

Pathogenesis of *Pasteurella multocida* in Rabbits by Intra-nasal and Intra-ocular infection

Al-Najjar S. Sarhad

Department of Pathology and Poultry, Collage of Veterinary Medicine, Baghdad University.

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Summary

In order to study the pathogenesis of the *Pasteurella multocida* in its natural rout of infection, this study was done to investigate and compare its pathogenesis after intranasal and intraocular infection. Thirty (30) rabbits were divided into three groups, the 1st group (n=12) infected with 240 CFU of *Pasteurella multocida* intra-nasal, the 2nd (n=12) infected with 240 CFU of *P. multocida* intra-ocular (eye drop), and the 3rd group (n=6) served as control negative. The animals were sacrificed if they did not die naturally at 24-48hr, 3, 6, 9, 30 day post-infection (p.i.). The results showed dullness of the animals with nasal and ocular discharge and some animals dead at 48hr and 72 hr p.i. (two animals from the 1st group and four animals from the 2nd group). The gross examination of the dead and sacrificed animals showed hemorrhage in the upper respiratory tract with fibrinous pleuropericaditis and emphysematous lung in the 1st group, while the 2nd group showed eye opacity and in some animal's eye swelling in addition to the feature noticed in the 1st group. *Pasteurella multocida* was isolated from the organs of all the infected animals. The histopathological changes in the 1st group were more intense than the 2nd group and concentrated at the upper and lower respiratory tract as acute hemorrhagic tracheitis with fibrinous pneumonia, while the kidney showed hypercellularity of the glomeruli with cellular degeneration of renal tubule and lymphocytic hepatitis, also there is focal gliosis. Conclusion, intranasal infection was the effective route and the main pathological changes observed in the respiratory system characterized by fibrinous pleuropneumonia.

Keywords: *Pasteurella multocida*, Rabbit, intranasal, intraocular

E. mail: aloubaidysarhad@yahoo.com

امراضية جرثومة *Pasturella multocida* في الارانب بالاصابة في داخل الانف و العين سرهد النجار

الخلاصة

اعدت هذه الدراسة لغرض معرفة امراضية جرثومة الباستوريلا مالتوسيدا في الاصابة الطبيعية والمقارنة بين الاصابة التجريبية عن طريق التنفس و عن طريق العين باعتبارها الطرق الطبيعية للاصابة. قسمت ثلاثون (30) ارنبا الى ثلاث مجاميع, المجموعة الاولى (12) ارنبا اصيبت عن طريق التنفس ب (240) CFU من جراثيم الباستوريلا مالتوسيدا, المجموعة الثانية (12) ارنبا اصيبت عن طريق التقطير بالعين ب (240) CFU من جراثيم الباستوريلا مالتوسيدا, والمجموعة الثالثة (6) ارانب عدت كمجموعة سيطرة. أجريت الصفة التشريحية على الحيوانات الهالكة والمقتولة بعد 24, 48 ساعة وكذلك 3, 6, 9, 30 يوم بعد الاصابة. اوضحت النتائج ظهور الخمول والافرازات الانفية والعينية وموت بعض الحيوانات بعد 48-72 ساعة بعد الاصابة. وبين الفحص العياني للحيوانات الهالكة والمقتولة وجود

نزف في المجرى التنفسي العلوي مع التهاب الخلب و التامور الليفي مع وجود النفاخ في الرئة في حيوانات المجموعة الاولى اما حيوانات المجموعة الثانية عانت من عتامة وتورم العين في بعض الحيوانات أضافا لما وجد في حيوانات المجموعة الاولى وقد تم عزل جرثومة الباستوريلا مالتوسيدا من كافة الاعضاء الداخلية للحيوانات المصابة وكانت الافات النسيجية المرضية في حيوانات المجموعة الاولى اكثر شدة من المجموعة الثانية حيث تركزت في المجرى التنفسي العلوي والسفلي والتمثلة بالتهاب الرغامي النزفي مع التهاب الرئة الليفي, اما الكلية فظهرت زريدة خلوية الكبيبة مع تنكس ظهارة النبيبات الكلوية بالاضافة الى التهاب الكبد و الدباق البؤري في متن الدماغ. نستنتج من ذلك بأن الإصابة عن طريق التنفس هي الأكثر تأثيرا وتركزت الغيرات المرضية في الجهاز التنفسي حيث تمثلت بالتهاب الرئة والجنب الليفي.

Introduction

The Gram-negative bacterium *Pasteurella multocida* exhibits a broad host range including most mammals, birds and also humans (1 and 2). Pasteurellosis is one of the most significant bacterial diseases of rabbits and one of the major causes of considerable economic loss in large production units throughout the world (3 and 4). The disease is characterized by various clinical syndromes, e.g. respiratory distress, genital affections, abscesses and septicemia, but infection by *P. multocida* can also appear without any clinical signs manifested (5). The variability in clinical signs as well as the course of the disease may be influenced by different *P. multocida* virulence factors such as a capsule, fimbriae, lipopolysaccharides (endotoxin), dermonecrotin, neuraminidase etc. (6, 7, 8, 9 and 10).

Pasteurellosis in laboratory rabbits can cause acute epizootics of pneumonia with high morbidity and mortality. Chronic infection may result in rhinitis, bronchopneumonia, otitis media, pyometra, abscessation and encephalomyelitis (11).

The aim of the study was the comparison of the pathogenicity by two types of infections route.

Materials and methods:-

- 1- *Pasteurella multocida* was obtained from Al-Kindy center for Veterinary Drug and Vaccines production, Baghdad, Iraq.
- 2- Experimental animals (rabbits) (30) were kept in animal house 2 weeks before the experiment for adaptation.
- 3- Histopathological slides were done according to(12)
- 4- Bacterial count was done according to Miles and Misra (13).
- 5- Experimental design: The animals were divided randomly into three groups:
 - a- The 1st group (12 rabbits) were infected with 240 CFU of *P. multocida* through. The nasal orifice.

- b- The 2nd group (12 rabbits) were infected with 240 CFU of *P. multocida* by eye drop.
- c- Control negative group (6 rabbits) were injected of with sterile normal saline through nose.

The animals were sacrificed at 24 and 48 hr, 3, 6, 9, and 30 days post infection if not dead.

6- Bacterial isolation and culture were done on blood agar and trypton soya agar

7- Samples for pathological examination were taken.

Results

Clinical signs:The clinical signs observed were anoxia, dullness, fever, congestion of the conjunctiva, rapid respiration and nasal discharge, in the nasal infected group, while the ocular infected group showed enlargement of eye and inflammation in addition to the other signs observed on the 1st group. Some of the animals dead at 16 hr and 3, 4 days post infection.

Bacterial isolation: The results showed that *Pasteurella multocida* was isolated from most of the examined internal organs of infected rabbits. The degree of bacterial isolates showed variations (very heavy, heavy, moderate, mild and very mild) according to periods of infection and organs. Lung, liver, spleen, lymph node, heart blood and kidney are the main organs which showed more extensive bacterial isolation (table: 1 and 2).

Pathological examination: Gross examination :-

The 1st group (nasal infected group):There was hemorrhage in the lumen of the upper respiratory tract (larynx and trachea) at 16 hr to day 2 post infection (fig:1), there was white fibrinous exudates on the pericardium and pleura which reveled a fibrinous pleuropiricarditis with few white spotted foci on the lung surface with emphysematous areas (fig: 2) as well as solid pneumonic areas with adhesion between plural surface and the thoracic wall, while the liver and spleen were enlarged with rounded and maningeal congestion.

The 2nd group (ocular infected group): Reveled eye opacity, congestion and excessive lacremation during 24 hr and also same changes appeared in the 1st group purities, congestion and thickening of menengies occurred at day 3 post infection.

Histopathological results:

First group:

Lung :- The microscopic examination of the lung tissue at 16 hrs showed thickening of the alveolar wall due to congestion of the blood capillaries and edematous fluid in the alveolar lumen (fig: 3) while at day 2 post infection, the inflammatory cells mainly neutrophils and lymphocyte appeared in section in addition to the prior lesion and in 2nd to the end of the experiment the blood vessels in the lung reveals hypertrophy of the tunica media and hyperplasia of

the tunica intima (endothelial lining) in addition to the congestion (fig: 4) and fibrinous exudates appear in the lung.

Trachea: - At 16 hrs post infection there was hemorrhage in the lumen of the trachea with congestion of the submucosa, while at day two. There was hemorrhages in the sub mucosa and also in the lumen of the trachea with inflammatory cells infiltration mainly neutrophils and lymphocytes (fig: 5) and at days 3 and 4 post infection the epithelial lining of the trachea suffered from sloughing and desquamation and there was inflammatory exudates in the lumen consisting of neutrophils and lymphocytes in addition to tissue debris (figures 6 and 7)

Liver :- At 16 hr to day 2 there was blood vessels congestion mainly central vein and at day 3 there was inflammatory cells infiltrating liver parenchyma between hepatocytes and also around the central vein consisting mainly from neutrophils, lymphocytes and macrophage (fig: 8) and at day 6 there was multifocal aggregation of inflammatory cells mainly lymphocyte (figure 9).

Kidney: - At 16 hrs there was congestion of blood vessels and at the second day post infection reveals hypercellularity of the glomeruli with congestion of blood capillaries of the glomerular tuft (fig: 10) and also between renal tubules with mild proliferation of endothelial lining of B.V between renal tubules while at day 4 post infected in addition to the congestion there is hemorrhage between renal tubules, and lymphocytes infiltration in proximal renal tubules (fig: 11) and at day 6 post infection the lymphocytes and macrophages infiltrated between the collecting ducts with vacuolar degeneration of the epithelial lining of the renal tubules.

Heart :- At days 3 to 6 there was inflammatory cells infiltration between muscles fibers, and there was thickening of the endocardium layer due to hyperplasia of the endothelial cells lining and also fibrinous exudates in addition to inflammatory cells infiltration (fig: 12).

Brain:- 16 hrs post infection revealed perivascular edema and at day 2 to 3 post infection there was congestion of blood vessels in the meninges and inflammatory cells mainly lymphocyte (fig: 13) and also there was focal gliosis at day 4-6 pi. (fig: 14).

Spleen: Showed congestion and atrophic white pulp at day 4 pi.

Other tissue: The intestine showed inflammatory cells infiltration on the lamina propria and also in the lumen of the intestine and also the stomach showed inflammatory cells aggregation in the sub-mucosa.

Second group:-

Lung: At day 3 to 6 post infection the lung tissue revealed a severe congestion and edema, thickening of the inter alveolar wall and at days 9 to 29 post infection there was thickening of the inter alveolar septa due to inflammatory cells and fibrinous exudates (fig: 15), in addition to focal aggregation of inflammatory cells mainly lymphocytes in some part of the lung.

Trachea: - The trachea showed congestion at day 9 post infection, the submucosa infiltrated with inflammatory cells mainly lymphocytes and neutrophils.

Liver: Reveled severe congestion of blood vessel mainly central vein also the sinusoid that filled with blood with severe infiltration of polymorph-nuclear cells between swollen hepatocytes at day 3 to 9 post infection (fig: 16) while in day 29 post infection the neutrophils replaced by lymphocytes and macrophages with Kupffer cells proliferation.

Kidney: - At days 3 to 9 post infection the renal tubule epithelia suffered from acute cellular degeneration with hyper cellularity of the glomeruli and also there is moderate hyperplasia of the endothelial lining of the blood capillaries between renal tubules and diffuse inflammatory cells infiltration mainly lymphocyte between renal tubules and interstitial tissue (fig: 17), in addition to atrophy of some glomeruli (fig: 18).

Heart: Reveled inflammatory cells infiltration between muscles fiber at days 9 to 29 post infection (fig: 19).

Brain: - At days 6 to 9 post infection the brain tissue reveled congestion of blood vessels with perivascular edema and congestion of meninges , with inflammatory cells infiltration (fig: 20)

Spleen: - Moderate lymphoid hyperplasia of the white pulp.

Discussion

Bacterial isolation: The results of study showed that the *Pasteurella multocida* isolated from different organs indicate the bacteremia and wide spread of the microorganism in the body and that's agreed with (14) who made experimental infection with *Pasteurella multocida* of field isolates to mice with intra peritoneal infection and isolated the bacteria from different organs (3 and 4). Bacteremia allows bacteria to spread widely in the body and permits them to reach tissue particularly suitable to their multiplication (15).

Pathological examination: The gross lesion of lung showed congestion and consolidation also there was adhesion between lung surface and pleura as well as with thoracic cavity, the lung showed foamy content which related to intra alveolar edema, this lesions agreed with (16 and 17).

The main histopathological changes have been seen in examined organ such as (liver, lungs, kidney, spleen and lymph node), there were extensive congestion, degeneration also these lesions associated with extensive infiltration of Inflammatory cells due to leukocytic recruitment as effect of bacterial infection on cell activation, and these recruited cells especially neutrophils were responsible for inducing parenchymal cell injury in the organs (18, 19, 20 and 21). The entire lesions noticed in all examined organs especially thickened connective tissue surrounding the blood vessels were agreed with (22) in their study of three elephants carcasses that die due to pasteurellosis. Present result revealed a fibrinous bronchopneumonia in the both groups that's agreed with (23) which reveals that the lung lesions comprised a fibrinous

bronchopneumonia with variable sized areas of coagulative necrosis, extensive deposition of fibrin and massive dilatation and edema of the interlobular and pleural lymphatic, bronchopneumonia may be due to the erogenous route of infection (24). Hemorrhagic tracheitis was observed in this study due to attachment and proliferation of *Pasteurella* on the surface epithelium, While the ultra structural changes detected by study of (25) were deciliation or clumping of cilia of ciliated epithelium, cellular swelling, vacuolation and sloughing. The subepithelial capillaries showed congestion, intravascular fibrin deposition, platelets aggregation and endothelial injury. *Pasteurella multocida* was observed attached to the injured endothelial cells. Neutrophils, mast cells, vacuolated monocytes and macrophages infiltrated the lamina propria and between the degenerated epithelial cells.

Table (1): bacterial isolation from rabbit's organs of the 1st group

	liver	lung	Spleen	Heart and blood	and kidney
Hrs16-2 days	+	++	++	++	++
3	+++	++++	++	++	++
6	++++	++++	++	+++	+++
9	++++	++++	+	+++	+++
30	+++	+++	+	++	++

Table (2): bacterial isolation from rabbit's organs of the 2nd group

	liver	lung	Spleen	Heart and blood	and Kidney
Hrs16-2 days	+++	+++	++	++	+
3	++++	+++++	++	+++	++
6	+++++	+++++	++	+++	++
9	+++++	+++++	+	++++	++
30	+++	+++	+	++	++

+++++ very heavy +++ moderate + very mild
 +++ heavy ++ mild - negative

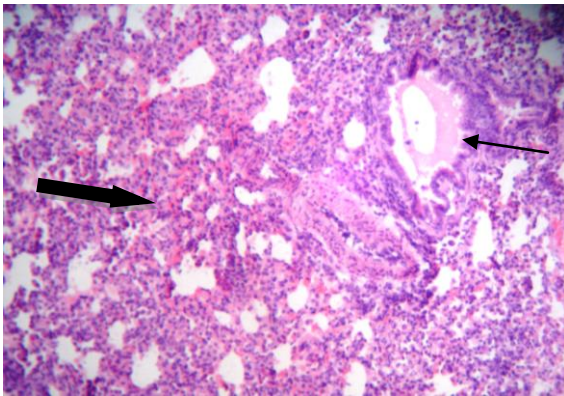


Fig (3):lung at 16hr P.I. showed congestion of the capillaries (←) with odema in the lumen(←) (H&EX10).

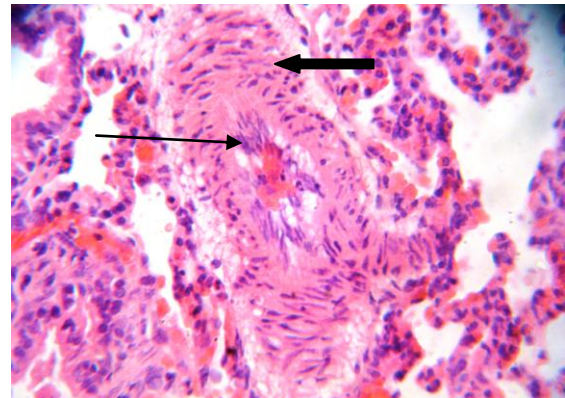


Fig (4): lung at day 6 pi. reveals hyperatrophy of the tunice maedia (←) & hyper plasia of the tunica antina (→) (H&EX40).

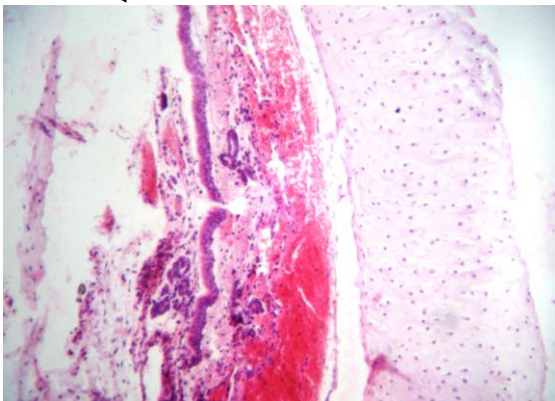


Fig (5): trachea at 16 hr pi. showed hemorrhages in the sub mucosa & also in the lumen with inflammatory cells infiltration mainly neutrophils & lymphocytes (H&EX10).

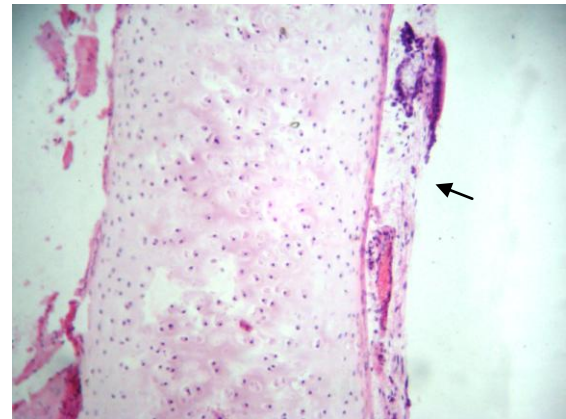


Fig (6): trachea at day 3 showed sloughing and desquamation of the epithelial lining (→) (H&EX10).

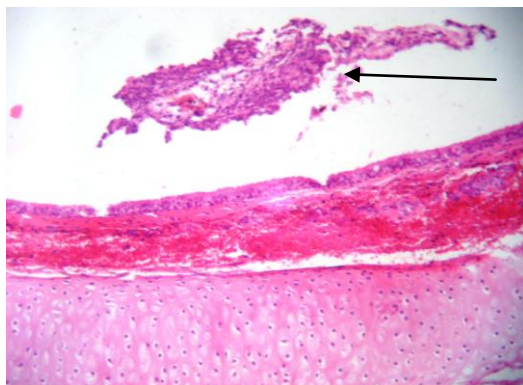


Fig (7): trachea at day 3 pi. showed inflammatory exudates in the lumen consist from neutrophils & lympho cytes in addition to tissue debris (←) (H&EX10).

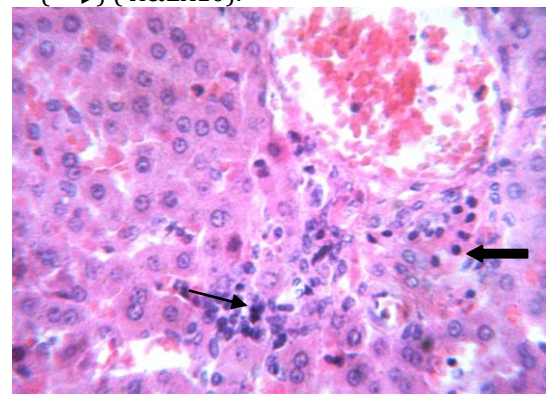


Fig (8) liver at day 3 pi. showed inflammatory cells infiltrated between hepatocytes and also around the central vein consist mainly from lymphocytes (→) and macrophage (←) (H&EX40).

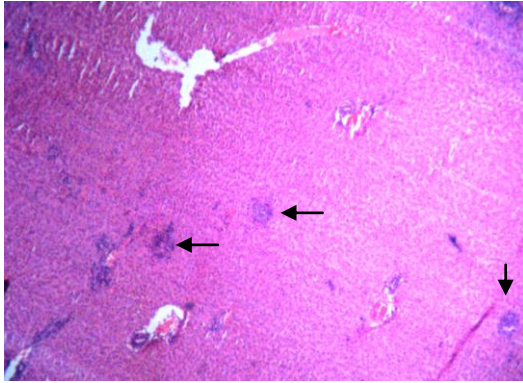


Fig (9): liver at day 6 reveals focal aggregation of inflammatory cells mainly lymphocyte (←) (H&EX10).

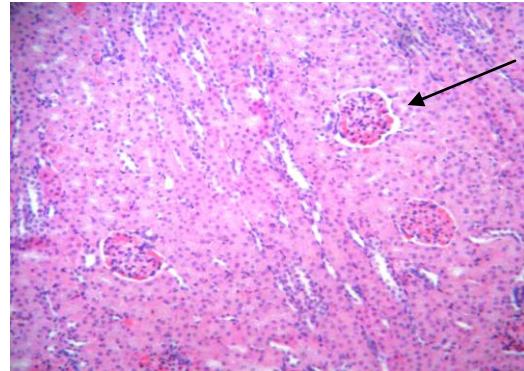


Fig (10): Kidney at 16 hr showed hypercellularity of the glomeruli (←) with congestion of blood capillaries of the glomerular tuft (H&EX10).

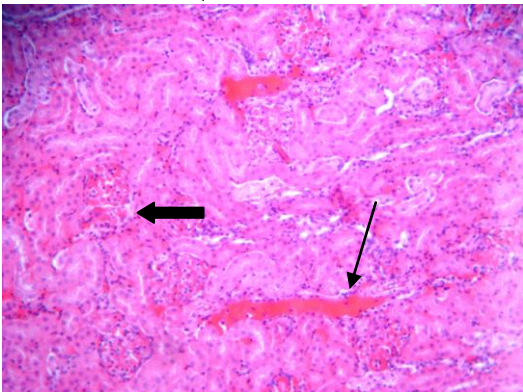


Fig (11): kidney at day 4 pi. revealed hemorrhage between renal tubules (←), and lymphocytes infiltration in proximal renal tubules (←) (H&EX10).

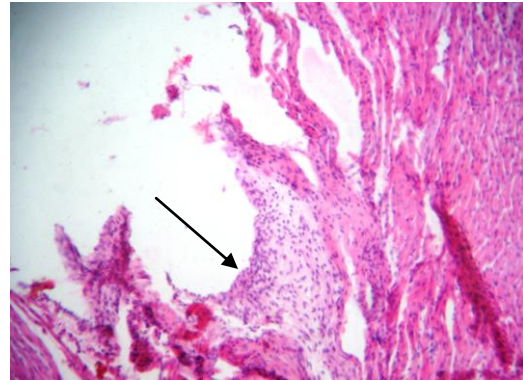


Fig (12): heart at day 6 pi. showed thickening of the endocardium layer (→) due to hyperplasia of the endothelial cells lining & also fibrinous exudates in addition to inflammatory cells infiltration(H&EX10).

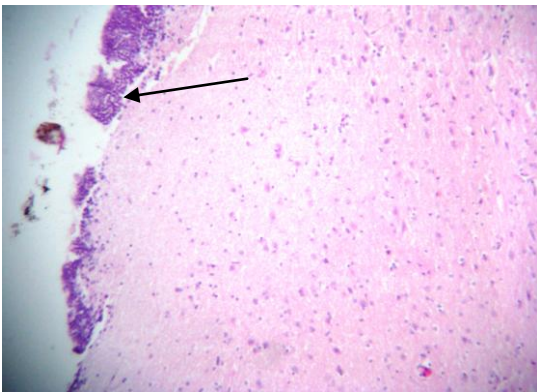


Fig (13): brain at day 2 pi. reveals lymphocytic infiltration in the meninges (←) (H&EX10).

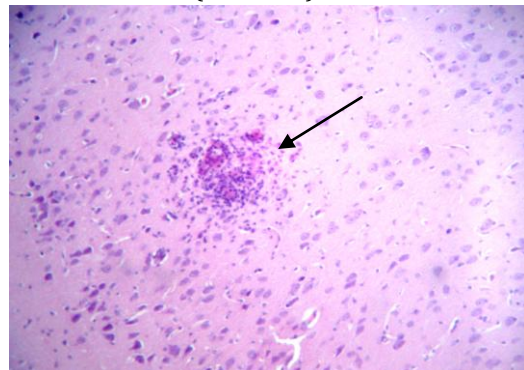


Fig (14):brain at day 6 pi. showed microgliosis (←) (H&EX10).

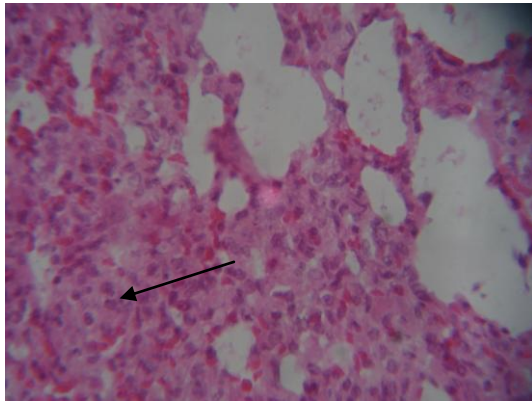


Fig (15): lung at day 6 pi. reveals thickening of the inter- alveolar wall due to inflammatory cells & fibranous exudates (←) (H&EX10).

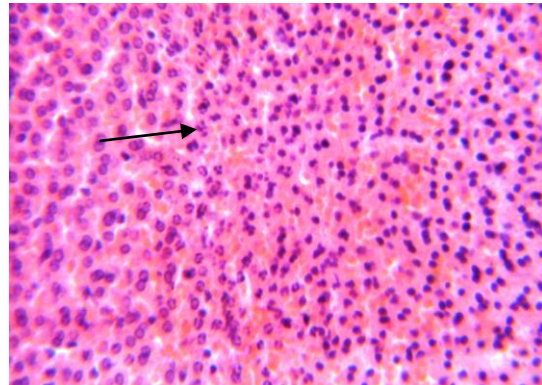


Fig (16): liver at day 3 pi. showed sever infiltration of polymorph-nuclear cells (→) between swollen hepatocytes (H&EX40).

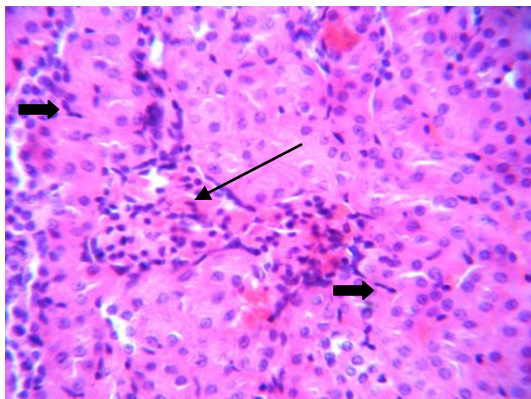


Fig (17):kidney atday 3 pi. showed hyper cellularity of the glomerli (←) with moderate hyperplasia of the endothelial lining of the capillaries between renal tubules (→) (H&EX40).

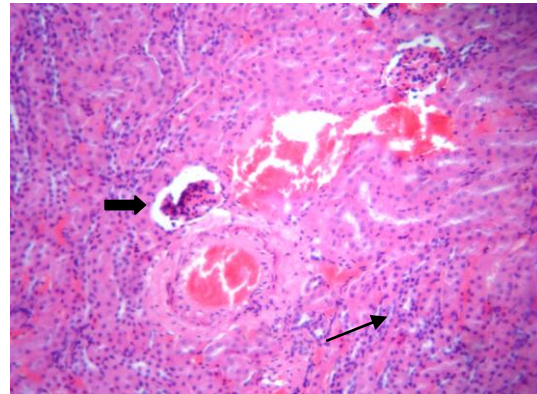


Fig (18): kidney at day 9 pi. showed inflammatory cells infiltration mainly lymphocyte between renal tubules (→) in addition to atrophy of some glomerali (→) (H&EX10).

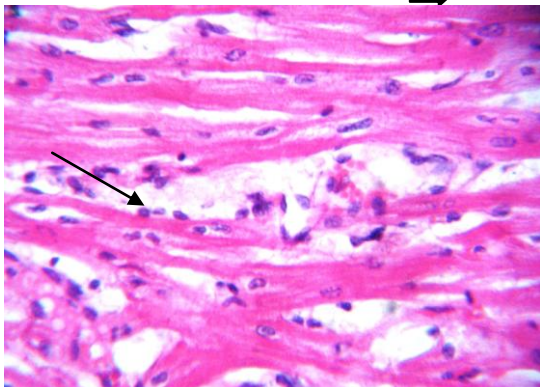


Fig (19): heart at day 9 pi. revel inflammatory cells infiltration between muscles fiber (→). (H&EX10).

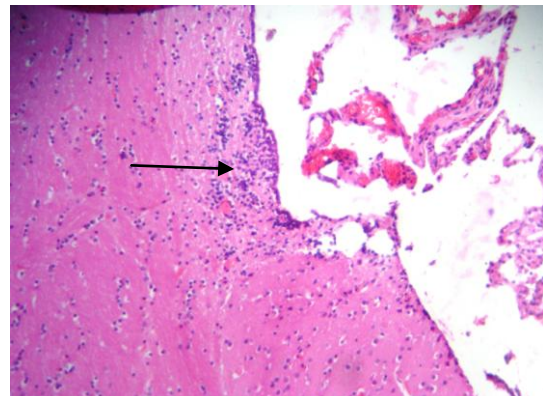


Fig (20): brain at day 9 pi. showed inflammatory cells infiltration (→) (H&EX10).

Nakamine (26) obtained two *P. multocida* isolates from muscovy ducks in Japan and carried out pathogenicity test in weaner rabbits and they died within 24 hrs, Histopathological study was carried out to observe the microscopic alteration induced by the organism in the tissue, They observed that multiple necrosis and bacterial aggregates were prominent in several organs, particularly in the liver. This result agreed with present observation in the liver which characterized by focal aggregations of lymphocyte in the liver parenchyma. Hyalourenase enzyme associated normally with invasive mechanism of bacteria (27)

Bhat (16) observed gross and microscopical changes in the tissues of pigeons which were died *P. multocida* infection. Postmortem examination revealed the liver and spleen were enlarged and mottled having hemorrhagic spots. Histopathologically the lesions in lungs showed edema and congestion. The liver revealed focal hepatitis surrounding the engorged vessels, as noticed by study. Das and Bhagman (28) found two isolates of *P. multocida* of sheep to be pathogenic to rabbits, on postmortem examination lesions including congestion of lungs, liver and kidney, hyperemia of intestine, congestion of mesenteric blood vessels, enteritis, congestion and haemorrhages on the myocardium and hemorrhagic tracheitis. That's agreed with our results.

Sujatha (29) recovered 30 *P. multocida* isolates from outbreaks in pig farm. Histopathological studies were carried out of vital organs of pig (heart, lung, liver kidney and intestine) to study the microscopic alterations induced by the organisms. Heart revealed congestion and hemorrhages. Congestion and edema were noticed in the lungs. Desquamations of superficial epithelial and mild congestion were seen in the intestine. Liver showed sever congestion, hemorrhages and degenerative changes in hepatic cell. Sever congestion and intertubular hemorrhages were noticed in kidneys.

Lesions of the acute form of the disease are typically of septicemia, including multiple hemorrhages through the serous surface in the myocardium, liver, and some time the lung. There may be a tracheitis, sub-acute cases shoe multiple necrotic lesions in the liver and spleen (30).

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