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Research Article

A morphological and histological study on Lyssa of Golden Jackal *(Canis aureus)*

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Abstract

This study aimed to reveal the topographic location, its measurements, and microscopic and macroscopic structures of Golden Jackal's Lyssa by using macro anatomical and histological methods. For this study, the tongues of three dead Golden jackals were first dissected to expose the lyssa. Then, lyssa were photographed macro anatomically, and measurements were made with the FIJI® program on the photographs. Additionally, for histological examination, the samples obtained from the lyssa were stained with Masson's Trichrome stain after undergoing histological procedures. In the topographic and macroscopic examination, Lyssa was located on the ventral surface of the tongue between the lingual frenulum and the apex linguae; its front half was visible just under the mucosa, and its shape was fusiform. In the histological examination, the lyssa was surrounded by a thick external connective tissue capsule from the outside, the fat tissue mass formed the ventral part of the structure within the capsule, and the muscle tissue mass formed the dorsal part, there was a thin internal connective tissue capsule separating these two tissues from each other. As a result, it was determined that the location and histological structure of Lyssa in the Golden Jackal were similar to its localization and histological structure in camels, dogs, and cats. Still, its shape was different from those of these animals. In addition, histologically, the external connective tissue capsule has branches extending into both the muscle and fat tissue mass, and the thin internal connective tissue capsule separates these two tissues.

Keywords: Golden jackal, Lyssa, Anatomy, Histology, Morphology

Introductıon

Jackals, which are small to medium-sized carnivores, consist of three species: the black-backed jackal (*Canis mesomelas*), the side-striped jackal (*Canis adustus*), and the golden jackal (*Canis aureus*). Golden jackals live in North Africa and south-central Eurasia, more closely related to wolves and coyotes, while the other two species are associated with each other (Hanlon, 2013). There are infrequent scientific studies on the anatomical structures of the Golden Jackal in the literature (Stan, 2017).

The lyssa, one of the anatomical structures, has attracted the attention of researchers since it was thought to be the cause of rabies disease in the ancient world (Capellari H, Egerbacher M, 2001). It described that the lyssa, derived from neuroectoderm, was a morphological and supporting structure situated the ventral surface of the apex the tongue in dog, cat (Konig et al., 2004; Besoluk et al., 2006; Shoeib et al., 2014; Sultana et al., 2017), swine (Capellari H, Egerbacher M, 2001), camel (Shoeib et al., 2014) and pangolins (Prapong et al., 2009). In carnivores, it was reported that the shape of the lyssa varies from strip to rod, depending on the animal species (Besoluk et al., 2006; Evans HE and De Lahunta, 2013; Shoeib et al., 2014). In literature (Konig et al., 2004; Besoluk et al., 2006; Capellari H, Egerbacher M, 2001; Shoeib et al., 2014) It described this anatomical structure as histologically composed of outer connective sheats, adipose tissue, blood vessels, the striated muscle, and nerve fibers. Capellari et al. (2001) claimed that when the striated muscle fibers contract, the volume of the lyssa reduces; on the contrary, when the fibers relax, the lyssa becomes undulated.

The topography, histology, and morphometry of the lyssa were extensively investigated in various domestic animals (Besoluk et al., 2006; Capellari H, Egerbacher M, 2001; Shoeib et al., 2014; Sultana et al., 2017) and few wild animals (Prapong et al., 2009) However, no literature data about the jackal's lyssa was found in the literature review. Therefore, this study was planned to reveal the topography, morphological, and histological structures of the golden jackal's lyssa.

Material and methods

Ethical approval

The dead animals used in this study were delivered to the Faculty of Veterinary Medicine, Kyrgyz-Turkish Manas University, with the date 18.01.2022/27.02.2022 of the Bugu-Enye Public Foundation. For this research, an application was made to the Animal Experiments Local Ethic Committee of 2024/03.

Animal Materials

This study was carried out on the heads of three adult dead Golden jackals of both sexes obtained from a Kyrgyz inspection institution. One of these heads belonged to males weighing 12 kg, while the other two belonged to female jackals weighing 11kg and 12 kg, respectively. The animals were immediately conveyed to the anatomy laboratory of the Veterinary Faculty of Kyrgyz-Turkish Manas University, and the common carotid artery was opened to insert a cannula to clean the arterial system. Then, the arterial system was given 10 % formaldehyde for fixation via the canal and kept in 10 % formalin and 1% glycerin solution for preservation. For the anatomical study, the lower chin with the tongue was separated from the heads at the level of the temporomandibular joint. After the lyssa was revealed, incising the ventral surface of the tongue medially, it was taken the length and width measurements of the lyssa using a vernier caliper and was photographed with a Canon® 50D camera. Based on the measurements in these pictures, the average tongue length, lyssa length, and thickness over five repetitions were calculated in the FIJI® program (Schneider et al. 2012). Then, the losses were transferred into containers with 10% formalin for histological examination. The Animal Care Committee of Kyrgyz-Turkish Manas University approved the use of dead jackal cadavers for this study. Nomina Anatomica Veterinaria (I.C.V.G.A.N, 2012) was followed for the nomenclature.

Light microscopy

For histological examination, The samples of the lyssa were taken from the middle ventral surface of the tongue. The sample blocks were first fixed in buffered formalin and cleaned in the running water for 24 hours. Then the specimens were dehydrated in graded alcohols (50-100 %), cleared in xylene, and embedded in paraffin for blocks. The serial sections were cut at 5-7 µm thickness. The sections were stained with Masson's Trichrome (H&E) for general histological structure (Bradbury & Gordon, 1990)

Results

Topographically, the lyssa is situated along the median plane of this area from the lingual frenulum to the free tip of the tongue in all cadavers (Fig1. A, the arrow). The lyssa of jackals was seen to be squeezed between two styloglossus muscles on the ventral surface of the tongue in the dissection (Fig1.C). While the caudal two-thirds were not visible because they were located deep in this region, the cranial third could be seen just under the mucosa of the ventral surface of the free end of the tongue (Fig. 1. C). Macroanatomically, the lyssa had a fusiform appearance (Fig. 1. C), a hard composition, and a pinkish-white color. The back half of this

fusiform was thin and made of connective tissue, the middle was swollen and stiff, and the front half was thinner than the middle and thicker than the back. The caudal third of the lyssa was composed of the connective tissue (Fig.1. D and B, The arrowhead).

The measurements of the lyssa are presented in table (1) below.

Table 1. The lyssa measurements of Golden Jackals

Cadavers	Live weight (Kg)	Tongue length (mm)	The lyssa length (mm)	The lyssa width (mm)
1	12	85.09	63.8	5.6
2	12	84.62	34.1	4.4
3	11	81.80	33.0	4.1

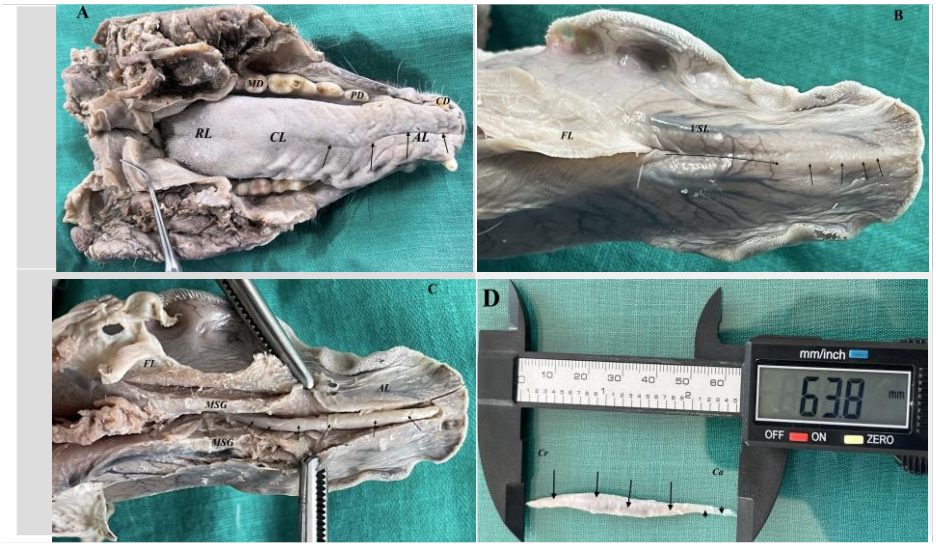

Fig1. Macroskopic views of the lyssa in Golden jackal. The dorsal surface of the tongue. *RL*= The root tongue, *CL*= The body tongue, *AL*=The apex of tongue, *CD*=The canin tooth, *PD*= The premolar tooth, *MD*= The molar tooth, The arrow= The lyssa. **B.** The ventral surface of the tongue. FL= The lingual frenulum, VSL= The sublingual vein, The arrow= The median groove of the tongue. **C**. The dissected ventral surface of the tongue. *FL*= The lingual frenulum, *MSG*= The styloglossus muscle, *AL*= The apex of tongue, The arrow= The lyssa. D. The dissected the lyssa. *Cr*= Cranial, *Ca*= Caudal, *The arrow*= The lyssa.

Histologically, the transverse section of the Jackal's Lyssa was encircled by the thick, dense connective tissue capsule. The capsule was mainly composed of bundles of collagen fibers (Fig. A/ CT). In the total body of Jackal's lyssa, the adipose tissue (Fig2. A/ AT) is situated ventral

to the striated muscle bundles (Fig2. A, B, C and D/ star) and enclosed by the thin internal connective capsule (Fig2. A/İC). The thin internal capsule separated the fat tissue mass from the dorsal muscle tissue mass (Fig2. A/İC). In addition, the external connective tissue capsule at the ventromedian plan split into several septa (Fig. 2. A/arrowhead), which penetrates the fat tissue of the lyssa, ending at the midline level of the fat tissue as connective tissue mass and partly divides the fat tissue (Fig2. A/rhomboid). Moreover, The muscle tissue mass, surrounded by the external connective tissue capsule, is located dorsal to the lyssa and the fatty tissue mass. The tissue mass consisted of scattered muscle fibers (Fig. A/star), irregular connective bundle (Fig 2. A, B, C, and D/ arrow), blood vessels, and nerve fibers (Fig 2. A, B /AT, and star). In addition, it was seen that the external connective capsule sent the short septa (Fig. 2.sc), which divided the muscle tissue mass. The course of the muscle bundles in the muscle compartments was observed to be parallel to the long axis of the lyssa.

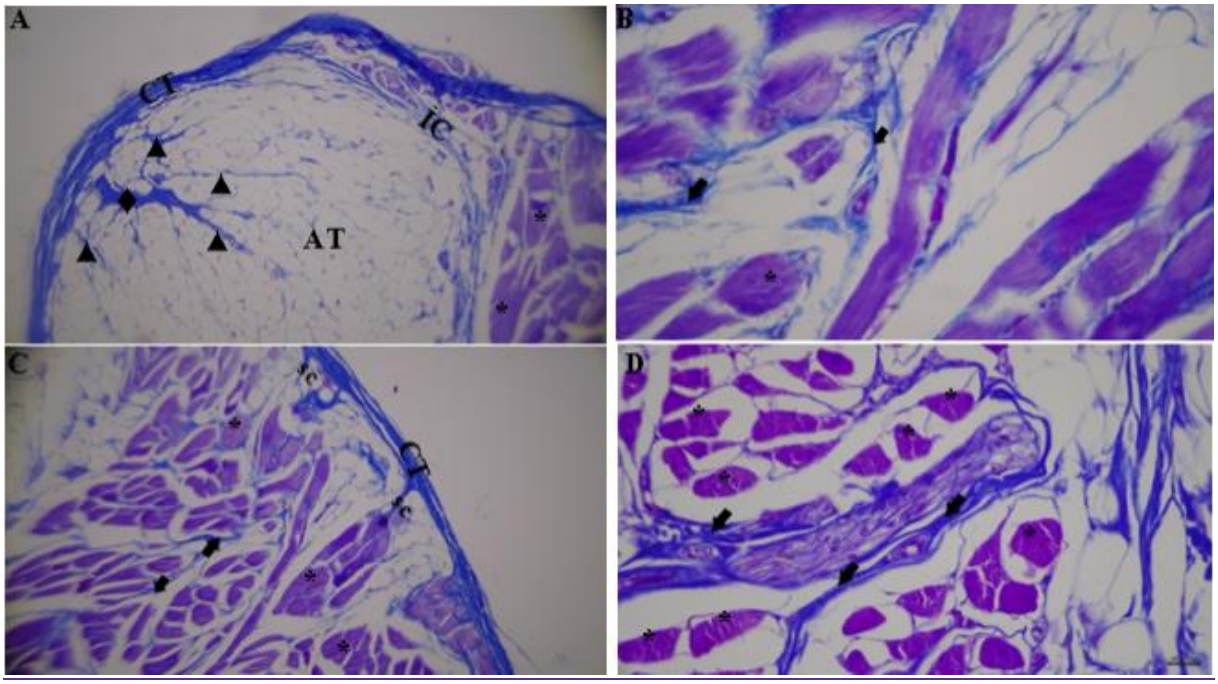

Fig2 (A,B,C,D). Histological views of the Lyssa in Golden jackal's. AT =Adipose tissue, CT =External connective capsule, İC= Internal capsule, Arrowhead =septa of adipose tissue, Stars= Muscle bundle, Arrow= Irregular connective bundle, Rhomboid= the connective tissue mass, sc=short connective septa

Discussion

The lyssa has been reported in the literature as a structure consisting of fat, striated muscle, connective tissue, nerve fibers, and capillaries (Capelleri et al., 2001; Eurell and Frappier, 2006; Beşoluk et al., 2006; Shoeib et al., 2014). In the study, the microscopic finding obtained from

Jackal's Lyssa was consistent with the in this literature data. In addition, some sources have reported that cartilage tissue does participate in the formation of the lyssa (Nickel et al., 1979; Schaller, 1992; Budras et al., 1994). Contrary to this literature report, the research findings show that Golden jackal's lyssa has no cartilaginous stated by Capellari et al. (2001), Eurell and Frappier (2006), Beşoluk et al. (2006) and Shoebid et al. (2014).

This study's histological finding was that the lyssa was surrounded by a connective tissue sheath, as depicted in the carnivore (Capelleri et al., 2001; Beşoluk et al., 2006; Shoeib et al., 2014) and the camel (Shoeib et al., 2014). However, muscle spindles, which Kamil et al. (2006) reported to be within the connective tissue sheath, were not found in this study.

As reported in the literature in cats, dogs (Capelleri et al., 2001; Beşoluk et al., 2006), and pangolins (Prapong et al., 2009) , in this study conducted in the jackal, it was observed that the lyssa was located in the median line of the ventral part of the tongue and between the two syloglosus muscles. In the current study, the cranial part of the lyssa was observed to be located just under the mucosa on the ventral surface of the tongue. The result was in agreement with that mentioned by the carnivore (Capelleri et al., 2001; Beşoluk et al., 2006; Shoeib et al., 2014), the crab-eating racon (Correa et al., 2012) and the camel (Shoebid et al., 2014)

Although it is reported in the literature that the lyssa is ridge-shaped in a camel (Kassem et al., 1984; Shoeib et al., 2014) and rod-shaped in a dog (Capelleri et al., 2001; Shoeib et al., 2014), spiral-shaped in a cat (Beşoluk et al., 2006) and J-shaped in a dog (Beşoluk et al., 2006), in this study, it was seen to be fusiform, it was observed that the findings obtained in the study were different in terms of shape from the literature reports on other animals.

As a result, it was determined that the location and histological structure of the lyssa in the Golden jackal was similar to its localization and histological structure in camels, dogs, and cats. Still, its shape was different from these animals. In addition, histologically, it has been observed that the external connective tissue capsule has branches extending into both the muscle and fat tissue mass and the internal connective tissue mass separating these two tissues from each other.

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