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Using of Diaphonization for Study of Domestic Pig's Auditory Tube

Nikolay Tsandev[1], Angel Vodenicharov[1], Ivailo Stefanov[2]*

[1] *Department of Veterinary Anatomy, Histology and Embryology,*
[2] *Department of Anatomy, Faculty of Medicine, Trakia University of Stara Zagora, Bulgaria*

* Corresponding author e-mail: drcandev@abv.bg

By diaphonization the cartilage and bone in the pig's auditory tube are visualized "*in situ*", with aim to estimate its suitability for that animal species. The study was carried on 16 head halves from 8 six months old (4 males and 4 females), 90-100 kg/b.w. pigs, slaughtered for meat consumption in licensed abattoir. For staining of cartilage part in blue – Alcian blue 8G was used, and in violet-red for the bone section – Alizarin Red S, respectively. Trypsin was used for achieving of transparence. Excellent results were obtained, as both cartilage and bone parts were very clearly delineated in three-dimensional images. The transparency enhanced precise and attraction of visualization the received specimens. In conclusion, the data obtained give enough detailed information for investigation structure of pig's auditory tube. The prepared durable specimens could be used many times from specialists in their work.

Key words: auditory tube, diaphonization, pig

Introduction

As an organ connecting the pharynx (*nasopharynx*) with the middle ear, the auditory tube (*tuba auditiva*) plays an important role not only in the drainage and ventilation of this part of the ear but is also a natural way for the pharyngitis to pass into the auris media cavity, causing otitis media. From here, it is possible for the inflammation to spread to the meninges [1, 2, 3, 5]. The tube consists of bone (*pars ossea tubae auditivae*) and cartilaginous (*pars cartilaginea tubae auditivae*) parts [4].

Diaphonization is a method that is widely used to visualize in a definite way the bone and cartilage tissues in different parts or in the whole vertebrates. The structures stained by a special method with different colors allow detailed research with different purposes, in which very accurate data and useful information about them are obtained [7].

This method allows to study the bone and cartilage elements of the skeleton in fish, amphibians, reptiles, as well as parts of the skeleton of mammals of different sizes [6]. The latter authors also used diaphonization to study in detail the structure of the tooth root canal.

The lack of more specific data on both parts of the auditory tube in domestic pigs, and in particular on its cartilaginous part, as well as the growing importance of this animal species as a model in biomedical research relevant to humans [8], motivates us to undertake the present study.

In this study, we used diaphonization for more precise differentiation and subsequent measurement of the bone and cartilage part of a transparent native preparation from auditory tube in a domestic pig. We believe that this is a suitable alternative method that allows conducting a precise morphometric study without the risk of mechanical damage to the studied structures**.**

Materials and Methods

Animals

The study was conducted on 16 halves of heads from 8 pigs at six months of age (4 males and 4 females), 90 – 100 kg/b.w., of the breed Bulgarian white × Landrace, slaughtered for local consumption in a licensed slaughterhouse, in accordance with the Bulgarian legislation.

Experimental design

Fixation: Immediately after the machine halving of the heads, they were placed in containers and transferred in a cooler bag to the Department of Veterinary Anatomy, Histology and Embryology of Stara Zagora. After rinsing on tap water for 30 minutes, the preparations were placed in distilled water for 10 minutes. Immediately after that they were immersed in buffered 10% formaldehyde at pH 8.0 for 97 hours.

Water rinsing: After fixation, the samples were placed for rinsing under tap water for 4 hours and then placed in distilled water for 10 minutes.

Bleaching: The tissues were further depigmented in a solution of 0.25% hydrogen peroxide and 0.5% potassium hydroxide (KOH) for 2 hours, followed by rinsing with distilled water for 30 minutes.

Dehydration: Placing the samples in 90° ethanol until they sink to the bottom of the container.

Alcian blue 8 GS staining: The halves were then placed for 24 hours at room temperature in a solution of Alcian blue 8G, dissolved in 95° ethanol, mixed with glacial acetic acid with pH 1.6 (90 mg Alcian blue 8G, 480 ml 95° ethanol and 120 ml glacial acetic acid) to stain the cartilage structures.

Rinsing in distilled water and processing in sodium borate solution: Removed from this solution, the halves were placed in a solution of 700 ml of distilled water and 300 ml of saturated sodium borate solution for 12 hours to neutralize the acid reaction.

Treatment with 1,5% Trypsin solution: To achieve transparency, the preparations were placed in a pre-prepared 1.5% solution of trypsin with saturated sodium borate solution – 3 parts and 7 parts distilled water for 72 hours.

Alizarin Red S staining: The Alizarin Red S working solution was prepared by adding 600 ml of 0.5% potassium hydroxide to 600 mg of Alizarin Red S. The samples are kept in this solution for 30 hours at 25°C.

Additional processing in 0.5% potassium hydroxide and glycerol: The preparations were then placed sequentially in a mixture of 0.5% potassium hydroxide and glycerol in the ratio as follows – 1:1 for 48 hours at room temperature, 1:2 for 72 hours at room temperature.

Pure glycerol: Finally, the preparations were placed in pure glycerol for 7 days at 28°C.

The samples were then removed from the solution, carefully drained, and placed on glass, illuminated with additional light from below for better visualization of the cartilage from the bone structures. The length of the auditory tube in the bone canal was carefully measured with a digital caliper with an accuracy of 0.01 mm and photographed with a digital camera. The measurements of the width and height of the cartilage part of the auditory tube were done by a steroscope (MBS-10).

Results

The position and views of treated auditory tubes with clear distinct outlines of both cartilaginous and osseous parts are show on **Fig. 1** and **Fig. 2**.

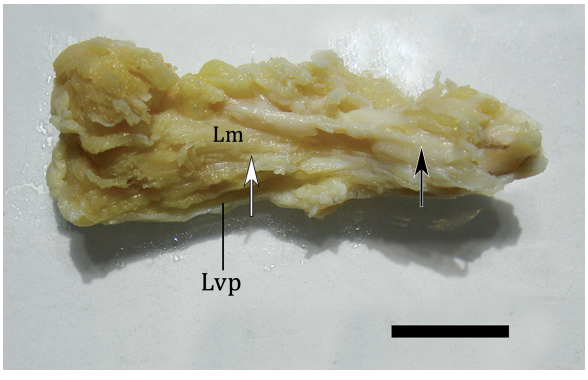
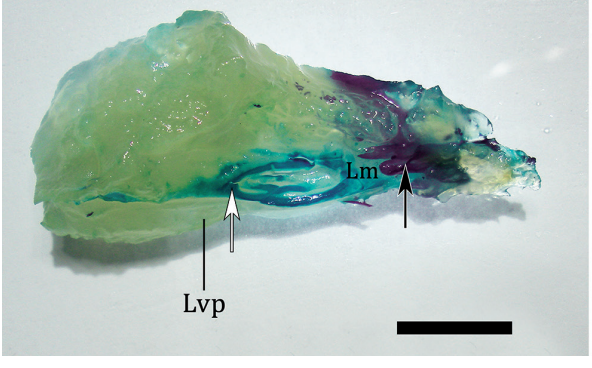


Fig.1. Medial view of a right auditory tube of a female pig – native preparation; **white arrow** – *pars cartilaginea tubae auditive*, **black arrow** – *pars ossea tubae auditive,* **Lm** – *lamina medialis*, **Lvp** – *m. tensor veli palatini.* Bar = 1 cm.



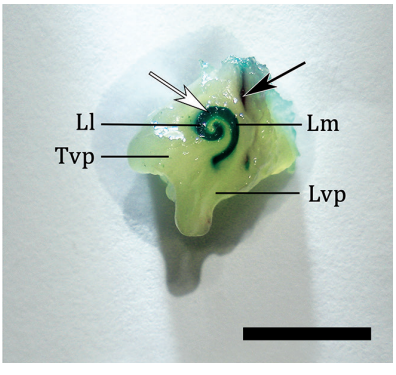


Fig.2. Left: Medial view of a right auditory tube of a female pig – diaphonized preparation (left)); **white arrow** – *pars cartilaginea tubae auditive*, **black arrow** – *pars ossea tubae auditive,* **Lm** – *lamina medialis*, **Lvp** – *m. tensor veli palatini.* Bar = 1 cm. **Right:** Transversal cut of a right auditory tube trough its bone part (right); **white arrow** – *pars cartilaginea tubae auditive*, **black arrow** – *pars ossea tubae auditive,* **Ll**– *lamina lateralis,* **Lm** – *lamina medialis*, **Tvp** – *m. tensor veli palatini,* **Lvp** – *m. tensor veli palatini.* Bar = 1 cm.

The measurements with a steroscope showed that the values of the width and height of cartilage part of left auditory tube in males the width varies from 2.10 to 3.05 mm (ẋ-2.61 mm), height – from 3.65 to 3.90 mm (ẋ-3.76 mm), and in females – the width varies from 2.80 to 3.40 mm (ẋ-3.14 mm), height – from 3.60 to 4.40 mm (ẋ-4.0 mm), in both sexes are higher than those of the right tube in males the width varies from 2.25 to 2.70 mm (ẋ-2.50 mm), height – from 2.80 to 3.10 mm (ẋ-2.96 mm), and in females – the width was 2.45-3.15 mm (ẋ-2.79 mm), and height – 3.25-3.80 mm (ẋ-3.61 mm). In male pigs, the measured parameters showed lower values compared to those in females.

The length of the auditory tube in the bone canal was measured with a digital caliper. Measurements of the length of the left and right tubes in both sexes showed similar values. In male pigs the length of right tube was 10.73-11.80 mm (ẋ-11.17 mm), and the left tube 9.60-11.84 mm (ẋ-10.97 mm),in female pigs – 10.52-12.40 mm (ẋ-11.5 mm), and 10.15-11.93 mm (ẋ-11.27 mm), respectively.

Discussion

Diaphonization is a method that is widely used to study the bone and cartilage elements of the skeleton in fish, amphibians, reptiles, as well as parts of the skeleton of mammals of different sizes [6]. The latter authors also used diaphonization to study in detail the structure of the tooth root canal. However, there is no information about the application of this method in studying the morphological features of the auditory tube in animals and humans. In the current study, the method of diaphonization was used for the first time in order to visualize the cartilage and bone parts of porcine auditory tube without the risk of mechanical damage of the studied structures. This method allowed precise measurements of the width and height of the cartilage part of auditory tube and the length of the bony part of the tube. We showed that the diaphonization can be very useful in studying the anatomy details of bony and cartilaginous structures of the animal and human body.

Conclusion

The data obtained give enough detailed information for investigation the peculiarities in position, form and structure of pig's auditory tube. The prepared specimens can be stored for unlimited period of time and they could be used many times from both medical and biological specialists in their work.

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