

Original Research Paper

# Hormonal and Histopathological Alterations in Pituitary Glands and Reproductive Organs of Male and Female Mice Orally Inoculated with *Pasteurella Multocida* Type B: 2 and its Lipopolysaccharides

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**Abstract:** Oral infections of mice as animal model with *Pasteurella multocida* type B: 2 and its lipopolysaccharides is liable to cause detrimental alterations in the pituitary glands, which results in disruption of reproductive hormonal levels as well as organ functions in the male and female animals; thereby impeding the fertility of at risk animal population. Therefore, this study was designed to evaluate the histopathological modifications in the pituitary glands, reproductive organs and the associated hormonal levels in male and female mice inoculated orally with *P. multocida* type B: 2 and its lipopolysaccharides. Forty eight mice were divided into three groups consisting of sixteen mice each (8 male and 8 female in separate cages). Group 1 served as the control group and were inoculated orally with 0.4 mL Phosphate Buffer Saline (PBS), group 2 were inoculated orally with 0.4 mL of 10<sup>9</sup> Colony Forming Unit (CFU) of *P. multocida* type B: 2 while group 3 were inoculated orally with 0.4 mL of LPS from 10<sup>9</sup> of colony *P. multocida* type B: 2. There were significant differences (p<0.05) in the lesions (oedema, haemorrhage, degeneration and necrosis) observed in the testes, ovaries and the pituitary glands amongst the three groups. Similarly, there were significant differences in the levels of testosterone, progesterone and estrogen (p<0.05) in the male and female mice inoculated orally with *P. multocida* type B: 2 and its lipopolysaccharide. There is paucity of information on changes related to reproductive hormones, pituitary glands and reproductive organs of male and female mice following oral inoculation with *P. multocida* type B: 2 and its endotoxin. Hence, the sex hormonal levels could be used to assess the immune statuses of at risk animal population and proffer amenable Veterinary medical care to afflicted animal population.

**Keywords:** *Pasteurella Multocida*, Lipopolysaccharide, Pituitary Glands, Reproductive Organs, Hormonal

## Introduction

Haemorrhagic Septicaemia (HS) is an important disease in African and Asian countries including Malaysia caused by *Pasteurella multocida* type B: 2

which has high mortality and morbidity (Jamal *et al.*, 2005; OIE, 2008). It can be described as acute, fatal septicaemic disease caused by specific serotypes of *Pasteurella multocida*. Based on the component of the outer cells layer, *P. multocida* is grouped into 5 capsular

types which are A, B, D, E and F (Shafarin *et al.*, 2009). Serotypes B: 2 and E: 2 are the most recognized in Asia and Africa based on Carter and Hendlestone system (OIE, 2008). HS is predominantly found in cattle and buffaloes whereas buffaloes are more susceptible than cattle (Saharee, 2006; Abubakar and Zamri, 2011). Younger animals were reported to succumb to HS more readily than older animals (Boyce *et al.*, 2010; Harper *et al.*, 2011). The important feature of *P. multocida* type B: 2 that is able to cause HS is its capability to synthesize the enzyme hyaluronidase (OIE, 2008). The clinical signs include rapid course, increase in temperature, hypersalivation, loud and stertorous breathing due to oedematous swelling, petechial haemorrhages in the throat and brisket region. It also involves nasal discharge, swellings in the submandibular region until brisket area and death within 24 h (Saharee, 2006), unless they are treated at the very early stage of the disease (Moffatt *et al.*, 2010). Morbidity and mortality in HS depends on the immunological status of the individual or groups of animals with the disease (Boyce *et al.*, 2010).

The organism is a small gram negative, non-motile and non-spore-forming bacterium. They are coccobacilli with bipolar staining abilities mostly taking up Leishman or methylene blue stain which make it distinguishable from other Pasteurella organisms (Jamal *et al.*, 2005). The colony is one millimetre in diameter with slightly raised centre and with a glistening lustre (Harper *et al.*, 2011). *P. multocida* type B: 2 produces oxidase, catalase, indole and trifling quantity of nitrates. It does not produce hydrogen sulphate or urase and fail to utilize citrate or liquefy gelatine and grows aerobically in an ordinary nutrient agar (OIE, 2008).

The gram negative bacteria *P. multocida* type B: 2 is composed of Lipopolysaccharide (LPS) structure. The LPS is an important component of the outer cell wall of the organism (Eckersall and Bell, 2010; Moffatt *et al.*, 2010; Harper *et al.*, 2011). They are released during multiplication or bacterial deaths that lead to an inflammatory reaction. It represents the endotoxins of the organism and in principle responsible for toxicity in HS and also plays an important role in the pathogenesis of the disease (Raetz *et al.*, 2002; Jesse *et al.*, 2013).

Rainwater and rivers also play an important role in disease transmission, assisting in the dissemination and transfer of infection to areas downstream (Khaleel *et al.*, 2013). Rivers and streams have been thought to be responsible for the transmission of haemorrhagic septicemia through carcasses washed down from one village upstream to another further down the rivers (Khaleel *et al.*, 2013). In fact HS has been associated with the monsoon seasons (Abdullah *et al.*, 2013a) and dumping of infected carcasses into streams and rivers.

Khaleel *et al.* (2014) Stated that there is dissemination and multiplication of *P. multocida* B: 2 in vital organs after intranasal and subcutaneous challenge in mice.

According to a previous study of HS by (OIE, 2008) using mice as animal model, oral inoculation of *P. multocida* type B: 2 in mice produced pathological lesions and also intraperitoneal inoculation of *P. multocida* type B: 2 and its LPS in male mice showed significant changes in male reproductive organs. However, there is paucity of knowledge on information regarding changes in the reproductive hormones, pituitary glands and reproductive organs of male and female mice after oral inoculation with *P. multocida* type B: 2 and its endotoxin. Therefore, this study was designed to evaluate the histopathological changes in the reproductive organs, pituitary glands and reproductive hormones of the male and female mice following oral inoculation with *P. multocida* type B: 2 and its Lipopolysaccharides (LPS).

## Materials and Methods

### Experimental Mice

Forty eight healthy ICR male and female mice aged 3 weeks old were used in this study. All the mice were obtained from Institute of Cancer Research (ICR) and were kept at the Animal Research Center, Universiti Putra Malaysia (UPM) and housed in plastic cages. Six plastic cages were used, where each cage consist of 8 mice. The mice were fed with commercial pellet and provided water ad libitum. All the mice were observed for one week for acclimatization to the new environment and to make sure they were healthy prior to the experiment. All procedures and experiments illustrated were undertaken under a project license approved by Animal Utilization Protocol Committee, Faculty of veterinary medicine, Universiti Putra Malaysia, with reference number: PM/IACUC/FYP-2013/FPV.043 and FPV/FYP/2013/059

### Inoculums

The wild type *P. multocida* type B: 2 that were used in this study was obtained from stock culture from Veterinary Research Institute Ipoh Perak. Identification of *P. multocida* type B: 2 was done using the Gram staining method and biochemical characterization of oxidase, urea broth, Sulphur Indole Motility (SIM), Triple Sugar Iron (TSI) and citrate test, to confirmed the isolate as *P. multocida* type B: 2. The pure stock culture that was stored on nutrient agar slants were subcultured on 5% horse blood agar and incubated at 37°C for 18 h. A single colony of the *P. multocida* type B: 2 was selected to grow on Brain Heart Infusion (BHI) broth, incubated in shaker incubator at 37°C for 24 h before the concentration was determined using McFarland Nephelometer Barium Sulfate Standards.

### Preparation of $10^9$ CFU of *P. multocida* Type B: 2

Preparation of  $10^9$  Colony Forming Unit (CFU) of *P. multocida* was done by adding distilled water onto pure

cultures of *P. multocida* before the bacteria was transferred into sterile test tubes. These sterile test tubes were then compared with McFarland standard to determine the  $10^9$  CFU of *P. multocida*.

#### *LPS Extraction from $10^9$ of Colony of P. Multocida Type B: 2*

The Lipopolysaccharides (LPS) of *P. multocida* type B: 2 was extracted using Intron Biotechnology® LPS extraction kit. In this study,  $10^9$  CFU of *P. multocida* type B: 2 was prepared for LPS extraction. The bacteria were first harvested by centrifugation in room temperature at 13,000 rpm for 30 sec. Then, 1 mL of lysis buffer was added and vortex vigorously. 200  $\mu$ L of choloform was later added and vortex vigorously for 10-20 sec before it was incubated for 5 min in room temperature. It was then centrifuged at 13,000 rpm for 10 min at 4°C and 400  $\mu$ L of the supernatant was transferred into a new 1.5 mL tube. About 800  $\mu$ L of purification buffer was added and mixed well with the transferred supernatant after which it was incubated at -20°C for 10 min. It was then centrifuged again at 13,000 rpm for 15 min at 4°C. The LPS pellet was obtained after the excess supernatant was discarded before the LPS pellet was washed with 1ml of 70% ethanol, which was then dried completely. Finally, 70  $\mu$ L of 10 mM Tris-HCl buffer of pH 8.0 was added to the LPS pellet whereby it was dissolved by 2 min of boiling.

#### *Study Designs*

Forty eight mice were divided into three groups of sixteen mice each; of eight Male (M) and eight Female (F) mice each. Group 1 served as the negative control group and were inoculated orally with 0.4 mL Phosphate Buffer Saline (PBS), group 2 were inoculated orally with 0.4 mL of  $10^9$  Colony Forming Unit (CFU) *P. multocida* type B: 2 and group 3 were inoculated orally with 0.4 mL of LPS from  $10^9$  of colony *P. multocida* type B: 2. After inoculation, all the groups were observed for 10 days. Mice that showed severe clinical signs, such as laboured breathing, reduction in responsiveness and those with closed eyes were euthanized by cervical dislocation to minimize suffering and blood was collected via cardiac venipuncture to determine the reproductive hormones concentrations using radioimmunoassay technique. On day 10, all the survived mice were also euthanized using the same technique. Post-mortem was conducted on all the euthanized mice, the organs of interest were the brain, testis, ovaries and pituitary glands. These organs were collected and put into 10% formalin before being processed. Tissue was processed into paraffin blocks and each section was routinely stained with standard hematoxylin and eosin (H and E) stain for histopathological study. Gross pathological lesions were examined during the post-mortem.

#### *Histopathology Lesions Scoring*

The histological slide of each organ was examined under light microscope and the histopathological lesions were scored by taking six different views using 400 times magnification. The lesions were classified into 4 different scores, which are “0” for normal, “1” for mild, “2” for moderate and “3” for severe lesions. The lesions were considered as “normal” if less than 30% of the field was affected, mild if only 30% of the field was affected, moderate if 60% of the field was affected and severe if more than 60% of the field was affected. Lesions that were scored are necrosis and degeneration, oedema, haemorrhage as well as presence of inflammatory cells.

#### *Blood Collection for Reproductive Hormone Analysis*

One ml of blood was collected from the mice via intracardiac venipuncture using plain tube after 10 days of experiment. The blood sample collected was used for the determination of the reproductive hormones specifically for testosterone, estrogen and progesterone concentrations. Then, the blood was centrifuged at 15,000 rpm for 10 min to separate the serum and was transferred into the Eppendorf tubes. After that, the testosterone, progesterone and estrogen level were analyze using Radioimmunoassay kits by Beckman Coulter.

#### *Radioimmunoassay Technique for the Determination of Testosterone*

To determine hormone concentration, radioimmunoassay of testosterone was conducted using serum from the blood sample collected from each mouse. Firstly, the addition step was performed where 50 microlitre of calibrator mixed with 500 microlitre of tracer and added to antibody coated tubes. After that, cover tubes was incubated for 1 h at 37°C in water bath. The last step was counting count bound per minute using Wallac Wizard Gamma Counter model 1470.

#### *Radioimmunoassay Technique for the Determination of Estrogen and Progesterone*

The female reproductive hormones were analyzed were estrogen and progesterone. These hormones were analyzed using Radioimmunoassay (RIA) by Beckman Coulter. Serum was used for hormone analysis. First, 100  $\mu$ L of calibrator, control or plasma from the mice blood were mixed with 500  $\mu$ L of the tracer. Then, the mixture was incubated at 18- 25°C with vibration at 350 rpm. The incubation duration for estrogen was 3 h while for progesterone only 1 h. After that, counting step was done by count Bound of cpm (B) and Total cpm (T) for 1 min using automatic gamma counter machine by Wallac Wizard Gamma Counter model 1470.

### Statistical Analysis

SPSS version 20 was used to analyze the data collected. The lesions scoring were analyzed using Kruskal-Wallis test. Comparisons between groups were considered significant at  $p < 0.05$  using Mann-Whitney test.

## Results

### Histopathological Findings

In the male mice group, the testis and pituitary gland were examined for histopathological changes. The lesions examined were the presence of necrosis and degeneration, oedema, haemorrhages and inflammatory cells. The result showed that there were significant differences ( $p < 0.05$ ) in the lesions scored amongst the three groups of male mice (Fig. 1). The mean concentration of testosterone in Group 2 was twofold higher compared to Group 1 and Group 3 (Fig. 2). Figure 5 to 10 showed the histopathological alterations in the testis and pituitary gland of male mice inoculated orally with *P. multocida* type B: 2 and its lipopolysaccharide.

Furthermore, in the female mice group, the ovaries and pituitary gland were evaluated for histopathological alterations. The lesions evaluated were necrosis and degeneration, oedema, haemorrhages and inflammatory cells. The result showed that there were significant differences ( $p < 0.05$ ) in the lesions scored amongst the three groups of female mice; the control (PBS) and the treatments groups 2 and 3 except in oedema where there was no significant alterations (Table 1 to 4). The results showed significant increase ( $p < 0.05$ ) in progesterone concentrations in group 3 compared to group 2 and 1. There was a significant decrease ( $p < 0.05$ ) in the concentrations of estrogen in group 2 compared to groups 1 and 3. The progesterone and estrogen concentrations in the female mice infected with PBS, *P. multocida* type B: 2 and LPS are shown in Fig. 3 and 4. Additionally, Figure 11 to 19 showed the histopathological alterations in the ovaries and pituitary gland of female mice inoculated orally with *P. multocida* type B: 2 and its lipopolysaccharide.

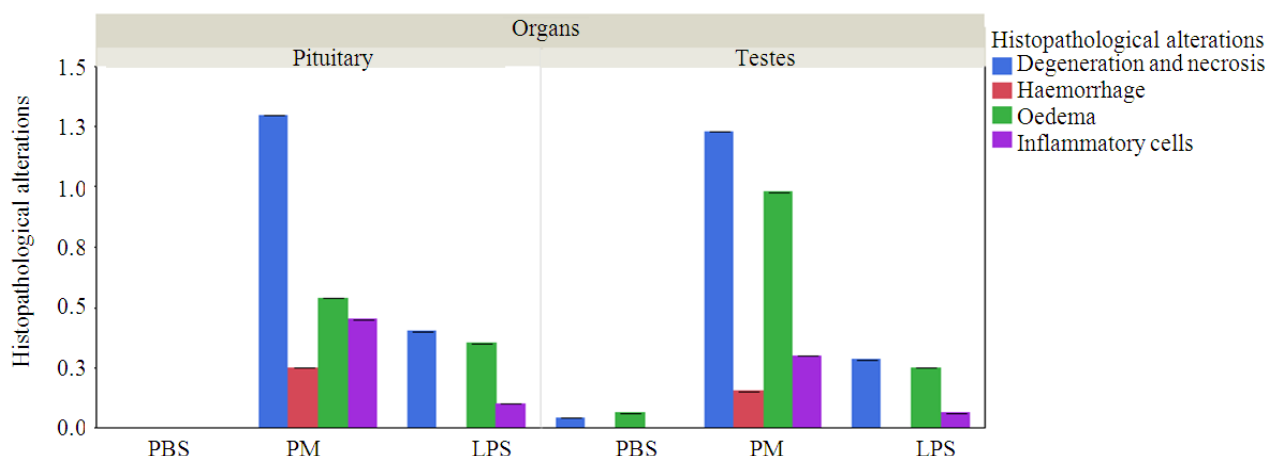


Fig. 1. Histopathological changes in the pituitary gland and testes in the PBS, PM and LPS groups of male mice; PBS = Phosphate Buffered Saline; PM = *P. Multocida* type B: 2; LPS = Lipopolysaccharide

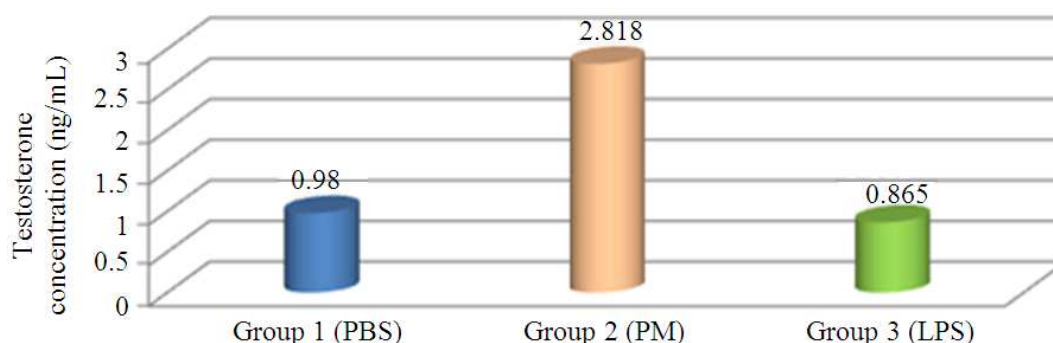


Fig. 2. Mean testosterone concentration in the various groups of the male mice; Group 1 = Control group; Group 2 and 3 are the inoculated groups; PBS = Phosphate Buffered Saline; PM = *P. Multocida* type B: 2; LPS = Lipopolysaccharide

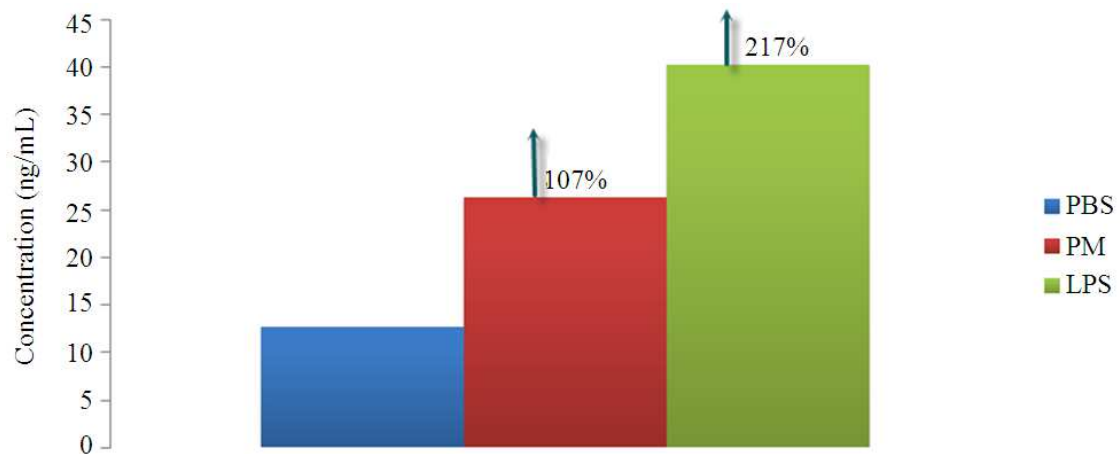


Fig. 3. Mean progesterone concentration in female mice; PBS = Phosphate Buffered Saline; PM = *P. Multocida* type B: 2; LPS = Lipopolysaccharide

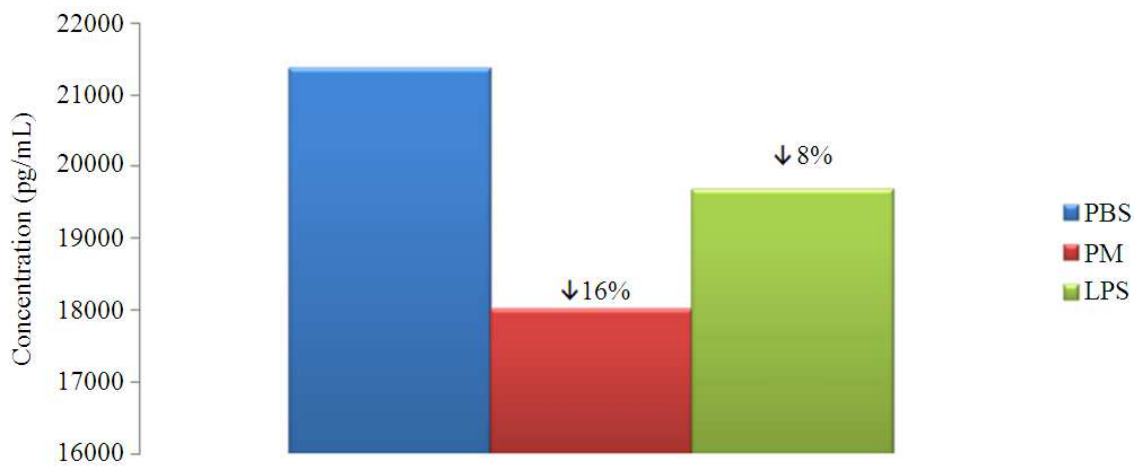


Fig. 4. Mean Estrogen concentration in female mice; PBS = Phosphate Buffered Saline; PM = *P. Multocida* type B: 2; LPS = Lipopolysaccharide

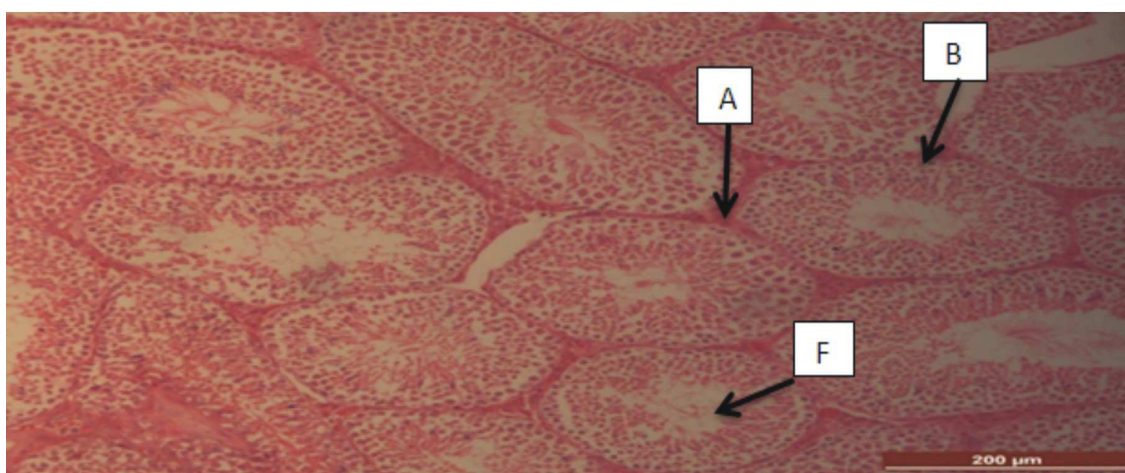


Fig. 5. Photomicrograph section of the testis of male mice in group 1. Leydig cells (A); Sertoli cell (B) and lumen of seminiferous tubules (F); H&E  $\times$ 200

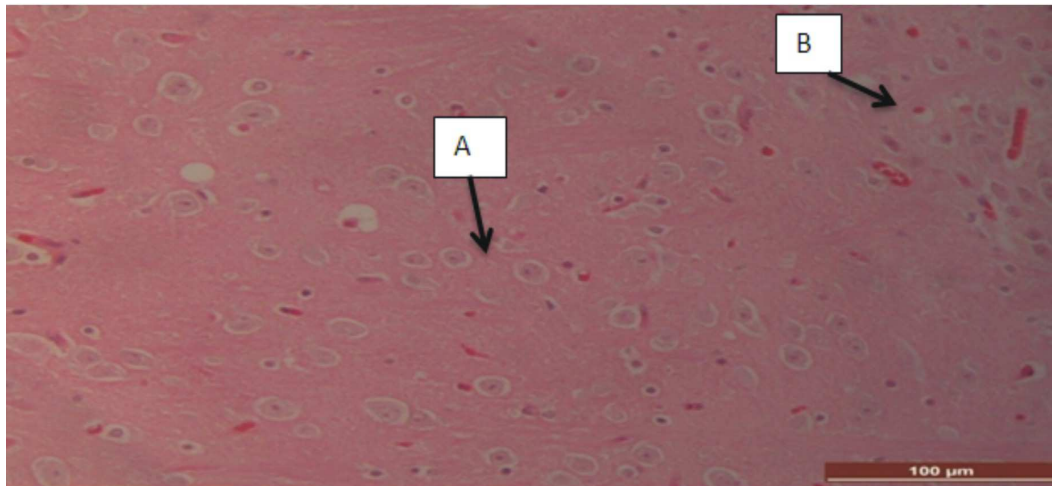


Fig. 6. Photomicrograph section of the pituitary gland of the male mice in group 2; H&E  $\times 200$

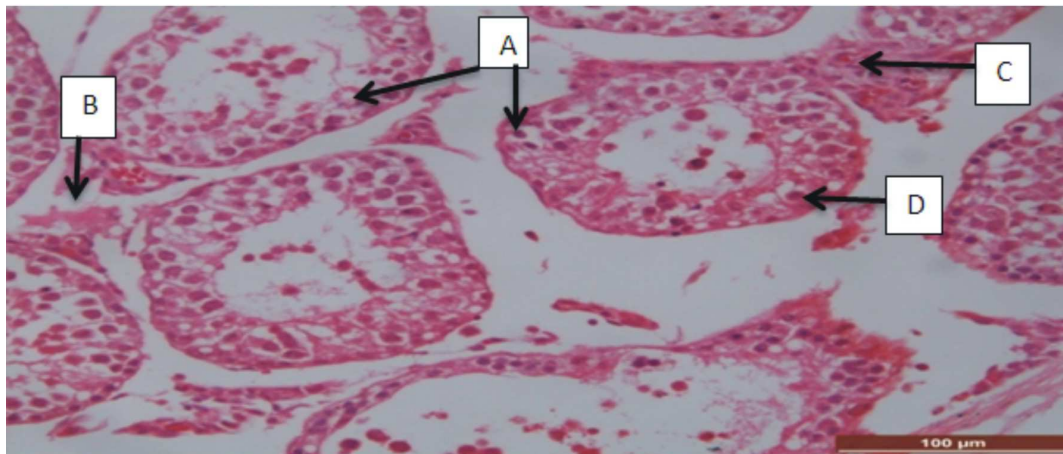


Fig. 7. Photomicrograph section of testis of the male mice in group 2. Area of necrosis and degeneration (A); Oedema (B); Haemorrhage (C) and inflammatory cells (D); H&E  $\times 200$

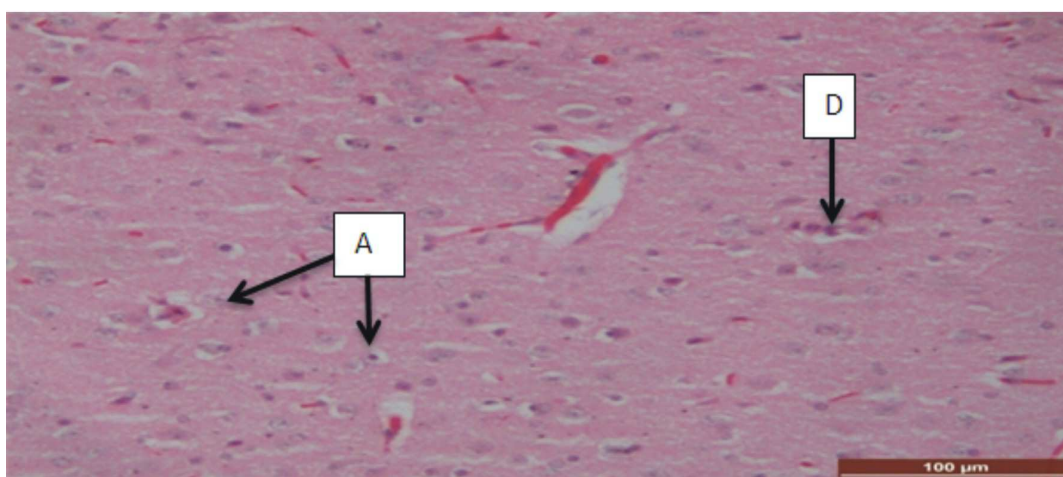


Fig. 8. Photomicrograph section of pituitary gland of male mice in group 2. Area of necrosis and degeneration (A) and inflammatory cells (D); H&E  $\times 200$

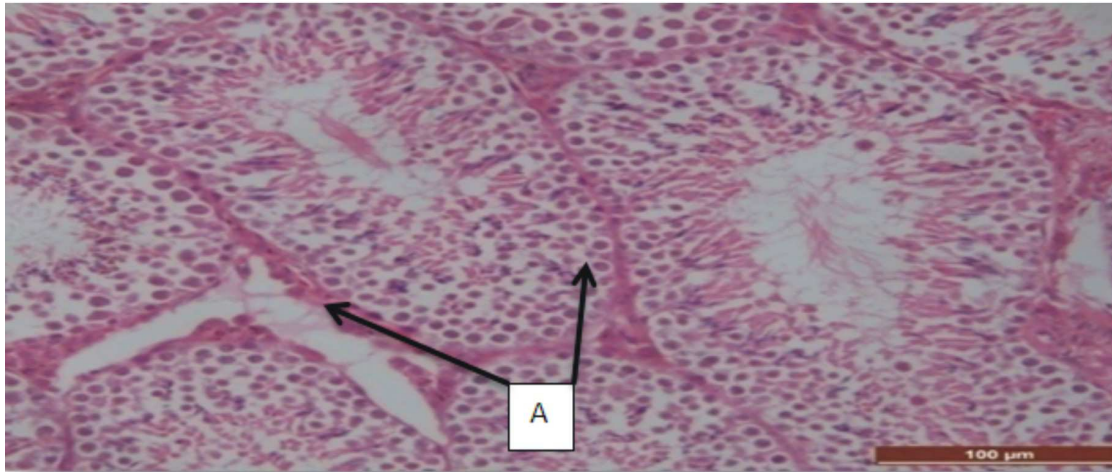


Fig. 9. Photomicrograph section of testis of male mice in group 3, showing area of necrosis and degeneration (A); H&E  $\times 200$

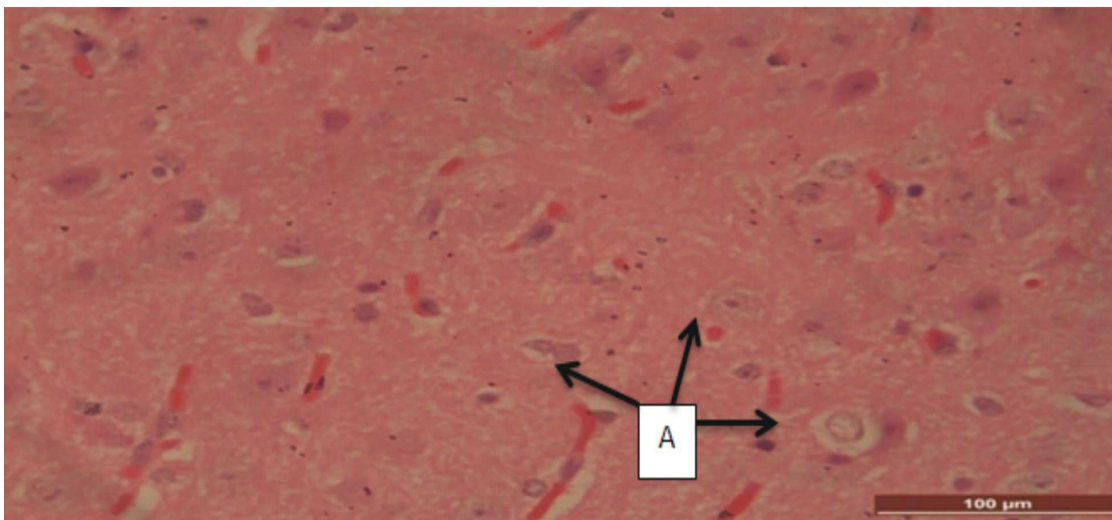


Fig. 10. Photomicrograph section of pituitary gland of group male mice showing area of necrosis and degeneration (A), H&E  $\times 200$

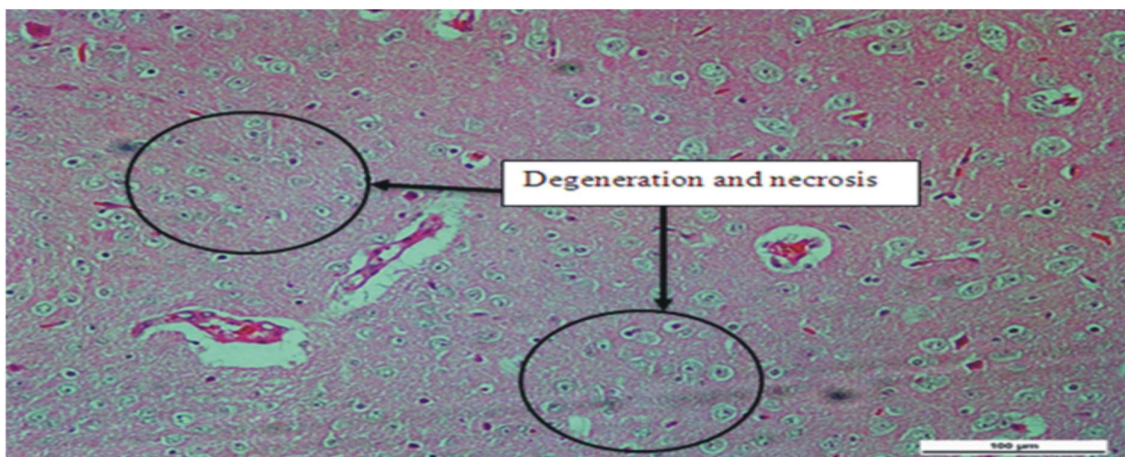


Fig. 11. Photomicrograph of a section of pituitary gland of female mice infected with *P. multocida* type B: 2 which showed swelling of the cells and some pyknosis indicating degeneration and necrosis, H&E  $\times 200$

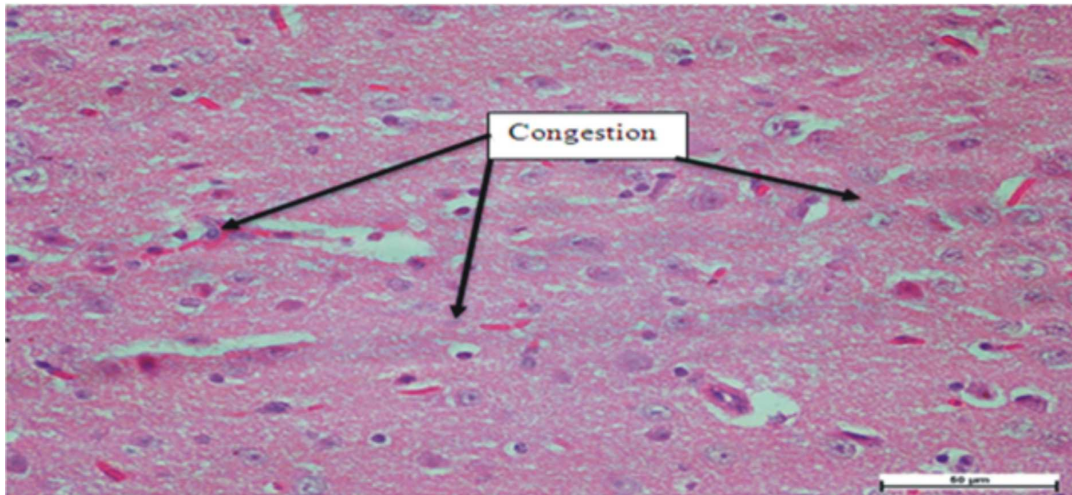


Fig. 12. Photomicrograph section of the pituitary gland which showed congested blood vessels in female mice inoculated with *P. multocida* type B; H&E  $\times 200$

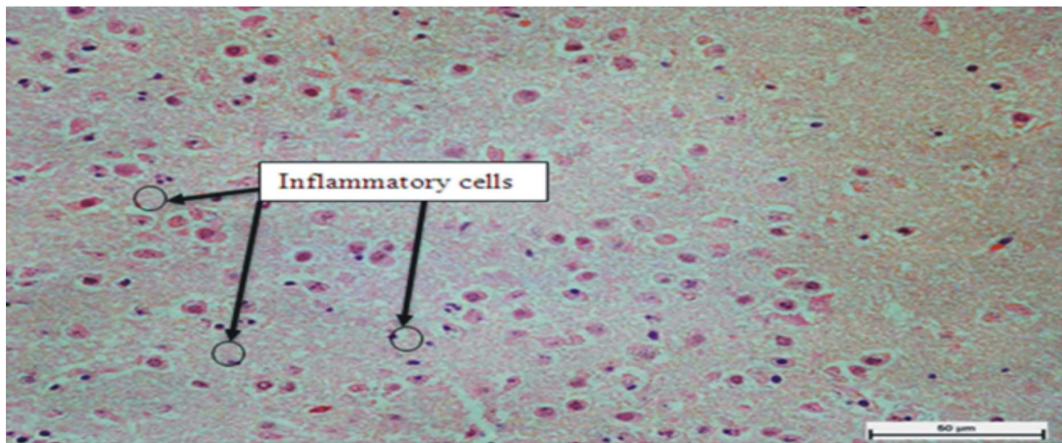


Fig. 13. Photomicrograph section of the pituitary gland which showed infiltrations of inflammatory cells in female mice infected with *P. multocida* type B; H&E  $\times 200$

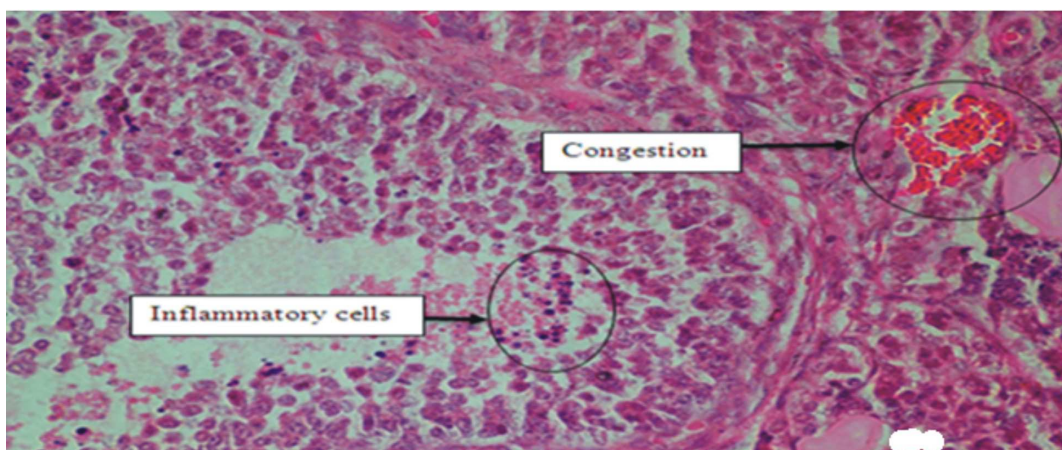


Fig. 14. Photomicrograph section of the ovary which showed infiltrations of inflammatory cells with severe congestions of the blood vessels in female mice inoculated with *P. multocida* type B; H&E  $\times 200$



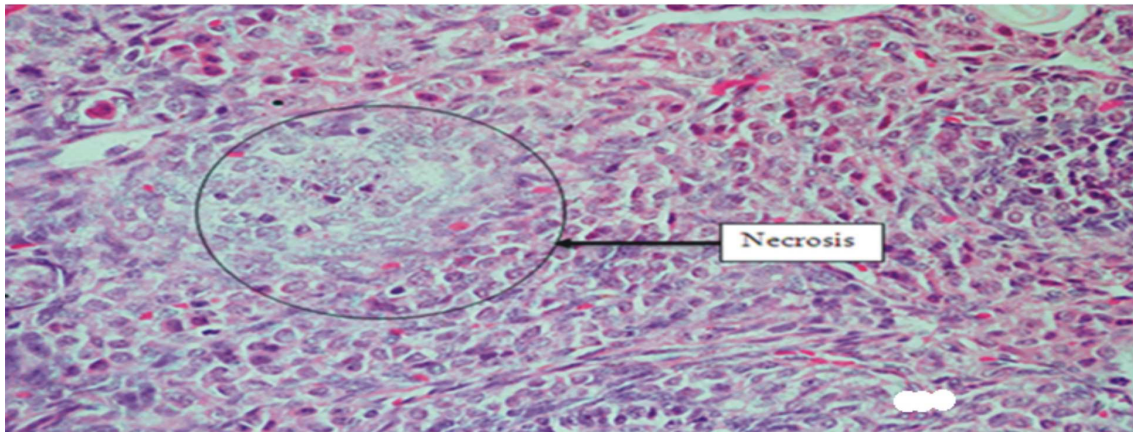


Fig. 15. Photomicrograph section which showed necrosis in some parts of the ovary in female mice inoculated with *P. multocida* type B; H&E  $\times 200$

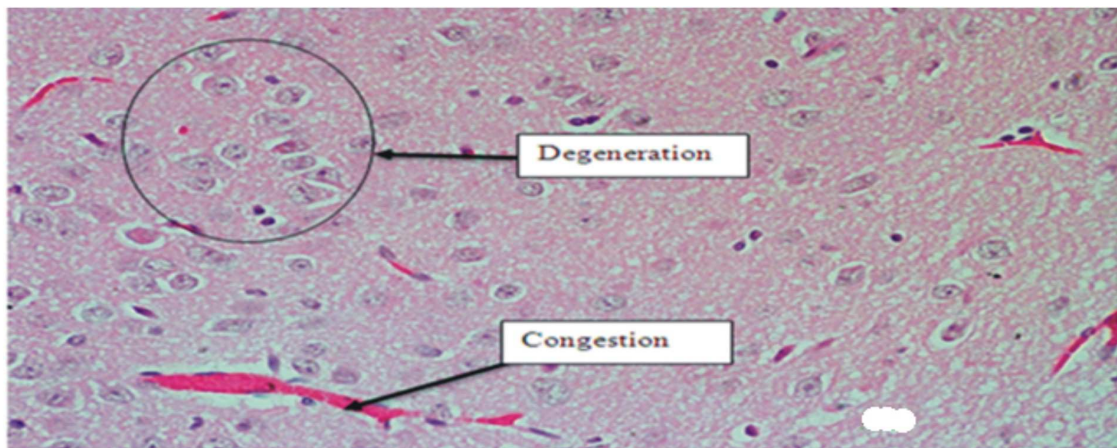


Fig. 16. Photomicrograph section of the pituitary gland which showed congestion of the blood vessels and the ballooning of the cells in female mice inoculated with LPS; H&E  $\times 200$

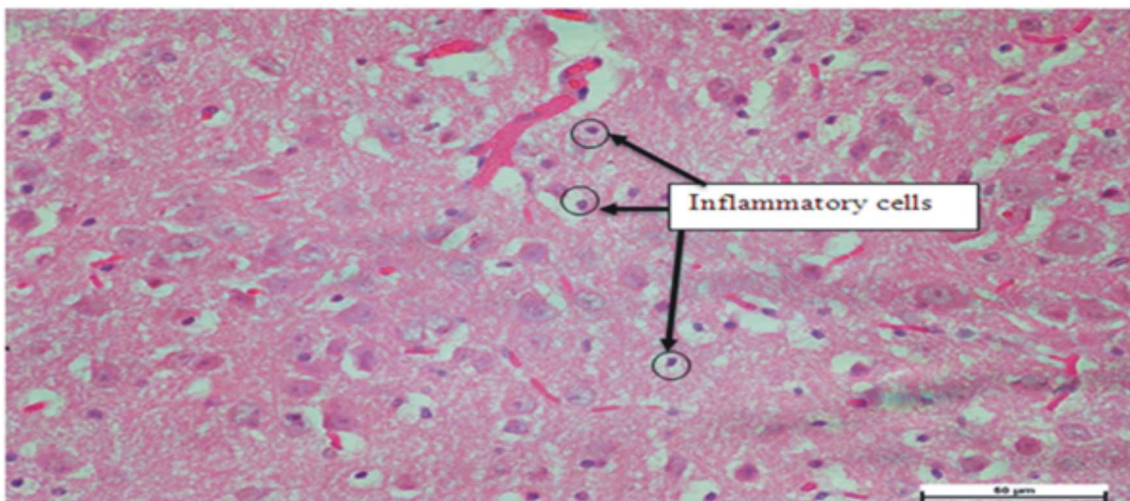


Fig. 17. Photomicrograph section of the pituitary gland which showed the presence of inflammatory cells in female mice inoculated with LPS; H&E  $\times 200$

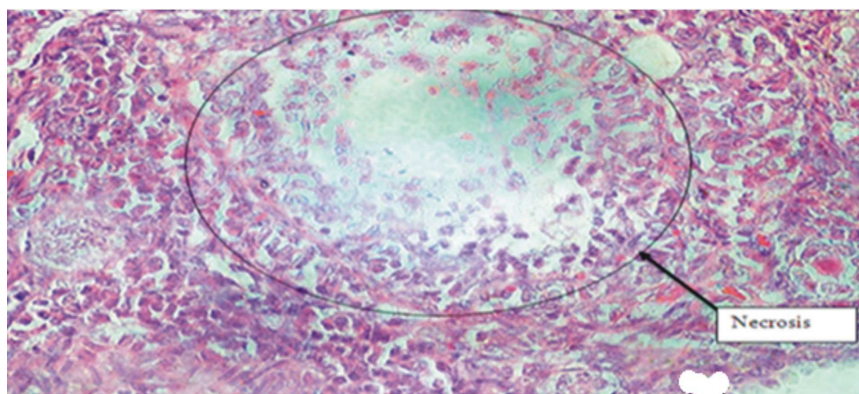


Fig. 18. Photomicrograph section of the Ovary which showed necrosis with some degree of degeneration in female mice inoculated with LPS; H&E x200

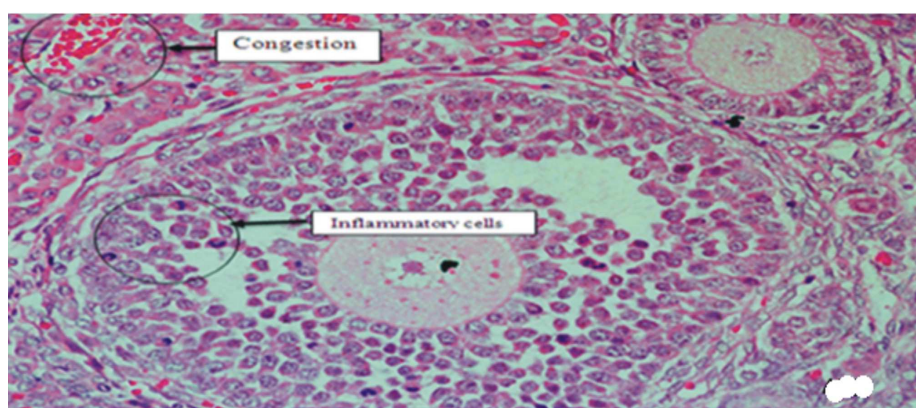


Fig. 19. Photomicrograph section of the Ovary which showed infiltrations of inflammatory cells and the congested blood vessels in the female mice inoculated with LPS; H&E x200

Table 1. Kruskal-wallis test comparing scores of histopathological lesions in pituitary glands between groups in the female mice

	Necrosis and degeneration	Oedema	Haemorrhage	Inflammatory cells
Chi-square	16.720	0.000	8.285	14.822
df	2.0000	2.000	2.000	2.0000
P-value	0.000*	1.000	0.000*	0.000*

\*Significant value  $p < 0.05$ , parameters with significant differences are further analyzed by mann-whitney test

Table 2. Mann-whitney test on comparison of histopathological lesions scoring in pituitary glands in the female mice

Groups	Necrosis and degeneration <sup>1</sup>	Haemorrhage <sup>2</sup>	Inflammatory cells <sup>3</sup>
PBS	4.75 <sup>a</sup>	7.25 <sup>a</sup>	5.00 <sup>a</sup>
PM	18.00 <sup>b</sup>	15.00 <sup>b</sup>	16.86 <sup>b</sup>
LPS	14.00 <sup>b</sup>	14.13 <sup>b</sup>	12.50 <sup>b</sup>

Means ranks<sup>a, b, c</sup> with different superscript in the same column are significantly different at ( $p < 0.05$ ) between groups; <sup>1, 2, 3</sup> $p = 0.000$  (from kruskal wallis test); Group 1 = Control or Phosphate Buffer Saline (PBS); Group 2 = *P. multocida* type B: 2 (PM); Group 3 = Lipopolysaccharide (LPS)

Table 3. Kruskal-wallis test comparing scores of histopathological lesions in ovaries between groups in the female mice

	Necrosis and degeneration	Oedema	Haemorrhage	Inflammatory cells
Chi-square	16.243	0.000	2.025	5.934
df	2.000	2.000	2.000	2.000
P-value	0.000*	1.000	0.363	0.051*

\*Significant value  $p < 0.05$ , parameters with significant differences are further analyzed by mann-whitney test

Table 4. Mann-whitney test on comparison of histopathological lesion scoring in ovaries in the female mice

Groups	Necrosis and degeneration <sup>1</sup>
PBS	5.56 <sup>a</sup>
PM	18.50 <sup>b</sup>
LPS	11.29 <sup>c</sup>

Means ranks <sup>a, b, c</sup> with different superscript in the same column are significantly different at ( $p < 0.05$ ) between groups; <sup>1</sup>  $p = 0.000$  (from kruskal wallis test); Group 1 = Control or Phosphate Buffer Saline (PBS); Group 2 = *P. multocida* type B: 2 (PM); Group 3 = Lipopolysaccharide (LPS)

## Discussion

This study reports the effects of *P. multocida* type B: 2 and its lipopolysaccharides on reproductive hormones concentrations and histopathological changes in the pituitary glands and reproductive organs in a mouse model.

Oral inoculation with *P. multocida* type B: 2 and its lipopolysaccharides into mice produced histopathological lesions in the testes. The lesions were more detrimental following inoculation of *P. multocida* type B: 2 relative to its LPS. These could be associated with the pathogenesis and the severity of infections induced by *P. multocida* type B: 2 and its lipopolysaccharides. These findings were similar to the study conducted by (OIE, 2008) following oral inoculation of the bacteria in mice. Similarly, the histopathological lesion in the pituitary gland produced significant changes after oral inoculation of the bacteria compared to the LPS and the most obvious lesion were degeneration and necrosis. This lesion could be associated with the pathogenesis and the severity of the disease in this group of mice. Thus, it may be considered that the bacteria or its endotoxin has affinity towards the hormone producing cells in pituitary glands and also the cells in the testes and ovaries which perchance can activate the infiltration of inflammatory cells to the respective organs. Meanwhile, the endotoxin (LPS) of the bacteria may have direct effects on the cells and may cause septicemic phase. These findings are in consensus with those of (Eckersall and Bell, 2010).

Testosterone concentration was twofold higher in the *P. multocida* type B: 2 group when compared to the other groups which are not consistent with the histopathological changes of the testes and pituitary gland. The increased level of testosterone production in this group of mice could be related to the reduction in the immune functions of inoculated male mice. This finding was similar to the study conducted by (Kharb and Charan, 2012) which reported testosterone depletion and testosterone receptor antagonism as responsible for prevention of the depression of immune function. However, in the current study the inoculation of the male mice with LPS indicated drastic reduction in the production of testosterone in

this group. On the other hand, inoculation of animals with LPS from these bacteria could be associated with increased immune functions in this group of animals and therefore could stand the chance of a better candidate for vaccine production. Furthermore, inoculation with LPS may induce testicular tissue which may contribute to the low testosterone concentration, as the hormone producing cells have been destroyed or as a result of disruption of the hypo-pituitogonadal axis. In another study by (Abdullah *et al.*, 2013b) showed that low immune performance in male mice has also been correlated with high target organ responsiveness to testosterone.

The mucosal surfaces of the pituitary gland and the female reproductive organs such as the ovaries represent the main area of contact of microbial agents and their endotoxin (LPS) with the body's immune system. The pituitary gland and the ovaries have perchance the most specialized immune system, since it has the twin task of providing incessant defense against the potential pathogens while providing an amenable milieu for the sperm viability. These conditions are maintained by the specific regulation of the immune system by the ovarian hormones estrogen and progesterone. In the current study, female reproductive hormones, progesterone and estrogen concentration levels do showed some changes in the infected female mice with *P. multocida* type B: 2 and its endotoxin. The higher concentrations of progesterone and lower concentrations of estrogen induced by both *P. multocida* type B: 2 and its LPS may occur because of the damages at the cellular levels observed in hormone producing cells in both ovaries and pituitary glands which contribute to the changes in reproductive hormone production. These findings are similar to the study conducted by (Angele *et al.*, 2000).

Based on the changes that occur in the female mice reproductive hormones, there would perhaps be some uncertainty regarding the reproductive status of the infected or carrier animals. The fertility potentials of these animals may be affected and they may be unable to be pregnant as there are disruptions in the reproductive hormonal levels. In the current study, female mice inoculated with both *P. multocida* type B: 2 and LPS indicated increased concentrations of progesterone. The increased progesterone concentrations in these groups of

mice could be indicative of enhanced infections in the female animals if the level of progesterone happened to be amplified by any means. This finding was in agreement to the study conducted by (Charu *et al.*, 2000). Furthermore, the lower concentrations of estrogen induced by both inoculation of *P. multocida* type B: 2 and its LPS in the female mice seems to decrease the susceptibility of the female mice to infections, this could be due to the correlations between the decrease in the concentration of estrogen and the levels of the severity in the histopathological alterations in the pituitary glands and the ovaries of the female mice. This finding was also similar to the study conducted by (Khuder *et al.*, 2012).

## Conclusion

In conclusion, inoculation of *P. multocida* type B: 2 and its endotoxin via oral route developed significant histopathological changes in the pituitary glands, testes and the ovaries. Similarly, there was significant alteration in the levels of the male and female reproductive hormones due to *P. multocida* type B: 2 and its LPS which are imperative considering the potential effects of immune responses of the individual animals. Therefore, the sex hormonal levels in animals could be used to assess the immune statuses of at risk animal population and proffer amenable Veterinary medical care to afflicted animal population.

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## Conflict of Interest

The researchers have no conflict of interest.

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## Author's Contributions

AWH, AAS and FFJA conceptualized and supervised the research. AMMA, YA, AT, KM, LA, MAS, RZ and MJBS collected samples, drafted the manuscript and ran all statistical tests. All authors have read and approved the manuscript.

## Ethics

All procedures and experiments illustrated were undertaken under a project license approved by Animal Utilization Protocol Committee, Faculty of veterinary

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