



The Labial and Zygomatic Salivary Glands in Mixed Breed Dogs in Trinidad: Anatomical Location, Histological Features and Histochemical Characteristics

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ABSTRACT

The objective of this investigation was to give detailed descriptions of the anatomical location, histological features and histochemical characteristics of the labial and zygomatic salivary glands in mixed breed dogs. This study was performed on five heads of adult mixed breed dogs of both sexes. The heads were dissected to detect in situ position of the labial and zygomatic salivary glands. The glands were dissected and examined grossly. Samples of the glands were taken, processed and stained using hematoxylin and eosin and Masson's Trichrome for histological examination as well as Periodic Acid-Schiff, Alcian Blue (pH 2.5 and 1.0) and a combination of Periodic Acid-Schiff and Alcian Blue (pH 2.5 and 1.0) techniques for histochemical examination. The labial and zygomatic salivary glands were located in the lower lip and in the orbit respectively and they were surrounded by fibrous capsules containing collagen fibers. They were minor, compound, mixed tubuloalveolar glands. They composed of mucous acini, mucous acini with serous demilunes and isolated serous acini. The secretion of the glands (chiefly mucous) consisted of neutral mucins, acid carboxylated mucins and acid sulphated mucins. The duct system of the glands was intralobular (intercalated and striated ducts) and interlobular ducts. The anatomical location as well histological and histochemical structures of the labial and zygomatic salivary glands were important to classify the glands and their secretion as well as to give veterinarians knowledge during clinical examination of the oral and orbital regions, and to recognize normal and pathological conditions.

Keywords: Anatomy, Dog, Labial, Salivary glands, Zygomatic

INTRODUCTION

The salivary glands are the accessory structures of the gastrointestinal tract and their exocrine secretion (saliva) prepare ingesta for digestion as it contains mucopolysaccharides, water, enzymes and lubricating glycoprotein (Adnyane et al., 2010; Al-Abbad, 2011). They are developed at various locations with different structures and secretions which are either serous, mucous or seromucous (Jaskoll et al., 2002). The major salivary glands include parotid, mandibular and sublingual, while the minor salivary glands include the lingual, buccal and labial salivary glands, which are found in the lingual, buccal and labial mucosa respectively (Kimura et al., 1998; Popovici et al., 2003; Barone, 2009; Miclăuş, 2012). The diversity of structures and secretions of the salivary glands depends on the animal's feeding habits (Tandler and Phillips, 1998). Zygomatic and molar glands are major salivary glands which are present in carnivores and cat respectively (Dyce et al., 2004; Mohammadpour, 2010). However, in other animals the zygomatic glands are classified either as minor (Dellman and Eurell, 1998) or major (Evans and Christensen, 1979). Tumors of salivary glands have been reported in the horse, cattle, sheep, goat, dog and cat (Head, 1976; Carberry et al., 1988). Pleomorphic labial gland adenoma was recorded in a dog (Izawa et al., 2017). Zygomatic gland mucocele associated with exophthalmos is less common in dogs (McGill et al., 2009 and Cannon et al., 2011); however, it was reported in a cat and ferret (Miller and Pickett, 1989; Speakman et al., 1997). Zygomatic salivary glands tumors in the orbit of the dog were recorded previously (Buyukmihiç et al., 1975; Attali-Soussay et al., 2001). There is no available study documenting the histomorphological and histochemical features of the labial and zygomatic salivary glands in mixed breed dogs. It is important for veterinarians to recognize normal and pathological conditions during examination and investigation of the oral and orbital cavities of dogs. Moreover, the labial gland is used in autologous transplant to stimulate the lubrication of the ocular surface and the zygomatic salivary glands problems can cause exophthalmos.

So the present study aimed to indicate the anatomical location, histological aspect and histochemical features of the labial and zygomatic salivary glands of mixed breed dogs.

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MATERIALS AND METHODS

A total of five apparently healthy adult mixed breed dogs (3 males and 2 females) were used for the current study. Fresh euthanized dogs with pentobarbital (1ml/4.5 kg, IV) were obtained from the Trinidad and Tobago Society for the Prevention of Cruelty to Animals (TTSPCA) and transferred to the anatomy laboratory, School of Veterinary Medicine, The University of the West Indies, Trinidad and Tobago. The head of each dog was dissected by removing the bony structures such as the zygomatic arch and mandible, followed by the removal of the masseter, temporalis and pterygoid muscles. The labial and zygomatic glands were dissected and excised from the adhering tissues. The position of the glands and their related structures were described and photographed using a Sony 12 megapixel digital camera (Sony Coporation, Japan). Tissue specimens were taken from the labial and zygomatic glands and fixed in 10% neutral buffered formalin for 24-48 hours, then dehydrated in ascending grades of ethanol, cleared in xylene and then embedded in paraffin blocks. Sections of 5µm thickness were prepared using a microtome and stained by hematoxylin eosin for general histological examination and Masson's trichrome stain for detecting collagen fibers (Culling et al., 1985). Periodic Acid-Schiff (PAS), Alcian Blue (AB pH 2.5 and 1.0) and AB-PAS (pH 2.5 and 1.0) techniques were used to detect and differentiate the types of the secretion of the labial and zygomatic salivary glands (Culling et al, 1985; Cunha et al., 2016). The stained sections were observed under a light microscope (Olympus BX40 with an Olympus DP 15 megapixel digital camera, Japan) at different magnifications and photomicrographs were taken.

Ethical approval

The ethical approval from the University of the West Indies, St. Augustine, Trinidad and Tobago ethical committee (CEC906/02/19) was obtained.

RESULTS

Anatomical location

The labial salivary gland of the mixed breed dogs was a lobulated gland located in the lower lip. It extended from the lower second premolar tooth to the distal edge of the lower molar tooth. The gland was located within the connective tissue, deeply embedded in the mucosal lining of the vestibular cavity (Figure 1). The zygomatic salivary gland was present as a pyramidal, lobulated organ in each side of the head. It was located ventrolaterally in the orbit, under the rostral end of the zygomatic arch and dorsolaterally to the pterygoideus muscles. It was covered by adipose tissue and encapsulated. The medial surface of the gland was slightly grooved by the maxillary vessels and nerve (Figure 1).

Histological features

The zygomatic and labial salivary glands of the mixed breed dogs were enclosed in a well-developed capsule containing collagen fibers. Interlobular connective tissue septae were originated from the tunica albuginea dividing the gland into various sized lobules (Figure 2). The labial and zygomatic salivary glands were compound, exocrine, tubuloacinar mixed glands containing mucous acini, mucous acini with serous demilunes (mixed seromucous units) and sporadic serous acini. The mucous secretory units, which secrete only mucous, were predominant. The mucous cells had basally located, flattened nuclei with foamy cytoplasm. The serous demilunes with cells had rounded central nuclei and were capped as a half-moon on the mucous cells. The sporadic serous acini showed rounded nuclei with dark cytoplasm. Myoepithelial cells and were present as spindle-shaped cells incorporated between the basement membrane and epithelium of the secretory cells. The ducts of the labial and zygomatic salivary glands were intralobular and interlobular. The intralobular ducts consisted of intercalated and striated ducts which were lined by cuboidal epithelium and tall columnar cells with basal striation respectively. The interlobular ducts were the main excretory ducts in the labial and zygomatic salivary glands. They were lined by stratified columnar epithelium and they were situated in the connective tissue septum between the gland's lobules (Figure 2).

Histochemical characteristics

The histochemical evaluation of the labial and zygomatic salivary glands showed that the mucous secretory units and serous demilunes in the seromucous acini and sporadic serous clusters as well as the striated ducts were positive to the PAS stain and looked magenta red due to the presence of the neutral mucins (Table 1 and figures 3 and 4). The mucous secreting cells were positive to AB (pH 2.5) due to the presence of the acid mucins with strongly staining of acid carboxylated mucins (Figures 3 and 4) and to the AB (pH1.0) due to the presence of the acid sulphated mucins and looked blue (Figures 3 and 4). The combination of AB-PAS showed that the mucous cells stained reddish purple at pH 2.5 (Figures 3, 4 and 5 and bluish purple at pH 1.0 (Figures 3, 4 and 5). On the other hand, the reaction of the serous demilunes (Figures 3 and 5) were negative to AB (pH 2.5 and 1.0).

Table 1. Histochemical features of the mucous secretory units and serous demilunes of the labial and zygomatic salivary glands in the mixed breed dogs in Trinidad

Technique / Gland	Labial salivary gland		Zygomatic salivary gland	
	Mucous secreting cells	Serous demilunes	Mucous secreting cells	Serous demilunes
PAS	+	+	+	+
Alcian Blue (pH 2.5)	+	-	+	-
Alcian Blue (pH 1.0)	+	-	+	-
AB-PAS (pH 2.5)	+	-	+	-
AB-PAS (1.0)	+	-	+	-

PAS: Periodic Acid-Schiff; AB: Alcian Blue; +: positive staining; -: negative staining

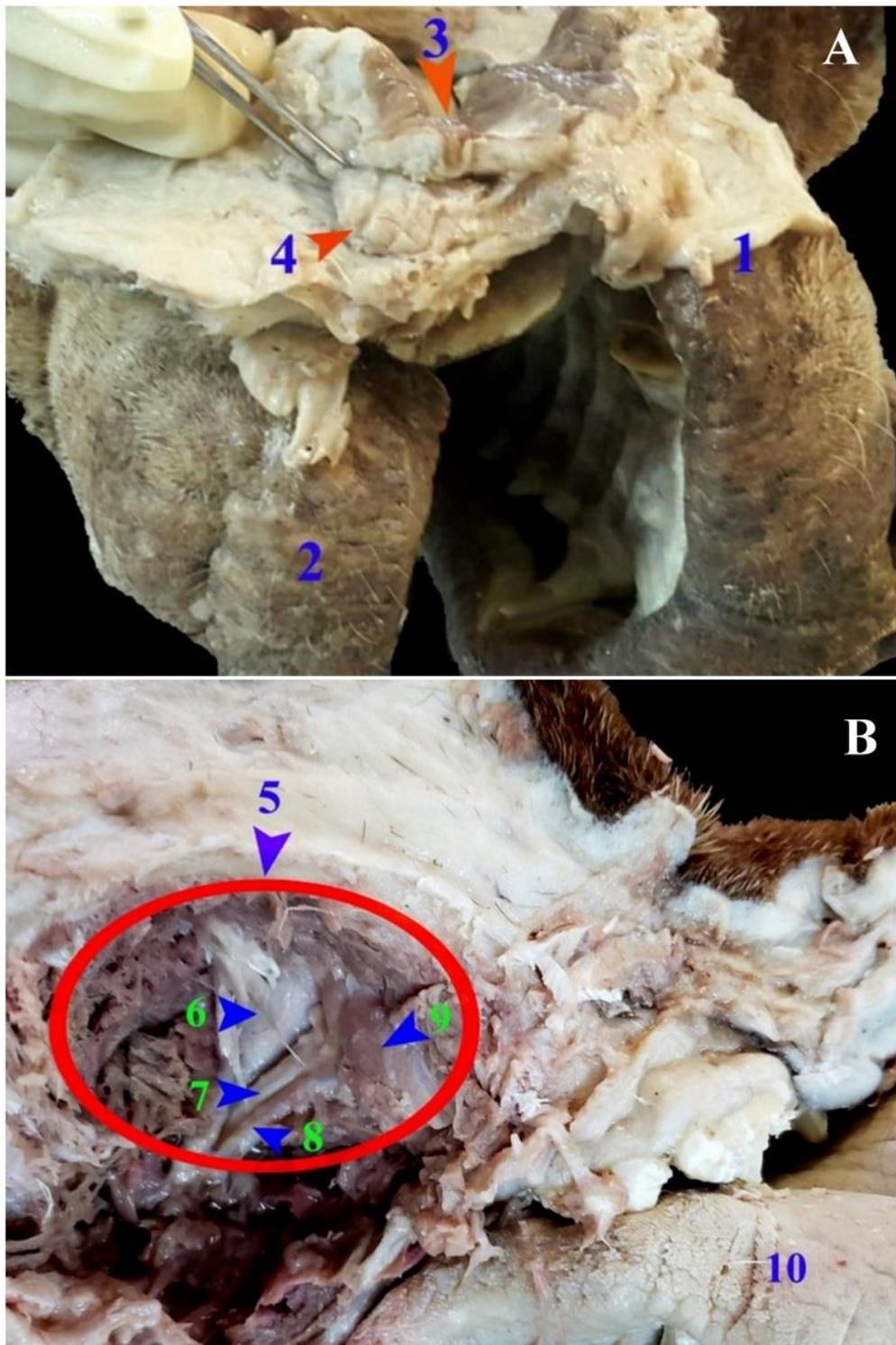


Figure 1. Photographs showing the location of the labial (A) and zygomatic (B) salivary glands in the mixed breed dog. 1: Upper lip; 2: Lower lip; 3: Labial commissure; 4: Labial salivary gland; 5: Orbital cavity; 6: Eye ball; 7: Maxillary nerve; 8: Maxillary artery; 9: Zygomatic salivary gland; 10: Tongue.

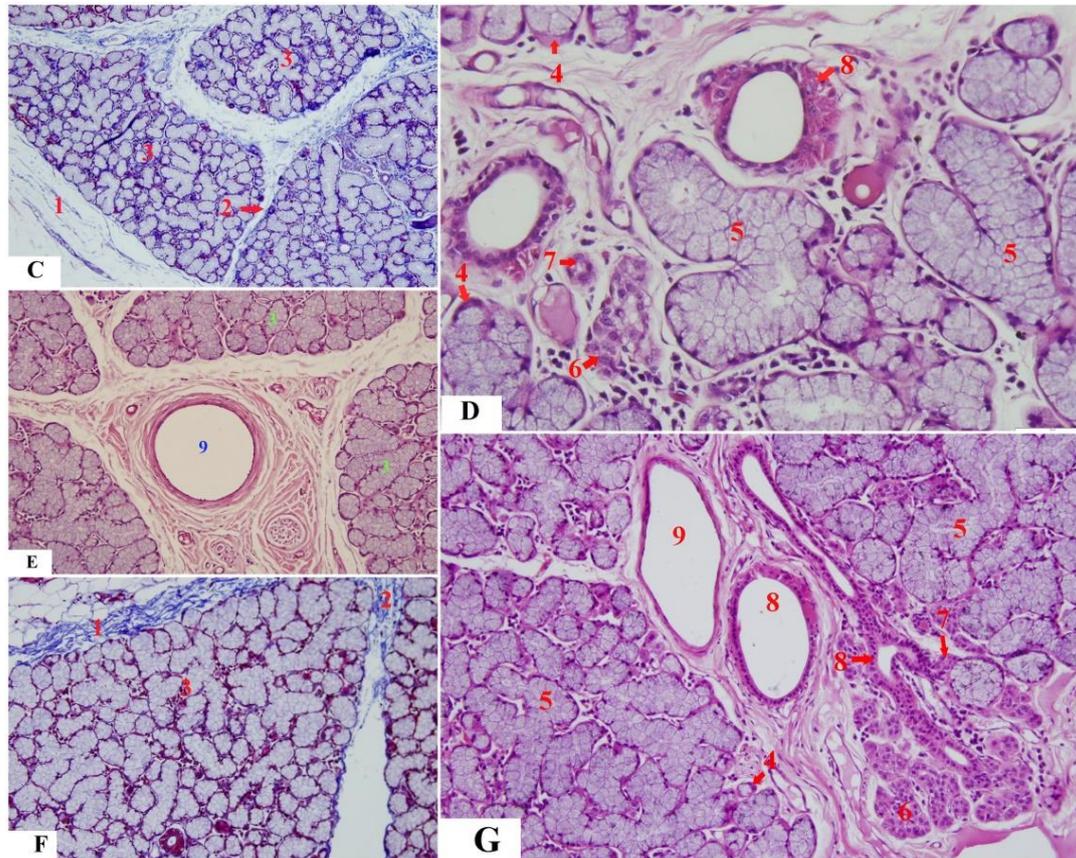


Figure 2. Photomicrographs showing the general histology of the labial (C, E and D) and zygomatic (F and G) salivary glands of the mixed breed dog stained with H & E (DX40; E x 20 and GX20) and Masson's Trichrome stain (CX10 and FX20). 1: Connective tissue capsule containing collagen fibers; 2: Interlobular septum; 3: Gland lobules; 4: Serous demilunes; 5: Mucous secretory units; 6: Serous acini clusters; 7: Intercalated duct; 8: Striated duct; 9: Interlobular duct.

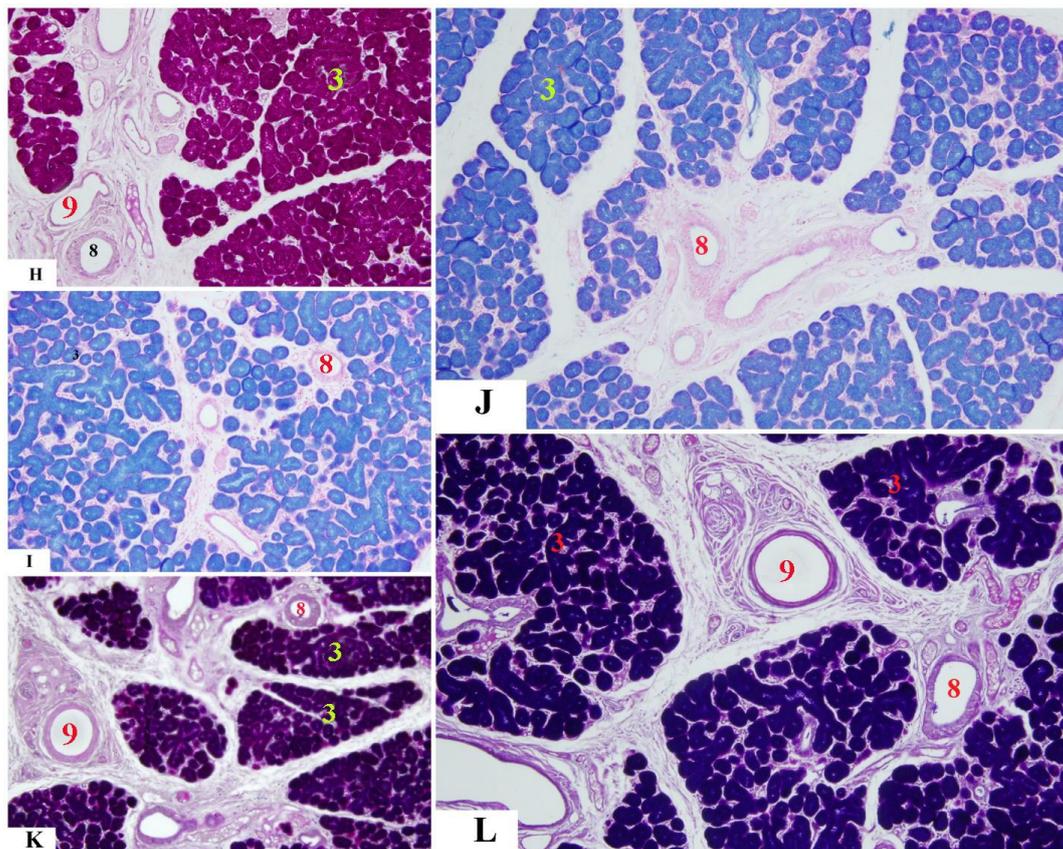


Figure 3. Photomicrographs showing the histochemical characteristics of the mucous secreting cells of the labial salivary gland of the mixed breed dog stained with PAS (HX10), AB (pH 2.5, I X10 and pH 1.0, JX10) and AB-PAS (pH 2.5, KX10 and pH 1.0, LX10). 3: Gland lobules; 7: Intercalated duct; 8: Striated duct; 9: Interlobular duct.

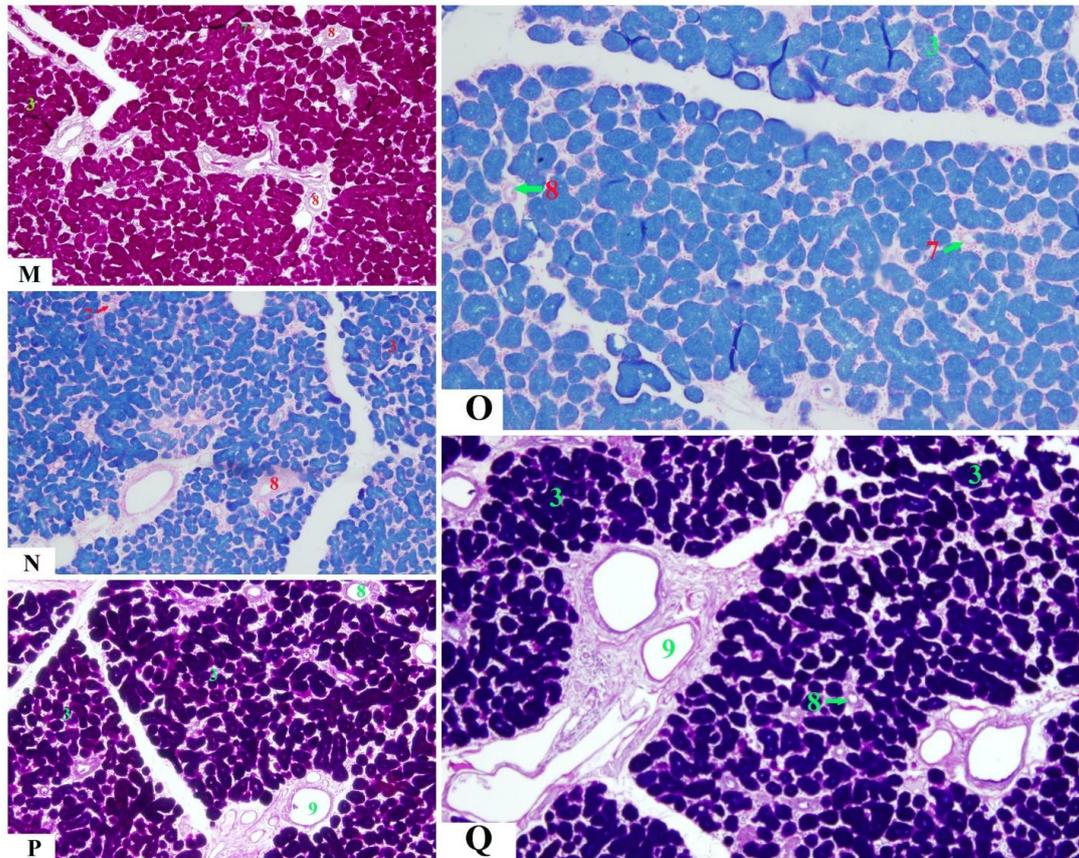


Figure 4. Photomicrographs showing the histochemical characteristics of the mucous secreting cells of the zygomatic salivary gland of the mixed breed dog stained with PAS (MX10), AB (pH 2.5, N X10 and pH 1.0, OX10) and AB-PAS (pH 2.5, PX10 and pH 1.0, QX10). 3: Gland lobules; 8: Striated duct; 9: Interlobular duct.

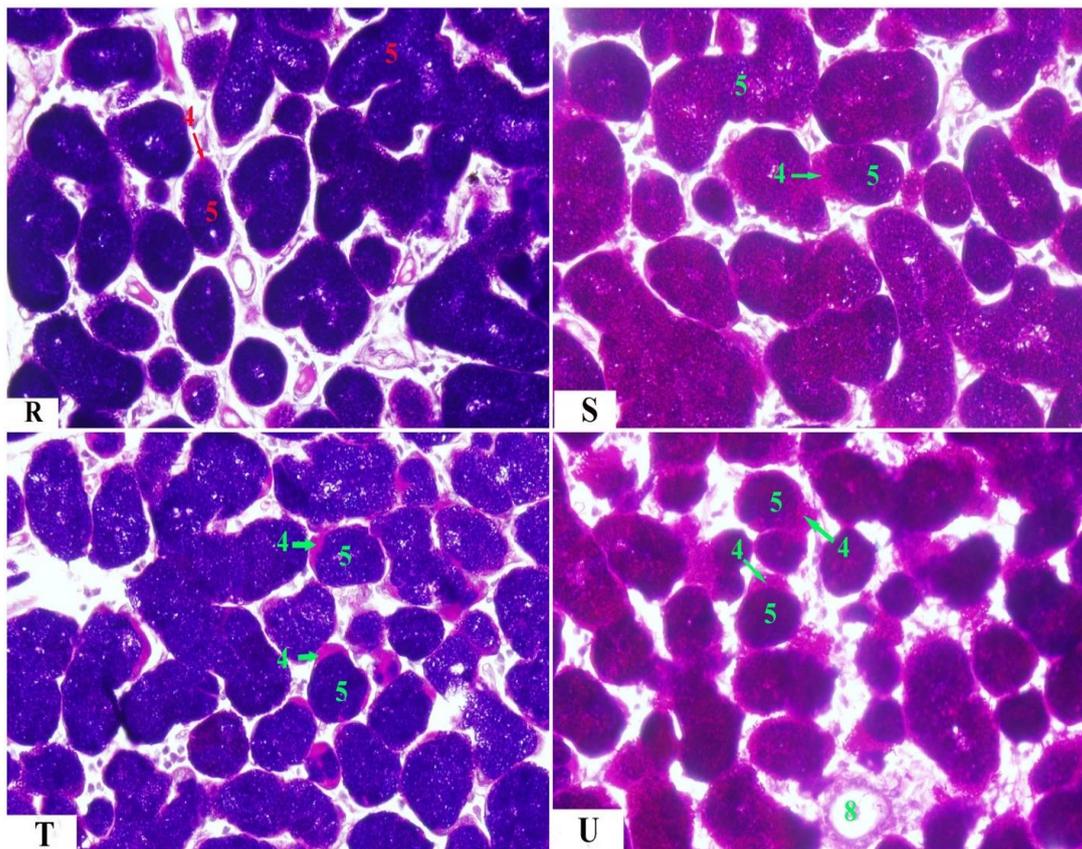


Figure 5. Photomicrographs showing the histochemical characteristics of the mucous secreting cells and serous demilunes of the labial salivary gland (R and S) and zygomatic salivary gland (T and U) of the mixed breed dog stained with AB-PAS (pH 1.0, R and TX40 and pH 2.5, S and UX40). 4: Serous demilunes; 5: Mucous secretory units; 8: Striated duct.

DISCUSSION

There was not much available literature reporting on the labial and zygomatic salivary glands of mixed breed dog in Trinidad. However, the zygomatic salivary gland of the Philippine non-descript dog and the labial salivary gland of the dog (Maala et al., 2008; Cunha et al., 2016) respectively were described.

The present results as well as that in dog (Angélico et al., 2011; Rocha, 2012 and Cunha et al., 2016) stated that the labial salivary gland was easily palpable in the lower lip between the lower second premolar and the second molar teeth. Moreover, the zygomatic salivary gland of the mixed breed dog was a pyramidal shaped gland located inside the orbit. Similar result was observed in mesaticephalic dog (Diesem, 1975). However, Maala et al. (2008) reported the same location but the gland was irregular in shape in the Philippine non-descript dog.

The labial and zygomatic salivary glands were surrounded by a connective tissue capsule with collagen fibers; a similar result is noticed in the labial salivary gland of the dog (Rocha, 2012; Cunha et al., 2016), zygomatic salivary gland of the dog (Maala et al., 2008), labial gland of the buffalo (Jabbar, 2010) and molar salivary gland in domestic cat (Mohammadpour, 2010). On the other hand, the minor salivary glands are lack of connective tissue capsule (Bacha and Bacha, 2003). Moreover, the labial and zygomatic salivary glands were divided into lobules by the connective tissue which surrounded and intermingled the gland as mentioned in the rat (Hand et al., 1999), labial salivary gland of the dog (Cunha et al., 2016), zygomatic salivary gland of the dog (Maala et al., 2008), labial salivary gland of the buffalo (Jabbar, 2010) and molar salivary gland in domestic cat (Mohammadpour, 2010).

Similar to the observations in the labial salivary gland in the dog (Rocha, 2012; Cunha et al., 2016), zygomatic salivary gland in the dog (Dellmann and Eurell, 1998; Maala et al., 2008; Gomi et al., 2017), zygomatic salivary gland in the Leopard and cat (Sadeghinezhad et al., 2016), molar salivary gland in the domestic cat (Mohammadpour, 2010) and Weber's salivary gland of the rabbit (Haddao and Yasear, 2018), the secretion of the labial and zygomatic salivary glands of the mixed breed dog was mixed and primarily mucous. However, the secretion is classified as mucous in the dog (Banks, 1991). However, the labial salivary gland is serous glands in the buffalo (Jabbar, 2010) and in the Giant rat (Asojo and Aire, 1983). On the other hand, the labial gland has mucoserous acini capped by serous demilunes in the camel (Taib and Jarrar, 1987). On the other hand, the secretion of the zygomatic gland of the Japanese serow is chiefly serous (Tsuchimoto et al., 1984).

The current study as well as Cunha et al. (2016) and Gomi et al. (2017) in the dog and Jabbar (2010) in the buffalo stated that the secretions of the labial and zygomatic salivary glands pass via the intralobular (intercalated and striated) and interlobular ducts. However, Frappier (2006) mentioned that the minor salivary glands are lacking in striated ducts. However, the intercalated duct was absent in the zygomatic salivary gland, while the intralobular and interlobular ducts were present; the former ones appeared as oval ducts, lined by cuboidal epithelium, without basal striations in dog (Dellmann and Eurell, 1998; Maala et al., 2008) and in the labial gland of the camel (Taib and Jarrar, 1987). On the other hand, there are no intercalated or striated ducts in molar salivary gland in domestic cat (Mohammadpour, 2010). The striated duct is absent in the Weber's salivary gland of the rabbit (Haddao and Yasear, 2018).

In the samples of the current study of the labial and zygomatic salivary glands of the mixed breed dog showed that the mucous secretory units and serous demilunes secrete neutral mucins as they were positive to PAS stain. All acid mucous of the mucous secretory units was positive to AB (pH 2.5) with strongly staining of acid mucins, while acid, sulphated mucins were stained by AB (pH 1.0). Similar results were observed in the labial salivary gland in the dog (Giudice et al., 2005; Cunha et al., 2016), zygomatic salivary gland in the dog (Sozmen et al., 1999; Maala et al., 2008) and labial salivary gland of the buffalo (Jabbar, 2010) and camel (Taib and Jarrar, 1987). However, the mucous acini of the weber's salivary gland of the rabbit were negative to PAS and AB (1.0) while the mucous acini and serous cells were positive to PAS (Haddao and Yasear, 2018).

The current study showed that the combination of PAS-AB stain showed that the mucous cells of the labial and zygomatic salivary glands stained bluish purple at pH 1.0 and reddish purple at pH 2.5 which indicated that mucous cells secrete neutral mucins more than acidic mucins. Moreover, the mucous cells secrete large amounts of acidic sulphated mucins; similar results were reported in the zygomatic salivary of the dog (Sozmen et al., 1999). However, Maala et al. (2008) stated that the zygomatic salivary gland secretes small amount of acidic sulphated mucins. On the other hand, the mixed mucins of the mucous cells of the zygomatic salivary glands are more acidic in leopard and cat (Sadeghinezhad et al., 2016). However, the weber's salivary gland secretes acidic mucins from the mucous cells and neutral mucins from the serous cells (Haddao and Yasear, 2018).

The mucin of the labial salivary glands was characterized by its high viscosity, low solubility and stickiness which made them able to lubricate the mucosa of the food passage and prevent its drying. Furthermore, it facilitates the passage of the food from the mouth to the esophagus by coating food boluses and keeping the integrity while protecting the mucosa of the pharynx and esophagus from mechanical injury. Similar findings were mentioned by Samar et al. (1995), Maala et al. (2008), Munyala et al. (2009) and Cunha et al. (2016).

CONCLUSION

The labial and zygomatic salivary glands of mixed breed dogs were tubuloacinar with mixed seromucous secretions. The duct system consists of intercalated, striated and interlobular ducts in the labial salivary and zygomatic salivary glands. The secretions of the glands were composed of neutral, acid carboxylated and acid sulphated mucins.

DECLARATIONS

Competing interests

The author declared that there is no conflict of interests.

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