HISTOPATHOLOGY POST INTRAPERITONEAL INFECTION WITH *Providencia rettgeri* IN MICE

Zainab Ismail Ibrahim

Pathology and Poultrym Diseases Department, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq.

(Received 9 November 2015 , Accepted 24 December 2015)

Keywords: *P.rettgeri*, Catheterization, Bile.

ABSTRACT

The *Providencia rettgeri* (*P.rettgeri*) is an opportunistic pathogen of human and animals and has a clinical importance as a nosocomial pathogen causing infections in the urinary system after catheterization and in immunocompromised patients less than human feces, bile and sputum**.** To estimati the pathogenic role of *P.rettgeri* in mice post-infection intraperitonial infection (I/P). thirty mice from both sexes, aged 6-7 weeks and weight 25-30 gm. were infected I/P by a whole bacterial suspension of *P.rettgeri* 0.5 ml (containing $1x10^8$ CFU/ml), the negative control group injected I/P with (0.5 ml) PBS,five mice were sacrificed after (24, 48hrs. and 4,7 and 14 days) post-inoculation, also (n=5) were considered as negative control group, for histopathological examination. The histopathological changes at 24,48hrs until 4 days; recorded mild to moderate acute inflammatory cells reaction, edema, fibrinous exudate. At 7&14 days there was also necrosis and focal aggregation of mononuclear cells. Strong pathogenic role of *P.rettegri* in induction acute inflammatory response in experimental lab mice.

INTRODUCTION

The genus *Providencia* are gram-negative opportunistic pathogens that have been isolated from a wide variety of environments and many living beings ranging from humans, insects, sea turtles and shark mouths (1, 2).*P.rettgeri*, have been isolated from human stool samples both as part of the natural human gut flora and as the cause of traveler's diarrhea (3),also has been isolated from nosocomial urinary tract infections(4),prominent among the microorganisms isolated from healthy and ill captive reptiles. These bacteria can remain dormant and become invasive when

conditions decrease the immune resistance of the host and/or follow primary viral infection.

*Providenciarettgeri*are urease positive, as are some strains of *P. stuartii*. *P. rettgeri*and *P. stuartii*were originally subdivided based on urease production (5).In contrast to *P. stuartii*and *P. rettgeri*, *P. alcalifaciens*is an invasive enteric pathogen and implicated as a cause of diarrheal disease (6).The experiment was designed to improve the pathogenicity of *Providenciarettgeri* by studying the main histopathological changes that occurred post intraperitoneal infection with apathogenic strain of *P. rettgeri*in white mice.

MATERIALS AND METHODS

1- Bacterial isolate: was obtained from the College of Science , University of Baghdad .Diagnosed and purified on their selective media (MacConkey agar, Xylose-Lysine-Deoxycholate agar), grown in nutrient broth in order to estimate the CFU/ml (effective dose)(7).

2- Lab animals: Thirty white mice were taken from (Biotechnology lab center of Al-Nahrin university), weight about 20-30 gm., they were housed and adapted in the animal house of the College Veterinary Medicine, University of Baghdad.

3-Histopathologic examination was according to (8).Tissues were obtained from liver, kidney, lung, intestine, brain, muscles and skin.

4- Experimental design: Thirty white mice were divided randomly into five groups (in each group five mice) according to the time of sacrificing post-infection at 24,48hours.,4days,7and 14days injected intraperitonealy with 0.5 ml ($1x10^8$ CFU/ml).Sixth group was treated with sterile PBS as negative control group.

RESULTS

Histopathological examination:

the prominent features of *P.rettger*i infection different tissues revealed acute inflammatory reaction at 24, 48 hours and 4 days extended to day 7 post-infection; characterized by diffuse infiltration of polymorphoneutrophils (PMNs) sometimes in

small focal aggregations, congestion of blood vessels (dilated and filled with blood and few PMNs,fibrin with edema,also there are variable lesions according to the duration of infection:

At 24 and 48 hours post infection;

Mostly the internal organs(liver, kidney, lung, intestine, brain, muscles and skin) appeared acute inflammatory response, predominantly consisted from PMN sinfiltration, presence of fibrin, congestion of blood vessels and contained PMNs(Figure-1), abscess formation also at 48 hrs spots infection (Figure-2).In kidney severe acute cell swelling of tubular lining epithelial cells (Figure-3)and desquamation of epithelial cells, the glomerular tufts showed bluish-spots may represented the infected bacterial colonies, diffuse infiltration of neutrophils or focal aggregations in interstitial tissue. The pulmonary tissue showed acute bronchitis and bronchiolitis, characterized by sloughing of epithelial lining cells with infiltration of PMNs and fibrin, severe hemorrhage and congested blood vessels (Figure-4).At 48 hours the site of injection and adjacent sites of the skin appeared with severe acute suppurative dermatitis (Figure-5).

At 4 days;

The same histopathologic changes that occurred post 24 and 48 hours from infection were showed in liver, kidney, lung, intestine, muscles and skin,also acutemeningitis seen was characterized by thickening of meninges due to infiltration of inflammatory cells mainly PMNs and congestion of blood vessels (Figure-6),also there is focal aggregation of glial cells (gliosis) in brain parenchyma (Figure-7).

At 7 and 14 days;

Also seen periglomerulitis characterized by infiltration of PMNs and mononuclear cells mainly lymphocytes,the latter focally infiltrated between renal tubules and necrosis of renal epithelial cells (Figure-8) and presence of protein acious material (hyaline casts)in the lumen of renal tubules.The intestine showed enteritis characterized by infiltration of mononuclear cells in lamina properia (Figure-9).The infected skeletal muscles were severely inflamed necrotized, with hyalinization of muscle fibers and atrophy (Figure-10).

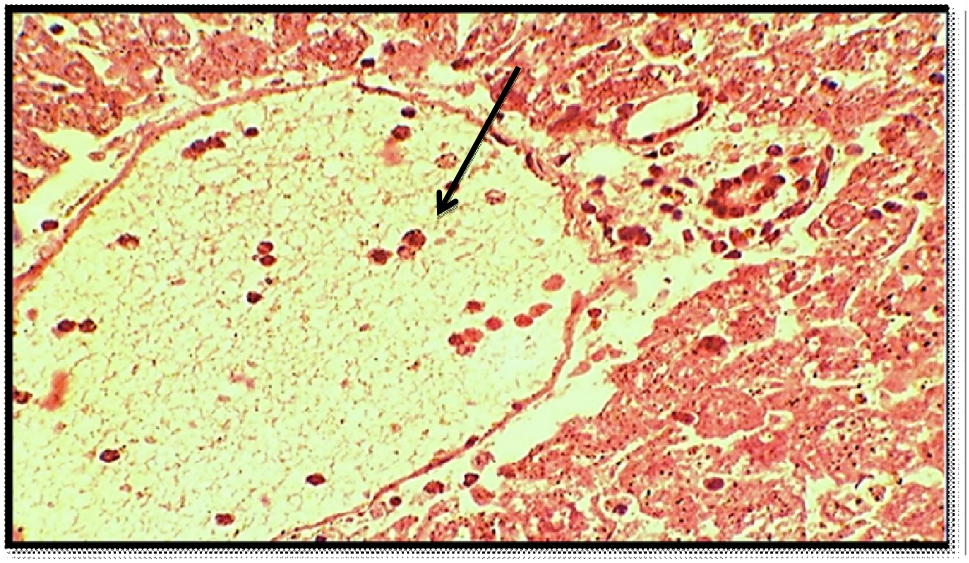

Figure-1: microphotograph of liver at 24 hours post infection; presence of neutrophils and fibrinin the dilatedportal vein (→), and in the stroma of portal area (H&E stain,40X).

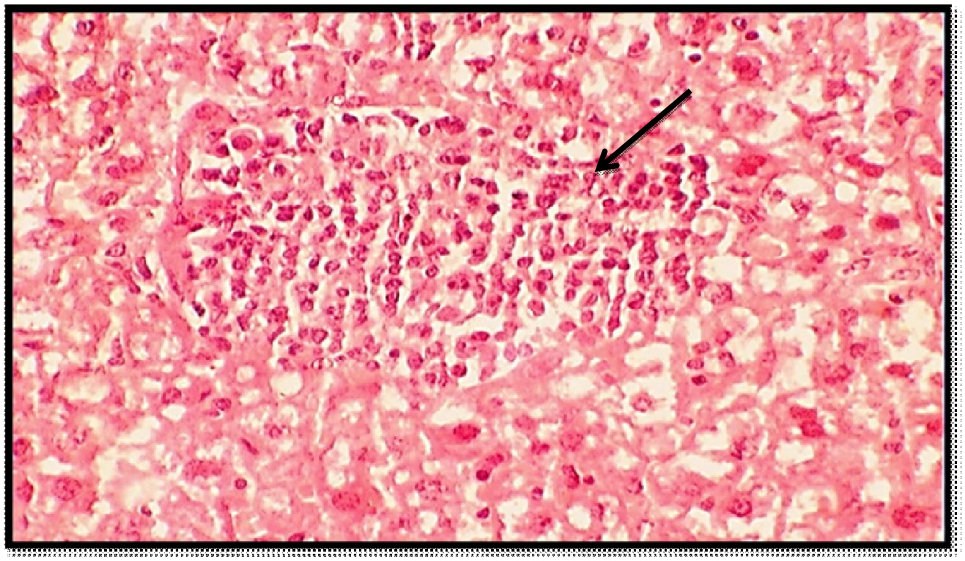

Figure-2:microphotograph of liver at 48 hours post infection;with abscess (→) in hepatic parenchyma (H&E stain,40X).

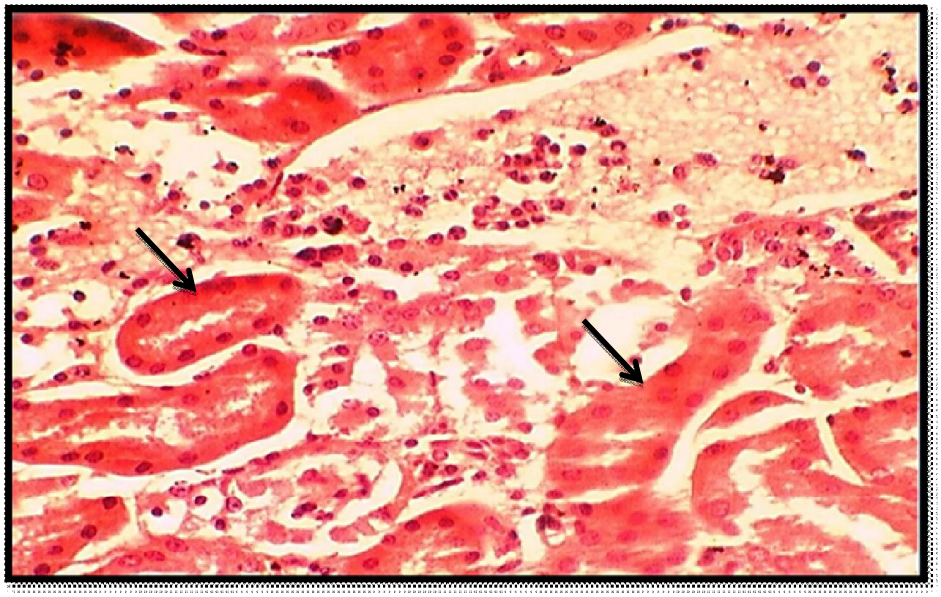

Figure-3:microphotograph ofkidney at 24 hours post infection; with acute cell swelling of lining epithelial cells (➞), severe congestion of renal blood vesselswhich contained neutrophilsand fibrin fibrils (H&E stain,40X).

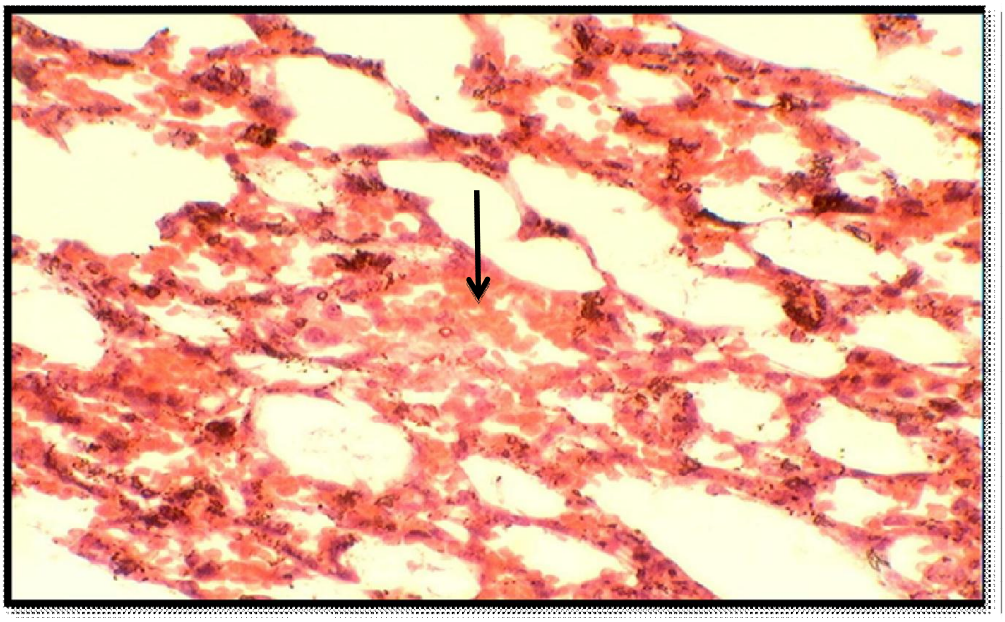

Figure-4:microphotograph oflung at 48 hours post infection; severe hemorrhage (➞) (H&E stain,40X).

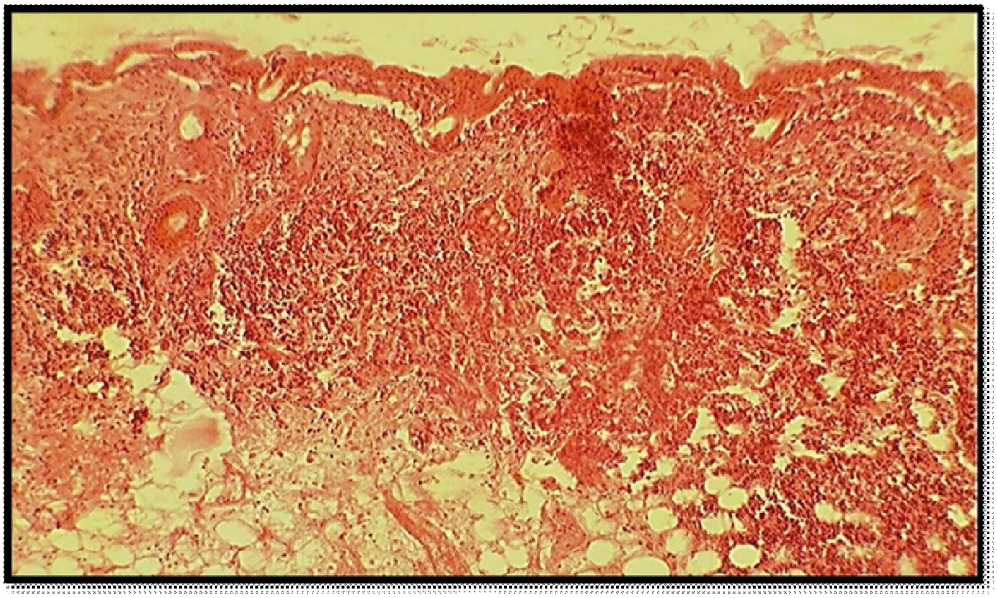

Figure-5: microphotograph ofskin at 48 hours post infection; showed acute suppurative dermatitis (H&E stain,40X).

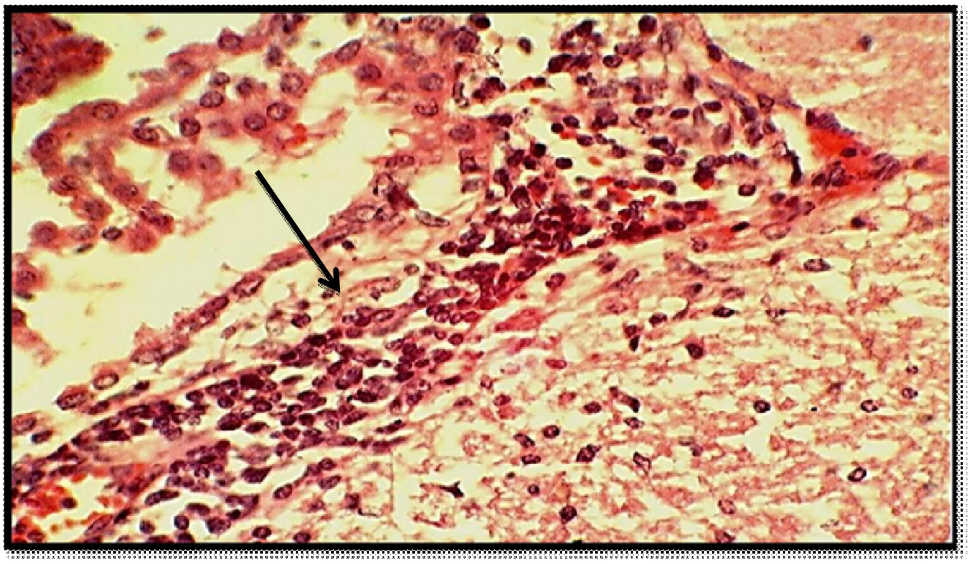

Figure-6: microphotograph ofbrain at day 4post infection; acute suppurated meningitis (⟷) (H&E stain,40X).

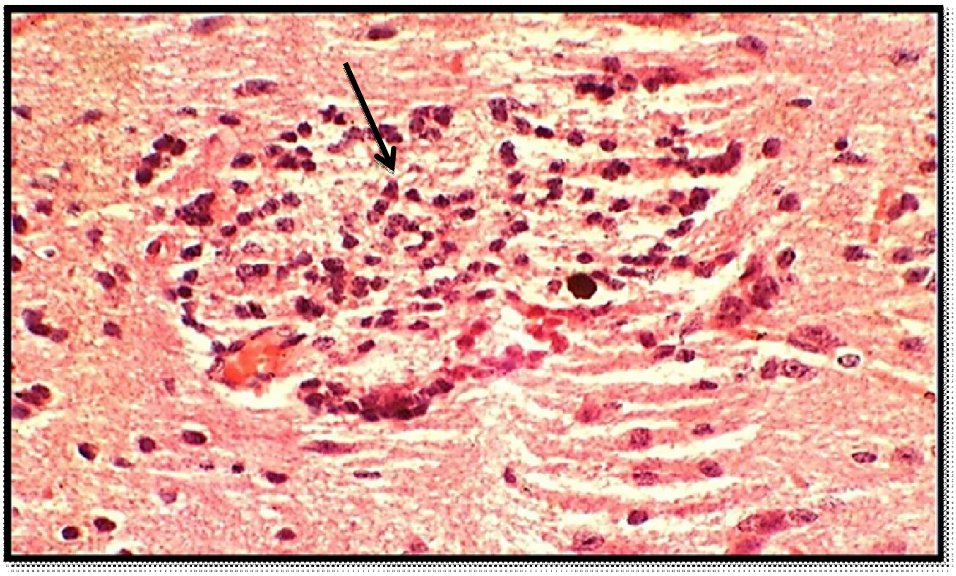

Figure-7: microphotograph in brain at 48 hours post infection; gliosiswith abscess (⟶)(H&E stain,40X).

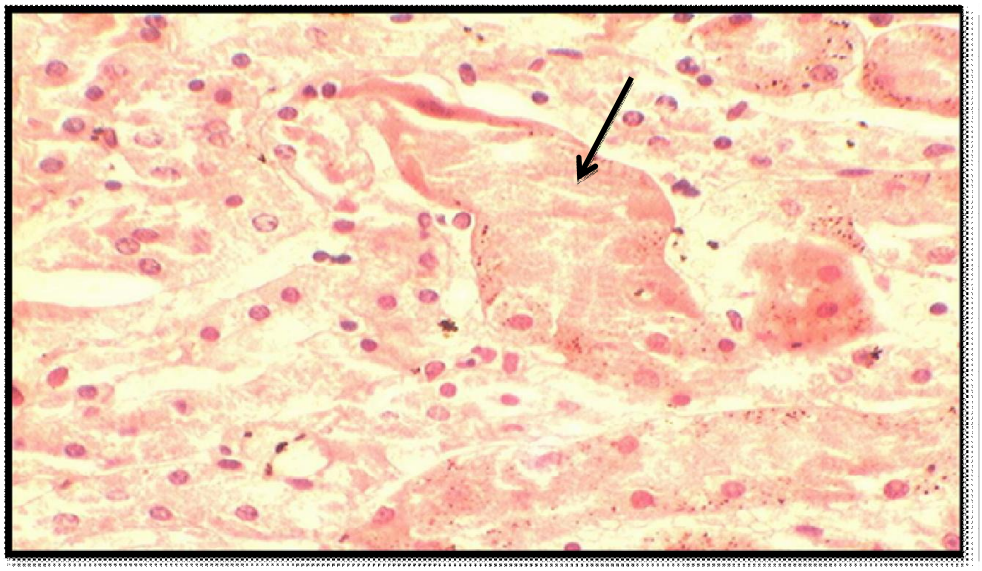

Figure-8: microphotograph ofkidney at 7&14 days post infection; showed necrosis (⟶) of renal tubular epithelial lining cells (H&E stain,40X)

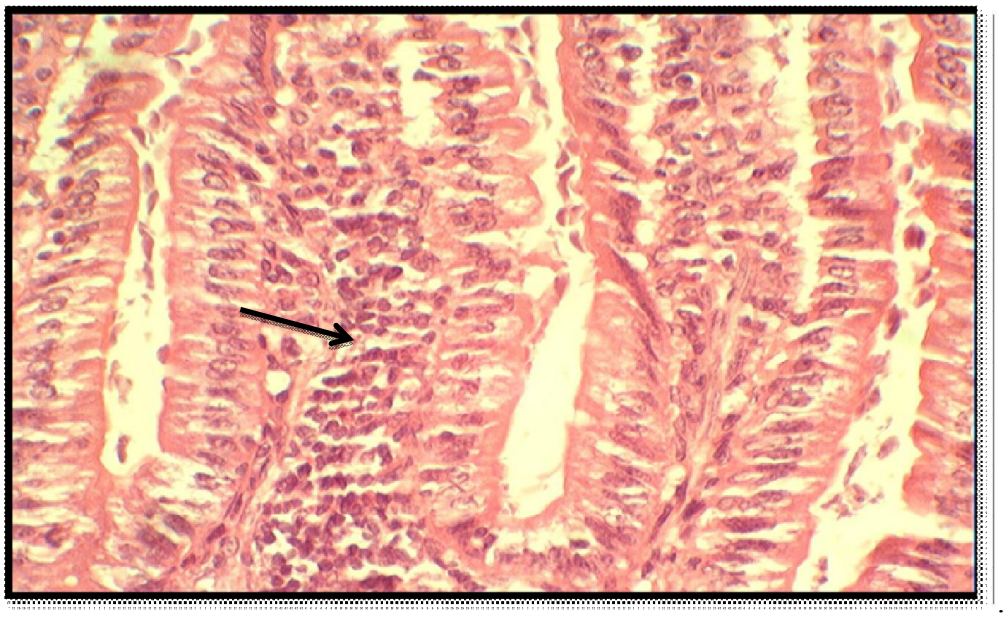

Figure-9: microphotograph ofintestine at 7&14 days post infection; showed heavy infiltration of mononuclear cells (⟶) in the lamina properia(H&E stain,40X).

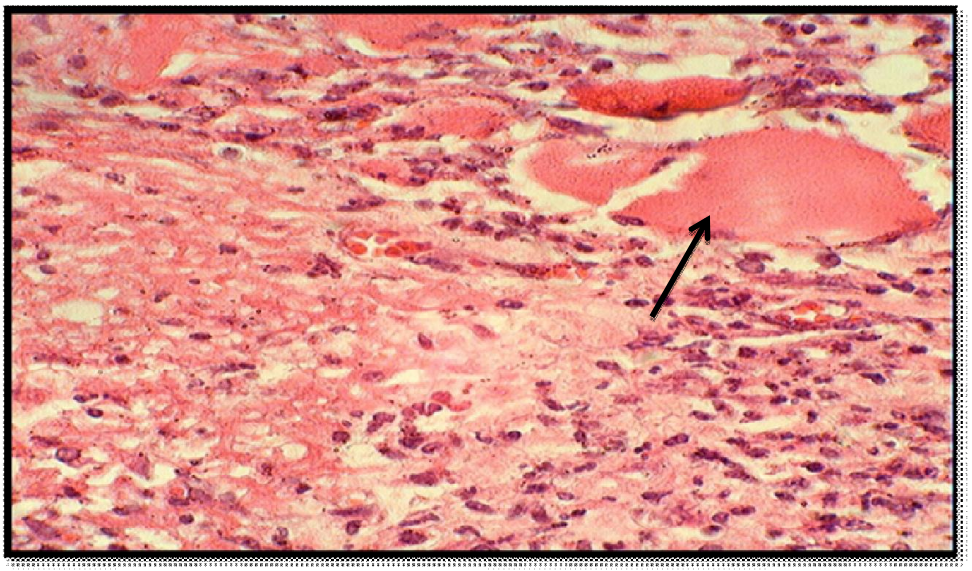

Figure-10: Massiveatrophic necrosis of skeletal muscles (left side) and heavyinfiltration of PMNs,with hyalinizationafter 14 days of infection (⟶)(H&E stain, 400X).

DISCUSSION

The current results explained the serious pathogenic effects of *P.rettgeri* post intraperitonial infection in mice at different times ;involved most of the internal organs in which the histopathological changes were severe acute inflammatory reaction (9) exclusively during 24-48 hours post infection; characterized by

infiltration of polymorphneutrophilic cells(PMNs) and fibrinous exudate that mayexpressed the strong virulence factors of the present bacterial straininactivationof the complement components to induce humoral immune responses increased the migration of PMNs in order to phagocytize the foreign agents (bacteria) from the site of injury, evenly caused liquaifactive abscesses lesions, that agreed with (10**)** who reported the same histopathological changes from congestion and edema, hemorrhage, infiltration of PMNs (signs of acute inflammation).*P.rettgeri* is a gram-negative, urea-splitting organism which has been known to cause urinary trac tinfections and bacteremia, especially in immunosuppressed patients (11). (12)they reported the causative agents of bacteremia in hospitalized patients was due to *P. rettgeri*and *P. stuartii*so *Providencia* bacteremia was primarily occurred; in elderly patients with cerebrovascular disease, indwelling urinary catheter which lead to UTIs,and more fatal in cases with primary bacteremia, and frequently occurred with poly microbial infection, making the selection of appropriate empirical antibiotic therapy difficult.

The epithelial cells of internal organs appeared with severed generation, acute cell swelling, vacuolation even necrosis in other tubules that may explained the toxic injury (O2 deprivation by the action of free radicals (super oxide)that released by LPS from sick cells (infected cells) (13).Urease production is not characteristic of all *Providencia* species, only *P. rettgeri* strains producing urease (14) that reported from the researches deals with UTI and that clear in current study from the renal pathological changes; degeneration and necrosis of lining epithelial cells of renal tubules especially at 7-14 days post infection.

Conclusions:

The current result srevealed the important histopathologic changes of acute inflammatory response caused by *P. rettgri* post intraperitonial inoculation.

Acknowledgment:

to whom support me with their advices and help.

التغيرات المرضيةالنسيجية بعد الخمج داخل الخلب بجراثيم *Providenciarettgeri* في الفئران البيضاء

زينب اسماعيل ابراهيم

فرع الامراض والدواجن ، كلية الطب البيطري-جامعة بغداد ، بغداد ، العراق .

الخلاصة

تعتبر بكتريا(*P.rettgeri*) *Providencia rettgeri*من الجراثيم الانتهازية في الانسان والحيوان على حد سواء لما لها من اهمية سريرية في قابليتها على احداث الخمج البكتيري الانتهازي في الجهاز البولي عند استخدام القسطرة البولية وفي حالات نقص المناعة المكتسب مع قلتها في البراز والمرارة والقشع،لذاكان الهدف من الدراسةهو التعرف على الدور الخمجي لبكتريا*P. rettgeri*في احداث التغيرات المرضية النسيجية في الفئران البيضاء المخمجة بها داخل الخلب. تم استخدام ثلاثون فارا اعمارها بين 6-7 اسابيع واوزانها 25-30 غرام ومن كلا الجنسين قسمت عشوائيا الى خمسة مجاميع حسب فترات التضحية بالحيوانات بعد تعريضها للخمج البكتيري بجرعة 0.5 مل ($1x^{8}10$خلية بكتيرية/مل) داخل الخلب، و المجموعة السادسة تم حقنها داخل الخلب بالمحلول الملحي الفسلجي المعقم كمجموعة سيطرة سالبة. تم التضحية بالحيوانات بعد انتهاء الفترات الزمنية للخمج البكتيري (24 و48 ساعة وكذلك 4 و7 و14 يوما). اظهرت نتائج الخمج البكتيري خلال الفترات24، 48 ساعة وحتى 4 ايام ارتشاح الخلايا الالتهابية الحادة في الاعضاء الداخلية وتجمع السوائل الخزبية مع نضجة ليفينية في الانسجة الخلالية للاعضاء الداخلية وغشاء الخلب، كما لوحظ نخر نسيجي مع تجمعات الخلايا وحيدة النواةخلال الفترتين 7 و 14 يوما.نستنتج مما سبق لضراوة الشديدة لبكتريا *P.rettgeri*في احداث الطور الحاد من الاستجابة الالتهابية في الفئران البيضاء والمصابة بها داخل الخلب.

REFERENCES

1-Foti, M.Giacopello, C.Bottari, T.Fisichella, V.Rinaldo, D. andMammina, C. (2009). Antibiotic resistance of gram negatives isolates from loggerhead sea turtles (Carettacaretta) in the central Mediterranean Sea, Mar. *Pollut. Bull*. 58:1363-1366.

2-Nascimento, J.A.,Ventura, R.F.,Batista, J.E. Souza, M.M.,Hazin, F.H. Pontes-Filho, N.T., Lima-Filho, J.V. (2010). Recovery and screening for antibiotic susceptibility of potential bacterial pathogens from the oral cavity of shark species involved in attacks on humans in Recife, *Brazil, J. Med. Microbiol*. 59: 941-947.

3-Yoh, M. Matsuyama, J. Ohnishi, M. Takagi, K. Miyagi, H. Mori, K. Park, K. Ono, T. Honda. T.(2005).Importance of *Providencia*species as a major cause of travellers'diarrhoea, *J. Med. Microbiol*.54: 1077-1082.

4-Broomfield, R. J., Morgan, S. D., Khan, A. & Stickler, D. J. (2009). Crystalline bacterial biofilm formation on urinary catheters by ureaseproducing urinary tract pathogens: a simple method of control. *J MedMicrobiol,*58, 1367–1375.

5-Penner, J. L., N. A. Hinton, G. R. Whiteley, and HennessyJ. N. (1976).Variation in urease activity of endemic hospital strains of Proteus rettgeri and Providenciastuartii. *J. Infect. Dis.* 134:370–376.

6-Albert, M. J., Faruque, A. S. and Mahalanabis.D. (1998). Associationof Providenciaalcalifaciens with diarrhea in children. *J. Clin. Microbiol*. 36:1433–1435.

7-Luna, L.G. (1969).Manual of histological staining methods of the armed forces institute of Pathology. 3rd Ed. Mcg raw-Hill, New york.

8-Miles, A.A. and Misra,S.S. (1939). The estimation of bacterial power of the blood.Hygiene,VIII:732-749.

9-Greemwood, D., Slack, R.C.B and Peuthere, J.F. Medical Microbiology (A guide to microbial infections, pathogenesis, immunity, labotratory diagnosis and control).6th Ed, 2002,PP:208.

10-Obayes, H. S. and Abd, F. G. (2013).Pathogenesis of Providenciarettgeri in mice Journal of Babylon University-Pure and *Applied Sciences*, 8 (21): 2785 - 2800.

11-O'Hara, C. M., Brenner, F. W. & Miller, J. M. (**2000**).Classification, identification, and clinical significance of Proteus, Providencia, andMorganella.*ClinMicrobiol Rev*, 13, 534–546.

12-Choi, H. K., Kim, Y. K., Kim, H. Y., Park, J, E., and Uh, Y. (2015).Clinical and microbiological features of *Providencia*bacteremia: experience at a tertiary care hospital, *Korean J Intern Med*;30:219-225.

13-Yokoyama, H.; Mizukami, T.; Kamegaya, Y.; Fukuda, M.; Okamura, Y.; Matsumoto, M.; Kato, S. and Ishii, H. (1998).Formation of super oxide anion in the hepatic sinusoid after lipopolysaccharide challenge.Alcohol.*Clin. End Res*., 22: 1335-65.

14-Brenner, D. J., Farmer J. J. III, Fanning,G. R. Steigerwalt,A. G. Klykken, P.Wathen, H. G. Hickman,F. W. and Ewing.W. H. (1978). Deoxyribonucleic acid relatedness of Proteus and *Providencia species*. Int. J. Syst. *Bacteriol.* 28:269–282