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Haematological and Histopathological Vicissitudes Following Oral Inoculation of Graded Doses of *Pasteurella multocida* Type B: 2 and its Lipopolysaccharide in Mice

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Abstract

Pasteurella multocida type B: 2 is responsible for major animal diseases of economic importance in both developed and developing countries. Haemorrhagic Septicaemia (HS) could inflict devastating effects on blood tissues and organs in the host animal. Therefore, the current study aims to investigate the haematological and clinico- pathological responses in mice following oral inoculation of graded doses of *Pasteurella multocida* type B: 2 and its lipopolysaccharide. Sixty healthy Balb c mice were placed in twelve plastic cages each one containing five mice. The mice were divided into three major groups (A, B and C). Group A is the control group (n=10) and these were inoculated with 0.4 ml of PBS pH 7.4 orally. The treatment groups (B; n=25 and C; n=25) were inoculated with *P. multocida* type B: 2 and its lipopolysaccharide respectively. The mice in group B and C were further divided into five subgroups. The subgroups were designated based on the graded doses as $B10^1$, $B10^3$, $B10^5$, $B10^7$ and $B10^9$ for *Pasteurella multocida* and $C10^1$, $C10^3$, $C10^5$, $C10^7$ and $C10^9$ for LPS respectively. The mice were observed for 120 hours post- inoculation. The clinical signs (Ruffled fur, Ocular discharges, Level of alertness and Laboured breathing) were significantly different ($p<0.001$) in mice inoculated orally with variable doses of *Pasteurella multocida* type B: 2 and its LPS. RBC, PCV, haemoglobin concentrations, PT, APTT, Thrombocyte, WBC, Lymphocytes, monocytes, plasma proteins, band and segmented neutrophils were significantly different ($p<0.0001$) in mice inoculated with graded doses of *Pasteurella multocida* type B: 2 and its LPS. Inflammatory cells, degeneration, necrosis and congestion were significantly different ($p<0.0001$) in mice inoculated with graded doses of both *Pasteurella multocida* type B: 2 and its LPS. In conclusion, 10^9 cfu of *Pasteurella multocida* type B: 2 and its lipopolysaccharide have devastating effects on organs and blood tissues.

Keywords: *Pasteurella multocida type* B: 2; Lipopolysaccharides; Vital organs; Blood; Oral route; Mice

Introduction

The Gram-negative bacterium *Pasteurella multocida* is of substantial economic significance in the livestock industry around the world and it is an opportunistic human pathogen [1]. Haemorrhagic Septicaemia (HS) is an acute high mortality systemic disease of cattle and water buffaloes [2,3] leading to huge economic loss in the bovine industry particularly in South East Asia [4,5]. However, in the context of susceptibility buffaloes were found to be more susceptible to the disease in comparison to others [6-8]. The common HS serotypes which have been reported to be responsible of recurrent outbreaks in Asia are the serotypes B: 2 [1,5,9]. In Malaysia, the stressful condition is during the raining season where most outbreaks occurred; the development of the disease in animals has been frequently reported to occur following exposure of the susceptible hosts to infections usually occurring by the inhalation or ingestion of the bacterium [9-11]. The disease is characterized by a rapid increase in the body temperature, respiratory rate, loud and stertorous breathing, profuse salivation, severe depression, anorexia and finally the animal dies within 24 hours [3,6,8,12]. The pathological modifications include generalized lymphadenopathy, acute fibrinous pneumonia, proctitis, acute colitis, hemorrhagic typhilitis, submandibular and brisket edema [13-15].

In most cases the histopathological lesions observed via different routes of inoculation were similar and classical to what was observed in natural HS infection [1,5,16,17]. Pathogenesis of *Pasteurella multocida* is a complex interaction between host specific factors and specific bacterial virulence factors [18]. There are varieties of virulence factors identified in *Pasteurella multocida* type B: 2 which include lipopolysaccharide, capsule, surface adhesions, iron regulated and iron acquisition proteins [16,19]. Lipopolysaccharide (LPS) is the primary antigen for the identification of strains located in the Outer Membrane Proteins (OMPs) of *Pasteurella multocida* type B: 2 and it is an important virulence factor by having a dominant role during the host immune-histopathological responses [20]. The immuno-histopathological changes of endotoxin-producing bacterial infections represent an unrestrained over-reaction by the host immune system to the endotoxins [21]. In pathogens, LPS plays an imperative role in the disease process by interacting directly with innate host immune defenses leading to the activation of a range of host immune cells, which

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can result in immuno-histopathological changes in the vital organs and blood tissue of death hosts [22]. A recent study of experimental nature has confirmed the development of typical HS following inoculation of *Pasteurella multocida* type B: 2 and its LPS in mice and calves [3,5,8].

There is no documentation on haematological and histopathological changes in the host inoculated orally with graded doses of *Pasteurella multocida* type B: 2 and its LPS. Therefore, the present study was set to evaluate the haematological and histopathological changes in different vital organs and blood tissues of mice following inoculation with graded doses of *Pasteurella multocida* type B: 2 and its LPS via the oral route. The current findings might improve the vaccines, vaccination, control and the preventative methods that we have employed in curtailing this important disease in Malaysia.

Material and Methods

Animals

Sixty healthy Balb c mice of eight to ten weeks old of both sexes were enrolled in this study. They were obtained from the Institute of Cancer Research (ICR) and kept at the Animal Resource Centre, Universiti Putra Malaysia. The animals were confirmed negative for *Pasteurella multocida* following culture of peripheral blood for bacterial isolations; the mice were housed in plastic cages and provided with water and pellet ad libitum. Five mice were kept in each plastic cage. The mice were observed for 2 weeks prior to the experiment to make sure that they were healthy and acclimatized.

Inoculums

Throughout the experiments, two types of inoculums were used; the whole cell and Lipopolysaccharide (LPS) extracted from *P. multocida* type B: 2.

Wild type *Pasteurella multocida* serotype B: 2

The wild-type *Pasteurella multocida* type B: 2 used in this study were obtained from stock culture. It was isolated from a previous outbreak of Hemorrhagic septicemia (HS) in the state of Kelantan, Malaysia. Identification of *Pasteurella multocida* type B: 2 were made using the Gram-staining method and biochemical characterization of oxidase, urea broth, and Sulphur Indole Motility (SIM), Triple Sugar Iron (TSI) and citrate tests. The isolate was confirmed to be *Pasteurella multocida* type B: 2 by the Veterinary Research Institute (VRI) Ipoh, Perak. Pure stock culture that was stored on nutrient agar slants was sub-cultured onto 5% horse blood agar and incubated at 37°C for 18 hours. A single colony of *Pasteurella multocida* type B: 2 was selected and grown in Brain Heart Infusion broth (BHI), incubated in shaker incubator at 37°C for 24 hours before the concentration was determined by McFarland Nephelometer Barium Sulfate Standards.

The Lipopolysaccharide (LPS) of *P. multocida* B: 2

The LPS Extraction Kit (Intron Biotechnology) was used to prepare the inoculums of LPS. For this experiment, LPS was extracted from different dosage of bacteria (10^1, 10^3, 10^5, 10^7 and 10^9) cfu. The whole cells were centrifuged for approximately 30 sec at 13,000 rpm at room temperature. Then the supernatant was removed and 1 mL of lysed buffer was added and vortexed vigorously to lyse the bacterial cells. This was followed by adding 200 µL of chloroform and vortexed vigorously. The mixture was incubated for 5 min at room temperature and centrifuged at 13,000 rpm for 10 min at 4°C. Then, 400 µL of the supernatant was transferred into a new 1.5 mL centrifuge tube and 800 µL of purification buffer was added. The mixture was incubated for 10 min at -20°C. This was followed by another centrifugation at 13,000 rpm for 15 min at 4°C. Lastly, the LPS pellet was washed with 1ml of 70% ethanol and dried completely. Following that, 70 µL of 10 mM Tris- HCl (pH 8.0) (Sigma®) was added into the LPS pellet and was dissolved by boiling for 1 min. The LPS extraction obtained was subjected to SDS-PAGE to confirm that no protein was present in the extracted LPS.

Experimental design in mouse model

Sixty healthy Balb c mice were placed in twelve plastic cages each one containing five mice. The mice were divided into three major groups (A, B and C). Group A is the control group (n=10) and these were inoculated with 0.4 ml of PBS pH 7.4 orally. The treatment groups (B; n=25 and C; n=25) were inoculated with *Pasteurella multocida* type B: 2 and its lipopolysaccharide respectively. The mice in group B and C were further divided into five subgroups. The subgroups were designated based on the graded doses as $B10^1$, $B10^3$, $B10^5$, $B10^7$ and $B10^9$ for *Pasteurella multocida* and $C10^1$, $C10^3$, $C10^5$, $C10^7$ and $C10^9$ for LPS respectively. After 120 hours of inoculation, the clinical signs and mortality rates were observed. Mice showed severe clinical signs and survived mice after 120 hours post-inoculation were sacrificed via cervical dislocation approach and post-mortem examination was performed. 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: UPM/IACUC/AUP-R017/2014.

Histopathology

After 120 hours of post inoculation, the mice were euthanized by cervical dislocation and the visceral organs (intestine, kidney, lungs, spleen and liver) were collected in 10% buffered formalin, processed, sectioned and stained with H&E and the histopathological lesions were observed using light microscopy.

Lesions scoring and statistical analysis

Histopathological changes observed (degeneration and necrosis, inflammation and congestion) were scored based on the following categorization; 0 (normal), 1: Mild (less than 1/3 of field involved), 2: Moderate (between 1/3 and 2/3 of field involved) and 3: Severe (more than 2/3 of the field involved). Six microscopic fields were examined for each lesion per slide and the mean ± standard error was calculated for each organ based on the different lesions observed.

Evaluation of clinical signs

Clinical signs such as ruffled fur, laboured breathing, alertness and ocular discharges were assessed for a period of 120 hours. In summary, the clinical signs of the twelve groups were scored on scale of 0-3 based on the presence of the following clinical signs: ruffled fur, laboured breathing, alertness and ocular discharges. The score 0 represent no abnormality of clinical signs observed, 1 for mild (30% of abnormality), 2 for moderate (60% of abnormality), 3 for severe (more than 60% of abnormality).

Statistical Analysis

All the data were analyzed using JMP® 11. NC: SAS Institute Inc. software Version. The data were considered significant at $p<0.05$.

Results

Inflammatory cells, congestion, degeneration and necrosis were

significantly different ($p<0.0001$) in mice inoculated with graded doses of both *Pasteurella multocida* type B: 2 and its LPS (Figures 1 and 2). The clinical signs (Ruffled fur, Ocular discharges, Level of alertness and Laboured breathing) were significantly different ($p<0.001$) in mice inoculated orally with variable doses of *Pasteurella multocida* type B: 2 and its LPS. Red Blood Cells (RBC), Packed Cell Volume (PCV), Haemoglobin Concentrations (Hb), Prothrombin time (PT), Activated Partial Thromboplastin Time (APTT), Thrombocyte, White blood cells (WBC), Lymphocytes, monocytes, band and segmented neutrophils, plasma proteins were significantly different ($p<0.0001$) in

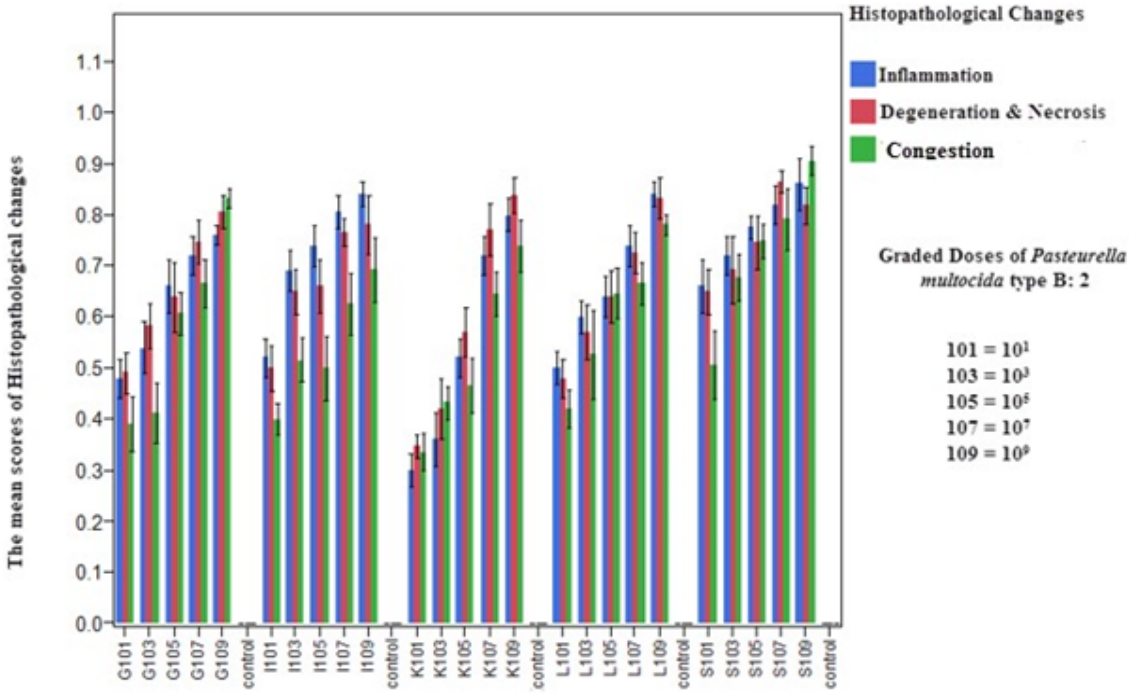

Figure 1: Histopathological changes in the Lungs (G), Intestines (I), Kidney (K), Liver (L), and Spleen (S) in mice inoculated with graded doses of *Pasteurella multocida* type B: 2 phosphate buffered saline (control group).

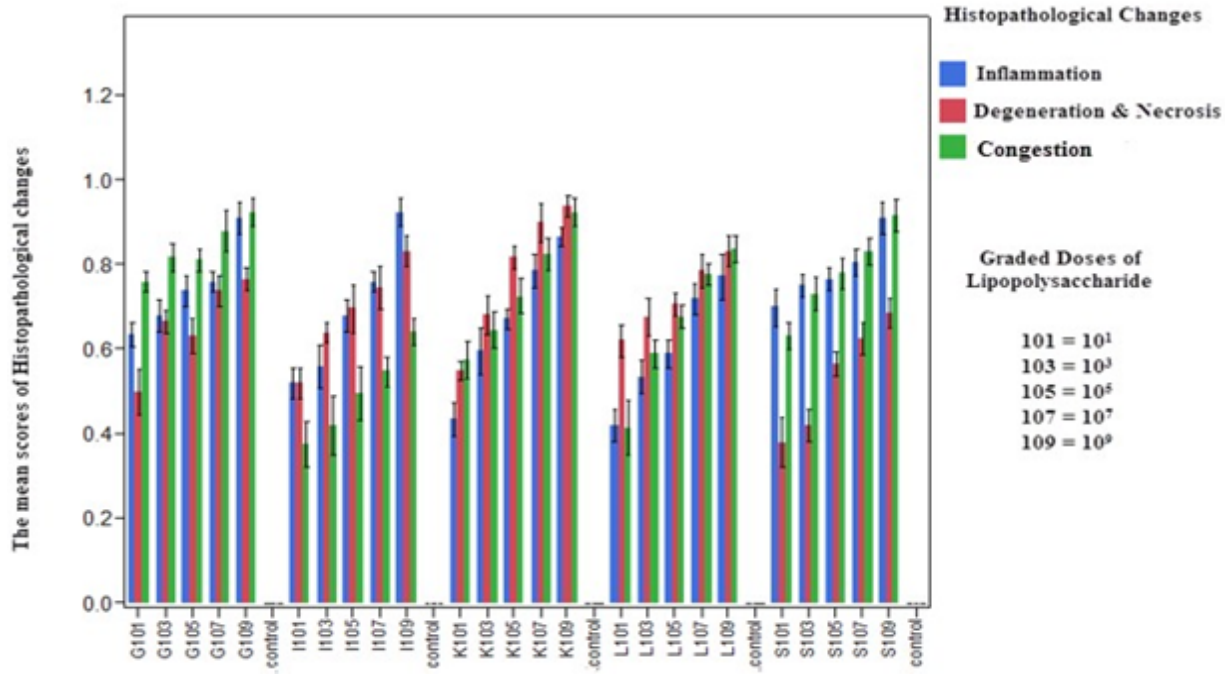

Figure 2: Histopathological changes in the Lungs (G), Intestines (I), Kidney (K), Liver (L), and Spleen (S) in mice inoculated with graded doses of LPS and phosphate buffered saline (control group).

mice inoculated with graded doses of *Pasteurella multocida* type B: 2 and its LPS (Tables 1-4).

Discussion

In Malaysia, the large ruminant sector is endangered by Haemorrhagic Septicaemia (HS) caused by *Pasteurella multocida* type B: 2. The protein toxin from *Pasteurella multocida* type B: 2 experimentally induces all of the major symptoms of Haemorrhagic Septicaemia (HS) in cattle and water buffaloes. The lipopolysaccharide (LPS) has a role in disease processes as they act at an interface between the host and pathogen [23]. To the researchers knowledge this is the first study to comprehensively evaluate the haematological and histopathological changes in mice following 120 hours of inoculation with graded doses of *Pasteurella multocida* type B: 2 and its LPS through the oral routes.

The commonly used route of inoculation of *Pasteurella multocida* type B: 2 and its LPS employed by most researchers in Balb c mice is the intraperitoneal route [23]. Other routes that are used, less commonly, include subcutaneous and intramuscular [4]. The routes of inoculation used in these studies differ in most cases with other research, in our research we used graded doses of *Pasteurella multocida* type B: 2 and it's LPS via the oral route and observed the mice for 120 hours post inoculation. The clinical signs observed which include ruffled fur, laboured breathing, closure of the eyes and also discharge from the eye were more severe in groups inoculated with 109 cfu of *Pasteurella multocida* type B: 2 and it's LPS through the oral routes compared to the control and other treatment groups. These findings were similar to those reported by Jesse et al. [5] which described an experiment following intraperitoneal inoculation with the same organism. The present study showed that mice can succumb to experimental HS infection following oral inoculation of graded doses of *Pasteurella multocida* type B: 2 and its LPS, and the clinical signs observed were similar and classical to what was observed in natural HS infection in other research work [21]. All the groups of mice inoculated with *Pasteurella multocida* type B: 2 and its LPS did not died during the experiment period of 120 hours. Kharb and Charan [16] reported that regardless of the route of inoculation, mortality in mice was 80% in all the groups they studied. The absence of death in the present study could be due to the protracted time period and the route of inoculation with the graded doses of *Pasteurella multocida* type B: 2 and its LPS [24].

Like the LPS of other Gram-negative pathogens, *Pasteurella multocida* LPS has endotoxic properties, particularly in buffalo, where the injection of purified LPS from a serogroup B strain was able to mimic the clinical signs of bovine haemorrhagic septicaemia [25]. Oral inoculation of *Pasteurella multocida* type B: 2 and its LPS into Balb c mice in the present study was able to produce histopathological changes

PM	Control	10^1 (cfu)	10^3(cfu)	10^5 (cfu)	10^7 (cfu)	10^9 (cfu)
Ruffled fur	0.0 ± 0.05^d	2.02 ± 0.05^c	2.08 ± 0.05^c	2.5 ± 0.05^b	2.9 ± 0.05^a	2.9 ± 0.05^a
Ocular discharges	0.0 ± 0.0611^d	0.06 ± 0.0611^d	0.64 ± 0.0611^c	1.42 ± 0.0611^b	2.4 ± 0.0611^a	2.5 ± 0.0611^a
Level of alertness	0.0 ± 0.054^f	0.6 ± 0.054^e	1.2 ± 0.054^d	1.8 ± 0.054^c	2.08 ± 0.054^b	2.4 ± 0.054^a
Laboured breathing	0.0 ± 0.058^d	1.4 ± 0.058^c	1.7 ± 0.058^c	2.4 ± 0.058^b	2.8 ± 0.058^a	2.9 ± 0.058^a

Values with different superscript in a row are significantly different (P<0.05); PM: *Pasteurella multocida* type B: 2; cfu: Colony Forming Unit

Table 1: Modifications in Clinical Signs in mice following oral inoculation of graded doses of *Pasteurella multocida* type B: 2 after 120 hours of inoculation.

LPS	Control	10^1 (cfu)	10^3 (cfu)	10^5 (cfu)	10^7 (cfu)	10^9 (cfu)
Ruffled fur	0.0 ± 0.055^f	0.48 ± 0.055^e	1.02 ± 0.055^d	1.5 ± 0.055^c	2.14 ± 0.055^b	2.5 ± 0.055^a
Ocular discharges	0.0 ± 0.044^e	0.0 ± 0.044^e	0.22 ± 0.044^d	0.56 ± 0.044^c	1.12 ± 0.044^b	1.68 ± 0.044^a
Level of alertness	0.0 ± 0.04^f	0.78 ± 0.04^e	1.46 ± 0.04^d	1.84 ± 0.04^c	2.46 ± 0.04^b	2.72 ± 0.04^a
Laboured breathing	0.0 ± 0.032^f	0.56 ± 0.032^e	0.78 ± 0.032^d	1.3 ± 0.032^c	1.8 ± 0.032^b	2.4 ± 0.032^a

Values with different superscript in a row are significantly different (P<0.05); LPS: LPS of *Pasteurella multocida* type B: 2; cfu: Colony Forming Unit.

Table 2: Modifications in clinical signs in mice following oral inoculation of graded doses of LPS after 120 hours of inoculation.

PM Parameters	Control	10^1 (cfu)	10^3 (cfu)	10^5 (cfu)	10^7 (cfu)	10^9 (cfu)
RBC (1012/L)	9.26 ± 0.047^a	8.89 ± 0.047^b	8.67 ± 0.047^c	8.47 ± 0.047^c	8.25 ± 0.047^d	8.05 ± 0.047^e
Hemoglobin(g/L)	155.61 ± 0.243^a	147.66 ± 0.243^b	143.76 ± 0.243^c	139.84 ± 0.243^d	137.02 ± 0.243^e	134.82 ± 0.243^f
PCV (L/L)	0.46 ± 0.005^a	0.414 ± 0.005^b	0.392 ± 0.005^c	0.368 ± 0.005^d	0.318 ± 0.005^e	0.306 ± 0.005^e
WBC ($\times 10^9$/L)	7.72 ± 0.042^a	5.2 ± 0.042^b	4.92 ± 0.042^c	4.2 ± 0.042^d	3.92 ± 0.042^e	3.1 ± 0.042^f
BNeutrophil ($\times 10^9$/L)	0.15 ± 0.007^f	0.19 ± 0.007^e	0.22 ± 0.007^d	0.32 ± 0.007^c	0.42 ± 0.007^b	0.52 ± 0.007^a
SNeutrophil ($\times 10^9$/L)	19 ± 0.462^f	37.4 ± 0.462^e	44.3 ± 0.462^d	56.5 ± 0.462^c	62.6 ± 0.462^b	71.04 ± 0.462^a
Lympho ($\times 10^9$/L)	5.58 ± 0.0097^a	4.92 ± 0.0097^b	4.51 ± 0.0097^c	3.52 ± 0.0097^d	2.92 ± 0.0097^e	2.62 ± 0.0097^f
Monocytes ($\times 10^9$/L)	0.52 ± 0.0069^a	0.53 ± 0.0069^a	0.48 ± 0.0069^b	0.43 ± 0.0069^c	0.33 ± 0.0069^d	0.27 ± 0.0069^e
Eosinophils ($\times 10^9$/L)	0.320 ± 0.0061^f	0.362 ± 0.0061^e	0.408 ± 0.0061^d	0.490 ± 0.0061^c	0.532 ± 0.0061^b	0.67 ± 0.0061^a
Plasma Protein (g/L)	62.66 ± 0.475^e	68.90 ± 0.475^d	70.20 ± 0.475^d	74.70 ± 0.475^c	79.64 ± 0.475^b	87.20 ± 0.475^a
PT (seconds)	26.46 ± 0.361^f	37.72 ± 0.361^e	43.50 ± 0.361^d	49.66 ± 0.361^c	56.56 ± 0.361^b	63.34 ± 0.361^a
APTT (seconds)	53.30 ± 0.516^f	63.12 ± 0.516^e	69.30 ± 0.516^d	77.96 ± 0.516^c	84.12 ± 0.516^b	97.34 ± 0.516^a
Thrombo ($\times 10^9$/L)	407.54 ± 1.12^f	549.10 ± 1.12^e	634.72 ± 1.12^d	707.56 ± 1.12^c	779.34 ± 1.12^b	837.80 ± 1.12^a

Values with different superscript in a row are significantly different (P<0.05); PM: *Pasteurella multocida* type B: 2; PCV: Packed Cell Volume; WBC: White Blood Cells; BNeutrophils: Band Neutrophils; SNeutrophils: Segmented neutrophils; Lympho: Lymphocytes; PT: Prothrombin Time; APTT: Activated Partial Thromboplastin Time; Thrombo: Thrombocytes; cfu: Colony Forming Unit.

Table 3: Modifications in hematological parameters in mice following oral inoculation of graded doses of *Pasteurella multocida* type B: 2 after 120 hours of inoculation.

LPS Parameters	Control	10^1 (cfu)	10^3 (cfu)	10^5 (cfu)	10^7 (cfu)	10^9 (cfu)
RBC (1012/L)	9.32 ± 0.036[a]	8.57 ± 0.036[b]	8.37 ± 0.036[c]	8.12 ± 0.036[d]	7.89 ± 0.036[e]	7.69 ± 0.036[f]
Hemoglobin(g/L)	155.62 ± 0.233[a]	142.44 ± 0.233[b]	136.96 ± 0.233[c]	132.16 ± 0.233[d]	129.14 ± 0.233[e]	125.48 ± 0.233[f]
PCV (L/L)	0.46 ± 0.0043[a]	0.398 ± 0.0043[b]	0.384 ± 0.0043[b]	0.352 ± 0.0043[c]	0.312 ± 0.0043[d]	0.282 ± 0.0043[e]
WBC (×10^9/L)	7.72 ± 0.031[a]	4.92 ± 0.031[b]	3.90 ± 0.031[c]	2.94 ± 0.031[d]	2.20 ± 0.031[e]	1.92 ± 0.031[f]
BNeutrophil (×10^9/L)	0.15 ± 0.0058[f]	0.213 ± 0.0058[e]	0.319 ± 0.0058[d]	0.423 ± 0.0058[c]	0.525 ± 0.0058[b]	0.621 ± 0.0058[a]
SNeutrophil (×10^9/L)	19.06 ± 0.44[f]	41.22 ± 0.44[e]	54.30 ± 0.44[d]	61.66 ± 0.44[c]	76.20 ± 0.44[b]	84.24 ± 0.44[a]
Lympho (×10^9/L)	5.580 ± 0.0186[a]	3.974 ± 0.0186[b]	3.148 ± 0.0186[c]	2.330 ± 0.0186[d]	1.920 ± 0.0186[e]	0.92 ± 0.0186[f]
Monocytes (×10^9/L)	0.530 ± 0.0066[a]	0.430 ± 0.0066[b]	0.382 ± 0.0066[c]	0.30 ± 0.0066[d]	0.220 ± 0.0066[e]	0.130 ± 0.0066[f]
Eosinophils (×10^9/L)	0.326 ± 0.0068[f]	0.38 ± 0.0068[e]	0.47 ± 0.0068[d]	0.60 ± 0.0068[c]	0.72 ± 0.0068[b]	0.83 ± 0.0068[a]
Plasma Protein (g/L)	62.72 ± 0.467[f]	68.58 ± 0.467[e]	70.72 ± 0.467[d]	77.54 ± 0.467[c]	84.14 ± 0.467[b]	95.66 ± 0.467[a]
PT (seconds)	26.50 ± 0.278[f]	41.90 ± 0.278[e]	49.66 ± 0.278[d]	58.96 ± 0.278[c]	67.10 ± 0.278[b]	75.70 ± 0.278[a]
APTT (seconds)	53.30 ± 0.451[f]	67.94 ± 0.451[e]	76.56 ± 0.451[d]	88.20 ± 0.451[c]	103.60 ± 0.451[b]	117.54 ± 0.451[a]
Thrombo (×10^9/L)	407.54 ± 1.097[f]	583.22 ± 1.097[e]	691.58 ± 1.097[d]	763.42 ± 1.097[c]	871.22 ± 1.097[b]	917.60 ± 1.097[a]

Values with different superscript in a row are significantly different (P<0.05); LPS: Lipopolysaccharide; PCV: Packed Cell Volume; WBC: White Blood Cells; BNeutrophils: Band Neutrophils; SNeutrophils: Segmented neutrophils; Lympho: Lymphocytes; PT: Prothrombin Time; APTT: Activated Partial Thromboplastin Time; Thrombo: Thrombocytes; cfu: Colony Forming Unit.

Table 4: Modifications in hematological parameters in mice following oral inoculation of graded doses of LPS after 120 hours of inoculation.

in the vital organs. These findings were similar to those reported in the experimental infection of mice and buffalo calves via oral exposure [15,26-29]. However, the use of graded doses of *Pasteurella multocida* type B: 2 and its LPS as used in this study was not previously reported in mice following oral inoculation. HS is a deadly infection and the extent of lesion manifestation depends on the duration of the disease and the dose of bacterial inoculums [16]. In the study, mice inoculated with 10^9 cfu of *Pasteurella multocida* and its LPS showed extensive histopathological changes in most organs and the most common lesions observed were inflammation, necrosis, degeneration and congestion. This showed that *Pasteurella multocida* type B:2 and its LPS were more severe in the 10^9 cfu, perhaps due to the effective doses of the bacteria and its immunogen. Nevertheless, the strong effect of LPS in this study produces an extensive histopathological changes in the vital organs in the treatment groups of mice which were inoculated with LPS extracted from 10^9 cfu of *Pasteurella multocida* type B: 2. This could be due to the sufficient dose of 10^9 cfu of LPS that was inoculated via oral route to produce the HS histopathological lesions. It is also possible that the LPS dose of 10^9 cfu which was inoculated via the oral route was able to produce its endotoxic effects to alter the histopathological response in the Balb c mice. A dose of 10^9 cfu of LPS inoculation through oral route produces histopathological changes in the vital organs that mimic a response more closely associated with clinical septicaemia in the present study. Some studies denoted that endotoxin or LPS is needed to be infused continuously since endotoxin is rapidly cleared by the mononuclear phagocytic system [3,5,8,15,28]. Abdullah et al. [29] observed that single intravenous injection of LPS was tolerated easily by mice without any observable pathology or evidence of ill health. They also stated that repeated intraperitoneal injections of LPS in mice at 8 hours intervals caused the mice to become ill [30]. For infection to occur, the bacterial pathogen must have the capability to penetrate the mucus membrane of the buccal cavity and the epithelial layers of the digestive system and multiply whilst evading the host immune system. If the infection is to result in disease and cause cellular changes, the pathogen might also interact with the host in a way which might result in disturbance of homeostasis [16]. In the present study, the infection of Balb c mice orally with graded doses of *Pasteurella multocida* type B: 2 and its LPS was able to mimic the natural scenario and it was observed that the bacteria and its LPS were successfully recovered and they were able to produce haematological and histopathological changes in the mice through oral routes of inoculation. The haematological and histopathological changes observed were also similar with the mice and calves inoculated with this bacteria and its LPS [15,24]. Besides that, the histopathological lesions were also similar to those of the cattle infected naturally and experimentally with *Pasteurella multocida* type B: 2 [12,31,32]. In a related study, Jesse et al. [5,8] observed that calves and mice inoculated intramuscularly and intravenously developed classical HS with the presence of degeneration, necrosis, hemorrhage and inflammatory cells. It was observed that the severity of histopathological lesions in the present study was moderate to severe following oral routes of inoculation of10^9 cfu of the bacteria. Similar observations were made by Abdullah et al. [29], Rhoades et al. [33] and Horadagoda et al. [34] following oral inoculation of *Pasteurella multocida* type B: 2 in mice. The least commonly observed lesion at histopathology was congestion. These lesions were mild following inoculation with 10^1 and 10^3 cfu and mild to moderate on inoculation with 10^5 and 10^7 cfu and severe with dose of 10^9 cfu in all the organs. Jesse et al. [5,8] reported a similar finding in calves and mice inoculated with *Pasteurella multocida* type B: 2 and its LPS in which thrombosis was mild in all the groups.

Haematological changes caused by bacterial infections are first detected during routine blood count. However, an animal's defensive mechanisms can react quite differently to different bacteria; therefore, there is no singular pattern in complete blood count that indicates a bacterial infection. Nevertheless, there are few abnormalities that are suggestive of bacterial infection such as neutrophilia with a left shift being the hallmark of acute inflammation [35,36]. Results from the present study revealed significant reduction in the red blood cell counts in all treated groups. This is consistent with Walton et al. [37] whose findings concluded that inflammation is able to reduce red blood cell count. As part of the bacteriostatic mechanisms, inflammatory mediators such as tumor necrosis factor up-regulate ferritin and transfer macrophage receptors and thus, promote iron storage in the mononuclear phagocytic system. The shifting of iron to storage plus the use of iron by bacteria makes iron less available as erythroid precursors thus, leading to anaemia [37].

Neutrophilic leukocytes are critical component of the host system, forming the first line of cellular defense against invading organisms. Neutrophils normally are released from bone marrow as mature cells, which after a brief period in circulation transmigrate through the vascular endothelium into tissues. Their primary function is ingestion

and killing of bacteria [35,36]. To perform its functions, neutrophils employ mediators that promote inflammation and eliminate invading microorganisms. Mounting evidences indicate that neutrophils are not only end-stage effectors of the inflammatory response, but also modulate the immune response [37]. Increased peripheral blood neutrophil counts or neutrophilia reflects physiological, pathological or xenobiotic induced states [15,24]. Total blood and circulating neutrophil pools are increased in the present study in the treatment groups inoculated with graded doses of *Pasteurella multocida* type B: 2 and its LPS. Neutrophilia with a left shift is the classic response to inflammation, accompanied by lymphopenia, reflecting endogenous steroids release. The decrease in monocytes in the present study may reflect a response to endogenous mediators of inflammation [15,24,35,36]. In the present study, inoculation of whole cells and its LPS resulted in significant increase in neutrophil counts, which is consistent with observations by other workers [35-37]. The increased neutrophils count in the present study denotes HS [15,24]. Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) are coagulation profiles. The protracted time period or the increase in PT and APTT in the current study was perchance associated to the degree of infections produce by the graded doses of *Pasteurella multocida* and its LPS via the oral routes. This findings agrees with a study conducted on dengue fever which also produced a protracted PT and APTT [38,39]. The increase in eosinophils and Plasma proteins in the present study could be related with the infections induced by inoculation of graded doses of *Pasteurella multocida* and its LPS. This is similar to the study conducted by Faez et al. [15] and Lin et al. [24] on mice and calves infected with 10^9 cfu of *Pasteurella multocida* and its LPS via the intraperitoneal routes. Furthermore, the increase in eosinophils in the present study could perchance be associated with the congestion observed in the treatment groups. Jesse et al. [5,8] reported a similar finding in calves and mice inoculated with *Pasteurella multocida* type B: 2 and its LPS in which thrombosis was mild in all the groups [40].

Conclusion

In conclusion, this result showed the possible effect of 10^9 cfu of *Pasteurella multocida* and its LPS in causing devastating effects on vital organs and blood tissues of susceptible animals compared to the treatment groups.

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