

Pathological Changes of Immunized Rabbits with *proteus vulgaris* Fimbriae Antigen

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Summary

The study was carried to investigate pathological effects of fimbrial antigen of *proteus vulgaris* 20 rabbit has been divided into (4) equal groups The first group immunized subcutaneous twice with (01) ml of pv fimberial Ag (200 μ /ml) with 2 week interval the second group was treated as the 1st group but in a dose containing 100 μ /ml. Third and fourth group considered as positive and negative control groups respectively. After 45 days post immunization first second and third groups were challenged with 01ml of bacteria suspension contain (10⁷x5cfu/ml) of virulent *pvulgaris*. All rabbits of the third group died during 24-48 hr post challenge with severe congestion in all internal organs associated with necrotic foci mainly in liver and kidney at days 3-20 post challenge immunized infected rabbits (died and survival) were sacrificed histopathologically the third group showed focal necrosis and polymorphonuclear cells (PMNCs) infiltration in liver parenchyma pulmonary edema with severe congestion and depletion of spleen Mild pathological changes were revealed in the 1st and 2nd immunized groups characterized by kupffer cell proliferation dilation of sinusoids with mononuclear cells (MNCs) aggregation (mainly lymphocyte) around blood vessels lymphoid hyperplasia in spleen with hyperplasia of goblet cells and secretion of mucin in the intestine mainly in the first group fimberial antigen considered as effective immunogen for protecting rabbits against *p vulgaris* infection and it is synchronized with dose of this antigen.

Keywords: fimbria, *p.vulgaris*, rabbits, pathology

التغيرات المرضية في الأرناب الممنعة بمستضد الخمل لجرثومة *Proteus vulgaris*

انعام بدر فالح

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الخلاصة

إجري البحث لدراسة التأثيرات المرضية لمستضد الخمل لجرثومة *P.vulgaris* وقد تم استخدام 20 أرناب قسمت بالتساوي إلى أربع مجموعات واستخدم في التمنيع مستضد الخمل *P.vulgaris*. المجموعة الاولى منعت مرتين بـ 0.1 مل تحت الجلد من مستضد الخمل الحاوي على 200 مايكروغرام / مل وبفاصل زمني مقداره إسبوعين بين الجرعتين والمجموعة الثانية منعت مرتين بـ 0.1 مل من المستضد نفسه الحاوي على 100 مايكروغرام / مل تحت الجلد وبفاصل إسبوعين بين الجرعتين المجموعتين الثالثة والرابعة اعتبرت مجموعة سيطرة موجبة وسالبة على التوالي بعد 45 يوم من التمنيع حقنت المجاميع الثلاثة الاولى بجرعة التحدي وبالغلة 01 مل من العالق الجرثومي الحاوي على 5×10 من جرثومة *P.vulgaris* الضارية هلكت جميع حيوانات المجموعة الثالثة مع وجود تغيرات مرضية عيانية حادة تميزت بالاحتقان الشديد في الأعضاء الداخلية مع بؤر نخرية لا سيما في متن الكبد والكلية مقارنة بالحيوانات الممنعة التي أظهرت تغيرات مرضية متوسطة الشدة وقد أجريت الصفة التشريحية لها خلال 3-20 يوم. أظهر الفحص المرضي النسجي في المجموعة الثالثة نخر بؤري مع إرتشاح العدلات في متن الكبد والرئة إضافة إلى الخبز الرئوي مع نفاذ اللب الأبيض في الطحال بينما كانت الآفات المرضية قليلة في الحيوانات الممنعة بعد إعطائها جرعة التحدي إذ تميزت بتوسع الجيبانيات مع إرتشاح خلايا كوفر وتكثف لمفي خلوي حول الأوعية الدموية مع فرط تنسج لمفي في الطحال إضافة إلى فرط تنسج الخلايا الكأسية مع زيادة إفراز المخاط ولا سيما في أمعاء حيوانات المجموعة الأولى. يعد مستضد الخمل مسضد فعال لحماية الأرناب ضد الاصابه بجرثائم المتقلبات الشائعة وله تأثير تناغمي مع جرعة المستضد.

Introduction

Proteus vulagris is a rod-shaped Grams (-) negative bacterium that inhabits the intestinal tracts of humans and animals which can found in soil water and fecal matter (1). *Proteus* bacilli have developed several virulence factors such as adherence due to the presence of fimbriae or a fimbrial adhesion invasiveness swarming phenomenon hemolytic activity urea hydrolysis proteolysis and endotoxicity (23). These factors enabling them to colonized and survive in a higher numbers (4). Several types of fimbria which are potentially involved in adhesion to the uroepithelium (5) and other serious complications including the formation of kidney and bladder stones (6) wound infection fever septicemia and invasion of host immunity by immunoglobulin protease (2) it may also cause respiratory infection that persist even after antibiotic (7). Urinary tract infections by *proteus* bacteria accounts 1-2% in healthy women and 5% of hospital-acquired infection; also are more in male than female in neonatal population and are more common in persons aged 20-50 years old (87). This study was intended to reveal the pathological changes after the immunization by fimbriae antigens of *proteus vulgaris* in rabbits.

Materials and Methods

Proteus vulgaris isolate: The bacterial isolate was supplied from zoonotic unite/veterinary Medicine College/Baghdad University; which maintained in urea base agar for preparation the fimbriae antigen (9) and the fimbriae protein concentration was measured by Biurate method (10).

Experimental animals: Twenty local breed between 1-15 kg/bw healthy rabbit of both sex were used; which derived into four equal groups (each one five animal).

Immunization: The first group was immunized by 200µg/ml/animal subcutaneously (S/C) of fimbriae antigens; the second group immunized by 100µg/ml S/C of fimbriae antigens. The third group was considered as control positive while the fourth group gave 01ml of phosphate buffer saline as a control negative. After two weeks the animals of the first and second groups gave a same dose of immunization as a booster doses.

The animals (first second and third groups) were challenged after 45 days from the first immunization at a dose 1ml/rabbit (5×10^7 cfu/ml) (11) and between (3-20)days all animals were sacrificed and tissue specimens from liver lung spleen kidneys and intestine were fixed in 10% formalin saline for histopathology section which was done according to (12).

Results

-Macroscopic examination: Immunized animals: The internal organs of immunized rabbits were examined the main gross lesions were revealed as light congestion with some consolidating foci in the lung parenchyma (Fig1) liver showed moderate enlargement and congestion mainly 20 day post challenge (Fig2) while spleen showed moderate to severe splenomegaly mainly 20 day post challenge specially when compared the first group with control group (Fig3). No significant gross lesion were seen in the kidney and intestine.

Control animals: All rabbits of third (positive control) were dead during 24-48 hr post challenge There were severe congestion and swelling in selected organs (liver, lung, kidney and intestine) see, (Fig4) with presence of some necrotic foci mainly in kidney (Fig5).

-Microscopic examination:

1st immunized group: The lung sections showed at 3 days post challenge severe congestion of blood capillaries with polymorphonuclear cell infiltration (Fig6). The same lesion appeared at 20 days post challenge together with increase thickening of interalveolar septa due to congestion and MNCs infiltration (Fig7). Histopathological examination of liver at 3 days post challenge revealed slight dilation of sinusoids with few number of mononuclear cell aggregation around blood vessels and bile duct (Fig8), there was also increase in number of Kupffer cells with subcapsular hemorrhage while main lesion at 20 days post challenge was moderate Kupffer cell proliferation with vacuolar degenerative changes

of hepatocytes (Fig9). Kidney showed no clear pathological changes at 3 days post challenge while the characteristic lesion at 20 days post challenge was MNCS infiltration (mainly lymphocyte) between renal tubules (Fig10). No clear pathological changes were seen in the spleen at 3 days post challenge but the results at 20 days post challenge revealed prominence lymphoid hyperplasia of white pulp (Fig11). The intestine showed at 3 days post challenge hypertrophy of goblet cells with mucin production in the lumen together with inflammatory cells and slight sloughing of some epithelial mucosal layer (Fig12), but the main lesion at 20 days post challenge was MNCS infiltration in lamina propria of mucosa (Fig13).

2nd immunized group: There were fibrinous materials within edema in the lumen of alveoli together with congestion of capillary blood vessels and infiltration of inflammatory cells at 3 days post challenge (Fig14), but the result at 20 days post challenge showed only lymphocytic aggregation in the wall of bronchioles. There were severe dilation and congestion of sinusoids with Kupffer cells proliferation mainly at 3 days post challenge (Fig15) while no clear pathological changes observed in the liver at 20 days post challenge. Moderate to severe cellular degeneration appear in the kidney tissue at 3 days post challenge characterized by vacuolation of renal epithelial lining with sloughing of epithelial lining in some tubules (Fig16). The microscopic lesion in spleen at 3 days post challenge characterized by slight congestion of red pulp with slight depletion of white pulp (Fig17) but the results at 20 days post challenge showed lymphoid hyperplasia in the periarteriolar sheath (Fig18). Also there was hypertrophy of goblet cell with mucin secretion in the lumen as well as lymphocytic proliferation in the subepithelial and inter epithelial layer of intestinal mucosa mainly at 20 days post challenge (Fig19).

3rd non immunized group: The main lesion in lung revealed presence of proteinaceous material homogenous pink in color fill the lumen of alveoli (edema) together with congestion of capillary blood vessels and presence of inflammatory cells in alveoli lumen at 3 days post challenge (Fig20). Liver showed various pathological changes ranging from acute cellular degeneration characterized by vacuolation of hepatocyte with fatty changes together with focal area of necrosis (pyknosis of nuclei of the hepatocyte or disappearance) as well as neutrophil and MNCs infiltration associated with dilation of sinusoids (Fig21). The result of kidney sections characterized by acute cellular swelling with sloughing and necrosis of epithelia of renal tubules (Fig 22) together with cellular infiltration mainly around glomeruli (Fig23). Pathological changes of positive control spleen were severe congestion and dilation of vascular sinuses of red pulp with PMNCs infiltrate (Fig24) with slight depletion of white pulp. No clear lesion were seen in intestine except presence of congestion and slight cellular infiltration in the mucosa.

Discussion

Enterobacteriaceae of which proteus is a member when invade the blood stream, endotoxin a component of bacterial cell walls apparently triggers a cascade of host inflammatory responses that can causes sepsis and leads to major detriment effects (8), also the proteus bacteria are able to evoke pathological event in different region of the body(4) and the various componants of the membrane (liped bilayer lipoproteins polysaccharides and lipopolyscharides) inter play with the host to determine virulence and the inoculum size have a positive correlation to the risk of infection; also fimberiae facilitate adherence and enhance the capacity of the organism to produce the disease (8). These factors explain our result that a severe pathological effects was shown in the control positive group compared with the immunized groups which showed a synchronized immune cells infiltration with increase the immunized dose in the vital internal organs. On the same hand; the effect of interleukine 6 (IL-6) and interlukine 8 (IL-8) secretion initiates apoptosis and epithelial cell desqamation and the bacterial production of urease increase the risk of bacteremia and sepsis (1) that may be summarized in the control group which suffer from a severe epithelial cells sloughing of the urinary tubules (13); in addition the vili on the surface of the bacterium have a specific chemicals located on the tips enable the organism to attach the selected host tissue sites (8) and the proteases hemolysins amino acid deaminases and swarming growth enabling them to colonized and survived in a higher numders (4). The urea hydrolysis by urease causes severe tissue necrosis and inflammation at the site of infection; although fimbriae and flagella are immunogenic and aid in the persistence of this pathogen in the host (14);that may agree with our results which shown a moderate to severe mononuclear cells (mostly lymphocytes) infiltrations in vital internal organs specially in liver kidney and lungs; also spleen shown marked hyperplasia of lymphoid follicles in the immunized groups compared to control group that shown intensity and extent of inflammation accompanied by neutrophilic interstitial nephritis and in some cases the infection extending into the surrounding renal parenchyma. The main serum protein that bind to p vulgaris cells were shown to be albumin immune globulin G and complement C₃ in addition an unidentified proteins of low molecular weight (15). Also the survival and multiplication of intracellular bacteria specially in the blood lymphocyte may be of importance for development of infection in higher organism (16) Our conclusion of this study that show the immunization by *proteus vulgaris* fimbriae antigens can be repair the pathological effects of the virulence strain infection of this bacteria and this effects which synctronized with the dose of immunization.

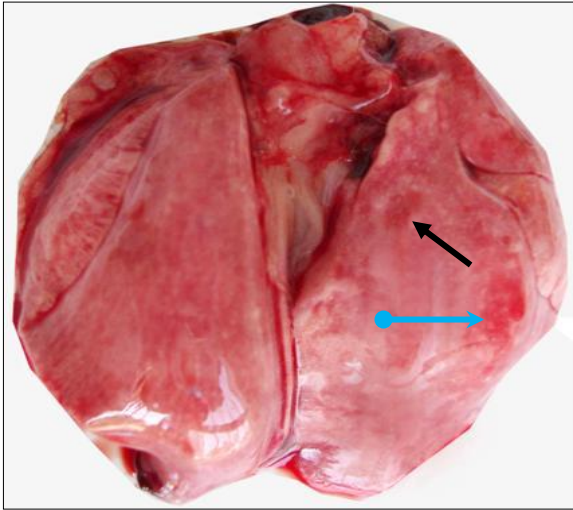


Fig:1 Gross rabbit lung of 1st immunized group at 20days post challenge show slight → congestion with some consolidating foci →

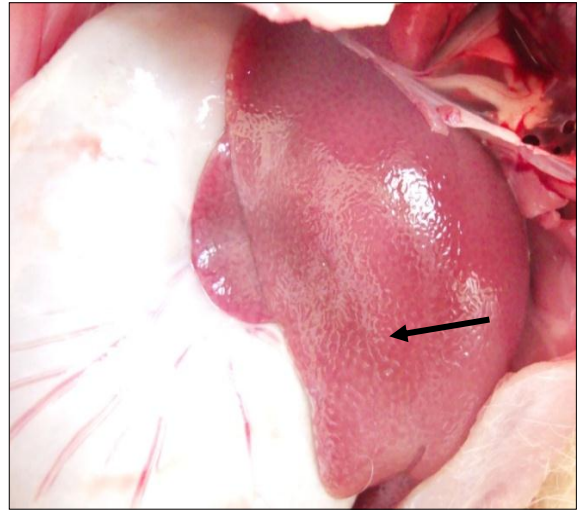


Fig:2 Gross rabbit liver of 2nd immunized group at 20 days post challenge show moderate enlargement with lobular pattern on its surface. →

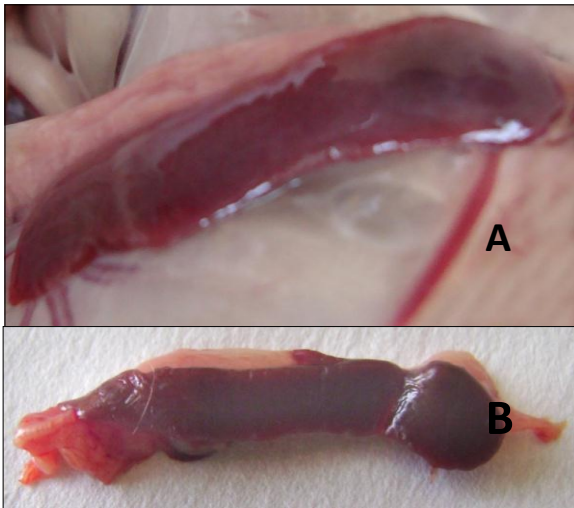


Fig:3 Gross rabbit Spleen of 1st immunized group at 20 days post challenge show severe enlargement (splenomegaly) **A** when compared to the control group **B**.

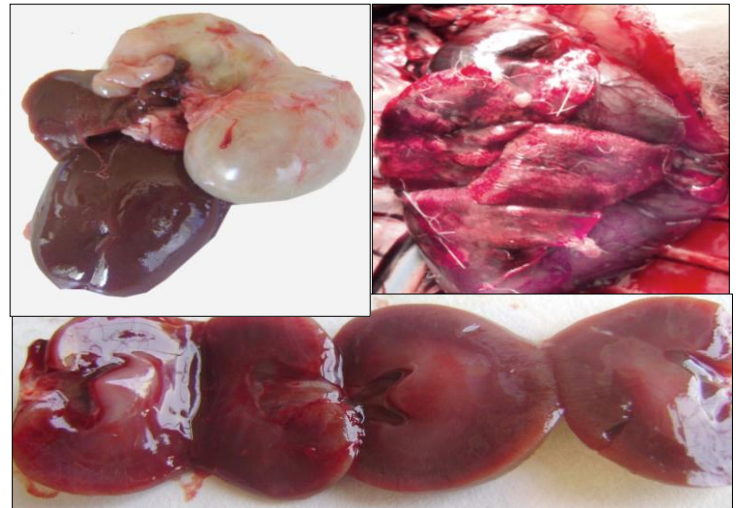


Fig:4 Gross rabbit lung, liver and kidney of 3rd (positive control) group at 24-48hr. post challenge show severe congestion in these organs with multiple consolidating foci mainly in lung parenchyma.

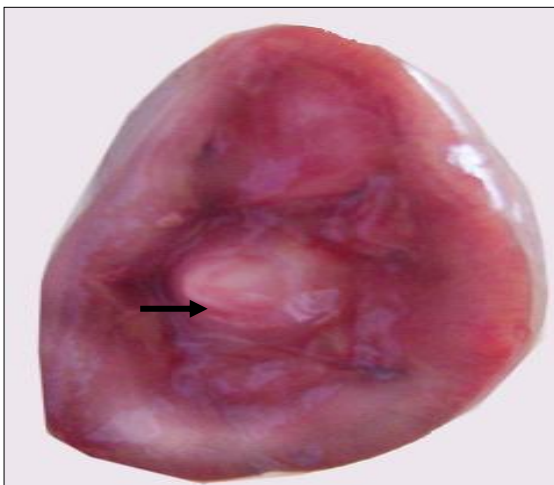


Fig:5 Gross rabbit kidney of 3rd (positive control) group at 24- 48 hr. post challenge show focal raised necrotic foci. →

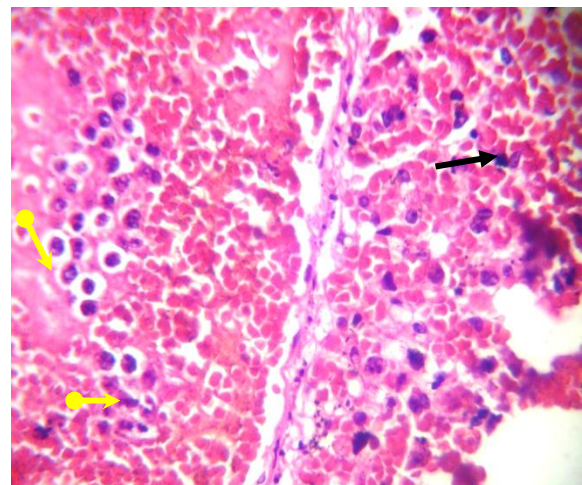


Fig 6: Microscopic section of rabbit lung from 1st immunized group at 3 days post challenge show severe congestion of blood capillaries → with PMNCS infiltration → (H&E X20).

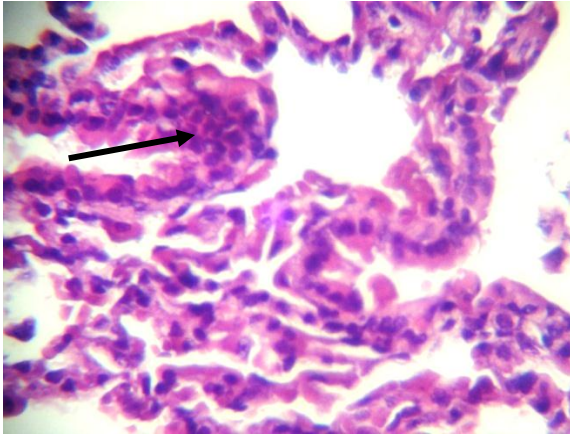


Fig 7: Microscopic section of lung from 1st Immunized group at 20 days post challenge show increase thickening of internal septa with MNCS infiltration → (H&E X20).

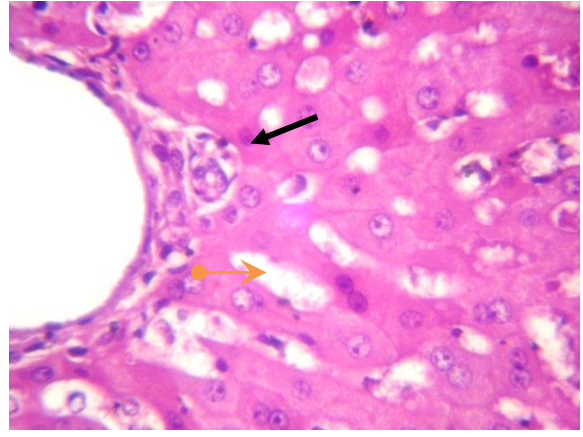


Fig 8: Microscopic section of liver from 1st immunized group at 3 days post challenge show slight dilation of sinusoids → with few MNCS infiltration around blood vessels & bile ducts → (H&E X20).

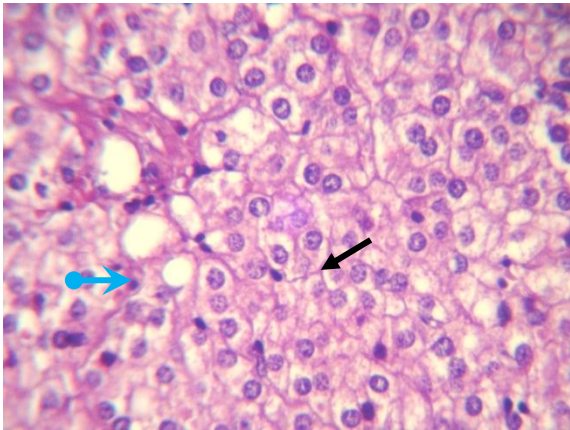


Fig 9: Microscopic section of liver from 1st immunized group at 20 days post challenge show moderate cellular swelling → and vacuolation of hepatocyte → (H&E X20).

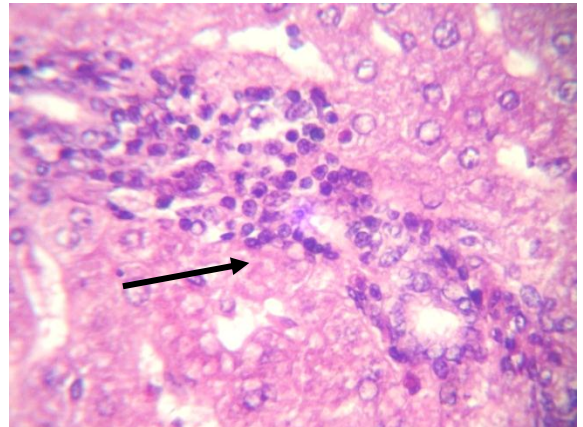


Fig 10: Microscopic section of kidney from 1st immunized group at 20 days post challenge show MNCS infiltration mainly lymphocyte between renal tubules → (H&E X20).

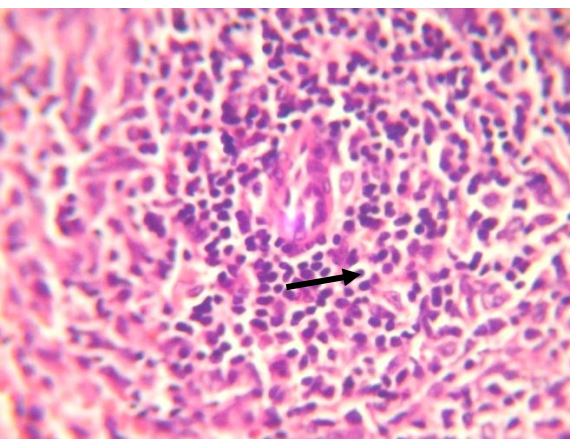


Fig 11: Microscopic section of spleen from 1st immunized group at 20 days post challenge show lymphoid hyperplasia of white pulp → (H&E X20).

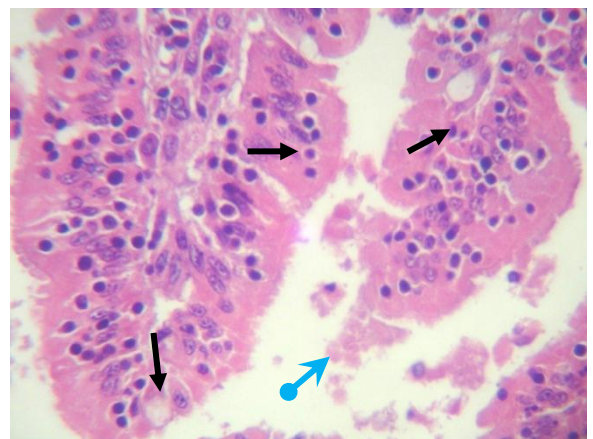


Fig 12: Microscopic section of intestine from 1st immunized group at 3 days post challenge show slight hypertrophy of goblet cells → and slight sloughing of epithelium mucosal layer → (H&E X20).

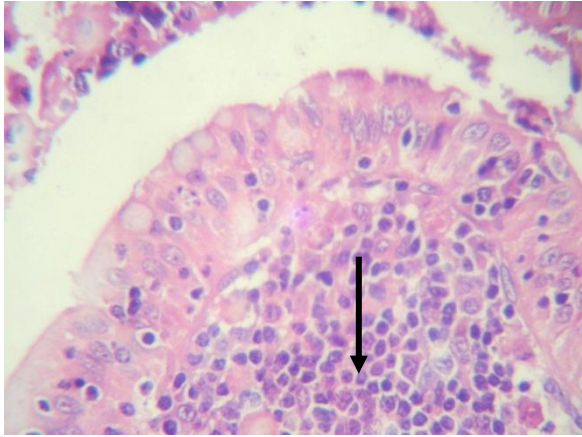


Fig 13: Microscopic section of intestine from 1st immunized group at 20 days post challenge show MNCs infiltration in lamina propria of intestinal villi → (H&E X20).

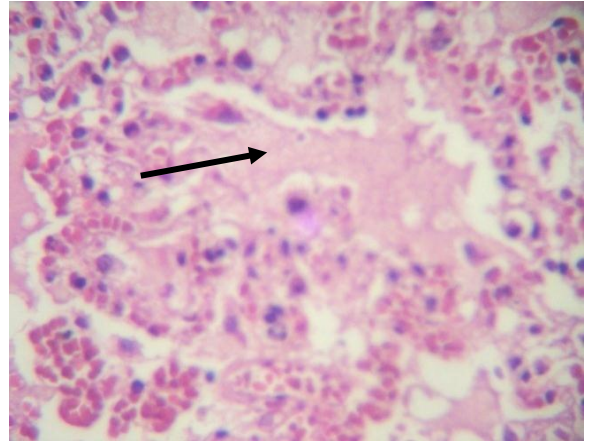


Fig 14: Microscopic section of lung from 2nd immunized group at 3 days post challenge show fibrinous material within edema in lumen of alveoli → (H&E X20).

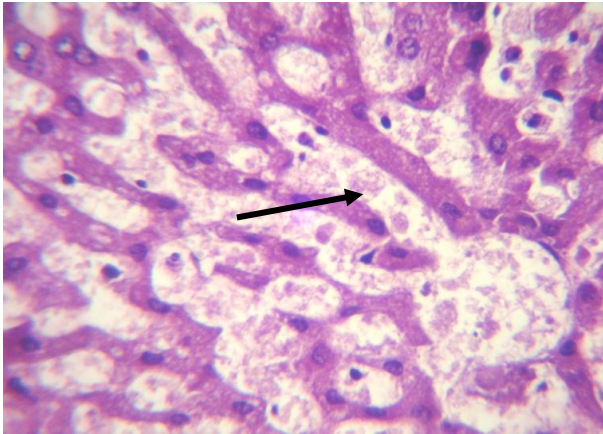


Fig 15: Microscopic section of liver from 2nd immunized group at 3 days post challenge show severe dilation of sinusoids with kupffer cells infiltration → (H&E X20).

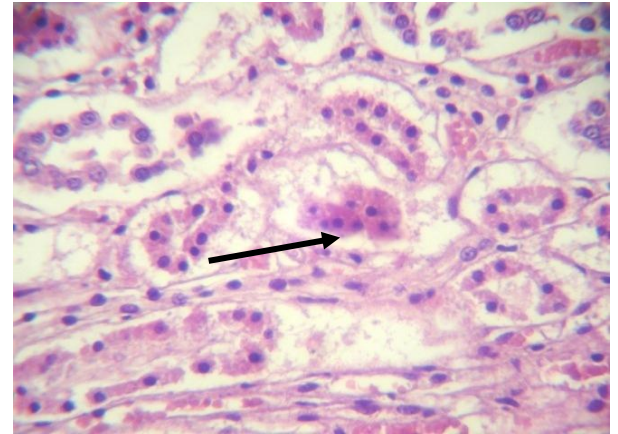


Fig 16: Microscopic section kidney from 1st immunized group at 3 days post challenge show slight sloughing of epithelial lining of tubules → (H&E X20).

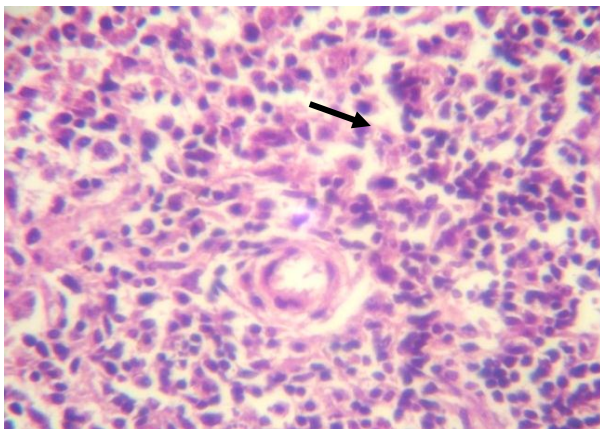


Fig 17: Microscopic section of spleen from 2nd immunized group at 3 days post challenge show slight congestion of red pulp with slight depletion of white pulp → (H&E X20).

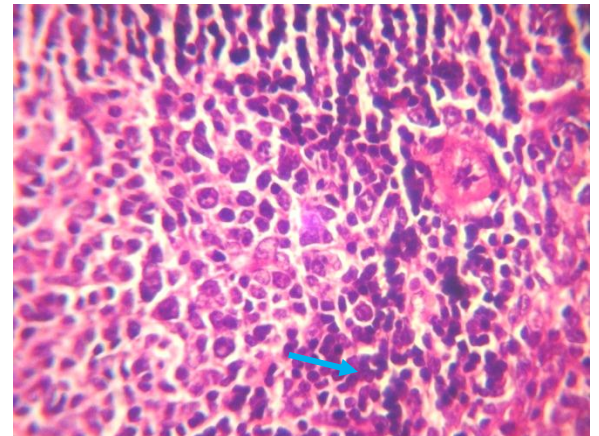


Fig 18: Microscopic lesion of spleen from 2nd immunized group at 20 post at 20 days post challenge show lymphoid hyperplasia in the periarteriolar sheath → (H&E X20).

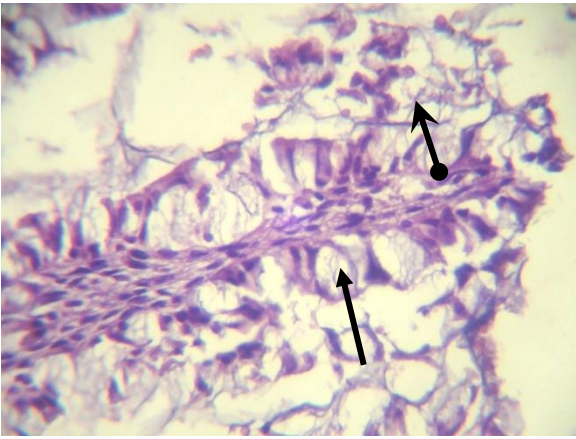


Fig 19: Microscopic section of intestine from 2nd immunized group at 20 post at 20 days post challenge show sever hyper trophy of goblet cells → with mucin secretion & lymphocyte proliferation in the sub epithelial layer ● → (H&E X20).

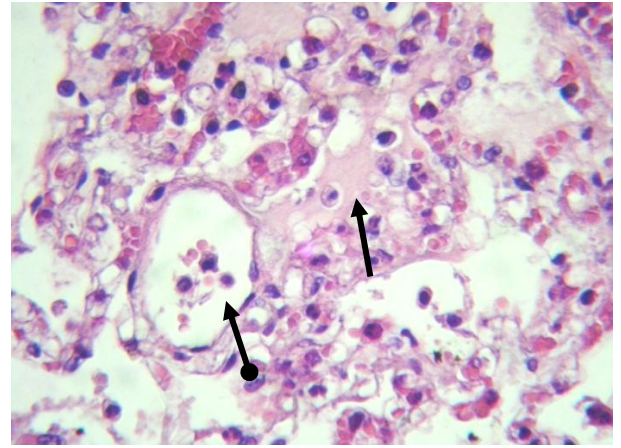


Fig 20: Microscopic section of lung from third group during 24-48 hrs show homogenous portentous substances in the alveolar lumen → with cellular infiltration in the alveoli & blood vessel ● → (H&E X20).

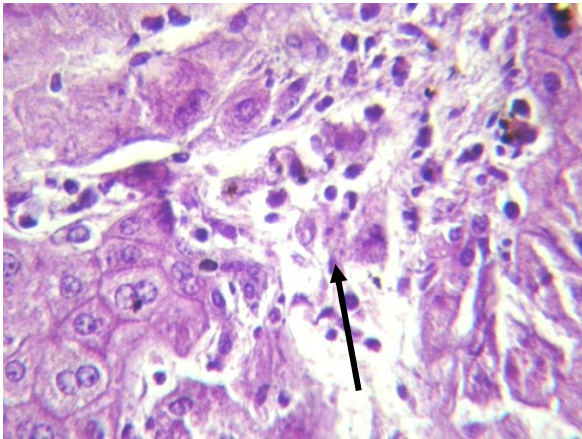


Fig 21: Microscopic lesion of liver from third group during 21-48 hrs show focal area of necrosis with PMNCS infiltration & sinusoids dilation → (H&E X40).

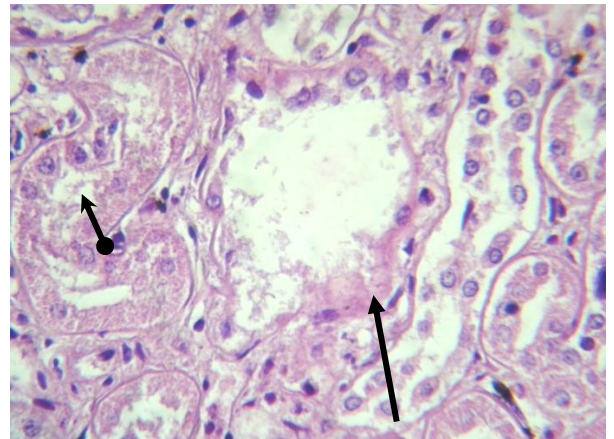


Fig 22: Microscopic section of kidney from third group during 24-48 hrs show cellular swelling ● → with sloughing & necrosis of epithelial lining tubule. → (H&E X40).

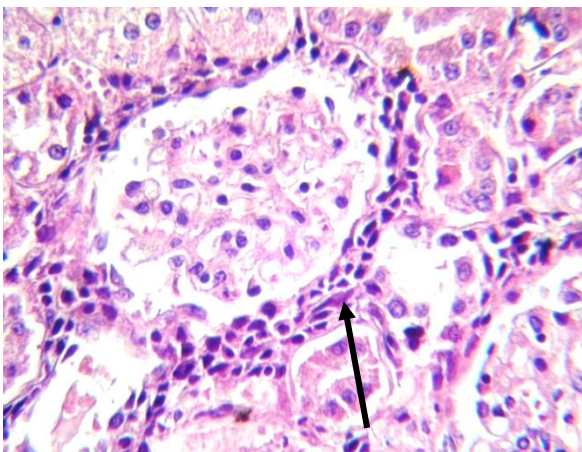


Fig 23: Microscopic section of kidney from third group during 24 - 48 hrs show cellular infiltration mainly around glomeruli → (H&E X20)

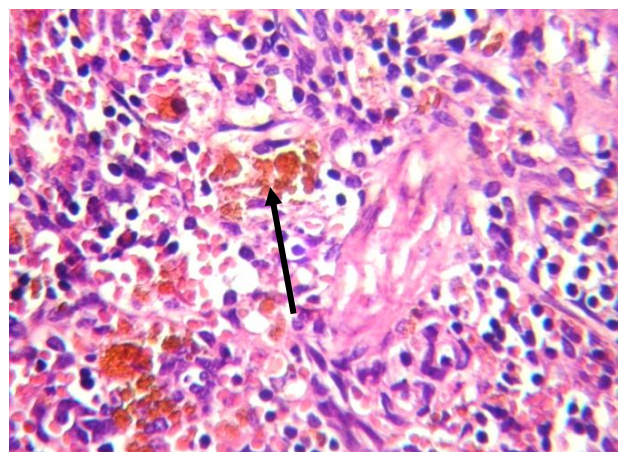


Fig 24: Microscopic section of spleen from third group during 24 - 48 hrs. post challenge show severe congestion → and dilation of vascular sinuses of red pulp with PMNCs infiltration (H&E X20).

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