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LIGHT AND SCANNING ELECTRON MICROSCOPIC STRUCTURE OF THE GUTTURAL POUCH

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LIGHT AND SCANNING ELECTRON MICROSCOPIC STRUCTURE OF THE GUTTURAL POUCH

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A project paper submitted to the

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CERTIFICATION

It is hereby certified that I have read this project paper entitled "Light and Scanning Electron Microscopic Structure of The Guttural Pouch", by Siti Syahadah BintiSaadand in my opinion it is satisfactory in term of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999- Final Year Project.

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DEDICATIONS

Foremost Syukur Alhamdulillahi Rabbil'alamin to Allah S.W.T. who had created me and made all things possible throughout the course of this project,

Especially dedicated to my beloved family,

My father,

Saad Bin Yahaya

My late mother,

Siti Rudziah Binti Abd Ghani

My siblings; Mohd Syazuan, Mohd Syahmi, Siti Syazana, Siti Syazni

To My friends

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

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

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Categorically I would like state that his research would not been possible without the kind contribution of Polis Di Raja Malaysia who had kindly donated two horses for this study and to whom I will always be grateful.

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Last but not least, my most heartfelt gratitude to my family; my parents and siblings and friends for their love and support throughout my studies. Not to forget the continous support of Azeef Izzuddin and my student room committee.

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LIST OF ABBREBIATIONS

H & E stain	: Hematoxylin & Eosin
E	: Epithelium
EP	: Epithelial cells
C	: Ciliated cells
SG	: Secretory glands
BV	: Blood vessels
MU	: Mucous droplets
SEM	: Scanning electron micrograph
LC	: Lateral compartment
MC	: Medial compartment
ICA	: Internal carotid artery

ABSTRAK

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

STRUKTUR MIKROSKOPIC CAHAYA DAN IMBASAN ELETRON KANTUNG GUTURAL

Oleh

Siti Syahadah Binti Saad

2016

Penyelia: Dato' Dr. Tengku Azmi Tengku Ibrahim

Penyelia Bersama:

Dr. Nurul Hayah Binti Khairuddin

Dr. Lau Seng Fong

Walaupun setakat ini fungsi kantung guttural dalam kuda masih tidak dapat ditakrifkan, diverticula ini mendapat perhatian veterinar dari sudut jangkitan kulat. Dirujuk sebagai mikosis kantung gutural, ia mengakibatkan hakisan pada arteri carotid dalaman yang berdekatan, menyebabkan pendarahan yang boleh membawa maut. Kajian ini bertujuan untuk mengenal pasti laluan munasabah untuk kulat

daripada kantung gutural masuk ke dalam arteri karotid. Dua kuda yang dimatikan secara berperikemanusiaan telah digunakan dalam kajian ini dan kantung gutural dibuka melalui Segitiga Viborg. Spesimen selaput kantung gutural daripada compartment tepi, tengah dan bumbung telah diambil dan diproses untuk mikroskopi cahaya dan imbasan electron. Pemeriksaan mikroskopik cahaya mendedahkan epitelium mukosa kantung gutural adalah epitelium berlapis palsu bersilia dengan kehadiran sel goblet. Di dalam tisu perantara yang didapat di bawah epitelium tersebut terdapat banyak kapilari, venul dan kelenjar mukus. Di bawah mikroskop electron, titisan mukus didapati hadir di dalam kelenjar dalam tisu perantara, di anatara sel epitelial dan di atas permukaan mukosa kantung gutural. Dalam hal ini, terdapat kemungkinan bahawa mukus di dalam tisu penghubung dibawa melalui ruang antara sel epitelia dan dirembeskan di permukaan mukosa kantung gutural. Melalui pemerhatian ini, kulat daripada kantung gutural berkemungkinan melalui laluan yang sama sepertimana mukus tetapi dengan arah bertentangan untuk mencapai tisu perantara. Kulat tersebut akan memasuki arteri carotid dalaman melalui kapilari dan venules yang berdinding lapisan tunggal dalam tisu penghubung yang terdapat di bawah epitelium.

Kata kunci: ***mikosis kantung gutural, laluan, mikroskopi cahaya dan elektron imbasan, ruang antara sel, kapilari dan venul.***

ABSTRACT

Abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfilment of the course VPD 4901 project.

Light and Scanning Electron Microscopic Structure

of the Guttural Pouch

By

Siti Syahadah Binti Saad

2017

Supervisor: Dato' Dr. Tengku Azmi Tengku Ibrahim

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Although to date the functional significance of the guttural pouches in the horse is still not well defined this outpouching command considerable veterinary attention from the point of view of fungal infection. The latter referred to as guttural pouch mycosis resulted in erosion of the adjacent internal carotid artery leading to fatal haemorrhage. This study on the light and scanning electron microscopic structure of the guttural pouch is aimed at identifying the possible passageway for the fungus from the guttural pouch into the carotid artery. Two horses euthanized humanely were used in this study and the guttural pouches were approached via the

Viborg's triangle. Samples of the membranous guttural pouch from the lateral, medial and the roof were collected and processed for light and scanning electron microscopy. Light microscopic examination revealed the guttural pouch mucosal epithelium is pseudostratified columnar and ciliated interspersed with goblet cells. In the loose connective tissue below the epithelium are numerous capillaries, venules and mucous glands. Under the scanning electron microscope mucus droplets are present in glands in the underlying connective tissue of the epithelium, between contiguous epithelial cells and on the mucosal surface of the guttural pouch. In this respect, there is a possibility that mucus in the connective tissue are carried through intercellular spaces between contiguous epithelial cells and subsequently deposited on the guttural pouch mucosal surface. Based on this observation fungus from the guttural pouch could follow the same passageway as that of mucous but in the opposite direction to reach the underlying connective tissue. The fungus would gain entry into the internal carotid artery via the numerous single layer walled capillaries and venules in the connective tissue below the epithelium.

Keywords: ***guttural pouch mycosis, passageway, light and scanning electron microscopy, intercellular space, capillaries and venules.***

CHAPTER 1

1.0 INTRODUCTION

Guttural pouches are paired outpouchings of the Eustachian tubes which connect the pharynx to the middle ear. Not all animals possess the guttural pouches. They are present in horses and other members of the order Perissodactyla which also known as odd-toed ungulates. It also found in some bats, hyraxes and South America forest mouse. The guttural pouches which are located in parotid region extend from the base of the skull and the atlas bone to the nasopharynx (Lepage, *et al.,* 2004). Each pouch is connected to the nasopharynx via a cartilaginous flap which open during swallowing. Based on a study, the opening of the auditory tube the shape of the guttural pouch is describe funnel-shaped (Manglai *et al.,* 2000).

The guttural pouches are bound laterally by the parotid and mandibular salivary glands as well as caudal belly of digastricus muscles. The petrous part of the temporal bone, tympanic bulla and auditory meatus make up the dorsal boundary of the pouches while its floor lies on the pharynx. Each guttural pouch consists of a large medial and a small lateral compartments which is divided by the stylohyoid bone. Based on a study by Dyce *et al.* (2010) the guttural pouches in adult horses each has a capacity of 300 to 500 ml with the lateral compartment being about one third of the medial compartment's capacity.

The functional significance of the guttural pouch is still unclear although a number have been proposed without any conclusive evidences to support the claims. The functions proposed include the pouches' involvement in the physiology of

swallowing, maintenance of equilibrium across tympanic membrane and a rather remote possibility as a floatation device (Hodgson, 1998; Sasaki *et al.,* 1999). A plausible functional significance put forward recently by several studies suggested the guttural pouches having an important role in selective brain cooling in the horses. In this respect Baptiste (1998) is of the opinion that the guttural pouch contributes to regulation of temperature of the arterial blood, cooling the brain thereby protecting this sensitive organ from thermal shock and keeping it below body temperature. Furthermore, the author also proposed that the guttural pouch act in conjunction with the intracranial cavernous venous sinuses to cool the blood supply to the brain, particularly during exercise. In spite of the interest in its functional significance the guttural pouch is however of considerable importance veterinary practice and of major concern is damage to the adjacent internal carotid artery which in this case is the guttural pouch mycosis invariably leading to fatal haemorrhage.

Although this study is focussed on the light and scanning electron microscopic structure of the guttural pouches it is however aimed at providing an understanding on the possible passageway of mycotic infection from the guttural pouch into the carotid artery which to date is poorly understood. The light microscope provides the histological structure of the wall of the guttural pouch while the scanning electron microscope provides a three-dimensional appearance of the wall of the guttural pouch. The combined light and scanning electron microscopic structures would provide a more comprehensive information on the structural organization of the guttural which could possibly be related to the possible passageway of mycotic infection from the guttural pouch before it reach the carotid artery.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 : Guttural Pouch

Guttural pouches are air-filled outpouchings of the Eustachian tubes located in parotid region which extend from the base of the skull and the atlas bone to the nasopharynx (Lepage *et al.,* 2004). It can be found in horses and other members of the order Perissodactyla, which also known as odd-toed ungulates. The guttural pouches are located between the base of the skull and the atlas dorsally and the pharynx and oesophagus ventrally (Dyce *et al.,* 2010). It consist of two pouches (right and left) each attaining a capacity of about 300 to 500 ml. Equids is the species which have the largest guttural pouches in proportion to its body size compared to other mammals (Baptiste, 1998). Thus, it occupy a wide area of the caudal portion of the head.

2.2 : Anatomy & Associated Structures

Dyce *et al.* (2010) stated that the guttural pouch is formed by the escape of mucosa lining of the auditory tube through a ventral slit between medial and lateral supporting cartilage forming a diverticulum. It has also been described that the pterygoid muscles, parotid and mandibular glands bounded the guttural pouch laterally. The right and left pouches are not connected but in contact with each other medially in which they are separated by a thin layer of areolar tissue rostrally and the rectus capitis ventralis and longus capitis muscles caudally (Rush and Mair, 2004). The floor of the guttural pouch lies on the pharynx where it is moulded into the stylohyoid bone forming a ridge dividing the pouch into lateral and medial

compartments (Dyce *et al.,* 2010). The pouches are connected to pharynx via an opening known as pharyngeal orifice. Its funnel-shaped opening is located in the pharynx dorsa laterally. In other words, the orifice formed an oblique slit which is located rostral and ventral to the dorsal pharyngeal recess while the medial lamina is a fibrocartilage. (Freeman and Hardy 2012).

Other neighbouring structures directly related to the guttural pouch include several cranial nerves and arteries which abut on the guttural pouch as they pass to and from the foramen lacerum (Dyce *et al*., 2010). The important structures that lies in the medial compartment includes the internal carotid artery, cranial cervical ganglion, cervical sympathetic trunk, the vagus, glossopharyngeal, hypoglossal and the spinal accessory nerves which are all contained in a fold of mucous membrane along the caudal wall of the pouches. Beneath the mucosa on the floor of the medial compartment is where the cranial laryngeal nerve and the pharyngeal branch of the vagus nerve pass. (Sisson, 1975). The lateral compartment has been mentioned to have a close associations with external carotid, maxillary and superficial arteries, as well as the facial and mandibular nerves. (Hayah, 2011). The facial nerve lies in the submucosa of the dorsal part of lateral compartment (Mair *et al*., 2014). The external carotid artery runs along the wall of the lateral compartment and branched intot he caudal auricular artery and superficial temporal artery (Freeman and Hardy 2012), and it continues as the maxillary artery (Hinchcliff *et al*., 2004). The internal maxillary artery and vein cross the wall of the lateral compartment the area where the pouch lies beneath Viborg's triangle (Mair *et al.*, 2014). Specifically, the maxillary artery runs dorsally in the lateral compartment. While the maxillary vein lies lateral to the external

carotid artery and slightly deep into the muscles. Part of the digastric muscle can be seen along the ventrolateral wall of the lateral compartment (Hinchcliff *et al.,* 2004). The mandibular nerve, a branch of the trigeminal nerve (CN V), emerges from the foramen lacerum, passes close to the muscular process of the petrous part of the temporal bone and continues rostrally along the roof of the lateral compartment of the guttural pouch (Freeman and Hardy 2012).

2.3 : Epithelium of the Guttural Pouch

Previous histological studies revealed that the guttural pouch is lined by ciliated epithelium containing numerous goblet cells which suggest the ability of the epithelium in clearing foreign substances (Freeman, 1991; Rooney, 1997).

In the study by Manglai *et al.* (2000) a similar characteristic feature of the epithelium lining were observed in the guttural pouch of adults horses and foals. The study also revealed variation in number of goblet cells and thickness of the mucosal epithelium in different regions of the pouch suggesting a difference in the clearance ability of the epithelium in different parts of the pouch.

2.4 : Function of Guttural Pouch

Many functions have been suggested in relation to the guttural pouches which includes its function in the bray mechanism (Ghazi *et al.,* 2012), physiology of swallowing (Hodgson, 1998; Sasaki *et al.,* 1999) and also several studies that suggest its role in selective brain cooling mechanism, in this species (Mitchell *et al.,* 2006 ; Baptiste *et al.,* 2000).

In some mammalian species, selective brain cooling mediated by carotid rete a specialized anatomy where it cooled the brain arterial blood via heat exchange with cold venous blood where it is not possessed by the equids (Jessen, 2001; Baker & Hayward, 1968). According to Baptiste (1998), the guttural pouch protect the brain from thermal shock by keeping it below body temperature through it's function in cooling the circulation to the brain. He proposed that the guttural pouch contributes in regulation of the arterial blood temperature in conjuction with the intracranial cavernous venous sinuses to cool the blood supply to the brain, particularly during exercise. However, at this point of time, the precise functions of these structures are remain uncertain and mystery.

2.5 : Disease of Guttural Pouch

Guttural pouch is of considerable significance as it is susceptible to potentially life-threatening diseases caused by bacterial and fungal infections. Important clinical guttural pouch conditions are injury of specific nerves and arteries and the acoustic system. (Freeman and Hardy 2012). However the most common diseases that affect the guttural pouch includes emphysema, tympany and guttural pouch mycosis (Hardy & Léveillé, 2003).

2.5.1 : Guttural Pouch Mycosis

Guttural pouch mycosis is a diphtheroid-necrotic inflammation of the mucosa of the guttural pouch which commonly occur on one or occasionally both guttural pouches (Markus *et al.,* 2005). This disease commonly occur in adult horses however

it has also been reported in animals as young as six or 12 months of age (Lepage *et al.,* 2004).

Several microbiological investigations carried out on horses affected with guttural pouch mycosis frequently demonstrated the presence of Aspergillus spp the most common being *Aspergillus nidulans, Aspergillus flavus and Aspergillus fumigates;* hence it was postulated these microorganism as the aetiological agent of the disease (Cook *et al.*, 1968). Other studies revealed *Aspergillus fumigatus, Aspergillus niger, and Aspergillus versicolor* as demonstrated from cultures of samples obtained from mycotic horses (Ludwig *et al.,* 2005). Lepage (2007) reported that Aspergillus spp is a normal flora in the equine respiratory tract and exist as decomposers in soil and infection established in immune-suppressed host. Infection usually occur in the caudal dorsal area of the medial compartment and invariably affect the internal carotid artery (Cook 1968; Cook *et al.,* 1968; Church *et al.,* 1986; Caron *et al.,* 1987; Greet 1987).

Common clinical signs shown by the affected horses include dysphagia, epistaxis and occasionally purulent nasal discharge (Robinson & Sprayberry, 2008). Currently diagnosis of this disease is mainly relied upon endoscopy performed only when clinical signs are present (Lepage, *et al.,* 2004). Endoscopic examination is characterized by plaques of dark yellow to black necrotic material which are found in the dorsal part of the medial compartment. (Constable *et al.,* 2016). This disease may result in a fatal haemorrhage or even irreversible neurological signs, if left untreated. The principal of treatment of guttural pouch mycosis is only to prevent fatal haemorrhage which can be achieved through surgical ligation, occlusion with intra-

arterial ballons or transarterial coil embolization. It has also have been recommended that administration of antifungal drugs is an approach in the treatment of this disease (Robinson & Sprayberry, 2008).

CHAPTER 3

3.0 MATERIALS AND METHODS

3.1 Experimental Animals

A total of two adult female horses were used in this study. The animals were humanely euthanized for reasons unrelated to the guttural pouches by intravenous administration of Pentobarbital, 1mg/kg. The heads were separated from the body at the level of the 3rdcervical vertebrae. Each guttural pouch was approached via the Viborg's triangle.

3.2 Light Microscopy

Pieces of the tissues from the medial and lateral compartments of both pouches were obtained from the following parts: roof, floor, and body. Additionally the internal carotid artery together with part of the membrane of guttural pouch were also sampled. Being a thin membranous structure the samples were initially spread out on cut out pieces of papers and to prevent dehydration the samples were covered with a few drops of 10% buffered formalin fixative. Using sharp blades pieces of the membrane measuring 0.5 cm by 0.5 cm were obtained and dropped into specimen bottles containing the above fixative and fixed for 24 hours. Samples were then processed using the automatic tissue processor (Histokinette) and embedded in liquid paraffin. 4um thick sections were obtained using the rotatory microtome and stained with hematoxylin and eosin. Samples were examined and photographed under a photomicroscope.

3.3 Scanning Electron Microscopy

Parts of the samples collected for light microscopy were also used for scanning electron microscopy. Samples of the guttural pouch were initially fixed in 4% glutaraldehyde in 0.1M Sodium cacodylate buffer for 24 hours. Samples were then washed thrice in the same buffer, post-fixed in 1% aqueous Osmium tetroxide and dehydrated in ascending concentrations of grades of alcohol (30%, 50%, 75%, 85%, 90%, 95% and 100%). The dehydrated samples were then critically point dried in liquid nitrogen and mounted on specimen stubs with their mucosal surface facing upwards. Samples were then sputter-coated with platinum, examined and photographed using the field emission scanning electron microscope.

CHAPTER 4

4.0 RESULTS

Light Microscopy

The light microscopic revealed that the epithelium of the guttural pouch is lined by an epithelium which is pseudostratified columnar and ciliated. (Figure 1). This epithelium comprised of tall and low columnar cell where the nuclei of the tall columnar cells were located at a higher levels compared to that of the low columnar. However, base of both cell types were on the same basement membrane thus giving the epithelium of the guttural pouch a false stratified appearance. The mucosal surface were thrown into folds or elevations which made the mucosal surface appear of variable heights and non-isometric (Figure 2).

Figure 1 : Light micrograph of pseudostratified columnar epithelium lining the guttural pouch (E) with thin-walled blood vessels (BV) in the underlying connective tissue. x40. H & E stain.

Figure 2 : Auditory tube diverticulum of horse. epithelium (E) and mucosal fold (F). Mucous-secretory glands (GL) and blood vessels (BV) are present in the connective tissue. x10 . H & E stain.

Numerous goblet cells were interspersed between the columnar epithelial cells (Figure 3). In the deeper underlying connective tissue there were well developed mucous-secreting glands identified by the flat nuclei of the acinar cells which are disposed towards the basement membrane (Figure 4)

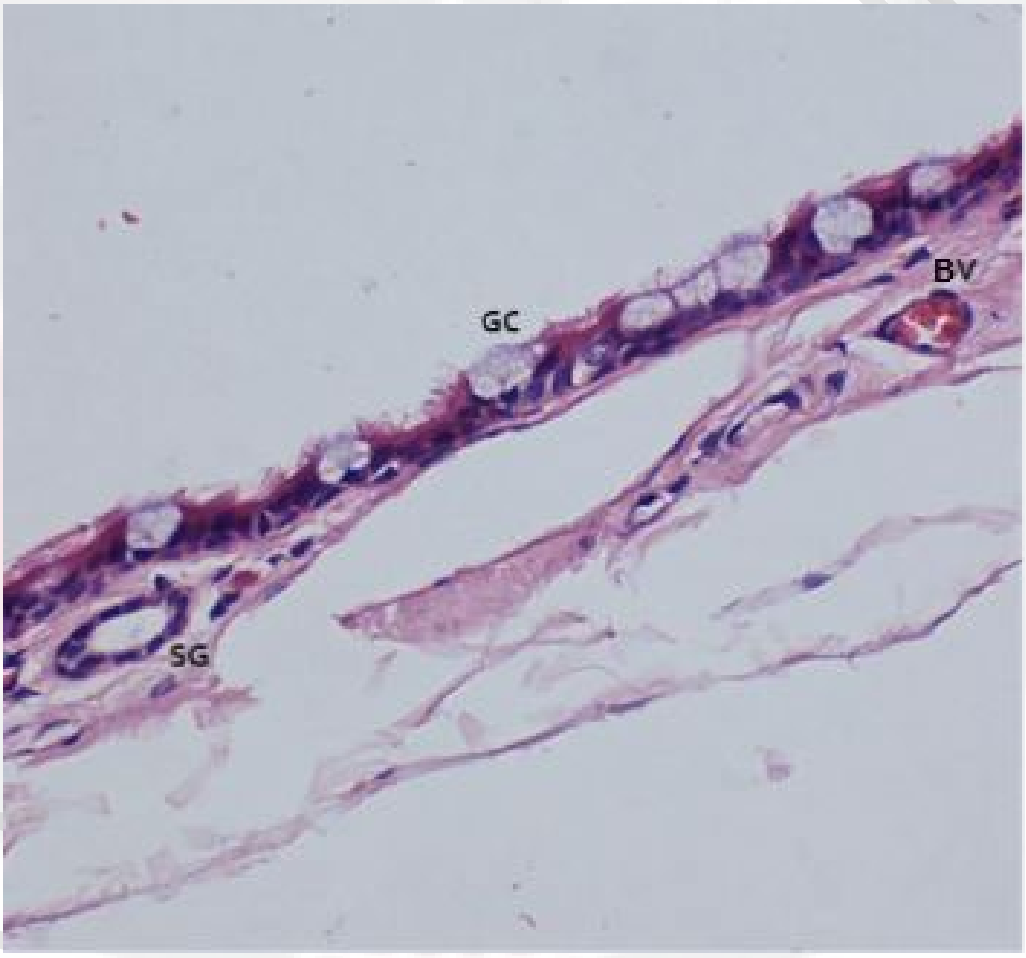


Figure 3 : Numerous goblet cells (GC) are scattered throughout the epithelium of the guttural pouch. x20. H & E stain.

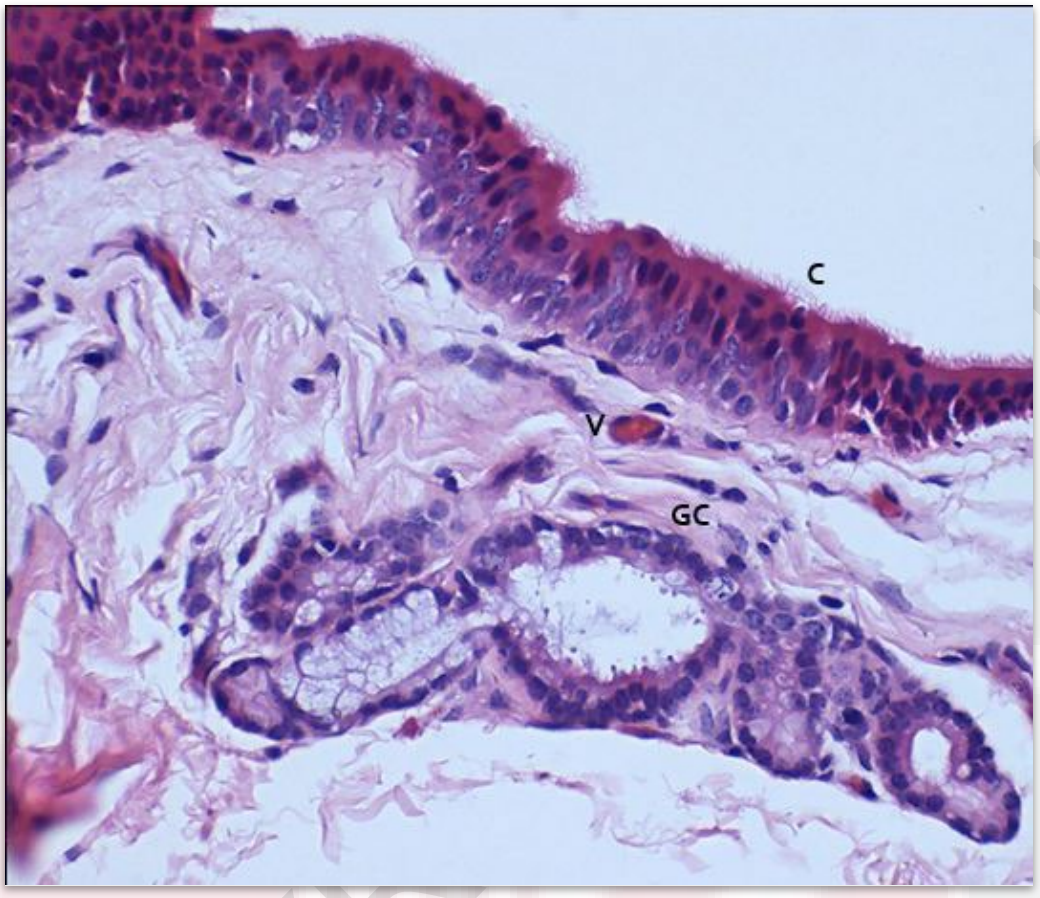


Figure 4 : Light micrograph of the ciliated (C) epithelium of guttural pouch and well developed mucus secretory glands in the deeper layer of the underlying connective tissue. A number of thin-walled vein (V) in the connective tissue (SG). X40. H & E stain.

The close association of the internal carotid with the epithelium of the guttural pouch is shown in Figure 5. The epithelium of the guttural pouch is separated from the carotid artery by only a very loose connective tissue. Unlike the epithelium on other parts of the guttural pouch the epithelium close to the carotid artery was very flat and thin.

Figure 5 : Light micrograph showing the close association between the epithelium of the guttural pouch (E) and the internal carotid artery (ICA) separated by a very loose connective tissue. The epithelium is flat and not thrown into folded X4. H & E stain.

Scanning Electron Microscopy

Under the scanning electron microscope the mucosal surface of the guttural pouch were observed to be thrown into folds (Figure 6). Under higher magnification, two types of cells were observed lining the mucosal surface – ciliated cell with the cilia covering the entire mucosal surface except on the surface of the second cell type which were covered with microvilli (Figure 7).

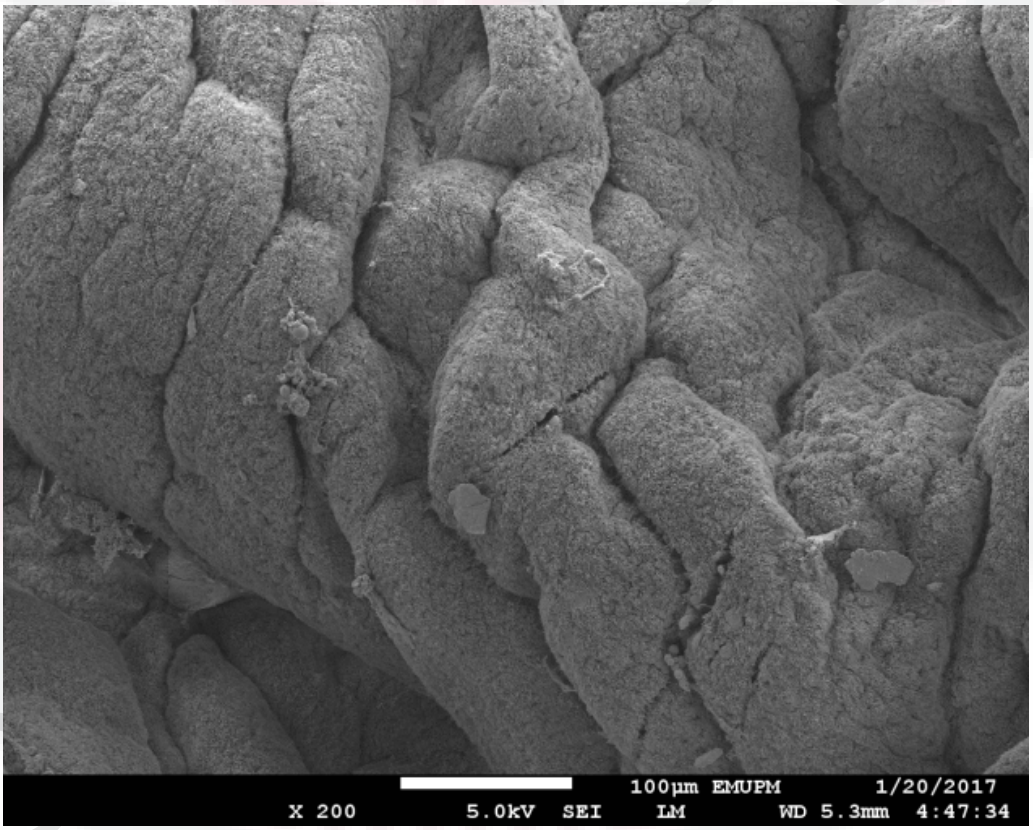


Figure 6 : Low power scanning electron micrograph of mucosal surfaces of the guttural pouch viewed under showing the folds on the mucosal surface. x200

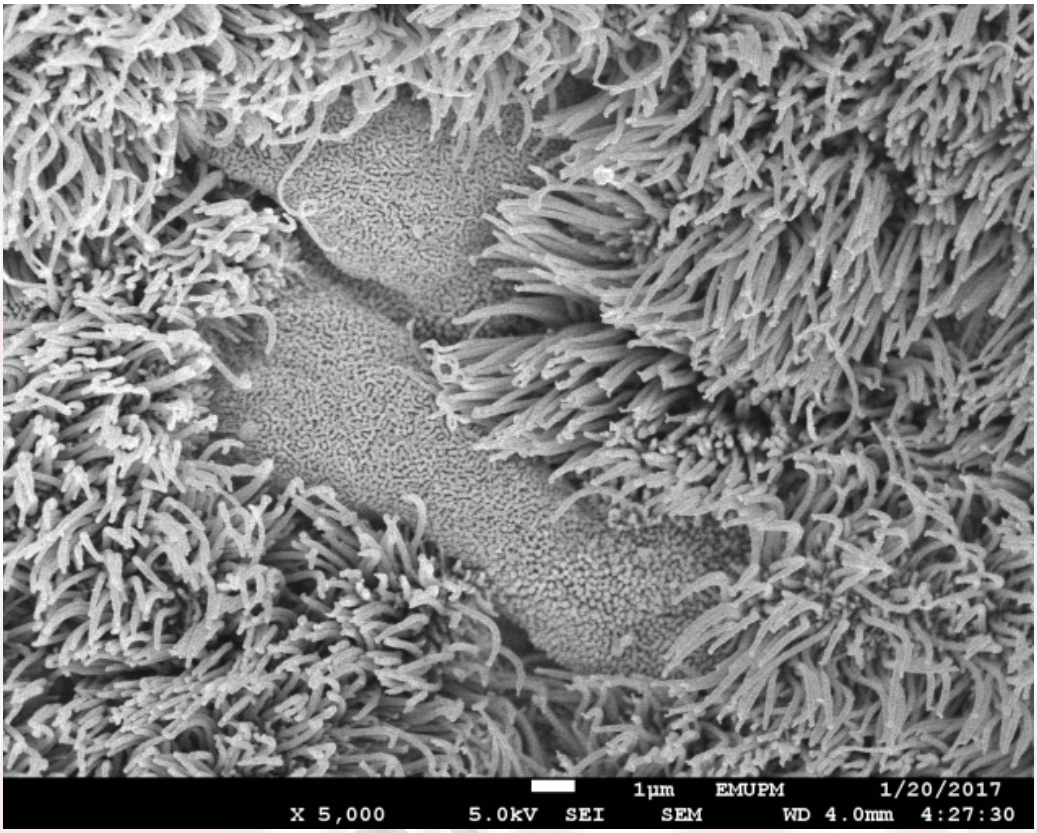


Figure 7 : Higher magnification scanning electron micrograph showing cilia covering the mucosal surface except for cells with microvilli. x5000

The presence of cilia on the mucosal surface varies in different regions of the guttural pouch. Cilia were most numerous on the lateral and medial compartment of the guttural pouch while the mucosal surface of the roof of the guttural pouch the cilia were very scanty (Figure 8).

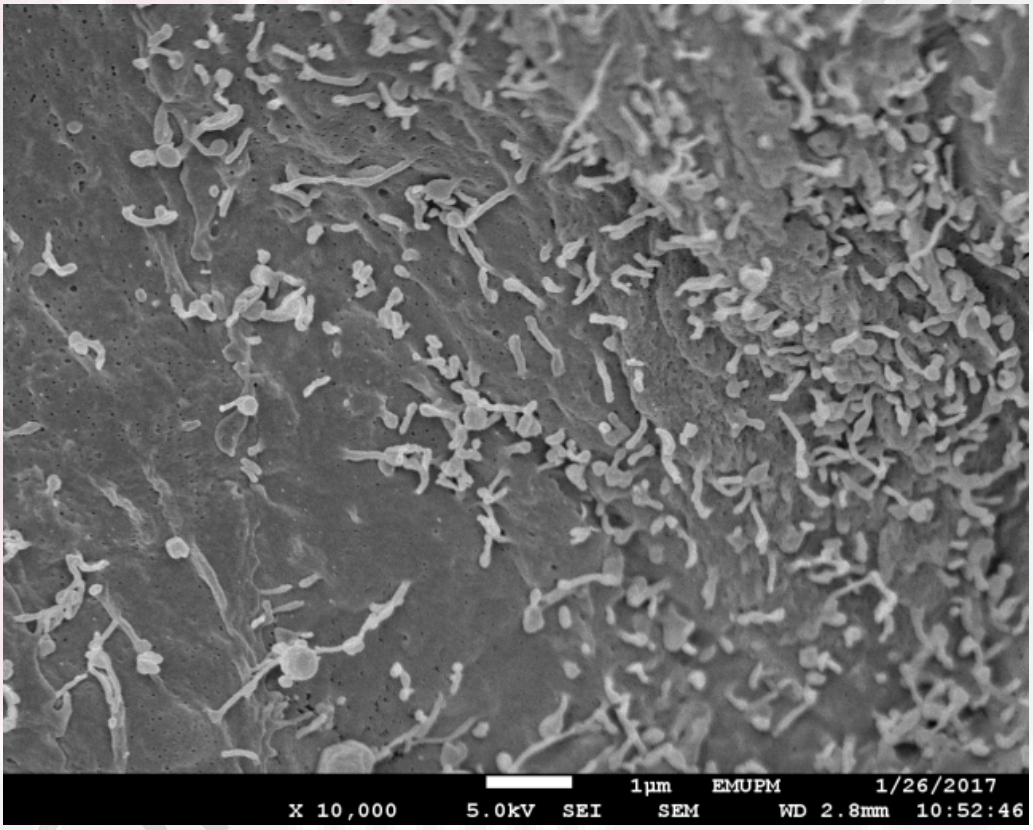


Figure 8: Scanning electron micrograph of themucosal surface of the roof of guttural pouch showin a low incidence of cilia (C) . x10000

In the deeper layer of the connective tissue below the epithelium of the guttural pouch were prominent mucous glands with large mucus droplets in their acinar cells (Figure 9). Mucus droplets were also observe in between contiguous epithelial cells (Figure 10). Additionally, numerous mucous droplets were observed deposited on the mucosal surface of the guttural pouch (Figure 11).

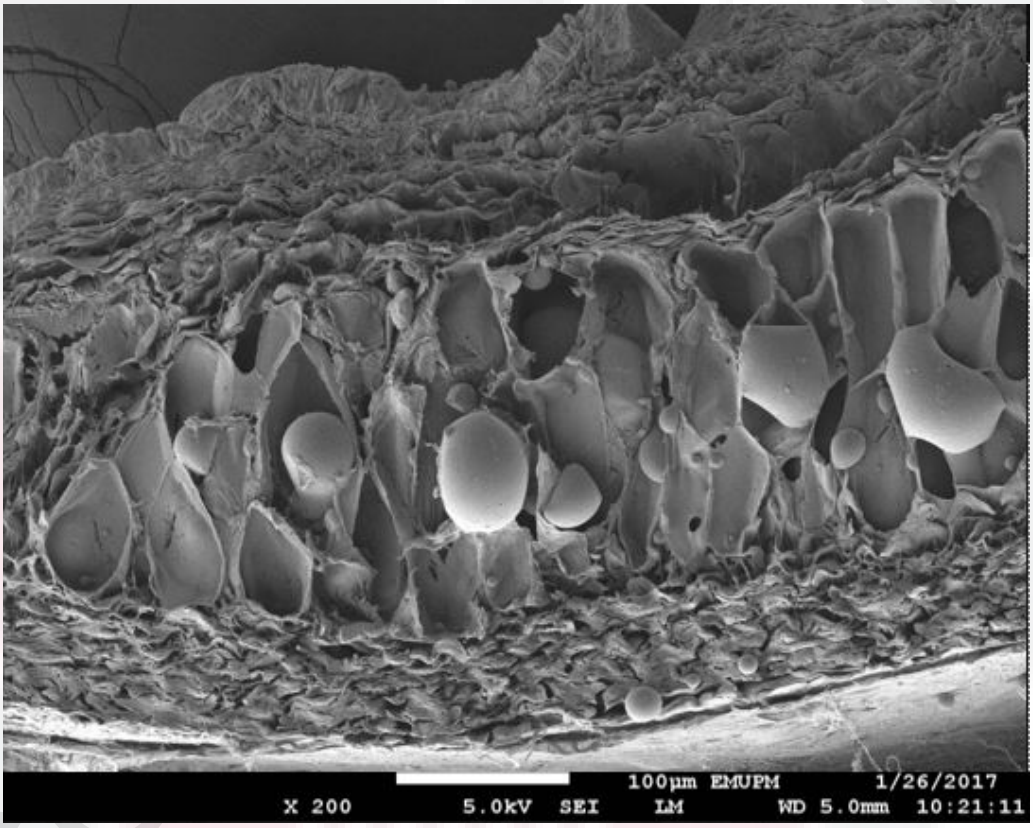


Figure 9: Scanning electron micrograph of mucus glands in the deeper layer of the connective containing large mucus droplets (mu). ep = epitheliumx200

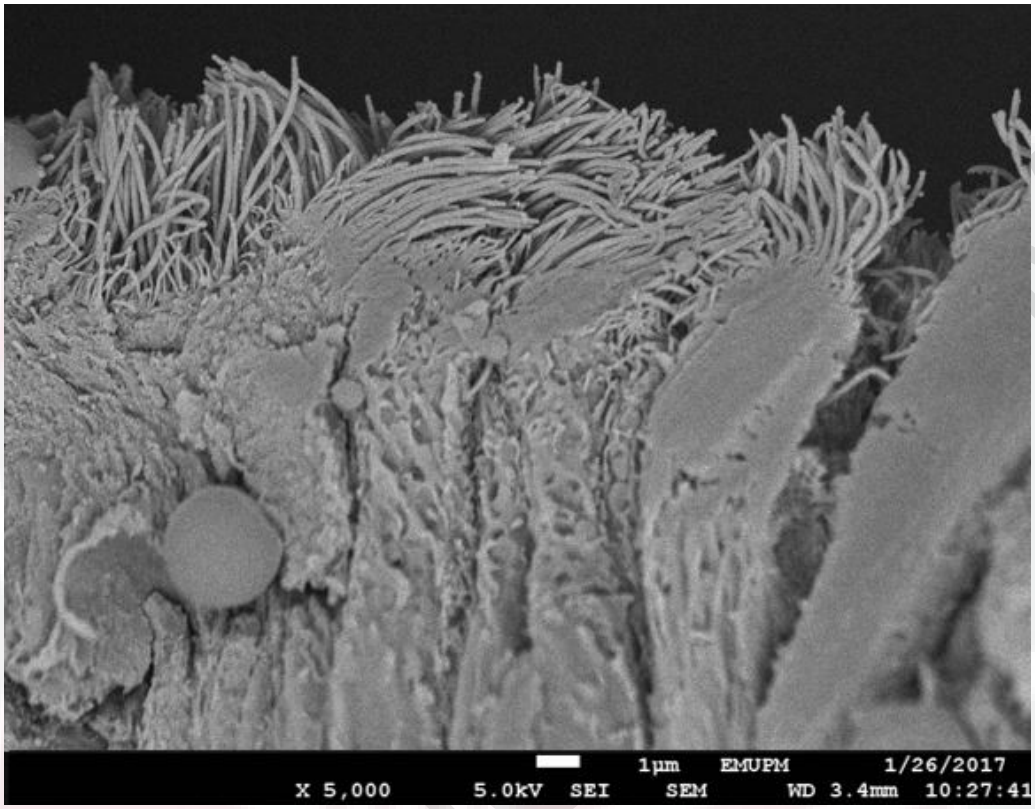


Figure 10: Scanning electron micrograph of a mucus droplet (mu) in between contiguous epithelial cells (ep) x5000

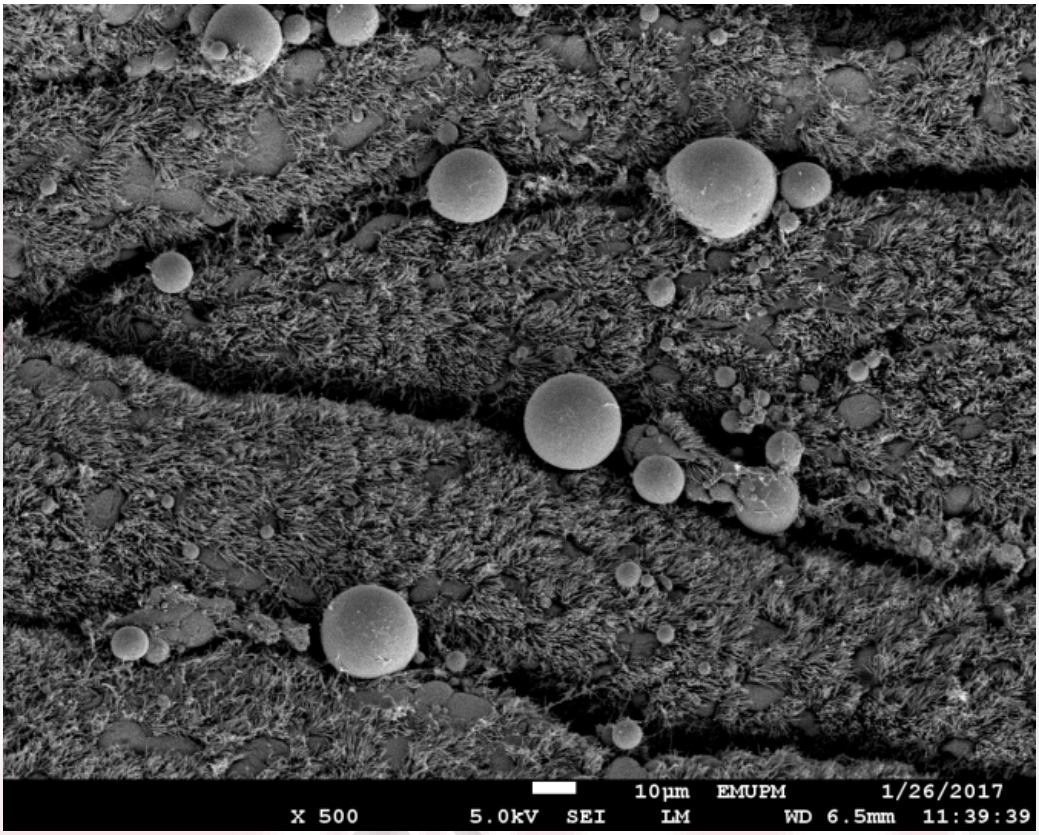


Figure 11 : Scanning electron micrograph of mucous droplets (mu)of variable sizes on the mucosal surface of the guttural pouch. x500

CHAPTER 5

5.0 DISCUSSION

Although a number of functions of the guttural pouch have been suggested (Ghazi *et al.,* 2012 ; Hodgson, 1998; Sasaki *et al.,* 1999 ; Mitchell *et al.,* 2006 ; Baptiste *et al.,* 2000) none had really been universally accepted due to lack of scientific evidence to substantiate the functions proposed. Thus the functional significance this membranous outpouching of the Eustachian tube remained unclear till to date. The guttural pouch is however of considerable veterinary importance from the point of view that guttural pouch of stabled horses are very prone to mycotic infection. This condition invariably lead to fatal haemorrhage brought about by erosion of the wall of the internal carotid artery (Lepage *et al., 2004)*. In this respect it is rather intriguing as to how the mycotic infection from the mucosal surface of the guttural pouch could find its way into the internal carotid artery. Thus, in the present study although the focus is on the light and scanning electron microscopic structure of the guttural pouch it is in fact directed towards identifying the possible passageway for the fungal infection from the mucosal surface of the guttural pouch to reach the internal carotid artery. Thus, the histological structure of the guttural pouch complemented by its structural organizational as provided by the scanning electron microscopic could possibly provide an insight on the passageway of this infection.

The light microscope revealed that the epithelium of the guttural pouch is pseudostratified columnar and ciliated which concurred with that reported by Parillo *et al.* (2009). On the basis of this finding the epithelium made up of ciliated cells and mucus produced by the goblet cells is protective in function with the ability associated

with foreign substances clearance as suggested in a number of previous studies (Freeman, 1991 ; Rooney, 1997).

In the present study samples were obtained from different regions of the guttural pouch – lateral, medial, roof and floor. Differences in the prominence of cilia in different regions of the mucosal surfaces of guttural pouch could possibly suggest that the clearance ability of these mucosal surfaces varies according to regions which in agreement with results reported by Manglai *et al.* (2000). The incidence of cilia was especially high in lateral and medial compartment of the guttural pouch and this could be associated with maximum protective function as these regions have direct exposed to the incoming air into the pouches compared to dorsum or roof of the pouches.

Both the light and scanning electron microscopes confirmed that there were prominent mucous glands in the deeper underlying connective tissue of the guttural pouch. The scanning electron microscope further revealed that there are mucus droplets in glands in the underlying connective tissue, in the intercellular space between contiguous epithelial cells and on the mucosal surface of the guttural pouch. The presence of mucus droplets in/on all these three locations could be an indication that mucus produced by glands in the deeper layer of the underlying tissue passes through the intercellular space between contiguous epithelial cells to be deposited on the surface of the mucosal guttural pouch to complement the protective function of the ciliated cells. Based on the passage of mucus droplets from the glands in the underlying connective tissue through the intercellular space to the mucosal surface of the guttural pouch it is tempting to speculate that the passage of mycotic infection is in the opposite direction from the mucosal surface of the guttural pouch to the

underlying connective tissue through the same intercellular space between contiguous epithelial cells.

At this juncture the question arises as to how the mycotic infection in the connective tissue could gain entry into the lumen of the internal carotid artery. As observed under the light microscope the connective tissue below the epithelium of the guttural pouch is a very loose type with numerous capillaries and venules. Although grossly the internal carotid appear as a single artery without any collateral the capillaries in the connective tissue are in all probability arise from the internal carotid artery which are not visible grossly. The mycotic infection could gain entry into the lumen of the capillaries with relative ease as capillaries are single layered blood channels. As the capillaries arise from the internal carotid artery mycotic infection reaches the internal carotid artery through the ramification of vascular channels in the membranous connective tissue.

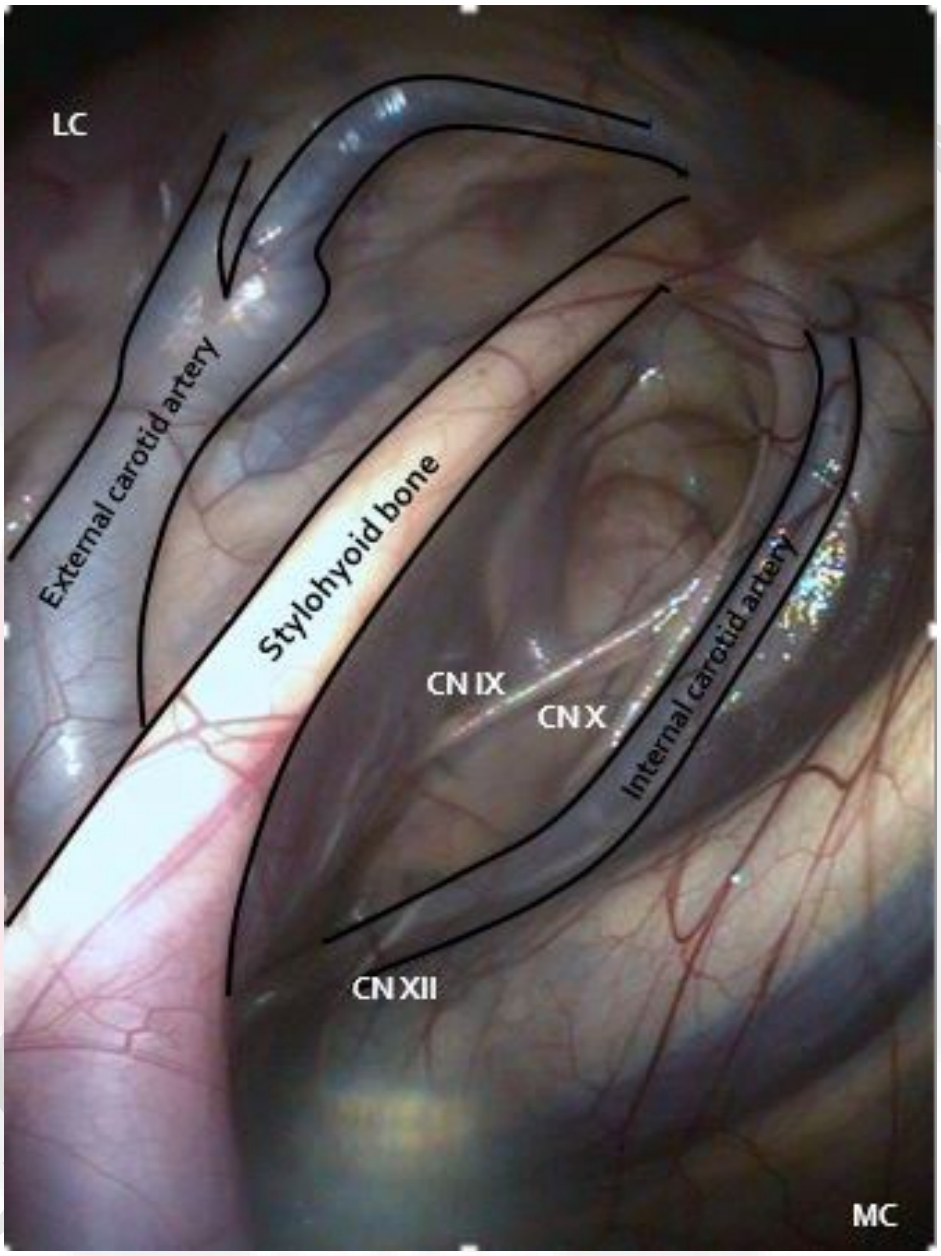
6.0 CONCLUSION

From the present study on the light and scanning electron microscopic structure of the guttural pouch it can be concluded that a possible passageway of mycotic infection from the mucosal surface of the guttural pouch into the internal carotid artery is through the intercellular spaces between contiguous epithelial cells and subsequently into capillaries in the underlying connective tissue. As these capillaries arise from the internal carotid artery passage the mycotic infection would be through vascular channel to reach the internal carotid artery.

7.0 RECOMMENDATION

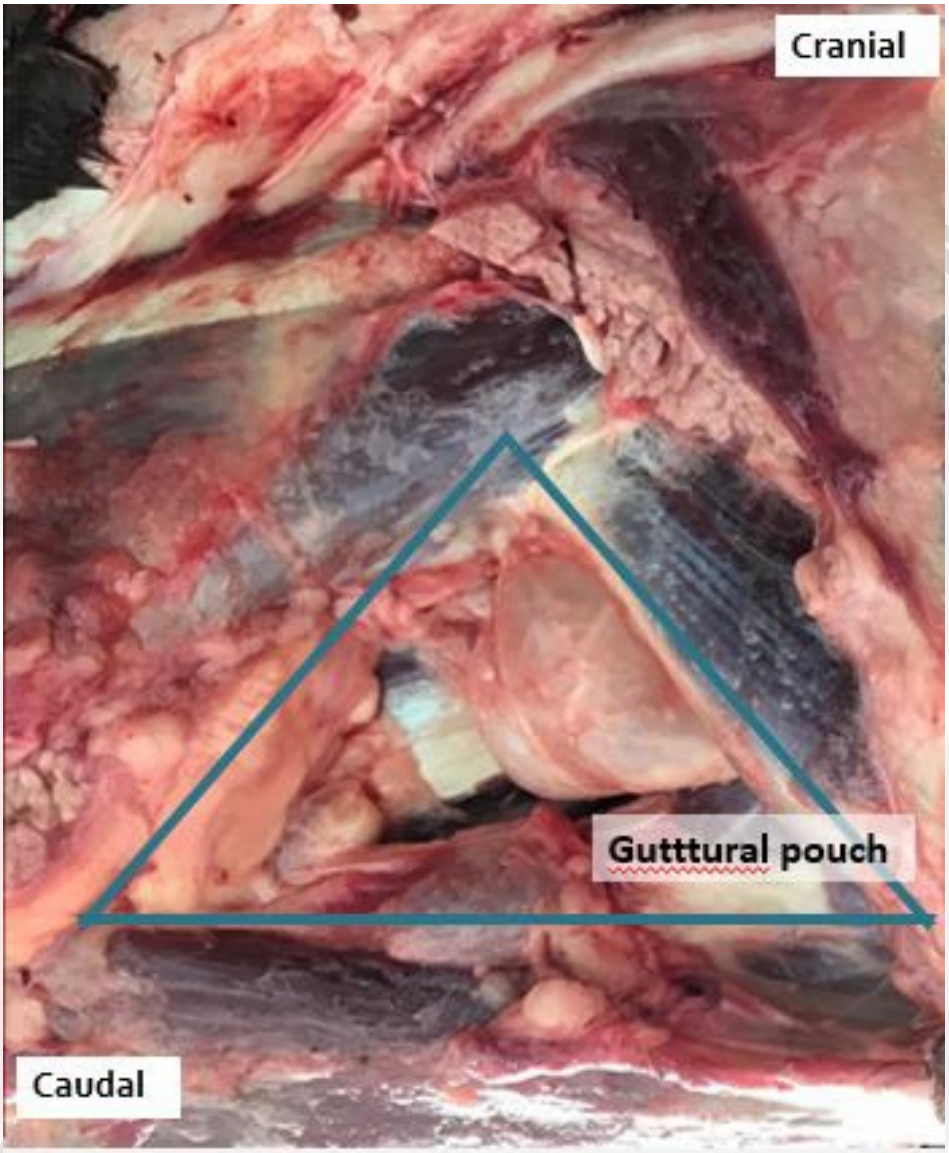
It is recommended that this study on the light and scanning electron microscopic structure which could provide an insight on the possible passage of mycotic infection from the mucosal surface of the guttural pouch into the carotid artery should be complemented by a transmission electron microscopic study. A study at the ultrastructural level would provide a better image of the intercellular space in between contiguous cells to support that the passage of mucus from the underlying connective tissue to the mucosal surface is indeed through the intercellular space of epithelial cells. Preferably the study at the ultrastructural level should be on a mycotic infected horse. The presence of the fungus in the intercellular space and in the connective tissue capillaries would conclusively support that the passageway of the mycotic infection from the mucosal surface of the guttural pouch into the internal carotid is indeed through the intercellular between contiguous epithelial cells and capillaries.

8.0 APPENDICES



Appendix 1 :

Endoscopic image of the guttural pouch. Each guttural pouch consist of lateral and medial compartment which divided by the stylohyoid bone. The lateral compartment (LC) is in close associations with the external carotid, maxillary and superficial temporal arteries. While the medial compartment (MC) is intimately related to the internal carotid artery and several cranial nerves.



Appendix 2 :

Guttural pouch located in the Viborg's Triangle which bounded rostrally by the vertical border of the mandibular ramus, dorsally by the tendinous insertion of the sternomandibularis muscle and ventrally by the linguofacial vein.

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