



**UNIVERSITI PUTRA MALAYSIA**

***ISOLATION AND IDENTIFICATION OF STREPTOCOCCUS EQUI  
EQUI IN GUTTURAL POUCH IN HORSES***

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**ISOLATION AND IDENTIFICATION OF STREPTOCOCCUS EQUI EQUI IN  
GUTTURAL POUCH IN HORSES**

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**A project paper submitted to the  
Faculty of Veterinary Medicine, University Putra Malaysia  
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DEGREE OF DOCTOR OF VETERINARY MEDICINE  
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**CERTIFICATION**

It is hereby certified that I have read this project paper entitled “Isolation and Identification of *Streptococcus equi equi* in Guttural Pouch in Horses”, by Norwahidah binti Alias and in my opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of the requirement for the course VPD 4999 –Final Year Project.

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## DEDICATION

I wish to dedicate this Final Year Project to my mother Kamariah bt Mohd,

my father, Alias bin Ali

my sisters, Noraslinda and Norafizah

Thank you for your endless support throughout my project.

Also to Dr. Zainita Mohd Noor

for the continuous words of motivation, guidance and attention.

My best friends, Siti Hawa, Farasiha and Hidayah

for their love and care

May this be your inspiration and motivation for your future endeavours

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**ABSTRACT**

An abstract of the project paper presented to the Faculty of the Veterinary Medicine in partial fulfillment of the course VPD 4999 –Project.

**ISOLATION AND IDENTIFICATION OF STREPTOCOCCUS EQUI  
EQUI IN GUTTURAL POUCH IN HORSES**

by

**Norwahidah bt Alias****2015****Supervisor: Assoc. Prof. Dr. Md Sabri Mohd Yusoff****Co- supervisor: Dr. Nurul Hayah Khairuddin**

Guttural pouch is a paired extension of auditory tube which is known to be one of the structures of upper respiratory tract in horses. Any changes in the microflora along the respiratory tract can lead to respiratory disease under certain circumstances. There is limited information on bacteria isolates in guttural pouch of horses from tropical countries and this study was conducted to identify *Streptococcus equi equi* and other normal flora in the guttural pouch of horses. A total of 4 euthanized horses were used in this study and surgical approach of Viborg's triangle technique was performed to get access to the



guttural pouch. A sterile PBS was instilled into the guttural pouch to obtain the samples. The samples were cultured onto blood agar and McConkey agar and identified by a series of biochemical test. Seventeen isolates obtained which comprised of 9 species of bacteria and they were identified as *Staphylococcus aureus* (17.65%), *Klebsiella pneumonia* (17.65%), *Staphylococcus intermedius* (11.76%), *Pasturella spp.* (11.76%), *Corynebacterium spp.* (11.76%), *Actinomyces spp.* (11.76%) and *Pasturella caballi* (5.88%), *Moraxella equi* (5.88%), and *Rhodococcus spp.* (5.88%). However, *Streptococcus equi equi* was not among the isolates obtained. The Polymerase Chain Reaction (PCR) employed confirmed, negativity for *Streptococcus equi equi*. Based on the findings of this study, it was concluded that several bacterial flora reside in the guttural pouch in apparently healthy horses and majority of bacteria are similar with those in upper respiratory tract reported in temperate countries which could become pathogenic in immunocompromised or stressed horses.

**Keywords:** Horses, immunocompromised, lavage, respiratory tract, Viborg's triangle.

**ABSTRAK****PENGASINGAN DAN PENGENALPASTIAN STREPTOCOCCUS EQUI EQUI  
DI DALAM KANTUNG GARAU KUDA**

oleh

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Kantung garau dikenali sebagai sepasang tiub auditori lanjutan dan merupakan salah satu struktur di dalam sistem pernafasan di kuda. Setiap perubahan pada mikro flora di sepanjang saluran pernafasan kuda dapat menyebabkan terjadinya masalah pada sistem pernafasan pada kuda dalam keadaan tertentu. Maklumat mengenai bakteria di dalam kantung garau adalah sangat kurang dilaporkan oleh negara-negara tropika. Kajian ini dijalankan untuk mengenalpasti kehadiran bakteria *Streptococcus equi equi* dan bakteria lain di dalam kantung garau. Sejumlah 4 ekor kuda eutanasia digunakan di dalam kajian ini. Teknik yang digunakan adalah Segi tiga Viborg untuk mendapatkan kantung garau. Larutan phosphate buffer dimasukkan ke dalam kantung garau untuk mendapatkan

sampel air daripada kantung garau. Sampel tersebut dikultur pada agar darah dan agar McConkey dan identiti bakteria dikenalpasti melalui ujian biokimia. Sebanyak 17 bakteria dijumpai yang terdiri daripada 9 jenis bakteria iaitu *Staphylococcus aureus* (17.65%), *Klebsiella pneumonia* (17.65%), *Staphylococcus intermedius* (11.76%), *Pasturella spp.* (11.76%), *Corynebacterium spp.* (11.76%), *Actinomyces spp.* (11.76%) and *Pasturella caballi* (5.88%), *Moraxella equi* (5.88%), and *Rhodococcus spp.* (5.88%). Walau bagaimanapun, tiada *Streptococcus equi equi* ditemui daripada sampel kantung garau. Ini dipastikan lagi dengan keputusan negatif pada kaedah PCR (Reaksi Rantai Polimer). Terdapat pelbagai normal bakteria yang mendiami kantung garau kuda yang sihat dan kebanyakan daripadanya adalah bakteria yang sama dengan bakteria pada saluran pernafasan kuda yang banyak dilaporkan oleh negara –negara beriklim sederhana. Bakteria –bakteria ini akan menyerang sekiranya haiwan mengalami tekanan dan imunisasinya terganggu.

**Kata kunci** : kuda, saluran pernafasan, imunisasi, Segi tiga Viborgs, *lavage*

## 1.0 INTRODUCTION

Guttural pouch is one of the structure of upper respiratory tract in horses as well as others such as nasal passages, nasopharynx, larynx and trachea. Horses are obligate nasal breathers due to their anatomical structure of complete separation of nasopharynx and oropharynx. Thus, it is important to keep the airways structures in good condition and function as normal to maintain the health of the horse and for them to achieve their best performance and athletic activity.

Respiratory tract has abundance of normal flora such as aerobes, anaerobes bacteria and fungus. The normal microflora will flare up under some circumstances and compromised the local immunity of the airway structure and lead to disease. However, *Streptococcus equi* subspecies *equi* which is known to cause strangles (Hardy *et al.*, 2005) is not one of normal flora of the airway tract in horses. It is from purulent discharges of infected horse or fomites which enters via the mouth or nostrils of susceptible horses (Sweeney *et al.*, 2005; Waller, 2014). Then, the bacteria will attach to the cells in the crypt of the tonsils and adjacent lymphoid nodules. (Taylor and Wilson, 2006). Besides, the pharyngeal opening of the guttural pouches are open during swallowing and dilatation of the openings can exposed the pouches to many pathogenic organisms and will lead to infection. (Edwards and Greet, 2007).

Strangles is an infectious respiratory disease of horses and a worldwide problem due to its causal agent which is difficult to eliminate and create persistence infection. The

source of infection can be from the apparently healthy horse who carry the organism in the guttural pouch without showing any clinical sign, horses that recently recover from the disease and apparently sick horses. (Sweeney *et al.*, 2005). Abscessation of retropharyngeal lymph nodes due to accumulation of extracellular organisms, degenerating neutrophils and necrotic tissue will then rupture into guttural pouch causing empyema. (Taylor and Wilson, 2006). Then purulent materials will drain into nostrils, giving the clinical signs of nasal discharges associated with strangles. Enlarged lymph nodes will compress the pharynx, larynx and trachea and results in the obstruction of the respiratory tract.

There are numerous literatures available on existence of the *Streptococcus equi* subspecies *equi* in guttural pouch of horses in temperate countries (Taylor *et al.*, 2006; Webb *et al.*, 2012; Parillo *et al.*, 2007; Sweeney *et al.*, 2005). However, there is lack of literature on *Streptococcus equi* subspecies *equi* and normal bacterial flora in guttural pouch of horses from the tropical countries. Therefore, this study is beneficial to detect asymptomatic carrier to provide future control and management of the disease. Other than that, it is important to determine bacterial flora in the airway tract as they will flare up and cause disease in immune compromised horses.

The objectives of this project were:

1. To isolate and identify *Streptococcus equi* subspecies *equi* in guttural pouch of horses.
2. To isolate and identify the bacterial flora population in the guttural pouch of horses.
3. To study the histological structure of the guttural pouch in horses.

Hypothesis of this study were:

1. *Streptococcus equi equi* are presence in guttural pouch in horses.
2. Bacteria flora in the guttural pouch are similar to those bacterial flora from the respiratory tract.

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