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Applied anatomy and morphology of minor salivary glands in commonly used experimental animals

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This study aimed to describe the anatomy and histology of minor salivary glands (MSGs) in commonly used experimental animals. Oral mucosal tissues containing MSGs were dissected and sampled. The sialomucin nature was examined and the expression patterns of aquaporin (AQP) family were assessed. MSGs were widely distributed beneath the oral mucosa in all four species, mainly as mucous glands, with mixed seromucinous glands in dogs and miniature pigs. Serous glands were found in the anterior lingual glands of miniature pigs. The duct system typically included terminal and excretory ducts, with cuboidal, columnar, or pseudostratified columnar epithelium seen in the ducts of rabbits and miniature pigs. The secretion in rats, pigs, and dogs was primarily neutral sialomucin, while rabbits exhibited a dominance of acid sialomucin. The mRNA expressions of AQP1, 3–5, and 8 were detected in the buccal glands of all species, with AQP5 being the most dominant. This study provided detailed insights into the anatomical distribution and histological characteristics of MSGs in rats, rabbits, miniature pigs, and dogs. These findings offer a fundamental basis for veterinary research related to MSGs and could aid in the development of relevant animal models for future studies.

Keywords Minor salivary glands, Experimental animal, Anatomy, Histology, Sialomucin, Aquaporin

The minor salivary glands (MSGs, *glandulae salivariae minores*) are widely present in the submucosal layer of the oral cavity and serve as important components of oral structure and function. The role of human MSGs in oral cavity function and defense has been thoroughly investigated¹and their significance in the diagnosis and treatment of various diseases in humans has gained attention. For instance, biopsy of labial MSGs has become crucial for classifying and determining the diagnosis of primary Sjögren's syndrome²and autotransplantation of labial or buccal MSGs has been confirmed as an effective surgical technique for treating dry eye diseases^{3,4}. Further research on clinical application of MSGs, such as synergy of stem cells to MSG transplantation for the treatment of severe dry eye, often requires experimental verification in animal models. Therefore, a systematic understanding of MSGs in experimental animals is necessary. Despite extensive studies on the major salivary glands in various animal models⁵the distribution and histological features of MSGs in commonly used experimental animals remain largely unknown.

¹Various imaging techniques, such as scintigraphy, computed tomography, and magnetic resonance imaging, have been employed to investigate the distribution and function of salivary glands in animals^{6–8}. These examinations effectively visualize the major salivary glands but do not provide clear visualization of MSGs. To this end, this study dissected and sampled the oral mucosa with MSGs from fresh cadavers of rats, rabbits, miniature pigs, and dogs according to anatomical regions to illustrate the approximate distributions of various MSGs. Additionally, we conducted a detailed examination of the histological structures of MSGs, assessed the properties of sialomucin in MSGs, and explored the differential expression patterns of the aquaporin (AQP) family in MSGs among different species. The objective of this study was to establish a foundation for the development of MSG disease models, MSG autotransplantation models, and veterinary medicine approaches for treating MSG diseases.

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Results

Topographic anatomy of MSGs

The anatomical dissection revealed a significant similarity in the distribution of minor salivary glands (MSGs) beneath the oral mucosa among the four species studied (Fig. 1). In all species, MSGs were found in varying amounts in the buccal, soft palatal, and oropharynx (consisting of glossopalatine arch, uvula and tonsillar pillars) regions, beneath the oral mucosa. No MSGs was observed in upper or lower labial regions of all four species.

In rats, MSGs were approximately divided into five groups based on anatomical locations: anterior buccal glands (*glandulae buccales ventrales*), posterior buccal glands (*glandulae buccales dorsales*), minor sublingual glands (*glandulae linguales*), soft palatal glands (*glandulae palatinae*), and oropharyngeal glands (*glandulae oralis pharyngis*). All MSGs in rats were located just beneath the oral mucosa, on the surface of the muscular layer. Unlike human buccal glands, the buccal glands in rats were not present throughout the entire buccal mucosa but were only observed at the level of the maxillary vestibular sulcus. These buccal glands could be further divided into two separate groups (anterior and posterior). No MSGs were found in the lower buccal mucosa or the middle area of the upper buccal mucosa by examination of serial sections. The minor sublingual glands were located in the lingual sulcus, accompanying the main ducts of the submandibular and major sublingual glands, which is consistent with previous research⁹.

In rabbits, MSGs were approximately divided into four groups: superior buccal glands (*glandulae buccales superior*), inferior buccal glands (*glandulae buccales inferior*), soft palatal glands, and oropharyngeal glands. The soft palatal glands and oropharyngeal glands were located shallowly beneath the oral mucosa, while the buccal glands were distributed at a relatively deeper level, between the submucosal layer and the superficial muscular layer. Additionally, there was a portion of the buccal mucosa where MSGs were not present, as the submucosal glands were only found at the level of the upper and lower vestibular sulcus regions.

In miniature pigs, MSGs were divided into five groups: buccal glands (*glandulae buccales*), anterior lingual glands (*glandulae linguales ventrales*), retromolar glands (*glandulae molares*), soft palatal glands, and oropharyngeal glands, which was similar to the division of MSGs in humans. Except for the anterior lingual glands, most of the MSGs were located deep within the superficial muscular layer, with thick and hard textured connective tissues above them. The anterior lingual glands were relatively small in number and located beneath the oral mucosa on bilateral sides of the lingual frenulum. The buccal glands were distributed throughout the entire buccal mucosa at a lower density. Submucosal glands were observed at bilateral mandibular retromolar regions.

In dogs, MSGs were divided into six groups: buccal glands, anterior lingual glands, retromolar glands, soft palatal glands, oropharyngeal glands, and inferior buccal glands, which is unique to dogs¹⁰. The inferior buccal



Fig. 1. Illustrations of the location of minor salivary glands in rats (**a**), rabbits (**b**), miniature pigs (**c**) and dogs (**d**). 1, anterior buccal glands; 2, minor sublingual glands; 3, posterior buccal glands; 4, 7, 12 and 18 are soft palatal glands; 5, 8, 13 and 19 are oropharyngeal glands; 6, 10 and 16 are buccal glands; 9 and 14 are anterior lingual glands; 11 and 17 are retromolar glands; 15, inferior buccal glands.

glands were continuously distributed from the mandibular third premolar to the retromolar region, located beneath the mandibular gingival mucosa and lower vestibular sulcus mucosa. They were independent of the buccal glands located beneath the buccal mucosa. The inferior buccal glands were covered by a thin layer of mucosa and could be clearly palpated as oval-shaped objects resembling human labial glands (Figure S1a). Small papillae (*papilla ductus*) were observed at the openings of the ducts on the mucosal surface of the inferior buccal glands (Figure S1b). The remaining MSGs were located on the surface of the muscular layer, and the mucosal connective tissues above them had a soft texture. No submucosal glands were found under the gingival mucosa or buccal mucosa at the level of the upper vestibular sulcus.

Histological analysis of MSGs

Different groups of MSGs in rats were composed of similarly structured mucous acini (*acinus mucosa*) without serous demilune (Fig. 2). The acini were elongated and tortuous, and so could be termed "tubulo-acini". Myoepithelial cells lied between the basal lamina and the acinar and ductal cells. The buccal glands (Fig. 2a), soft palatal glands (Fig. 2c) and oropharyngeal glands (Fig. 2d) in rats shared the same duct system. An abrupt transition was observed between the tubulo-acini and the simple flat epithelium of the terminal ducts. Multiple terminal ducts converged into one excretory duct, with the simple flat epithelium transitioning into stratified



Fig. 2. Histological features of MSGs in SD rats. The buccal (**a**), minor sublingual glands (**b**), soft palatal (**c**) and oropharyngeal glands (**d**) in rats were mucous. The acini were elongated and tortuous and so could be termed "tubulo-acini" (*). The duct system of the buccal, soft palatal and oropharyngeal glands were composed of the simple flat epithelium of the terminal ducts (single arrow) and excretory ducts (double arrow). The simple cuboidal epithelium (empty arrow) of the intercalated ducts were observed in the duct system of minor sublingual glands. *, tubulo-acini; A, acini; D, duct; E, epithelium layer of oral mucosa.

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squamous epithelium, which opened on the mucosal surface. The duct system of the minor sublingual glands exhibited differences (Fig. 2b). The intercalated, striated, and excretory ducts were observed, characterized by simple cuboidal epithelium, simple columnar epithelium, and stratified or pseudostratified columnar epithelium, respectively. These ducts joined to form one main duct, with the orifice to the main ducts of the major sublingual (Bartholin's duct) or submandibular glands (Wharton's duct). This difference in the duct system has also been confirmed in a previous study⁹.

The MSGs of rabbits were composed of mucous acini, without the presence of serous demilunes (Fig. 3). Myoepithelial cells were positioned between the tubulo-acini and the basement membrane. The duct system of most MSGs consisted of terminal ducts with simple flat epithelium and excretory ducts with stratified squamous epithelium. However, in the buccal glands (Fig. 3a), the ducts were composed of simple cuboidal epithelium and stratified cuboidal epithelium.



Fig. 3. Histological features of MSGs in rabbits. The buccal (**a**), soft palatal (**b**) and oropharyngeal glands (**c**) in rabbits were mucous. The duct system of the buccal glands was composed of simple cuboidal epithelium (single empty arrow) and stratified cuboidal epithelium (double empty arrow). The duct system of rest MSGs were the terminal ducts (single black arrow) and excretory ducts (double black arrow). *, tubulo-acini; A, acini; D, duct; E, epithelium layer of oral mucosa.

Histological composition varied among different MSGs in miniature pigs (Fig. 4). The anterior lingual glands (Fig. 4a) were purely composed of serous acini (*acinus serosa*). The buccal (Fig. 4d) and oropharyngeal (Fig. 4e) glands were classified as mixed seromucinous glands (*glandula seromucosa*). The retromolar (Fig. 4b) and soft palatal (Fig. 4c) glands were mucous glands (*glandula mucosa*). In terms of the duct systems, the retromolar and soft palatal glands were composed of terminal ducts with simple flat epithelium and excretory ducts with stratified squamous epithelium. On the other hand, the buccal and oropharyngeal glands possessed intercalated, striated, and excretory ducts.

The MSGs of dogs were composed of mucous acini, except for the inferior buccal glands (Fig. 5). Occasionally, glandular lobules composed of serous acini could be found among the mucous lobules in the inferior buccal glands (Fig. 5a), indicating that these glands should be identified as mixed and primarily mucous glands. Different groups of MSGs shared the same duct system, which consisted of terminal ducts with simple flat epithelium and excretory ducts with stratified squamous epithelium.

Sialomucin properties of MSGs across species

The properties of sialomucin were assessed using AB-PAS staining to evaluate the composition of the secretion in MSGs across the four species studied. The secretion was found to be predominantly composed of neutral (periodic acid Schiff positive) and acid (alcian blue positive) mucopolysaccharides (Fig. 6). In rats, miniature pigs, and dogs, the secretion consisted of a high proportion of neutral sialomucin and a low proportion of acid sialomucin, resulting in varying degrees of purple staining. Conversely, in rabbits, the secretion was stained bluish violet and primarily composed of acid sialomucin.

Differential expressions of AQP family in buccal glands among species

There are substantial differences between species in the expression patterns of AQP family. Previous studies have highlighted the importance of AQP1, AQP3, AQP4, AQP5, and AQP8 in salivary glands, with AQP5 being particularly noteworthy. The buccal glands were selected to represent the expression patterns of AQPs, as they were presented in all four species (Fig. 7). In the buccal glands of rats, rabbits, miniature pigs and dogs, mRNA expressions of AQP1, AQP3, AQP4, AQP5, and AQP8 were detected at varying levels, with CT value ranging from 15 to 35. Among the buccal glands of the four species, AQP5 exhibited the lowest CT value, indicating its dominant expression.



Fig. 4. Histological features of MSGs in miniature pigs. The anterior lingual glands (**a**) were serous, the retromolar (**b**) and soft palatal (**c**) glands were mucous, and the buccal (**d**) and oropharyngeal glands (**e**) were mixed seromucinous glands. The duct system of the retromolar and soft palatal glands were composed of terminal ducts (single black arrow) and excretory ducts (double black arrow). The duct system of the buccal and oropharyngeal glands was composed of simple cuboidal epithelium (single empty arrow), simple columnar epithelial (double empty arrow) and stratified or pseudostratified columnar epithelium (triple empty arrow). *, tubulo-acini; M, mucous acini; S, serous acini; D, duct; E, epithelium layer of oral mucosa.

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Fig. 5. Histological features of MSGs in dogs. The inferior buccal glands (**a**) were composed of high portion of mucous lobules and low portion of serous lobules, the anterior lingual (**b**), retromolar (**c**), soft palatal (**d**), buccal (**f**) and oropharyngeal (**g**) glands were mucous. The duct system of all MSGs was composed of terminal ducts (single black arrow) and excretory ducts (double black arrow). *, tubulo-acini; M, mucous acini; S, serous acini; D, duct.



Fig. 6. The AB-PAS staining of mucous MSGs of all species. The acid mucopolysaccharides were stained in blue by alcian blue, and the neutral mucopolysaccharides were stained in purplish red by periodic acid Schiff. In rats, miniature pigs and dogs, the sialomucin was composed of high proportion of neutral sialomucin and low proportion of acid sialomucin. In rabbits, the sialomucin was mainly composed of acid sialomucin.



Fig. 7. The mRNA expressions of AQPs in buccal glands of all species. Different mRNA levels of AQP1, $3 \sim 5$ and 8 were detected in all four species. AQP5 presented the lowest CT value, which indicating its dominated expression in all four species.

Discussion

The pathophysiology, morphology and illness development of minor salivary glands have gradually been recognized since the 1950s^{11–13}. Fundamental research on human minor salivary glands has been deepened over time. However, research on the salivary glands of experimental animals has primarily focused on the major salivary glands, leaving the structure and function of minor salivary glands largely unclear. Currently, detailed information regarding the histologic and ultrastructural features of MSGs in rats is available⁹. A review discussing the histology and physiology of major and minor salivary glands in mice has also been published⁵. Although scintigraphy has provided some insight into the function and location of salivary glands in rabbits, the visualization of MSGs remains challenging due to unsatisfactory resolution¹⁴. There is currently no systematic report available on the morphology of MSGs in miniature pigs. The anatomy and imaging features of major salivary glands in dogs have been described using MRI and tissue dissection¹⁵labial and buccal minor salivary glands (referred to as inferior buccal glands in this study) have been well reconstructed in three dimensions based on microCT data¹⁰the histology of soft palatal glands has been mentioned in a descriptive microanatomical study of mesaticephalic dog breeds¹⁶but the remaining MSGs in dogs have not been thoroughly elucidated.

This study presented the anatomical distribution and histological features of submucosal minor salivary glands in the oral cavities of rats, rabbits, miniature pigs and dogs. Firstly, consistent with previous studies^{9,14,17} no labial glands were observed beneath the upper or lower lips in all species. The labial and buccal minor salivary glands in dogs¹⁰ were separated by anatomical location into buccal regions and were named as inferior buccal glands in this study, as they distributed from the mandibular third premolar to retromolar region under the gingival and buccal mucosa. Secondly, the buccal glands were presented in all four species. They tended to cluster in some specific areas (such as the anterior and posterior buccal region in rats), while scattering with lower density and smaller size in the rest areas. Except for miniature pigs, their buccal glands were uniformly distributed throughout the entire buccal mucosa, similar to the distribution in humans. The minor salivary glands beneath the mucosa of glossopalatine arch, uvula and tonsillar pillars displayed anatomical proximity and shared the same histological composition within each species. Consequently, they were grouped and referred to as oropharyngeal glands in this study.

Histological analysis and AB-PAS staining revealed that the majority of MSGs were mucous glands, except for the anterior lingual glands of miniature pigs which were serous, the buccal and oropharyngeal glands of miniature pigs which were mixed seromucous, and the inferior buccal glands of dogs which were also mixed seromucous. The soft palatal glands were evaluated to mixed seromucous glands with misidentifying myoepithelial cells for serous demilune¹⁶. The results of our study did not support that evaluation, based on the AB-PAS staining intensity and the histology of the cells surrounding the mucous acini. Further confirmation this discrepancy requires immunohistochemical evidence. The nature of sialomucin were evaluated by AB-PAS staining. The specific genes encoding mucins among species were failed to be identified, since gene and protein sequencing of animal mucins (especially rabbits and dogs) remained to be accomplished currently.

AQPs are a family of water-specific channel proteins that contributes to fast transmembrane water transport, and play a significant role in salivary secretion¹⁸. The expression patterns of AQPs differ from species, and remain largely unknown in MSGs of commonly used experimental animals. Previous studies have confirmed that AQP1, AQP3, AQP4, AQP5, and AQP8 were expressed in salivary glands of various species, and they were detected in this study¹⁹. AQP5, in particular, has been shown to be the prominent member responsible for rapid water movement across the plasma membrane in major salivary glands²⁰. Similarly, our results revealed that AQP5 was the dominant molecular expressed in the buccal glands of all four species studied. Moreover, mRNA expressions of AQP1, AQP3, AQP4, and AQP8 were detected in the buccal glands of all four species. It is important to note that this study involved the detection of entire salivary gland tissues in vitro. The total RNA used for RT-PCR was extracted from the entire buccal gland tissues. Although efforts were made to remove the surrounding tissue as much as possible, the salivary gland tissues still contained acini, duct cells, myoepithelial cells. It should be acknowledged that AQPs are also expressed in non-acinar

cells above, and further investigation is needed to determine their specific localization^{21,22}. In addition, mRNA expression of AQPs may not be closely related to protein levels and the function of salivary glands²³.

This study has limitations. The separation of oral submucous minor salivary glands into groups based on anatomical findings and tissue serial sections might have resulted in inaccurate boundaries. The use of optical coherence tomography or microCT could potentially provide a more precise reproduction of their actual distribution. Furthermore, the ventral or dorsal lingual glands were not included in this study. It has been documented that von Ebner's glands (serous glands)⁹ and Weber's glands (mixed glands)²⁴ were present beneath the dorsal lingual mucosa in rats, while reports on the rest of the species are lacking.

In conclusion, this study provided a description of the anatomical distribution and histological features of minor salivary glands in rats, rabbits, miniature pigs, and dogs. It also evaluated the sialomucin properties of minor salivary glands and revealed the dominance of AQP5 expression in minor salivary glands across the species studied. This study might stimulate further research on the establishment of minor salivary gland related animal models and the study of minor salivary gland diseases.

Materials and methods

Animals

Sprague-Dawley rats (*rattus norvegicus*), New Zealand white rabbits (*oryctolagus cuniculus*), Guangxi Bama miniature pigs (*sus scrofa*) and Beagle dogs (*canis lupus familiaris*) are commonly used and readily available laboratory animals. Three fresh adult animal cadavers of each species without any artificial manipulation at oral and maxillofacial regions or systemic administration of experimental drug, were obtained from Peking University Laboratory Animal Center. All subjects were euthanized by narcotic overdose. The study protocol was approved by the Ethics Committee of Peking University Health Science Center (LA2016316) and adhered to the ARRIVE guidelines as well as the relevant national laws on the protection of animals.

Anatomical dissection of the minor salivary glands

Tissues of oral mucosa with MSGs were dissected deep to superficial muscular layer to ensure the inclusion of all possible MSGs. Samples were collected according to anatomical regions, with each sample within the same region continuously numbered. All samples were immediately fixed with 10% formalin and embedded in paraffin and then sectioned for histological examination. Afterwards, approximate distributions of each glands identified were depicted on a basally projected diagram of the head skeleton.

Histological staining

The specimens were sliced into 4 µm sections, then were rehydrated. The sections were stained with hematoxylin and eosin (H&E; Solarbio Science & Technology, Beijing, China), and alcian blue-periodic acid Schiff (AB-PAS; Solarbio Science & Technology, Beijing, China). For HE staining, paraffin sections were dewaxed with xylene and were rehydrated with gradient alcohol, then stained with hematoxylin for 1 min and eosin for 5 min. The sections were finally dehydrated in a gradient and sealed with a neutral resin. For alcian blue (AB) staining, the sections were rehydrated using xylene and gradient alcohol. After staining with alcian blue solution (pH 2.5) for 20 min, the sections were stained with hematoxylin for 1 min. The sections were finally dehydrated in a gradient and sealed with a neutral resin. For periodic acid-Schiff (PAS) staining, the sections were rehydrated using xylene and gradient alcohol. After oxidation with a 1% periodic acid solution for 10 min, the sections were treated with Schiff reagent in the dark for 20 min. The sections were finally dehydrated in a gradient and sealed with a neutral resin. Duration of staining process was maintained consistent among different species and among different MSGs. The stained sections were then photographed under a light microscope (Leica, Heidelberg, Germany).

Real-time polymerase chain reaction (RT-PCR)

Before total RNA extraction, fibrous connective tissue surrounding the salivary glands was removed as clean as possible. Total RNA of MSGs was extracted by using the E.Z.N.A Total RNA Kit (Omega Bio-tek, Norcross, GA, USA) and was reversely transcribed to cDNA by using the First-Strand cDNA Synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA). The obtained cDNA (2μ L) was amplified with Fast Start Universal SYBR Green Master (Roche, Basel, Switzerland) and detected by the Quant Studio 6 Flex Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). GAPDH was selected as the internal control. Primer sequences are shown in the Table 1. Cycle threshold (CT) values were used to compare the differential expressions between AQP molecules of single gland. CT values ranged from 15 to 35 was considered significant, with values exceeding 35 being considered as gene was not expressed.

Anatomical nomenclature

The anatomical terms that are applied in this article are derived from the sixth edition of Nomina Anatomica Veterinaria. When textually describing the results of our anatomical study, the English terms of the various anatomical structures are followed by the Latin terms between brackets the first time a structure is mentioned. Solely, English terminology is then applied in the further elaboration of the structure to increase the readability of the text.

Gene	Species	Forward (5'-3')	Reverse (5'-3')
AQP1	rat	CTGTGGTGGCTGAGTTCCTG	ATTTCGGCCAAGTGAGTTCTC
AQP3	rat	CACACAATAAGGGCTGCTGTG	GAGATGCTCCACATCCGCTAC
AQP4	rat	TTCCTTTAGGCGACGTTTGAG	CTCTGCTTTGGACTCAGCATTG
AQP5	rat	CCTTCTTCAAGGCGGTGTTC	CCCAGGCCAAAGAAGACGAA
AQP8	rat	GGGGAAGGAGACCAACATGG	AGCCCAAAAGTTCCACCACA
GAPDH	rat	ACCACAGTCCATGCCATCAC	TCCACCACCCTGTTGCTGTA
AQP1	rabbit	ACCACTGGATCTTCTGGGTGG	CATCTCCACCCTGGAGTTGA
AQP3	rabbit	ACATCCGCTACCGGCTGCT	GCCAGGTTGATGGTGAGGAA
AQP4	rabbit	GTGACAGACCCGCAGCAAG	TGTTCCACCCCAGTTGATGG
AQP5	rabbit	GCCACCTTGTGGGGGATCTAC	CATAGGAGCCCTTGACGACG
AQP8	rabbit	TGACCCTAGGCAGCAGGTAT	AAGTGTCCGCCACTGATGTT
GAPDH	rabbit	GAATCCACTGGCGTCTTCAC	CGTTGCTGACAATCTTGAGAGA
AQP1	swine	CGGTGATCACGCACAACTTC	TTTGGGCTTCATCTCCACCC
AQP3	swine	CAAGCTGCCCGTCTATACCC	GCCGATGAACTGGTCGAAGA
AQP4	swine	CCGGCGGCCTTTATGAGTAT	TTCTGTTGTCATCCGCCTCC
AQP5	swine	TCCATTGGCCTGTCTGTCAC	CAGCCGGTGAAGTAGATCCC
AQP8	swine	GAGTTTGGGGGGCATCAAGGT	TCTCAATGACCGACAGGCAG
GAPDH	swine	CGTCCCTGAGACACGATGGT	CCCGATGCGGCCAAAT
AQP1	canine	TGGGCATCGAGATCATTGGC	GATACCGCAGCCTGTGTAGT
AQP3	canine	ATCGGCACTTCCATGGGTTT	TACACGAATACGCCCGCAAT
AQP4	canine	GACCATGGTGCAGTGCTTTG	TGCTGCGACGTAGAAGACAG
AQP5	canine	CGTGGGGATCTACTTCACCG	GGGAGTTGGGGAATAGCAGG
AQP8	canine	AAACATCAGTGGAGGACATTTCAA	GCTCCTGGACTGTCACAAAGG
GAPDH	canine	CACTGGCCTGCAGGAATACA	GTACTCATCAGCAGCAGCCA

 Table 1. Primer sequences used for real-time PCR.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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