International Journal of Current Research in
Biosciences and Plant Biology
Volume 5 • Number 11 (November-2018) • ISSN: 2349-8080 (Online)
Journal homepage: www.ijcrbp.com

Original Research Article **doi:** https://doi.org/10.20546/ijcrbp.2018.511.002

Pathological Studies on Infertility in Bucks as a Sign to Some Bacterial Infection

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Article Info

Date of Acceptance:
20 October 2018

Date of Publication:
06 November 2018

Keywords

Bucks
Infertility
Pasteurella multocida
Resistant bacteria
Staphylococcus aureus

ABSTRACT

A total of 26.7% *Pasteurella multocida* and 36.7% *Staphylococcus species* were isolated from thirty naturally infected rabbit cases in the Egyptian field for a year. All *P. multocida* isolates identified as serogroups A and 36.4% were *S. aureus*. Using multiplex PCR upon different isolates, the putative virulence genes (*nan*B) were detected in 83.3% of *P. multocida* isolates, while (*fnb*B, *seb*) genes were detected in 90% and 100% of *staphylococci* isolates, respectively. The isolated pathogens used to induce an organized experimental study which goal for evaluation the breeding status and fertility of thirty bucks either post recovery or chronic diseased infected cases in both treated and non treated groups, samples were collected at 35 and 45 days post infection (PI). Blood samples were collected for CBC, testosterone hormone, superoxide dismutase and glutathione analysis. Semen samples were collected and examined microscopically. Brief pathological examination for testis, prostate, liver, lungs and kidneys, were achieved microscopically and computerized by using Image analysis software program for the testis slides image. Johnson score analysis was done to examine and evaluate the efficacy of testis for spermatogenesis. Moreover we evaluated the antimicrobial sensitivity/resistance of bacteria in bucks which given variable results. Additionally, the susceptibility of *P. multocida* and *S. aureus* to (penicillin and gentamicin), respectively indicates that they remain reliable choices for preventing and treating the post infection infertility.

Introduction

Pasteurella multocida is a worldwide distributed veterinary pathogen, causing a wide range of diseases in domestic animals. It is divided into 5 capsular serovars (A, B, D, E and F) according to the presence of capsular antigens. In general, strains possessing a capsule are more virulent than their a

capsular variants (Boyce et al., 2010) capsular types A, D and F have been reported in rabbits (Massacci et al., 2018).

Lungs were the most commonly and severely affected organ in case of *P. multocida* infection. The prominent lesion in intranasal infected rabbits was fibrin accumulation. The pathological scoring of affected organs were congestion, consolidation with fibrin purulent exudates on lungs exterior and thoracic cavity Leghari et al. (2016), rhinitis with purulent nasal discharge, pneumonia, otitis media, pyometra, abscesses, and septicemia were the prominent noticeable clinical manifestations (Lennox, 2012). Rabbits may develop orchitis and / or epididymitis exhibiting decreased fertility and enlarged firm testicles (James et al., 2002).

Staphylococcus aureus is an adaptive opportunistic pathogen, capable to persist and replicate under various conditions. In rabbits, problems of staphylococcosis arise when *S. aureus* bacteria infect small dermal lesions and invade subcutaneous tissue (Georgieva et al., 2016), provoking a number of lesions including visceral abscesses, pod dermatitis, and mastitis. Occasionally, abscesses in internal organs, most commonly in liver and lungs were observed (Bisnoa and Stevens, 1996).

Urogenital tract infections in males are one of the common and significant etiological factors causing infertility. The bacterial infection as *Staphylococcus* spp. has been detected at the male reproductive system that exerts detrimental effect on sperm activity. Previous studies indicated that staphylococci not only affect the sperm activity but also impact the secretory capacity of the epididymis, prostate and seminal vesicles (Marconi et al., 2009).

It has been demonstrated that *S. aureus* infection significantly interferes with semen quality and activity. It deteriorates the volume of semen and the concentration of sperm as well as the motility, morphology, and vitality of sperm. Therefore, a causative relationship may exist between staphylococcal infection and male infertility. A previous study reported a 20.6% infection of *S. aureus* in the semen samples from males with fertility problems. More importantly, *S. aureus* infection was found to be closely related to poor semen quality and reduced sperm motility (Onemu and Ibeh, 2001). Some molecular studies have been developed to identify virulence and resistant genes in microorganism, which thus recommended in many publications as tools for the identification (Chantratita et al., 2016; Schuster et al., 2017).

Current control measures of rabbits are still mainly based on antimicrobial therapy (Sellyei et al., 2009). Isolates of microorganism are usually susceptible to antibiotics, however, the emergence of resistant strains has been reported in rabbits, this finding has raised concerns, since antibiotic resistance in pathogenic bacteria from food-producing animals is recognized as an emerging issue (Ferreira et al., 2012).

Aim of the work

This work has been aimed to evaluate the dangerous effects of both *P. multocida and S. aureus* on male rabbits genitalia and how far it develops into infertility in either post recovery or chronic cases through pathological and biochemical assessment with full bacteriological studies on the previously mentioned isolates.

Materials and methods

Bacteriological examination

Sampling: Specimens were collected from 30 different male rabbits (bucks) with clinical manifestations of both diseases (Pasteurellosis and staphylococcosis) from Sharkia / Egypt during the period from August 2017 to February 2018.

Isolation and identification: The isolates came from diagnostic submissions for post mortem examination and were obtained from lungs affected by respiratory disease, skin with abscesses to liver in case of septicemia. Briefly, the primary isolation

from tissues was carried out on blood agar plates (Blood Agar Base) supplemented with 5% sheep red blood cells, after incubation at 37°C for 24 h. *Pasteurella* suspected colonies were identified using standard biochemical procedures (API20NE, BioMérieux, Mary l'Etoile, France) (Collins et al.,1981). On the other side, the traditional methods of isolation and identification of *Staphylococci* were used as mentioned in ISO/ IEC 6888-2003. In addition, the Integral System Stafilococchi Kit (Liofilchem) was used for specific confirmation and biochemical identification of *Staphylococcus* isolates.

Antimicrobial susceptibility testing: The susceptibility of identified strains to a panel of five commonly used antimicrobial agents was performed by the standard Kirby–Bauer disc diffusion method for all isolates (Bauer et al., 1966). Integral System Stafilococchi Kit was used as a recent technique for compare the results in *Staphylococci*. The results were interpreted according to the criteria recommended by the Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing (CLSI, 2015).

Molecular detection of antibiotic resistance and virulence genes

DNA extraction: DNA extraction from samples was performed using the QIAamp DNA Mini kit (Qiagen, Germany, GmbH) with modifications from the manufacturer's recommendations. Briefly, 200 µl of the sample suspension was incubated with 10 µl of proteinase K and 200 µl of lysis buffer at 56^{O}C for 10 min. After incubation, 200 µl of 100% ethanol was added to the lysate. The sample was then washed and centrifuged following the manufacturer's recommendations. Nucleic acid was eluted with 100 µl of elution buffer provided in the kit.

Oligonucleotide Primer: Primers used were supplied from Metabion (Germany) are listed in Table (1).

PCR amplification: Primers were utilized in a 25-µl reaction containing 12.5 µl of EmeraldAmp Max PCR Master Mix (Takara, Japan), 1 µl of each primer of 20 pmol concentration, 4.5 µl of water, and 6 µl of DNA template. The reaction was performed in an Applied biosystem 2720 thermal cycler.

Analysis of the PCR Products: The products of PCR were separated by electrophoresis on 1.5% agarose gel (Applichem, Germany, GmbH) in 1x TBE buffer at room temperature using gradients of 5V/cm. For gel analysis, 15 µl of the products was loaded in each gel slot. Gelpilot 100 bp (Qiagen, Germany, GmbH) was used to determine the fragment sizes. The gel was photographed by a gel documentation system (Alpha Innotech, Biometra) and the data was analyzed through computer software.

Experimental animals: Thirty mature healthy white New Zealand fertile male rabbits (bucks), six months age were obtained from rabbits farm of faculty of agriculture, Zagazig university, with an average weight 4.5 kg then were caged separately in grow-out batteries with access to water and commercial feed *ad libitum*.

Challenge of rabbits with P. multocida: The rabbits were inoculated S/C with 0.1 ml of inoculum having 1×10^{7} colony forming units (CFUs)/ ml and with normal saline in control group according to Leghari et al. (2016).

Challenge of rabbits with S. aureus: The rabbits were inoculated S/C with 0.1 ml of inoculum having 8×10^{8} colony forming units (CFUs)/ ml and with normal saline in control group as done by Georgieva et al. (2016).

Experimental design: Thirty health mature fertile male rabbits (bucks), six months age were randomly allotted into five equal groups, first group, bucks were kept as a control, meanwhile induction of infection with previously isolated strains from naturally infected rabbits cases with *P. multocida* and *S. aureus* consequently were applied in the other four groups, groups (2 and 3) were infected groups

without treatments, while groups (4 and 5) were infected and treated with chosen antibiotic drug after sensitivity test for 45 days. Samples were collected twice, at 35 and 45 days post infection (PI) (the end of the experiment) Table (4).

Biochemical examination

Applied drugs

Depo-Pen: Benzathine penicillin 1.2 Million I.U. obtained from Medical Union Pharmaceuticals used in a dose 40000IU/ kg body weight (Ivey and Morriesy, 2000).

Gentacure: Each 1 ml contains gentamicin sulphate 156 mg obtained from Pharma Swede Company and used in a dose of 5mg/kg body weight intramuscular (recommended dose).

Laboratory assay

Collection of samples: Five ml of venous blood samples were collected from the ear vein of all rabbits at 35 and 45 days PI. The first sample was 1 ml of blood collected on EDTA for hematological examination. The second blood sample was allowed to clot. Clear serum samples were obtained by centrifugation of blood samples at 3000 rpm for 20 min. Serum samples were put in dry clean capped tubes and kept in deep freeze at -20ºC for biochemical analysis.

Biochemical and hematological evaluation

Red blood cell (RBCs) and White blood cell (WBCs) counts were counted by hemocytometer according to Stoskopf (1993). Blood film was prepared according to the method described by Lucky (1977). Blood hemoglobin (Hb) was assessed by cyanometahemoglobin method (Drubkin, 1964). In addition, M.C.V. Mean Corpuscular Volume, M.C.H. Mean Corpuscular hemoglobin and M.C.H.C. Mean Corpuscular hemoglobin concentration were calculated according to the formula mentioned by Dacie and lewis (1975).

M.C.V. = (PCV / RBCs) × 10 as m/mm3.
M.C.H. = (HB content gm/100ml/ RBCs) × 10 as m/mm3.
M.C.H.C. = (HB content gm/100ml / PCV) × 100 as %.

Glutathione peroxidase GPx: GPx activity was assayed by the method Pagila et al. (1967).

Superoxide dismutase (SOD): SOD activity was assayed according to Wooliams et al. (1983) using kits purchased from Randox, UK.

Male fertility evaluation

Semen collection: Semen was collected from bucks using a rabbit AV and a teaser female (Hafez, 1970). The collection was achieved at 35 and 45 days PI. for the routine evaluation of both live sperm and sperm abnormalities percentage using eosin aniline stain and before the slaughter immediately for enzyme determination.

Semen analysis: It was done according to method described by Williams et al. (1990) for examination of sperm cell concentration, motility, abnormalities and live /dead ratio.

Measurement of serum testosterone level: Serum testosterone levels were estimated according to Burtis and Ashwood (1994) using active testosterone enzyme immunoassay (EIA) DSL-10-4000 kit obtained from Diagnostic Systems Laboratories Inc.

Pathological examination

Tissue specimens: Specimen from testis, prostate, liver and kidneys were collected from sacrificed rabbits at 35 and 45 days PI and fixed in 10% buffered neutral formalin. Paraffin sections 5 micron thick were prepared and stained with hematoxylin and eosin stain (Survarna et al., 2013) and examined microscopically.

Johnson score analysis: It was done to examine and evaluate the efficacy of testis for

spermatogenesis (Johnson, 1970) in grade from 1 to 10 was given to each somniferous tubule cross section according to range from no cells to complete spermatogenesis.

Image analysis: By using computerized software program for analysis of the testis slides image and assessment of somniferous tubules efficacy. About 5 different sections of somniferous tubules that were round or nearly round were chosen randomly and measured for each rabbit. The tubular diameter and lumen, height of somniferous tubule epithelium and height of interstitial tissues were measured at ×40 and ×100 magnifications using image analyzer Leica (DMLB) and Leica Qwin software.

Statistical analysis

Statistical analysis was performed using the analysis of variance (ANOVA). Duncan's Multiple Range Duncan (1955) was used to determine differences among treatments mean at significance level of 0.05. All statistics were run on the computer using the SPSS program (SPSS, 2004).

Results and discussion

Bacteriological results

Bacterial strains: A total number of thirty male rabbits (bucks), were collected randomly from four different localities of Sharkia governorate and necropsied for both bacteriological and pathological study. The positive bacterial cultures were 8/30 (26.7%) for *Pasteurella* and 11/30 (36.7%) *Staphylococcus spp.*, respectively Table (2). These results are agree with (Langan et al., 2000; Rougier et al., 2006) and in Italy Martino and Luzi (2008) detected (21.9%) and (6.2%) for eachmicrobe, respectively.

Serological typing of bacteria isolates: In this study capsular types A and D were detected using PCR as confirmatory test among *Pasteurella* isolates, all isolates identified as *P. multocida* serogroup A 10/10 (100%) Fig.(II) (E,F). In China (95.7%) (Zhangcheng et al., 2018), in Iran (76.7%) (Khamesipour et al., 2014) were isolated type A, while Massacci et al. (2018) and Jaglic et al. (2004) detected type A, D (51.3%, 23%) and (58.4%, 8.3%), respectively.

Using Integral system stafilococchi kit: ISSK for typing *staphylococci spp.* isolates which revealed 4/11(36.4%) isolates were identified as *S. aureus*, this result was nearly agreed with Indrawattana et al. (2018) (28.4%) in Thailand, while Williams et al. (2007) detected (15%) *S. aureus.*

Antimicrobial susceptibility testing: The ability of micro-organisms to cause disease depends on a combination of virulence factors with regards to the antibiotic susceptibility of bacteria involved in buck diseases. Phenotypic diversity of antibiotic sensitivity of all isolates (*P. multocida* and *Staphylococcus spp.*) demonstrated high resistance to doxycycline, tetracycline and ciprofloxacin (100%). Among the *Staphylococcus spp.* isolates, nine were resistant to penicillin 9/11 (81.8%) and four were resistant to gentamicin 4/11 (36.3%). In Thailand, Indrawattana et al. (2018) recorded that *Staphylococci spp* isolates were resistant to penicillin (37.5%)and gentamicin (12.5%),while in Trinidad, Dziva et al. (2015) recorded that resistance of isolates against penicillin (49.2%) and gentamicin (16.9%).Table (3).

On another side, all *P. multocida* isolates were resistant to gentamicin 8/8(100%), while three were resistant to penicillin 3/8(37.5%), the results are agree with Freshwater (2008) recorded high susceptibility of *P. multocida* isolates to penicillin, while disagree with Balakrishnan et al. (2012) who recorded that all *P. multocida* isolates showed sensitivity to Gentamicin, Ciprofloxacin and Doxycycline while all isolates were resistant to Penicillin (Table 3).

Distribution of virulence and resistant genes between different isolates: With regard to PCR results for ten *Staphylococcus spp.* isolates Table (5), all isolates were containing (*seb)* gene 10/10 (100%) that indicating virulence factor for exotoxin superantigens of the organism is highly correlated

with fibronectin-binding protein factor (*fnb*B) gene 9/10 (90%) Fig.I (A, B) (Viana et al., 2015). This result was in consistent with several authors (Cao et al., 2012; Mudili et al., 2015 and Crosby et al., 2016).

Phenotypic resistance of six *S. aureus* group 5 to penicillin and gentamycin antibiotics were explained by the presence of *bla*Z and *aac(6´) aph(2´´)* resistance genes among the examined isolates 6/6 (100%) and 1/6 (16.7%), respectively. An obvious finding in this study was that, the results of phenotypic resistant pattern of some isolates against certain antibiotic by disc diffusion method were found in parallel with the results of PCR for detection of their relevant antibiotic resistance genes by PCR as shown in Fig.I (C,D), the same results recorded by Indrawattana et al. (2018)in Thailand.

Among the six *P. multocida* isolates Table (6), neuraminidase (*nan*Bgene) virulence gene was found in 5/6 (83.3%) of the isolates Fig.(II)(G), the same results recorded by Li et al. (2018) (82.6%) in China, Furian et al. (2016) detected *nan*B gene in (99%) of *P. multocida* type A.

All six *P. multocida* isolates from experimental group4 show distribution of (*bla*ROB1and *aph*1) resistant genes for penicillin and gentamycin antibiotics which were exhibited in 2/6 (33.3%) and 6/6 (100%), respectively Fig.(II)(J,H), the same results recorded by Huang et al. (2008) who detected *aph*1 gene in all isolates, in Iran (40%) of isolates have *bla*ROB1 gene (Khamesipour et al., 2014).

Biochemical and hematological results

The results of hematological parameters presented in Tables (7, 8) showed that rabbits infected with *P. multocida* and *S. aureus* showed significant decrease in RBCs, HB and PCV comparable to the mean values of RBCs, Hb and pcv% in control group. Same changes in hematology were reported by Mohamed (2009) Praveena et al. (2010) they mentioned that there is information concerning anaemia, caused after *P. multocida* infection in chickens and mice related to hemolytic activity of different strains of this pathogen. Also, there are available reports on RBCs, HB, and PCV in rabbits, infected with *P. multocida* (Petrova et al., 2017; Leghari et al., 2016; Khalil and Saif, 2014). All above mentioned data reported a decrease in red blood cell counts after experimentally infection with *P. multocida* in rabbits.

Drop in the RBC after bacterial inoculation could be due to toxins released by *P. multocida* and partly due to reduced feed intake by the infected animals. the decrease in Hb and pcv% may related to the decrease the release of iron from macrophages to plasma cells, then reduced red cell life span and inadequate erythropoietin response to anemia (Hoffbr and Pettit 1993).

Our findings in agreement with Petrov and Mircheva (2013) and Dimitrova et al. (2000), as they reported a reduction of these parameters in rabbits with *S. aureus* infection and they assumed that due to the damage or complication of the hematopoietic function, caused by the *S. aureus*. Moreover, in the present study the rabbits infected with Pasteurellosis and treated by penicillin showed significant improvement in blood picture that due to the bactericidal effect of penicillin on *Pasteurella.* Penicillin has bactericidal action through binding of the beta-lactam ring to DD-transpeptidase, inhibiting its cross-linking activity and preventing new cell wall formation. Without a cell wall, a bacterial cell is vulnerable to outside water and molecular pressures, and quickly dies (Laurence et al., 1996). Our findings were in accordance with that recorded by Sandhu and Dean (1980) stated that a single dose of parentrally penicillin was significantly reduced mortality in white pekin ducklings experimentally infected with *Pasteurella*. Moreover, Petridou et al. (2010) stated that lambs infected with *P.multocida* and treated with penicillin showed rapid response to treatment. Also, Satyaprakash et al. (2010), Corbel (2012) and Antoni et al. (2016) reported that penicillin was the treatment of choice for *P. multocida* infection.

In the present study rabbits infected with staphylococcosis and treated by gentamicin revealed significant improvement in hematological findings. Gentamicin is a bactericidal aminoglycosides antibiotic that works by irreversibly binding to the 30s subunit of bacterial ribosome, interrupting protein synthesis in the bacteria (Laurence et al., 1996). Our results were in accordance with that recorded by Asseray et al. (2002) mentioned that gentamicin is the best aminoglycoside used for treatment of methicillin – resistant *S.aureus* infection. Also, Worlitzsch et al. (2001) stated that gentamicin well suited for treatment of localized chronic *S. aureus* infections because it reduce the level of hemolysis caused by *S. aureus* by decreasing bacterial numbers.

In the present study, infection with *P. multocida* and *S. aureus* in rabbits increased the levels of white blood cells and the similar findings have also been reported by Numata et al. (1998) and Carrigan et al. (2004) reported that leukocytosis was the feature of increased levels of white blood cells in blood stream occurred as a result of activation of bone marrow by *P. multocida* antigens.

Spermatozoa are highly specialized haploid cells produced in the testes of adult males, and spermatogenesis is a complex series of maturational and transformational processes that occur in the somniferous tubules of testis (Patton and Battaglia, 2005). According to WHO criteria, sperm number, motility, viability and morphology are used to assess the function of spermatozoa (World Health Organization, 2010). In the present study rabbits infected with *P. multocida* and *S. aureus* showed impaired male reproductive function by reducing the sperm count and volume or changing sperm motility and morphology. Semen analysis in Tables (11, 12) revealed that infection with *P. multocida* and *S. aureus* lower in motility, sperm cell count, live sperm %, while, they were significantly higher in the total primary sperm abnormalities %, than the control group. While; treatment with either penicillin or gentamicin corrected these adverse effects This was agree with the finding of Abdoli and Movahedin (2012) and Terpsidis et al. (2009) who found that sperm parameters decreased in male rats infected with *T. gondii* and concluded that toxoplasmosis can affect main reproductive, also Marconi et al. (2009), Onemu and Ibeh (2014) and Filipiak et al. (2015) mentioned that *S. aureus* infection significantly interferes with semen quality and activity. It deteriorates the volume of semen and the concentration of sperm as well as the motility, morphology, and vitality of sperm, also Kaur and Prabha (2013) and Prabha et al. (2009) explained the mechanism whereby staphylococci modulate sperm activity due to presence of sperm agglutinating factor in staphylococci so that *S. aureus* can adhere to the sperm head as well as sperm tail and agglutinate mouse spermatozoa and implicated the protein in control of sperm motility and survival. Berktas et al. (2008) raised another point of view that, rather than the direct interaction between bacteria and sperm, that based on alteration in genital micro environment due to over-consumption of energy by the bacteria led to the loss of sperm motility.

In the present study serum testosterone level was significantly decreased in the infected groups with *P. multocida* and *S. aureus* but it come again to the normal level in treated groups (Tables 9, 10). Our results were agree with that reported by Marconi et al. (2009) who stated that Staphylococcal infection in male reproductive organs and accessory glands has detrimental effect on sperm activity, staphylococci not only affect the sperm activity but also impact the secretary capacity of the epididymis, seminal vesicles, and prostate. Testosterone is a steroid hormone that is mainly produced in testis Flegr et al. (2008). Testosterone is required for the maturation of male germ cells and sperm production and quality (Attiaet al., 1993; Walker, 2009). Our results in accordance with that of Abdoli and Movahedin (2012) and Rui et al. (2009) demonstrated that serum free testosterone decreased in male patients with acute toxoplasmosis and they concluded that bacterial infections induce apoptosis of spermatogenic cell, especially that the apoptosis of spermatocyte may be related to the decrease in local testosterone level. A good semen quality is a main target requested from the male reproduction.

This is in need for a physiological boundary of reactive oxygen species (ROS) to accomplish its role. Whereas, high levels of ROS is related with the present of sperm fertilizing capability (Capucho et al., 2012). So ROS combined with total antioxidants capacity could predict fertility in male. Oxidative stress in the semen occurs when the level of ROS is greater than the antioxidant (Jones et al., 1997). The oxidative enzymes, such as SOD and GPx act protectively against oxidative damage of cells and to remove free radicals and products of their decomposition. Lack or limited ability of an organism to inhibit uncontrolled reactions of free radicals resulted in development of many diseases (Pitchumoni and Doraiswamy, 1998; Dhalla et al., 2000). In the present study the infected groups with *P. multocida* and *S. aureus* showed decline in the production of glutathione as well as a significant decrease in superoxide (Tables 9, 10) induced oxidative stress in animal. Oxidative stress is an imbalance between the free radical production and antioxidant defense systems of the body (Yao et al., 2006). The results also showed that treatment with penicillin and gentamicin reduced ROS generation and improved semen quality. Our results were in accordance with that reported by Mallikarjuna et al. (2006) who reported that bacterial lipopolysaccharide induced acute inflammation which resulted in decreased the activities of testicular antioxidant enzymes such as superoxide dismutase and glutathione peroxides. Also, Aitken (1999) mentioned that the oxidative stress is a major causal factor in altered steroid genesis, spermatogenesis, and perhaps male infertility during endotoxin-induced acute inflammation.

Clinical findings and pathological results

Clinical findings

The clinical findings in rabbits inoculated with *P. multocida* and *Staph. aureus* showed marked differences than control and treated groups. Marked decrease of sexual activities, behavior and desire among rabbits of groups (2 and 3), while it's less noticeable in groups (4 and 5), gradual rise of body temperature within first two weeks PI among rabbits of groups (2 and 3) that was in complete accordance with that mentioned by Georgieva et al. (2016). Respiratory manifestations (sneezing with catarrhal to purulent discharge as a thick yellowish gray matter) among rabbits of group (2) were noticeable. No clinical signs were detectable in groups (1, 4 and 5) those could be attributed to the effect of penicillin on rabbits infected with pasteurollosis showed significant remission of clinical signs of *Pasteurella* infection. Jaslow et al. (1981) and Worlitzsch et al. (2001) who mentioned the role of gentamicin in treatment of localized chronic *S. aureus* infections.

Pathological results

Macroscopical results: Rabbits of group (2) sacrificed at 35 days PI showed congested nostrils with thick sticky discharge, catarrhal, purulent and fibro purulent exudates probably originating from inflamed left lung concomitant pleurisy were noticeable. Congested lungs, liver and hearts Fig.(A) which appeared mild in rabbits of group (4), while at 45 days PI the previous changes became milder in group (2) and disappeared completely in group (4). Three rabbits out of six showed subcutaneous disseminated abscesses after overlying skin removal Fig.(B). Rabbits of group (3) sacrificed at 35 days PI showed congestion of upper respiratory tract without discharge, subcutaneous small abscesses were noticeable, no marked changes among rabbits of group (5) while at 45 days PI two rabbits out of six of group (3) showed spreading diffuse subcutaneous swelling (phlegmon) Fig. (C) that mainly affected the dorsal posterior region and appeared as white gray diffuse caseated matter which not appeared in rabbits of group (5) this sign was similar to that described by Georgieva et al. (2016) and could be attributed to the limited extent and activity of *Staph. aureus* causing acute suppurative inflammation (phlegmon) (Lowy, 1998). Only few cases showed mild orchitis specially in group (2) than that in group (3) and completely disappeared in groups (4 and 5). Lungs were apparently normal except in some rabbits of group (4 and 5) that showed small focal mild congested areas which could be attributed to the

action of both penicillin on *Staph. aureus* and gentamycin on *P. multocida* (Neut et al., 2015) in minimizing the pathological alteration due to both of them.

Microscopical results: Rabbits of group (2) sacrificed at 35 days PI showed different pathological changes in some organs when compared with other groups. Livers showed different degrees of congestion without or with leucocytic cells infiltration and fibrosis Fig.(1),while at 45 days PI livers showed focal caseation with leucocytic cells infiltration Figs. (2 and 3). Livers of rabbits of group (3) at 35 and 45 days PI showed focal areas of leucocytic cells infiltration Fig.(4), most rabbits' livers in groups (2 and 3) showed some degenerative changes from mild to moderate hydropic degeneration. Lungs was the most affected organ in both groups (2 and 3) that showed severe congestion, variable degrees of emphysema from mild to severe leading to alveolar rupture in severely affected cases with colonization of bacilli in its tissue which was clear among rabbits of group (2) at 35 days PI Fig. (5 and 6). Congestion was also noticed in lungs of rabbits of group (3) specially at 35 days PI, mild to moderate emphysema and inter alveolar hemorrhage were detected Fig.(7) , while kidney was the less affected organ in both groups (2 and 3) focal leucocytic cells infiltration with fibroblast was noticed in some rabbits of group (2) at 35days PI Fig.(8) with or without congestion while few cases showed mild degenerative changes in some renal tubules at 45 days PI. Some cases among rabbits of group (3) showed only cystic dilation of its renal tubules Fig.(9) at 45 days PI. Normal renal tissues were seen in group (4 and 5) at both 35 and 45 days PI. Testis of rabbits of group (2) at 35 days PI showed intertubular invasion with bacilli either sporadic or colonized without or with fibroblasts and leucocytic cells aggregation Fig. (10), while at 45 days PI rabbit's testis of the same group showed vacuolation of some spermatocytes and atrophy of some somniferous tubules with interstitial edema (Asterisk) Fig.(11). Testis of rabbits of group (3) at 35 days PI showed congestion of testicular blood vessels Fig.(12) with mild degeneration of some somniferous tubules, while at 45 days PI. testis showed mild to severe atrophy of some somniferous tubules without or with destruction of other tubules beside focal or diffuse interstitial edema Fig. (13). Other rabbit's testis showed vacuolation of some spermatogonia with disappearance of spermatogenesis beside interstitial edema (Asterisk) Fig.(14). There were no microscopical changes noticed in testis of rabbits of groups (1, 4 and 5). Prostate of rabbits of group (2) at 35 days PI showed focal leucocytic cells infiltration with fibrosis among prostatic acini Fig. (15) with or without congestion of prostatic blood vessels, while at 45 days PI. Interstitial edema (Asterisk) was the main noticeable lesion in all rabbits Fig. (16). prostates of rabbits of group (3) were more effected and showed mild congestion with or without disappearing the acinal folds which sometimes filled with homogenus esinophilic material Fig. (17), while at 45 days PI. Some rabbits showed thickening and fibrosis of the acinal wall without or with fusion of some acinal folds Fig. (18). The degree of the virulence of particular strains together with the influence of various stressors that suppress the animals' immunity may play an important role in clinical manifestation and pathological alterations of rabbits pasteurellosis Delong and Manning (1994) and Webster (1924). Most of our results were in harmony with those described by Zoran Jaglic et al. (2011) who noticed hyperemia, hemorrhage, edema, inflammatory cell infiltrates and degenerative changes in parenchymatous organs of rabbits infected with *P. multocida*. Although septicemic pasteurellosis in rabbits is fatal Webster(1924), moderate to severe signs of a respiratory disease were observed in this study. This could be attributed to the nature of challenged strain and it's virulence degree in addition to route of administration (SlC). Male genitalia was clearly affected specially in chronic cases as in groups (2 and 3) and post cure (recovery rabbits) as in groups (4 and 5), further indicates a high invasiveness of the tested avian strains. This also is supported by the fact that, besides the blood and parenchymatous organs, *P. multocida* was very often re-isolated from urine and rectal swabs. The presence of *P. multocida* in urine from bacteremic

rabbits was also reported by Ruble et al. (1999); however, its frequency in the rectal swabs is, to our knowledge, unusual. Our microscopical lesions of fresh sacrificed rabbits from which *Staph. aureus* was isolated were in partial accordance with those obtained with Abdel Gawad et al. (2004), who described signs of septicemia including congestion with pectichial hemorrhages in internal organs as liver, lung and kidneys, beside abscess in lungs and liver observed in adult rabbits. In few cases, while rabbits of group (5) showed minimize to no losses and lesions from this disease that could be explained on a base of applied filed treatment with antibiotics in affected rabbits for three successive days gave a satisfactory results, due to highly sensitive isolates to gentamycin.

Johnson score analysis results: Rabbits in different groups showed different grades of Johnson score that began with normal full spermatogenesis (score 10) to only spermatogonia present (score 3) as shown in plate (V). The highest percentage of control rabbits (35.7%) had modified Johnson score (10) at 35days PI, while the highest percentage (39.6%) of rabbits at 45 days PI had modified Johnson score (10). The highest percentage of rabbits (36.1%) of group (2) at 35th day had modified Johnson score (4), while the highest percentage (26.7%) of rabbits at 45th day had modified Johnson score(5). Rabbits of group (3) at 35th day showed highest percentage (34.4%) with modified Johnson score (4) while at 45th day, the highest percentage (36.2%) at modified Johnson score (5).The highest percentage of rabbits of group (4) was (27.6%) had modified Johnson score(7) at 35days PI, while the highest percentage (31.9%) of rabbits at 45 days PI had modified Johnson score(9) and finally the highest percentage (35.5%) of rabbits at 35 days PI had modified Johnson score (8) and (38%) at modified Johnson score 8 at 45 days PI. All recorded in Table (13) Grades of modified Johnson score could be explained on a base of role of inflammation induced by the direct action of both isolates on the somniferous tubules ,the ability of the developing male germ cells and mature sperm to avoid the immune system of the host and the ability of inflammation arising outside the male reproductive tract to inhibit male fertility are among the most intriguing conundrums of male reproductive function and also the ability of spermatogenic cells to evade the host immune system. One important consideration must be very different immunological environments of the testis, where sperm develop, and the epididymis, where sperm mature and are stored. Compared with the elaborate blood-testis barrier, the tight junctions of the epididymis are much less effective. Unlike the somniferous epithelium, immune cells are commonly observed within the epithelium, and can even be found within the lumen, of the epididymis (Hedger, 2013).

Image analysis: The highest tubular diameter was detected in rabbits of group (1) at 35 and 45 days PI. Rabbits of groups (3 and 2) consequently showed highly significant decrease in the highest of tubular diameter when compared with those of rabbits of groups (1, 5 and 4) consequently at 35 and 45 days PI. Tubular lumen was highly significantly increase in rabbits of groups (2 and 3) consequently and of significant increase in groups (4 and 5) consequently when compared with those of rabbits of group (1) at 35 days PI and when compared with those of groups (1 and 5) at 45 days PI. Thickness of interstitial tissue was significantly increased in both groups (2 and 3) consequently when compared with groups (1 and 5) at 35 days PI, while there was significant increase in group (2) when compared with other groups at 45 days PI. Finally the highest of germinal epithelium was significantly decrease in groups (4 and 5) consequently and highly significant decrease in groups (2 and 3) consequently when compared with group (1) at 35 days PI. While rabbits of group (5) showed significant decrease in comparison with those of group (1) and rabbits of groups (2, 3 and 4) showed highly significant decrease in germinal epithelium highest when compared with those of group (1) at 45 days PI (Table 14). These results were matching with our microscopical results of testis and at the same time confirm those obtained by Johnson score and semin analysis on a base of orchitis and epididemitis induced by both previously challenged isolates (James et al., 2002; Megan et al., 2015; Georgieva et al ., 2016).

Table 1. Primers sequences, target genes, amplicon sizes and cycling conditions.

Target gene	Isolates	Primers sequences	Amplified segment (bp)	Primary denaturation °C/min	Amplification (35 cycles)			Final extension °C/min	Reference
					Secondary denaturation °C/sec	Annealing °C/sec	Extension °C/sec		
fnbB	*S. aureus*	GTAACAGCTAATGGTCGAATTGATACT CAAGTTCGATAGGAGTACTATGTTC	524	94/5	94/30	55/40	72/45	72/10	Tristan et al. (2003)
blaZ		ACTTCAACACCTGCTGCTTTC TGACCACTTTTATCAGCAACC	173	94/5	94/30	54/30	72/30	72/7	Duran et al. (2012)
Aac (6') *aph (2')*		GAAGTACGCAGAAGAGA ACATGGCAAGCTCTAGGA	491	94/5	94/30	54/40	72/45	72/10	
seb		GTATGGTGGTGTAACTGAGC CCAAATAGTGACGAGTTAGG	164	94/5	94/30	57/30	72/30	72/7	Mehrotra et al. (2000)
blaROB1	*P. multocida*	AATAACCCTTGCCCCAATTC TCGCTTATCAGGTGTGCTTG	685	94/5	94/30	60/40	72/45	72/10	Klima et al. (2014)
aphA1		TTATGCCTCTTCCGACCATC GAGAAAACTCACCGAGGCAG	489	94/5	94/30	54/40	72/45	72/10	
nanB		GTCCTATAAAGTGACGCCGA ACAGCAAAGGAAGACTGTCC	554	94/5	94/30	50/40	72/45	72/10	Sarangi et al. (2014)
Serogroup A		TGC-CAA-AAT-CGC-AGT-GAG TTG-CCA-TCA-TTG-TCA-GTG	1044	94/5	94/30	55/40	72/60	72/10	OIE (2012)
Serogroup B		TA-CAA-AAG-AAA-GAC-TAG-GAG-CCC CAT-CTA-CCC-ACT-CAA-CCA-TAT-CAG	657	94/5	94/30	55/40	72/45	72/10	

Table 2. The prevalence of bacterial isolates.

Type of isolates (30 isolates)	No. of bacterial isolates (%)		Total (%)
	P. multocida	***Staphylococci***	
Lung	4	1	7
Liver	2	2	4
Abscess	2	8	8
Total (%)	8 (26.7%)	11 (36.7%)	19(63.3%)

Table 3. Phenotypic resistance profile of *P. multocida* and *Staphylococci* isolates.

Resistance pattern*	***P. multocida* (8)**	***Staphylococci* (11)**
Doxycycline	8/8 (100%)	11/11 (100%)
Tetracycline	8/8 (100%)	11/11 (100%)
Ciprofloxacin	8/8 (100%)	11/11 (100%)
Penicillin	3/8 (37.5%)	9/11 (81.8%)
Gentamicin	8/8 (100%)	4/11 (36.3%)

(*) The percentage was calculated according to the total no. of *P. multocida* and *Staphylococci* isolates.

Table 4. Experimental design.

Groups	No. of experimental rabbits	Treatment	Dose	Samples collection	
				35th day	45th day
Group(1)	6	----------	----------	3	3
Group(2)	6	*P.multocida*	$1x10^7$cfu	3	3
Group(3)	6	*S. aureus*	$8x10^8$cfu	3	3
Group(4)	6	*P. multocida* + Penicillin	40000IU/kg body weight	3	3
Group(5)	6	*S. aureus* + Gentamycin	5mg/kg bodyweight	3	3

Table 5. Genotypic characterization of some virulence and resistance gene of *Staphylococci*.

Genes / No. of positive samples	Virulence genes		Resistance gene	
	fnbB	***seb***	***blaZ***	***aac(6')aph (2'')***
Random isolates	8	7	------	-----
Group 3	1	1	------	------
Group 5	1	1	6	1
Total (%)	10/10 (100%)	9/10 (90%)	6/6 (100%)	1/6 (16.7%)

Table 6. Genotypic characterization of some virulence and resistance gene of *P. multocida*.

Genes / No. of positive samples	Virulence genes			Resistance gene	
	nanB	**Serogroup A capsule**	**Serogroup D capsule**	***blaROB1***	***aphA1***
Random isolates	3	8	-----	-----	-----
Group 2	1	1	-----	-----	-----
Group 4	1	1	------	2	6
Total (%)	5/6 (83.3%)	10/10 (100%)	0/10 (0%)	2/6 (33.3%)	6/6 (100%)

Table 7. The effect of infection with *P. multocida* and *S. aureus* and treatment with either penicillin or gentamicin on heamatological parameters in blood of male rabbits at 35 days PI.

Group	RBCs×10^6 mm	HB(g/dl)^3	PCV%	WBCs×10^3 mm^3	MCV(fl)	MCH(pg)	MCHC%
G1	5.6±0.32 ^{a}	13.1±0.52^a	38±1 ^{a}	10.4±0.8^b	62.7±1.2	20.1±1.2	34±0.48
G2	4.2±0.47^b	10.4±0.86^b	33.3±0.92^{bc}	14.2±1 ^{a}	61.5±1.7	20.4±0.67	31.2±0.46
G3	3.9±0.29^b	11.2±0.55^{ab}	31.9±0.89^c	13.7±0.65^a	60.8±1.5	19.5±1.2	32.6±1.3
G4	5.4±0.32^a	12.5±0.44^{ab}	36.5±0.86^{ab}	11.8±0.50^{ab}	61.8±1	21±1.8	33.8±1
G5	5.2±0.51^a	12±1^{ab}	34.6±1.3^{ab}	11.4±1.2^{ab}	59.6±1.6	19.3±0.50	32.5±1.1

Different letters at the same column means that there was a significant change at $p<0.05$.

Table 8. The effect of infection with *P. multocida* and *S. aureus* and treatment with either penicillin or gentamicin on heamatological parameters in blood of male rabbits at 45 days PI.

Group	RBCs×10^6 mm	HB(g/dl)^3	PCV%	WBCs×10^3 mm^3	MCV(fl)	MCH(pg)	MCHC%
G1	5.9±0.29^a	13.6±0.81^a	38.4±1.2^a	10.1±0.86^b	57.5±1.8	20.8±1.2	34.2±1
G2	4.1±0.32^b	10.4±0.73^b	31.3±0.49^b	15.6±0.72^a	60.5±2	19.6±0.86	31.6±1.5
G3	3.9±0.20^b	10.1±0.63^b	32.9±1.6^b	14.2±0.55^a	62.9±1.6	20.7±0.95	32.7±1.3
G4	5.5±0.45^a	12.7±1^{ab}	38.6±1.7^a	11.1±0.20^b	61±2.2	21.5±1.5	33.8±1.8
G5	5.2±0.43^a	13.1±1.4^{ab}	37±1.4^a	11.2±0.72^b	62.5±2	21±1.2	34±1.8

Different letters at the same column means that there was a significant change at $p<0.05$.

Table 9. The effect of infection with *P. multocida* and *S. aureus* and treatment with either penicillin or gentamicin ons ome enzymes biomarkers in blood of male rabbits at 35 days PI.

Group	GSH-Px (M/g %)	SOD/ml blood
G1	6.3±0.43 ^{a}	2.8±0.23 ^{a}
G2	4±0.32 ^{b}	1.9±0.20 ^{b}
G3	4.3±0.37 ^{b}	2.1±0.20 ^{b}
G4	5.9±0.40 ^{a}	2.3±0.32 ^{ab}
G5	6.1±0.17 ^{a}	2.4±0.14 ^{ab}

Different letters at the same column means that there was a significant change at $p<0.05$.

Table 10. The effect of infection with *P. multocida* and *S. aureus* and treatment with either penicillin or gentamicin on some enzymes biomarkers in blood of male rabbits at 45 days PI.

Group	GSH-Px (M/g %)	SOD/ml blood
G1	6.3±0.29 ^{a}	2.9±0.26 ^{a}
G2	3.8±0.37 ^{b}	1.8±0.21 ^{b}
G3	4.2±0.43^b	2±0.14 ^{b}
G4	6±0.17 ^{a}	2.6± ^{a}
G5	5.8± 0.20 ^{a}	2.8±0.24 ^{a}

Different letters at the same column means that there was a significant change at $p<0.05$.

Table 11. The effect of infection with *P. multocida* and *S. aureus* and treatment with either penicillin or gentamicin on male fertility at 35 days PI.

Group	Sperm cell count (sp.c.c x 10^6 /ml)	Volume (ml)	Sperm abnormalities (%)	Live (%)	Sperm mortility (%)	Serum testosterone level (ng/ml
G1	142.6±4.3 ^{a}	0.54±0.05 ^{a}	9.4±0.60 ^{b}	97.1±2 ^{a}	87.9±1.5 ^{a}	0.59±0.023 ^{a}
G2	101.6± 3.4 ^{b}	0.39±0.026 ^{b}	22.6±1.3 ^{a}	79.5±2.3 ^{b}	71.9±2.2 ^{b}	0.45±0.023 ^{b}
G3	106.6±4.4 ^{b}	0.35±0.035 ^{b}	21.7±1.4 ^{a}	78.2±1.2 ^{b}	74.3±3.6 ^{b}	0.47±0.031 ^{b}
G5	135±2.8 ^{a}	0.47±0.043 ^{a}	11.2±0.40^{b}	87.7±1.4 ^{ab}	83.8±1.9 ^{ab}	0.56±0.027 ^{a}
G5	130.2±3.2 ^{a}	0.53±0.037 ^{a}	10.9±1 ^{b}	89.4±2.1 ^{ab}	82.5±1.5 ^{ab}	0.57±0.032 ^{a}

Different letters at the same column means that there was a significant change at $p<0.05$.

Table 12. The effect of infection with *P. multocida* and *S. aureus* and treatment with either penicillin or gentamicin on male fertility at 45 days PI.

Group	Sperm cell count (sp.c.c x 10^6 /ml)	Volume (ml)	Sperm abnormalities (%)	Live (%)	Sperm mortility (%)	Serum testosterone level (ng/ml
G1	153.6±2.6 ^{a}	0.56±0.020 ^{a}	10.7±0.60 ^{b}	92.4±1.4 ^{a}	90.6±1.6 ^{a}	0.59±0.035 ^{a}
G2	106.6± 4.3 ^{b}	0.36±0.035 ^{b}	22.6±1.7 ^{a}	80.1±2.3 ^{b}	76±1.8 ^{b}	0.46±0.049 ^{b}
G3	108.3±4.8 ^{b}	0.38±0.043 ^{b}	21.8±2.2 ^{a}	77.6±2.7 ^{b}	77.3±1.7 ^{b}	0.48±0.040 ^{b}
G5	150±2.8 ^{a}	0.51±0.032 ^{a}	11±0.75 ^{b}	89.8±0.90 ^{a}	88.6±1.8 ^{a}	0.57±0.014 ^{a}
G5	151.3±3.2 ^{a}	0.54±0.052 ^{a}	10.5±0.76 ^{b}	90.7±1.2 ^{a}	89.7±1.3 ^{a}	0.58±0.023 ^{a}

Different letters at the same column means that there was a significant change at $p<0.05$.

Table 13. Modified Johnson score (3-10) at 35th day and 45th day of the experiment.

Score → Group↓	Time of scarification (at)	3	4	5	6	7	8	9	10
Group(1)	35thdayPI	0	0	0	1.5%	11.8%	23.%	28%	35.7%
	45th day PI	0	0	0	3.1%	3.6%	22.4%	31.3%	39.6%
Group(2)	35th day PI	12.3%	36.1%	23.8%	22.5%	1.2%	3.1%	1%	0
	45th day PI	6.2%	37.4%	26.7%	13.9%	12.5%	3.3%	0	0
Group(3)	35th day PI	0.5%	34.4%	31%	13.6%	19.4%	0	0.8%	0.3%
	45th day PI	0	18%	36.2%	28.6%	14.3%	1.2%	1.7%	0
Group(4)	35th day PI	1.3%	5.8%	6.7%	23.3%	27.6%	11.2%	13%	11.1%
	45th day PI	0	2.1%	4.3%	9.8%	19.4%	23.2%	31.9%	9.3%
Group(5)	35th day PI	0%	7.5%	15.4%	13.3%	11.2%	35.5%	15.8%	1.3%
	45th day PI	0%	5.9%	2.4%	5.3%	22.2%	38%	19.2%	7%

Table 14. Comparison of testicular histopathology among the different experimental groups.

Group / Point of comparison	Time of scarification (at)	Group (1)	Group (2)	Group (3)	Group (4)	Group (5)
Tubular Diameter	35th day PI	387.78±7.63^{a}	353.32±4.62^{b}	354.06±3.44^{b}	375.48±4.03^{a}	383.88±4.03^{a}
(micron)	45th day PI	394.82±5.46^{a}	347.3±4.25^{c}	365.42±1.85^{b}	385.22±2.81^{a}	385.52±3.60^{a}
Tubular Lumen	35th day PI	62.98±3.16^{d}	98.5±2.03^{a}	89.12±1.74^{b}	71.46±2.4^{c}	66.46±2.91^{c}
(micron)	45th day PI	70.54±1.34^{d}	96.86±1.13^{a}	88.14±183^{b}	78.40±2.01^{c}	69.96±2.60^{d}
Thickness of Interstitial	35th day PI	127.93±1.80^{b}	135.38±2.00^{a}	135.04±2.05^{a}	129.64±2.64^{ab}	128.90±1.03^{b}
tissue (micron)	45th day PI	130.18±3.08^{b}	146.62±9.23^{a}	129.52±2.14^{b}	130.64±2.37^{b}	129.88±4.5^{b}
Highest of germinal	35th day PI	17.79±1.34^{a}	8.16±1.00^{c}	8.40±1.00^{c}	11.82±0.47^{b}	13.20±1.03^{b}
epithelium (micron)	45th day PI	18.34±0.80^{a}	8.06±0.85^{c}	10.260±o.99^{c}	10.70±0.73^{c}	14.16±0.96^{b}

PI: Post Infection.

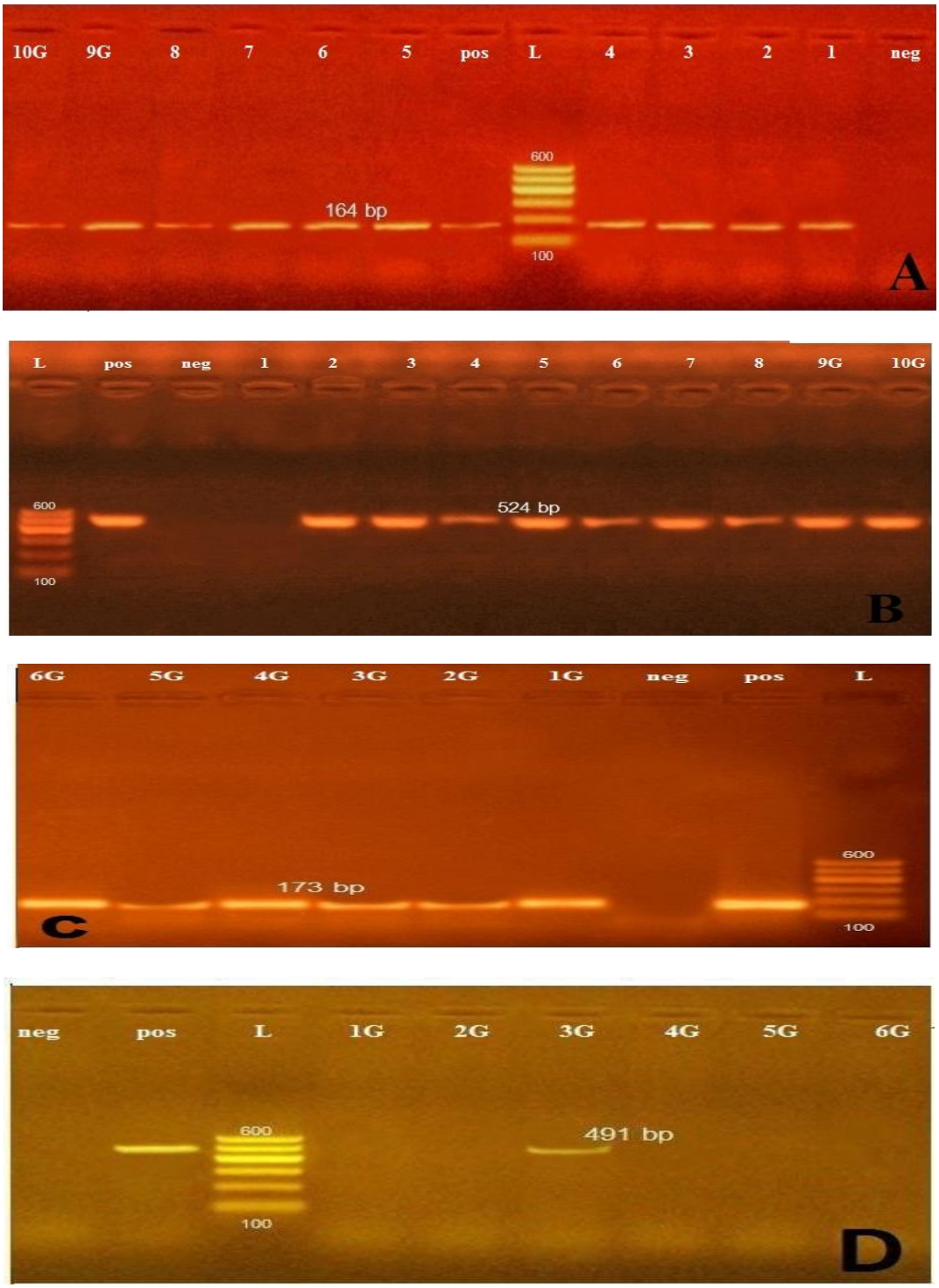

Fig. (I): Showing agarose gel electrophoresis of PCR amplified products for *Staphylococci* isolates (A): *seb gene at 164bp*, (B): *fnb*B gene at 524 bp, (C): *bla*Z gene at 173bp, (D): *aac(6´)aph(2´´)*gene at 491bp, Lane L:DNA molecular size marker (100 bp), lane (pos): positive control and lane (neg): negative control. The size in basepairs (bp) of each PCR product is indicated on the bands.

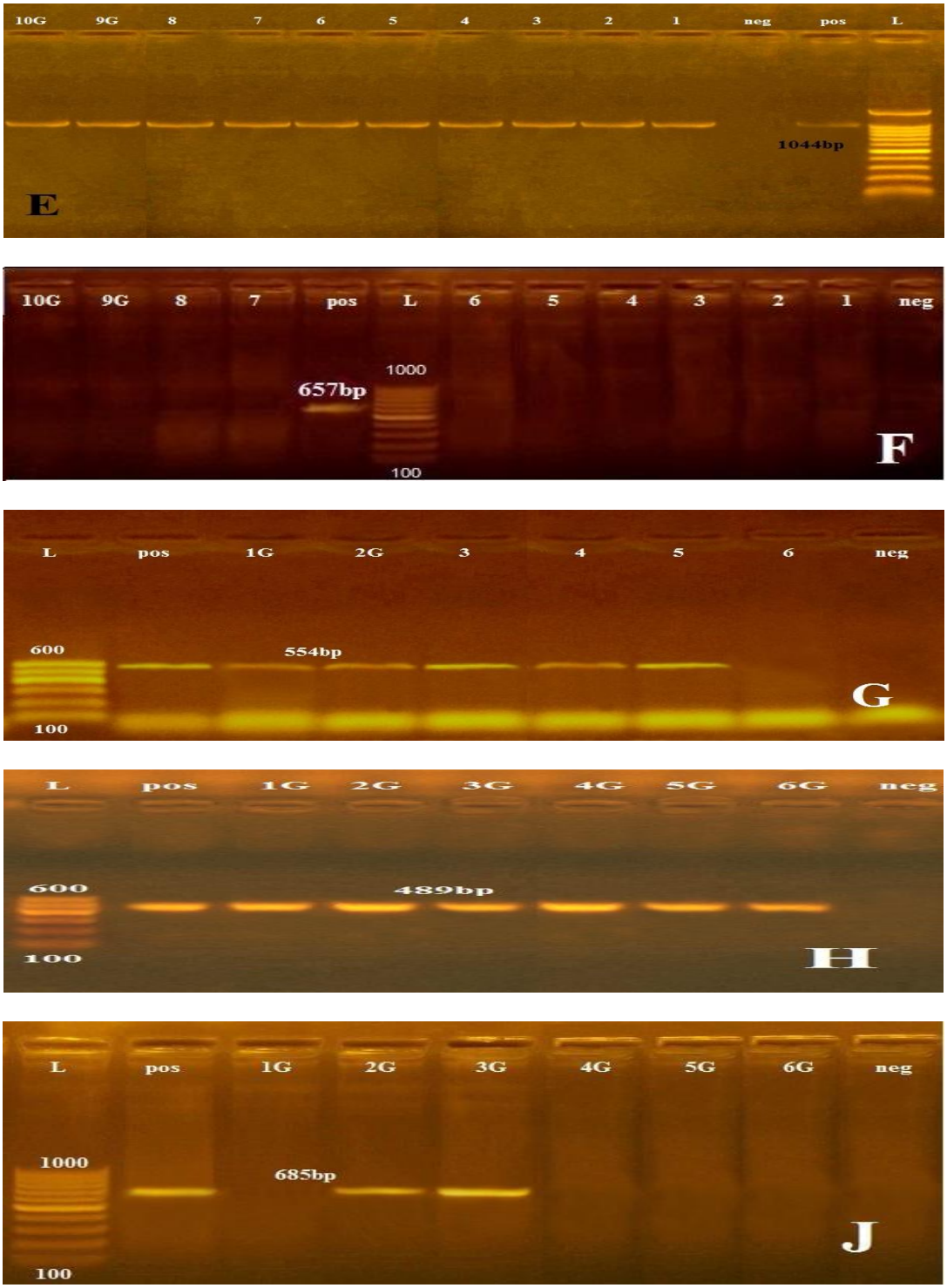

Fig. (II): Showing agarose gel electrophoresis of PCR amplified products of *P. multocida* isolates (E): *P. multocida type A gene at 1044bp*, (F): *P. multocida type D* gene at 657bp, (G): *nan*B gene at 554bp, (H):*aph*A1gene at 489bp, (J):*bla*ROB1gene at 685bp, Lane L:DNA molecular size marker (100 bp), lane (pos): positive control and lane (neg): negative control. The size in base pairs (bp) of each PCR product is indicated on the bands.

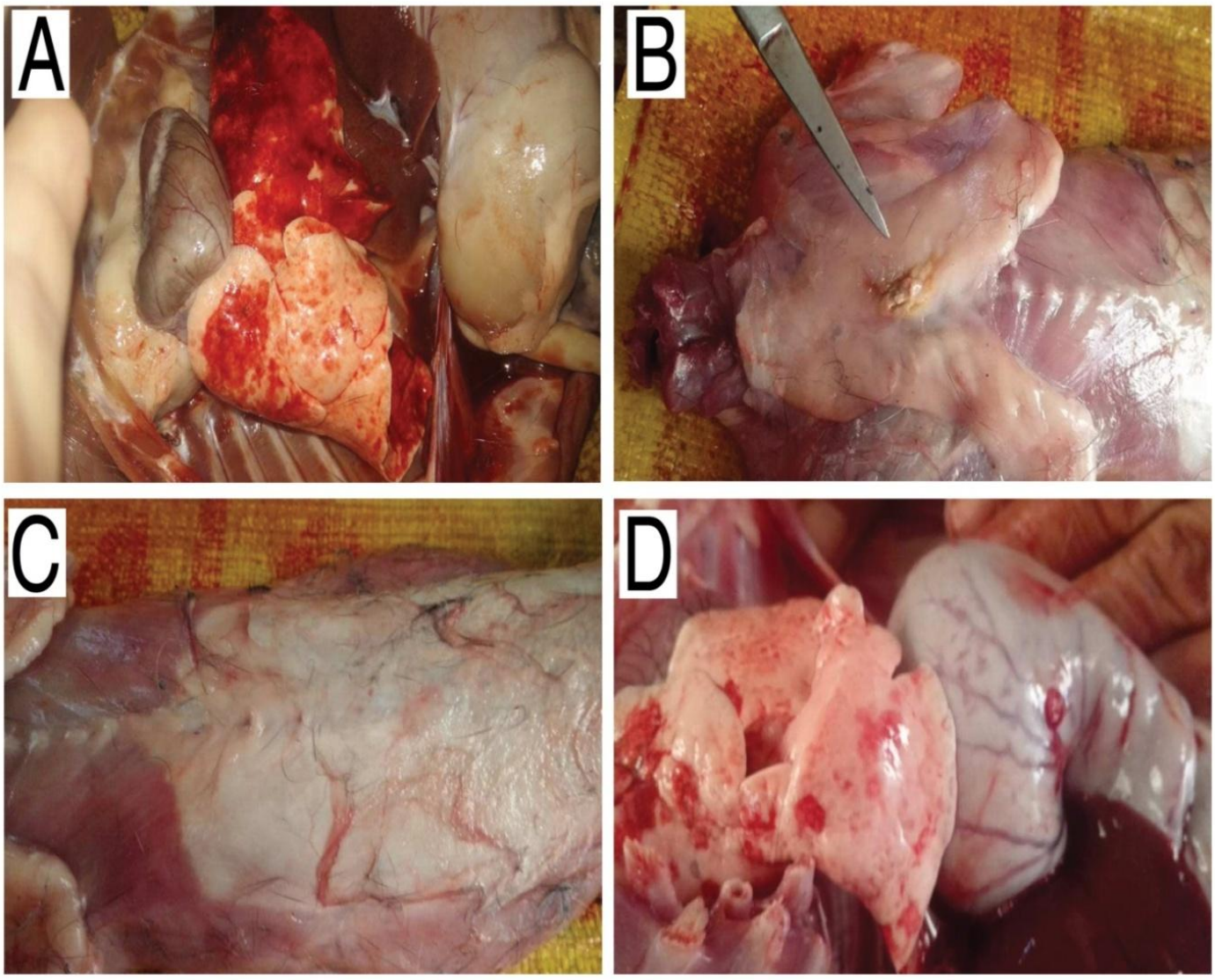

Plate I:

Fig. (A): Unilateral severely congested lung and mild congested liver has appeared in a rabbit of group.(2) at 35 days PI.

Fig. (B): Subcutaneous abscess of about 3.5cm diameter in the dorsal interscapular area has appeared in one rabbit of group(2) at 45 days PI.

Fig. (C): Spreading diffuse subcutaneous swelling (phlegmon) that affected the dorsal posterior region has appeared in one rabbit of group.(3) at 45 days PI.

Fig. (D): Small focal areas of congestion in lung of one rabbit of group(4) at 35 days PI

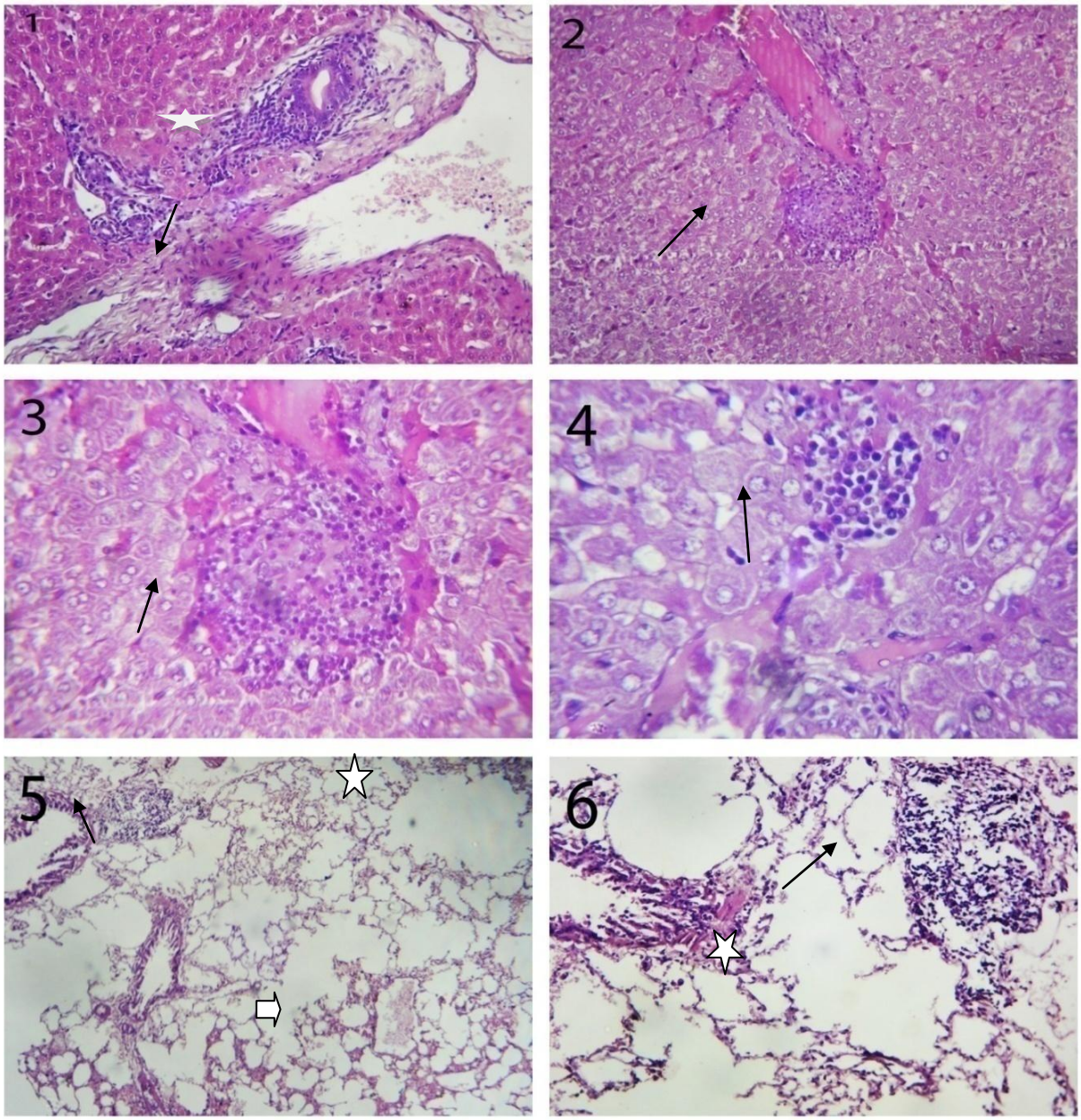

Plate II:

Fig. (1): Photomicrograph of liver, gp (2), 35 days PI. Showing periductal leucocytic cells infiltration (star) with perivascular fibrosis (arrow) (H&E×100).

Fig. (2): Photomicrograph of liver, gp (2), 45 days PI. showing focal perivascular caseation infiltrated with leucocytic cells (arrow) (H&E ×40).

Fig. (3): High power of the previous figure to illustrate the previous details (arrow) (H&E ×100).

Fig. (4): Photomicrograph of liver, gp (3), 35 days PI showing focal lymphocytic cells infiltration (arrow) (H&E×100).

Fig. (5): Photomicrograph of lung, gp (2), 35 days PI showing focal area of colonized bacilli (black arrow) with mild congestion (white arrow) and severe emphysema (star) (H&E×40).

Fig. (6): High power of the previous figure to show the colonized bacilli (arrow) and emphysema (star) (H&E×100).

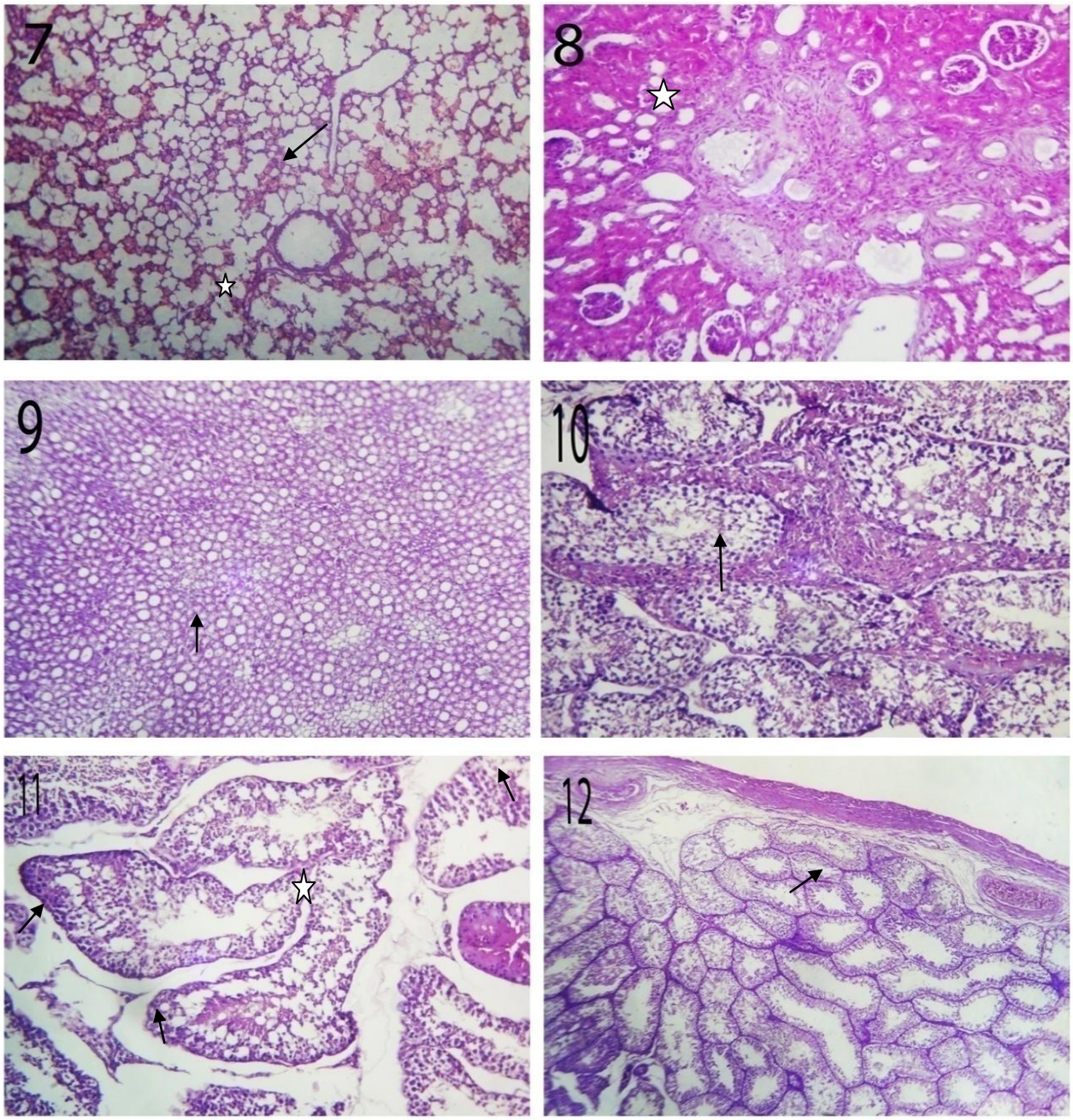

Plate III:

Fig. (7): Photomicrograph of lung, gp (3), 35 days PI showing interalveolar hemorrhage (arrow) with mild emphysema (star) (H&E ×100).

Fig. (8): Photomicrograph of kidney, gp (2), 35 days PI showing focal inter tubular fibrosis (star) (H&E×100).

Fig. (9): Photomicrograph of kidney, gp (3), 45 days PI showing, diffuse cystic dilation of the renal tubules in the renal medulla (arrow) (H&E×40)**.**

Fig. (10): Photomicrograph of testis, gp. (2), 35 days PI showing intertubular sporadic bacilli with fibroblasts and few leucocytic cells aggregation (arrow) (H & E ×100).

Fig. (11): Photomicrograph of testis, gp (2), 45 days PI showing vacuolation of some spermatocytes (arrow) and atrophy of some somniferous tubules with interstitial edema (asterisk) (star) (H&E ×100).

Fig. (12): Photomicrograph of testis, gp (3), 35 days PI showing congestion of some blood vessels (arrow) (H & E × 40)

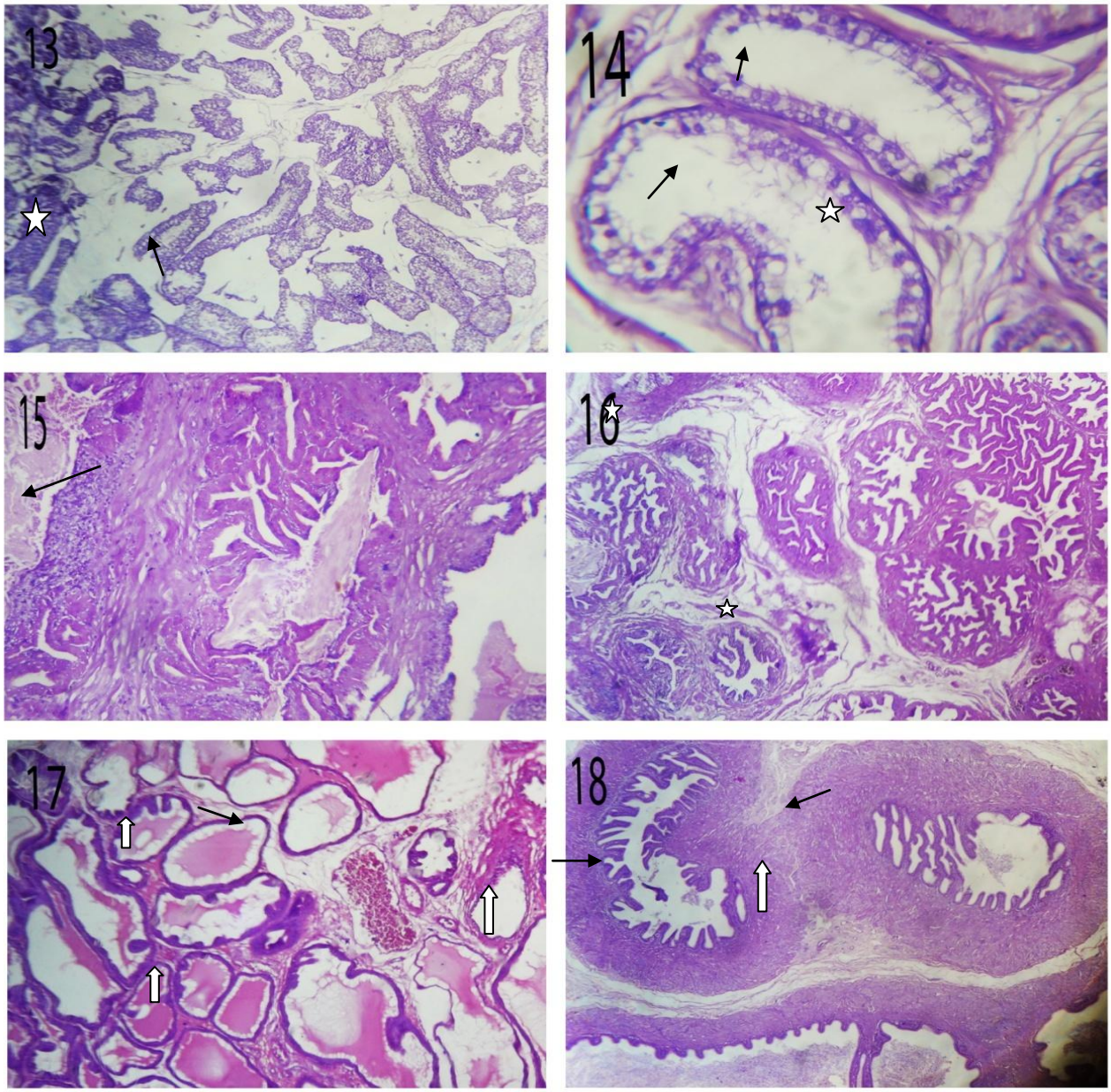

Plate IV:

Fig. (13): Photomicrograph of testis, of gp**(3),** 45 days PI. showing severe atrophy of some somniferous tubules (arrow)with destruction of others beside diffuse interstitial edema (asterisk) (star) ((H&E ×40)

Fig. (14): Photomicrograph of testis of gp**(3)**,45 days PI. Showing Vacuolation of some spermatogonia (arrow) with disappearance of spermatogenesis beside interstitial edema (asterisk) (star)((H&E ×100)

Fig. (15): Photomicrograph of prostate of gp**(2),** 35 days PI. showing focal leucocytic cells infiltration with fibrosis (arrow) among prostatic acini (H&E ×100).

Fig. (16): Photomicrograph of prostate of gp**(2),** 45days PI showing interstitial edema (Asterisk) (star) (H&E ×100).

Fig. (17): Photomicrograph of prostate, of gp**(3),** 35 days PI showing congestion of prostatic blood vessels (black arrow) with disappearing the acinal folds which filled with homogenous esinophilic material (white arrow) (H&E×100).

Fig. (18): Photomicrograph of prostate of gp**(3),45** days PI showing thickening and fibrosis of the acinal wall (white arrows)with fusion of some acinal folds (black arrows) (H&E ×100)

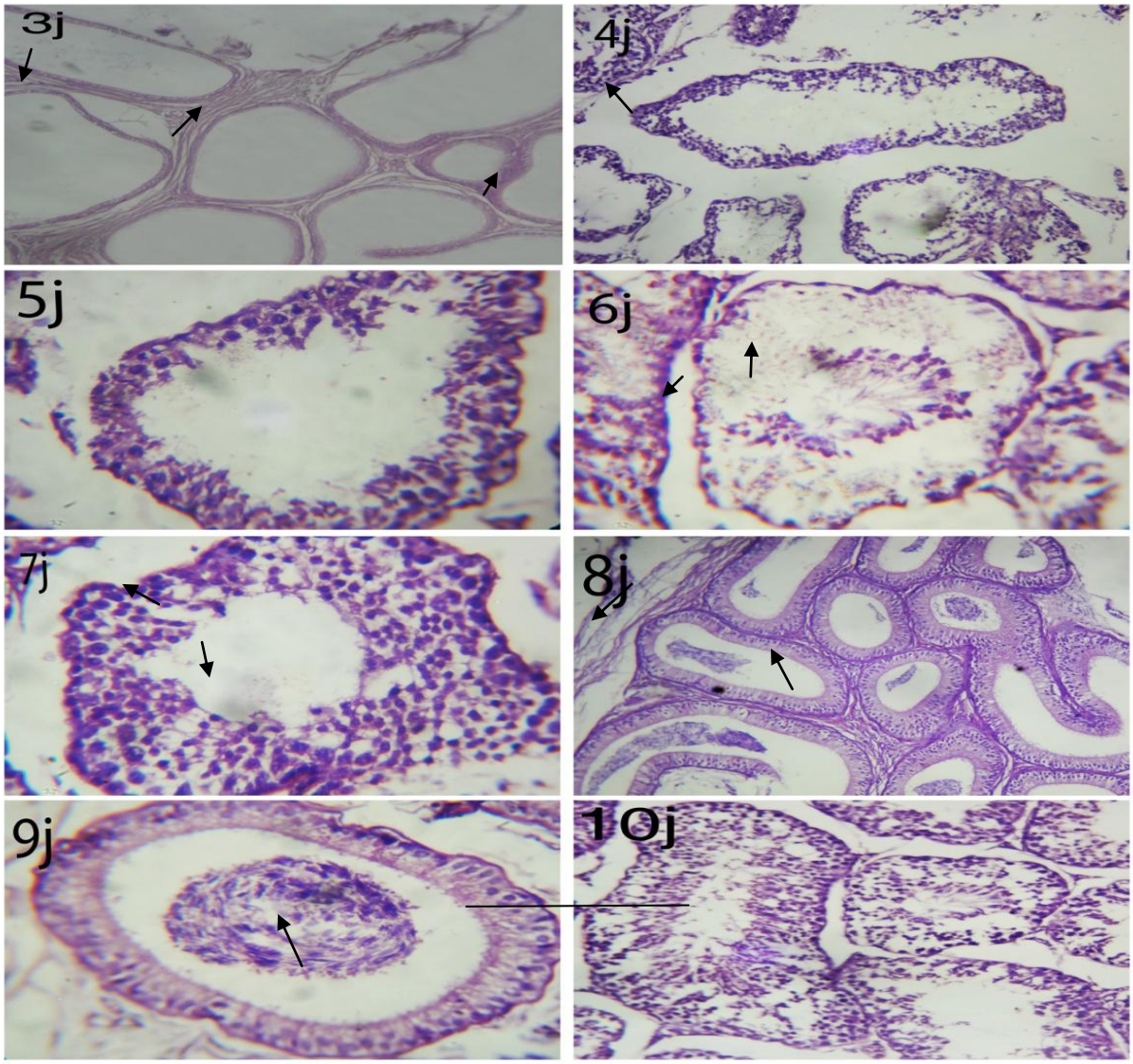

Plate V: Johnson score grades, (3-10)

Fig. (3j): Photomicrograph of somniferous tubules of score(3) showing presence of spermatogonia only(arrow) (H&E ×100).

Fig. (4j): Photomicrograph of somniferous tubule of score (4)showing presence of few spermatocytes only (arrow) (H&E×100).

Fig. (5j): Photomicrograph of somniferous tubule of score (5) showing presence of no spermatozoa or spermatocytes (H&E ×100).

Fig. (6j): Photomicrograph of somniferous tubule of score (6) showing presence of few spermatide only (arrows) (H&E×100).

Fig. (7j): Photomicrograph of somniferous tubule of score(7) showing presence of many spermatide (arrows) but no spermatozoa (H&E ×100)

Fig. (8j): Photomicrograph of somniferous tubules of score (8) showing presence of only few spermatozoa (arrows) (H&E ×40)

Fig. (9j): Photomicrograph of somniferous tubule of score(9) showing presence of many spermatozoa with disorganized spermatogenesis(arrow) (H&E ×100).

Fig. (10j): Photomicrograph of somniferous tubules of score (10) showing normal full spermatogenesis (line) (H&E ×100).

Conclusion

Both Pasteurella multocida and staph aureus microorganisms had a negative effect on bucks urogenital organs causing a pathological changes on these sex organs beside others causing orchitis, lowered semen quality and other changes leading to infertility which could be avoided with early administration of chosen antibiotics.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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How to cite this article:
Mowafy, R. E., Megahed, H. M., El Oksh, A. S., 2018. Pathological studies on infertility in bucks as a sign to some bacterial infection. Int. J. Curr. Res. Biosci. Plant Biol. 5(11), 7-33.
doi: https://doi.org/10.20546/ijcrbp.2018.511.002