



UNIVERSITI PUTRA MALAYSIA

***DETERMINATION OF SEPTICAEMIA CAUSATIVE AGENTS
IN RUMINANTS***

BATRISYIA SYAZANA BINTI FARIDON

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**DETERMINATION OF SEPTICAEMIA CAUSATIVE AGENTS
IN RUMINANTS**

By

BATRISYIA SYAZANA BINTI FARIDON

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
Fulfilment of the Requirements for the Degree of Master of Science**

August 2021

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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Chair : Associate Prof. Annas Salleh, DVM, PhD
Faculty : Veterinary Medicine

Domestic ruminant of economic importance includes buffalo, cattle, goats, sheep, and deer. These animals are known for their roles in conversion of fibrous feed into animal protein for human consumption. Over the years, despite remarkable enhancement has been made in regards to ruminant production, the diseases incidence also goes along in increasing manner. Recent studies of common health problems in ruminants in Malaysia showed that septicaemia as the leading threat to ruminant's sustainability as septicaemia covered 63.3% health problems involving circulatory system. The study also depicted *Escherichia coli*, *Pasteurella* sp., and *Klebsiella pneumoniae* as the most important bacteria that contributes to septicaemia or bacterial pneumonia in ruminants. Despite this, no study was conducted to understand common pathogens and risk factors of septicaemia in veterinary medicine. Septicaemia is the presence of pathogen and its toxin in the bloodstream of a host, potentially leading to acute death of the host. It could be caused by wide range of pathogens, particularly bacteria with numerous potential points of entry and risk factors. The objectives of this study were to (1) identify and analyze the common microorganisms, points of entry, and risk factors associated with septicaemia in ruminants, (2) isolate, identify, and perform phylogenetic analysis of common bacteria causing septicaemia in ruminants, and (3) analyze the antibiotic susceptibility profiles of these bacteria.

This study involved two parts; a retrospective study and a prospective study. In the retrospective study, data of post-mortem cases of small ruminants (sheep, goat, and deer) and large ruminants (cattle, buffalo, and gaur) diagnosed with septicaemia at the Post-mortem Laboratory, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM) between the years of 2007 and 2017 were used for analysis. It was later observed that septicaemia was more important in the small ruminants therefore the prospective study was conducted only in the small ruminants. A total of 20 carcasses of small ruminants with septicaemia submitted to the Post-mortem Laboratory, UPM between the year 2018 and 2019 were diagnosed with septicaemia based on isolation of similar microorganism in more than three internal organs (lung, liver, heart, or kidney).

For the retrospective study, it was observed that the three most commonly isolated microorganisms in cases of septicaemia in small ruminants are *E. coli* (66.2%), *K. pneumoniae* (23.2%) and *Pasteurella* sp. (10.6%). On the other hand, *E. coli* (50.9%), *K. pneumoniae* (40%), *Staphylococcus aureus* (9.1%) are the most commonly isolated septicaemia-causing bacteria in large ruminants. For both small and large ruminants, gastrointestinal tract has been found to be the most common point of entry for septicaemia, which was statistically significant ($p < 0.05$) compared to respiratory tract, reproductive tract, integumentary, and other body systems. Underlying infection in small and large ruminant is the significant ($p < 0.05$) risk factor for septicaemia.

In the prospective study, *E. coli* was found to be the most common microorganism associated with septicaemia with a total of 24 isolates (29.6 %), seven isolates of *S. aureus* (8.6 %), and six isolates of *K. pneumoniae* (7.4 %) compared to all other 26 different microorganisms with a total of 81 isolates. *Escherichia coli* was confirmed as the most important bacteria species in causing septicaemia in ruminants. Antibiotic susceptibility tests showed that most isolates of *E. coli* were resistant towards Tetracycline (30 µg) involving 17 isolates. This is followed by resistance towards Ampicillin (10 µg) and Amoxicillin (10 µg) involving 16 *E. coli* isolates, and Streptomycin (10 µg) with nine isolates. *Escherichia coli* is the most common bacterial isolated from cases of septicaemia especially those originated from the gastrointestinal tract and the respiratory tract. The antibiotic sensitivity test showed high percentage of resistance that requires strategies to prevent emerging of multiple-drug resistance microorganism in the near future.

Keywords: Septicaemia, ruminants, point of entry, risk factors, microorganism, antibiotic sensitivity

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENENTUAN AGEN PENYEBAB SEPTISEMIA DALAM RUMINAN

Oleh

BATRISYIA SYAZANA BINTI FARIDON

Ogos 2021

Pengerusi : Prof. Madya Annas Salleh, DVM, Ph.D
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Ruminan domestik yang berkepentingan ekonomi termasuk kerbau, lembu, kambing, biri-biri, dan rusa. Haiwan-haiwan ini dikenali untuk peranan mereka dalam menukarkan makanan berserat kepada protein haiwan untuk kegunaan manusia. Selama bertahun-tahun, walaupun perkembangan luar biasa telah berlaku berkaitan dengan produksi ruminan, insiden penyakit juga turut meningkat. Kajian terkini mengenai masalah kesihatan biasa dalam ruminan di Malaysia menunjukkan bahawa septisemia sebagai ancaman utama kepada kemampuan ruminan kerana septisemia meliputi 63.3% masalah kesihatan yang melibatkan sistem peredaran darah. Kajian itu juga menggambarkan *E. coli*, *Pasteurella* sp., dan *K. pneumoniae* sebagai bakteria terpenting yang menyumbang kepada septisemia atau pneumonia bakteria dalam ruminan. Walaupun begitu, tiada kajian yang dijalankan untuk memahami patogen lazim dan faktor risiko septisemia dalam perubatan veterinar. Septisemia ialah kehadiran patogen dan toksinnya dalam aliran darah perumah, yang berpotensi membawa kepada kematian akut perumah. Ia boleh disebabkan oleh pelbagai jenis patogen, terutamanya bakteria dengan banyak potensi tempat masuk dan faktor risiko. Objektif kajian ini adalah untuk (1) mengenal pasti dan menganalisis mikroorganismabiasa, tempat masuk, dan faktor risiko yang berkaitan dengan septisemia dalam ruminan, (2) mengasingkan, mengenal pasti, dan melakukan analisis filogenetik bakteria biasa yang menyebabkan septisemia dalam ruminan, dan (3) menganalisis profil kerentanan antibiotik bakteria ini.

Kajian ini melibatkan dua bahagian; suatu kajian retrospektif dan satu kajian prospektif. Dalam kajian retrospektif, data kes post-mortem ruminan kecil (biri-biri, kambing, dan rusa) dan ruminan besar (lembu, kerbau, dan seladang) yang telah didiagnosis dengan septisemia di Makmal Post-mortem, Fakulti Perubatan Veterinar, Universiti Putra Malaysia (UPM) di antara tahun 2007 dan 2017 telah digunakan untuk tujuan analisa. Oleh sebab kemudiannya telah diperhatikan bahawa septisemia lebih penting dalam ruminan kecil, jadi kajian secara prospektif cuma dilakukan dalam ruminan kecil sahaja. Keseluruhannya, sebanyak 20 karkas ruminan kecil dengan ruminan telah dihantar ke Makmal Post-mortem, UPM, di antara tahun 2018 dan 2019 telah digunakan untuk

mengenalpasti mikroorganisma lazim yang berkait dengan septisemia. Karkas-karkas ini telah didiagnosis dengan septisemia berdasarkan pengasingan mikroorganisma yang sama dalam lebih dari tiga organ dalaman (paru-paru, hati, jantung, dan ginjal).

Untuk kajian retrospektif, ia telah diperhatikan bahawa tiga bakteria yang paling lazim diasingkan dalam kes-kes septisemia dalam ruminan kecil ialah *E. coli* (66.2%), *Klebsiella pneumoniae* (23.2%), dan *Pasteurella* sp. (10.6%). Di sebaliknya, *E. coli* (50.9%), *K. pneumoniae* (40%), *Staphylococcus aureus* (9.1%) merupakan bakteria penyebab septisemia yang paling lazim diasingkan dalam ruminan besar. Untuk kedua-dua ruminan besar dan kecil, saluran gastrousus telah dikenalpasti sebagai titik kemasukan septisemia yang lazim ($p < 0.05$) berbanding saluran pernafasan, saluran reproduktif, integument, dan lain-lain. Jangkitan tersirat dalam ruminan kecil dan besar merupakan faktor risiko untuk septisemia yang ketara ($p < 0.05$).

Dalam kajian proseptif, *E. coli* telah dilihat sebagai mikroorganisma paling lazim yang berhubungkait dengan septisemia dengan sebanyak 24 isolat (29.6%), tujuh isolat *S. aureus* (8.6%), dan enam isolat *K. pneumoniae* (7.4%) berbanding dengan kesemua 26 mikroorganisma lain dengan keseluruhan 81 isolat. *Escherichia coli* telah dikenalpasti sebagai spesis bakteria paling penting dalam menyebabkan septisemia dalam ruminan. Ujian kerentanan antibiotik menunjukkan bahawa kebanyakan isolate *E. coli* rintang Tetracycline (30 μ g) melibatkan 17 isolat. Ini diikuti dengan kerintangan terhadap Amoxycillin (10 μ g) yang melibatkan 16 isolat *E. coli*, dan Streptomycin (10 μ g) dengan sembilan isolat. *Escherichia coli* ialah bakteria yang paling lazimnya diasingkan dari kes-kes septisemia terutamanya yang berasal dari saluran gastrousus dan saluran pernafasan. Ujian kerentanan antibiotik menunjukkan peratusan kerintangan yang tinggi yang memerlukan strategi untuk mencegah kemunculan mikroorganisma yang rentang ubat dalam jangka masa yang terdekat.

Kata kunci: Septisemia, ruminan, titik kemasukan, faktor risiko, mikroorganisma, kerentanan antibiotik

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LIST OF ABBREVIATIONS

°C	degree Celcius
µg	microgram
µl	microlitre
ADP	adenosine diphosphate
AEEC	attaching and effacing <i>E. coli</i>
aEPEC	atypical enteropathogenic <i>E. coli</i>
BHI	brain-heart infusion
CLSI	Clinical and Laboratory Standards Institute
DNA	deoxyribonucleic acid
<i>E. coli</i>	<i>Escherichia coli</i>
EAEC	enteroaggregative <i>E. coli</i>
EHEC	enterohaemorrhagic <i>E. coli</i>
EPEC	enteropathogenic <i>E. coli</i>
ETEC	enterotoxigenic <i>E. coli</i>
ESBL	extended-spectrum beta- lactamases
g	gram
LPS	lipopolysaccharide
MDR	multiple drug resistant
µg	miligram
MHC	Major histocompatibility complex
ml	mililiter
NaCl	sodium chloride
MODS	multiple organ dysfunction syndrome
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
PCR	polymerase chain reaction
NCBI	National Center for Biotechnology Information
rDNA	ribosomal DNA
qSOFA	quick sequential organ failure assessment
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SAGs	super antigens
SIM	sulphide indole-motility
SOFA	Sequential Organ Failure Assessment
SIRS	Systemic Inflammatory Response Syndrome
sp.	Species
SPSS	Statistical Packages for the Social Sciences
STEC	Shiga toxin-producing <i>E. coli</i>
UPM	Universiti Putra Malaysia
TSI	Triple sugar iron
UPGMA	unweighted pair group method with averages
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

Septicaemia is the presence of microorganism and its toxins in the blood circulation. It is most commonly caused by bacteria, although other microorganism could also cause the condition. Septicaemia frequently incites sepsis, which is a form of systemic inflammatory response (SIRS) by the host due to infection. Both of these conditions were reported as important health conditions in both human and veterinary medicine. In human, cases of septicaemia increase approximately 9% between the year 1997 and 2000 despite the rapid advancement in antimicrobial treatment, medical intervention, diagnosis, and research (Angus *et al.*, 2001; Martin *et al.*, 2003). Incidence of sepsis shows an increasing trend and it was identified as the third leading cause of death among the infectious diseases' category (Bone *et al.*, 1997). A study observed that septicaemia is the leading cause of death in medical and surgical intensive care unit wards (Bone *et al.*, 1997).

In veterinary medicine, study on septicaemic diseases typically focuses on single disease entity, such as haemorrhagic septicaemia in cattle and buffalo, fowl cholera in chicken, or colisepticaemia in various animal species. This has led to lack of general information pertaining to septicaemia in animals. A study by Fecteau *et al.*, (2009) explained systemic disease associated with the presence of pathogenic bacteria and its toxins in the blood referred to as septicaemia. In contrast to bacteremia, septicaemia suggested that the animal has clinical signs of diseases as compared to just simply a bacterial infection in the blood. Main bacterial pathogens commonly associated with septicaemia in calves are found to be *E. coli*. Despite that, other bacterial species such as *Salmonella*, *Campylobacter*, *Klebsiella* and different *Staphylococcus* species were also found to be isolated from blood samples of septicaemic calves. When born in heavily contaminated environment, instance of colonization of gastrointestinal tract with virulent bacteria will brought about worst- case scenario as normal adult flora was not fully established. Death from respiratory failure in both calves and adult cattle was commonly associated with severe endotoxemia.

It was previously reported that septicaemia and sepsis are among the most important health issues in small animals, especially those caused by *Pasteurella multocida* B:2 and *Escherichia coli* (Maniam *et al.*, 2020), but such study is lacking in ruminants. Septicaemia and sepsis in animals are not well-studied, largely due to the difficulty in their diagnosis. Unlike in human where diagnosis and progression of sepsis are made based on clinical data and the sequential organ failure assessment (SOFA) score (Ioakeimidou *et al.*, 2017; Liu *et al.*, 2019), in farm animals this is extremely difficult to perform due to the limitations in diagnosing and monitoring the health status of animals in the field. Previous studies investigated the prevalence of these pathogens in animals, but not the prevalence of sepsis caused by them. It was observed that prevalence of extended- spectrum beta-lactamase (ESBLs)-producing *E. coli* isolated from cattle in Japan was 12.5% (Hiroi *et al.*, 2011). Meanwhile, the prevalence of *E. coli* and *K. pneumoniae* were 19.1% and 6.7% respectively in dairy cattle in Korea (Nam *et al.*, 2009) and 17% for *P. multocida* in calves in Scotland (Hotchkiss *et al.*, 2010). Post-

mortem examination or necropsy is a technique practiced by human medical practitioners or veterinarians in order to determine the cause of death of in human or animals. It should be noted that post- mortem examination may not always provide confirmatory diagnosis, but it is useful in order to rule out differential diagnoses. Previous retrospective study involving companion animals has proved that septicaemia is the most important health problem among cats and dogs (Rathiyaler *et al.*, 2017). However, detailed information pertaining to the aetiological agents of septicaemia, routes of entry of the pathogens into the hosts, and risk factors were not available for the ruminant species. We seek to employ similar retrospective study design in order to gather information on the various history, common microorganisms and risk factors of the diagnosed septicaemia cases.

Peracute to acute death following septicaemia was largely incriminated to significant alteration and failure of vital organs to function (Osterbur *et al.*, 2014). This is termed as multiple organs dysfunction syndrome (MODS). The term septic shock is used to refer to the condition where the host completely loss its ability to maintain homeostasis due to MODS (Osterbur *et al.*, 2014). In cases of bacterial septicaemia and bacterial sepsis, it is believed that MODS occurs largely due to the effect of bacterial toxin in the blood circulation. This bacterial toxin, especially the endotoxin of Gram-negative bacteria causes damage to the cell wall which leads to loss of cellular injury and failure to function normally (Remick, 2007). In human and animals, intervention of septicaemia could not be performed rapidly because of lacking in rapid tool to diagnose this condition (Silverstein, 2006). Recent study suggested high prevalence of antibiotic resistant Gram-negative bacteria in septicaemia cats and dogs (Rathiyaler, 2020), but no such information is available for ruminants. Due to the extremely limited background knowledge pertaining to septicaemia in ruminants, therefore, the objectives of this study were:

1. to identify and analyze the common microorganisms, points of entry, and risk factors associated with septicaemia in ruminants.
2. to isolate, identify, and phylogenetically analyse the common septicaemic- causing bacteria in ruminants.
3. to determine the sensitivity and resistance of common septicaemic-causing bacteria towards commonly used antibiotics.

Hypotheses:

Ho1: Any bacteria can cause septicaemia in ruminants and all point of entry gives the same incidence of septicaemia occurrence among the ruminants.

Ha1: Gram-negative bacteria predominates in causing septicaemia among ruminants and the common risk factor associated with septicaemia is underlying infection originated from gastrointestinal tract.

Ho2: Commonly isolated bacteria from septicaemic ruminants do not have any zoonotic potential.

Ha2: Commonly isolated bacteria from septicaemic ruminants have zoonotic potential.

Ho3: Septicaemic-causing bacteria are sensitive towards commonly used antibiotics.

Ha3: Septicaemic-causing bacteria are resistance towards commonly used antibiotics.



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