

AN ABSTRACT OF THE THESIS OF

Ismael Concha-Albornoz for the degree of Master of Science in Veterinary Science presented on March 12, 2010.

Title: Anatomy of the Osseous External Acoustic Meatus, Middle Ear and Surrounding Soft Tissue in Llamas (*Lama glama*)

Abstract approved:

Kathy Magnusson

Susanne M Stieger-Vanegas

Llamas (*Lama glama*) appear to have predisposing anatomical features for developing otitis media such as a long and narrow external acoustic meatus and a trabecular tympanic bulla. However, there is limited information available about the morphology of the ear in this species. The aim of this study was to evaluate the osseous structures of external acoustic meatus, tympanic cavity and tympanic bulla using CT, and the soft tissue surrounding the ear using dissections. Ten heads were collected from healthy llamas slaughtered for meat production. Using a CT scanner with slices acquired at 1 mm, measurements of the bony structures of the external and middle ear of each head were obtained. The surrounding soft tissue was examined using dissection, a 6-inch protractor and a digital caliper. The osseous external acoustic meatus was ventrally curved with an obtuse angle facing ventrally. Its narrowest portion was located medially at the level of the tympanic annulus. The conformation of the tympanic bulla was the most different in appearance compared to other domestic animals. It was divided into caudo-lateral and caudo-medial processes, body, apex, and stylohyoid fossa to study its morphometry. The interior of the tympanic bulla had a honeycombed structure with pneumatized cells similar to the human's mastoid process. The nerves, vessels, muscles

and tendons had the general distribution of those structures in herbivorous domestic animals. The present study supplied new information about the shape and measurements of the osseous external and middle ear and surrounding soft tissue in adult llamas. This study also supplied specific landmarks of the location of these structures in relationship with each other. Based on our observations and measurements, a new surgical approach to perform a tympanic bulla osteotomy was suggested to treat otitis media in llamas.

© Copyright by Ismael Concha-Albornoz

March 12, 2010
All Rights Reserved

Anatomy of the Osseous External Acoustic Meatus, Middle Ear and Surrounding Soft
Tissue in Llamas (*Lama glama*)

by
Ismael Concha-Albornoz

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented March 12, 2010
Commencement June, 2010

Master of Science thesis of Ismael Concha-Albornoz presented on March 12, 2010.

APPROVED:

Co-Major Professor, representing Veterinary Science

Co-Major Professor, representing Veterinary Science

Dean of the College of Veterinary Medicine

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Ismael Concha-Albornoz, Author

ACKNOWLEDGEMENTS

I would like to thank all my family in Chile especially my parents, Olga and Ernesto. Their education, imagination and artistic talent proved that everything is possible with hard work and creativity. Thank you also to my wife, Paola, for her patience, unconditional understanding and love in these years of our adventure in Oregon. Thank you to my children, Benjamin and Catalina, for giving love, endless energy and happiness to my life.

I would like to express sincere appreciation and thanks to the members of my Committee: Dr. Kathy Magnusson, Dr. Susanne Stieger-Vanegas, Dr. Terri Clark, Dr. Christopher Cebra, and special gratitude and thanks to Dr. Karen Timm. Their help and advices were critical to finishing this study.

Lastly, I would like to thank all of our friends who have contributed their support, friendship, advices, encouragement and company during this journey.

TABLE OF CONTENTS

Chapter 1- Introduction.....	2
Chapter 2- Literature Review.....	5
2.1. General anatomy of the external and middle ear and surrounding soft tissue structures.....	5
2.2. Studies of the ear anatomy using diagnostic imaging techniques.....	6
2.3. Otitis externa and media in bovines and humans.....	7
2.4. Surgical treatment for ear disease.....	8
2.5. Ear anatomy and otitis in South American Camelids (SAC).....	10
Chapter 3- Hypothesis.....	12
Chapter 4- Material and Methods.....	13
4.1. Sample collection.....	13
4.2. CT images.....	13
4.3. Preservation protocol.....	14
4.4. Latex injection.....	15
4.5. Dissection.....	15
4.6. Statistics.....	17
Chapter 5- Results.....	19
5.1. CT Images.....	19
5.1.1. Osseous external acoustic meatus (OEAM).....	21
5.1.2. Tympanic cavity (TC).....	22
5.1.3. Tympanic bulla (TB).....	23
5.1.4. Measurements of the osseous external acoustic meatus, tympanic cavity and tympanic bulla using CT.....	27
5.2. Dissections.....	30
5.2.1. Skin.....	30
5.2.2. Parotid salivary gland.....	30
5.2.3. Nerves.....	32
5.2.4. Veins.....	33
5.2.5. Arteries.....	35
5.2.6. Muscles and tendons.....	37
5.2.7. Bony structures and foramina.....	38
5.2.8. Dorso-rostral quadrant.....	41
5.2.9. Dorso-caudal quadrant.....	42
5.2.10. Ventro-rostral quadrant.....	43
5.2.11. Ventro-caudal quadrant.....	47
5.2.12. Measurements obtained during dissection.....	48
5.3. Statistical analysis.....	51

TABLE OF CONTENTS (Continued)

Chapter 6- Discussion	53
6.1. Discussion regarding the bony structures evaluated on CT images	53
6.1.1. Osseous external acoustic meatus (OEAM).....	53
6.1.2. Tympanic cavity (TC)	55
6.1.3. Tympanic bulla (TB)	56
6.2. Discussion of soft tissue and bony structures found during dissection	57
6.2.1. Skin.....	57
6.2.2. Parotid salivary gland.....	57
6.2.3. Nerves.....	58
6.2.4. Vessels.....	59
6.2.5. Muscles, tendons and bony structures.....	60
6.2.6. Quadrants and measurements	61
6.3. Proposal for a surgical approach to lateral tympanic bulla osteotomy in llamas...61	
Chapter 7- Conclusions	71
Bibliography.....	73

LIST OF FIGURES

<u>FIGURE</u>		<u>PAGE</u>
1a	Positioning of the protractor to describe the spatial orientation of the structures, with the osseous external acoustic meatus porus as a center point.....	16
1b	Quadrants: DR: dorso-rostral; DC: dorso-caudal; VR: ventro-rostral; VC: ventro-caudal.....	17
2a	Ventral view of the right tympanic bulla of the llama.....	20
2b	Lateral view of the left tympanic bulla of the llama.....	20
3	Axial CT images of the right osseous external acoustic meatus.....	21
4	Coronal reconstructed CT image of the right external acoustic meatus showing the lateral diameter (OEAM6).....	22
5	Axial CT image of the right ear at the level of the head of the malleus (HM).....	23
6	Right axial CT image demonstrating the dorso-ventral height (CLp1), proximal medio-lateral width (CLp2), and distal medio-lateral width (CLp3) of the caudo-lateral process of the tympanic bulla.....	24
7	Right axial CT images demonstrating a- caudo-proximal width (B1) and caudo-distal width (B2) immediately rostral to the stylohyoid fossa.....	25
8	Right reconstructed coronal CT image of the tympanic bulla showing the caudo-rostral length (CRL) and caudo-rostral angle of the caudo lateral process & body of the tympanic bulla.....	26
9	Left parotid salivary gland and surrounding soft tissue of the llama.....	32
10	Nerves related with the tympanic bulla of the llama. Left view.	33
11	Veins surrounding the tympanic bulla of the llama. Left view.....	35
12	Arteries surrounding the tympanic bulla of the llama. Left view.....	37

LIST OF FIGURES (Continued)

<u>FIGURE</u>		<u>PAGE</u>
13	Muscles, tendons and ligaments surrounding the tympanic bulla in the llama. Left view.....	38
14	Bony structures and foramina surrounding the tympanic bulla in the llama. Left view.....	39
15	The general anatomical panorama and the structures distributed in the quadrants DR dorso-rostral, DC dorso-caudal, VR ventro-rostral and VC ventro-caudal in a left view of the head.....	40
16	Deep dissection demonstrating the structures located in the dorso-rostral quadrant.....	41
17	Deep dissection showing some of the structures found in the dorso-caudal quadrant.....	41
18	Superficial dissection of the structures found in the ventro-rostral quadrant.....	44
19	Deep dissection of the structures found in the ventro-rostral quadrant after dissection and reflection of the superficial part of the parotid gland.....	45
20	Deepest dissection of the structures found in the ventro-rostral quadrant after dissection and reflection of the superficial and deep portions of the parotid gland.....	46
21	Deep dissection of the structures found in the ventro-caudal quadrant after dissection and reflection of the superficial and deep portions of the parotid gland.....	48
22	Left lateral view of a llama head and neck showing the landmarks to be used during the lateral bulla osteotomy and the area to be clipped for the surgery.....	65
23	Left lateral view of a llama head and neck showing the location of the skin incision.....	65
24	Left lateral view of a llama head and neck showing the structures to be identified after reflection of the skin.....	66

LIST OF FIGURES (Continued)

<u>FIGURE</u>		<u>PAGE</u>
25	Left lateral view of a llama head and neck showing the caudal projection of the parotid gland separated from the jugular vein and sternomandibularis tendon.....	67
26	Left lateral view of a llama head and neck showing the tympanic bulla and related structures.....	68
27	Left tympanic bulla of a llama after the opening of the periosteum and external wall.....	69
28	Left lateral view of a llama head and neck showing the tympanic bulla after the opening of the periosteum and the angle to introduce the curette.	70

LIST OF TABLES

<u>TABLE</u>		<u>PAGE</u>
1	Width, height and angle of the osseous external acoustic meatus (OEAM)...	27
2	Measurements of the tympanic cavity (TC).....	28
3	Measurements of the tympanic bulla (TB).....	29
4	Measurements of the caudo-lateral process and body.....	30
5	Skin thickness.....	48
6	The parotid salivary gland and duct.....	49
7	The nerves.....	49
8	Orientation of the nerves and parotid salivary duct in degrees.....	50
9	Location of the vessel.....	50
10	Orientation of the vessels in degrees.....	51
11	Location of bony structures and foramina.....	51

**Anatomy of the Osseous External Acoustic Meatus, Middle Ear and Surrounding
Soft Tissue in Llamas (*Lama glama*)**

Chapter 1- Introduction

South American Camelids (SAC), especially llamas and alpacas, are valuable animals for their wool, as pets or working animals and, in some countries, as a food source. SAC, such as llamas, have predisposing anatomical factors for otitis, however there is scarce information available about ear anatomy of these species and further studies of surgical treatments for otitis media in llamas are needed.¹

Anatomically, the ear or vestibulocochlear organ is divided into three segments: the external, middle, and inner ear.² The external ear includes the pinna and the external acoustic meatus.³ The middle ear contains the tympanic cavity, which is defined by six walls.⁴ In domestic animals, the tympanic cavity is extended ventrally by a bulbous expansion of the tympanic portion of the temporal bone called the tympanic bulla.⁵ Studies using imaging techniques have provided important details about ear anatomy of certain species including camels,⁶ dogs,^{7,8} California sea lions,⁹ and humans.¹⁰⁻¹² Nevertheless, there are limited studies that include measurements of the external and middle ear. Recently, morphometric research using computed tomography (CT) was performed on the sheep ear.¹³ Other techniques, such as radiography or ultrasonography, cannot be used reliably to identify structures in the inner or middle ear.¹⁴

Some of the predisposing, primary, and perpetuating causes of otitis externa are canal conformation, external parasites, foreign bodies, bacteria, and yeast.¹⁴ Advanced clinical cases of otitis can lead to irreversible or fatal neural lesions.¹⁵ Most ear

infections in South American Camelids are not diagnosed until the disease process has advanced to chronic, severe otitis externa or media.¹⁶ It has been suggested that otitis media in alpacas occurs secondary to upper respiratory tract infections and that lateral bulla osteotomy is a suitable treatment option because the tympanic bulla in alpacas is difficult to access through the external acoustic meatus.¹⁶ Surgical management of otitis in SAC, however, can be difficult as the anatomical ear conformation in SAC is complex.¹⁷

The tympanic bulla in alpacas is difficult to reach surgically because of the long, narrow external acoustic meatus and the multi-compartmental shape of the tympanic bulla.¹ The osseous anatomy of the camelid ear is complex, making external otoscopic examination difficult.¹⁷ In humans, the middle ear is connected with the pneumatic portion of the temporal bone called the mastoid process. The human mastoid process is a multi-compartmental bone with numerous air cells directly connected with the tympanic cavity.¹⁸ In humans, inflammation and infection of the mastoid process, or mastoiditis, normally originates from otitis media.¹⁹ This pathology can lead to acute complications and can, without treatment, produce grave consequences.²⁰ The conformation of the human mastoid process appears to be similar to the structure of the multi-compartmental tympanic bulla of SAC.

Currently information about the anatomy of the external acoustic meatus, middle ear and surrounding soft tissues in llamas is scarce. No gross anatomical morphometric studies of these areas have been found. The objective of this study was to provide an overview of the normal anatomy of the osseous external acoustic meatus, tympanic

cavity, tympanic bulla and surrounding soft tissue of llamas (*Lama glama*) and to use this information to design a surgical approach to treat chronic otitis media in this species.

Chapter 2- Literature Review

2.1. General anatomy of the external and middle ear and surrounding soft tissue structures

The external ear includes the pinna or auricula formed by the auricular cartilage and the external acoustic meatus (EAM).²¹ In domestic animals, the external acoustic meatus has a distal cartilaginous origin on the concha auriculae, which is the lateral narrow orifice of the auricular cartilage. The annular cartilage joins with the bony portion of the EAM, formed by the tympanic part of the temporal bone. Therefore, the EAM is made up of the lateral cartilaginous part and the medial osseous portion and ends medially at the tympanic membrane or eardrum.³ The osseous external acoustic meatus porus is defined as the junction between the cartilaginous and bony portions of the osseous external acoustic meatus.²¹

The middle ear is composed of the tympanic cavity, auditory ossicles and auditory tube.^{2,3} The tympanic cavity is housed within the petrous and tympanic portions of the temporal bone. This cavity has six paries or walls, which are: dorsal (paries tegmentalis), caudo dorsal (paries mastoideus), caudo ventral (paries jugularis), medial (paries labyrinthicus), rostral (paries caroticus), and lateral (paries membranaceus).⁴ In domestic animals, the tympanic cavity is extends ventrally into a bulbous expansion of the tympanic portion of the temporal bone called the tympanic bulla (TB).⁵ This conformation creates three spaces in the tympanic cavity lined by mucous membrane: the

epitympanic recess situated dorsally, the atrium in the middle, and the large tympanic bulla ventrally.²² The function of the tympanic bulla is not known with certainty, but it has been suggested that it may improve the perception of sounds of very low and very high frequencies.⁵

The key soft tissue structures surrounding the external and middle ear are vessels, nerves, and glands. The most important vessels are the carotid, caudal auricular, facial, and superficial temporal arteries, and the retroarticular, maxillary, facial, and caudal auricular veins. The facial nerve and parotid salivary gland must also be considered.²³

2.2. Studies of the ear anatomy using diagnostic imaging techniques

Computed tomography (CT) is suitable for studying the anatomy in the external and middle ear.^{7,10,11} CT has been used in children to examine ear structures including the mastoid process and tympanic cavity, and also is an indispensable diagnostic modality for middle and inner ear examination.¹⁰ Alterations in the human external acoustic meatus, such as polyps or cancer of the temporal bone, can be detected using CT images.¹¹ Reconstructive surgery in dogs with external auditory canal atresia can also be performed using CT images as a diagnostic and planning tool.⁷ Morphometric analysis using CT images in ovine ears showed that the tympanic cavity and tympanic bulla are simple compartmental spaces without connection with the mastoid process.¹³ Ultrasonography has been successfully used in rabbits to evaluate the normal anatomy of the tympanic bulla, a good correlation between ultrasound images and gross dissections was present.²⁴

2.3. Otitis externa and media in bovines and humans

Otitis is classified according to the location of the inflammatory process in the external, middle or inner ear. Otitis externa is the most common ear disease encountered in veterinary practice.¹⁴ Inflammation of the ear skin and epithelium of the external acoustic meatus can affect cattle of all ages, in isolated cases, in an entire herd, or in entire regions.²⁵ The most common etiological agents in tropical and subtropical areas are rhabditiform nematodes and mites of the genus *Raillietia*.²⁶ The prevalence of otitis is highest in adult and Zebu cattle.²⁶

The pathogenesis of middle ear infections in calves is not fully understood. The extension of infection from the pharynx via the auditory tube (Eustachian tube) is the most common means of entry into the middle ear.²⁷ In bovines, chronic ear infection involving the middle ear can lead to inner ear dysfunction, paralysis of the facial and vestibulo cochlear nerves, as well as meningitis.²⁸ Ear infection in calves has been linked with concurrent respiratory diseases and mixed infections. The main bacterial agents of otitis in calves are: *Actinomyces spp.*, *Corynebacterium pseudotuberculosis*, *Escherichia coli*, *Haemophilus somnus*, *Pasteurella multocida*, *Mannheimia haemolytica*, *Pseudomonas spp.*, *Streptococcus spp.* and *Mycoplasma bovis*.²⁶ Sheep with otitis media often respond well to five days of antibiotic treatment, with the use of consecutive doses of procaine penicillin or trimethoprim-sulphonamide.²⁹⁻³¹

In humans, diseases of the middle ear region are among the most frequent ear diseases. Acute otitis media is typically caused by invasion of bacteria through the auditory tube into the tympanic cavity.³² Inflammation and infection of the mastoid process in humans normally originates from otitis media, and is called mastoiditis.¹⁹ This pathology can lead to acute complications, and without treatment, can produce grave consequences.²⁰ Although mastoiditis is a serious illness, most children recover well after mastoidectomy or treatment with intravenous antibiotics. Treating these otitis media episodes, however, may pose a larger public health problem in terms of antibiotic resistance in humans.³³

2.4. Surgical treatment for ear disease

Vertical canal ablation is used to improve the drainage and ventilation of the external acoustic meatus, and to preserve hearing in dogs. It includes total removal of lateral vertical canal tissue. Middle ear surgery is recommended when medical treatment is either no longer effective or deemed unlikely to be effective, because of chronicity or ineffective antibiotic treatment.¹⁴ Lateral or ventral bulla osteotomy in the dog can be performed with or without total ear canal ablation.¹⁴ Lateral bulla osteotomy (LBO) in dogs is often performed along with total ear canal ablation to expose and debride tissue and exudates deep within the tympanic cavity.

The following is a recommended technique:²³

Ear Canal Ablation

Step 1-Initial approach to the ear canal: Make a vertical incision directly over the ear canal from the intertragic notch to the ventral aspect of the ear canal. Make a transverse

incision below the tragus. Reflect the subcutaneous tissue with scissors until the entire lateral face of the vertical canal is exposed.

Step 2-Soft tissue dissection to free the ear canal: Create deep tunnels rostral and caudal to the ear canal with scissors. Closely dissect soft tissue from the ear canal to avoid possible damage to blood vessels medial to the pinna cartilage. Using scissors, begin cutting around the diseased ear canal. Be careful to avoid damage to the facial nerve located caudally.

Step 3-Facial nerve isolation, ear canal amputation: Carefully hold the facial nerve by retracting perineural tissue and dissect any attachments of the ear canal to free the nerve. Cut a medial peripheral branch of facial nerve to the ear canal and use it to locate the main trunk of the nerve. Cut the base of the ear canal pointing the sharp part of the blade away from the nerve. Remove the ear canal tissue.

Lateral Bulla Osteotomy

Step 4-Facial nerve protection, lateral bulla exposure: Using elevators, remove soft tissue from the rostral and caudal aspect of the tympanic bulla. Be careful with the facial nerve during this procedure. Keep elevation of soft tissue confined to the bulla to avoid damage to deeper vascular structures. Expose the entire lateral aspect of the bulla. Identify the maxillary artery located ventral to the bulla.

Step 5-Ventral bone slot in osseous ear canal, epithelium removal: Using ronguers, make a slot into the ventral aspect of the osseous external acoustic meatus, creating free ridges of epithelium of the osseous meatus. Create a larger slot in the ventral floor of the canal. Pick up the free rostral and caudal edges of epithelium and elevate it. During this procedure, identify the retroarticular vein rostral to the bulla.

Step 6-Ventrolateral tympanic bulla removal: Carefully elevate soft tissue from the caudal aspect of the bulla. Remove the caudal bony edge of the bulla using ronguers and free remaining epithelium from the osseous canal.

Step 7-Tympanic cavity evacuation and exploration: Remove the epithelium from the tympanic bulla until the tympanic cavity begins to be exposed. Obtain a sample of contents from the tympanic cavity for culture and susceptibility. Remove the floor of the bulla and clean any free edge. Keep track of the facial nerve during this procedure. Remove the epithelium from the rostral aspect of the tympanic cavity close to the entrance of the auditory tube. Be careful to avoid damage to the promontory and ossicles.

2.5. Ear anatomy and otitis in South American Camelids (SAC)

Possible predisposing anatomical factors for otitis in SAC include abnormal stenotic external ear, sigmoid shape of the external acoustic meatus, and the multi-compartmental disposition of the tympanic bulla in the middle ear.¹ A major challenge in treating otitis media and externa in SAC is the difference in anatomy, compared with dogs and cats.¹ The external acoustic meatus (EAM) in llamas is directed slightly rostral and ventral, and at a depth of approximately 1.8 cm bends ventrally at an angle of 120 degrees.¹⁵ The external orifice of the EAM is approximately 6 mm in diameter, narrowing to 4 mm at the bend, and then the canal is a further 1 cm in depth before the tympanic membrane is reached.¹⁵ The tympanic bulla in llamas is large, honeycombed and without a fundic cavity.¹⁵ It has been theorized that the multi-chambered middle ear cavities provide more than one resonant frequency when sound is transmitted thus allowing the animal to distinguish between resonant frequencies.¹⁷ In a recent CT study in alpacas the osseous EAM measured 6-7 mm in width, then narrowed to 3 mm wide near the tympanic membrane.¹⁷ No other descriptions of the anatomy of the ear of South American Camelids were found.

In SAC otitis media occurs more often as a result of upper respiratory infections and, in cases that are refractory to medical treatment, an appropriate option seems to be an entire ear canal ablation and lateral bulla osteotomy.¹⁶ Facial paralysis and Horner's syndrome are typically seen in camelids with ear infection.¹⁵ Surgeons who have attempted surgical treatment of chronic otitis media in these species have found it difficult because of the lack of anatomical studies of the complicated shape of the

external ear and tympanic bulla and of the surrounding soft tissue.¹⁻¹⁶

Chapter 3- Hypothesis

A morphometric description of the surrounding soft tissue of the external and middle ear, osseous external acoustic meatus, and tympanic bulla in llamas will help in describing a new surgical approach for lateral bulla osteotomy, which could be used as a treatment for chronic otitis media in llamas.

Chapter 4- Materials and Methods

4.1. Sample collection

Ten llama (*Lama glama*) heads were obtained from healthy animals with no evidence of external ear or surrounding tissue abnormalities. These animals were slaughtered for meat production and the heads were donated by Mount Angel Meat Company, Inc. located in Mount Angel, Oregon USA. The specimens were from six males and four females. The llamas were slaughtered with a captive bolt to the dorsal aspect of their skull. Each head was separated from the neck at the level of the atlanto-axial joint at the slaughterhouse. All animals were adults. Nine had all molars fully erupted and in wear. Female number two was approximately four years old, her third mandibular molars were erupted but not yet in wear.

4.2. CT images

Computed tomography (CT) of the ten fresh llama heads was performed using a 64 slice Toshiba Aquilion CT scanner (Toshiba America Medical systems, Inc., Tustin, California, USA), with 1 mm slice thickness. Axial (transverse), sagittal, and coronal (horizontal) images and 3D reconstructions were obtained. The external and middle ear were evaluated using a window width (WW 2500) and window level (WL 480). The heads were positioned to ensure the hard palate was parallel to the scanning table. This allows symmetrical comparison of the structures of the head. A pre-scan lateral and dorso-ventral image was obtained to ensure correct positioning. Measurements were

obtained using eFilm (eFilm 2.1, Merge healthcare, Milwaukee, WI).

Size of the head was determined on sagittal CT image at the level of the nasal septum by measurement of the length, or distance, from the rostral border of the incisive bone to the external occipital protuberance. Height of the cranial cavity, or distance from the dorsal internal cortex at the level of the sagittal crest to the internal cortex of the base of the basisphenoides, was obtained on an axial image at the level of the osseous external acoustic meatus. Multiple parameters of the osseous external acoustic meatus (OEAM), tympanic cavity (TC), and tympanic bulla (TB) were measured as shown in Figures 3-8 and listed in Tables 1-4.

4.3. Preservation protocol

All heads were treated with the following preservation protocol on the day of collection.

1. Heads were washed externally with warm water
2. Heads were drained and labeled in the right ear with the letter M for males; with consecutive numbers from M1 to M6. The same procedure was performed for females using the letter F (F1 to F4). This labeling was the same as was used for CT imaging.
3. The vascular system of each head was washed with the use of plastic tubes placed into the common carotid arteries and external jugular veins and 35 ml syringes. In this step, approximately 2 liters of warm water were infused through each head, using both arteries and veins in order to remove as much blood as possible. The

tubes in the external jugular veins were placed rostral to a valve located at the level of the caudal border of the mandible.

4. Fixation was performed via vascular injection of 1 liter of 10 % formalin solution, using the same tubes and syringes mentioned in the previous step. Then the specimens were wrapped with a cloth, which was soaked with the same solution of 10% formalin and placed in plastic bags and plastic boxes.
5. Heads were stored at 0 to 4 °C, for at least 72 hrs. before vascular injection of latex.

4.4. Latex injection

Vascular injection was performed using red latex via the common carotid arteries and blue latex via the external jugular veins in the llama heads. The same tubes and syringes mentioned during the preservation protocol were used. The average volumes of injection for male heads were 50 ml of latex for arteries and 70 ml for veins. For female heads the average volumes were 37.75 ml for arteries and 70 ml for veins. The injected heads were stored in a cold room (0–4° C) for at least 48 hrs. to allow the latex to harden before dissection .

4.5. Dissection

Each llama head was dissected using the lateral bulla osteotomy approach suggested in previous reports of surgical procedure in alpacas,¹⁶ and llamas,¹ and using the technique described in dogs for bulla osteotomy.²³ The goal was to describe the nerves, glands, and vascular structures in the proximity of the external and middle ear. This was performed to create a better anatomical panorama, which could contribute to a

new surgical approach to treat chronic otitis media in llamas. Measurements of bony structures, vessels, nerves, parotid salivary gland, and skin were taken in millimeters with the use of a 6-inch digital stainless steel caliper accurate to 0.01-mm. A 6-inch circular protractor was used to describe the route of vessels and nerves near the tympanic bulla. The horizontal line (0-180°) was parallel to a line traced from the external occipital protuberance to the rostral edge of the body of the incisive bone. The central point of the protractor was placed in the ventral border of the osseous external acoustic meatus porus as shown in Figures 1a and 1b. The 0° mark was always placed caudally. In addition, the structures were classified according to the dorso-rostral, dorso-caudal, ventro-rostral, and ventro-caudal quadrants (Figure 1b).

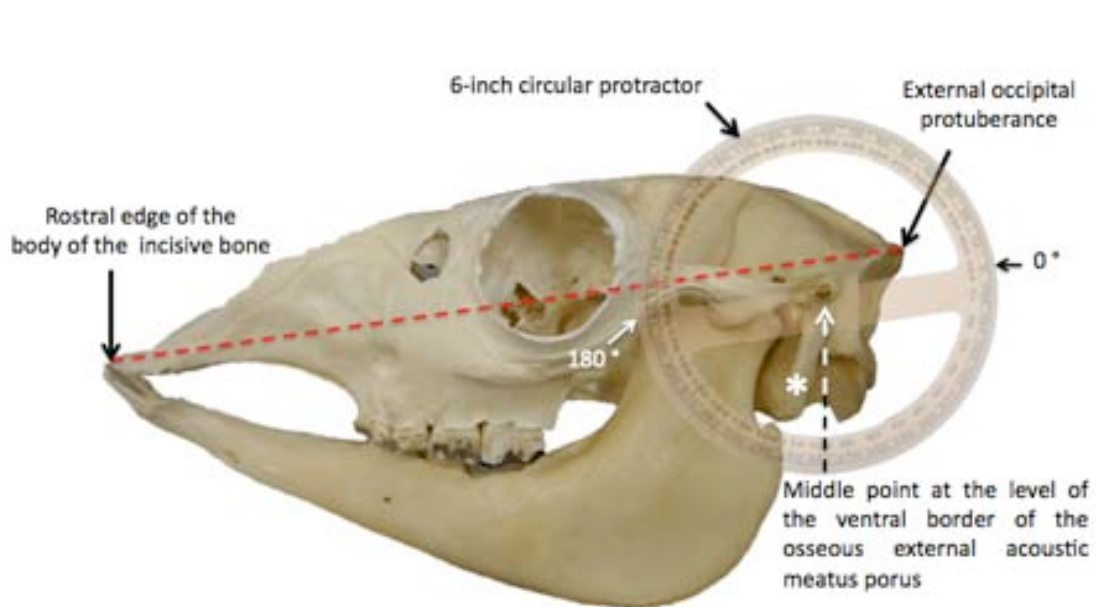


Figure 1a. Positioning of the protractor to describe the spatial orientation of the structures, with the osseous external acoustic meatus porus as a center point. The tympanic bulla is labeled with a *. The 0° mark of the protractor is situated caudally.

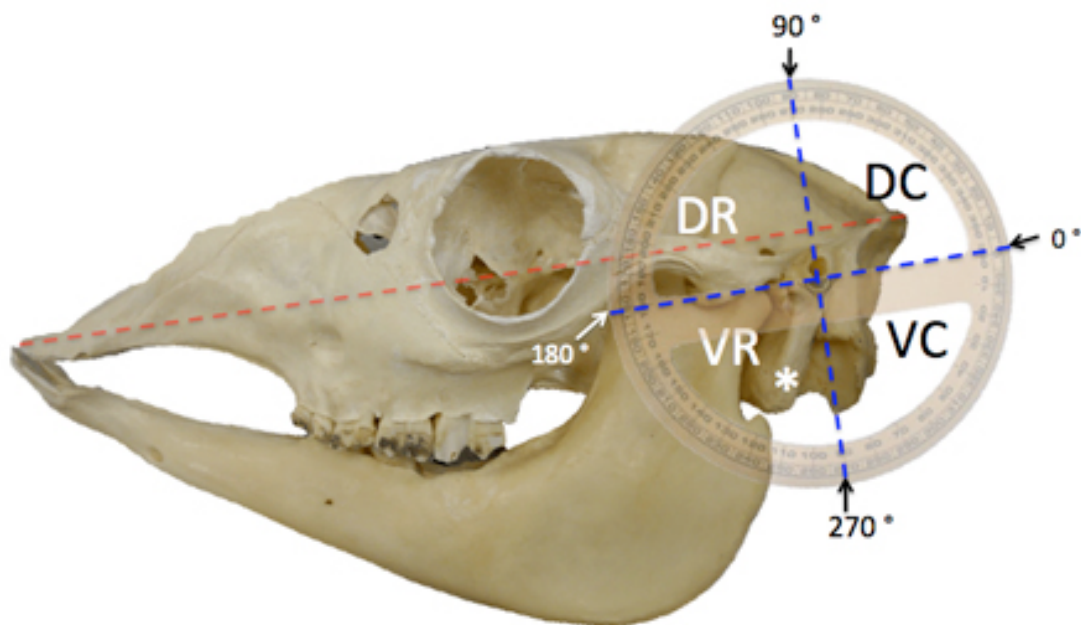


Figure 1b. Quadrants: DR: dorso-rostral; DC: dorso-caudal; VR: ventro-rostral; VC: ventro-caudal.

The structures described were in accordance with a subset of nerves and vessels mentioned in previous studies in alpacas¹⁶ and llamas.¹ A classical text of small ruminant anatomy also was used as a reference.³⁴ Anatomical illustrations of superficial and deep structures laterally surrounding the llama's ear were made based on the assessments made during the dissections. The same protractor and caliper utilized to measure the soft tissue during dissection were also used to produce the illustrations.

4.6. Statistics

The mean in mm (\bar{x}) and the standard deviation (SD) were obtained for each parameter measured in CT images and dissections. Measurements on both sides were compared by 2-way ANOVA for repeated measures with gender and side as the two factors. A two sample independent t-test was run for the comparison between the length

of the heads and the height of the cranial cavities. Because of the large numbers of the parameters tested there was concern that significant results would be found spuriously. A Bonferroni adjustment was used to control for false positives with the multiple comparisons. A p value less than 0.05 was used to evaluate for statistical significance. If a statistical significant different ($p < 0.05$) between the measurements of the females and males was noted, the values of the females and males were listed separately. The program SigmaStat was used to perform the ANOVA and the program Stata 11 was used for the t-test analysis.

Chapter 5- Results

5.1. CT Images

CT images of the osseous external acoustic meatus (OEAM), tympanic cavity (TC) and tympanic bulla (TB) were analyzed morphologically and morphometrically.

For the morphometric study of the complex structure of the tympanic bulla of llamas, this region was divided into caudo-lateral and caudo-medial processes, body and apex (Figure 2a, 2b).

Caudo-lateral (CLp) and caudo-medial processes (CMp) were caudal bony projections of the tympanic bulla separated from each other by the stylohyoid fossa. The body (B) was the bulbous portion of the TB situated rostrally to an imaginary line traced between the rostral border of CLp of the tympanic bulla and the rostral edge of the stylohyoid fossa. The pyramidal shape of the body was situated in such a way that the base faced the caudal processes and the apex was rostrally placed. The apex (A) corresponded to the muscular process of the tympanic portion of the temporal bone. The stylohyoid fossa (SF) was the depression for the tympanohyoid cartilage and its junction with the stylohyoid bone.

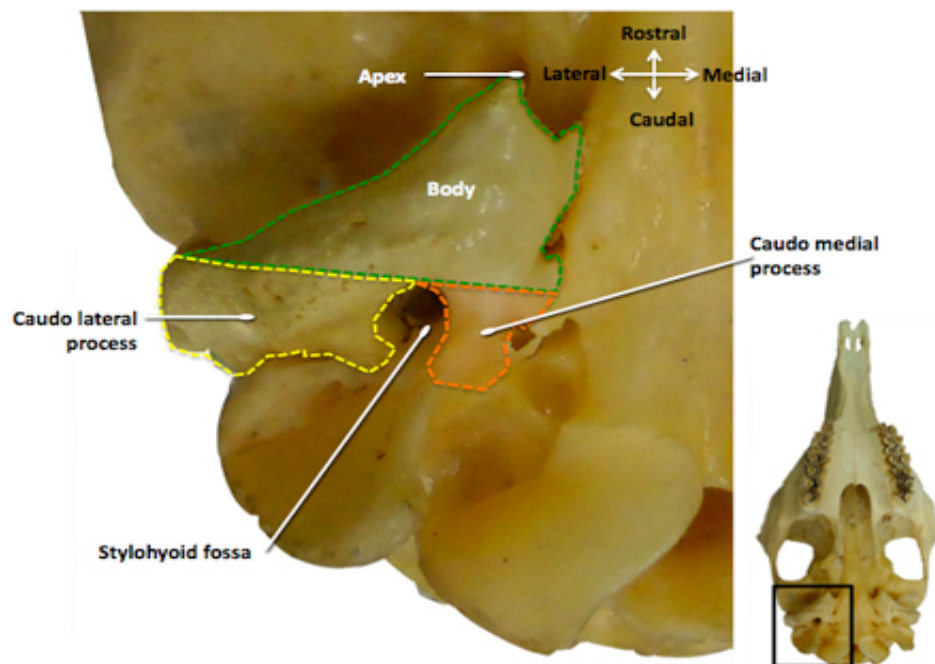


Figure 2a. Ventral view of the right tympanic bulla of the llama. Black box in inset image indicates location of higher magnification image.

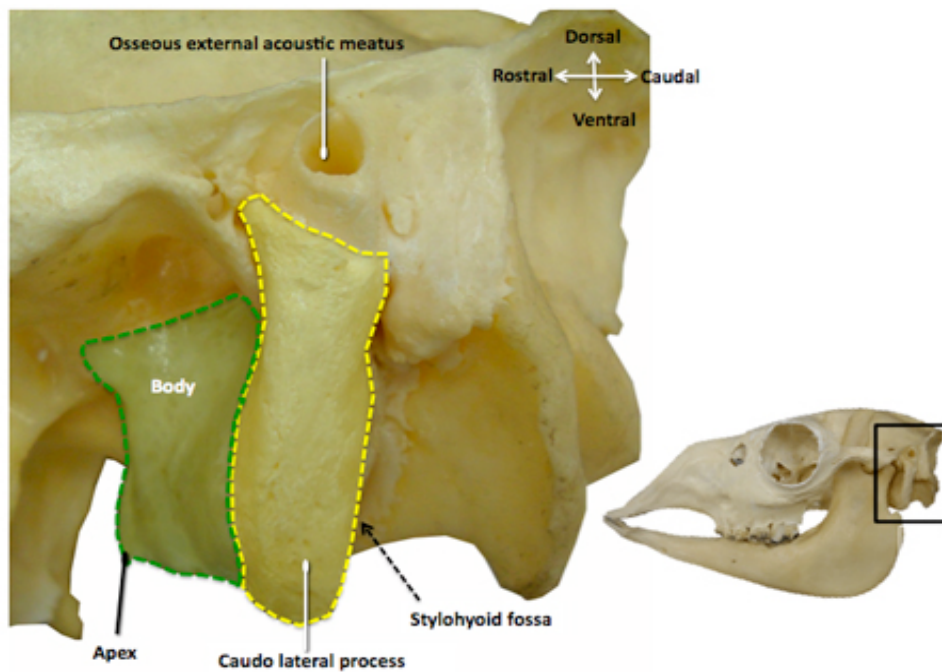


Figure 2b. Lateral view of the left tympanic bulla of the llama. Black box in inset image indicates location of higher magnification image. The mandible was removed in the magnified image to expose the body and apex of the tympanic bulla.

5.1.1. Osseous external acoustic meatus (OEAM)

The OEAM was surrounded by bony structures with air filled cells. The air compartments were most numerous and larger in size ventrally within the tympanic bulla. The largest measurement of diameter of the OEAM was located at the level of the joint with the cartilaginous portion of the canal and the smallest measurement was taken at the level of the incomplete bony ring or tympanic annulus, to which the tympanic membrane was attached. The OEAM was ventrally curved from the latero-dorsal to the ventro-medial aspect of the canal with an obtuse angle facing ventrally (Figures 3, 4, Table 1).

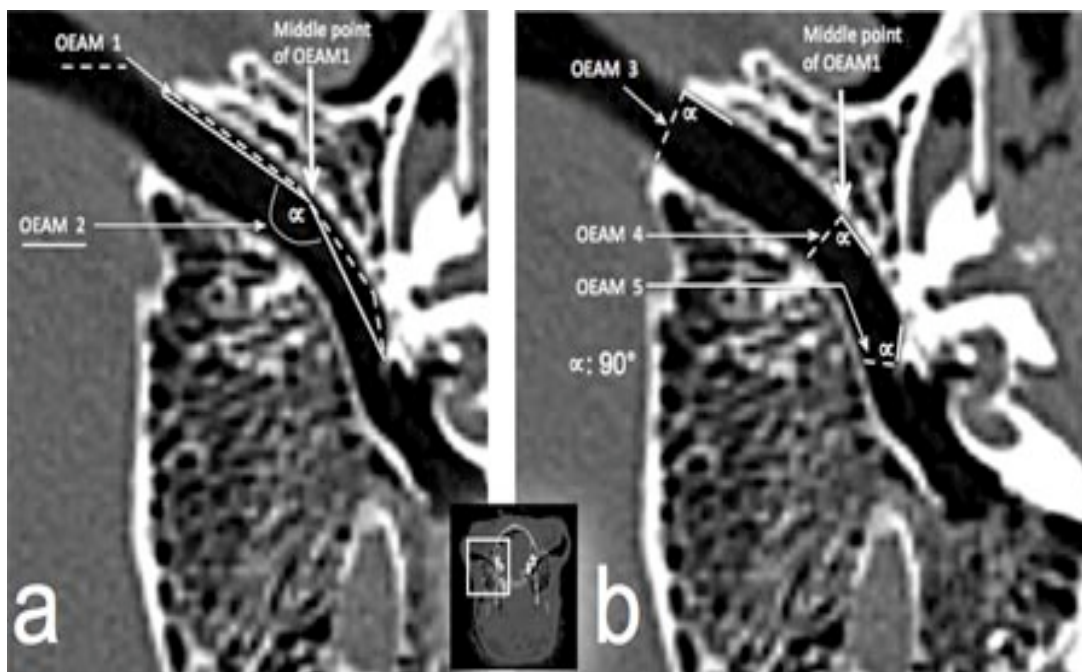


Figure 3: Axial CT images of the right osseous external acoustic meatus. a: The dashed line represents length (OEAM1) of the osseous external acoustic meatus and the solid line shows how the dorso-ventral angle (OEAM2) of the osseous external acoustic meatus was obtained. b: The image demonstrates landmarks for measurements of the lateral vertical diameter (OEAM3), the intermediate diameter (OEAM4), and medial diameter (OEAM5) of the osseous external acoustic meatus. White box in inset image indicates location of higher magnification image.

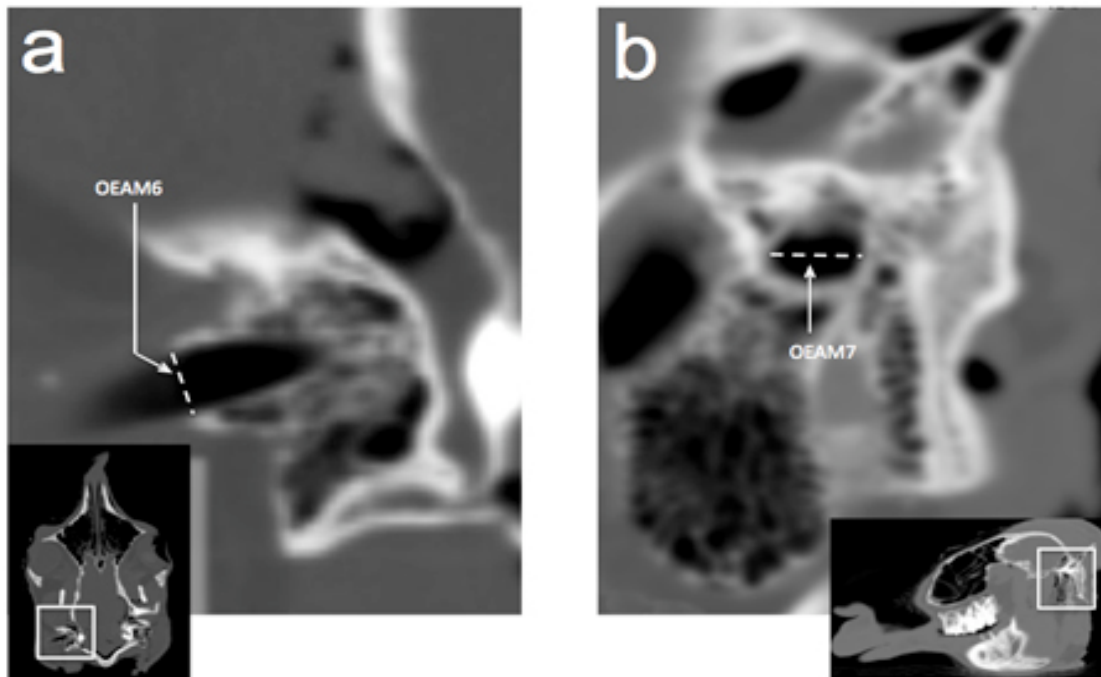


Figure 4: a- Coronal reconstructed CT image of the right osseous external acoustic meatus showing the lateral diameter (OEAM6). b- Sagittal reconstructed CT image showing the horizontal diameter (OEAM7) of the osseous external acoustic meatus. White boxes in inset images indicate location of higher magnification image.

5.1.2. Tympanic cavity (TC)

Due to the absence of a fundic cavity, the tympanic bulla was considered a different structure to be measured and not part of the tympanic cavity assessments. The largest of the TC diameters was the rostro-caudal (TC3), followed by the dorso-ventral (TC1), and the latero-medial (TC2) diameter. The walls of the tympanic cavity, structures and spaces, such as epitympanic recess, malleus, promontorius, auditory tube, and the communication area with the tympanic bulla in the ventral wall were clearly identified. The tympanic membrane was not identified in all cases (Figure 5, Table 2).

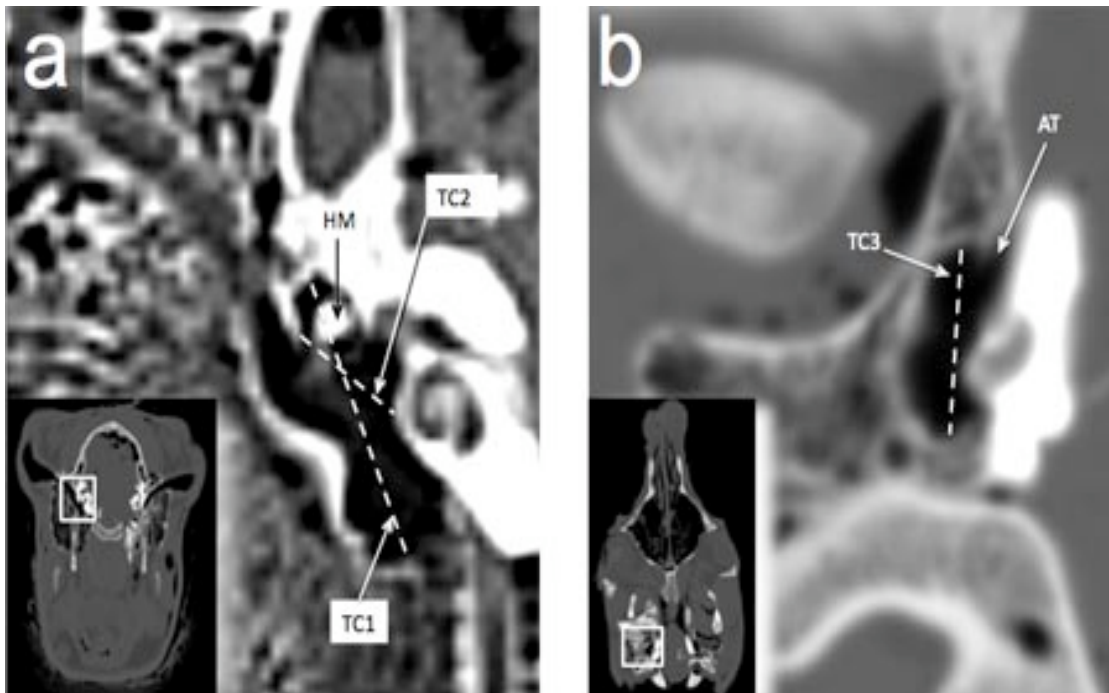


Figure 5: a: Axial CT image of the right tympanic cavity at the level of the head of the malleus (HM). The oblique dorso-ventral height (TC1) and the latero-medial width (TC2) are shown. b: Horizontal reconstructed CT image in which the rostro-caudal length (TC3) of the tympanic cavity is shown at the level of the maximum medial opening of the auditory tube (AT). White boxes in inset images indicate location of higher magnification image.

5.1.3. Tympanic bulla (TB)

The TB was a honeycombed bony structure with air filled cells throughout. The semi-cylindrical stylohyoid fossa was located in the caudal aspect of the TB. The stylohyoid fossa separated the TB into a larger caudo-lateral and a smaller caudo-medial process. The dorsal half of the fossa was occupied by the tympanohyoid cartilage and the ventral half by the stylohyoid bone. The joint between the cartilage and bone was located within the fossa. The body of the tympanic bulla had a conical shape with the base caudally oriented and communicating with both caudal processes. The rostral aspect was medially oriented. This conformation produced a caudo-rostral and latero-medial

orientation of the body relative to the sagittal plane of the head. The most rostral aspect of the body was the apex (Figures 6, 7, 8, Tables 3, 4).

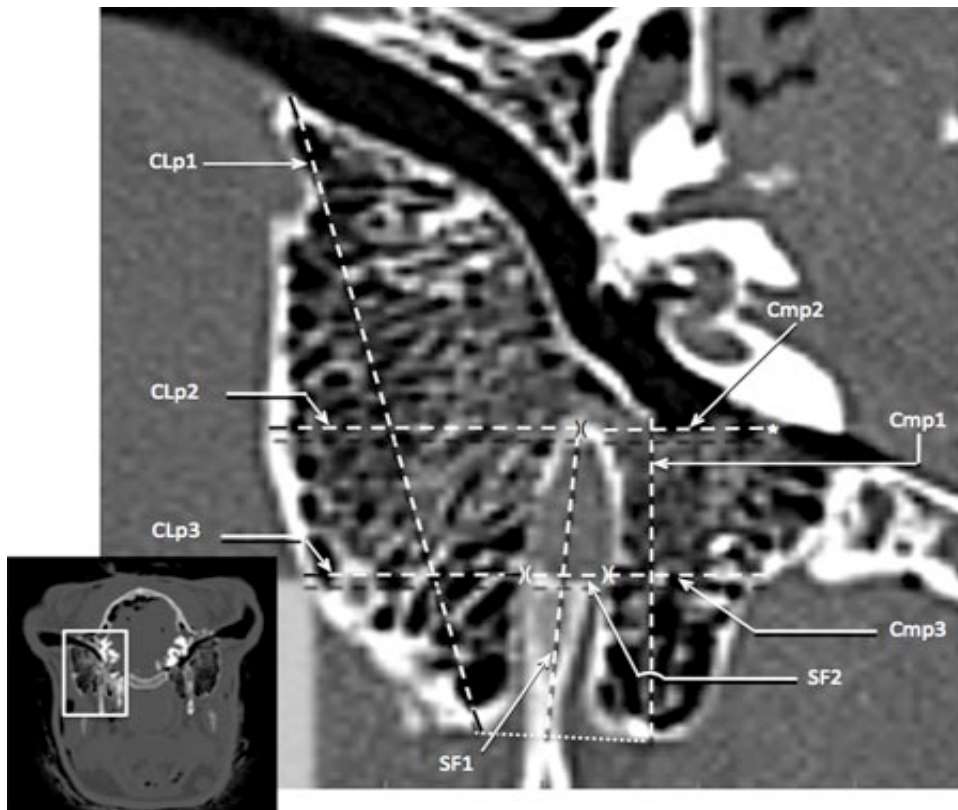


Figure 6: Right axial CT image demonstrating the dorso-ventral height (CLp1), proximal medio-lateral width (CLp2), and distal medio-lateral width (CLp3) of the caudo-lateral process of tympanic bulla; dorso-ventral height (Cmp1), proximal medio-lateral width (Cmp2), and distal medio-lateral width (Cmp3) of the caudo-medial process of tympanic bulla. Also shown are the dorso-ventral height (Sf1) and the medio-lateral diameter of the lumen (Sf2) of the stylohyoid fossa. White box in inset image indicates location of higher magnification image.

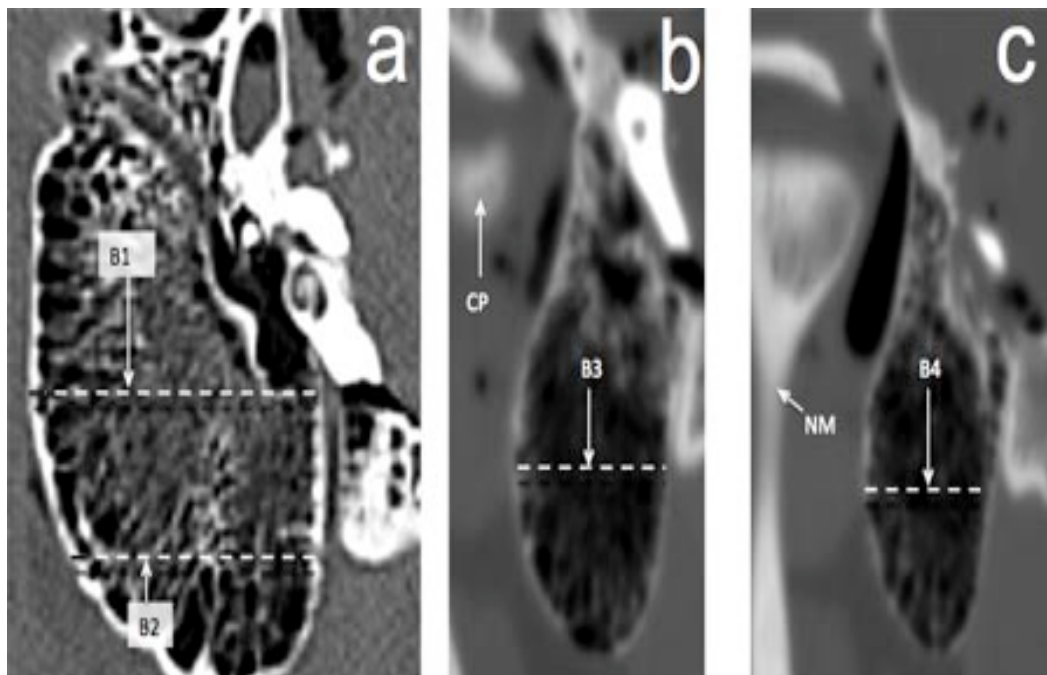


Figure 7: Right axial CT images of tympanic bulla demonstrating a- caudo-proximal width (B1) and caudo-distal width (B2) immediately rostral to the stylohyoid fossa. b- Intermediate width (B3) at the level of the caudal border of the condylar process of the mandible (CP). c- Rostral width (B4) at the level of the caudal border of the neck of the mandible (NM).

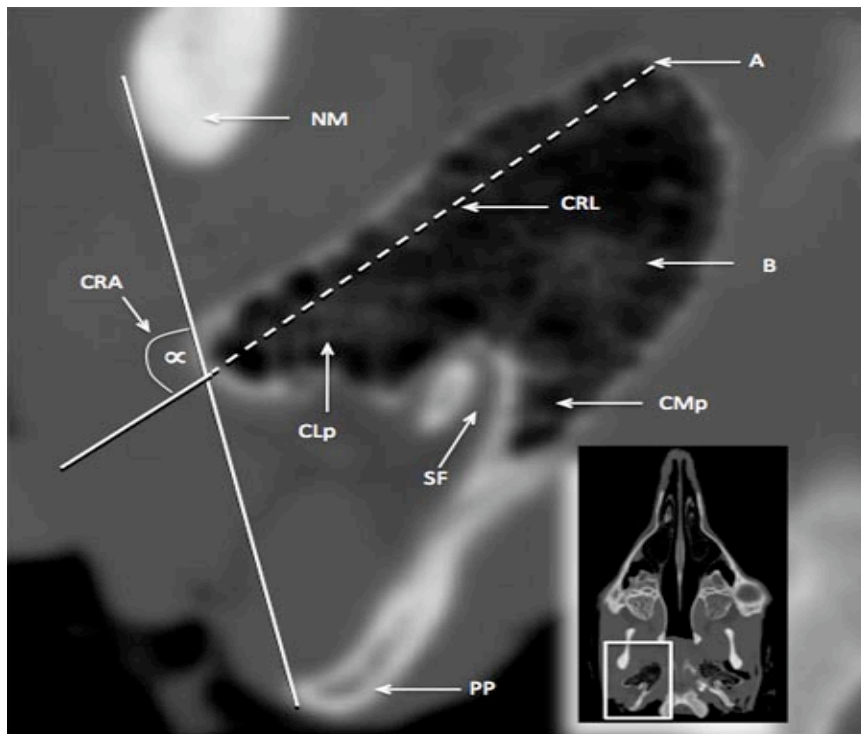


Figure 8: Right reconstructed coronal CT image of the tympanic bulla showing the caudo-rostral length (CRL) (dashed line) and caudo-rostral angle (CRA) of the caudo lateral process and body of the tympanic bulla at the level of the ventral border of the occipital condyle. The caudo-rostral angle is formed by the intersection of a line traced between the caudal border of the mandible (NM) and the paracondylar process (PP) and a second line traced from the most lateral extent of the caudo-lateral process (CLp) to the apex (A) of tympanic bulla. White box in inset image indicates location of higher magnification image.

- | | | | |
|------------|-----------------------|-----------|----------------------|
| A | Apex | SF | Stylohyoid fossa |
| B | Body | NM | Neck of the mandible |
| CMp | Caudo-medial process | PP | Paracondylar process |
| CLp | Caudo-lateral process | | |

5.1.4. Measurements of the osseous external acoustic meatus, tympanic cavity and tympanic bulla using CT

Table 1: Width, height and angle of the osseous external acoustic meatus (OEAM)

CT image used	Code	Landmarks for measurements	Average measurements \pm SD
Axial	OEAM1 ^a *	Length. Line following the dorsal margin of the osseous external acoustic meatus from the porus to the dorsal aspect of the tympanic annulus of tympanic membrane.	Male 25.4 \pm 1.9 mm Female 24.4 \pm 1.9 mm
	OEAM2 ^a	Dorso-ventral angle. Ventral angle between a straight line traced from the dorsal aspect of the external acoustic meatus porus to the middle point of OEAM-1, and a straight line traced from the middle point of OEAM-1 to the dorsal aspect of the tympanic annulus.	144 ^o \pm 9.7 ^o
	OEAM3 ^a	Lateral-vertical diameter. Vertical line from dorsal to ventral border of osseous external acoustic meatus porus, oriented in a 90-degree angle from the dorsal wall of the meatus.	4.7 \pm 0.8 mm
	OEAM4 ^a	Intermediate diameter. Line traced from the dorsal to the ventral wall of the osseous external acoustic meatus, oriented in a 90-degree angle from the dorsal wall at the half way point along OEAM-1.	4.4 \pm 1.0 mm
	OEAM5 ^a	Medial diameter. Line traced from the dorsal to the ventral wall of the osseous external acoustic meatus, oriented at an angle of 90 degrees from the dorsal wall at the level of the tympanic annulus of the tympanic membrane.	2.8 \pm 0.7 mm
Coronal	OEAM6 ^a	Horizontal-lateral diameter. Horizontal diameter of the osseous external acoustic meatus porus.	6.5 \pm 1.4 mm
Sagittal	OEAM7 ^a	Horizontal-medial diameter. Horizontal diameter of the osseous external acoustic meatus at the level of the tympanic annulus.	6.3 \pm 1.3 mm

^a Data from the right side of female 2 is missing because the area in study was fractured.

SD= standard deviation

* p < 0.05 for difference between males and females

Table 2: Measurements of the tympanic cavity (TC)

CT image used	Code	Landmarks for measurements	Average measurements \pm SD
	TC1 ^{a-b-c}	Oblique dorso-ventral height. Line traced from the epitympanic recess to the floor of the tympanic cavity.	13.4 \pm 0.6 mm
Axial	TC2 ^{a-b-c}	Medio-lateral width. Line traced from the promontory to the dorsal aspect of the tympanic annulus of the tympanic membrane.	5.4 \pm 0.8 mm
Coronal	TC3 ^{a-b-c}	Rostro-caudal length. Line traced from the rostral wall (paries caroticus) to the caudal wall (paries mastoideus) of TC at the level of the widest opening of the auditory tube.	14.1 \pm 0.9 mm

^a Data from the right side of female 2 is missing because the area in study was fractured.

^b Data from the right and left side of female 4 is missing for TC1 and TC2, and the right side for TC3 because the image in this area was blurry.

^c Data from the right side of male 5 is missing because the area in study was fractured.

SD= standard deviation

Table 3: Measurements of the tympanic bulla (TB)

Code	Landmarks for measurements	Average measurements ± SD
<u>Caudo-lateral process (CLp)</u>		
CLp1 ^a	Dorso-ventral height. Line traced from ventral border of the osseous external acoustic meatus porus to the most distal edge of CLp.	44.1 ± 2.7 mm
CLp2 ^a	Proximal medio-lateral width. Horizontal line traced from the top of the stylohyoid fossa (SF) to the lateral edge of the CLp external cortex at the level of the maximum depth of SF.	19.3 ± 2.3 mm
CLp3 ^a	Distal medio-lateral width. Line traced from the medial edge of CLp to the lateral edge of CLp at the level of the tympanohyoid cartilage and stylohyoid joint.	13.8 ± 2.4mm
<u>Caudo-medial process (CMp)</u>		
CMp1 ^{a-c}	Dorso-ventral height. Line traced from the floor of tympanic cavity to the distal edge of CMp at the level of the osseous external acoustic meatus.	22.3 ± 2.3 mm
CMp2 ^{a-c}	Proximal medio-lateral width. Line traced from the medial edge of CMp cortex of the petrous portion of the temporal bone to the top of the stylohyoid fossa.	12.1 ± 1.8 mm
CMp3 ^a	Distal medio-lateral width. Line traced from the medial edge of CMp at the level of the joint between the basisphenoides and CMp to the lateral edge of CMp.	10.2 ± 3.4 mm
<u>Stylohyoid fossa (SF)</u>		
SF1 ^a	Dorso-ventral height. Line traced from the top of SF to the most ventral edges of CLp and CMp,	21.6 ± 2.4 mm
SF2 ^a	Medio-lateral diameter of the lumen. Horizontal line traced at the level of the tympanohyoid cartilage and stylohyoid joint	5.1 ± 0.7 mm
<u>Body (B)</u>		
B1 ^{a-b-c}	Caudo-proximal width. Line traced from the medial edge of the body limit of the petrous portion of temporal bone to the lateral edge of the body.	24.6 ± 8.0 mm
B2 ^{a-b}	Caudo-distal width. Line traced rostral to the stylohyoid fossa and meatus from the medial edge of B at the ventral edge of the basisphenoid to the lateral edge of B.	25.9 ± 3.9 mm
B3 ^{a-b}	Intermediate width. Line traced caudal to the temporo-mandibular joint from the medial edge of the body at the ventral edge of the basisphenoid to the lateral edge of B	20.3 ± 2.2 mm
B4 ^{a-b}	Rostral width. Line traced caudal to the neck of the mandible from the medial edge of the body at the ventral edge of the presphenoid to the lateral edge of B.	13.1 ± 4.4 mm
^a Data from the right side of female 2 is missing because this area was fractured. ^b Data from the right side of female 4 is missing because this area was fractured. ^c Data from the right side of male 5 is missing because this area was fractured. SD= standard deviation		

Table 4: Measurements of the caudo-lateral process and body

Code	Description of the measurements obtained	Average measurements \pm SD
CRL	Caudo-rostral length. Line traced from the lateral border of CLp toward the apex of the tympanic bulla at the level of the ventral edge of the occipital condyle.	33.4 \pm 2.6 mm
CRA *	Caudo-rostral angle. Lateral rostral angle formed by the intersection of a line traced between the caudal border of the mandible and the paracondylar process and a second line traced from the most lateral extent of the caudo-lateral process to the apex of tympanic bulla.	Male 113° \pm 3.1° Female 120° \pm 4.1°

SD= standard deviation
* p < 0.05

5.2. Dissections

5.2.1. Skin

The skin was 1.4 \pm 0.4 mm at the level of the external acoustic meatus and 6.4 \pm 0.4 mm at the level of the middle third of the parotid gland.

5.2.2. Parotid salivary gland

The parotid gland was found at the junction of the head and the neck, similar to most domestic animals. In all dissected specimens a strong fascia wrapped around the gland. The ventral insertion of the parotidoauricularis muscle was in the middle third of the superficial surface of the parotid gland and the platysma muscle covered the ventral third of the gland. The dorsal border of the parotid salivary gland was ventral to the annular cartilage of the ear and was not associated with large nerves or vessels. However, rostrally and caudally glandular tissue overlapped the external acoustic meatus. Those projections were referred to as rostral and caudal horns respectively. The rostral horn was associated with the auricular and palpebral branches of the auriculopalpebral nerve and

the superficial temporal artery. The caudal horn was associated with the caudal auricular nerve, caudal auricular vessels and the great auricular nerve. The rostral border was located superficial to the masseter muscle and the temporomandibular joint. From dorsal to ventral, the superficial temporal vein, auriculotemporal nerve, transverse facial vessels, dorsal buccal branch, parotid duct, facial vessels, and the ventral buccal branch arose deep from the rostral border of the parotid gland. The ventral portion of the parotid salivary gland formed a sharp end or apex. The caudal border was cranial to the wing of the atlas and superficial to the paracondylar process of the occipital bone. The parotid salivary gland was associated dorsally with the great auricular nerve and ventrally with the maxillary vein and the tendon of the sternomandibularis muscle. The parotid duct appeared from the distal third of the rostral border of the gland, situated dorsal to the facial vessels and the ventral buccal branch. No accessory parotid salivary glands were found. The superficial temporal, caudal auricular, transverse facial, and facial arteries appeared to be the main sources of blood supply to the parotid gland (Figure 9). The measurements of the parotid salivary gland and duct are shown in Table 6.

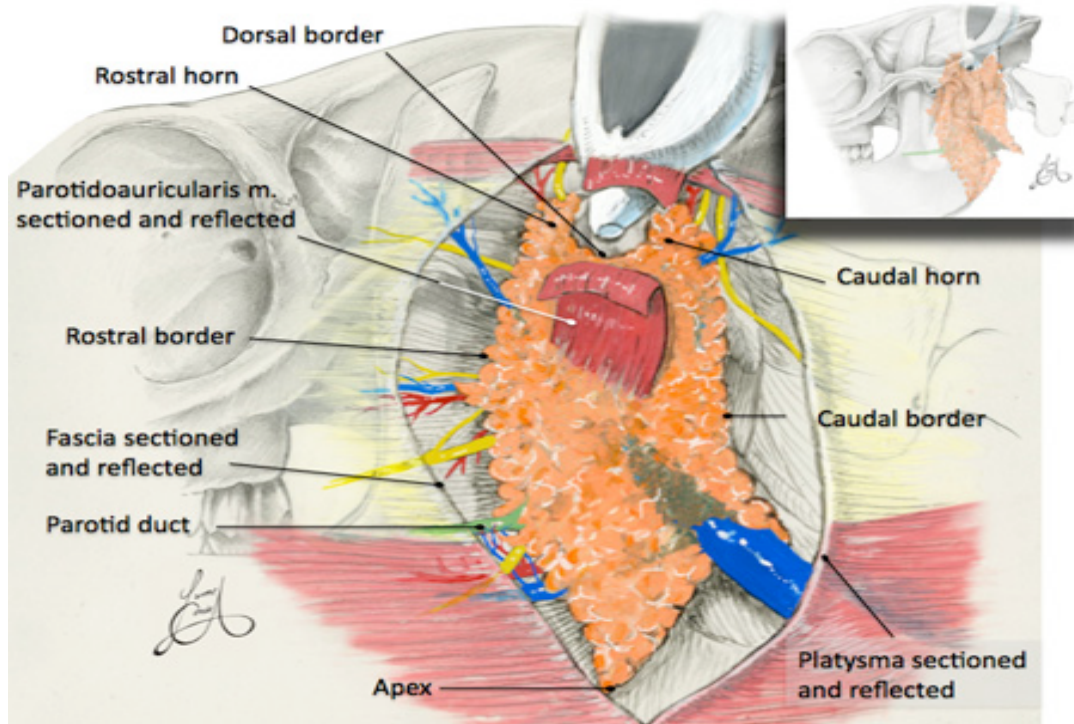


Figure 9. Left parotid salivary gland and surrounding soft tissue of the llama.

5.2.3. Nerves

The main nerves surrounding the tympanic bulla were branches of the facial nerve. The extra-cranial portion of the facial nerve exited the skull from the stylomastoid foramen and was immediately embedded in the parotid gland. Rostrally, the facial nerve gave rise to the auriculopalpebral nerve, which ascended from the base of the ear and divided into auricular and palpebral branches. The next rostral divisions of the facial nerve were the dorsal buccal branch, which turned rostrally over the masseter muscle, and the ventral buccal branch, which ran close to the ventral border of the mandible. The caudal auricular nerve was caudally situated with respect to the external acoustic meatus and ascended dorsally. Two minor divisions of the facial nerve, the internal auricular nerve (dorsally oriented) and the digastric branch were also identified. The

auriculotemporal nerve (a branch of trigeminal nerve) and its communicating branch to the dorsal buccal branch of the facial nerve were found between the neck of the mandible and the tympanic bulla. The great auricular nerve, a branch of the second cervical spinal nerve, was closely related to the caudal border of the parotid gland. The hypoglossal nerve was located ventral to the paracondylar process and the tympanic bulla (Figure 10). See Tables 7 and 8 for the measurements and orientations of the nerves.

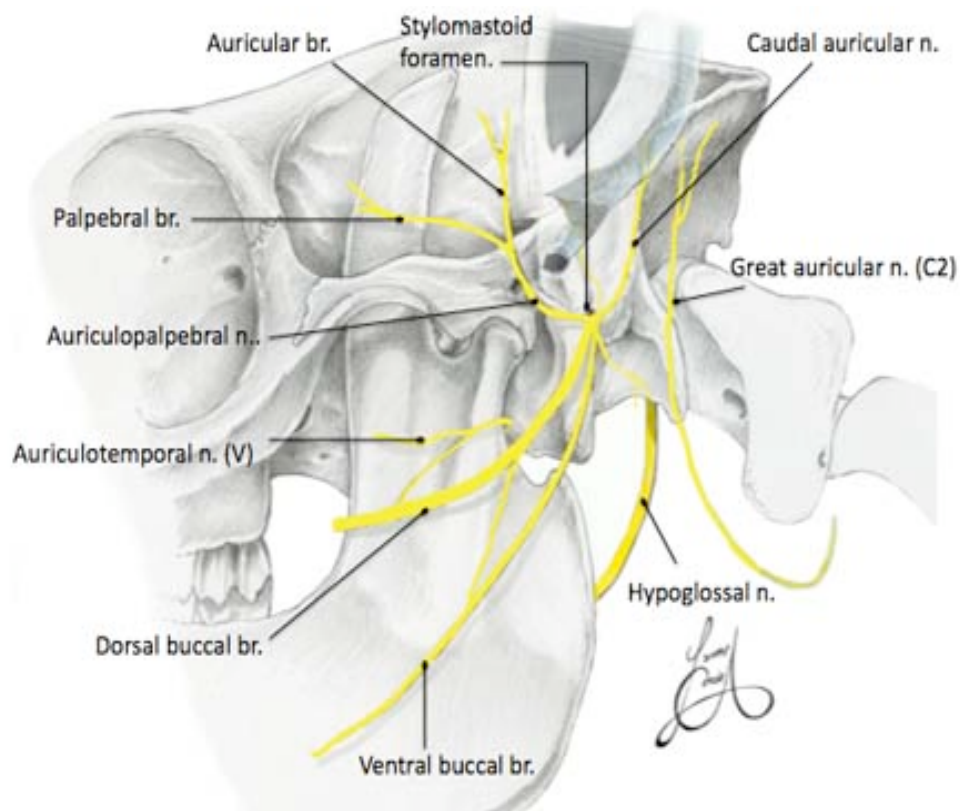


Figure 10. Nerves related to the tympanic bulla of the llama. Left view. The facial nerve exits the stylomastoid foramen and immediately branches.

5.2.4. Veins

The maxillary vein was the largest vein found surrounding the tympanic bulla. It coursed through the parotid salivary gland from the medial aspect at the level of the

temporomandibular joint to the angle of the mandible. It coursed obliquely rostro-caudal and dorso-ventral, was superficial to the tympanic bulla and received numerous tributary veins (Figure 11). Near the temporomandibular joint it received several veins from the plexus around the pharynx. Rostrally and caudally the maxillary vein received two or three shorts veins from the parotid salivary gland. A large venous sinus, located between the temporal bone and the temporal muscle, was found in all dissections and correlated with the CT images. This structure was called the external temporal sinus because of its location and communication with the temporal sinus, as well as the retroarticular vein. The retroarticular vein originated from the retroarticular foramen and connected the external temporal venous sinus with the maxillary vein. The retroarticular vein was situated rostral to the caudo-lateral process and body of the tympanic bulla and was embedded in the parotid salivary gland. The superficial temporal vein and the transverse facial vein joined in a short trunk before draining together into the maxillary vein, close to the neck of the mandible. At that point both veins were embedded in the parotid salivary gland. The facial vein ran horizontal, dorsal to the ventral border of the mandible, before draining into the maxillary vein, which was embedded in the parotid salivary gland. The caudal auricular vein began as a superficial vein from the caudal aspect of the ear, then was embedded in the parotid salivary gland and ran close to its caudal border, receiving blood from this organ. The caudal auricular vein ended in the maxillary vein at the level of the ventral portion of the parotid salivary gland. The measurements and orientation of the veins were shown in Figure 11, Tables 9 and 10.

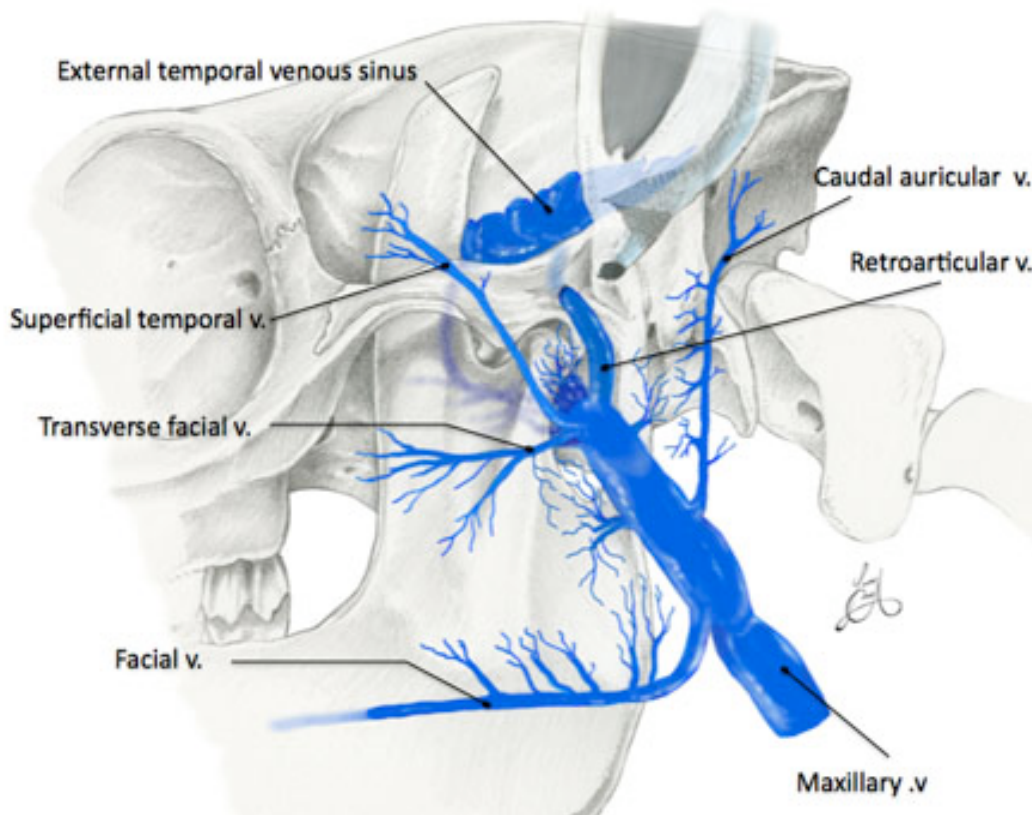


Figure 11. Veins surrounding the tympanic bulla of the llama. Left view.

5.2.5. Arteries

The main vessel that supplied blood to the tympanic bulla and surrounding areas was the external carotid artery. The external carotid artery was located deep to the digastricus muscle and superficial to the hypoglossal nerve. Two of its branches were important to the present study. One was the common trunk for the caudal auricular and facial arteries. The other was the superficial temporal artery, which gave rise dorsally to the transverse facial artery. The superficial temporal artery was rostral to the tympanic bulla. It was embedded in the parotid salivary gland and gave numerous branches to the rostral and dorsal aspect of this organ. After supplying the gland, the superficial temporal artery emerged from the rostral horn in conjunction with the auricular branch of the

auriculopalpebral nerve. The transverse facial artery originated from the superficial temporal artery and gave rise to several arteries supplying the parotid salivary gland. At its origin the transverse facial artery was situated in the parotid salivary gland and then ran rostrally and horizontally, lying in a groove between the superficial and deep portions of the masseter muscle, ventral to the zygomatic arch, and parallel to the auriculotemporal nerve. The facial and caudal auricular artery originated from a short common trunk of the external carotid artery. The facial artery contributed to the blood supply of the ventral portion of the gland. It turned laterally at the level of the angle of the mandible and emerged from the parotid salivary gland along with the facial vein. The caudal auricular artery was well developed, providing important blood supply to the parotid salivary gland. It ran caudal to the tympanic bulla, close and deep to the caudal border of this bony structure, and rostral to the paracondylar process. Near the external acoustic meatus the caudal auricular artery turned superficial and slightly caudal and emerged from the caudal horn of the gland along with the caudal auricular nerve (Figure 12). The measurements and orientation of the arteries are shown in Tables 9 and 10.

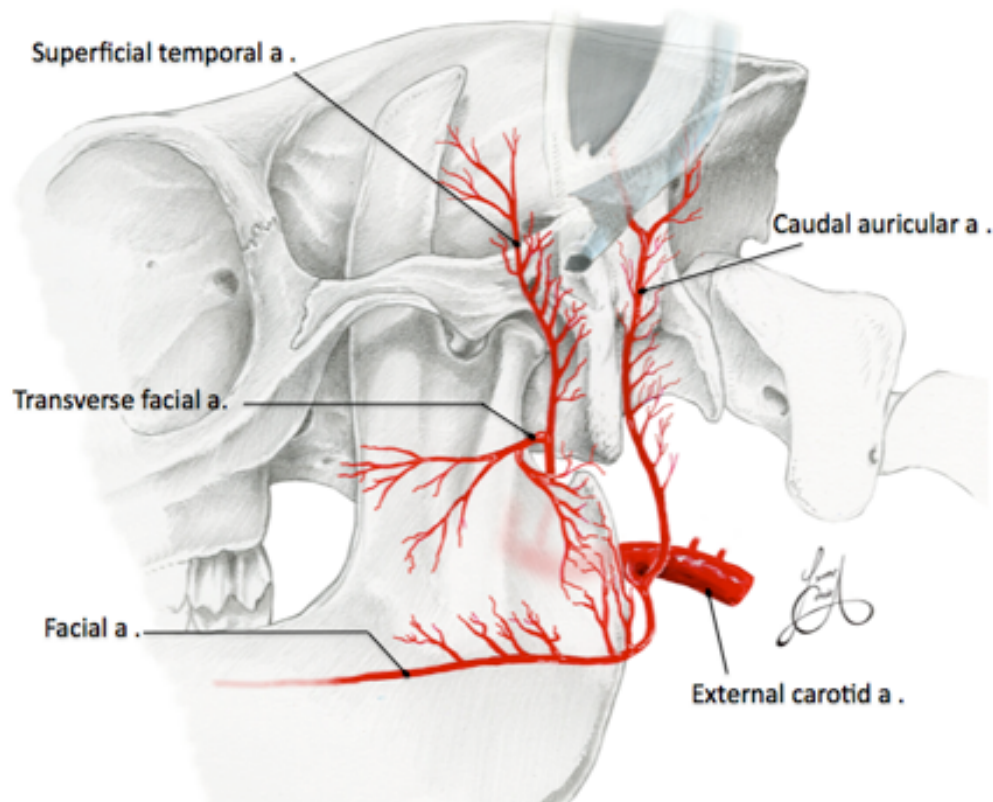


Figure 12. Arteries surrounding the tympanic bulla of the llama. Left view.

5.2.6. Muscles and tendons

The masseter was a well developed muscle in llamas, with superficial and deep portions. Between the bellies of the masseter muscle coursed the transverse facial vessels and the auriculotemporal nerve. The tendon of the sternomastoideus muscle, easily identified after reflecting the caudal border of the parotid salivary gland rostrally, inserted on the mastoid process very close to the stylomastoid foramen. The digastricus muscle ran from the paracondylar process of the occipital bone to the angle and the ventral border of the mandible. A tendinous intersection split the muscle into caudal and rostral bellies. Between this tendinous intersection and the mandible, the mandibular salivary duct, rostral portion of the mandibular salivary gland, and the facial artery were found.

The sternomandibularis tendon inserted on the caudal border and angular process of the mandible, as well as on the ventral border of the caudo-lateral process of the tympanic bulla. In its trajectory, it ran between the digastricus muscle and the maxillary vein. A ligament connected the ventral border of the tympanic bulla with the angular process of the mandible (Figure 13).

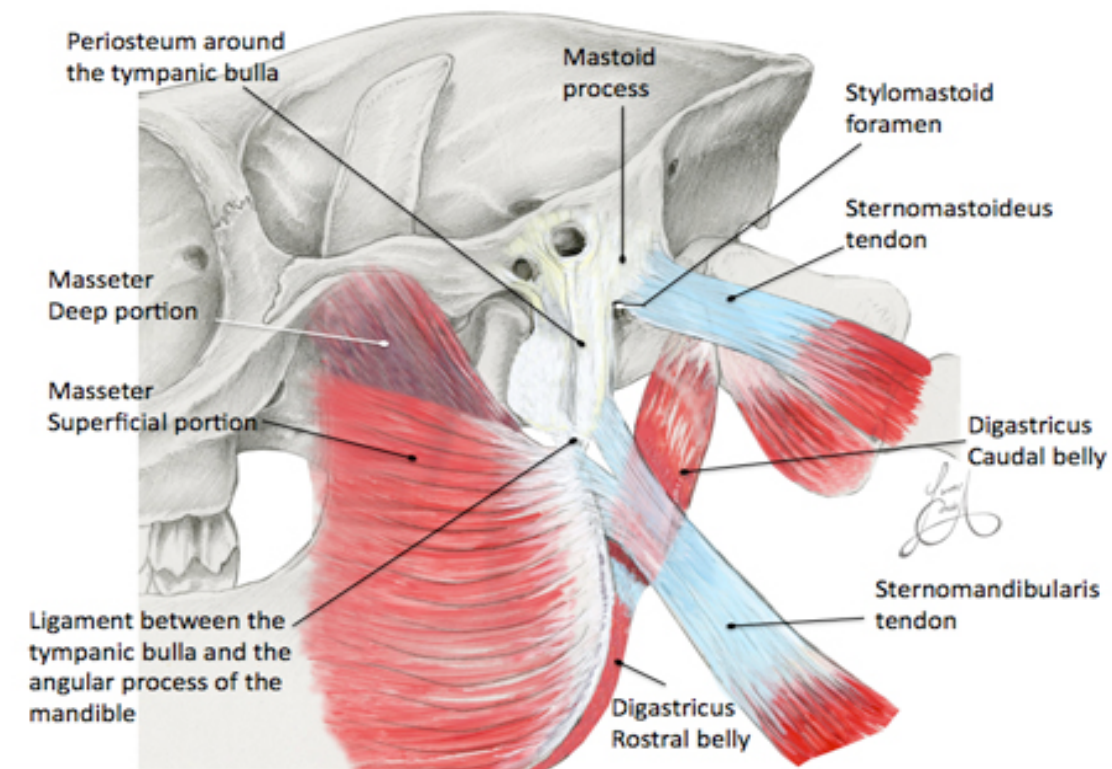


Figure 13. Muscles, tendons and ligaments surrounding the tympanic bulla in the llama. Left view.

5.2.7. Bony structures and foramina

The annular cartilage joined with the osseous external acoustic meatus porus. Rostral to the osseous external acoustic meatus and dorso-caudal to the temporomandibular joint was the retroarticular foramen, which contained the retroarticular vein. Slightly ventral to the retroarticular foramen and between the caudo-

lateral process of the tympanic bulla and the mastoid process lay the stylomastoid foramen. The ventral border of the tympanic bulla faced the angular process of the mandible. The paracondylar process was well developed. It arose close to the mastoid process and extended ventrally at the level of the ventral border of the tympanic bulla and the angular process of the mandible (Figure 14). The measurements of the bony structures and foramina are shown in Table 11.

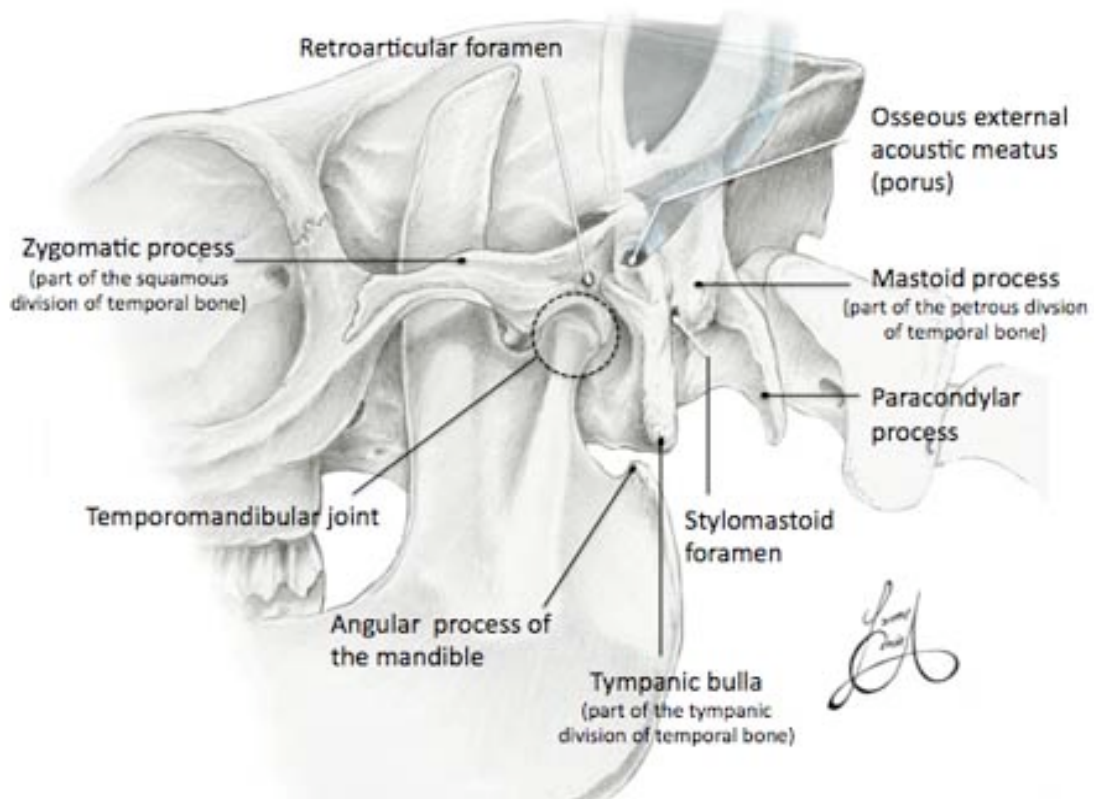


Figure 14. Bony structures and foramina surrounding the tympanic bulla in the llama. Left view.

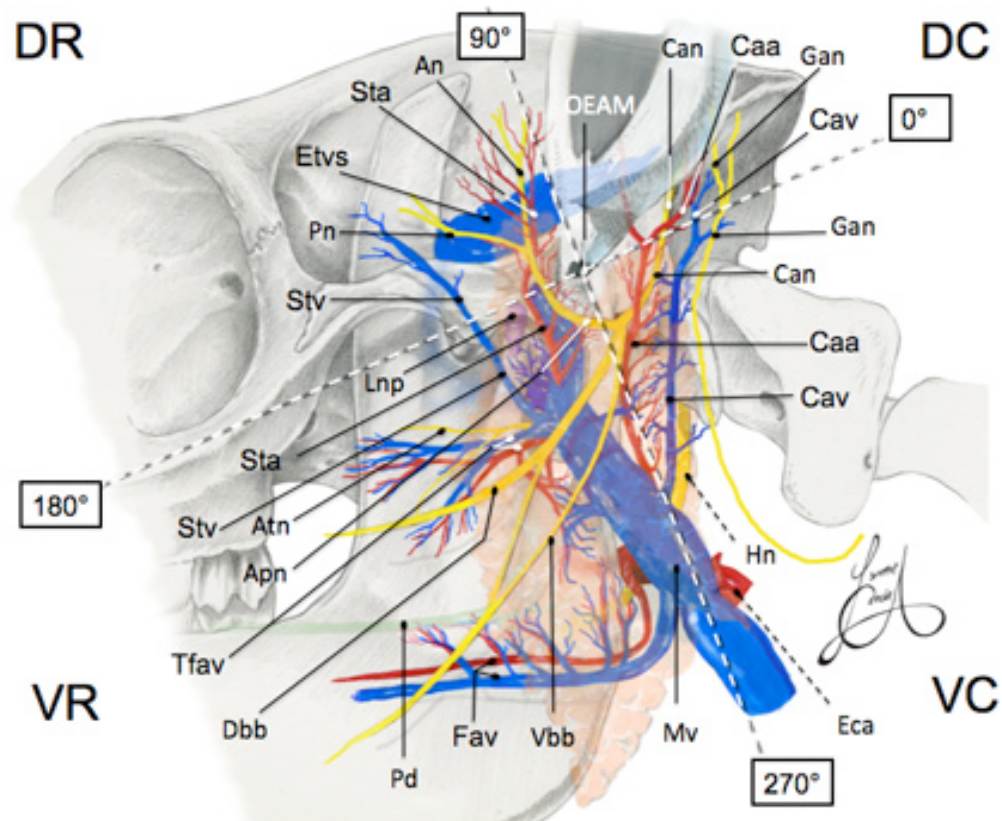


Figure 15. The general anatomical panorama and the structures distributed in the quadrants DR dorso-rostral, DC dorso-caudal, VR ventro-rostral and VC ventro-caudal in a left view of the head.

An	Auricular nerve	Hn	Hypoglossal nerve
Apn	Auriculopalpebral nerve	Lnp	Parotid lymph center
Atn	Auriculotemporal nerve	Mv	Maxillary vein
Caa	Caudal auricular artery	OEAM	Osseous external acoustic meatus
Can	Caudal auricular nerve	Pd	Parotid salivary duct
Cav	Caudal auricular vein	Pn	Palpebral nerve
Dbb	Dorsal buccal branch	Sta	Superficial temporal artery
Eca	External carotid artery	Stv	Superficial temporal vein
Etsvs	External temporal venous sinus	Tfav	Transverse facial vessels
Fav	Facial vessels	Vbb	Ventral buccal branch
Gan	Great auricular nerve		

5.2.8. Dorso-rostral quadrant

The auriculopalpebral nerve and its branches (auricular and palpebral), the superficial temporal artery with several branches to the temporal area, and the superficial temporal vein in the superficial fascia oriented toward the orbit were found in the dorso-rostral quadrant. The external temporal venous sinus was located deep to the temporalis muscle. The rostral horn of the parotid gland was closely related to the nerves and vessels. In this region, the parotid gland had a strong fascia attached to the ventral aspect of the ear canal cartilages and bone that made dissection difficult (Figure 16).

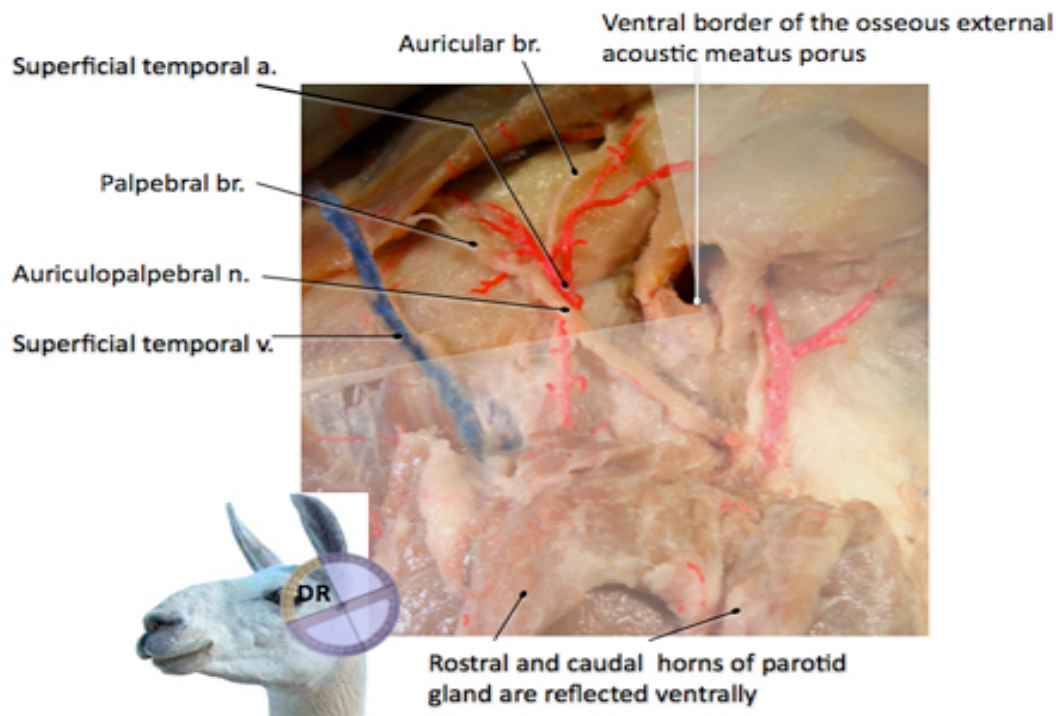


Figure 16. Deep dissection demonstrating the structures located in the dorso-rostral quadrant. Protractor in inset llama head image indicates location of higher magnification image.

5.2.9. Dorso-caudal quadrant

The caudal auricular artery, which gave rise to medial and lateral branches related with the auricular cartilage, was located in the dorso-caudal quadrant. The caudal auricular vein was found running dorsally within the caudal border of the parotid gland. Close to the caudal horn of the gland this vein turned superficially and caudally. The caudal auricular nerve was found closely related to the artery deep to the parotid tissue. The great auricular nerve was found caudal to the parotid gland. The caudal horn of the parotid gland covered the vessels and nerves in this area. The parotid fascia was strongly inserted on the ear canal cartilage and bones making the dissection laborious (Figure 17).

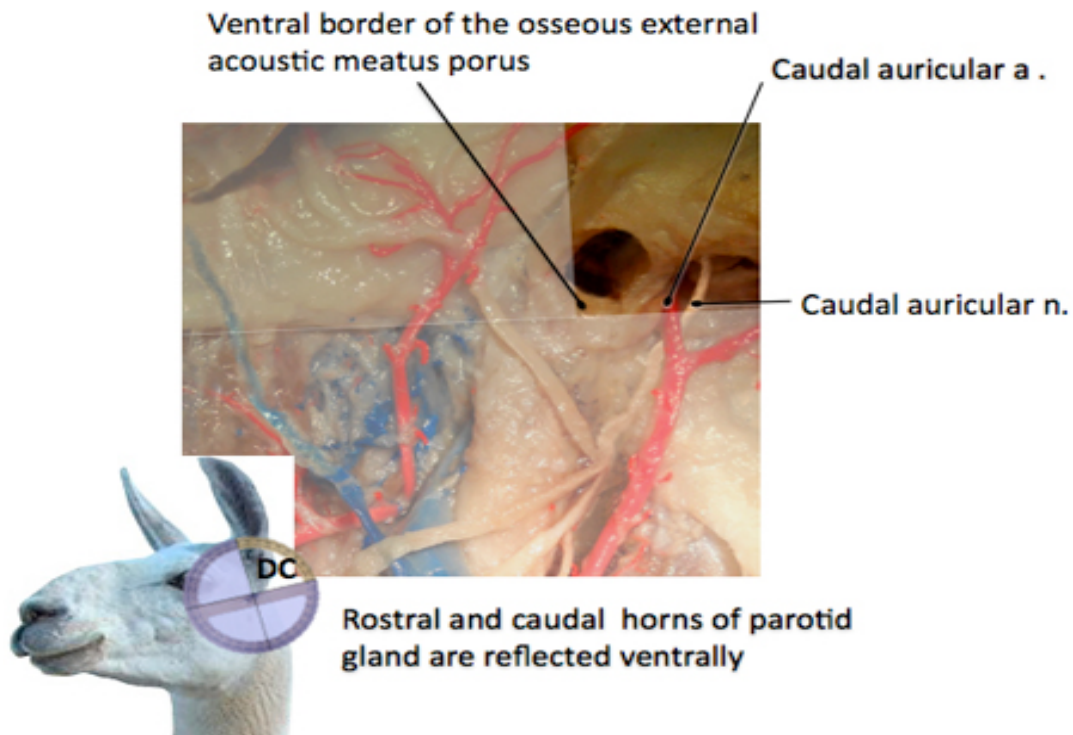


Figure 17. Deep dissection showing some of the structures found in the dorso-caudal quadrant. Protractor in inset llama head image indicates location of higher magnification image.

5.2.10. Ventro-rostral quadrant

The ventro-rostral quadrant was a broad and complex region. It contained the superficial temporal, transverse facial and facial arteries and veins. The retroarticular vein was found rostral to the tympanic bulla. The maxillary vein was covered by the parotid salivary gland and represented the main vessel in this area. It lay in an oblique dorso-ventral and rostro-caudal direction. The branches of the facial nerve in this quadrant were the auriculopalpebral, dorsal buccal and ventral buccal branches. The auriculotemporal nerve was encountered dorsal to the dorsal buccal branch. The parotid salivary duct was located between the dorsal buccal branch and the facial vessels. The parotid salivary gland was strongly attached to the vascular and nerve structures, as well as to the masseter muscle, making the dissection laborious and time consuming (Figures 18, 19, 20).

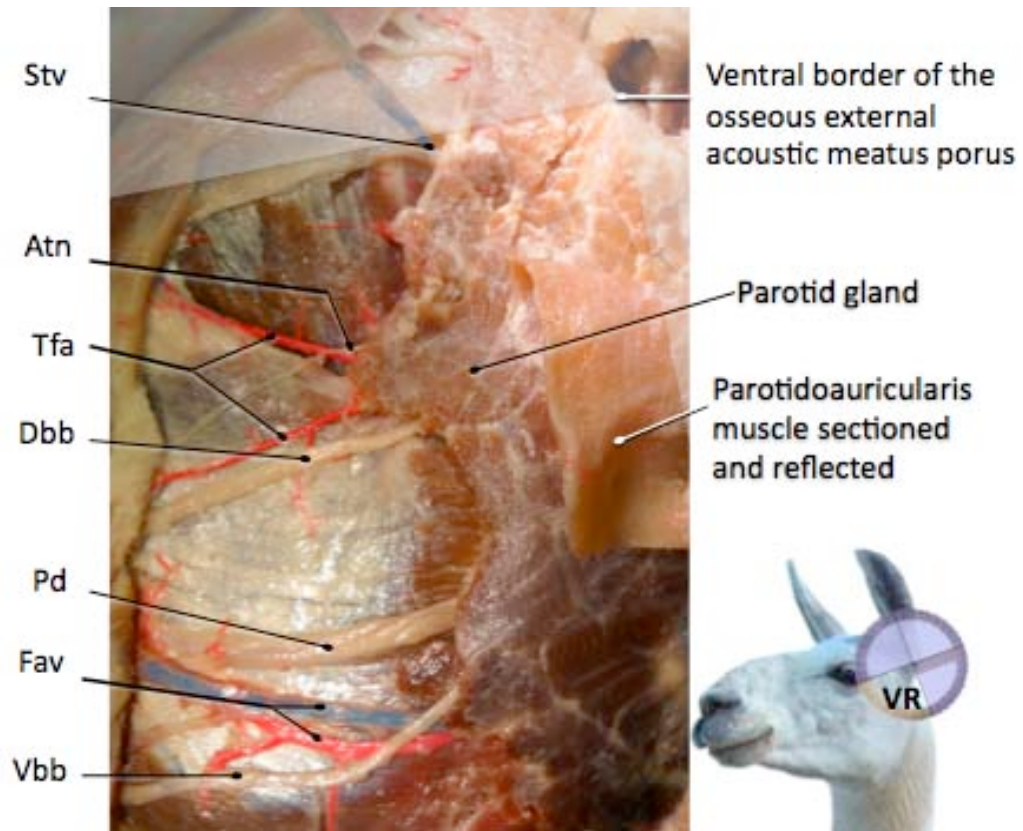


Figure 18. Superficial dissection of the structures found in the ventro-rostral quadrant. Left lateral view. Protractor in inset llama head image indicates location of higher magnification image.

Atn	Auriculotemporal nerve	Stv	Superficial temporal vein
Dbb	Dorsal buccal branch	Tfa	Transverse facial artery (branches)
Fav	Facial vessels	Vbb	Ventral buccal branch
Pd	Parotid salivary duct		

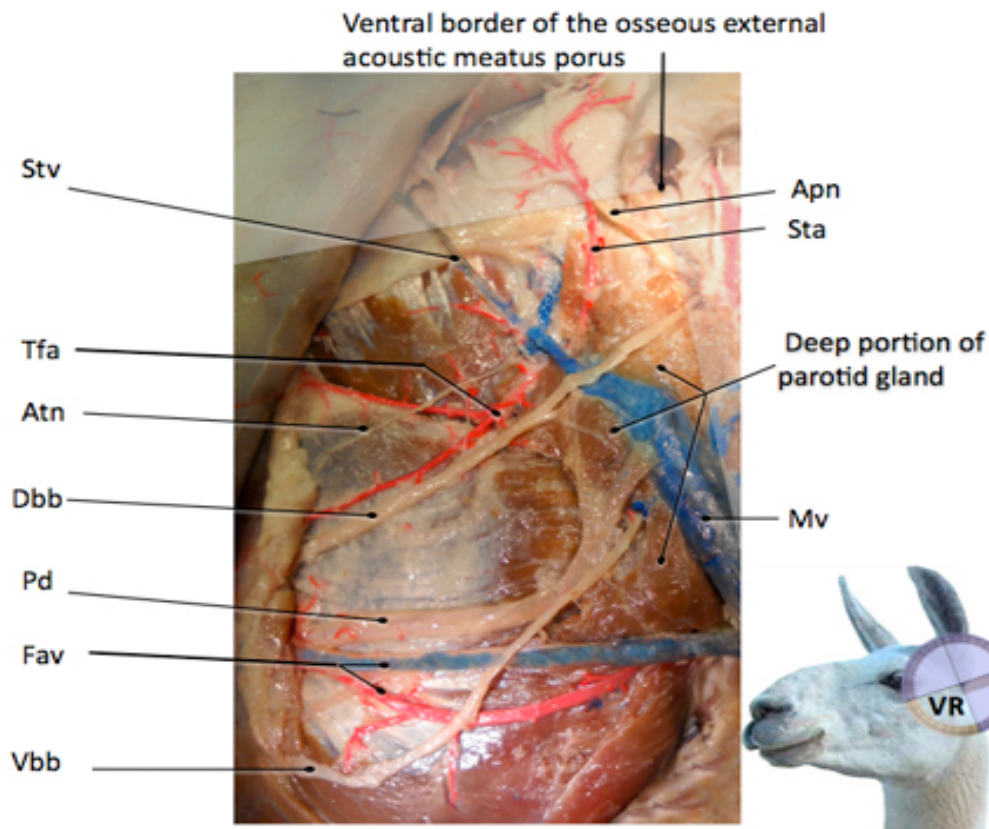


Figure 19. Deep dissection of the structures found in the ventro-rostral quadrant after dissection and reflection of the superficial part of the parotid gland. Left lateral view. Protractor in inset image indicates location of higher magnification image.

Atn	Auriculotemporal nerve	Pd	Parotid salivary duct
Apn	Auriculopalpebral nerve	Sta	Superficial temporal artery
Dbb	Dorsal buccal branch	Stv	Superficial temporal vein
Mv	Maxillary vein	Tfa	Transverse facial artery
Fav	Facial vessels	Vbb	Ventral buccal branch

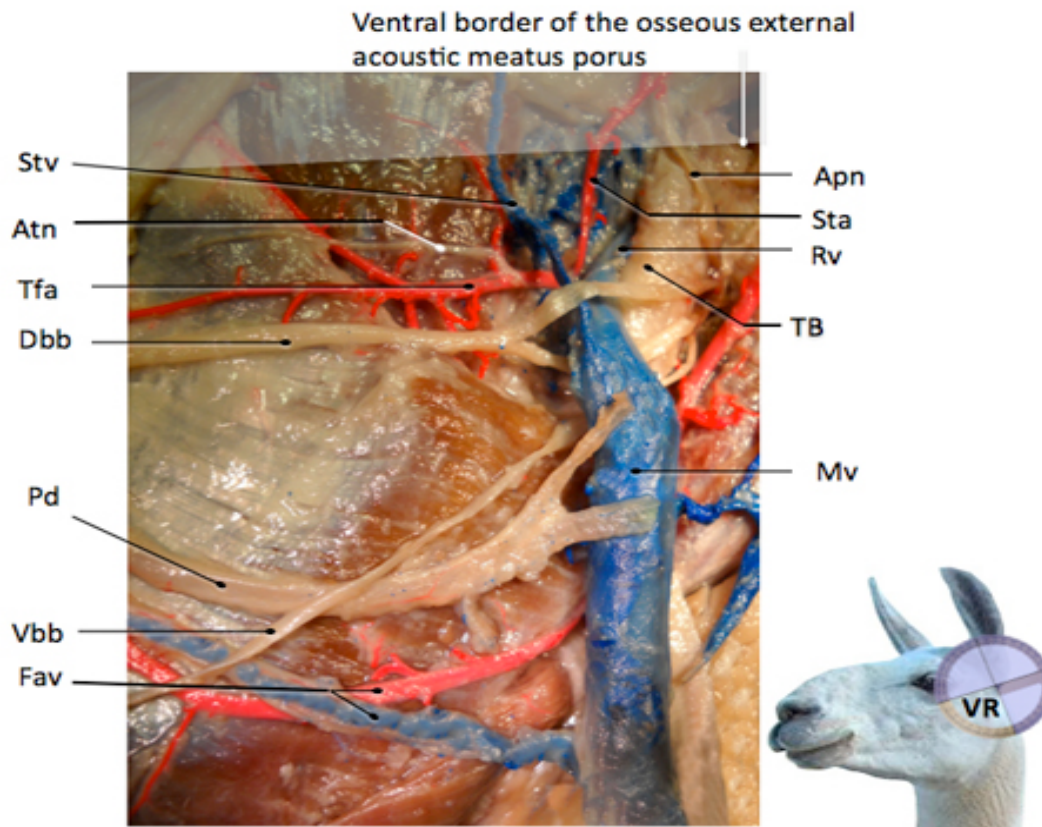


Figure 20. Deepest dissection of the structures found in the ventro-rostral quadrant after dissection and reflection of the superficial and deep portions of the parotid gland. Left lateral view. Protractor in inset image indicates location of higher magnification image.

Atn	Auriculotemporal nerve	Rv	Retroarticular vein
Apn	Auriculopalpebral nerve	Sta	Superficial temporal artery
Dbb	Buccal nerve	Stv	Superficial temporal vein
Fav	Facial vessels	TB	Tympanic bulla
Mv	Maxillary vein	Tfa	Transverse facial artery
Pd	Parotid salivary duct	Vbb	Ventral buccal branch

5.2.11. Ventro-caudal quadrant

The parotid salivary gland in this region had a less developed fascia. There were no large vessels or nerves running superficial to the parotid gland at this level. The great auricular nerve ran caudal to the caudal border of the gland. The caudal auricular nerve was found dorsally and deep to the gland. The caudal auricular vein was found closely related to the caudal border of the parotid gland. The caudal auricular artery was well developed. The ventral two-thirds of the artery was situated caudal and deep to the tympanic bulla, then the artery turned more superficially close to the origin of the facial nerve. The caudal belly of the digastricus muscle was identified by a deep dissection. The tendon of the sternomandibularis muscle was found between the maxillary vein and the caudal auricular artery toward the mandible and the tympanic bulla (Figure 21).

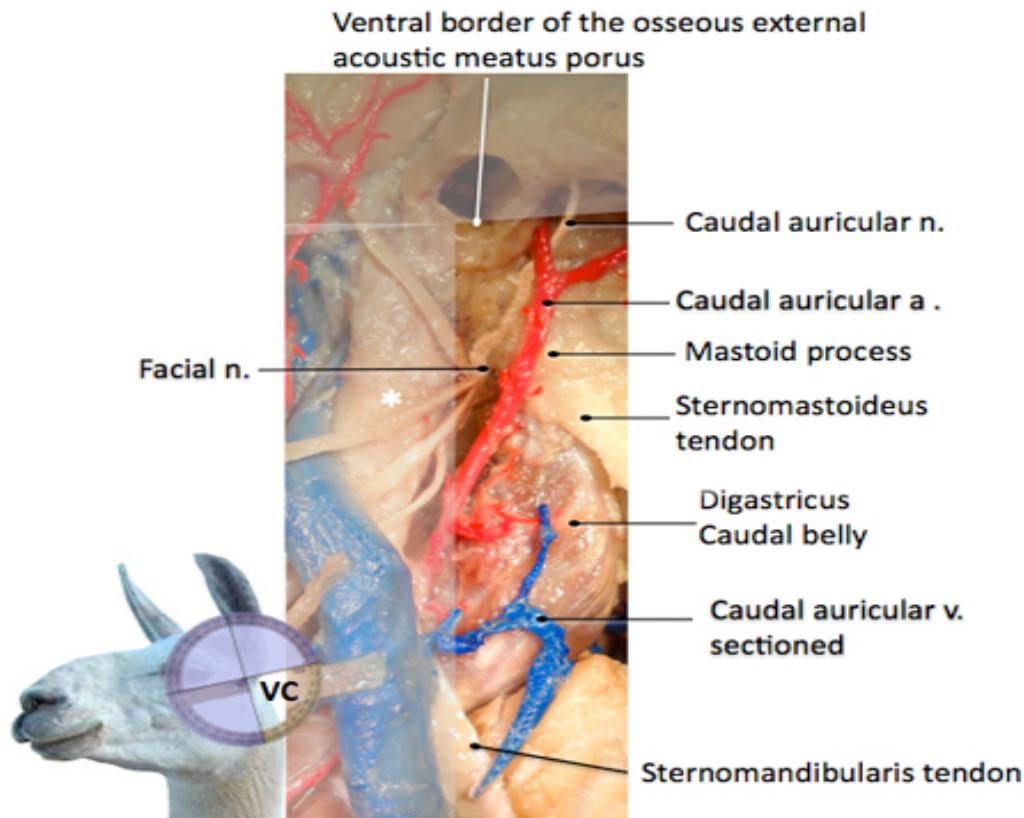


Figure 21. Deep dissection of the structures located in the ventro-caudal quadrant after dissection and reflection of the superficial and deep portions of the parotid gland. Left lateral view. * Shows the tympanic bulla. Protractor in inset image indicates location of higher magnification image.

5.2.12. Measurements obtained during dissection

All measurements from all animals were noted as means \pm standard deviation (SD). Where the measurements were significantly different the males and females values were listed. The general symbols used are: OEAMP: osseous external acoustic meatus porus; CLp: caudo-lateral process; TB: tympanic bulla; n= nerve; br= branch; a=artery; v= vein

Table 5: Skin thickness.

Code	Location of skin thickness measurements	Average measurements \pm SD
S1	At the lateral area of the cartilaginous external acoustic meatus	1.4 \pm 0.4 mm
S2	At the level of the middle third of the parotid gland	6.4 \pm 0.4 mm

Table 6: The parotid salivary gland and duct.

Code	Description of how the measurements were obtained	Average measurements \pm SD
Pd	Distance of the parotid salivary duct from the ventral border of the OEAMp to its emergence from the border of the parotid gland	74.2 \pm 2.6 mm
Pl *	Parotid salivary gland length from the dorsal border to the ventral apex of the gland.	Males 112.8 \pm 3.3 mm
		Females 97.3 \pm 2.6 mm
Pw	The widest distance of the gland from the caudal to the rostral border at the level of the middle third of the organ.	56.5 \pm 1.8 mm
* p < 0.05 for difference between males and females		

Table 7: The nerves.

Code	Description of how the measurements were obtained	Average measurements \pm SD
An	Auricular br. of auriculopalpebral n. Distance from the ventral border of the OEAMp to its emergence from the border of the parotid gland	15.5 \pm 2.4 mm
Pn	Palpebral br. of auriculopalpebral n. Distance from the ventral border of the OEAMp to its emergence from the border of the parotid gland	21.4 \pm 2.4 mm
APn	Auriculopalpebral n. Distance from the ventral border of the OEAMp to the nerve where it crosses the TB	10.8 \pm 1.2 mm
Dbb1	Dorsal buccal branch of facial n. Distance from the ventral border of the OEAMp to its emergence from the border of parotid gland	53.7 \pm 4.3 mm
Dbb2	Dorsal buccal branch of facial n. Distance from the ventral border of the OEAMp to the nerve when it crosses the TB	19.7 \pm 1.7 mm
Vbb1	Ventral buccal branch. Distance from the ventral border of the OEAMp to its emergence from the border of the parotid gland	87.6 \pm 2.7 mm
Vbb2	Ventral buccal branch. Distance from the ventral border of the OEAMp to the nerve when it crosses the maxillary vein	33.5 \pm 3.7 mm
Can	Caudal auricular n. Distance from the ventral border of the OEAMp to its emergence from the border of the parotid gland	11.8 \pm 1.3 mm
Atn	Auriculotemporal n. Distance from the ventral border of the OEAMp to its emergence from the border of the parotid gland	47.8 \pm 2.9 mm
Hn	Hypoglossal n. Distance from the ventral border of the OEAMp to the hypoglossal n.	22 \pm 1.4 mm
Gan	Great auricular n. Distance from the ventral border of the OEAMp, following an horizontal line, to the great auricular n.	15 \pm 7.8 mm

Table 8: Orientation of the nerves and parotid salivary duct in degrees.

Code	Structures measured	Average measurements \pm SD
An-o	Auricular br. of auriculopalpebral n.	115.8° \pm 6.9°
Pn-o	Palpebral br. of auriculopalpebral n.	148.8° \pm 6.9°
Dbb-o	Dorsal buccal branch	208.8° \pm 9.4°
Vbb-o	Ventral buccal branch	232° \pm 7.2°
Can-o	Caudal auricular n.	350.2° \pm 4.1°
Atn-o	Auriculotemporal n.	200.8° \pm 5.9°
Pd-o	Parotid salivary duct	229.5° \pm 6.3°

Table 9: Location of the vessels.

Code	Description of the location of the vessels relative to surrounding structures.	Average measurements \pm SD
Sta	Superficial temporal a. Distance from the ventral border of the OEAMp to its emergence from the border of the parotid gland	14.8 \pm 1.8 mm
Caa-1	Caudal auricular a. Distance from the ventral border of the OEAMp to its emergence from the border of the parotid gland	10.2 \pm 1.4 mm
Caa-2	Caudal auricular a. Distance from the lateral-caudal border of CLp of TB	1.6 \pm 0.5 mm
Rv	Retroarticular v. Distance from the lateral-rostral border of CLp of TB	0.7 \pm 0.4 mm
Mv*	Maxillary v. Distance from OEAMp to the vein at the level of the rostral border of CLp of TB	Male 31 \pm 1.7 mm Female 24.6 \pm 2.3 mm
Eca	External carotid a. Distance from the ventral border of the CLp of TB	21.7 \pm 2.1 mm
Fav*	Facial a. and v. Distance from the ventral border of the OEAMp. (The superior vessel was considered for this measurement)	Male 89.9 \pm 1.8 mm Female 85.2 \pm 1.1 mm
Tfav	Transverse facial a. and v. Distance from the ventral border of the OEAMp (The superior vessel was considered for this measurement)	49.4 \pm 3.3 mm

* p < 0.05 for difference between males and females

Table 10: Orientation of the vessels in degrees.

Code	Structures measured	Average measurements \pm SD
Sta-o	Superficial temporal a.	124.3° \pm 6.1°
Caa-o	Caudal auricular a.	350.3° \pm 5.3°
Fav-o	Facial a. and v.	233.8° \pm 5.8°
Tfav-o	Transverse facial a. and v.	207.8° \pm 8.4°

Table 11: Location of bony structures and foramina.

Code	Description of how the measurements were obtained	Average measurements \pm SD
Smf-1	Distance from the ventral border of OEAMp to the stylomastoid foramen	16.9 \pm 2.1 mm
Smf-2	Distance from the ventral border of CLp of TB to the stylomastoid foramen	22. \pm 0.8 mm
Raf-1	Distance from the ventral border of OEAMp to the retroarticular foramen	12.9 \pm 1.8 mm
Raf-2	Distance from the ventral border of CLp of TB to retroarticular foramen	30.1 \pm 1 mm
PcTb	Distance between caudal border of paracondylar process to the caudal border of TB	19.5 \pm 1 mm

5.3. Statistical analysis

The length of the head and the height of the cranial cavity did not vary significantly between males and females ($p \geq 0.05$).

CT measurements

CT measurements did not vary significantly between the right and left sides of the head ($p \geq 0.05$). Osseous external acoustic meatus length (OEAM 1) was significantly longer in males (25.4 \pm 1.9 mm) than in females (24.4 \pm 2 mm) ($p < 0.05$). The caudo-rostral angle (CRA) was significantly smaller in males (113 \pm 3.1 degrees) than in females (120 \pm 4.1 degree) ($p < 0.05$).

Dissection measurements

Measurements of tissue structures did not vary between right and left side of the head ($p \geq 0.05$). The maxillary vein (Mv) position was farther from the OEAMp in males (31 ± 1.7 mm) than in females (24.6 ± 2.3 mm) ($p < 0.05$). Facial artery and vein (Fav) position was farther from the ventral border of the OEAMp in males (89.9 ± 1.8 mm) than in females (85.2 ± 1.1 mm) ($p < 0.05$). The parotid salivary gland was longer (Pl) in males (112.8 ± 3.3 mm) than in females (97.3 ± 2.6 mm) ($p < 0.05$).

Chapter 6- Discussion

All of the males and three of the four females were adult animals with complete dental eruption and teeth in wear. In female F2 the caudal mandibular molars (m3) were erupted but not yet in wear, making this animal approximately four years old and an adult.³⁵ In llamas, growth factors of adult weight, thoracic circumference, adult height, and adult length in llamas are reached at 36 months of age or less,³⁶ supporting consideration of female F2 as an adult animal. Measurements of the length of the skull and the height of the cranial cavity between male and female demonstrated that there are no statistical differences. Of all the anatomical parameters measured the few that were statistically significantly different between males and females were longer in males.

6.1. Discussion regarding the bony structures evaluated on CT images

The osseous external acoustic meatus (OEAM) was observed intimately related to the tympanic bulla and was a long narrow canal with a curvature that bent ventrally. The epitympanic recess and the atrium of the tympanic cavity retained the general shape found in domestic animals.⁵ The tympanic bulla was quite different from other domestic animals, but similar to the dromedary.³⁷

6.1.1. Osseous external acoustic meatus (OEAM)

The analysis of the CT images showed that the OEAM had an average of length of 25.1 ± 1.9 mm as measured from the dorsal border of the osseous external acoustic meatus porus, following the dorsal wall until the tympanic annulus. The OEAM bent

ventrally approximately half way between the porus and the tympanic annulus and the angle of the OEAM curvature was 144 ± 9.7 degrees in these llamas. A previous report described the osseous external acoustic meatus as a canal, which bent ventrally at a depth of 18 mm, forming an angle of 120 degrees, however it is not clear where those measurements were obtained.¹⁵

The OEAM belongs to the tympanic division of the temporal bone with a clear demarcation from the lateral portion of the petrous division of the temporal bone. The OEAM was located caudal to the zygomatic process of the temporal bone (Figure 14). As in others species the OEAM was part of the tympanic division of the temporal bone,^{5, 21} and did not agree with a previous study, which described the osseous ear canal in llamas entering the petrous temporal bone immediately ventral to the zygomatic process.¹⁵ The current study demonstrated a long and narrow osseous external acoustic meatus in llamas with an ovoid lumen, narrowing in vertical diameter from the lateral to the medial side. This is in agreement with a previous study performed in alpacas.¹⁶ Another study of a skeletal preparation of one large male llama described an OEAM orifice with 6 mm diameter, narrowing to 4 mm at the bend.¹⁵ The lack of criteria where the measurements in that study were obtained makes it difficult to compare with the CT data of this study. The external vertical diameter of OEAM measured in average of 4.7 ± 0.8 mm and the horizontal diameter was 6.5 ± 1.4 mm. The intermediate vertical diameter at middle point of the length of the OEAM was 4.4 ± 1 mm. These values were similar to the previous study performed in llama.¹⁵ At the level of the tympanic annulus the average of the

diameter (latero-medial oriented) was 2.8 ± 0.7 mm and the horizontal diameter was 6.3 ± 1.3 mm.

6.1.2. Tympanic cavity (TC)

The tympanic cavity in llamas was housed within the petrous and tympanic portions of the temporal bone as described in other domestic animals.^{2,5} As the tympanic bulla did not have a fundic cavity in llamas, it was treated and measured as a separate structure and not as part of the tympanic cavity. The TC in llamas was observed as a latero-medially flattened cavity with an oblique axis in dorso-ventral and latero-medial orientation. The lengths of 15 mm described for the tympanic cavity in humans and 13.3 mm in sheep¹³ were similar to the 14.1 ± 0.9 mm observed in our study. The height of the human TC has been described as an average of 15 mm,¹³ this is similar to our measurements of 13.4 ± 0.6 mm. In sheep the height of the tympanic cavity has been described as an average of 18.9 mm,¹³ larger than the llamas in our study. However, this is not comparable as the study in sheep included the tympanic bulla in the height of the tympanic cavity. The malleus was observed without difficulty, its head reposed into the epitympanic recess, whereas the neck and the manubrium were in the atrium of the TC. The tympanic opening of the auditory tube was visualized rostral in the middle wall of the TC. The promontory, which is the prominence of the cochlea into the tympanic cavity, was visualized in the medial wall of TC with similar characteristics as in previous CT analysis in dogs⁸ and sheep.¹³

6.1.3. Tympanic bulla (TB)

The tympanic bulla of the llama was the bony structure with the most differences in comparison to other domestic animals, especially with those species with spherical bullae and a single fundic cavity such as dogs, cats, and sheep.^{2,3,5,13,14,23} In contrast, llamas have a multi-compartmental tympanic bulla with air cells surrounded by bony trabeculae as mentioned in previous studies in llamas¹⁵ and alpacas.^{16,17} This feature makes the interior of the TB of llamas a honey-combed structure, similar to the human mastoid process. The mastoid process can be affected in humans by infections that originate in the middle ear and lead to mastoiditis. These are treated, in some occasions, surgically by osteotomy, lavage and curettage of the bone.^{18-20,32,33} Thus, surgery in llamas will be more similar to mastoidectomy in the human than bulla osteotomy in dogs or cats.

To describe and measure the complex tympanic bulla in llamas, this bony structure was divided into four portions: caudal processes (lateral and medial), body and apex. The sagittal CT views demonstrated a quadrilateral shape of the TB located caudo-medial to the neck of the mandible. The coronal CT views showed the pyramidal shape of TB. It consisted of the small caudo-medial process of TB closely related with the paracondylar process of the occipital bone, the large caudo-lateral process connected to the osseous external acoustic meatus, and the stylohyoid fossa (Figure 2). Previous studies have mentioned that the entire bulla “drapes” over the proximal portions of the stylohyoid bone, creating the stylohyoid fossa in alpacas.¹⁷ That was not the case in llamas, in which only the proximal third of the stylohyoid bone was completely enveloped by the

TB. The distal two-thirds of the stylohyoid fossa were incompletely covered by the tympanic bulla, leaving a caudal aperture of the fossa.

6.2. Discussion of soft tissue and bony structures evaluated during dissection

Previous studies of the gross anatomy, or morphometric measurements of the soft tissue surrounding the ear in South American Camelids (SAC) are lacking. Two descriptions of a surgical approach to the tympanic bulla of SAC note the need for more anatomical knowledge and development of new surgical techniques in these species.^{1, 16} Key structures to consider in the surgical approach to the tympanic bulla in llamas are the parotid salivary gland, nerves, vessels, and the location of muscle tendons and relevant bony structures.

6.2.1. Skin

The skin in the parotid area showed a notable difference between the thicknesses observed at the lateral level of the cartilaginous external acoustic meatus (1.4 ± 0.4 mm) and the middle third of the parotid area (6.4 ± 0.4 mm). This should be considered during the incision in a surgical procedure in that area.

6.2.2. Parotid salivary gland

Beneath the sparse subcutaneous tissue laid the parotid salivary gland. The anatomy of the parotid salivary gland of llamas was similar to that classically described in domestic herbivores.³ The rostral and caudal horns of the superficial gland tissue overlaid the basal portion of the auricular and annular cartilages, and the dorsal concave

border was similar in conformation to the parotid salivary glands of dogs.^{2,38} The majority of the gland was pyramidal in shape and overlaid the caudal margin of the masseter muscle. In addition, the parotid salivary gland in llama had a ventral apex similar to the horse⁴ and goat.³⁴ The parotid salivary duct emerged from the rostral border of the gland and ran rostrally and superficially on the lateral surface of the masseter muscle, similar to the course in goats,³⁴ dogs,^{2,38} and the dromedary.³⁷ The facial nerve and several veins penetrated the gland similar to many domestic animals and humans.^{2-4,18,34,37,38}

6.2.3. Nerves

The facial nerve was the main cranial nerve found around the tympanic bulla. As in other domestic animals, as well as humans, the extra-cranial portion (free portion) of the facial nerve in llamas emerged from the skull through the stylomastoid foramen and was embedded in the parotid salivary gland tissue.^{3,39,40} Four main branches of the facial nerve were found in llamas: the auriculopalpebral n., dorsal buccal br., ventral buccal br., and caudal auricular n. and two minor branches, internal auricular n. and the digastric br. This distribution was similar to the general arrangement of the facial nerve in herbivores such as the cow,^{41,42} goat,³⁴ and dromedary.³⁷ The proximity of the facial nerve branches to the tympanic bulla made this nerve a key structure to be considered in a surgical approach to this bony structure.

6.2.4. Vessels

The retroarticular vein in llamas was proportionally to the head size more elongated in comparison to other domestic animals such as dogs.³⁸ The average distance found between the retroarticular vein and the middle third of the rostral border of the caudo-lateral process of the tympanic bulla was 0.7 ± 0.4 mm. Because the retroarticular vein was so closely related to the TB, this vessel was an important structure to be considered during surgery. A venous sinus located between the temporal bone and the temporal muscle, the external temporal venous sinus, was observed in llamas connected with the retroarticular vein through the retroarticular foramen. A different temporal sinus is described in dogs³⁸ and dromedary³⁷ between the tympanic and squamous division of the temporal bone as part of the cranial venous sinuses, this sinus empties into the retroarticular vein. The external temporal sinus in the llama has not been described in other domestic animals.

The distribution of the caudal auricular and the facial arteries originating from a common short trunk in llamas was similar to the dromedary.³⁷ In all ten llamas this trunk emerged directly from the external carotid artery, medial to the caudal margin of the mandible and ventral to the angular process of this bone. The immediate branching yielded a long portion of the caudal auricular artery before it reached the auricula, as compared with a shorter caudal auricular artery in other species such as dogs,^{39,43} horses,^{42,44} or cows.^{41,42} After its origin, the caudal auricular artery had a trajectory caudal to the tympanic bulla. The average distance between the middle third of the caudal border of the caudo-lateral process of the tympanic bulla and the caudal auricular artery

was 1.6 ± 0.5 mm. This short distance between the caudal auricular artery and the TB made this vessel a crucial structure to consider during a surgical approach.

6.2.5. Muscles, tendons and bony structures

Superficial and deep portions of the masseter muscle were clearly identified in llamas, even though no deep dissections were performed. This conformation was in agreement with the description of the masseter muscle in the dromedary.³⁷ The digastricus muscle showed a tendinous intersection between the caudal and rostral bellies, making the conformation of this muscle in llamas more similar to the dromedary,³⁷ cow,⁴¹ and horse⁴⁴ than to the dog.³⁸ The tendon of the sternomastoideus muscle was easily identified after reflecting the caudal border of the parotid salivary gland rostrally. This tendon could play an important role as a landmark during a surgical approach to the tympanic bulla because its insertion in the mastoid process was in proximity to the stylomastoid foramen. This feature could guide the surgeon and help to prevent damage to the facial nerve. The tendon of the sternomandibularis muscle was identified parallel and deep to the maxillary vein with a tight relationship to the parotid salivary gland fascia. Its insertion on the caudal border of the mandible was similar to the dromedary,³⁷ however, the sternomandibularis tendon in llamas differed as it also inserted on the angular process of the mandible and the ventral border of the tympanic bulla. This tendon could represent a good landmark to be followed to reach the ventral aspect of the tympanic bulla. It could also be a natural limit between the plane in which the surgeon could work and a deeper plane in which the facial artery, the mandibular salivary gland and its duct, and the origin of the caudal auricular artery were located.

6.2.6. Quadrants and measurements

The superficial temporal vein (Stv) and caudal auricular vein (Cav) were not measured because as superficial veins they showed high variability in positioning. The general locations were described in accordance with the quadrants. The parotid lymph center (Lnp) was not measured because of high variability in size and position, but it was generally located in the dorso-rostral quadrant. The newly described external temporal venous sinus (EtvS) was not measured because it was considered too far from the area studied. However, it was described in its relationship to the retroarticular vein.

The soft tissue description around the tympanic bulla in llamas, quadrant location, measurements, and orientations in degrees from the ventral border of the osseous external acoustic meatus porus, provided us with a good spatial anatomical panorama in order to create a new surgical approach to the tympanic bulla (Figure 15). The dorso-rostral and the dorso-caudal quadrants were narrow areas in which the parotid fascia was firmly inserted on the bone and cartilage. Two branches of the facial nerve and the superficial temporal and caudal auricular arteries were found in these quadrants. The ventro-rostral quadrant was a wide territory filled with several nerves, arteries and veins in addition to the parotid duct and lymph nodes. The parotid fascia was firmly anchored in the masseter muscle making the dissection laborious and time consuming. A surgical approach to the TB through the ventro-rostral quadrant could put at risk large branches of the facial nerve, as well as important vessels, with the consequences of paralysis and/or hemorrhage. The ventro-caudal quadrant contained the fascia around the caudal border of the parotid gland and its caudal projection loosely inserted in the neighboring tissue. The

origin of the main trunk of the extra-cranial portion of the facial nerve was found in the dorsal aspect of the ventro-caudal quadrant, rostral to the origin of the caudal belly of the digastricus muscle. The average distance between the caudal border of the paracondylar process (origin of the caudal belly of the digastricus muscle) and the stylomastoid foramen was 19.5 ± 1.0 mm. In humans a similar surgical landmark called the digastric ridge, is used during superficial parotidectomy.³⁸ No large branches of the facial nerve were identified running caudal to the tympanic bulla. The great auricular nerve was found related to the caudal border of the parotid salivary gland.

Using the results of this study, the following proposal for a surgical approach to the tympanic bulla in llamas was developed. Three fresh heads from alpacas without external head abnormalities were obtained from specimens admitted to the OSU Veterinary Diagnostic Laboratory and donated for research purposes. The alpaca heads were dissected using the proposed surgical procedure.

6.3. Proposal for a surgical approach to lateral tympanic bulla osteotomy in llamas

In severe cases of otitis media a combination of bulla osteotomy and canal ablation is recommended in small animals to remove the chronically diseased tissue of the meatus. This also facilitates future cleaning of the narrow external acoustic meatus, and access to the tympanic bulla to allow irrigation and lavage of exudates, debride the pathological epithelium, and treat any osteomyelitis.⁴⁵ The following is a proposed new approach to lateral bulla osteotomy for llamas, which could be combined with lateral ear canal ablation or total ear canal resection in animals with chronic otitis media and externa.

a- General anesthesia is required.

b- Area to be clipped. Clip hair from the caudal border of the orbit as the rostral border, to the middle of the temporal region, and from the lateral aspect of the ventral half of the pinna as the dorsal border, to the level of the axis as the caudal border, and to the level of the mandible and laryngeal region as the ventral border (Figure 22).

c- Body position. Place the patient in lateral recumbency with the diseased side up and with the head and neck extended and the pharynx elevated. Preferably place the head slightly higher than the rest of the body, to reduce the blood pressure in the veins of the head.

d- External meatus cleaning. Deep lavage of the external acoustic meatus using disinfecting solutions is necessary.

e- External landmarks. Following clipping and skin preparation with antiseptic solutions, the following external landmarks should be palpated and identified: ventral and caudal border of the mandible, angular process of the mandible, zygomatic arch, wing of the atlas, jugular groove, mastoid process, paracondylar process, cartilaginous ear canal, and the dorsal aspect of the tympanic bulla (Figure 22).

f- Incision. To make the “L” incision in the skin and subcutaneous tissue, trace a vertical line from the rostro-ventral aspect of the ear canal toward the caudal border of the mandible ventral to the level of the jugular groove. Then trace a horizontal line, caudally oriented, from the ventral edge of the vertical incision to the atlanto-axial joint at a level dorsal to the jugular groove. The skin thickness at the level of the ear canal would be expected to be approximately 1.4 mm and at the level of the middle third of the parotid salivary gland around 6.4 mm (Figure 23).

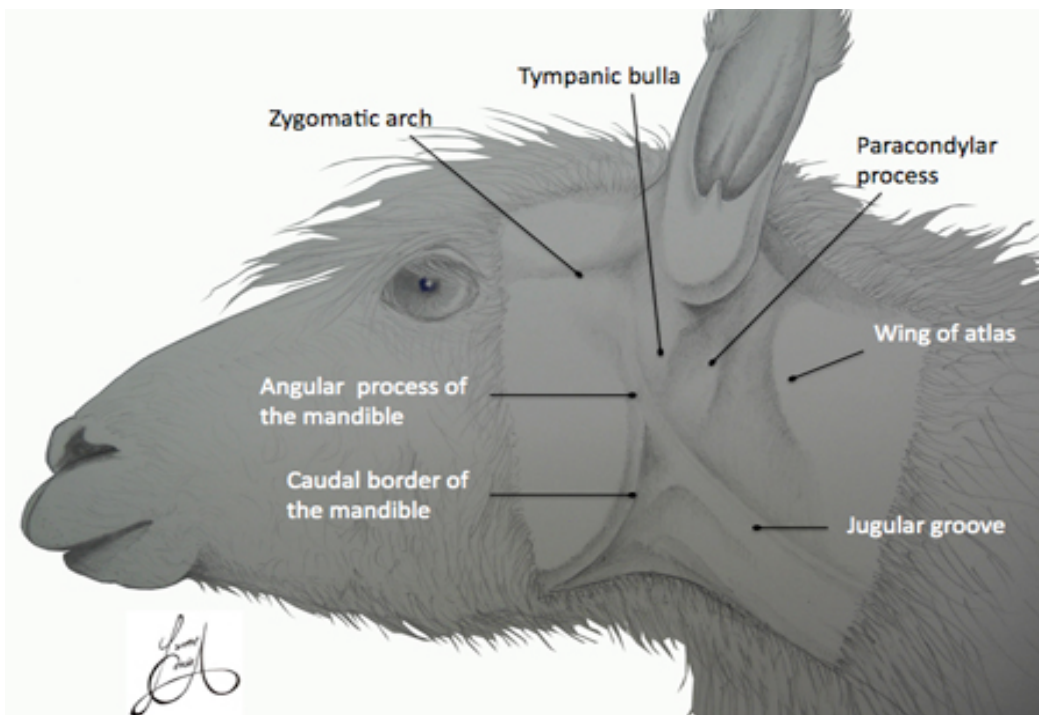


Figure 22. Left lateral view of a llama head and neck showing the landmarks to be used during the lateral bulla osteotomy and the area to be clipped for the surgery.

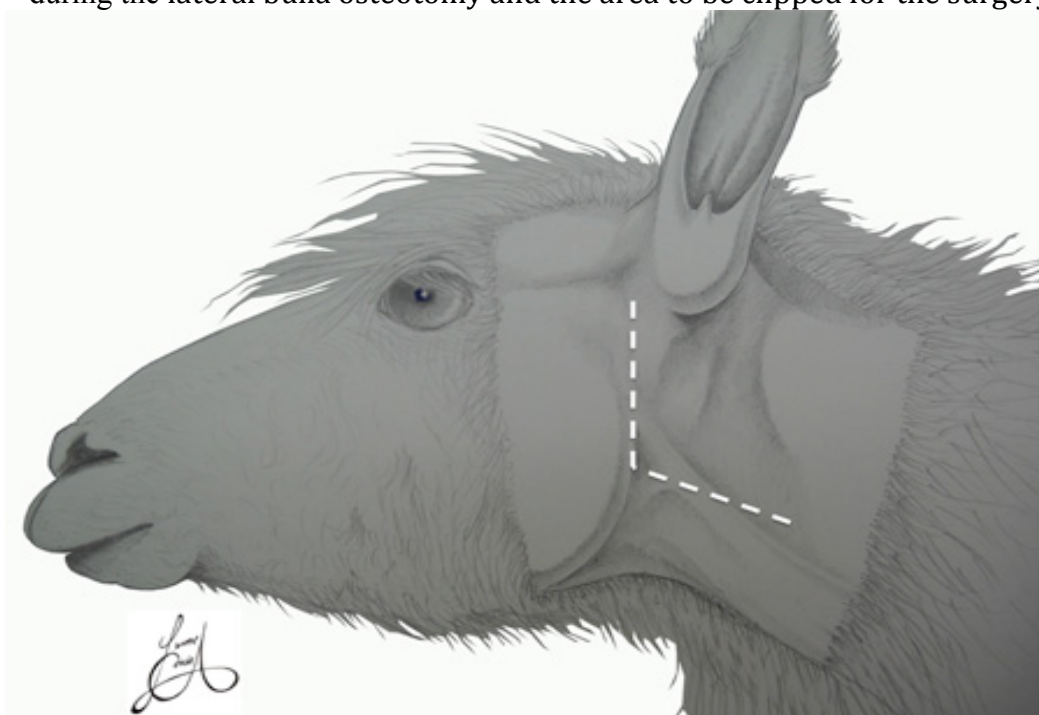


Figure 23. Left lateral view of a llama head and neck showing the location of the skin incision.

g- Skin reflection. Reflect the skin and subcutaneous tissue caudally, detaching the subcutaneous tissue from the deeper structures. With this procedure the surgeon should be able to visualize the caudal projection of the parotid salivary gland and the jugular vein, as well as the ventral portion of the parotidoauricularis muscle (Figure 24).

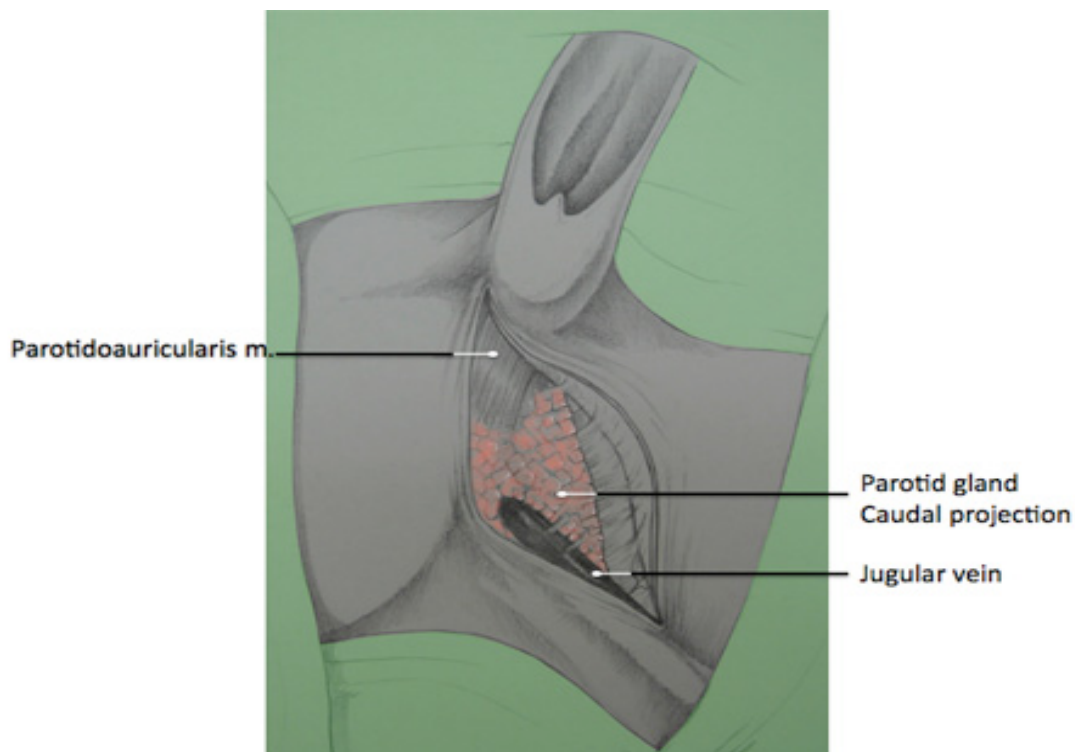


Figure 24. Left lateral view of a llama head and neck showing the structures to be identified after reflection of the skin.

h- Parotid salivary gland reflection. Using blunt dissection, carefully detach the caudal projection of the parotid salivary gland from the jugular vein and the tendon of the sternomandibularis muscle. Reflect the gland rostrally, avoiding excessive trauma to the gland. Visualize the great auricular nerve and reflect it caudally. Visualize or at least palpate the tendon of the sternomandibularis muscle, jugular vein, paracondylar process, the angular process of the mandible, the tympanic bulla and the tendon of the sternomastoideus muscle, which inserts on the mastoid process (Figure 25). If the parotid

salivary gland is accidentally traumatized, resection of a portion of the gland can be accomplished using a vertical line above the jugular vein, at the level of half the distance between the paracondylar process and the tympanic bulla. Incisions of the gland rostral to this point could cause injury to the facial nerve branches and/or maxillary vein.

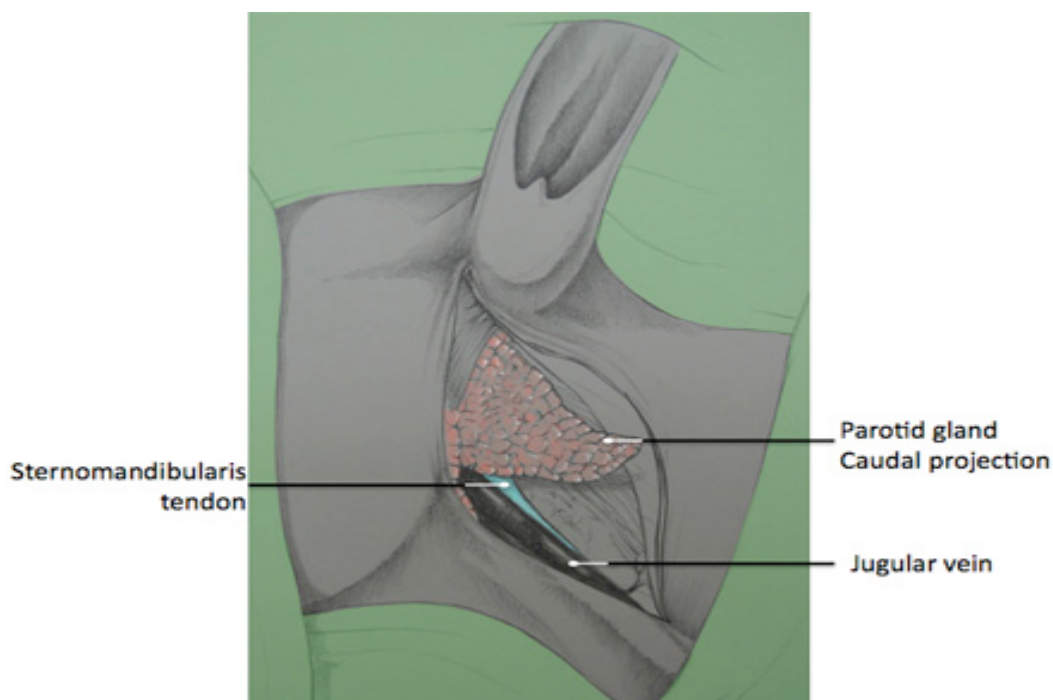


Figure 25. Left lateral view of a llama head and neck showing the caudal projection of the parotid gland separated from the jugular vein and sternomandibularis tendon.

i- Tympanic bulla approximation. Using a retractor, reflect the parotid salivary gland rostrally to visualize the tympanic bulla. Carefully detach the deep areas of the parotid gland from the tympanic bulla. Small vessels from the caudal auricular artery to the medial aspect of the parotid salivary gland can be ligated. This will expose the ventral two thirds of the caudo-lateral process of the tympanic bulla. Avoid dissection to further expose the dorsal third of the tympanic bulla close to the stylomastoid foramen in order

to prevent facial nerve damage. Expect the average total distance between the ventral border of the tympanic bulla and the stylomastoid foramen to be 22 mm. Follow the tendon of the sternomandibularis muscle rostro-dorsally until it joins with the tympanic bulla and mandible. This step will allow a safe rostro-ventral retraction of the maxillary vein. The sternomandibularis tendon is a good natural border between the tympanic bulla plane in which the surgeon will work and the deep structures, such as the caudal auricular, facial and external carotid arteries, mandibular salivary gland and its duct, digastricus muscle and hypoglossal nerve (Figure 26).

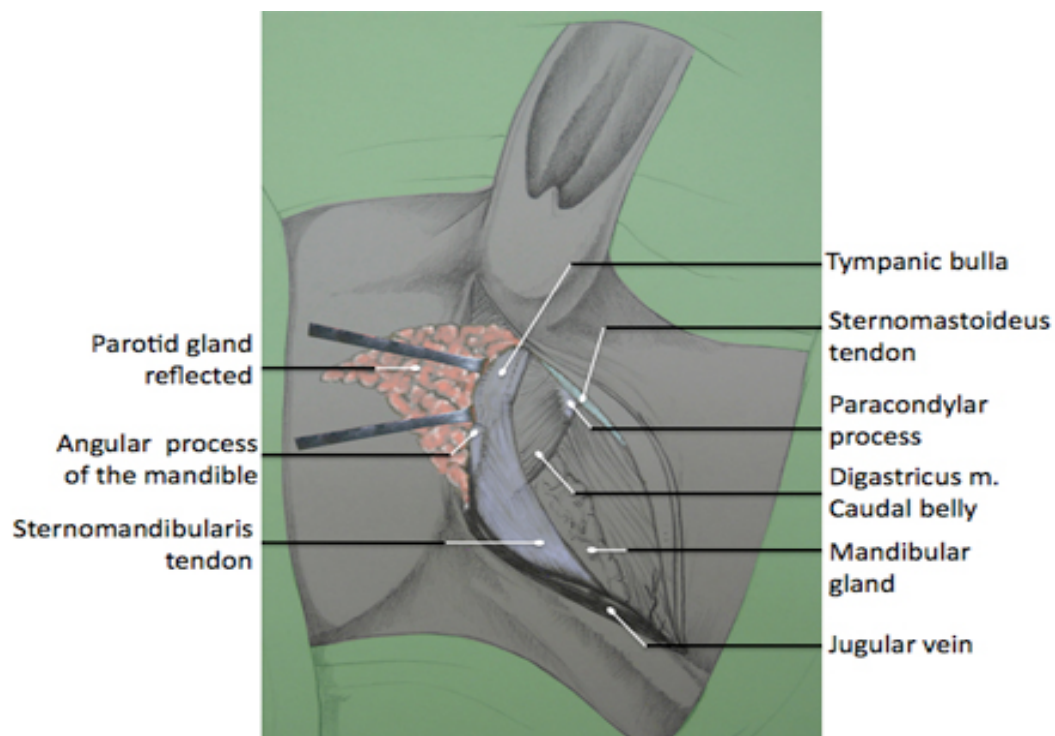


Figure 26. Left lateral view of a llama head and neck showing the tympanic bulla and related structures.

j- Tympanic bulla osteotomy. Incise the periosteum of the TB with a vertical incision in the middle of the caudo-lateral process of the tympanic bulla. Using a periosteal

elevator, elevate the periosteum rostrally and caudally, exposing the body and the caudal aspect of the tympanic bulla. Use great caution when retracting the retroarticular vein and the caudal auricular artery with the periosteum to avoid damaging them. Using a 3/16-inch Steinmann pin, perforate the external cortex of the ventral two-thirds of the caudo-lateral process of the tympanic bulla. Take a sample of the secretion inside the TB for further bacterial culture and sensitivity tests. Using a curette remove the trabecular osseous tissue and infected material. The angle found from the lateral border of the caudo-lateral process to the apex of the tympanic bulla was $113^{\circ} \pm 3^{\circ}$ in males and $120^{\circ} \pm 4^{\circ}$ in females (Figures 8, 27 and 28).

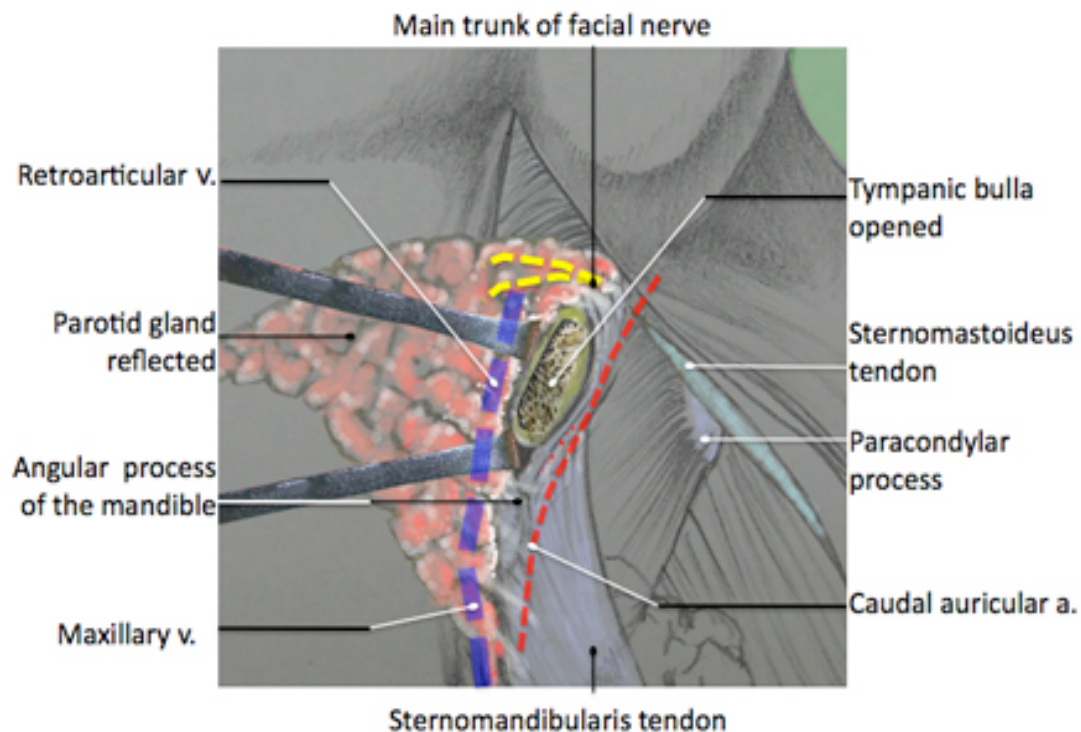


Figure 27. Left tympanic bulla of a llama after the opening of the periosteum and external wall. The dashed lines show the deep trajectory of the main trunk of facial nerve, maxillary and retroarticular veins, and caudal auricular artery.

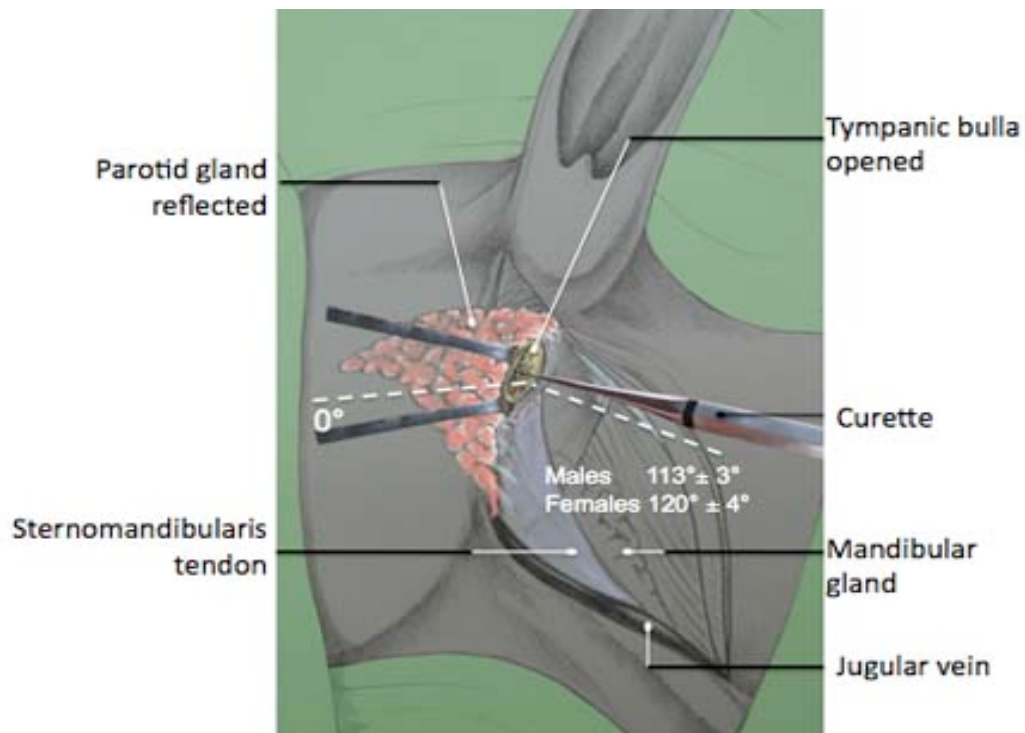


Figure 28. Left lateral view of a llama head and neck showing the tympanic bulla after the opening of the periosteum and the angle to introduce the curette. The curette is positioned horizontally on a line between the paracondylar process and the caudal margin of the neck of the mandible.

The bulla should be thoroughly lavaged. The decision should be made whether to place a drain for continued flushing after primary closure or to perform a partial closure leaving the tympanic bulla open to allow post surgical lavage and drainage.

Chapter 7- Conclusions

- The present study supplied new information about the shape size and location of the osseous external acoustic meatus, tympanic cavity and tympanic bulla in adult llamas. Included in this study are specific computed tomography descriptions and landmarks of the external and middle ear of llamas.
- The present study supplied new information about the gross anatomy of the soft tissue surrounding the osseous external acoustic meatus and tympanic bulla in llamas with specific descriptions and landmarks of the position of the vessels, nerves, and parotid salivary gland surrounding the ear.
- Head size in male and female llamas did not show a statistical significant difference. However, there were differences in the osseous external acoustic meatus length, the position of the maxillary vein and the facial vessels, as well as the length of the parotid salivary gland with the males being larger. The caudo-rostral angle of the tympanic bulla was in males smaller than in females.
- The anatomical descriptions and measurements obtained in this study lead to the production of numerous anatomical illustrations of the surrounding ear tissue in llamas.
- A theoretical surgical approach to the tympanic bulla is proposed based on the knowledge gained from the CT images and dissections of the soft tissue structures surrounding the llama ear.

- To prove the efficacy of this surgical approach to the tympanic bulla in the llama, it will be necessary to perform the procedure in live animals in the future.

Bibliography

1. Koenig JB, Watrous BJ, Kaneps AJ, Adams JG, Parker JE. Otitis media in a llama. *J Am Vet Med Assoc.* 2001; 218: 1619-1623.
2. Ellenport CR. The Ear. In: Saunders WB(ed): *Sisson and Grossman The Anatomy of Domestic Animals*. Philadelphia: Saunders Company, 1975; pp. 244-246.
3. König HE, Liebich HG. *Veterinary Anatomy of Domestic Mammals. Textbook and Colour Atlas.* 4th Edition. Stuttgart: Schattauer, 2009. Chapter 17.
4. Schaller O. *Illustrated Veterinary Anatomical Nomenclature.* Stuttgart: Ferdinand Enke Verlag, 1992.
5. Dyce KM, Sack WO, Weinsing CJG. *Textbook of Veterinary Anatomy.* 3rd Edition Philadelphia: Saunders, 2002. Chapters 3, 8 & 25.
6. Arencibia A, Rivero MA, Gil F, Ramirez JA, Corbera JA, Ramirez G, et al. Anatomy of the cranioencephalic structures of the camel (*Camelus dromedarius L.*) by imaging techniques: a magnetic resonance imaging study. *Anat Histol Embryol.* 2005; 34: 52-55.
7. Schmidt K, Piaia T, Bertolini G, De Lorenzi D. External auditory canal atresia of probable congenital origin in a dog. *J Small Anim Pract.* 2007; 48: 233-236.
8. Eom K, Kwak H, Kang H, Park S, Lee H, Kwon J, et al. Virtual CT otoscopy of the middle ear and ossicles in dogs. *Vet Radiol Ultrasound.* 2008; 49: 545-550.
9. Dennison SE, Schwarz T. Computed tomographic imaging of the normal immature California sea lion head (*Zalophus californianus*). *Vet Radiol Ultrasound.* 2008; 49: 557-563.
10. Kurilenkov G. Computed tomography of the temporal bones in children with developmental anomalies of the external and middle ear. *Vestn Rentgenol Radiol.* 2001;5: 4-8.
11. Zelikovich EI, Kurilenkov GV, Filipkin MA. Potential of temporal bone CT in the study of external acoustic meatus in health and pathology. *Vestn Otorinolaringol.* 2007;3: 7-13.

12. Seo Y, Ito T, Sasaki T, Nakagawara J, Nakamura H. Assessment of the anatomical relationship between the arcuate eminence and superior semicircular canal by computed tomography. *Neurol Med Chir (Tokyo)*. 2007; 47: 335-339; discussion 339-340.
13. Ayres VA, Lavinsky L, De Oliveira JA. Morphometric study of the external and middle ear anatomy in sheep: a possible model for ear experiments. *Clin Anat*. 2006; 19: 503-509.
14. Slatter D. *Textbook of Small Animal Surgery*. 3rd Edition. Philadelphia: Saunders, 2003. Chapters 122 & 123
15. Fowler ME. *Medicine and Surgery of South American Camelids*. Llama, Alpaca, Vicuña, Guanaco. 2nd Edition. Iowa: Iowa State University Press, 1998. p. 441.
16. Marriott MR, Dart AJ, Macpherson C, Hodgson DR. Total ear canal ablation and lateral bulla osteotomy in an alpaca. *Aust Vet J*. 1999; 77: 301-302.
17. Silver TI, Saskatoon SK. Computed tomographic evaluation of otitis in the alpaca. *ACVIM Forum Canadian VMA Convention 264* Montréal, QC. 2009.
18. Netter F. *Atlas of Human Anatomy*. 4th Edition. Philadelphia: Saunders, 2006. Chapter 1.
19. Flohr S, Schultz M. Mastoiditis--paleopathological evidence of a rarely reported disease. *Am J Phys Anthropol*. 2009; 138: 266-273.
20. Stalpers XL, Smink-Bol M, Verweij PE, Wesseling P, van Dijk GW. Fatal consequences of an ear infection. *Lancet*. 2009; 373: 1658.
21. *Nomenclatura Anatomica Veterinaria*. Zürich & Ithaca New York: International Committees on Veterinary Gross Anatomical Nomenclature, under the financial responsibility of the World Association of Veterinary Anatomists, 1994. p. 137.
22. Fubini S, Ducharme N. *Farm Animal Surgery*. Missouri: Saunders, 2004. pp. 505-506.
23. Smeak D, Inpanbutr N. Lateral approach to subtotal bulla osteotomy in Dogs: Pertinent anatomy and procedural details. 2005 [cited 2009 September 12]; Available from: <http://vet.osu.edu/LateralBullaOsteotomy.htm>
24. King AM, Hall J, Cranfield F, Sullivan M. Anatomy and ultrasonographic appearance of the tympanic bulla and associated structures in the rabbit. *Vet J*. 2007; 173: 512-521.

25. Bruyette DS, Lorenz MD. Otitis externa and otitis media: diagnostic and medical aspects. *Semin Vet Med Surg (Small Anim)*. 1993; 8: 3-9.
26. Duarte ER, Hamdan JS. Otitis in cattle, an aetiological review. *J Vet Med B Infect Dis Vet Public Health*. 2004; 51: 1-7.
27. Jensen R, Maki LR, Lauerman LH, Raths WR, Swift BL, Flack DE, et al. Cause and pathogenesis of middle ear infection in young feedlot cattle. *J Am Vet Med Assoc*. 1983; 182: 967-972.
28. Msolla P, Mmbuji WE, Kasuku AA. Field control of bovine parasitic otitis. *Trop Anim Health Prod*. 1987; 19: 179-183.
29. Martin WB, Aiten ID. *Diseases of Sheep*. Oxford: Blackwell Science, 2000. p. 233.
30. Sargison N. *Sheep Flock Health, a Planned Approach*. Oxford: Blackwell Science, 2008. pp. 337-338.
31. Scott P. *Sheep Medicine*. Iowa: Manson Publishing Ltd, 2007. pp. 177-1778.
32. Leskinen K, Jero J. Acute complications of otitis media in adults. *Clin Otolaryngol*. 2005; 30: 511-516.
33. Thompson PL, Gilbert RE, Long PF, Saxena S, Sharland M, Wong IC. Effect of antibiotics for otitis media on mastoiditis in children: a retrospective cohort study using the United kingdom general practice research database. *Pediatrics*. 2009; 123: 424-430.
34. Constantinescu G. *Guide to Regional Ruminant Anatomy Based on the Dissection of the Goat*. Iowa: Iowa State University Press, 2001.
35. Niehaus A. Dental disease in llamas and alpacas. *Vet Clin North Am Food Anim Pract*. 2009; 25: 281-293.
36. Smith BB, Timm KI, Reed PJ. Morphometric evaluation of growth in llamas (*Lama glama*) from birth to maturity. *J Am Vet Med Assoc*. 1992; 200: 1095-1100.
37. Smuts M, Bezuidenhout AJ. *Anatomy of the Dromedary*. Oxford: Oxford Science Publications, 1987. pp. 2-6, 62-8, 72-3, 146-7, 169-71, 189-192.
38. Evans H. *Miller's Anatomy of the Dog*. 3rd Edition. Saunders, 1993. p. 415.
39. Pasquini C, Spurgeon T, Pasquini S. *Anatomy of Domestic Animals. Systemic and Regional Approach*. Sudz Publishing, 1997. p. 460.

40. Oliver J, Lorenz M, Kornegay J. Handbook of Veterinary Neurology. Philadelphia: Saunders, 1997. Chapter 9.
41. Budras KD, Habel R. Bovine Anatomy. An Illustrated Text. Schlütersche, 2003. Chapters 3& 4.
42. Clayton H, Flood P. Color Atlas of Large Animal Applied Anatomy. Mosby-Wolfe, 1996. Chapter 2.
43. Done S, Goodoy P, Evans S, Strickland N. Color Atlas of Veterinary Anatomy. The Dog and Cat. 2nd Edition. Mosby Elsevier, 2009. Chapter 2.
44. Budras KD, Sack WO, Rock S. Anatomy of the Horse. An Illustrated Text. 4th Edition. Schlütersche, 2003. Chapter 3.
45. Bojrab MJ, Ellison GW, Slosum B. Current Techniques in Small Animal Surgery. 4th Edition. Maryland: Williams & Wilkins, 1998. pp. 102-109
