REPRODUCTIVE PATHOPHYSIOLOGY OF BUCKS POST-CHALLENGED WITH MANNHEIMIA HAEMOLYTICA A2 AND ITS IMMUNOGENS LIPOPOLYSACCHARIDE AND OUTER MEMBRANE PROTEIN

By

NUR AMIRA BINTI AZHAR

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REPRODUCTIVE PATHOPHYSIOLOGY OF BUCKS POST-CHALLENGED WITH *MANNHEIMIA HAEMOLYTICA* SEROTYPE A2 AND ITS LIPOPOLYSACCHARIDE AND OUTER MEMBRANE PROTEIN

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Nur Amira Binti Azhar

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Chairman : Jesse Faez Firdaus Bin Abdullah, PhD
Faculty : Institute of Tropical Agriculture and Food Security

Pneumonic mannheimiosis is a bacterial respiratory disease caused by *Mannheimia haemolytica* serotype A2 in sheep and goats. This disease had led to significant economic losses to the small ruminant industries worldwide including Malaysia, mainly due to its high mortality and morbidity rate. The *M. haemolytica* A2 is a Gram-negative, weakly haemolytic bacterium residing in the upper respiratory tract of healthy animals as commensals. It may eventually gain access to the lower respiratory tract when the immune system is compromised and cause further damage to the lung tissues. The pathogenicity of *M. haemolytica* A2 is triggered by its numerous virulence factors which include the lipopolysaccharide (LPS) and outer membrane protein (OMP), which in turn may trigger host systemic inflammatory responses. While its role in causing a respiratory infection is evident, its potential impact on the reproductive system remains uncovered. Hence, this study was conducted to investigate the potential impact of the infection from *M. haemolytica* A2 and its immunogens LPS and OMP on the reproductive functions of infected bucks.

A total of 16 clinically healthy, crossbred bucks aged 1.5-2 years old were used in this study. The bucks were randomly assigned into four groups of four bucks each. Bucks in Group 1 (control group) were intranasally inoculated with 2 mL of PBS pH 7.0, Group 2 with 2 mL of 1.2 X 10^9 CFU/mL *M. haemolytica* A2 intranasally, Group 3 with 2 mL of LPS endotoxin extracted from 1.2 X 10^9 CFU/mL *M. haemolytica* A2 intravenously and Group 4 with 2 mL of OMP extracted from 1.2 X 10^9 CFU/mL *M. haemolytica* A2 intramuscularly. Bucks were closely monitored for clinical responses post-challenge throughout the study, which include changes in temperature, heart rate, respiratory rate, rumen motility, lung sound, and presence of nasal discharge. Blood samples were obtained via jugular venipuncture into 5 mL plain tubes and the sera harvested at pre-determined intervals were used to determine the pro-inflammatory cytokines (IL1β, IL6, TNFα, and IFNγ), heat shock proteins

(HSP70, HSP90), toll-like receptors (TLR2, TLR4) and reproductive hormones (testosterone, LH) responses. Semen quality was also examined to determine the effect of treatments on bucks by evaluating the changes in sperm motility, viability, wave pattern, and abnormal morphology. Ejaculates were collected by using an electro-ejaculator and were analyzed promptly after collection. The experimental bucks were euthanized via exsanguination on Day 60 post-challenge and the organs were examined grossly at necropsy. Tissue samples from the vital organs (lung, liver), reproductive organs (testis, epididymis, vas deferens, prostate), and lymph nodes (bronchial, iliac) were also obtained and processed by using a common H&E protocol to be analyzed and scored under light microscopy.

All experimental bucks from Groups 1, 2, 3, and 4 survived until the end of the study. This study revealed that the administration of *M. haemolytica* A2 and its immunogens, LPS and OMP may induce clinical responses similar to natural infection of pneumonic mannheimiosis such as fever, increased heart rate and respiratory rate, rumen hypomotility and nasal discharge in experimental bucks. Serum analyses then showed a significant increase in serum IL1β, IL6, TNFα and IFNγ concentrations in all treatment groups, particularly in Group 3 bucks by 1h and were prominent within the first 12 hours post-challenge, indicating the activation of host systemic inflammatory responses. HSP70 and HSP90 levels were also increased post-challenge in Groups 2, 3 and 4 bucks with the highest fold increase recorded was a 2.5-fold increase in HSP90 level in Group 3. The serum levels of pro-inflammatory cytokines and heat shock proteins gradually decreased approaching Day 56 of this study. These increases were also accompanied by increased concentrations of TLR2 and TLR4, a vital receptor for OMP and LPS, respectively, as early as 1h post-challenge. The serum TLR2 in Group 4 and the serum TLR4 in Groups 2 and 3 remained higher than in Group 1 until the end of the study, signifying the activation of inflammatory responses by these immunogens that result in the release of pro-inflammatory cytokines.

Moreover, of both reproductive hormones examined in this study, only the testosterone hormone showed an evident decrease in serum concentrations, mainly in Groups 3 and 4 and a slight reduction in Group 2 as compared to Group 1 which lasted until day 56. In contrast, the only significant changes in serum concentration of LH were recorded at 1h and 12h in Groups 2 and 3, respectively, indicating that the administration of *M. haemolytica* A2 and its LPS and OMP in experimental bucks may influence the concentration of reproductive hormones to a varying degree. Likewise, a significant reduction in sperm motility, percentage viability, wave pattern and increased morphological abnormalities were observed as early as 1d post-challenge and were evident across groups, particularly in Group 3 within the first 35 days before it normalizes with Group 1 approaching Day 56 of the study. While the reproductive organs and lymph nodes appeared normal grossly at necropsy, the lung and liver of bucks from Groups 2, 3 and 4 displayed mild gross lesions. Histopathological analysis revealed changes in the reproductive organ which highlight the potential effect of *M. haemolytica* A2 on the reproductive functions of infected bucks. The lesion scoring of the reproductive organs, vital organs and lymph nodes ranged from mild to moderate lesions across the groups, mostly for necrosis and degeneration. These changes persisted until the end of a study, suggesting that it may require more than one spermatogenic cycle for infected bucks infected to regain optimal reproductive functions post-infection.

Therefore, the results obtained in this study indicate that besides affecting the respiratory system of infected animals as previously reported, the experimental administration of *M. haemolytica* A2 and its LPS and OMP induced inflammatory responses and may affect the reproductive functions of bucks by altering the reproductive hormone levels and sperm parameters. This would be the first study reporting the potential effect of *M. haemolytica* bacterial respiratory disease on the reproductive function of bucks, assisted by its immunogens LPS and OMP.

Keywords: Goat; Pneumonic mannheimiosis; Goat reproduction; Semen characteristics; Reproductive hormones; Histopathology; LPS endotoxin; OMP

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PATOFISIOLOGI REPRODUKTIF KAMBING JANTAN PASCA CABARAN DENGAN *Mannheimia haemolytica* A2 BERSERTA LIPOPOLISAKARIDA DAN PROTEIN MEMBRAN LUAR

Oleh

NUR AMIRA BINTI AZHAR

Oktober 2022

Pengerusi : Jesse Faez Firdaus Bin Abdullah, PhD
Fakulti : Institut Pertanian Tropika dan Sekuriti Makanan

Mannheimiosis pneumonik merupakan penyakit jangkitan pernafasan yang disebabkan oleh bakteria *Mannheimia haemolytica* serotaip A2 dalam biri-biri dan kambing. Penyakit ini telah menyebabkan kerugian ekonomi yang besar kepada industri ruminan kecil di seluruh dunia, termasuk Malaysia, disebabkan oleh kadar kematian dan morbiditi yang tinggi. *M. haemolytica* A2 adalah bakteria Gram-negatif, lemah hemolitik, yang tinggal di saluran pernafasan atas haiwan yang sihat sebagai komensal. Bakteria ini boleh mendapat akses ke dalam salur pernafasan bawah apabila sistem imun haiwan terganggu dan menyebabkan kerosakan selanjutnya pada tisu paru-paru. Sifat patogenik *M. haemolytica* A2 dicetuskan oleh faktor-faktor virulennya termasuk endotoksin lipopolisakarida (LPS) dan protein membran luar (OMP), yang seterusnya boleh mencetuskan tindak balas keradangan sistemik oleh haiwan yang dijankiti. Walaupun peranannya dalam menyebabkan jangkitan pernafasan adalah jelas, kesannya terhadap sistem pembiakan masih belum diketahui. Oleh itu, kajian ini dijalankan untuk menyiasat potensi kesan jangkitan dari *M. haemolytica* A2 dan imunogennya LPS dan OMP terhadap fungsi pembiakan kambing jantan yang dijangkiti pneumonia.

Sebanyak 16 kambing jantan dewasa kacukan yang sihat secara klinikal berusia 1.5-2 tahun digunakan dalam kajian ini. Semua kambing jantan dibahagi secara rawak kepada empat kumpulan dengan empat ekor haiwan dalam setiap kumpulan. Kambing jantan dalam Kumpulan 1 (kumpulan kawalan) telah disuntik secara intranasal dengan 2 mL PBS pH 7.0, Kumpulan 2 dengan 2 mL 1.2 X 10^9 CFU/mL *M. haemolytica* A2 secara intranasal, Kumpulan 3 dengan 2 mL endotoksin ekstrak LPS dari 1.2 X 10^9 CFU/mL *M. haemolytica* A2 secara intravena dan Kumpulan 4 dengan 2 mL ekstrak OMP dari 1.2 X 10^9 CFU/mL *M. haemolytica* A2 secara intramuskular. Kesemua kambing jantan dipantau dengan teliti untuk sebarang tindak balas klinikal selepas cabaran sepanjang kajian, yang merangkumi perubahan suhu, kadar denyutan jantung, kadar pernafasan, pergerakan rumen, bunyi paru-paru, dan discaj hidung. Sampel darah diperolehi melalui vena jugular ke dalam tiub 5 mL untuk menentukan perubahan dalam sitokin pro-radang (IL1β, IL6, TNFα, dan

IFNγ), protein kejutan haba (HSP70, HSP90), reseptor-seperti tol (TLR2, TLR4) dan hormon pembiakan (testosteron, LH) secara berkala mengikut masa yang telah ditentukan. Kualiti air mani juga diperiksa untuk menentukan kesan rawatan pada kambing jantan dengan menilai motiliti sperma, daya maju sperma, corak gelombang, dan morfologi yang tidak normal sepanjang kajian. Sampel ejakulasi dikumpulkan dengan menggunakan elektro-ejakulasi dan dianalisis dengan segera selepas proses pengumpulan. Semua kambing jantan dimatikan melalui penyembelihan pada hari ke-60 selepas cabaran untuk post-mortem dan organ-organ telah diperiksa secara kasar pada nekropsi. Sampel tisu dari organ-organ penting, organ pembiakan, dan nodus limfa juga diperolehi dan diproses dengan menggunakan protokol H&E biasa untuk dianalisis dan diberi skor secara mikroskopik.

Semua kambing jantan dari Kumpulan 1, 2, 3, dan 4 terselamat sehingga akhir kajian. Kajian ini mendedahkan bahawa cabaran *M. haemolytica* A2 dan imunogennya, LPS dan OMP boleh menyebabkan tindak balas klinikal yang serupa dengan jangkitan semula jadi mannheimiosis pneumonik seperti demam, peningkatan kadar denyutan jantung dan kadar pernafasan, hipomotiliti rumen dan discaj hidung pada kambing jantan. Analisis serum kemudian menunjukkan peningkatan ketara dalam kepekatan serum IL1β, IL6, TNFα dan IFNγ dalam semua kumpulan rawatan, terutamanya dalam Kumpulan 3 seawal jam pertama dan menonjol dalam tempoh 12 jam pertama selepas cabaran, yang menunjukkan pengaktifan tindak balas keradangan sistemik. Tahap HSP70 dan HSP90 juga meningkat selepas cabaran dalam Kumpulan 2, 3 dan 4 dengan peningkatan lipatan tertinggi yang dicatatkan ialah sebanyak 2.5 kali ganda dalam tahap HSP90 dalam Kumpulan 3. Tahap serum sitokin pro-radang dan protein kejutan haba secara beransur-ansur menurun menghampiri hari ke-56 kajian. Peningkatan ini juga disertai oleh peningkatan kepekatan TLR2 dan TLR4, reseptor penting untuk OMP dan LPS, seawal 1 jam selepas cabaran. Serum TLR2 dalam Kumpulan 4 dan serum TLR4 dalam Kumpulan 2 dan 3 kekal lebih tinggi daripada Kumpulan 1 sehingga akhir kajian. Peningkatan serum TLR menunjukkan pengaktifan tindak balas keradangan oleh imunogen ini yang mengakibatkan pembebasan sitokin pro-radang.

Selain itu, daripada kedua-dua hormon pembiakan yang diperiksa dalam kajian ini, hanya hormon testosteron menunjukkan penurunan yang ketara dalam kepekatan serum, terutamanya dalam Kumpulan 3 dan 4 dan sedikit pengurangan dalam Kumpulan 2 yang berlarutan sehingga hari ke-56. Sebaliknya, perubahan dalam kepekatan serum LH tidak ketara pada kebanyakan titik masa di semua kumpulan dengan perubahan ketara hanya direkodkan pada 1 jam dan 12 jam, masing -masing dalam Kumpulan 2 dan 3, yang menunjukkan bahawa cabaran *M. haemolytica* A2 dan LPS dan OMP dalam kambing jantan boleh mempengaruhi kepekatan hormon pembiakan ke tahap yang berbeza-beza. Perubahan dalam parameter air mani juga diperhatikan selepas cabaran yang mencerminkan kesan potensi rawatan terhadap kualiti air mani yang dihasilkan. Perubahan signifikan dalam motiliti sperma, peratusan daya maju sperma, keabnormalan morfologi dan corak gelombang diperhatikan seawal sehari selepas cabaran dan dapat diperhatikan dalam seluruh kumpulan, terutamanya dalam Kumpulan 3 dalam tempoh 35 hari pertama kajian ini sebelum ia kembali normal dengan Kumpulan 1 menghampiri hari ke-56 kajian.

Walaupun organ pembiakan dan nodus limfa kelihatan normal secara kasar, paru-paru dan hati bucks dari Kumpulan 2, 3 dan 4 menunjukkan lesi kasar ringan pada nekropsi. Perubahan histopatologi dalam organ pembiakan juga menyerlahkan kesan

M. haemolytica A2 terhadap fungsi pembiakan kambing jantan yang dijangkiti. Skor lesi organ pembiakan, organ penting dan nodus limfa berkisar dari lesi ringan hingga sederhana dalam semua kumpulan, kebanyakannya dicirikan sebagai nekrosis dan degenerasi. Perubahan ini berterusan sehingga akhir kajian, menunjukkan bahawa ia mungkin memerlukan lebih daripada satu kitaran spermatogenik untuk orang yang dijangkiti untuk mendapatkan semula fungsi pembiakan optimum selepas jangkitan.

Oleh itu, keputusan yang diperolehi dalam kajian ini menunjukkan bahawa selain menjejaskan sistem pernafasan haiwan yang dijangkiti seperti yang dilaporkan sebelum ini, cabaran *M. haemolytica* A2 dan LPS dan OMP boleh menjejaskan fungsi pembiakan kambing jantan dengan mengubah kepekatan hormon pembiakan dan parameter sperma dalam air mani. Ini adalah kajian pertama yang melaporkan kesan penyakit pernafasan bakteria *M. haemolytica* terhadap fungsi pembiakan kambing jantan yang dibantu oleh imunogen LPS dan OMP.

Kata kunci: Kambing; Mannheimiosis penumonik; Pembiakan kambing; Kualiti air mani; Hormon reproduktif, Histopatologi; Endotoksin LPS; OMP

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APPROVAL

I certify that a Thesis Examination Committee has met on (date of viva voce) to conduct the final examination of Nur Amira Binti Azhar on her thesis entitled "Reproductive Pathophysiology of Bucks Post-Challenged with *Mannheimia Haemolytica* A2 and Its Immunogens Lipopolysaccharide and Outer Membrane Protein" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the degree of Doctor of Philosophy.

Members of the Thesis Examination Committee were as follows:

Name of Chairperson, PhD
Title (e.g., Professor/Associate Professor/Ir; omit if irrelevant)
Name of Faculty
Universiti Putra Malaysia
(Chairman)

Name of Examiner 1, PhD
Title (e.g., Professor/Associate Professor/Ir; omit if irrelevant)
Name of Faculty
Universiti Putra Malaysia
(Internal Examiner)

Name of Examiner 2, PhD
Title (e.g., Professor/Associate Professor/Ir; omit if irrelevant)
Name of Faculty
Universiti Putra Malaysia
(Internal Examiner)

Name of External Examiner, PhD
Title (e.g., Professor/Associate Professor/Ir; omit if irrelevant)
Name of Department and/or Faculty
Name of Organisation (University/Institute)
Country
(External Examiner)

ZURIATI BINTI AHMAD ZULKARNAIN, PhD
Professor and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of the Supervisory Committee were as follows:

Faez Firdaus Jesse Abdullah, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Mohd Azmi Mohd Lila, PhD
Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Eric Lim Teik Chung, PhD
Senior Lecturer
Faculty of Agriculture
Universiti Putra Malaysia
(Member)

Annas Salleh, PhD
Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Wan Nor Fitri Wan Jaafar, PhD
Senior Lecturer
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

Declaration by Members of Supervisory Committee

This is to confirm that:

- the research conducted and the writing of this thesis was under our supervision;
- supervision responsibilities as stated in the Universiti Putra Malaysia (Graduate Studies) Rules 2003 (Revision 2012-2013) are adhered to.

Signature: ______________________________
Name of Chairman of Supervisory Committee: Professor Dr. Faez Firdaus Jesse Abdullah

Signature: ______________________________
Name of Member of Supervisory Committee: Professor Dato' Dr. Mohd Azmi Mohd Lila

Signature: ______________________________
Name of Member of Supervisory Committee: Dr Eric Lim Teik Chung

Signature: ______________________________
Name of Member of Supervisory Committee: Associate Professor Dr. Annas Salleh

Signature: ______________________________
Name of Member of Supervisory Committee: Dr. Wan Nor Fitri Wan Jaafar

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°C	degree celcius
µg	microgram
µL	microliter
ADV	adenovirus
ANOVA	analysis of variance
APP	acute phase protein
APV	alum-precipitated vaccine
bpm	beats per minute
BRD	bovine respiratory disease
cAMP	cyclic adenosine monophosphate
CD	cluster of differentiation
CFU	colony-forming unit
COPD	chronic obstructive pulmonary disease
DC	dendritic cell
DNA	deoxyribonucleic acid
ELISA	enzyme-linked immunoassay
FSH	follicle-stimulating hormone
g	gram
GnRH	gonadotropin-releasing hormone
HCl	hydrochloric acid
HDL	high-density lipoprotein
Hp	haptoglobin
HPA	hypothalamic-pituitary-adrenocortical
HPG	hypothalamic-pituitary-gonadal

HRP	horse radish peroxidase
HSP	heat shock protein
IFN	interferon
IL	interleukin
kDa	kilodalton
kg	kilogram
LBP	LPS binding protein
LH	luteinizing hormone
LKT	leukotoxin
LPS	lipopolysaccharide
MD	myeloid differentiation
mL	millilitre
MLP	murine lipoprotein
mM	millimolar
mRNA	messenger ribonucleic acid
MyD88	myeloid differentiation factor 88
NFkβ	nuclear factor kappa B
ng	nanogram
NK	nature killer
OAV	oil-adjuvanted vaccine
OD	optical density
OMP	outer membrane protein
P-450scc	cholesterol side-chain cleavage enzyme
PAL	peptidoglycan-associated lipoprotein
PAMP	pathogen-associated molecular protein

PCR	polymerase chain reaction
PGE_2	prostaglandin
pH	potential hydrogen
PRR	pathogen recognition receptor
rcf	relative centrifugal force
RNA	ribonucleic acid
ROS	reactive oxygen species
rpm	revolution per minute
RSV	respiratory syncytial virus
SAA	serum amyloid A
SD	standard deviation
SDS-PAGE	sodium dodecyl sulfate-polyacrylamide gel electrophoresis
SSL	self-sufficiency level
StAR	steroidogenic acute regulatory
TLR	toll-like receptor
TNF	tumor necrosis factor
xg	times gravity

CHAPTER 1

INTRODUCTION

1.1 Background of Study

The goats (Capra hircus) are among the earliest livestock domesticated by humans, dating to about 10,000 years ago (Zeder & Hesse, 2000). Since then, they have been known to serve an important role in the agricultural sector among small and large-scale farmers across continents (Zheng et al., 2020). Goats are regarded as versatile animals for their fairly low maintenance despite their high production yield for meat, milk, and leather (Pophiwa et al., 2020; Chakraborty et al., 2014). While being known as animals of high adaptability, goats remain susceptible to a number of diseases caused by infectious agents such as viruses, bacteria, or a combination of both.

One of the primary diseases infecting goats worldwide is pneumonia, a respiratory disease known to cause infection and inflammatory responses in one or both lungs among goats of all ages (Rico et al., 2017). This disease has been classified as an economically important disease as it may contribute to significant losses attributable to its high morbidity and mortality rate (Griffin et al., 2010). It negatively affects farm productivity, thus hindering the development of the small ruminant industry regardless of the increase in demand for livestock products (Purba et al., 2020; Shahudin et al., 2018). Hence, any losses in productivity due to diseases are undesirable.

Pneumonia in goats is predominantly caused by the Gram-negative bacteria, *Mannheimia haemolytica* serotype A2 (Sabri et al., 2013; Rice et al., 2008; Ali et al., 1992). The *M. haemolytica* serotype A2 is a non-motile, weakly haemolytic, facultatively anaerobic, and coccobacillus bacterium (Oppermann et al., 2017; Abdelsalam, 2008). It belongs to the Pasteurellaceae family along with *Pasteurella multocida* which causes haemorrhagic septicaemia in buffalos (Abdullah et al., 2017; Brogden et al., 1998). This opportunistic pathogen is a commensal in the upper respiratory tract of healthy animals. It would gain access to the lower respiratory tract when the defence system is compromised upon exposure to either one or a combination of predisposing factors which include viral infection, poor husbandry practices, overcrowding and transportation stress (Singh et al., 2011).

The transformation of *M. haemolytica* into a pathogenic entity is made possible by its numerous virulence factors, such as the lipopolysaccharide (LPS), leukotoxin (LKT), outer membrane protein (OMP), adhesins, capsule, neuraminidase and proteases (Singh et al. 2011). These virulence factors are classified as pathogen-associated molecular patterns (PAMPs) due to their ability to trigger the innate immune response in an infected host (Vahanan et al., 2008). Above all, the LPS endotoxin is denoted as the most important virulence factor of *M. haemolytica* (Davies & Lee, 2004). The LPS is localized at the outer membrane of most, if not all Gram-negative bacteria, along with the OMP (McClenahan et al., 2008). While the LPS is predominantly found on the outer leaflet of the outer membrane, the OMP is embedded in the outer membrane with its surface exposed to allow for bacterial adhesion on mucosal surfaces (Confer & Ayalew, 2013). The localization of the β-barrel OMP may suggest that it is essential for the survival and virulence of Gram-negative bacteria during bacterial pathogenesis (Piek & Kahler, 2012). These PAMPs, the LPS and OMP, would trigger inflammatory responses of the host when released during growth and rapid cell death (Wassef et al., 2004).

The LPS endotoxin is capable of imposing direct pulmonary damage by increasing the permeability of the endothelial cells, thus causing vascular leakage. It may also cause indirect damage by promoting the abundant release of pro-inflammatory cytokines, followed by neutrophil infiltration at the site of inflammation (Tadros & Frank, 2012; Lafleur et al., 2001). Studies have shown that LPS-induced inflammation triggers the upregulation of various cytokine production such as the tumour necrosis factor-alpha (TNFα), interleukin 1-beta (IL1β), interleukin-6 (IL6), and other chemotactic mediators both locally and systemically (Abdullah et al., 2017; Turner et al., 2014). Similar pro-inflammatory responses were also observed following the administration of OMP in buffalos suggesting the potential effects of OMP-induced inflammation (Chung et al., 2016).

Subsequently, increased production of pro-inflammatory cytokines due to LPS-induced inflammation could impair the biosynthesis of reproductive hormones such as the testosterone hormone by the Leydig cells of the testes and the Luteinizing hormone by the anterior pituitary gland (Faeza et al., 2019; de Oliveira Souza et al., 2011; Allen et al., 2004). Since the testosterone hormone is crucial for spermatogenesis and the development of accessory sex glands in males, changes in the concentration of this hormone would negatively affect both processes. This suggests that the inflammatory responses induced by *M. haemolytica* A2 and its immunogens LPS and OMP may alter the semen quality and later cause cellular changes in the reproductive system of affected bucks, which have not been addressed in previous studies.

The effects of *M. haemolytica* A2 and its LPS and OMP on the respiratory system have been well-established in numerous studies. However, the potential effects of pneumonic mannheimiosis on the reproductive pathophysiology remain unclear. It is noteworthy that inflammations and infections could impair the process of steroidogenesis and spermatogenesis in the testes which shed light on the possible effect of bacterial infection caused by *M. haemolytica* A2 and its immunogens LPS and OMP on the reproductive system and its impact on the reproductive functions of infected bucks. Therefore, this study was conducted to determine the pathophysiological responses, particularly in the reproductive system of bucks due to experimental infection of *Mannheimia haemolytica* A2 and its immunogens LPS and OMP. This study would fill in the knowledge gap regarding the adverse effects of bacterial respiratory diseases on the reproductive functions of bucks.

1.2 Problem Statement

Extensive studies on *M. haemolytica* A2 had been conducted in the past years in order to gain more insights into this disease-causing pathogen. Most studies have been focusing on its effects on the respiratory system, but its potential impact on the reproductive system remains unclear. Therefore, the effect of *M. haemolytica* A2 and its immunogens LPS and OMP on the pathophysiology of the reproductive system in small ruminants should be investigated to further understand this economically important disease and to fill the existing gap of knowledge.

1.3 Hypotheses

The hypotheses made for this study are as follow.

1. Experimental bucks would exhibit clinical responses associated with pneumonic mannheimiosis post-challenged with *M. haemolytica* A2 and its immunogens LPS and OMP.

2. Serum analyses would reveal alterations in pro-inflammatory cytokines (IL1β, IL6, TNFα, IFNγ) and heat shock proteins (HSP70 and HSP90) levels in experimental bucks post-challenged with *M. haemolytica* A2 and its immunogens LPS and OMP.
3. Alterations in reproductive hormones (testosterone and LH) levels and semen quality parameters of experimental bucks will be observed post-challenged with *M. haemolytica* A2 and its immunogens LPS and OMP.
4. Changes in serum concentration of TLR2 and TLR4 will be observed in experimental bucks challenged with *M. haemolytica* A2 and its immunogens LPS and OMP.
5. Histopathological changes in the respiratory organs, reproductive organs, and associated lymph nodes will be observed in bucks following *M. haemolytica* A2 and its immunogens LPS and OMP administration.

1.4 Objectives

1. To determine the clinical responses of experimental bucks challenged with *M. haemolytica* A2 and its immunogens LPS and OMP throughout the experimental period.
2. To evaluate the alterations of serum pro-inflammatory cytokines (IL1β, IL6, TNFα, IFNγ) and heat shock proteins (HSP70 and HSP90) in bucks post-challenged with *M. haemolytica* A2 and its LPS and OMP extracts.
3. To analyze any changes in reproductive hormone levels and semen quality parameters of experimental bucks post-challenged with M. haemolytica A2 and its immunogens LPS and OMP.

4. To study the changes in serum TLR2 and TLR4 protein levels in experimental bucks post-challenged with *M. haemolytica* A2 and its immunogens LPS and OMP in relation to inflammatory responses.
5. To examine the histopathological changes of the respiratory organs, reproductive organs, and its associated lymph nodes in experimental bucks following *M. haemolytica* A2 and its LPS and OMP extra1cts administration.

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