Anatomical, Histological and Radiological Studies of Parotid Salivary Glands of the Red Fox (Vulpes vulpes) with Comparison to Baladi Dog

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ABSTRACT

The present study was designed to investigate and compare the gross anatomy and histology of the parotid glands, with details of their ducts and radicals, in dogs and foxes, using contrast radiographs. It was applied on six heads for each apparently healthy, adult Baladi dog and red fox that was euthanatized by pentobarbital. All parotid salivary glands were dissected for morphological investigations. Two specimens were injected through the parotid papilla by colored latex neoprene to show the intra glandular distribution of the parotid duct and the other one was inoculated by Urographin for lateral radiograph. The parotid salivary gland appeared as a thin, unevenly triangular shaped gland, located ventrally to the base of the ear. Dorsal border of the gland was notched in the dog but not in the red fox. The parotid radicals had four main branches in the dog, while three major radicals were seen in red fox. Portions of the right and left lobes of parotid salivary glands from other three heads for each dog and fox were fixed in 10% neutral buffered formalin for histological and histochemical investigation. The parotid gland of the dog consisted of pure serous acini, while it consisted of both serous and mucous secretory units in the fox. The acinar cells in the dog revealed a weak positive reaction to the Alcian blue stain. On the contrary, acinar cells in the fox were particularly mucous adenomeres, displaying a significantly high positive reaction. Both secretory acinar cells and duct system lining cells in the dog and fox parotid gland showed weak positive reaction to Periodic Acid-Schiff stain. This work gave a detailed description of parotid salivary gland to help veterinarians in clinical diagnosis and surgical approach.

Key words: Parotid gland, Parotid radicals, Histology, Histochemistry, Dog, Red fox.

INTRODUCTION

Salivary glands play an important role in the lubrication of food and protection of the oral mucosa through the production of saliva, which plays an important role as an antimicrobial agent and helps in heat loss during painting (Eubanks and Woodruff 2010). The parotid salivary gland is the major salivary gland which is considered to be the natural reservoir of rabies virus, mainly in wild animals responsible for the transmission of that virus to humans (Acha and Szyfres 2003). The morphological considerations of parotid salivary glands in wild species can serve as an important tool for the efficient management of these species, mainly in zoos and animal houses (Souza Junior et al. 2014; Pereira and Faria Júnior 2018).

The veterinarians should be able to distinguish between normal and pathological conditions of parotid salivary glands during their examination, as there are several pathological conditions including inflammation, salivary calculi and rupture of salivary gland that can lead to submucosal swelling (runula) and development of neoplasms of the gland (Faustino and Dias Pereira 2007). Currently in veterinary medicine, different techniques are used for the diagnosis of diseases of the parotid salivary gland, such as conventional X-rays for radio-opaque sialoliths (Ryan et al. 2008); sonography for sialoceles, fine-needle aspiration for sialoceles and sialoadenitis (McGill et al. 2009; Park et al. 2009).

However, there is relatively little information in the literature about parotid salivary gland of red fox and its possible relation with that of dog. Therefore, the present study aimed to give detailed comparative information about morphological, histological and radiological aspects of the parotid salivary gland in fox and dog. Such information seems necessary for making a correct decision regarding surgical or medical interference of any affection of that gland, as well as for proper feeding and management of the animal after surgical intervention of the gland. This work is the first to describe the comparative anatomical, histological and radiological aspects of parotid salivary glands in the red fox and Baladi dog.

MATERIALS AND METHODS

Ethical approval for this study was obtained from the ethical standards set by the institutional animal care and use committee (Vet. CU.12/10/2021/353).

Animals

The present study was conducted on the parotid salivary glands obtained from both sides of six heads from each apparently healthy, adult Baladi dog and red fox of both sexes, with body weight varying from 7 to 12kg for dogs and 4 to 7kg for foxes. These animals were taken from the Western Egyptian desert. They were euthanatized with an overdose of pentobarbital (1mL/4.5kg IV).

Anatomical Study and Contrast Radiographic Imaging

The parotid salivary glands from six heads were dissected through the removal of skin showing their relation to the neighbouring structures, although their ducts were measured till reaching the parotid papilla in the oral cavity. The length and width of each gland were measured by using digital callipers. Two heads were injected through the parotid papilla with a coloured orange and green latex neoprene for illustrating the intra-glandular distribution. One head was inoculated with Urographin at parotid papilla and a lateral radiograph was obtained. The exposure factors were 100cm FFD, with 15mAs and 55kV (Rezk and Shaker 2017).

Histological Study

The parotid salivary gland samples were obtained from the right and left lobes of three heads for each dog and fox.

Light Microscopic Examination

Immediately after removal, the samples of parotid salivary glands were sectioned, and fixed in 10% neutral buffered formalin for 48 hrs. The fixed samples were dehydrated in ascending series of alcohol, cleaned in xylene, and embedded in paraffin wax. Then, 3-4μm thick sections were obtained by rotatory microtome and stained with Delafild’s iron Hematoxylin and Eosin (H&E) stain for general tissue structure studies (Bancroft and Gamble 2013).

Histochemical Examination

Paraffin sections were stained with Periodic Acid-Schiff (PAS) stain for neutral glycoprotein and with Alcian blue stain (pH 2.5) for acidic mucopolysaccharides (Bancroft and Gamble 2013).

Statistical Analysis

The data were presented as mean±SE. In order to ascertain the magnitude of variation in different parameters between the two species, the data were analyzed using a paired sample T-Test. Statistical significance was set at P<0.05.

RESULTS

Gross Anatomical Findings

The parotid salivary gland was partially covered laterally by the parotid-auricularis muscle. Its medial surface was related to the branches of the facial nerve, superficial temporal artery and the maxillary vein. The parotid gland (Figs. 1, 2, 3A & 3B/pg) was a thin, irregularly triangular shaped gland. It was located ventral to the wing of atlas and base of ear, and caudal to the ramus of the mandible. In the dog the mean length of the parotid gland was 6.0±0.5cm and mean width was 3.0±0.5cm, while in red fox the mean length of parotid gland was 3.0±0.5cm and the mean width was 2.0±0.5cm.

The parotid gland of the dog appeared as faint pinkish in color (Fig. 3A), while the red fox had a grayish-yellow colored gland (Fig. 3B). Each gland presented three borders (Figs. 3A & 3B); the rostral border was concave, indistinctly longer than the caudal one and related to small rounded parotid lymph node. The caudal border in both species was straight in contact with the maxillary vein. The dorsal border (Figs. 3A & 3B) was notched in the dog by the auricular cartilage, while such notch was absent in the red fox. The parotid gland had three angles in both species (Figs. 3A & 3B); these angles were pre-auricular, post-auricular, and masseteric. However, in dogs, the masseteric angle formed the apex of the gland; it was a thin elongated lobe, extending between the caudal borders of the masseter and rostral border of the mandibular salivary gland.

The parotid duct in the dog (Fig. 1A, 1C&3D/pd) emerged from the middle of the rostral border of the gland. It passed in a straight manner under the skin, at the lateral surface of masseter, ventral to dorsal buccal nerve and zygomatic muscle, then turned rostro-ventrally between the facial vein and facial artery, reaching the parotid papilla (Fig. 1B/pp) that opened at upper 4th cheek tooth; its length was 6.0±0.5cm. However, in red fox, the parotid duct (Fig. 2A, 2C&3C/pd) originated from the ventral aspect of the rostral border of the gland and passed in a curved manner at masseter muscle in between dorsal and ventral buccal branches of the facial nerve, and then passed straight rostrally beneath the zygomatic muscle, facial vein and artery, till it opened at parotid papilla (Fig. 2B/pp) between upper 3rd and 4th premolar tooth. Its length was 5.0±0.5cm.

There were four main parotid radicals in the dog, while only three principal radicals were seen in the red fox. The first one (dog and fox) (Figs. 3C & 3D/1) emerged at the rostral border of the gland. The second branch (dog and fox) (Figs. 3C & 3D/2) was the largest ramus that drained from the rostral lobe, body, and caudal lobe of the gland (Figs. 3C & 3D/2a, 2b, 2c). At the apex, one radical (Fig. 3C/3) emerged in the fox, while two small branches (Fig. 3D/3, 4) drained from the caudal border and apex of the gland in the dog.

Histological Observations

In the present study, each parotid salivary gland in the dog and the fox was surrounded by a connective tissue capsule, which appeared thicker in fox than that in the dog, and contained blood vessels. Septa originating from the connective tissue capsule divided the gland into lobes and lobules. These septa were thicker in the fox compared to those in the dog, and contained blood vessels and ducts (Figs. 4A and 4C). The parenchyma of the dog parotid gland consisted of pure serous acini. However, in the fox, the glandular parenchyma was composed of both serous and mucous secretory units. Serous secretory units had basally situated myoepithelial cells, and narrow star-shaped lumen, lined by truncated pyramidal cells that had
spherical basal nuclei. The basal part of cytoplasm appeared basophilic, while the apical part appeared acidophilic and contained zymogenic granules. The mucous secretory units had a wide lumen, lined by cuboidal mucous secreting cells resting on the basement membrane, and had flat nuclei and foamy cytoplasm (Fig. 4B and 4D).

In both dogs and foxes, the duct system of the parotid glands began with an intercalated duct lined by simple cuboidal cells, a striated duct lined by striated columnar cells, an interlobular duct lined by simple columnar cells, and finally a main excretory duct lined by stratified squamous cells near its opening in the oral cavity.

Concerning the secretion of the gland, the histochemical results of the present study revealed that the secretory acinar cells of parotid salivary glands in the dog exhibited weak positive reaction, while duct system lining cells showed negative reaction for Alcian blue stain (Fig. 5A). However, secretory acinar cells in the fox parotid gland, particularly mucous adenomeres, displayed a strong positive reaction, and duct system lining cells had a mild positive reaction for Alcian blue stain (Fig. 5B). There was a significant increase in the Alcian blue stain intensity in parotid gland parenchyma of the fox than that of the dog, as shown in Figure 6. Meanwhile, both secretory acinar cells and duct system lining cells of parotid glands in both species showed weak positive reaction to PAS stain (Figs. 5C and 5D).

DISCUSSION

Gross Anatomical Features

The parotid salivary glands in carnivorous animals, including the dog, cat, fox, and ferret are grossly similar to those in other mammalian species (Tadjalli et al. 2004). In the present study, the parotid salivary gland was a thin, irregularly triangular in shape and located ventrally to the wing of the atlas, caudal to the ramus of the mandible, and ventral to the base of the ear. These results are supported by those reported previously in dogs (Tadjalli et al. 2004; Dyce et al. 2010; Gaber et al. 2020) and crab-eating foxes (Pereira and Faria Júnior 2018). However, in the camel (Rezk and Shaker 2017) and sheep (Dehghani et al. 2000), it was irregularly rectangular, and regularly quadrilateral in the donkey (Maher et al. 2020). Moreover, in cattle (Al-Sadi 2013) it was elongated and thick but oval in goats (Tadjalli et al. 2002) and rounded in cats (Dyce et al. 2010).

This gland had three borders; the rostral border was indistinctly longer than the caudal one and related to small rounded parotid lymph node, and in contrast to cattle (Al-Sadi 2013), this border was concave. The caudal border in the dog and red fox was straight in contact with the maxillary vein, as has also been reported for donkeys (Maher et al. 2020), whereas it is convex dorsally and concave ventrally in the camel (Rezk and Shaker 2017).
Fig. 4: Photomicrograph of parotid salivary gland of a dog (A & B) and a fox (C & D). A: Dog parotid salivary gland showing thin connective tissue capsule (arrow) and septa (chevron) divided the gland into lobules (L) (H&E stain, X100). B: Dog parotid gland composed of pure serous acini (yellow arrow) and had spherical basal nuclei. Basally situated myoepithelial cell (orange arrow) was observed. Intercalated duct (ID) was observed (H&E stain, X1000). C: Fox parotid gland surrounded by thick connective tissue capsule (arrow) and septa (chevron) divided the gland into lobules (L) (H&E stain, X100). D: Fox parotid gland composed of serous acini (arrow) and mucous acini (chevron) (H&E stain, X1000).

Fig. 5: Histochemical examination of parotid salivary gland of dog (A & C) and fox (B & D). A: Dog parotid gland showed weak positive reaction of acinar cells (arrow) and negative reaction of duct lining cells (chevron) to Alcian blue stain (X400). B: Fox parotid gland showed strong positive reaction of acinar cells, especially mucous acini (arrow) and mild reaction of duct lining cells (chevron) to Alcian blue stain (X400). C: Dog parotid gland and D: Fox parotid gland showed weak positive reaction to PAS stain in both secretory acinar cells (arrow) and duct system lining cells (chevron) (X400).
In the dog, the parotid duct emerged from the middle of the rostral border of the gland, passed in a straight manner under the skin, at the lateral surface of masseter, then turned rostro-ventrally between the facial vein and facial artery, reaching the parotid papilla. These findings are supported by those reported previously (Tadjalli et al. 2004; Konig et al. 2009; Dyce et al. 2010; Gaber et al. 2020). However, in the red fox, parotid duct originated from the ventral aspect of the rostral border of the gland, then passed in a curved manner at masseter muscle in between dorsal and ventral buccal branches of the facial nerve and passed straight rostrally beneath the zygomatic muscle, facial vein and artery, till it opened at parotid papilla. These findings are similar to those found in other carnivorous mammals such as dogs (Mendonça et al. 2004), raccoons and coatis (Santos et al. 2012) and crab eating foxes (Pereira and Faria Júnior 2018).

Results of the present study have also revealed that parotid papilla opens at upper 4\textsuperscript{th} cheek tooth in the dog, while between upper 3\textsuperscript{rd} and 4\textsuperscript{th} premolar teeth in the fox. Tadjalli et al. (2004), Konig et al. (2009), Dyce et al. (2010) and Gaber et al. (2020) have observed that the parotid duct opens opposite to the third or fourth premolar tooth in dogs and the second premolar in cats. Moreover, Mursal (2016) reported that opening of the parotid duct was opposite to the fifth upper cheek tooth in the ox, and the second upper molar tooth in sheep and goats.

Our observations in red fox and dog were the first to describe anatomical features of the parotid radicals; there were four main branches in the dog, as in the camel (Rezk and Shaker 2017), while three principal radicals were seen in the red fox, as has been described in donkeys (Maher et al. 2020). The first one emerged at the rostral border of the gland. The second branch was the largest ramus that collected secretions from the rostral lobe, body, and caudal lobe of the gland. At the apex, one radical emerged in the fox, while two small branches in the dog drained the caudal border and apex of the gland.

**Histological Findings**

Histological findings in the present study revealed that the parotid salivary gland in the dog was surrounded by thin connective tissue capsule containing blood vessels. Septa emerging from the connective tissue capsule divided the gland into lobes and lobules. Gaber et al. (2020) described a similar histological structure of the dog parotid gland. The parenchyma of the dog parotid gland consisted of pure serous acini, which is in line with the findings of Gaber et al. (2020), who reported that the dog parotid salivary gland consisted mostly of pure serous acini producing mainly a serous secretion. Our results regarding the histological appearance of parotid gland in the dog are also similar to those described for humans and rodents (Amano et al. 2012), deer (Adnyane et al. 2010), goats and sheep (Elewa et al. 2010).

Histochemical results of the present study revealed that the secretory acinar cells of the dog parotid gland showed a weak positive reaction, while duct system lining cells showed a negative reaction for Alcian blue stain. These results contradict Gaber et al. (2020), who reported that both acinar cells and duct lining cells showed negative reactions to Alcian blue stain in the dog. The significant increase in the Alcian blue stain intensity in parotid gland
parenchyma in the fox may be attributed to the nature of food, because Gaber et al. (2020) have reported that dogs fed dry food mainly produce serous saliva, while dogs fed on meat diet secrete saliva with high mucous contents.

The secretory acinar cells and duct system lining cells of dog parotid gland showed weak positive reaction to PAS stain. These findings support the results of Gaber et al. (2020), who also stated that the secretory acini of the dog parotid gland exhibited a weak positive reaction, but the duct system exhibited a negative reaction to PAS stain.

Conclusión

The anatomical description of the salivary glands of wild carnivores improves the basic information for a veterinarian in clinical diagnosis and surgical interventions. According to histological observations in this study, the secretion of the dog parotid salivary gland is watery with little neutral mucin, while in the fox, parotid salivary gland secretion is mixed, predominantly mucous with a mixture of neutral and acid mucins, which depended on the eating habits of animals of the two species.

Author's Contribution

AMI and NAS designed the project, performed the anatomical representation of parotid glands and described their radical division. YHA performed histological and histochemical studies. All authors reviewed the manuscript and approved its final version.

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