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ANATOMIC AND HISTOLOGICAL INVESTIGATIONS OF THE EQUINE GUTTURAL POUCHES *(Diverticulum tubae auditivae)* - CLINICAL ADVISEMENTS

INVESTIGACIONES ANATÓMICAS E HISTOLOGICAS SOBRE LA BOLSA GUTURAL DEL EQUINO *(Diverticulum tubae auditivae)* - OBSERVACIONES CLINICAS

König Horst Erich[1*], Klaus-Dieter Budras[2], Johannes Seeger[3], Martina Fersterra[3], und Mircea-Constantin Sora[4].

[1]Institut für Anatomie- und Histologie der Veterinärmedizinischen Universität Wien, Austria. *E-mail: Horst.Koenig@vetmeduni.ac.at o: horst.koenig@vetmed.uni-leipzig.de
[2]Institut für Veterinär Anatomie der FU Berlin/Alemania.
[3]Veterinär-Anatomisches Institut der Universität Leipzig, Alemania.
[4]Zentrum für Anatomie und Zell Biologie der Medizinischen Universität Wien, Austria.

RESUMEN

Las bolsas guturales del caballo son convexidades pares del conducto auditivo. Cada bolsa gutural está constituida de un compartimento medial de mayor tamaño y uno lateral de menor tamaño. El pliegue neurovascular *(Plica neurovasculosa)* y el pliegue neural *(Plica accessoria neuralis)* están situados en el área caudal del compartimento medial. Estos pliegues albergan las siguientes estructuras: el nervio glosofaríngeo, nervio vago, nervio accesorio, nervio hiplogloso, el tronco simpático con el ganglio cervical craneal y la arteria carótida interna. El objetivo de esta investigación fue estudiar las bolsas guturales y su relación con las estructuras neurovasculares, lo que es de gran interés clínico. Se utilizaron cuatro cabezas de caballo y un cráneo, las que fueron seccionadas sagitalmente por su línea media. Una cabeza (de un caballo pequeño de 350 kg) fue utilizada para la producción de láminas plastinadas E12, de 2 mm de grosor, transparentes y duras, con buena calidad óptica para microscopio. En el pliegue neurovascular en el compartimento medial, se observaron el nervio glosofaríngeo, nervio vago, nervio accesorio, nervio hiplogloso, el tronco simpático con el ganglio cervical craneal y la arteria carótida interna. El pliegue neurovascular se bifurca hacia rostral en el pliegue neural que contiene los nervios glosofaríngeo e hipogloso. Se concluyó que las bolsas guturales tienen gran importancia en las enfermedades que afectan el tracto respiratorio. La inflamación de la mucosa por infecciones fúngicas puede conducir a la ulceración de las paredes de las arterias y una irritación de los nervios, generando diversos síntomas. Los nombres de *Plica neurovasculosa*, *Plica accessoria neuralis* y *Plica oclusiva* deberían ser incluidos en la Nómina Anatómica Veterinaria, ya que son estructuras de gran importancia en la planificación de una cirugía de mínima invasión.

Palabras clave: topografía, histología, anatomía clínica, nomenclatura anatómica, patología.

ABSTRACT

The guttural pouches of the horse are convexities of the paired auditory tuba. Each guttural pouch is built of a greater medial and a smaller lateral compartment. The neurovascular fold *(Plica neurovasculosa)* and the nervous fold *(Plica accessoria neuralis)* are situated in the caudal area of the medial compartment. These folds contain the following structures: the glossopharyngeal nerve, the vagal nerve, the accessorial nerve, the hypoglossal nerve, the sympathetic trunk with the cranial cervical ganglion and the internal carotid artery. This aim of this research was to study the guttural pouch

and the related neurovascular structures, which is of a great clinical interest. A skull and four horse heads, which were subjected to median-sagittal sections, were used. One horse head (of a small horse of 350 kg) was used for the production of E12 plastinated slices, 2 mm thin, transparent and hard, with good optical qualities at microscopic level. In the guttural pouch, the *Plica neurovasculosa* in the medial compartment, containing the glossopharyngeal nerve, the vagal nerve, the accessorial nerve, the hypoglossal nerve, the sympathetic trunk with the cranial cervical ganglion and the internal carotid artery could be observed. The *Plica neurovasculosa* bifurcate rostral into the *Plica accessoria neuralis* containing the glossopharyngeal and hypoglossal nerve. It was concluded that the guttural pouch is of great importance in diseases that affect the respiratory tract. The inflammation of the mucosa through fungal infection could erode arterial walls but also cause nervous irritation during infectious diseases, leading to different symptoms. The names of the *Plica neurovasculosa*, *Plica accesoria neuralis* and Plica oclusiva should be included into the Nomina Anatomica Veterinaria, as they are very important structures for planning minimally invasive surgery.

Key words: topography, histology, clinical anatomy, anatomical nomenclature, pathology.

INTRODUCTION

The guttural pouches of the horse are mucosal convexities of the auditory tuba. Like the nasal sinuses, the design of the guttural pouch is meant to reduce the specific weight of the head. Other species, like the cow, have a longer Pars perpendicularis of the pterygoid bone which will elongate caudo-ventral the tubular choane, being so unavoidable inflated. Due to the smaller pteryogoid bone in horse, there will be less space for a pneumatic cavity at the base of the skull (König, 1984). Each guttural pouch of the adult horse has a volume of 350 - 500 ml. The guttural pouch develops in the horse fetus and increases larger in the growing foal (Bierwirth-West, 1983; König, 1984; Liebich and König, 2009).The folds of the guttural pouch contain important neurovascular structures. The caudo-ventral fold contains neurovascular structures and therefore it is called *Plica neurovasculosa* (Berg and Budras, 2009). Diseases of the guttural pouch could affect these structures. Nearby the guttural pouch we will find lymph nodes. Any pathology of these lymph nodes can interest the guttural pouch (Grabner, 1984; Frey, 2006). The aim of this study is to investigate the guttural pouch and the related neurovascular structures, which can be of a great clinical interest. Concomitant we investigated histologically the mucosa of the guttural pouch.

MATERIALS AND METHODS

In this study were used a skull and four horse heads, which were sectioned median-sagittal. Concomitant we investigated allot of specimens from the anatomical collection of the Vienna, Leipzig and Berlin anatomic institute. The specimens were selected after exclusion of any disease of the guttural pouch. On the skull we investigated the tympanic cavity from the medial bony entrance of the auditory tuba *(Ostium tympanicum tubae auditivae)*. On the lateral side the entrance is covered by the *Tympanohyoideum* and the *Processus muscularis* of the temporal bone.

One horse head (a small horse of 350 kg) was use for the production of transparent E12 plastinated slices. The head was removed, from euthanasia, at the level of the second cervical vertebra and frozen at -81°C for a week. In a next step the frozen head was cut serial into 2 mm transversal slices. The slices were plastinated according to the standard E12 protocol (Sora, 2007). The thin 2 mm slices produced were transparent and hard, with good optical qualities. The finished E12 slices provided excellent anatomic detail down to the microscopic level.

For formalin specimens, after euthanasia, we used two horse heads removed at the level of the second cervical vertebra. The heads were fixed by means of injection of 4% formalin into the carotid artery. The fixed specimens were frozen for a week and then sectioned median-sagittal. On each specimen the guttural pouch was dissected and by removing the mucosa of the guttural pouch we exposed the neurovascular structures running in the folds. Each step of our dissection was documented by photography using a Canon EOS 1000D digital camera.

From an unfixed specimen we removed samples from the auditory tuba, the lateral and medial compartment of the guttural pouch and from the neurovascular fold, for histological examination. These samples were fixed with 4% buffered formalin and then embedded in paraffin. By using a microtome (Leica RM2265, Germany) we produced 5 µm slices, which were mounted on protein-glycerin covered glass slide and stained with HE (Mulisch and Welsch, 2010). The mucosa samples were studied microscopically and selected areas were documented with a digital camera in jpeg format.

RESULTS

The guttural pouch of the horse is a mucosal convexity of the auditory tuba. The surface projection of rostral entrance of the auditory tuba, which constitutes the pharyngeal entrance to the guttural poach, is placed in the lateral corner of the horse eye. The Eustachian tube extends herself over 10 cm on the skull basis to reach the bony entrance of the auditory tuba *(Ostium tympanicum tubae auditivae)*. The Eustachian tube ends in the tympanic cavity at the level of the temporomandibular joint. The cartilaginous aperture frames the auditory tuba. The aperture is positioned dorsoventral and has an elongate shape. The auditory tube has a ventrolateral slot hole through which the respiratory mucosa is put over and builds a pouch with a capacity of 0.5 liter. The mucosa of the auditory tube (Figs. 1, 2) the lateral and medial compartment of the guttural pouch (Fig. 3, 4) is built up by lots of longitudinal folds. These folds range through the entire guttural pouch and bifurcate in the depth (Fig. 4). The folds are built of elastic fiber and smooth muscle bundles. A characteristic multi-row ciliated epithelium covers the entire guttural pouch and the auditory tuba. The apical kinocilia (Figs. 2, 5) are evident, but the goblet cells are not good differentiated.

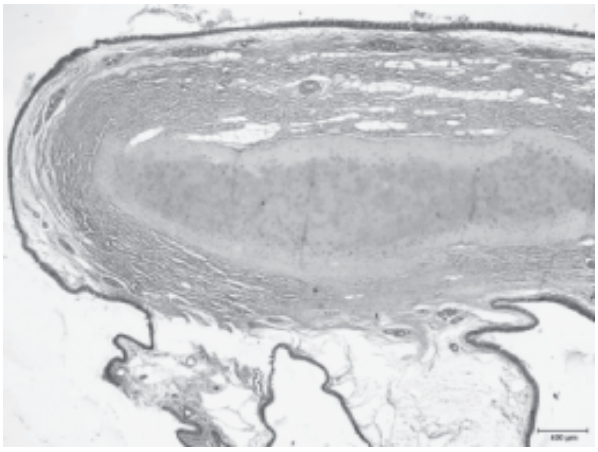

Fig. 1. Auditory tuba showing the hyaline cartilage surrounded by collagen fibers. Some glands are located in the sub mucosa.
Fig. 1. Tuba auditiva mostrando el cartílago hialino rodeado por fibras de colágeno. Algunas glándulas están localizadas en la submucosa.

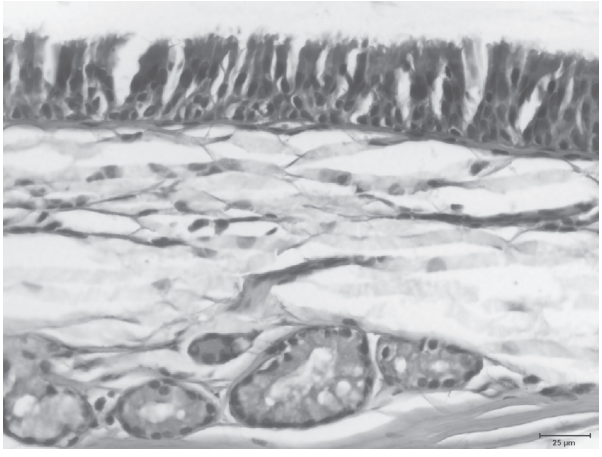

Fig. 2. Ciliate epithelium of the auditory tuba, presenting mixed glands in the sub mucosa.
Fig. 2. Epitelio ciliado de la tuba auditiva presentando glándulas del tipo mixta en la submucosa.

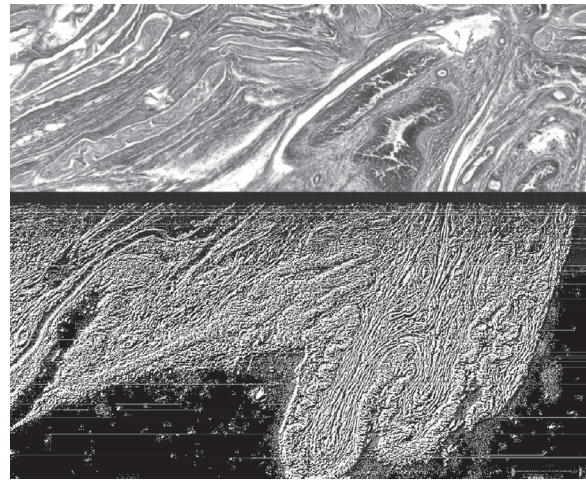

Fig. 3. Medial compartment of the guttural pouch with *Plica nervosa* in the left corner. Bifurcated folds are shown in the right side.
Fig. 3. Compartimento medial de la bolsa gutural con *Plica nervosa* en el lado izquierdo. La bifurcación de los pliegues se muestra en el lado derecho.

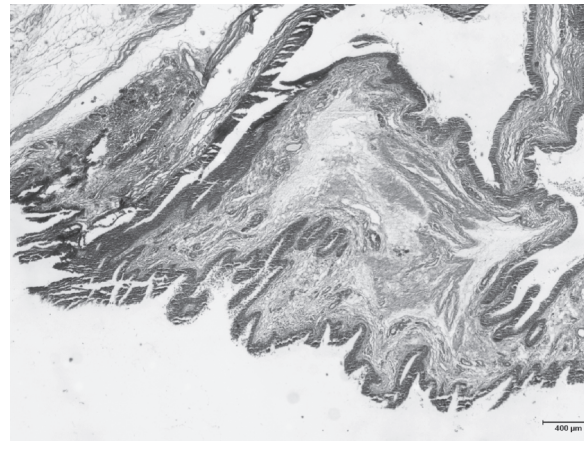

Fig. 4. Caudal area of the medial compartment with two big folds.
Fig. 4. Área caudal del compartimento medial con dos grandes pliegues.

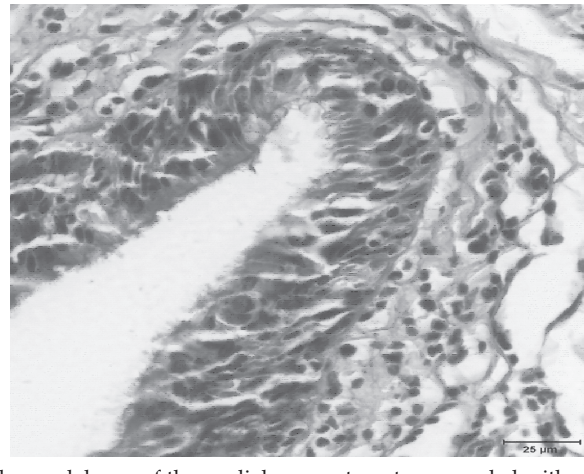

Fig. 5. Ciliate epithelia of the caudal area of the medial compartment surrounded with connective tissue.
Fig. 5. Epitelio ciliado del área caudal del compartimento medial rodeado por tejido conectivo.

The shaped like slot entrance to the auditory tube, which is the nasopharyngeal access to the guttural pouch, has a length of 20 mm in the foal, 45 mm in midsize horses and 75 mm in big horses. The guttural pouch meats caudoventral the stylohyoid bone, dividing into a medial and a lateral compartment. Between the right and left guttural pouch, in the dorsal side of the skull basis, lie *Musculus rectus capitis ventralis* and *Musculus longus capitis*. These muscles insert on the muscular tuberculum of the basisphenoidal bone and produce a flexion in the atlanto-occipital joint. In front of these muscles both guttural pouches contact each other, so that they are actually separated only by a double mucosal layer. These mucosal layers are subordinate by elastic and collagen fibers. In the sub mucosal layer we can find lymphatic nodules and few mixed glands. The thickness of the guttural pouch wall is about 0.5 mm.

In the caudal area of the medial compartment we find the neurovascular fold. In the rostral side of this fold a smaller fold diverts, containing lateral the hypoglossal nerve and medial the glossopharyngeal nerve with his lingual and pharyngeal branch. This fold is called *Plica accessoria neuralis*. The external carotid artery runs between the glossopharyngeal nerve (medial) and hypoglossal nerve (lateral) outside the guttural pouch (Figs. 6, 7, 8, 9).

The facial nerves together with the *Chorda tympani* run over the roof of the lateral compartment of the guttural pouch. Besides this compartment we find the maxillary artery and vein. The medial retropharyngeal lymph nodules lie ventral to the medial compartment of the guttural pouch and the lateral retropharyngeal lymph nodes are located caudoventral to the medial compartment.

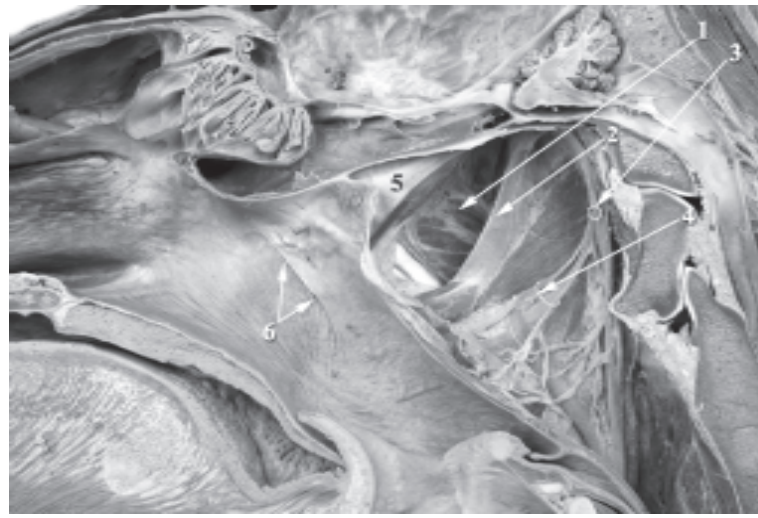

Fig. 6. Neurovascular structures next to the guttural pouch. 1. Lateral compartment of the guttural pouch, 2. *Stylohyoideum*, 3. *Plica neurovasculosa*, 4. *Plica accessoria nervosa*, 5. Auditory tuba, 6. *Ostium pharyngeum tubae auditivae*.
Fig. 6. Estructuras neurovasculares adyacentes a la bolsa gutural. 1. Compartimento lateral de la bolsa gutural, 2. Stylohyoideo, 3. Pliegue neurovascular, 4. Pliegue nervioso accesorio, 5. Conducto auditivo, 6. Apertura faringea del conducto auditivo.

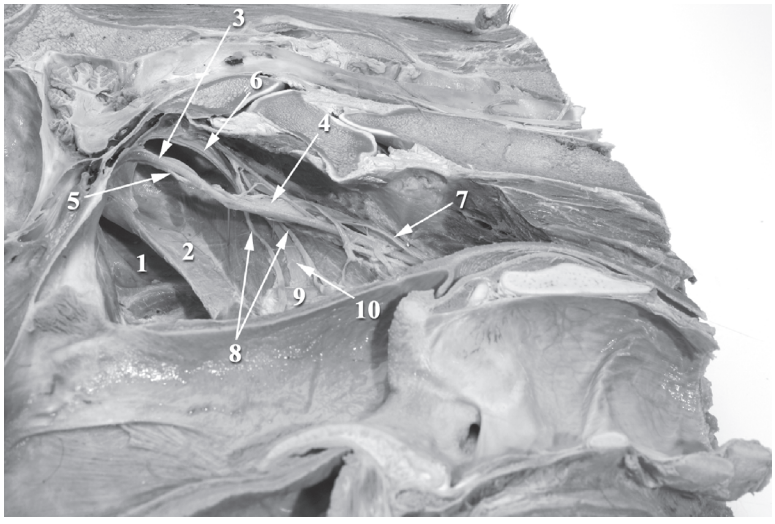

Fig. 7. Vessels and nervous structures in the medial compartment. 1. Lateral compartment of the guttural pouch, 2. *Stylohyoideum,* 3. Internal carotid artery, 4. Cranial cervical ganglion, 5. Internal carotidal nerve, 6. Vagal nerve, 7. Accessorial nerve, 8. Glossopharyngeal nerve, 9. External carotid artery, 10. Hypoglossal nerve.
Fig. 7. Vasos y estructuras nerviosas del compartimento medial. 1. Compartimento lateral de la bolsa gutural, 2. Stylohyoideo, 3. Arteria carótida interna, 4. Ganglio cervical craneal, 5. Nervio carotideo interno, 6. Nervio vago, 7. Nervio accesorio, 8. Nervio glosofaringeo, 9. Arteria carótida externa, 10. Nervio hipoglosso.

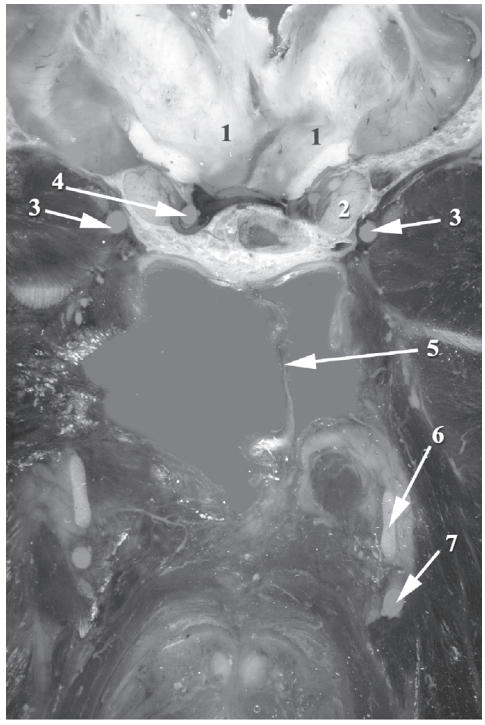

Fig. 8. Plastinated cross section of the guttural pouch. 1. Hypothalamus, 2 Maxillary nerve, 3. Internal carotid artery, 4. Cerebral carotid artery, 5. Dividing wall between the guttural pouch compartments, 6. *Stylohyoideum*, 7. External carotid artery.
Fig. 8. Sección transversal de bolsa gutural plastinada. 1. Hipotálamo, 2. Nervio maxilar, 3. Arteria carótida interna, 4. Arteria carótida cerebral, 5. Pared divisoria entre los compartimentos de la bolsa gutural, 6. Stylohyoideo, 7. Arteria carótida externa.

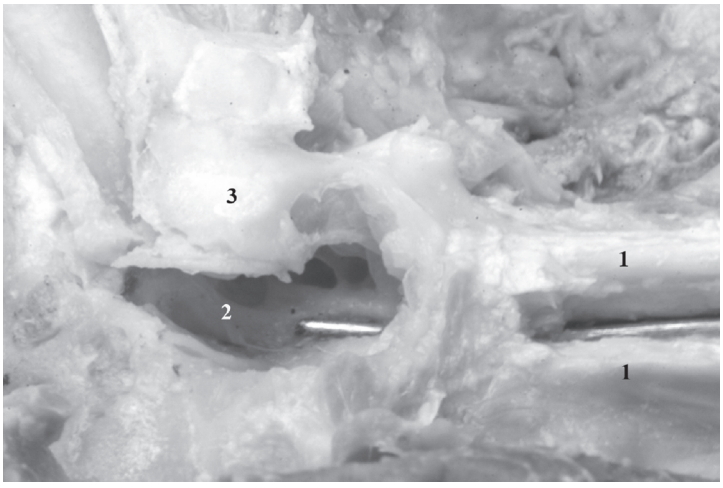

Fig. 9. Probe inserted into the tympanic opening of the auditory tuba. 1. Auditory tuba, 2. Tympanic cavity, 3. Styloid processus.
Fig. 9. Sonda insertada en la abertura timpánica de la tuba auditiva. 1. Tuba auditiva, 2. Cavidad timpánica, 3. Proceso stiloideo.

DISCUSSION

The guttural pouch has been described in literature since the beginning of the veterinary science. The first to describe this structure was the French anatomist Claude Bourgelat in 1764 (Richter, 1923). The guttural pouch is only present in horse and mule, and therefore different authors had a controversial interpretation of its function (Richter, 1923). This structure does not influence the horse hearing, swimming or his phonetic ability.

The authors believe that the presence of the guttural pouch could be related with the reduction of the horse head weight. Animals with a high weight head need a pneumatic chamber at the skull base. In cows we can find such a structure at the level of the choane and in addition the paranasal sinuses are greater as in horse. The choane area in horse is very tight and therefore the guttural pouch is situated at the skull base (König, 1984). Some authors indicated that the air which inflows the guttural pouch could cool done the blood temperature of the internal carotid. Even this hypothesis was considered ungrounded (Wissdorf et al., 2002; Baptiste, 1998; Baptiste et al., 2000) and the authors of the present article do not find any anatomical indication which could sustain this theory. Although the internal carotid runs through the neurovascular fold of the guttural pouch, there is no indication of the existence of a heat exchange system between the warm blood and the air system of the guttural pouch, especially as the artery is covered by the mucosa. There is no evidence that such a cooling system could function, as we do not find a bifurcation of the internal carotid into a Rete mirabile. Only the ruminant, the cat and the pig have a *Rete mirabile* as a cooling system for the arteries which supply the brain with blood.

Additionally there is no significant temperature difference between the blood and the air in the guttural pouch. It is known that the mucosa of the tympanic cavity can resorb air, but nobody demonstrated that such a resorb could be done in the guttural pouch. Even if this could be theoretical possible the exchange is to slow and volumetric insignificant. The swallow act moves the tuba opening and so there is a low air movement in order to equalize the pressure (Engelke, 1995), but it would be insufficient for cooling the blood flow and inside the guttural pouch there is body temperature.

Authors who sustain the cooling theory (Baptiste, 1998; Baptiste et al., 2000; McConaghy, 1995) did not consider the arterio-venous coupling, between the internal carotid and the venous system. Before entering the skull the internal carotid is coupled with the ventral petrosus sinus. The venous blood in this sinus has a lower temperature than the arterial blood. Inside the skull the arteries which irrigate the brain will pass through the cavernous sinus. Veins which come from the sub mucosa of the nasal pharynx end in the cavernous sinus. During breading this veins will transport blood with a lower temperature and therefore the blood temperature inside the sinus will be lower than in the arteries, building so a cooling system for the arteries, similar to the *Rete mirabile* (Mitchell et al., 2006; v. Engelhardt, 2010).

The guttural pouch with its warm and saturated air inside, consist a wonderful ambiance for germ growth, ending in respiratory diseases (Grabner,

1984). Fungal infections of the guttural pouch could lead to destruction of the mucosa and even erode the arterial wall, ending with a deadly epistaxis (Berg and Budras , 2009; König et al., 2010). The fold structure of the guttural pouch mucosa is a further condition which could favor germinal growth. The internal carotid artery is the most endangered structure as she has tide relations with the guttural pouch mucosa. Fungal infections could spread through the auditory tuba into the middle ear (Frey, 2006).

Nervous structures which are embedded in the folds of the guttural pouch are irritated during infectious diseases, leading to different symptoms; facial nerve: paralysis; glossopharyngeal nerve: pharyngeal dysphagia; vagal nerve: pharyngeal dysphagia, laryngeal skirl, dysfunction of thoracic and abdominal organs; accessory nerve: oblique posture of the neck; hypoglossal nerve: tongue paralysis/ atrophy; sympathetic trunk: Horner syndrome (Budras and Röck, 2009; Kipar and Freese, 1992). Even the vestibulocochlear nerve could be affected although he has no direct contact with the guttural pouch (Berg and Budras, 2009; Wissdorf et al., 2002).

The lymph nodules groups around the guttural pouch could be infected in young animals and lead to abscess, which can penetrate into the guttural pouch. In young horses we can remark lesions of the head flexors; described as rupture of the insertion of the ventral rectus capitis and longus capitis muscle. This type of lesion can lead to bleeding into the guttural pouch (Vandenhoven, 2010).

Endoscopic surgery uses for the guttural pouch access the naso-pharynx and the auditory tuba. (König and Liebich, 2009; McCarthy, 1990; Constantinescu et al., 1997; Dyce et al., 2009; Wissdorf et al., 2002, König et al., 2010). Surgeons can orientate themself by inspecting the direct access of the auditory tuba. The pharyngeal opening of the auditory tuba lies at the level of the lateral corner of the horse eye and has a caliber between 20 - 75 mm. During the swallow act we can see a mucosal fold through the opening of the auditory tuba, which corresponds to the Eustachian tube and is related to the guttural pouch (Skoda, 1911; Sisson, 1975). This fold is very important for orientation during endoscopic surgery when accessing the guttural pouch and is called *Plica oclusiva* described by Skoda, 1911. The access of the guttural pouch through the lateral wall over the Viborgs triangle is only an exception (Otto et al., 1995; Fey, 2006; Wissdorf et al., 2002).

The name of the fold of the guttural pouch *Plica neurovasculosa, Plica accesoria neuralis* and *Plica occlusiva* are not mentioned neither in the Nomina Anatomica Veterinaria NAV (2005) nor in the illustrated Nomina (Schaller, 1992).

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