Egypt. J. Comp. Path &Clinic Path. Vol.26 No.1 2013 ; 130- 145
ISSN 1110-7537

Pathogenesis of *Enterobacteriaceae* Isolated from Commercial Chicken Eggs in Broilers

By

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ABSTRACT

Enteric bacterial infections in poultry pose a threat to intestinal health and can contributed to poor feed efficiency and livability of a flock. A variety of enteric bacterial diseases are recognized in poultry. In our study we investigated the pathogenesis of four bacterial strains ***E coli, E Sakazakii, E. fergusonii, and P. mirabilis*** belonging to Enterobacteriacea isolated from chicken egg samples and cloacal swabs from Elminya and Beni-Suef governorates. They used to reproduce its pathogenesis in one hundred and twenty Hybrid broiler chicks divided into 4 groups each one of 30 chicks in number infected at 12th day of age, all groups were inoculated by 0.5 ml s/c Enterobacteriaceae isolates with different infective dose of each bacteria isolate using McFarland standard. Clinical signs, postmortem lesions, and histopathological findings were recorded. Generally, these bacterial isolates induced signs that differed in severity and pathobiology.

This study point out the pathogenicity of the used isolates to chickens, induced signs, pathological lesions and their effect on broiler productivity.

INTRODUCTION

E. coli is a complicating factor for other disease conditions including swollen head syndrome (SHS), and Chronic Respiratory Disease (CRD) that affect mainly bird performance specially in

growing birds as poor feed conversion and increase rate of downgraded carcasses ***(Shane, 1983)***. Enterobacteriaceae is a family of rod-shaped, aerobic, facultative anaerobic bacteria. The Enterobacteriaceae family is subdivided into 8 tribes including: Escherichieae, Edwardsielleae, Salmonelleae, Citrobactereae, Klebsielleae, Proteeae, Yersineae, and Erwineae. ***Cecilia Rosario Cortés et al (2004)*** reported that E .coli were the most common bacterium that recovered from all samples except the sawdust and fertile eggs collected from the nest. Fertile egg contamination at breeder farm level was found to be minimal. ***Shalaby and Abd El-Hamid (1987)*** reported that the isolated E. coli in prevalence of 44.5% from hatching eggs which responsible for embryonic mortalities in hatcheries. ***Israa and Majeed (2011)*** conducted to detect E.coli in hatching eggs and premises in poultry hatcheries. Results revealed isolation of E.coli, Klebsiella sp. Proteus sp. and pseudomonas sp. ***Ramnoff, (1960).*** Fertilized eggs contaminated with micro-organisms may result in weak chicks, poor chick growth and low feed conversion rate. ***Jones et al (2011)*** studied the prevalence of coliforms, Salmonella, Listeria and Campylobacter associated with eggs and the environment of conventional cage and free range egg production flocks of laying hens. ***Ardrey et al, (1968)*** isolated E coli from droppings contaminated eggs of layers at the level of 2.7%.Colibacillosis is an infectious disease caused by the bacterium Escherichia coli and is seen in poultry flocks worldwide. E. coli can cause an infection under the skin, known as cellulitis, and is commonly associated with respiratory disease in birds, which in severe cases leads to septicaemia and death. Avian colibacillosis primarily affects broiler chickens between the ages of 3 and 6 weeks as well as one day-old chicks and layers and is responsible for a significant proportion of the mortality found in poultry flocks. This mortality, treatment of the disease and decreased feed conversion efficiency result in significant costs to the poultry industry ***(Radwan, 2000).*** *Proteus sp., Enterobacter sp., Pseudomonas sp., Klebsiella sp., Staphylococcus sp., Streptococcus sp., Clostridium sp., Bacillus cereus, S. typhimurium and Enterococcus* has been isolated from hatching eggs. However, the most common isolated bacterium is *E. coli.* ***(Sarma et al., 1985).***

Ibrahim (1997) found that, in the experimental infection of chicks with E coli at 1, 7 and 11 days of age , The main clinical symptoms were respiratory signs (40%), and

pasty vent (30%). The mortality rate during the experiment was 27.5% at different intervals. The P.M. lesions were congestion and enlargement of the liver and gizzard with air saculitis and cellulitis.

This study was done to investigate the pathogenicity of some Enterobacteriaceae isolates isolated from chickens and eggs through different Performance parameters including; clinical signs and /or mortality, postmortem lesions, food consumption and body weight, as well as histopathological examination.

MATERIALS AND METHODS

I- Experimental Chicks:

One hundred and fifty 1 day old broiler hybrid chicks were purchased from Cairo Co. and raised on non medicated diet free from anticoccidial and antibiotic on deep litter. Feed and water were consumed ad-libitum with continous source of light.

II-Bacterial-isolates

E. coli, E. Sakazakii, E. fergusonii, and P. mirabilis previously isolated according to ***Amer et.al. (2013)***. The tested bacterial strains were used in the following dose/ chicks using McFarland standard **Table (1)** Subcutaneously inoculations of 0.5 ml suspension of previously mentioned isolates, then the birds were kept under daily observation for clinical signs, mortality, and post mortem lesions ***Marilda et al. (1990).***

Table (1) the infected dose of tested bacterial strains

Bacterial type	McFarland standard	Number of bacterial Colonies
E coli	2	$6X10^8$ CFU/ml
E. Sakazakii	2	$6X10^8$ CFU/ml
E .fergusonii	1	$3 X10^8$ CFU/ml
P. Mirabilis	2	$6X10^8$ CFU/ml

III- Experiment design

The 150 one day old broiler hybrid chicks were divided into 5 groups and the Fifth group (30 chicks) kept as control –ve non infected group **Table (2)** .

Table (2) groups of infected chicks

Groups	Number	Infected groups
1	30	chicks infected with *E.coli* isolate
2	30	chicks infected with *E.Sakazakii* isolate
3	30	chicks infected with *E. fergusonii* isolate
4	30	chicks infected with *P. mirabilis* isolate
5	30	Control -ve

IV- Chicken performance

Daily observation of all groups including; the clinical signs and /or mortality, postmortem lesions, food consumption and body weight were recorded.

V- Histopathological examination

Tissue samples were taken from each group at 3rd ,6th , 9th, 12th , and 15th day post infection (dpi) from liver and intestine of 3 sacrificed birds from infected and control chicken groups immediately after cervical dislocation. All the specimens were fixed in 10% formol saline for processing. Sections of 5 µm thick were routinely stained with hematoxyline and eosin and examined microscopically for histopathological lesions **Bancroft and Stevens (1996)**.

RESULTS & DISCUSSION

This experiment was carried out to investigate the pathogenicity of Enterobacteriacea isolates from commercial chicken eggs, both table and fertilized type. Enterobacteriaceae used in this study includes *E coli, E Sakazakii, E. fergusonii and P. mirabilis.*

Chicken performance results

Both clinical signs and postmortem lesions were recorded for **Group 1 (infected with *E.coli* isolate).** At 1st dpi, the clinical signs were dullness, depression, white and brown diarrhea in 5 (20%) chicks. These clinical signs gradually developed to respiratory signs as coughing, sneezing, Ralls and also brown diarrhea in 10 chicks from 2nd to 6th dpi. with estimation of performance parameters which tabulated in **Table(3)** and **figs (1,2),** these results agreed with ***Ibrahim (1997)*** who found that in the experimental infection of chicks with E coli at 1, 7 and 11 days of age. The main clinical symptoms were respiratory signs (40%), and pasty vent (30%). The mortality rate during the experiment was 27.5% at different intervals. Results of the current study were matched with those of ***Youssef et al (1983)*** who confirmed the pathogenicity of E coli serogroups O124:K22 in one-day-old chicks inoculated subcutaneously with 2.5×107 viable CFU/chick. The clinical signs observed for 18 hours post infection were depression, inappetance and diarrhea. The estimated performance parameters (Body weight, feed consumption, body weight gain and feed conversion rate) were lowered than control group as illustrated in **table(3).**

Fig (1): Weekly body weight gain /grams of group 1 and control

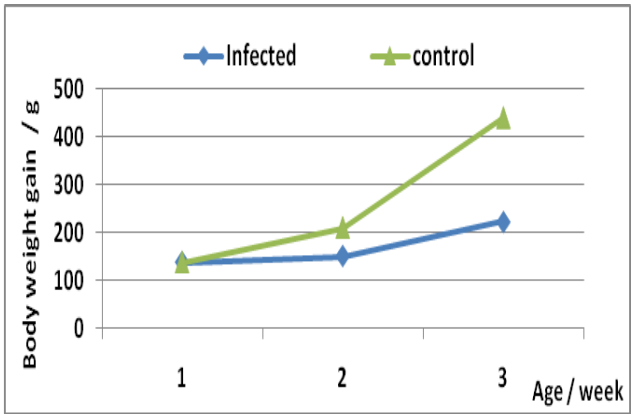


Fig (2): Weekly feed consumption/grams of group 1 and control

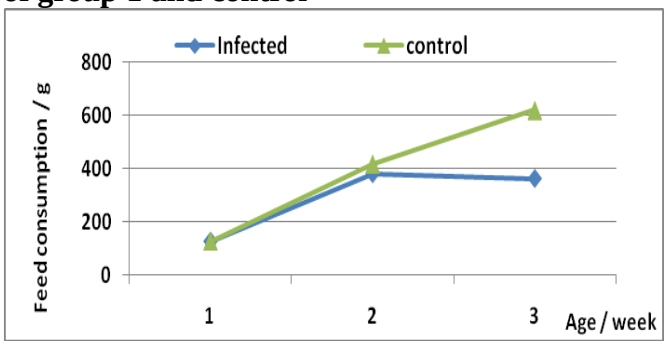


Table (3) Performance parameters of all infected groups and control

Group No	infection	Age / week	Mean Weekly body weight /gm	Mean Weekly feed consumption /gm	MeanWeekly body weight gain /gm	FCR
1	*E.coli*	1	90±48	126	138	0.9
		2	201 ± 69	382.2	151	2.5
		3	286 ± 103	364	223	1.6
2	*E. Sakazakii*	1	90 ± 48	126	138	0.9
		2	298 ± 70	382.2	156	2.5
		3	468 ± 88	453	265	1.7
3	*E. fergusonii*	1	90 ± 48	126	138	0.9
		2	303 ± 74	307.5	171.5	1.8
		3	457 ± 56	262.5	129	2
4	*P. mirabilis*	1	90±48	126	138	0.9
		2	294 ± 68	237.7	128	1.8
		3	503 ± 95	480.5	306	1.6
5	Control negative	1	90±48	126	138	0.9
		2	305±77	417.2	210	1.9
		3	616±125	620	440	1.4

Group 2 (infected with *E.Sakazakii* isolate) , The clinical signs were dullness , depression , sleepy and ruffled feather. These clinical signs gradually developed to become brown diarrhea, enlarged shank and coughing from 2nd to 4th dpi, but at 5th dpi one chick died. Estimation of performance parameters was tabulated in **Table(3)** and **fig (3,4).**

Fig (3): Weekly body weight gain of group 2 and control

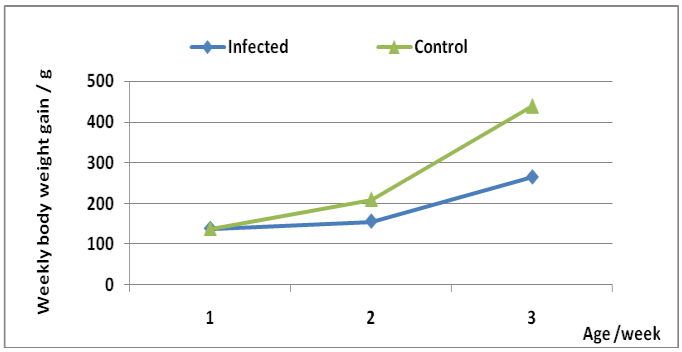
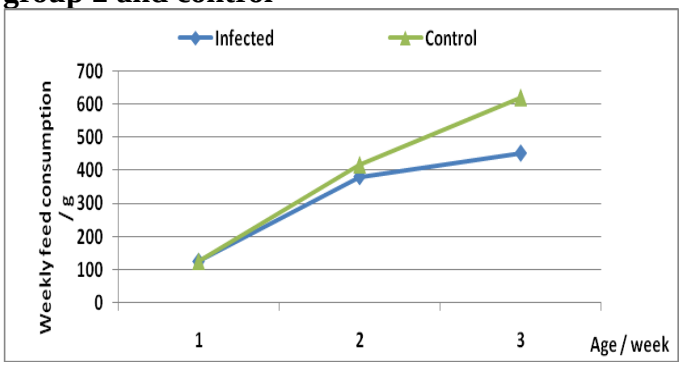


Fig (4): Weekly feed consumption of group 2 and control



The clinical signs and PM lesions were recorded for **Group 3 (infected with *E. fergusonii* isolate)** as following; at the 1st dpi, clinical signs described as dullness, depression, white and greenish diarrhea in 5 (17%) chicks, these clinical signs gradually developed to respiratory signs as coughing, sneezing and ralls from the 2nd to 7th dpi. Estimation of performance parameters was tabulated in **Table(3)** and **fig (5,6)** were; lower main weekly body weight (457 ± 56

g) , weekly feed consumption (262.5 g) , weekly body weight gain(129 g), and feed consumption rate (2) than the control group and these results were concomitant with ***Herráez et al (2005)*** who mentioned that *E.fergusonii* in Ostriches and chickens caused anorexia, prostration, and severe hemorrhagic diarrhea, dying 24 hr after the onset of clinical signs.

Fig (5): Weekly body weight gain of group 3 and control

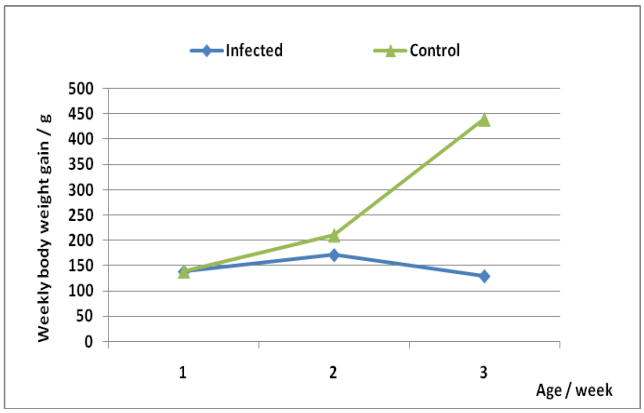
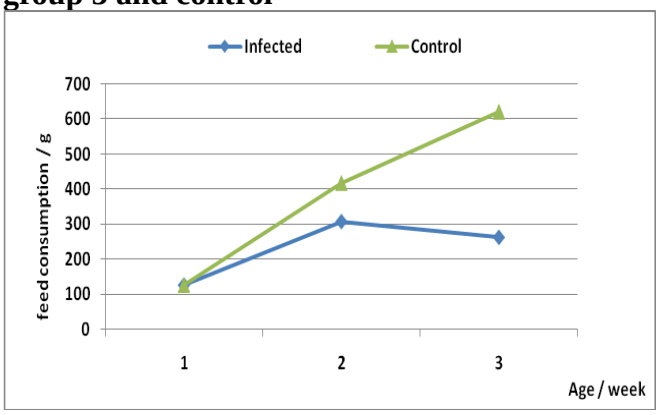


Fig (6): Weekly feed consumption of group 3 and control



The clinical signs and postmortem lesions were recorded for **Group 4 (infected with *P. mirabilis* isolate),** 1st day post infection, the clinical signs described as dullness, depression, and brown to orange diarrhea in 4 (13%) chicks, these clinical signs gradually developed to respiratory signs as coughing, sneezing, Ralls and also brown diarrhea from the 2nd to 7th dpi.With developing of signs at 8th till 11th dpi, one experimentally infected chicks died, other chicks in the same group suffered from brown diarrhea, recumbence, dullness, and depression. The performance parameters **Table (3)** and **fig (7,8)** resulted that group 4 had lower main weekly body weight (503 ± 95g) , weekly feed consumption(480.5 g) , weekly body weight gain(306g) and feed consumption rate(1.6) than control group , these results were explained when compared with the results of ***A.M.Abd - El Gwad and Thabet (2001)*** who reported that the experimental infection of P.mirabilis in one day old chicks by different route of inoculation revealed that yolk sac route was highly effective with mortality rate 55% while oral route of infection was almost less (20% mortality).

Fig (7): Weekly body weight gain of group 4 and control

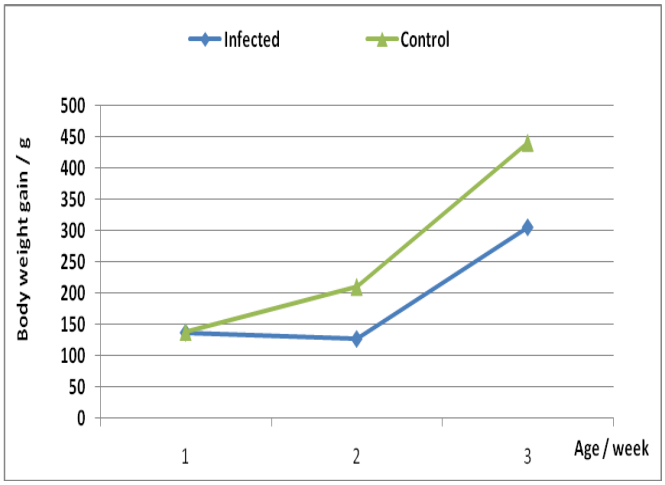
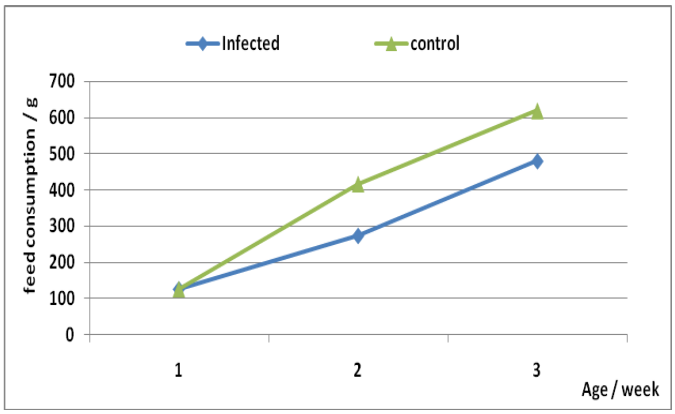


Fig (8): Weekly feed consumption of group 4 and control



Pathological results

Group 1 (infected with *E.coli* isolate);

Postmortem lesions: Congestion of liver, spleen, kidneys and lungs, pericarditis, perihepatitis and air sacculitis. The forementioned results coinsides with that given by ***Youssef et al (1983)*** who observed that the postmortem lesions were omphalitis, unabsorbed yolk sac, congestion of liver, spleen, kidneys and lungs, pericarditis, perihepatitis and air sacculitis. While ***Ibrahim (1997),*** found that the postmortem lesions were congestion and enlargement of the liver and gizzard with air saculitis and cellulitis.

Histopathological findings: In comparing with the normal hepatic histology of control negative non infected group, the examined liver showed vacuolated hepatocytes and disorganized hepatic cords at 3rd dpi**.** At 6th and 9th dpi, liver revealed focal areas of hepatic necrosis infiltrated by mononuclear cell infiltration **(plate 1A).** Congested hepatoportal blood vessels associated with portal edema were detected at the 12th dpi**.** Morover, at 15th dpi, liver revealed portal leucocytic cells infiltration **(plate 1B).**

Intestine of this group showed slight activation of mucous secreting glands, periglandular edema with few leucocytic cells infiltration at 3 dpi **(plate 1C),** sub mucosal leucocytic infiltration at 6th and 9th dpi **(plate 1D),** as well as necrotic glands at 12th dpi**,** and massive mucosal degeneration at 15th dpi**.** These results are in agreement with ***Eman Hassan et al (2012)*** who studied the histopathological examination of 12 days of age chickens of groups orally inoculated with o.4 ml of phosphate buffered saline (PBS) containing E coli (O_{78}) showed that liver central and portal veins were

moderately to markedly dilated and congested in almost all samples. Changes in the hepatic parenchyma varied from diffuse and marked vascular degeneration in which the nucleui were either pyknotic or karyolysed, Intestine showing diffused degeneration of the mucosa and desquamation of the epithelial cells that accumulate in the lumen. And with ***Esther-Maria Antao et al (2008)*** who stated that the histopathological picture of of E. coli in 1 day old chicks were perivascular as well as parenchymal lymphatic infiltrations in the liver tissue, presence of inflammatory cells in the spleen and pyknosis in the tubular epithelium as well as enlargement of the Bowman's capsule were observed in the infected kidney.

Group 2 (infected with *E. Sakazakii* isolates);

Postmortem lesions: Septicemic picture from the 1st to the 4th dpi represented by congested lung and Spleen, air sacculitis, hepatitis with streaks of hemorrhages on its surface , distended gall bladder, congested kidney , slight to moderate pericarditis, petechial hemorrhage on coronary fat, endocardium and on brisket muscle.

Histopathological findings: Liver microscopy revealed kupffer cells activation, vacuolization of hepatocytes, and large focal area of hepatic necrosis infiltrated with leucocytic cells infiltrations at 3rd and 6th dpi **(plate 2A)**. Portal tract permeation with leucocytic cells at 9th dpi **(plate 2B),** and central vein dilatation and congestion together with sinusoidal congestion at 12th and 15th dpi.

Massively damaged mucosa were recorded at 3rd and 6th dpi **(plate 2C).** At 9th dpi, massive sub mucosal gland necrosis **(plate 2D)** was noticed, while at 12th and 15th, sub mucosal leucocytic infiltrations were detected **.**

Group 3 (infected with *E. Fergusonii* isolates);

Postmortem lesions: Septicemic picture from the 1st to 4th dpi appeared as congested lung, air sacculitis and spleen, slight to moderate pericarditis, and enlarged distended ceci with gases. these results agreed with ***Herráez et al (2005)*** who mentioned that *E.fergusonii* in Ostriches and chickens on postmortem examination, the cecal mucosa showed locally extensive areas of hemorrhages and fibrino-necrotic typhlitis with a white-yellowish material covering the mucosal surface. Multiple serosal petechial hemorrhages and fibrinous peritonitis were

present.

Histopathological findings: Liver showed dilated and congested central vein at 3rd and 6th dpi. At 9th and 12th dpi, liver revealed portal tract congested vessels **(plate 3A).** At 15th dpi, there were cytoplasmic vacuolization of hepatocytes and focal area of hepatic necrosis infiltrated by leucocytic cells **(plate 3B).**

Submucosal mononuclear cells infiltration at 3rd and 6th dpi **(plate 3C)**, while at 9th, 12th , and 15th dpi, hyperactivity of the glands were recognized **(plate 3D).** These results mimic those given by ***Herráez et al (2005)*** who found that *E.fergusonii* infection in adult ostriches caused an intense mononuclear infiltration in the lamina propria and submucosa of the cecum and extensive superficial necrosis associated with fibrin and serocellular deposits. Several gram-negative bacterial colonies were observed within the necrotic areas.

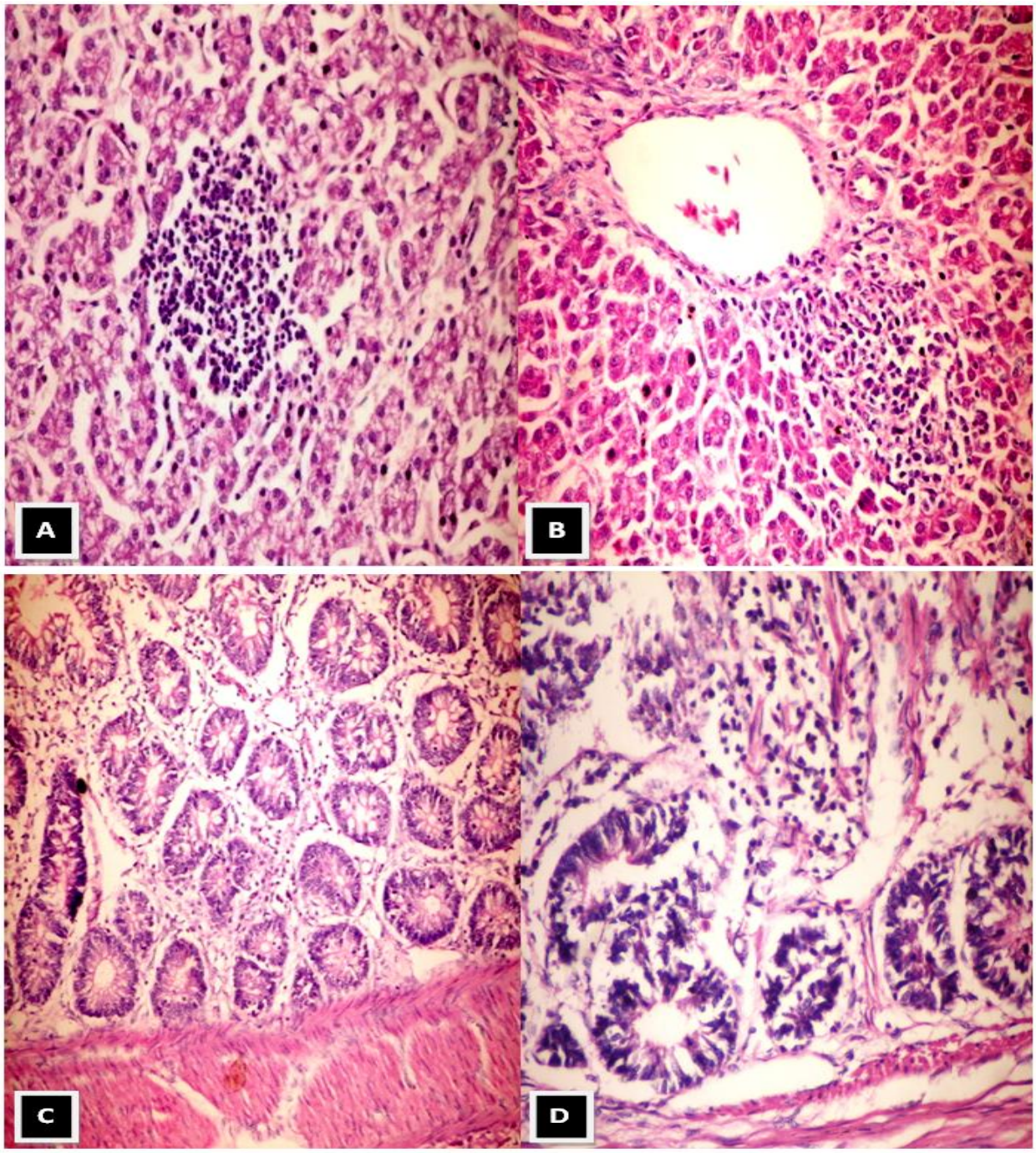
Group 4 (infected with *Pr.mirabilis* isolates) ;

Postmortem lesions: Severe septicemic picture from the 1st to 4th dpi appeared as congested lung and spleen, air sacculitis, distended gall bladder, congested kidney , severe pericarditis, diffuse petechial hemorrhage on coronary fat, endocardium and on brisket muscle,

Histopathological findings: Liver showed focal areas of necrosis infiltrated with leucocytic cells at 3rd , 6th , and 9th dpi **(plate 4A)**. And hyperplasia of epithelial lining bile duct, cholangitis, newly formed bile ductules, and massive portal leucocytic cell ifiltration at 12th and 15th dpi **(plate 4B).**

Intestinal showed necrosed glands at 3rd , 6th , and 9th dpi **(plate 4C).** And sub mucosal leucocytic infiltration at 12th and 15th dpi **(plate 4D).** These results matched well with ***Sah et al (1983)*** who descriped septicemic *P.*mirabilis infection in quail chicks.

Plate (1): Liver (A&B) and intestine (C&D) of *E. coli* infected chicken group



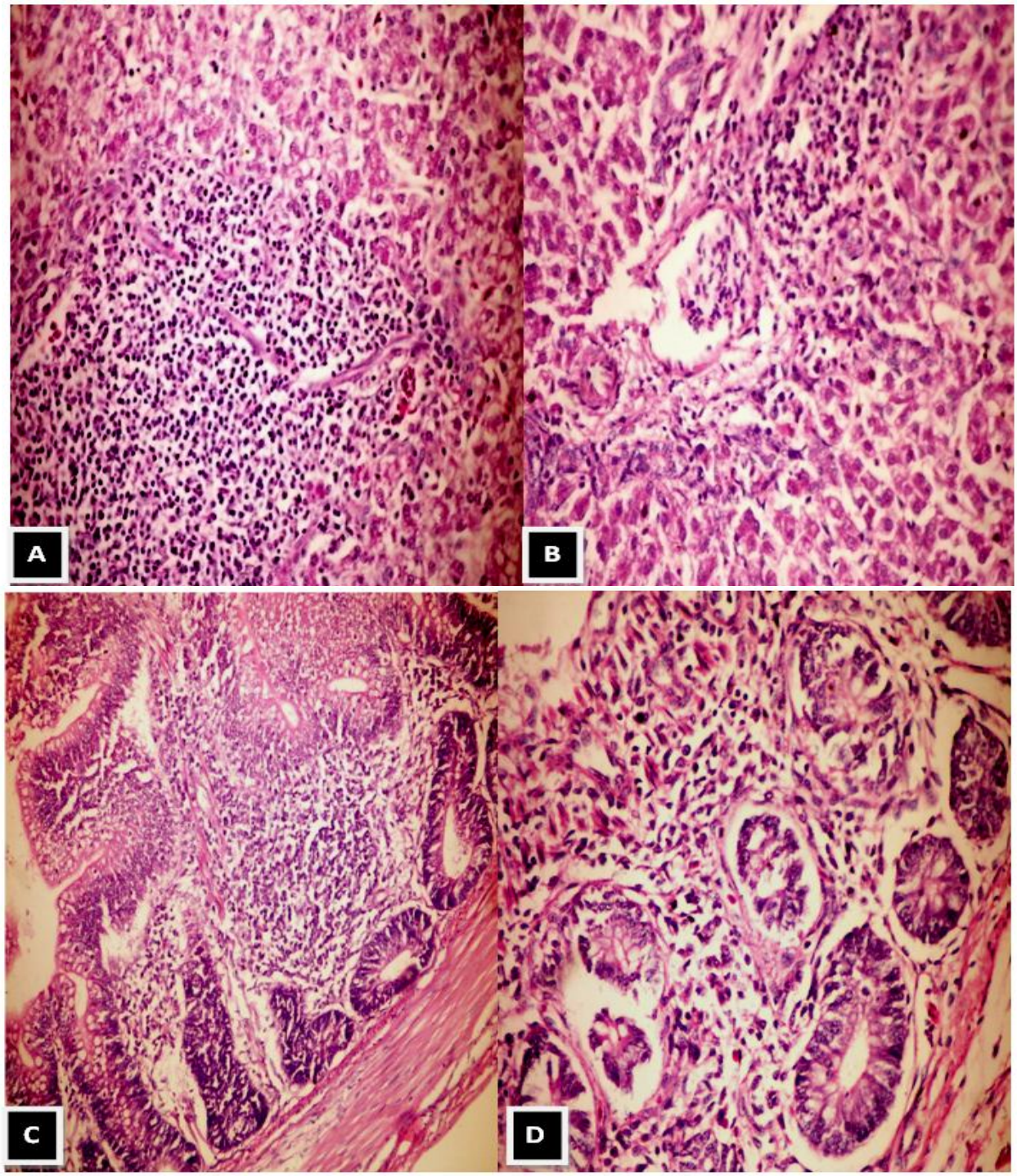
(A): Focal areas of hepatic necrosis infiltrated by mononuclear cells (H&EX 400)

(B): Portal leucocytic cell infiltration (H&E X 400).

(C): Slight activation of mucous secreting glands (H&E X 200).

(D): Sub mucosal leucocytic infiltration(H&E X 200).

Plate (2): Liver (A&B) and intestine (C&D) of *E. Sakazakii* infected chicken group



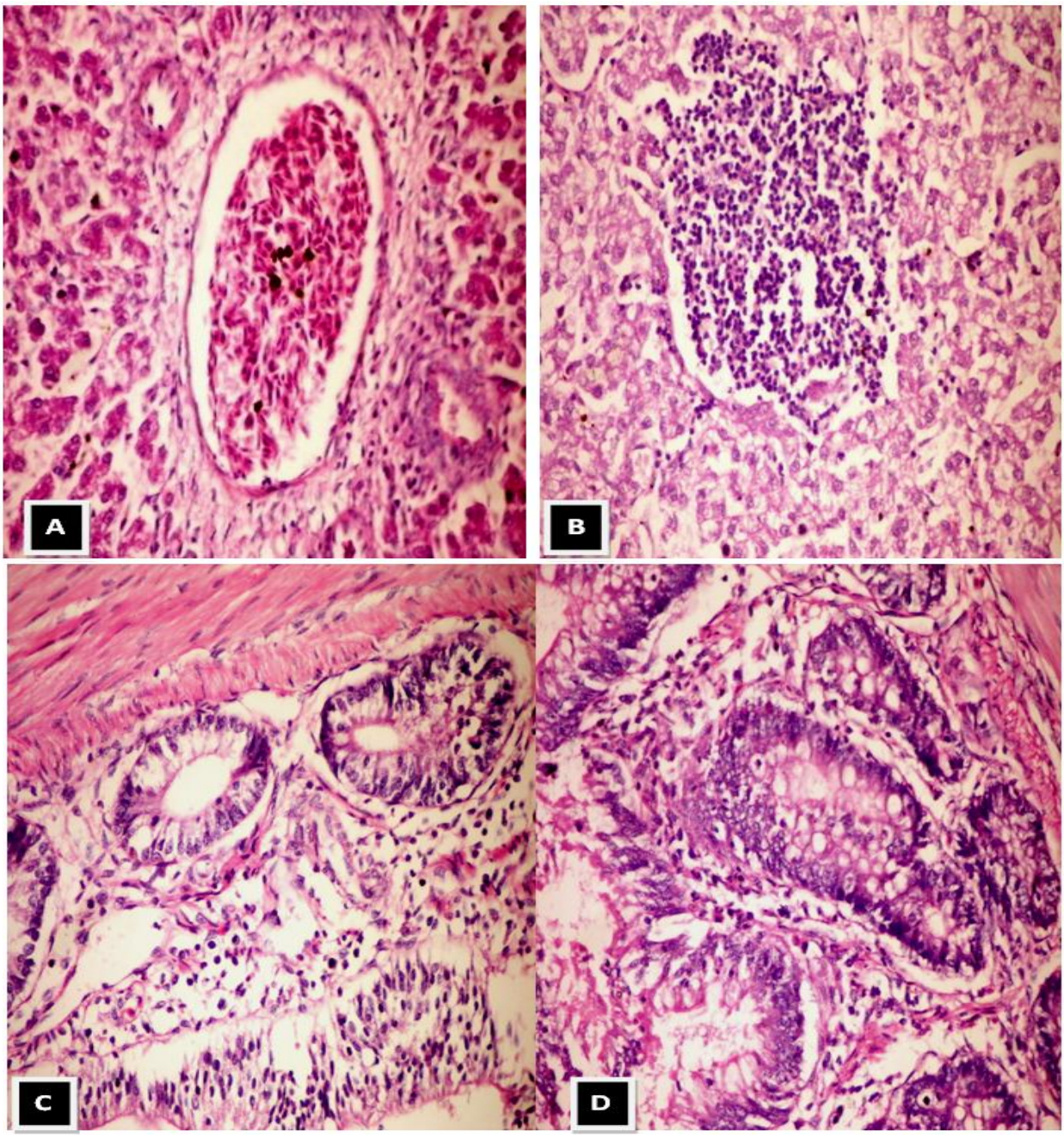
(A): Large focal area of hepatic necrosis infiltrated with leucocytic cells (H&E X 400).

(B): Portal tract permeation with leucocytic cell infiltrations (H&E X 400).

(C): Massively degenerated mucosa (H&E X 200).

(D): Massive sub mucosal gland necrosis (H&E X 200).

Plate (3): Liver (A&B) and intestine (C&D) of *E. Fergusonii* infected chicken group



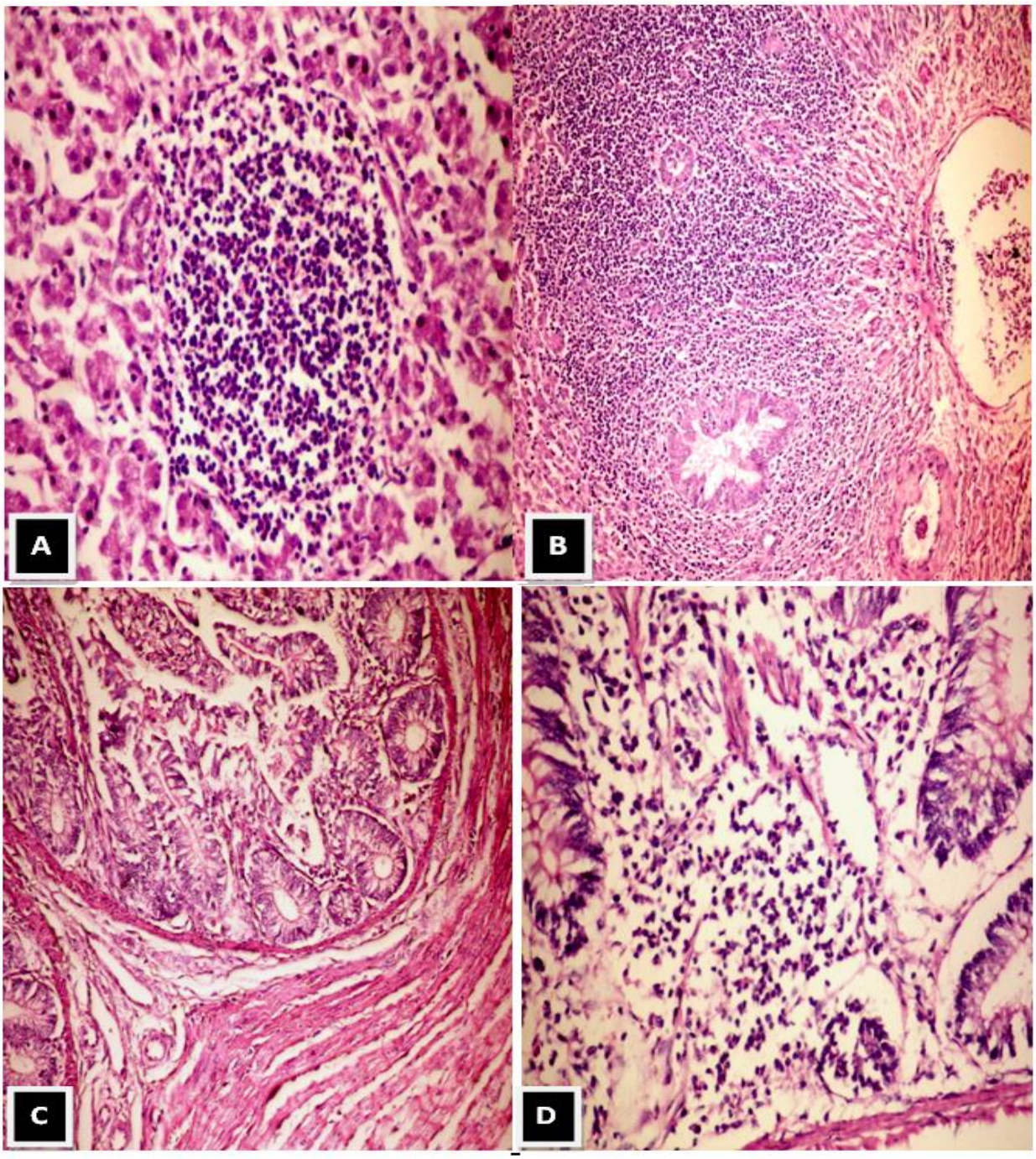
(A): Portal tract dilated and congested vessels (H&E X 400).

(B): Focal area of necrosis with leucocytic infiltration (H&E X 400)

(C): Sub mucosal mononuclear cells infiltration (H&E X 200).

(D): Hyperactivity of the glands (H&E X 200).

Plate (4): Liver (A&B), and intestine (C&D) of *Pr.mirabilis* infected chicken group



(A): Focal areas of hepatic necrosis infiltrated with leucocytic cells (H&E X 400).

(B): Hyperplasia of epithelial lining bile duct, cholangitis, newly formed bile ductules, and massive portal leucocytic cell ifiltration (H&E X 400).

(C): Severly necrotic glands (H&E X 200).

(D): Submucosal leucocytic infiltration (H&E X 200).

CONCLUSION

It could deduced that; the isolates of Enterobacteiacea were highly pathogenic. From the histopathological point of view, it is considered that *Pr.mirabilis* is the most pathogenic followed by *E. Sakazakii*, *E. Fergusonii, and E. coli* respectively. . Moreover, it is recommended to take these bacterial types in consideration in taking preventive and control measures from the poultry and public health points of view.

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