



**UNIVERSITI PUTRA MALAYSIA**

**TRANSMISSION ELECTRON MICROSCOPIC STRUCTURE OF THE  
GUTTURAL POUCH**

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FPV 2017 52**

**TRANSMISSION ELECTRON MICROSCOPIC STRUCTURE OF THE  
GUTTURAL POUCH**

**EMA ARZAIRIMA BINTI ARIFFIN**

**A project paper submitted to the**

**Faculty of Veterinary Medicine, Universiti Putra Malaysia**

**In partial fulfilment of the requirement for the**

**DEGREE OF DOCTOR OF VETERINARY MEDICINE**

**Universiti Putra Malaysia**

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## **CERTIFICATION**

I hereby certify that I have read this project paper entitled “Transmission Electron Microscopic Structure of the Guttural Pouch”, by Ema Arzairima Binti Ariffin and in my opinion it is satisfactory in term of the scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project

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## DEDICATIONS

This project paper is dedicated to Allah S.W.T who had created me and made all things possible throughout this project,

To my parents:

My father, Ariffin Bin Tawang

My mother, Zainab Binti Jusoh

My siblings:

Mohd Arzairimeen Bin Ariffin

Mohd Arzairiman Bin Ariffin

Enie Arzairinie Binti Ariffin

Ena Arzairina Binti Ariffin

Emira Arzaireen Binti Ariffin

Arzairie Hakeemi Bin Ariffin

My cat: Papi

My friends: Shruthy and Ashreen

And to all my teachers who have committed themselves towards the noble cause of education. Thank you for your continuous support, love and care.

May this be your inspiration and motivation for your future endeavours.

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First and foremost I would like to thank Allah S.W.T for giving me the strength and resilience, physically, mentally and spiritually throughout my life, that I give me the desire to seek knowledge in order to improve myself and to contribute towards the betterment of mankind and the society.

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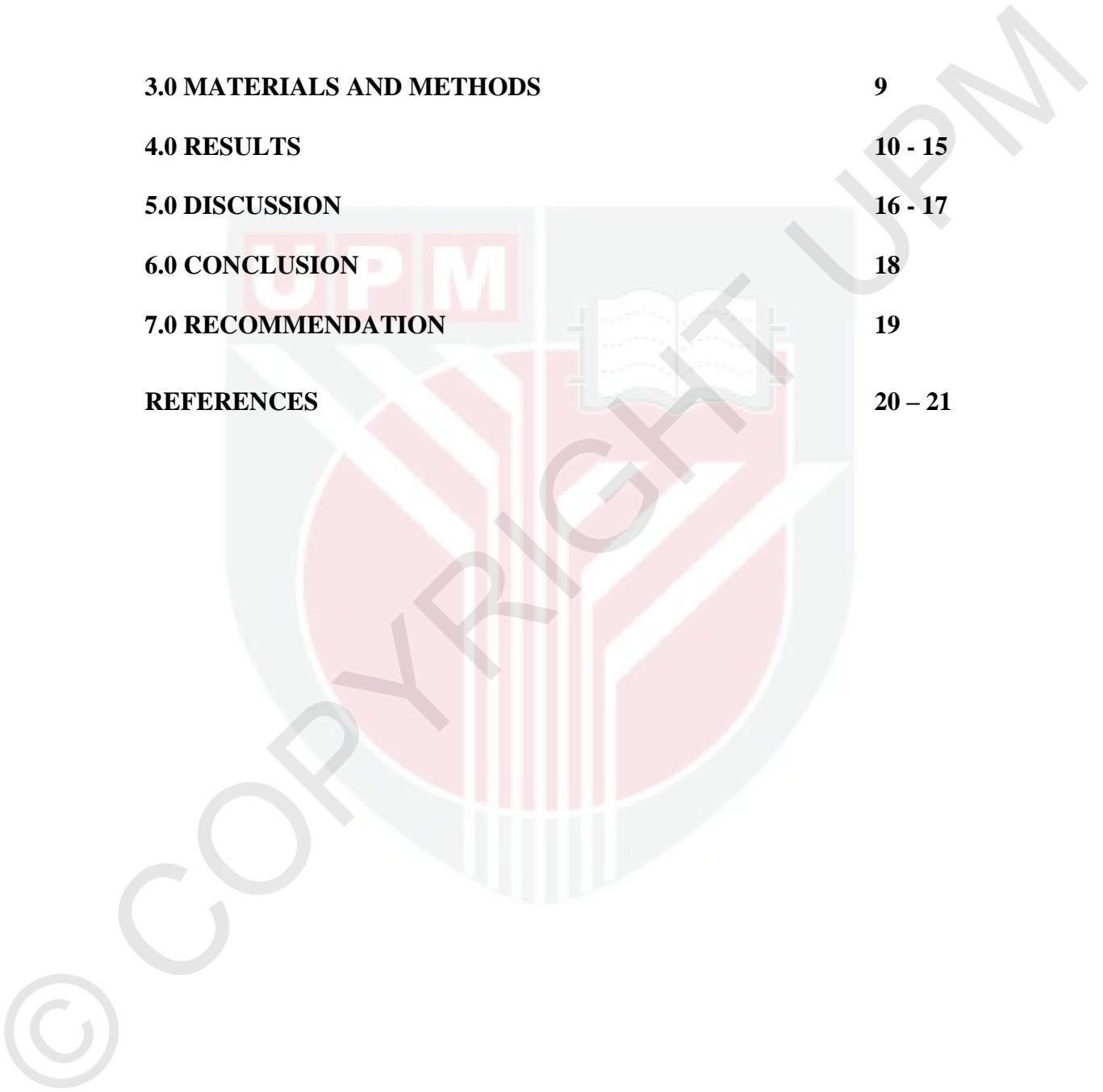
I would also like to thank En. Rafiuz Zaman Haroun and all staff of the Microscopy Unit, Institute of Bioscience, UPM for their kind assistance, guidance and knowledge sharing in preparing and processing of samples for electron microscopy during the conduct of research.

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the connective tissue below the epithelium



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**LIST OF ABBREVIATIONS**

%	Percent
Nm	Nanometer
µm	Micrometer
°C	Degree Celsius
BB	Basal body
C	Cilia
ECA	External carotid artery
GC	Goblet cell
GP	Guttural pouch
ICA	Internal carotid artery
L	Lumen
M	Molar
MV	Microvilli
N	Nucleus
SEM	Scanning electron microscope
SG	Secretory granule
TEM	Transmission electron microscope

TJ                      Tight junction

V                        Vacuole



## **ABSTRAK**

Abstrak kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek Ilmiah Tahun Akhir

### **STRUKTUR PAU GUTUR MENGGUNAKAN MIKROSKOP ELEKTRON**

#### **PANCARAN**

Oleh

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2017

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**Dr. Lau Seng Fong**

Kajian kepada struktur mikroskopik elektron pancaran pau gutur kuda ini adalah lanjutan daripada kajian mengenai struktur mikroskopik cahaya dan elektron imbasan yang dijalankan oleh Siti Syahadah Saad. Kedua-dua kajian ini bertujuan untuk mengenal pasti laluan yang mungkin digunakan oleh kulat dari pau gutur untuk memasuki arteri karotid dalaman. Jangkitan kulat, yang disebut sebagai mikosis pau gutur, membawa kepada hakisan arteri yang mengakibatkan pendarahan maut.

Dalam kajian ini, beberapa bahagian tisu yang sama digunakan dalam kajian terdahulu telah diproses untuk dikaji menggunakan mikroskop electron pancaran

Di bawah mikroskop elektron penghantaran, sel-sel epitelium dari pau gutur menunjukkan dua jenis pengubahsuaian pada permukaan sel. Jenis pertama ialah kehadiran silia dan mikrovili pada permukaan sel kolumnar yang tinggi dan juga titisan rembesan mukus. Namun, yang paling menarik adalah ruang interselular yang diperbesarkan antara sel-sel epitelium yang berdampingan yang berkemungkinan menjadi laluan kepada mukus daripada tisu penghubung di bahagian bawahbagi melalui epitelium yang akhirnya dikeluarkan pada permukaan mukosa pau gutur.

Hal ini akhirnya dibuktikan dalam kajian Siti Syahadah yang melakuka pemerhatian dengan menggunakan mikroskop electron imbasan. Oleh itu dalam kes mikosis pau gutur, kulat bergerak dalam arah yang bertentangan - dari pau gutur, melalui ruang interselular yang diperbesarkan dan juga melalui dinding kapilari tunggal yang terletak dibawah lapisan tisu penghubung untuk masuk ke dalam arteri karotid.

***Katakunci:*** mikroskop electron pancaran, mikosis pau gutur, epitelium berdampingan, ruang interselular yang diperbesarkan, laluan.

## **ABSTRACT**

Abstract of project paper presented to the Faculty of Veterinary Medicine in partial requirement for the course VPD 4999 – Final Year Project

### **TRANSMISSION ELECTRON MICROSCOPIC STRUCTURE OF THE GUTTURAL POUCH**

**By**

**EmaArzairimaBintiAriffin**

**2017**

**Supervisor: YBhg. Dato' Dr. Tengku Azmi Bin Tengku Ibrahim**

**Co-Supervisor: Dr. Nurul Hayah Binti Khairuddin**

**Dr. Lau Seng Fong**

This study on the transmission electron microscopic structure of the guttural pouch (GP) in the horse is complementary to another study on the light and scanning electron microscopic (SEM) structure of the GP concurrently being undertaken. Both studies are aimed at identifying the possible passageway utilised by a fungal infection from the GP into the internal carotid artery (ICA). Referred to as guttural pouch mycosis (GPM), the fungal infection erode the wall of the said artery resulting in fatal haemorrhage.

Under the transmission electron microscope (TEM), the epithelial cells of the GP showed two types of modification of their cell surfaces. The first type is the presence of cilia and microvilli on the surface of the tall columnar cell and mucus secretory cells respectively. The second type is the presence of tight junction between contiguous epithelial cells. However more interestingly is the enlarged intercellular space between contiguous epithelial cells; these spaces could be the passage through which mucus droplets from the underlying connective tissue transverse through the epithelium to be deposited on the surface of the mucosa of the GP. Observation on the passage of the mucus through the epithelial lining was demonstrated by SEM in the concurrent study. The present study which demonstrated the existence of distended intercellular spaces between contiguous epithelial cells lend support the passage of mucus droplets from the underlying connective tissue to the mucosal as hypothesised in the concurrent study. Thus based on the passage of the mucous droplets across the epithelial lining of the GP it can be concluded that the possible passage of the mycotic infection is similar to that of the mucous droplets but in the opposite direction. With numerous single layered wall capillaries in the connective tissue below the epithelium gaining entry into the capillaries would be with relative ease and hence on into the ICA.

**Keywords:** *transmission electron microscopy, guttural pouch mycosis, contiguous epithelial, enlarged intercellular space, passageway.*

## 1.0 INTRODUCTION

The equine guttural pouches (GP) are bilateral, air-filled ventral diverticula of the auditory tube, ranging in volume from approximately 300 – 500 ml (Dyce *et al.*, 1996). The horse is the only domesticated animal that has a GP (Manglaiet *al.*, 2000). Other than horses, a diverticulum of the auditory tube was first described in the *Perissodactyla*, for example equids, tapirs and rhinoceros (Fjeldborget *al.*, n.d).

The equine GP were first described in the year 1764 by the French veterinarian, Claude Bourgelat (Fjeldborget *al.*, n.d). The pouch is symmetrically separated into left and right chambers by a septum (Manglaiet *al.*, 2000) and each pouch is then subdivided into wider medial and narrower lateral compartment by the stylohyoid bone with a capacity ration of 2:1 (Carmalt, 2002). In addition to that, because of the localization, these pouches have unique and indicate relationship with several cranial nerve and major blood vessels that make the pouch susceptible to serious and sometimes fatal clinical condition (Çağdaş;haziroluet *al.*, 2011).

Some of the common diseases related to the GP is mycosis, empyema and tympany (Freeman, 2005). Guttural pouch mycosis (GPM) is a life-threatening disease of the horse due to fatal haemorrhage caused by fungal erosions of mainly the internal carotid artery (ICA) as well as the external carotid artery (ECA) and maxillary artery (Markus, 2005). The disease is not commonly seen in Malaysia;however, there was a report on atypical mycotic lesion in the guttural pouch of a Thoroughbred racehorse by Yiew et al., in a year of 2015. Yiewet *al.*, (2015) also mention that the most common fungus to invade the guttural pouch is *Aspergillus spp.*

A number of functions of the GP have been proposed, however, its specific, principal function is still unclear due to inadequate scientific evidence to substantiate the proposed functional significance. Thus, the debate on the actual function of the GP continued unabated.

### **1.1 OBJECTIVE**

To study the ultrastructure of the GP with a view to relate the findings with that of the light and scanning electron microscopic (SEM) study, towards identifying the possible passageway of mycotic infection from the GP to the ICA.

### **1.2 JUSTIFICATION**

A study on the guttural pouch observed at the ultrastructural level reveal more structural detail which otherwise cannot be seen with the light microscope. Thus, the ultrastructure study of the GP could shed some light on the possible passageway of mycotic infection from the GP to the ICA.

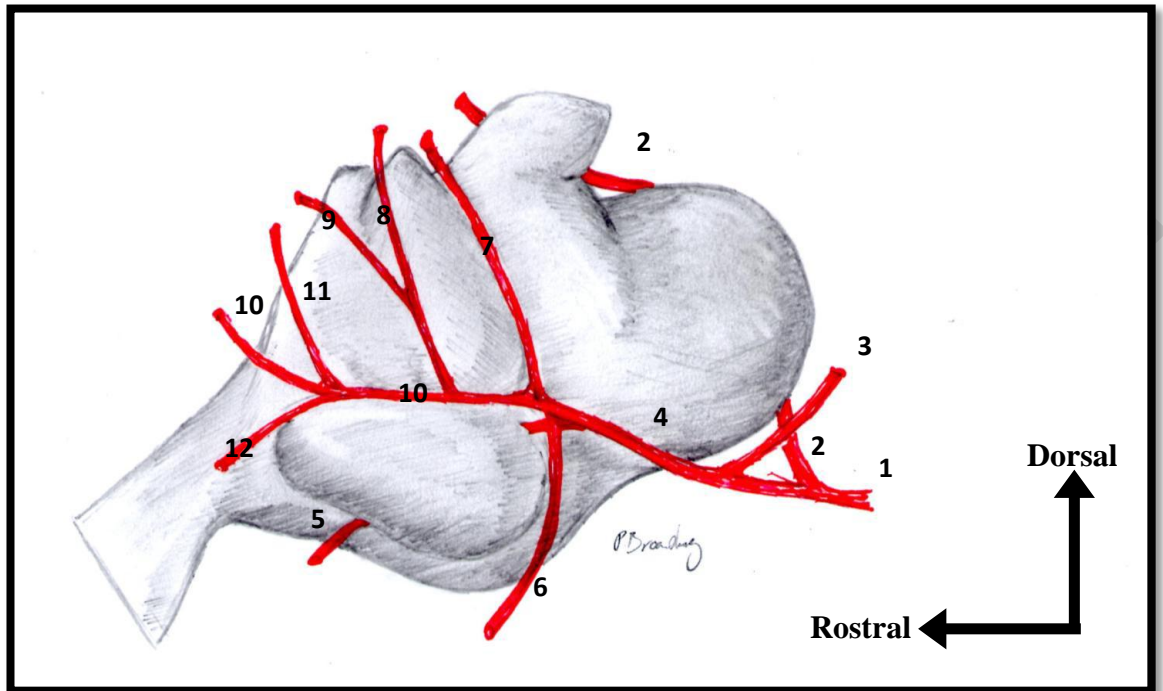
### **1.3 HYPOTHESIS**

The possible passageway of mycotic infection from the GP into the ICA is through the intercellular space between contiguous epithelial cells of the GP and via capillaries in the connective tissue to enter the ICA

## 2.0 LITERATURE REVIEW

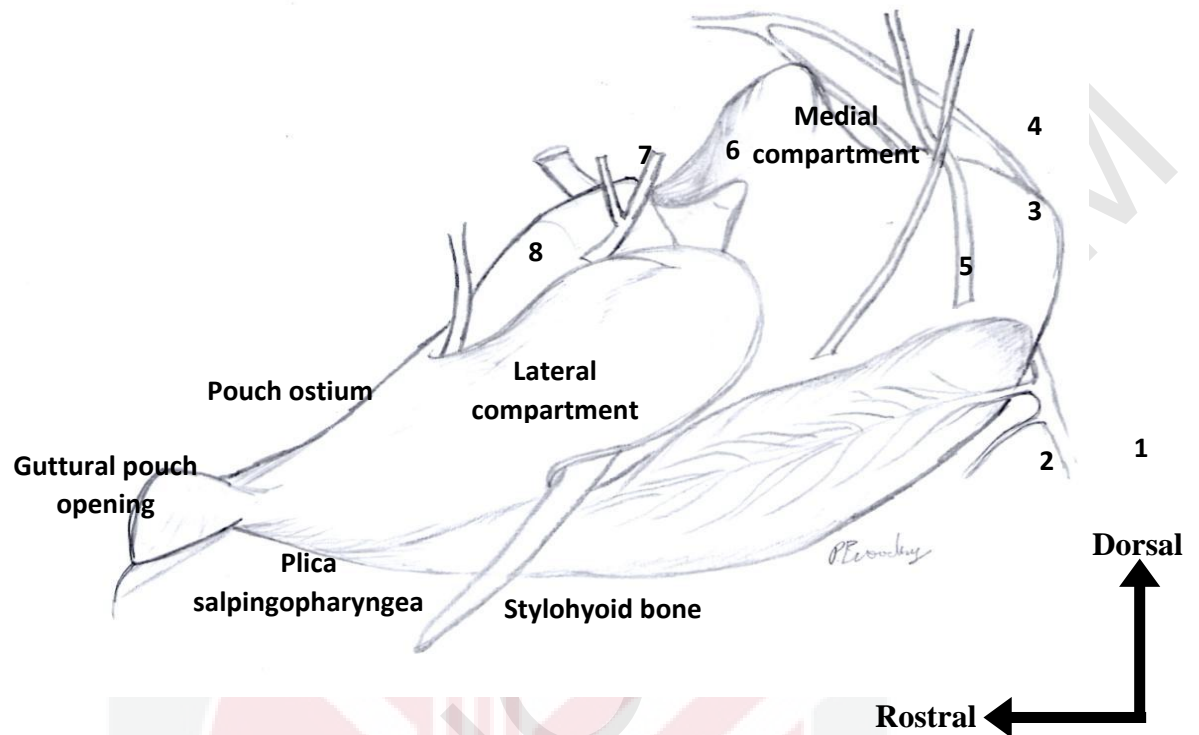
### 2.1 ANATOMY AND PHYSIOLOGY OF THE GUTTURAL POUCH

Guttural pouches are large diverticula of the Eustachian tubes, which connect the pharynx to the middle ear and is bilaterally air-filled ranging in volume from approximately 300 – 500ml (Dyce *et al.*, 2002). It can be found in horses and other Perissodactyla such as donkeys, tapirs and rhinoceros (Çağdaş;hazirolu *et al.*, 2011). According to Hardy *et al.*, (2003) each pouch is lined with a pseudostratified columnar and ciliated epithelium interspersed with goblet cells; the epithelium overlies several vascular, neural, and lymphoid structures. The pouches has an extension of basisphenoid bone rostrally, the pharynx, oesophagus and retropharyngeal lymph nodes ventrally, the atlanto-occipital joint caudally, lateral to the parotid and mandibular salivary glands and dorsal to the petrous temporal bone, tympanic bulla and auditory meatus (Pollock, 2007). The shape of the GPs largely determined by the many other important structures that closely related to each other physically. There are muscles, bones, blood vessels, nerves and lymph nodes (Baptiste *et al.*, 1996). In addition to that, some of the structures that of veterinary concern include the cranial nerves V, VII, IX, X, XI and XII, as well as the maxillary, internal and external carotid arteries that provide the principal arterial blood supply to head (Getty, 1975).



*Figure 1: Arteries associated with the guttural pouch.*

1 common carotid artery; 2 internal carotid artery; 3 occipital artery; 4 external carotid artery; 5 linguofacial trunk; 6 masseteric branch; 7 caudal auricular artery; 8 superficial temporal; 9 transverse facial; 10 maxillary artery; 11 middle meningeal; 12 inferior alveolar. (Source: Hayah, N. (2012) *A study of the anatomical variations of the carotid arterial tree in Equidae*)



*Figure 2: Nerves associated with the guttural pouch.*

1 vagosympathetic trunk; 2 cranial laryngeal nerve; 3 glossopharyngeal; 4 accessory nerve; 5 hypoglossal nerve; 6 facial nerve; 7 auriculo-temporal nerve; 8 mandibular nerve. (Source: Hayah, N. (2012) *A study of the anatomical variations of the carotid arterial tree in Equidae*)

## 2.2 FUNCTIONS OF THE GUTTURAL POUCH

The debate on the actual function of these pouches had continued unabated. Previously, many functions have been proposed which include pressure equilibration across the tympanic membrane, contribution to air warming, a resonating chamber for vocalization, and a floatation device (Hardy *et al.*, 2003). A more plausible

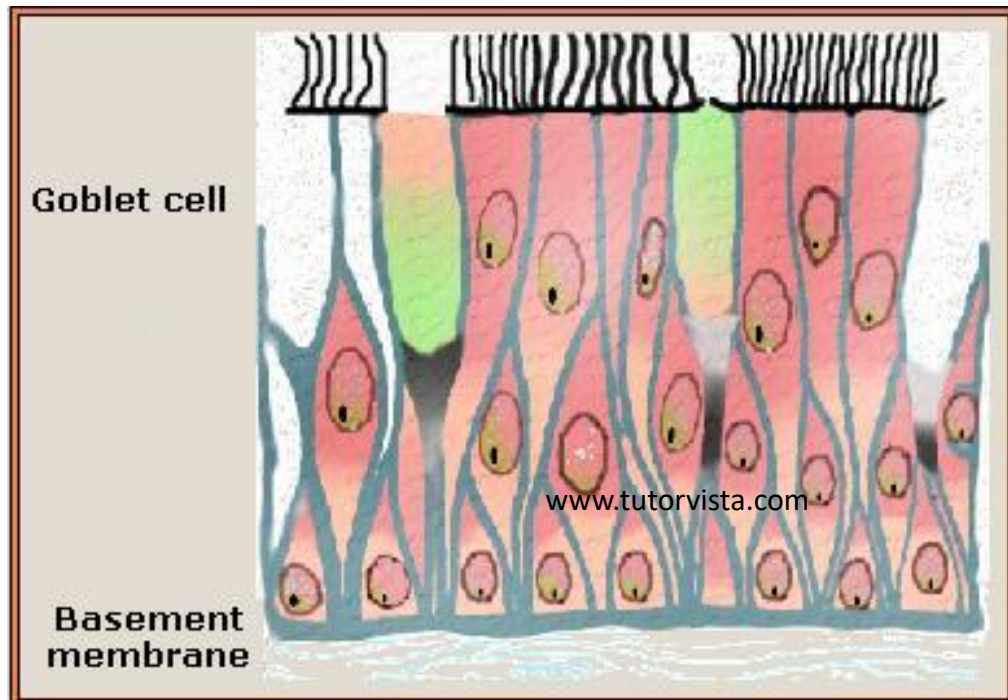
hypothesis had stated that it could play a role in cooling blood to the brain (Perkins *et al.*, 2003) by allowing air from the nasopharynx to ventilate the pouches and thus cool blood flowing within the (ICA). GP filling was previously reported to occur on expiration, but a subsequent study demonstrated that filling occurs on inspiration (Sellonet *al.*, 2007). Thus, a knowledge on the structure and function of the GP is important which could possibly shed some light on an important condition in the horse – the guttural pouch mycosis.

### **2.3 GUTTURAL POUCH MYCOSIS**

Guttural pouch mycosis was first described by Rivolta in 1868, who named the condition *Gutturomycesequi* and concluded based on the appearance of the fungus and the organism responsible to the lesion which is *Aspergillus sp.* fungus (Pollock, 2007). The predilection site for this fungus is in the dorsal aspect of the medial compartment of the pouch, which usually sit at or just below the sigmoid flexure of the ICA (Barakzai, 2006). Additionally, the fungus often erodes the major blood vessels that lie within the pouch, leading to a moderate or massive arterial epistaxis non-associated with exercise is a common presenting sign. According to Markus *et al.*, (2005) the aetiology of the disease is still unknown, but it appears probable that infection of the mucosa in the GP is a sequel to a primary arterial lesion; several surgical procedures have been developed to occlude the affected vessel to prevent haemorrhages.

## 2.4 HISTOLOGICAL STRUCTURE OF THE GUTTURAL POUCH

The GP are lined with pseudostratified ciliated epithelium with goblet cells (Pleasant *et al.*, 2016). The auditory diverticulum of domestic donkeys was composed of tunica mucosa and tunica submucosa in all different locations (Ghazi *et al.*, 2013). According to Manglai *et al.*, (1999) the lamina propria of the guttural pouch contain a capillary and PAS-positive glands that connected to the capillary tube even though there's branching of capillary in some parts, but it was primarily a single tube. Thus, they concluded that presence of secretory cells, such as goblet cells, and the cilia in the epithelium indicate that the guttural pouch mucosa has foreign substance clearance ability. Further studies also shows that there was a significant difference in the thickness of the lamina propria, thus conclude that the foreign substance clearance ability of the guttural pouch mucosa varies according to region. The numbers of goblet cells differed between sexes and among the various regions but the lamina propria-submucosa of auditory diverticulum on both sexes was formed by frame of connective tissue containing collagenous, reticular, elastic fibers, numerous diffuse or nodular lymphatic tissues, blood vessels, tubulo-acinar glands, adipose tissue, skeletal muscles, nerve bundles and parasympathetic ganglia (Ghazi *et al.*, 2013). The subepithelial glands were lined by mostly columnar epithelium with large proportions of neutral lipids along with mucus suggesting it to forms a waxy layer to retard dehydration (Baptiste *et al.*, 1994).



*Figure 3:* The mucosal lining of the guttural pouch.

The GP is lined by pseudostratified columnar and ciliated epithelium interspersed with goblet cells (Source: [www.tutorvista.com](http://www.tutorvista.com))

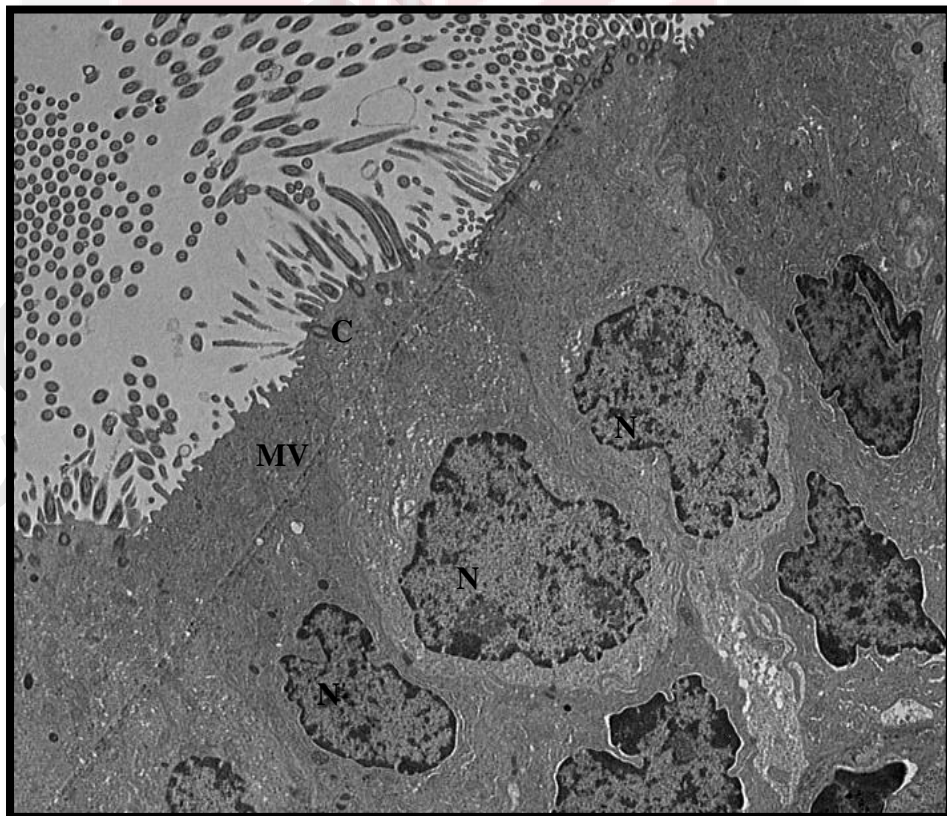
### 3.0 MATERIALS AND METHODS

Two horses obtained from Equine Unit, PDRM were euthanized using Dolethal, 1ml/kg of body weight, via the jugular vein. The head was then separated from the body at the 3<sup>rd</sup> cervical vertebrae.

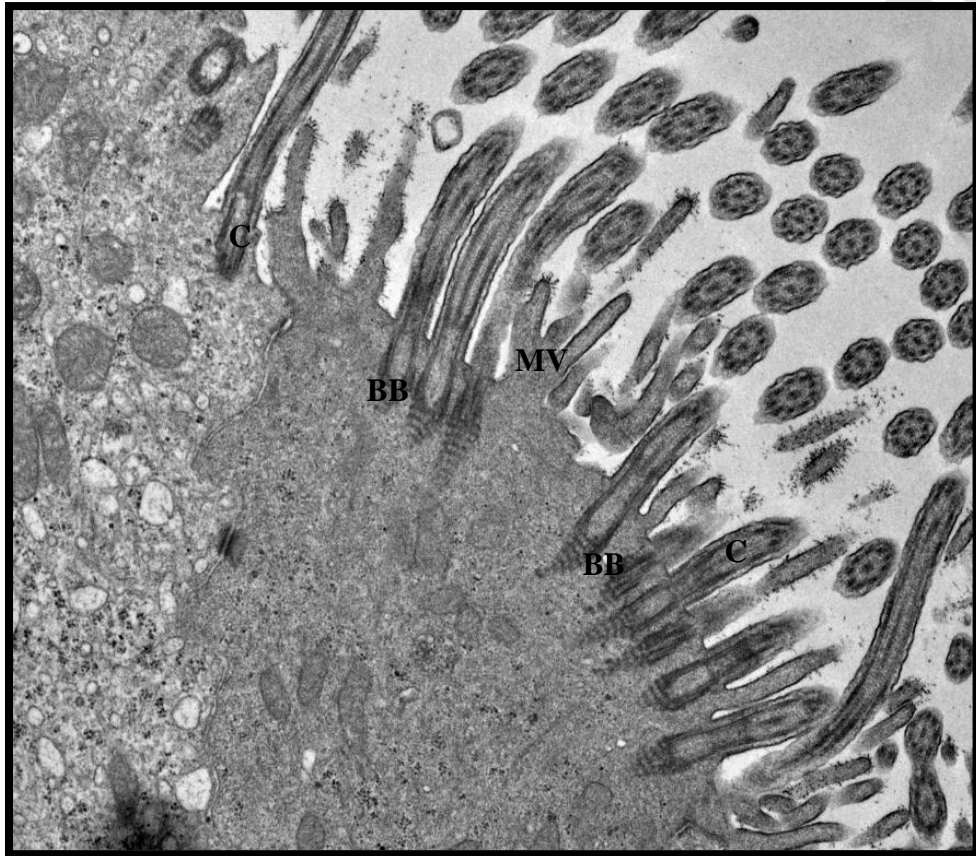
Through the Viborg's Triangle, samples of the GP were obtained and fixed in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer and post-fixed in 1% osmium tetroxide. Samples were then dehydrated in an ascending grades of concentrations of alcohol, starting from 30% through to 100% before embedded in resin and polymerised for 24 hours. Next, ultrathin section were obtained using the ultramicrotome and stained with uranyl acetate and lead citrate. Finally, stained section were examined and photographed under the transmission electron microscope (TEM).

#### 4.0 RESULTS

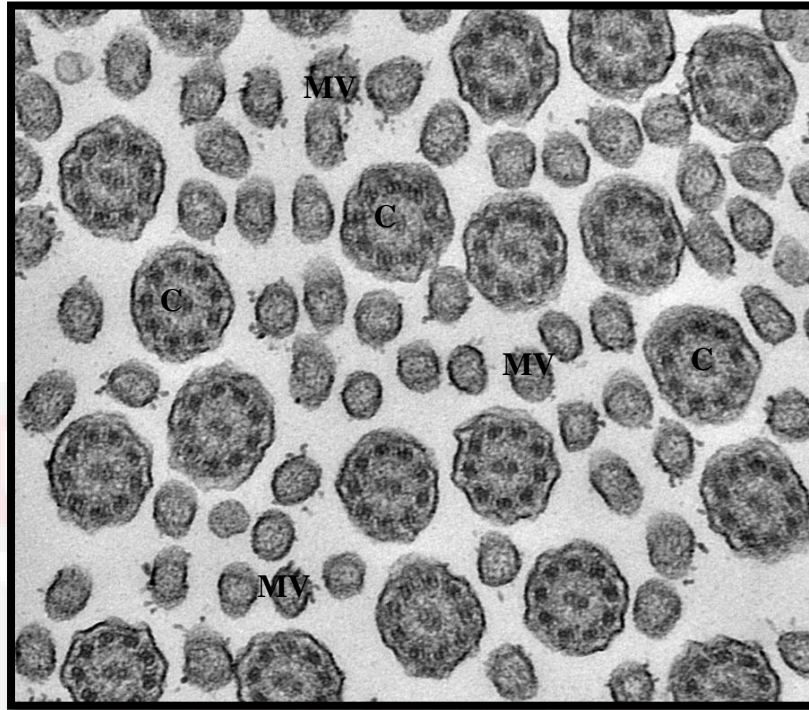
From the result observed under the TEM, Figure 4 showed that the mucosal layer of the GP consisted of pseudostratified with different surface modification of the epithelial cells consisting cilia and microvilli, which is associated with one of the function of the GP. Figure 5 showed a more detail structure of the cell surface modification of the GP, consisting cilia. There are also presence of a cross-section of cilia, showing the 9 + 2 microtubule arrangement, with 9 pairs at the periphery and a single central pair as shown in Figure 6. Figure 7 also showed the modification of the cell surface, consisting microvilli.



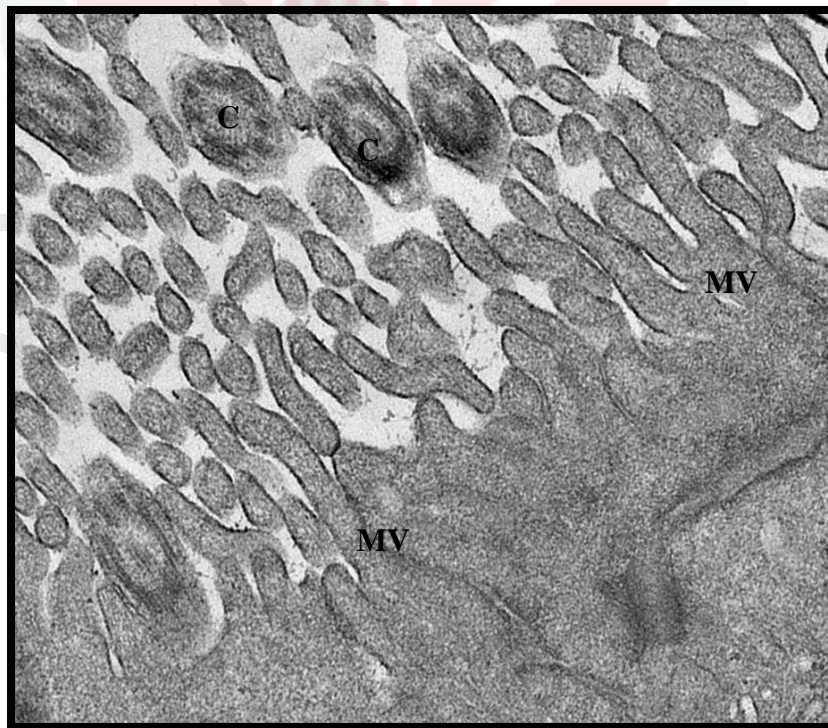
*Figure 4:* General view of pseudostratified epithelium of the GP showing different surface modifications of the epithelial cells.



*Figure 5:* Cell surface modification – Presence of cilia

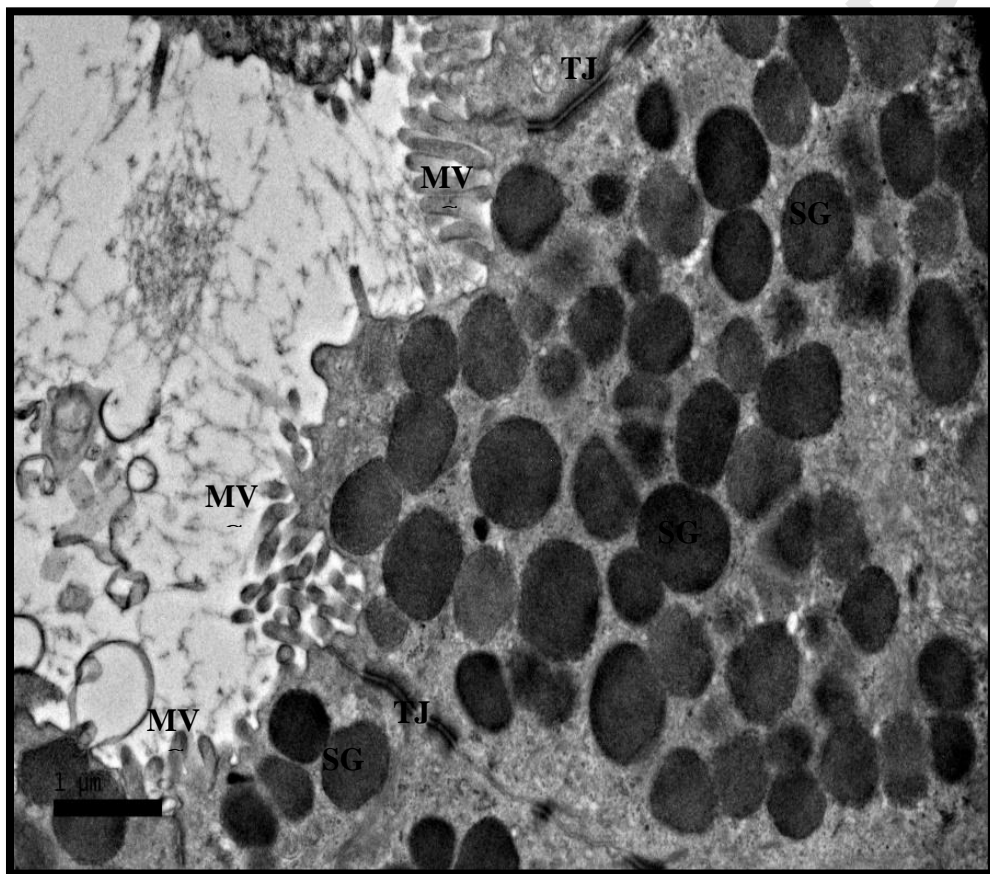


*Figure 6: Cross section of cilia*



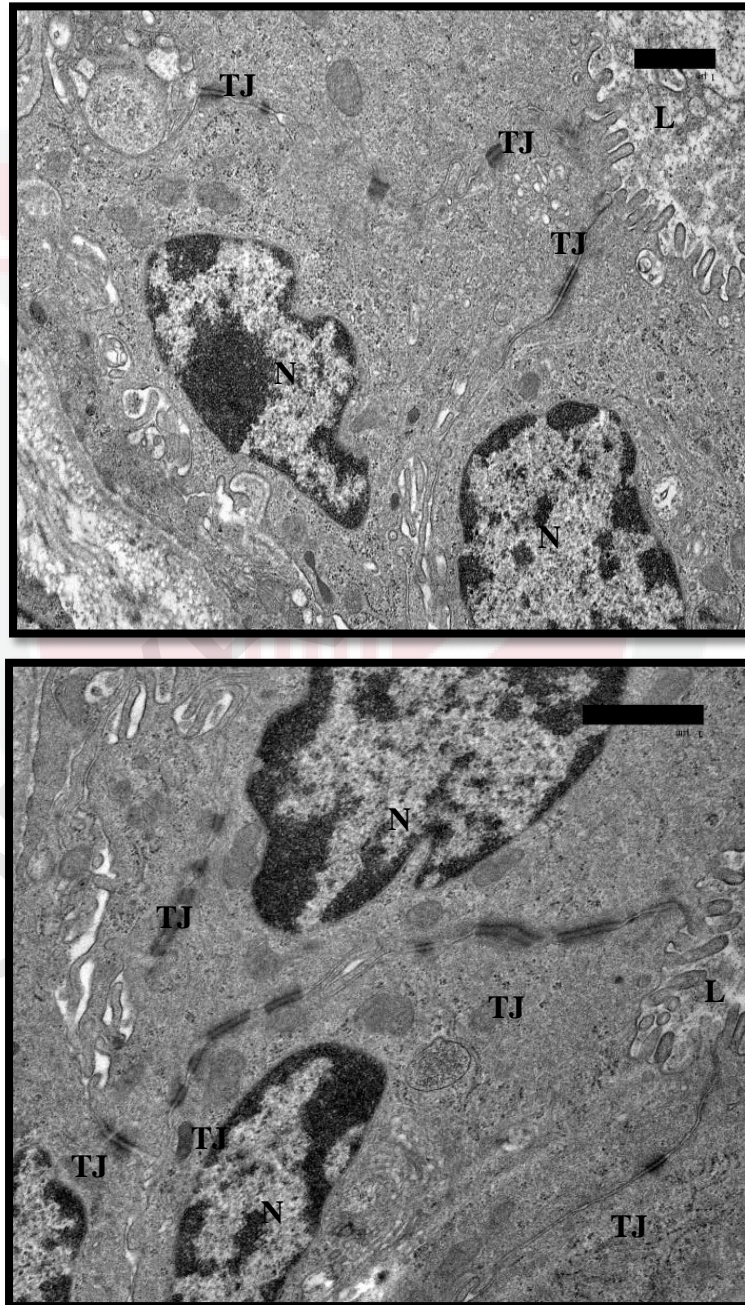
*Figure 7: Cell surface modification – Microvilli*

The next structure that can be found is the goblet cells with secretory granules that contains mucous (Figure 9) through which mucous is carried from the the passage through which mucus is carried from the underline of the connective tissue to the surface of the GP.



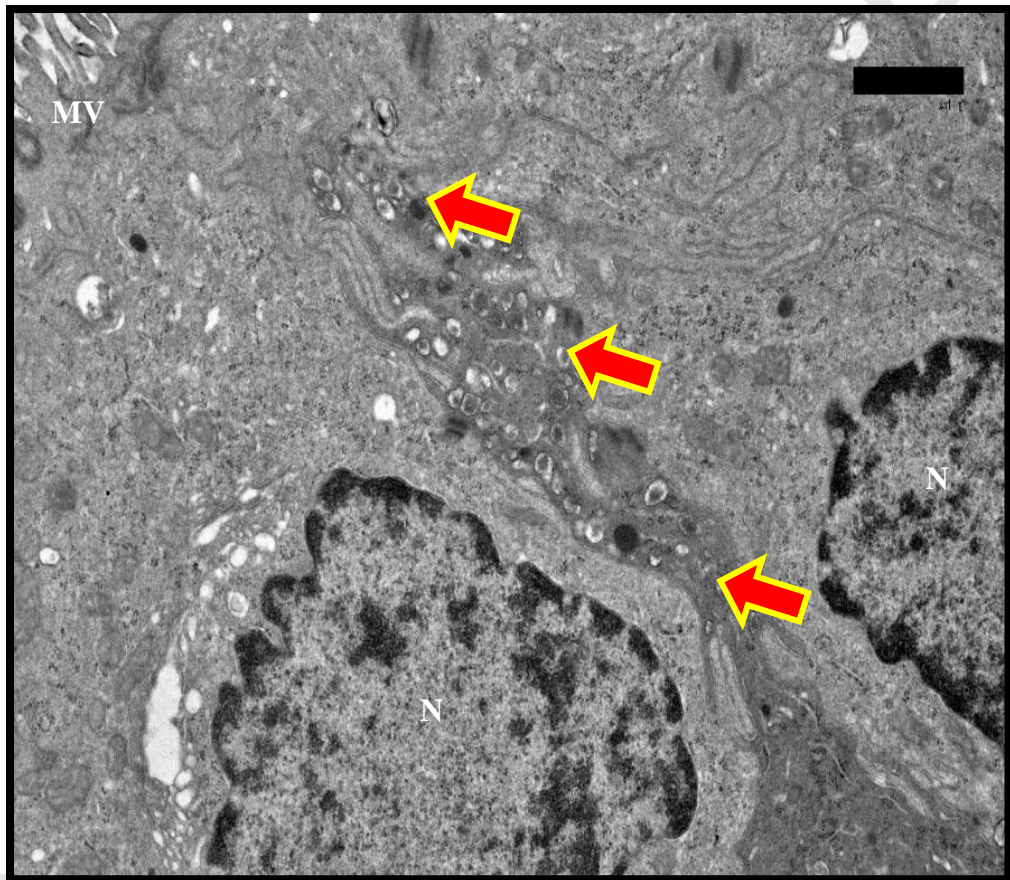
*Figure 8:* Goblet Cells with secretory granules

There are also presence of tight junctions in between the contiguous epithelial cells, to prevent the passage of materials or substances from the lumen of the guttural pouch to enter the connective tissue of the guttural pouch (Figure 10)



*Figure9:*Tight junctions between contiguous epithelial cells.

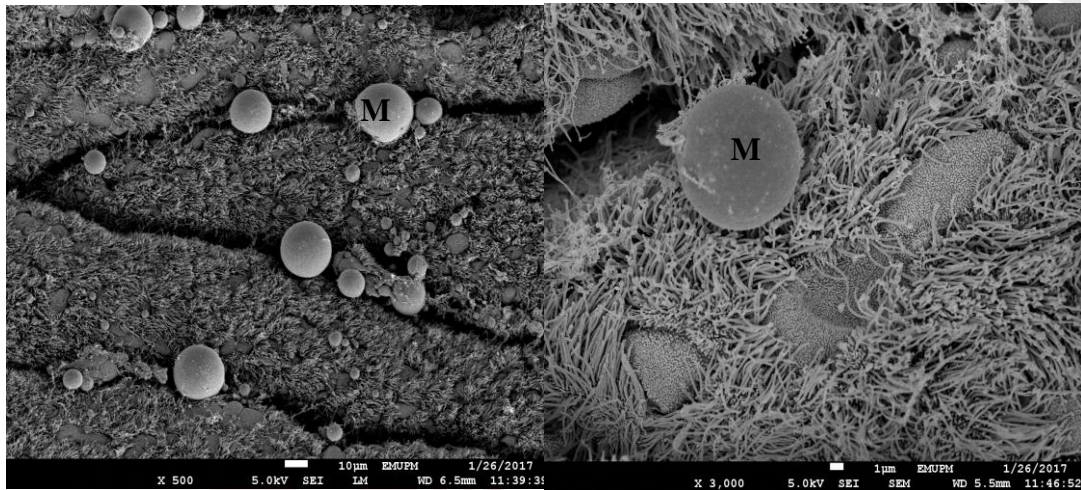
However, in between the contiguous epithelial cells, there is a presence of extended intercellular space, in which this might be the possible passageway through which mucous from the underlying connective tissue passes through epithelium to be deposited on the surface of the mucosa of the GP.



*Figure 10:* Dilated intercellular space from base to the lumen (red arrows) between contiguous epithelial cells.

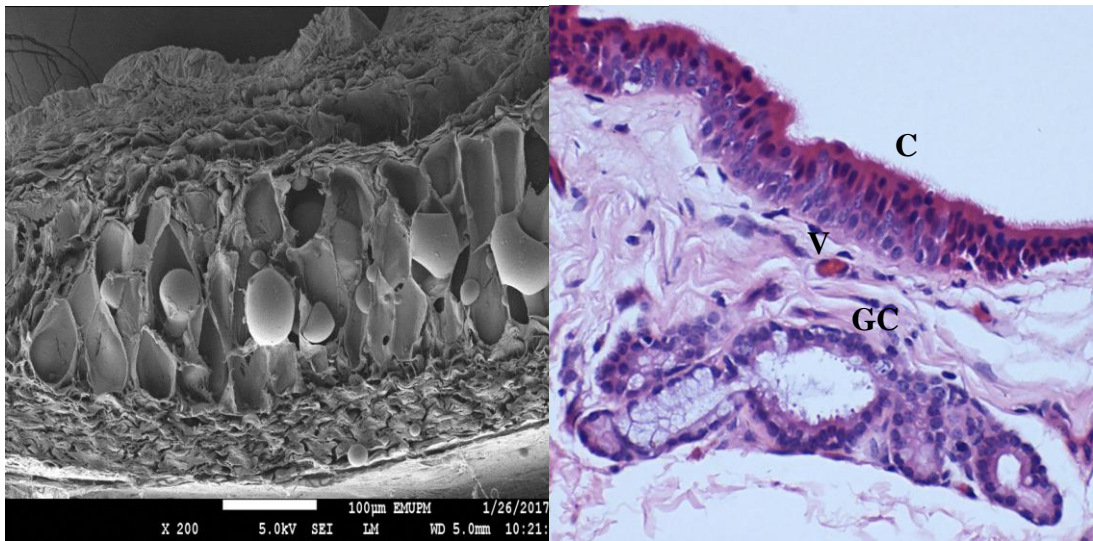
## 5.0 DISCUSSION

A previous study on the GP using light and scanning electron microscope revealed presence of the mucous on the surface of the GP as seen in figure 11.



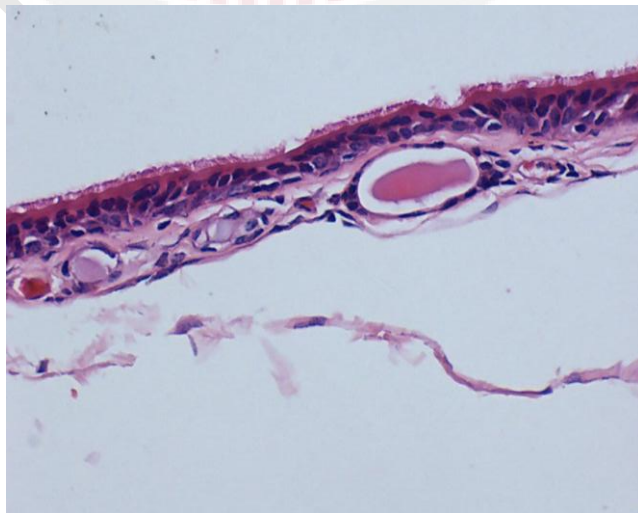
*Figure 11:* Mucous on the surface of the guttural pouch. (Source: SEM kindly donated by SitiSyahadah)

The mucous on the surface of the GP was originated from the underlying connective tissue and passes through the intercellular space between epithelial cells. This findings was demonstrated in the concurrent study using light and SEM (Figure 11). The intercellular space demondtrated in the present study could be the passageway for the mycotic infection from the GP to reach the underlying connectiove tissue (Figure 10).



*Figure 12:* Passage of mucous onto the surface of the guttural pouch. (Source: Light micrograph/SEM kindly donated by SitiSyhadah)

In the concurrent study it was demonstrated that there were numerous capillaries in the connective tissue below the epithelium of the GP. These capillaries being made up of a single layer of endothelial cells would be easily penetrated by the mycotiv infection through which it will gain entry into the ICA (Figure 13)



*Figure 13:*The presence of capillaries in the connective tissue below the epithelium(Source: Light micrograph kindly donated by SitiSyahadah)

## 6.0 CONCLUSION

The following conclusion could be drawn from the present study:

1. There was structural evidence to support that the possible passageway of mycotic infection from the GP into the ICA is through the intercellular spaces between epithelial cells and reach the ICA via capillaries in connective tissue below the GP epithelium.
2. The passage of mycotic infection from the GP into the ICA is opposite that of the mucous.

## 7.0 RECOMMENDATION

For future study, to substantiate the evidence for the presence of mycotic infection in the intercellular space and capillaries in the underlying connective tissue, it is recommended that an ultrastructural study of the GP of mycotic infected horse should be undertaken.

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