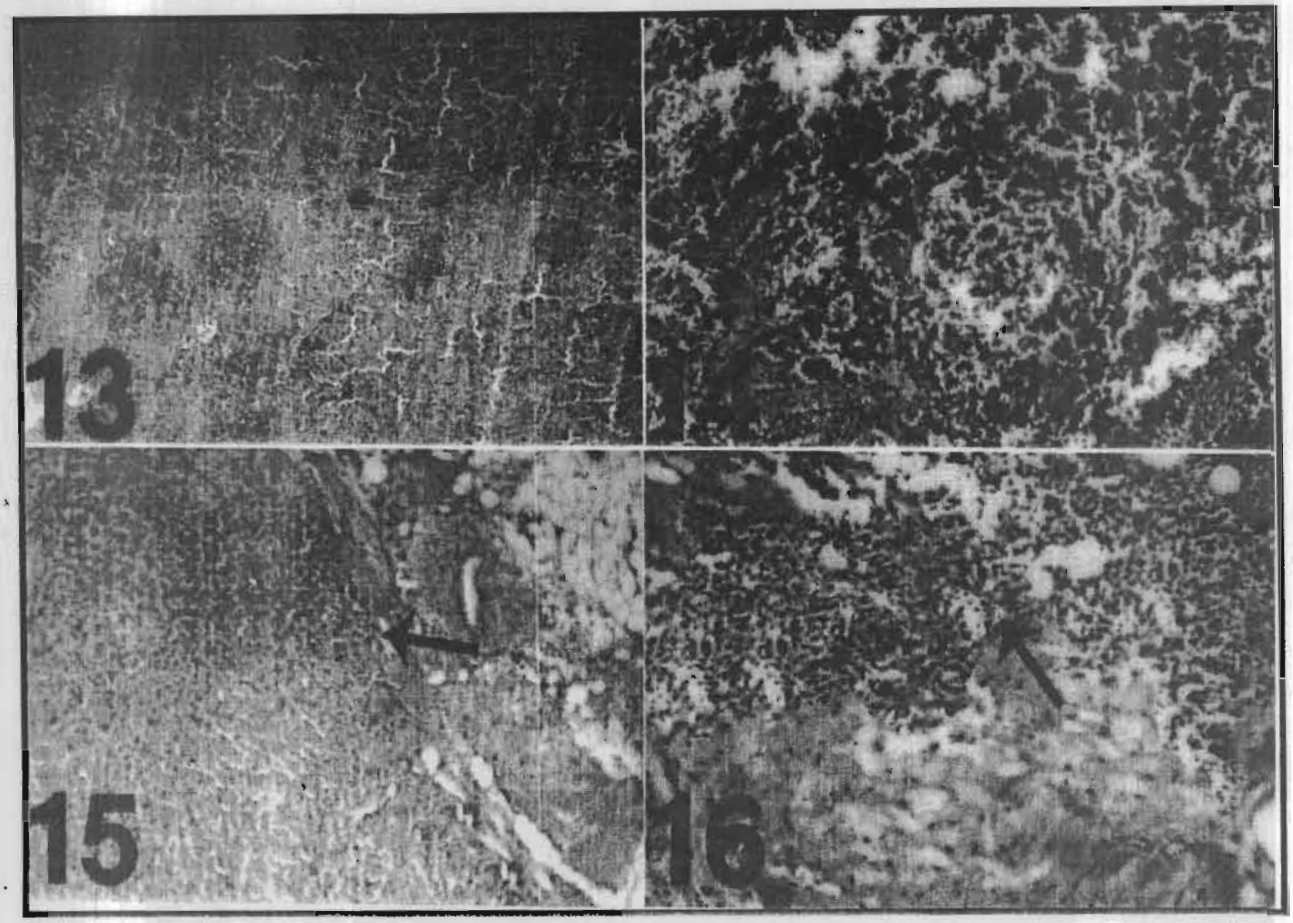
Fig.7. Severe desquamation of the intestinal villi which filled the lumina. H & E x 150.

Fig.8. Liver showing, severe congested blood vessels and hemorrhages. H & E x 300.



Figs.9-12. Liver showing

Fig.9. Large area of necrosis in addition to thickened capsules . H & E x 600.
Fig.10. The high power of the previous figure showing, infiltrated with fibroblasts and, roud cells. H & E x 1200
Fig.11. The necrotic areas highly infiltrated with round cells mainly heterophils. H & E x 200.
Fig.12. Hyperplasia of the epithelial lining of the bile ducts .. H & E x 600.



Figs.13-16. Spleen showing

Fig.13. Severe depletion of the white pulp H & E X 150 .
Fig.14. The high power of the previous figure . H & E X 600.
Fig 15. Subcapsular: infiltiration of round cells and cavitations of the white pulp. H & E X 300.
Fig 16. Infiltration of the red pulp with round cells. H & E X 600 .

DISCUSSION

Our work revealed that pigeons infected with *Campylobacter jejuni* ,suffered from depression, watery or bloody diarrhea,and loss of body weight and the same findings were cited previously ***(9)*** recording the same clinical signs in quails infected with *Campylobacter jejuni*. ***Synial et al.(16),*** found that the main clinical signs of chicken infected with campylobacter were watery and bloody diarrhea. Isolation of *Campylobacter jejuni* showed that only ten *Campylobacter jejuni* strains out of 100 examined cases were positive (10%), other recorded 19% ***(17)*** at Assuit province, Egypt. The lower percent of isolation may referred to the wide use of prophylactic medication

Campylobacter isolation from internal organs as intestine, gallbladder and liver were suitable sites for isolation ***(1,18)***. Inoculation of *Campylobacter jejuni* isolate to 20 days old pigeons showed that postmortem findings were confined to liver, spleen, kidnay and intestine similar findings were observed by others ***(1,19,20).***

Macroscopically, The intestine was congested with thickened wall and the lumina filled with watery or bloody fluid.In some casses erosions and ulcers were seen in mucosa . The liver was congested, enlarged with necrotic foci 0f about 1- 2 mm in diameter beside subcapsular hemorrhages. The spleen was slightly enlarged. Our results are concided with other investigators ***(9)***, they recorded the same lesions in liver and intestine only .Microscopically, the intestinal mucosa was thickened with hypertrophied of tunica muscularies. The epithelial lining was hyperplastic and transformed into undifferentiated cells with vacuolated cytoplasm and large vesicular nuclei(intestinal adenomatosis). The term intestinal adenomatosis in the infection with *Campylobacter jejuni* was mentioned early ***(21),*** and may be due to the continous replication of the microbe inside the epithelial lining ***(22)***. The mucosa and submucosa were highly infiltrated with infilamatory cells with decreased number of intestinal crypts which are similar to those previously described ***(23)***. The authers noticed a moderate mononuclear cells in mucosa and submucosa of chickens experimantly infected with *Campylobacter jejuni*.The intestinal crypts were atrophied and infiltrated with round cells beside hemorrhages and the number of goblet cells were decreased which may be changed to undiffretiated adenomatotic cells ***(22)***. In some casses the tunica muscularies and serosa were infiltrated with round cells and hemorrhages. Other casses showed severe desquamation of the intestinal villi which filled the lumina. The hepatic parenchyma revealed severe congested blood vessels and hemorrhages.Necrotic foci were focally replaced the hepatic tissue ,infiltrated with fibroblasts and roud cells in addition to thickened capsule. Other necrotic areas were highly infiltrated with round cells mainly heterophils. Hyperplastic of the epithelial lining of the bile ducts was seen. The spleen showed severe depletion of the white pulp .Subcapsular infiltiration of round cells was detected with cavitations of white pulp. Our results are consistent with those recorded in previous investigations ***(9)*** .In addition to the previous causes, also the main previous pathological findings may be due to the toxic activity of *Campylobacter jejuni*. It has been proved that the *Campylobacter jejuni* secrete enterotoxin similar to the LT-toxin of Vibrio cholerae causing thickness with hypertrophied of tunica muscularies.In addition to ,the cavitations of the white pulp may be due to the necrotic lymphocytes.

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الملخص العربى

دراسات باثولوجيه وبكتريولوجيه فى الحمام فى محافظه الشرقيه

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تم تجميع مائة حمامة تتراوح أعمارها بين ٢ الى ٤٠ اسبوع يعانون من الاسهال ويمثلون ٣٤ قطيع بمحافظة الشرقية وتم فحص الحمام اكلينيكيا وأجراء الصفة التشريحية والعزل البكتريولوجى والفحص الباثولوجى للأعضاء المصابة

واظهرت الدراسة عن ١٠ معزولات من الكمبيلوباكتر جوجوناى وتم التعرف عليها من خلال الشكل الظاهرى والتفاعلات البيوكيميائية .

وكانت نسبة الاصابة فى الحمام ١٠ % كما تم تصنيف البكتريا المعزولة I بايوتايب (٧معزولات) وبايو تايب IA (٣ معزولات)، وتم احداث العدوى الصناعية بالكمبيلوباكتر جوجوناى فى حمام عمر ٢٠-٣٠ يوم وكانت نسبة الاصابة والنفوق ٩٠ % ، ٣٠ % على التوالى٠

وكانت التغيرات الباثولوجيه عباره عن تضخم بالامعاء مع سائل مدمم بداخلها مع تضخم بالكبد ووجود بقع متنكرزه و بقع دمويه بالسطح الخارجى بالاضافه الى تضخم الطحال وبالفحص الميكروسكوبى وجد ان بالامعاء تضخم بالطبقات المختلفه بالجدار مع تكاثر بخلايا الامعاء وتحورها الى خلايا غدانيه مع ارتشاحات لكرات الدم البيضاء و وجود انزفه بين الخلايا ٠اما الكبد فحدث احلال لانسجته ببقع متنكرزه مع ارتشاحات دمويه مع كثره الخلايا الدفاعيه ٠اما الطحال فحدث نقص شديد للخلايا الليمفاويه٠

وقد اظهرت النتائج ان البكتريا المعزولة كانت عالية الحساسية للجنتاميسين و الكوليستين والترامبثوبريم ٠

ونظرا لقله الابحاث المسجله عن الدراسه الباثولوجيه والبكتريولوجيه عن الكامبيلوباكترفى الحمام قمنا باجراء هذا البحث فى محافظه الشرقيه مع ايجاد العلاج المناسب٠