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NOTE

Pathology

Pleomorphic adenoma of the mandibular salivary gland in a captive African pygmy hedgehog (*Atelerix albiventris*)

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ABSTRACT. A 3.9-year-old female African pygmy hedgehog (*Atelerix albiventris*) had a firm, tan-colored mass with an uneven surface arising from the mandibular salivary gland. A histopathologic examination revealed that the mass was composed of neoplastic proliferation of epithelial and spindle cells. The neoplastic spindle cells showed positive for vimentin, smooth muscle actin, calponin and cytokeratin 14 and, negative for cytokeratin 19, suggesting that spindle cells were derived from myoepithelial cells. Based on the histological findings and immunohistochemistry results, the mass was diagnosed as pleomorphic adenoma. Pleomorphic adenoma is the most common benign tumor found in human salivary glands, but it is rare in animals. To the best of our knowledge, this is the first report of pleomorphic adenoma in hedgehogs.

KEY WORDS: hedgehog, immunohistochemistry, pleomorphic adenoma, salivary gland

The African pygmy hedgehog (*Atelerix albiventris*) is a popular pet in many countries, and the interest in disease in this species is increasing. According to 3 retrospective studies on disease of African pygmy hedgehog, neoplasm is common, with tumor incidence rates of 53, 36 and 29%, respectively, and is known to be the main cause of death [4, 12, 13]. The average lifespan of captive African pygmy hedgehogs is 4 to 6 years, and the average age of individuals with tumors is 3.5 to 3.9 years, indicating that tumors mainly occur in adults [4, 11, 12, 17]. About 85% of hedgehog tumors are known as malignant, and most have a poor prognosis [3]. It has been reported that tumors occur at various anatomical locations, and among them, oral squamous cell carcinoma, soft tissue sarcoma, lymphoma (including alimentary form) and mammary gland adenocarcinoma are the most common [4, 12, 17]. On the other hand, 2 cases of salivary carcinoma, 1 case of mucoepidermoid carcinoma and 1 case of salivary adenoma have been reported as a tumor derived from hedgehog salivary glands [4, 10, 17].

Salivary gland tumors can show a wide variety of histologic features and are classified into various subtypes according to histologic morphology. In humans, the clinicopathological behavior and prognosis of each subtype have been studied, but they are not well known in animals [9]. Salivary gland tumors are uncommon in animals. Most of the reported cases are from dogs and cats, and reports from other animals are rare [2, 9]. Approximately 80% of human salivary gland tumors were benign, whereas 80% and 90% of salivary gland tumors in dogs and cats were malignant [9, 15]. In the hedgehog, 3 cases of salivary gland tumors of 4 cases reported so far were also malignant. This study reports on benign tumor arising from the salivary gland of the hedgehog.

A 470 g, 3.9-year-old, female African pygmy hedgehog presented a solitary mass in the left mandibular region. The hedgehog did not show any specific clinical symptoms, including clinical symptoms associated with the mass such as pain and heat. The mass was surgically excised for histopathology 8 days after the mass was first noticed by the owner. During the 8-day period, the mandibular mass was not rapidly enlarged and hedgehogs did not show additional clinical symptoms. The mass, measuring 3 × 2 × 2 cm, was firm and tan-colored with an uneven surface (Fig. 1). It was fixed in 10% neutral formalin, processed routinely, embedded in paraffin wax, cut 3 μm thick and stained with hematoxylin and eosin (HE). To identify the origin of neoplastic cells, immunohistochemistry (IHC) for calponin (1:200, rabbit polyclonal antibody; Abcam, Cambridge, U.K.), smooth muscle actin (SMA) (1:200, mouse monoclonal antibody; Dako, Santa Clara, CA, U.S.A.), cytokeratin (CK) 14 (1:200, rabbit polyclonal antibody; Abcam), CK19 (1:200, mouse monoclonal antibody; Novocastra, Leica Biosystems, Newcastle, U.K.) and vimentin (1:200, mouse monoclonal antibody; Dako) was performed. Deparaffinized, rehydrated sections were treated with 0.3% hydrogen peroxide for 1 hr to prevent endogenous peroxidase after heat-induced antigen retrieval. Sections were incubated overnight at 4°C with all primary antibodies and then incubated with horseradish peroxidase-conjugated secondary antibodies for 2 hr at 18–22°C.

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All sections were visualized as 3,3′-diaminobenzidine (DAB) solution and then counterstained with Mayer's hematoxylin.

Microscopically, the mass was encapsulated in a thin fibrous connective tissue, and normal salivary glands, ducts and adipose tissue were present in a part of around the mass. However, neoplastic cells were present near the surgical margin. The proliferation of cuboidal or columnar neoplastic epithelial cells and plump and spindle-shaped neoplastic cells was coexisted in the mass (Fig. 2A). Neoplastic epithelial cells formed similarly sized ducts, which is surrounded by spindle-shaped tumor cells showing irregular or streaming growth pattern (Fig. 2B). Duct-forming tumor cells had a moderate amount of eosinophilic cytoplasm and a round nucleus. Spindle-shaped tumor cells had a few or moderate amount of eosinophilic cytoplasm, and a few cells had clear cytoplasm. The nucleus of these cells was fusiform or elliptical. Both types of tumor cells showed mild pleomorphism and no invasiveness. There were 5 mitotic figures per 10 high-power fields. Inflammatory cells such as lymphocytes were infiltrated throughout the mass. Stromal components such as myxoid and chondroid were not clearly observed in the mass. Compared with positive control, Alcian Blue positive matrices were also rarely seen in the mass (Fig. 3). We presumed that spindle-shaped tumor cells might be derived from myoepithelial cells, so we performed IHC for markers that could show positive reactivity in myoepithelium. In order to confirm the accurate IHC results, we used a complex type of mammary gland adenoma tissue of a dog containing myoepithelial cell proliferation as a positive control for IHC. As a negative control, we used sections with only the primary antibody treatment omitted, and horseradish peroxidase-conjugated anti-rabbit IgG and anti-mouse IgG were used as secondary antibodies, respectively. As a result, spindle-shaped tumor cells displayed moderate to intense positivity to SMA (Fig. 4A), calponin (Fig. 4B) and CK14 (Fig. 4C) in their cytoplasm. Specifically, these markers were strongly expressed in the outer layer of the ducts in which myoepithelial cells are normally located. Vimentin was detected only in spindle-shaped tumor cells (Fig. 4D), while, immunoreactivity to CK19 was negative in spindle-shaped tumor cells but strongly positive only in luminal epithelial cells (Fig. 4E). In the positive control, myoepithelial cells were positive for SMA, calponin, CK 14, vimentin and negative for CK 19. This was consistent with the immunohistochemical characteristics of spindle-shaped tumor cells observed in our case. On the other hand, no specific signals were observed in the negative control. These immunophenotypes indicated that the spindle-shaped tumor cells were derived from myoepithelial cells. Based on histological findings and IHC results, the mass was diagnosed as pleomorphic adenoma.

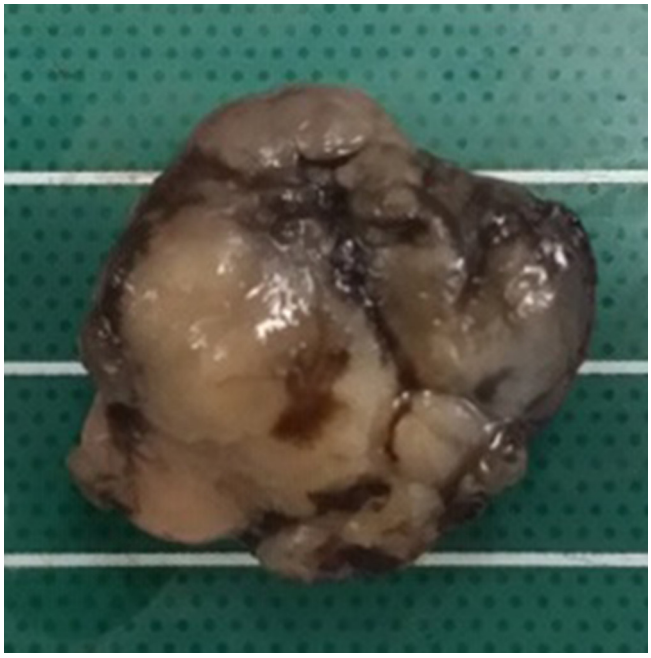

Fig. 1. Pleomorphic adenoma, mandibular salivary gland, African pygmy hedgehog (*Atelerix albiventris*). The mass was firm and tan-colored with an uneven surface.

Pleomorphic adenoma is a benign primary tumor in salivary glands. The origin of the term "pleomorphic" comes from various histologic features in which tumors are formed by mixing proliferation of luminal epithelial and myoepithelial cells with stroma in various aspects [2]. Therefore, this tumor has been also called "mixed tumor". In order to diagnose pleomorphic adenoma, it is necessary to confirm whether tumor cells are derived from myoepithelial cell through IHC. In general, myoepithelial cells have been reported to be positive for IHC for various markers including SMA, calponin, CK14, p63, and vimentin [1]. In particular, myoepithelial cells are able to express high molecular weight cytokeratins (CK5, CK6, CK7, CK14) [1], which can be used to differentiate other spindle-shaped cells, such as smooth muscle cells and myofibroblasts. Therefore, we performed IHC for CK14. Considering the results of IHC for vimentin, SMA, calponin, and CK14, we found that spindle-shaped tumor cells were derived from myoepithelial cells.

Pleomorphic adenoma is rarely reported in animals [2]. The occurrence of pleomorphic adenomas in salivary glands or oral cavity was not reported in a recent study of diseases in African pygmy hedgehogs [10]. To the best of our knowledge, this paper is the first description of pleomorphic adenoma in hedgehogs. On the other hand, pleomorphic adenoma is the most common tumor in human salivary glands [8]. Thus, histologic subtypes and prognostic factors have been studied only in human cases [8, 19]. In pleomorphic adenoma, epithelial cells may show ductal, basaloid or squamous growth patterns, and myoepithelial cells may show various forms such as spindle, stellate, polygonal, round and plasmacytoid shapes [7, 8]. In addition, various stromal components such as fibrous, myxoid, chondroid, and hyaline can coexist around the tumor cells resulting in pleomorphic adenoma having a wide variety of histological features [8]. Pleomorphic adenoma can be classified histologically as classic type in which the epithelial and myoepithelial cell and stroma are balanced, cellular (cell-rich) type, and stroma-rich type, depending on the proportions of the tumor cell population and the amount of stroma [5]. According to the classification, this case corresponds to the cellular type because most of the mass consisted of proliferation of tumor cells and stromal components were rarely observed in Alcian Blue stain. However, these histologic types do not seem to be closely related to prognosis [8].

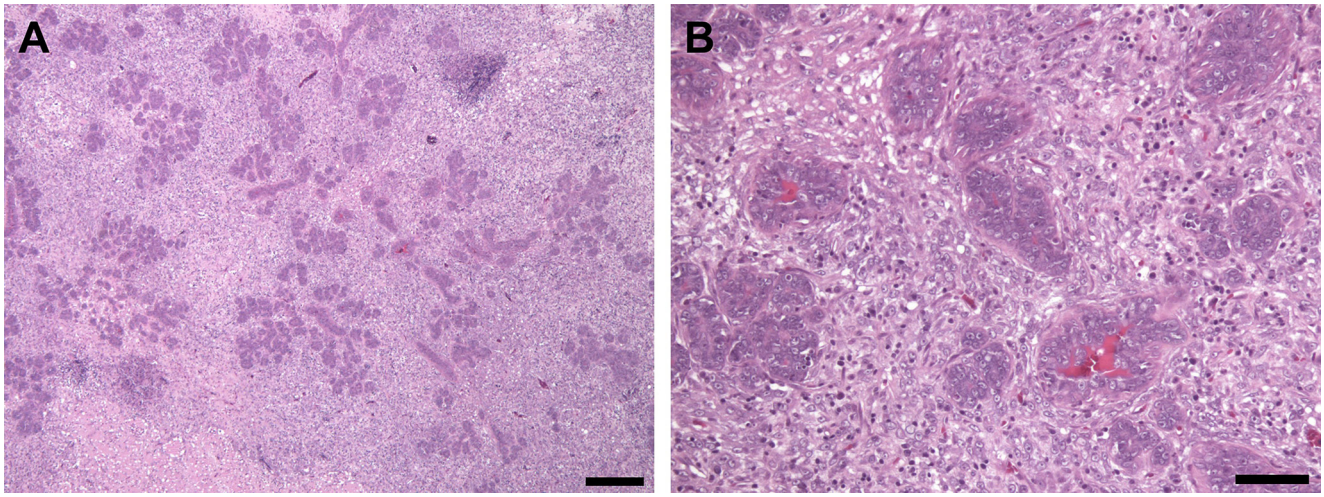

Fig. 2. Histology of pleomorphic adenoma in a African pygmy hedgehog (*Atelerix albiventris*). A. The tumor is composed of neoplastic proliferation of epithelial and spindle-shaped cells. H&E. Bar=400 μm. B. Neoplastic epithelial cells form ducts and spindle-shaped cells surround ducts showing irregular or streaming growth pattern. H&E. Bar=100 μm.

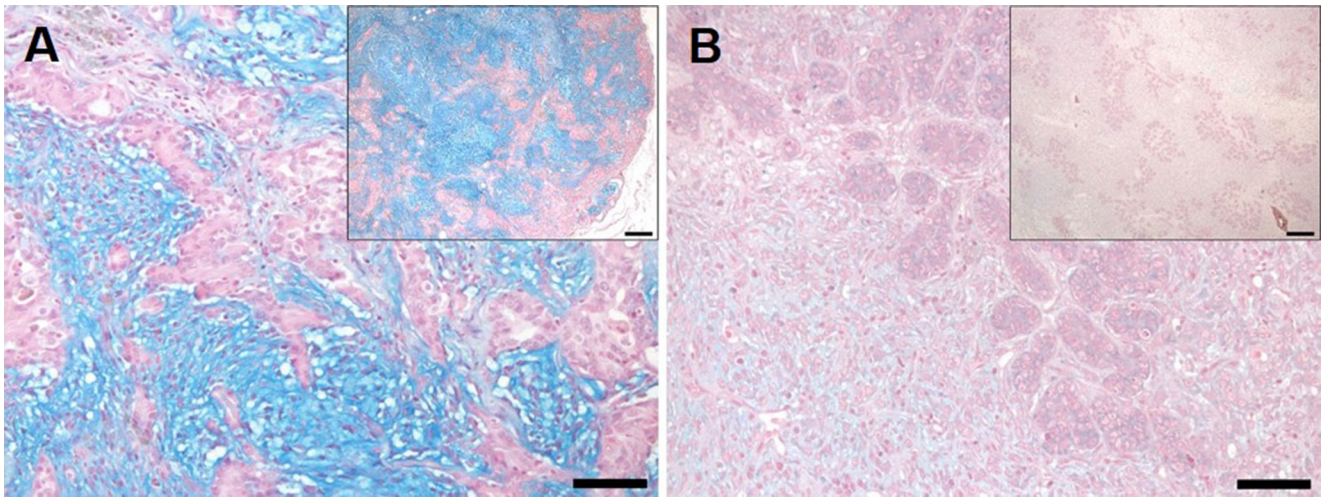

Fig. 3. Alcian Blue stain of pleomorphic adenoma in a African pygmy hedgehog (*Atelerix albiventris*). A. Positive control (mammary gland adenoma; complex type). B. Alcian Blue positive stroma were rarely observed in the mass. Bar=100 μm/400 μm for high/low magnifications (applies to A and B).

The prognosis for pleomorphic adenoma is highly correlated with progression to malignant tumor and complete resection. Pleomorphic adenoma generally develops slowly and expansively, but it can exhibit invasive growth into a capsule surrounding the tumor. Pleomorphic adenoma with malignant transformation are called "carcinoma ex pleomorphic adenoma", which can be observed mainly in ductal-type adenocarcinoma and myoepithelial carcinoma [6]. The incidence of pleomorphic adenoma with malignant transformation in humans is known to be approximately 5%, whereas in animals, 2 cases have been reported in the mandibular salivary glands of each dog and cat [9, 14, 18]. The extent of malignant transformation in the pleomorphic adenoma may be variable, so it is important to examine the different parts of the tumor histologically. In this case, histologic examination was performed on 8 different sites in the tumor and there were no suspicious histologic findings for malignant transformation, such as capsular invasion or high mitotic rate.

The prognosis for pleomorphic adenoma is generally favorable after complete resection but has been reported to recur up to 45% in humans [8, 16, 19]. The cause of recurrence is presumed to be the limitations of complete resection due to the capsular characteristics of this tumor, including incomplete capsule, capsule penetration, pseudopodium and satellite nodules [19]. Therefore, careful resection which includes the normal tissue around the tumor is necessary.

In this case, it seems that complete surgical resection was not performed considering the neoplastic cells present near the surgical margin on the microscope. However, on a follow-up study, no recurrence was noted since surgical removal for 14 months.

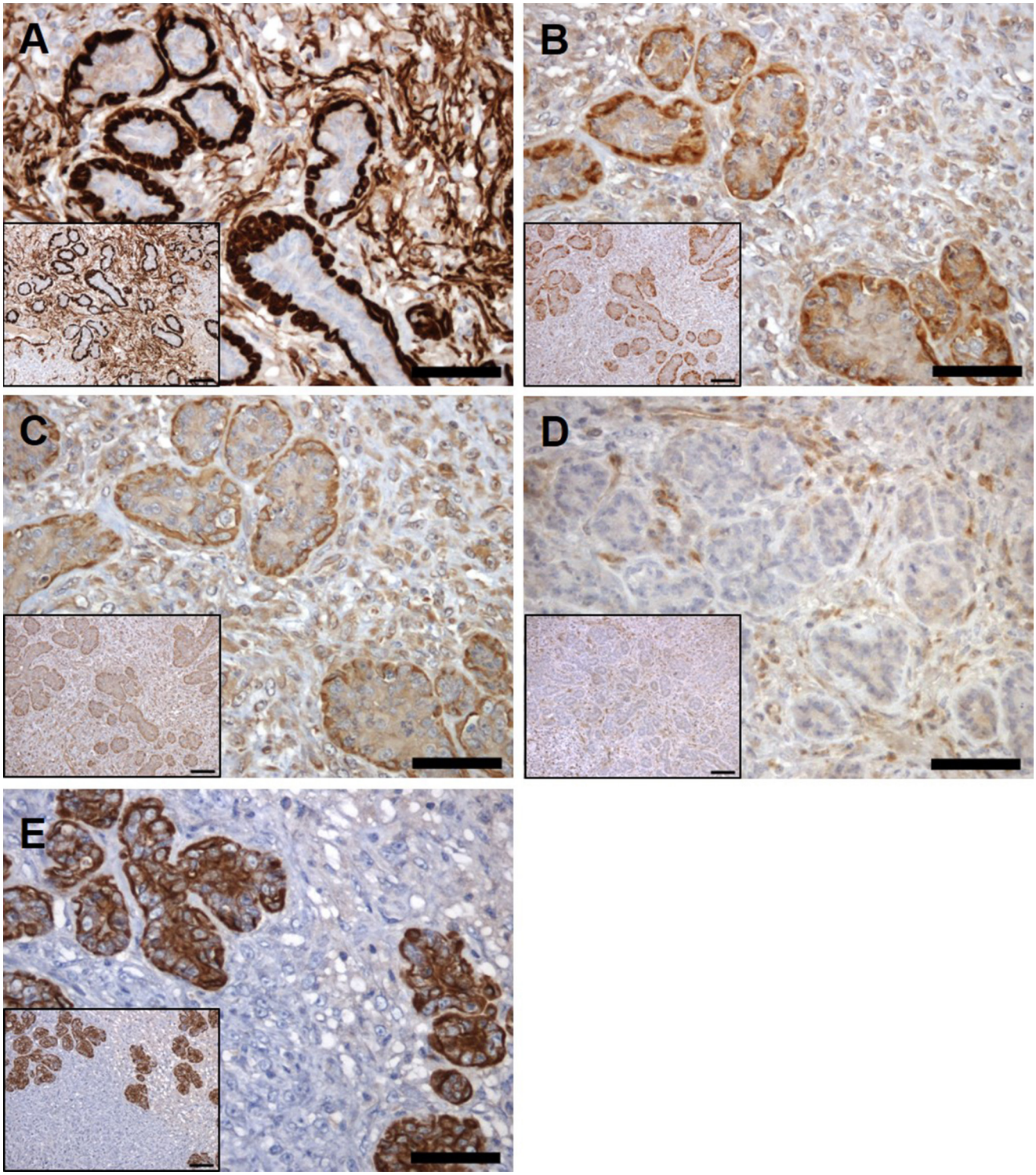

Fig. 4. Immunohistochemistry of pleomorphic adenoma in a African pygmy hedgehog (*Atelerix albiventris*). Spindle-shaped tumor cells and outer layer of the ducts (basal/myoepithelial cells) are positive for smooth muscle actin (A), calponin (B) and cytokeratin 14 (C). Spindle-shaped tumor cells are positive for vimentin (D). Immunoreactivity to cytokeratin 19 was negative in spindle-shaped tumor cells but positive in neoplastic epithelial cells (E). Bar=50 μm/100 μm for high/low magnifications (applies to A, B, C, D and E).

CONFLICT OF INTEREST. The authors declare no conflicts of interest.

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REFERENCES

1. Goldschmidt, M. H., Peña, L. and Zappulli, V. 2017. Tumor of the mammary gland, pp. 727–729. *In*: Tumors in Domestic Animals, 5th ed. (Meuten, D. J. ed.), Wiley Blackwell, Ames.
2. Head, K. W., Cullen, J. M., Dubielzig, R. R., Else, R. W., Misdorp, W., Patnaik, A. K., Tateyama, S. and van der Gaag, I. 2003. Histological classification of salivary gland tumors of domestic animals. pp. 59–62. *In*: Histological Classification of Tumors of the Alimentary System of Domestic Animals, 2nd ed. (Yvonne Schulman, F. ed.), Armed Forces Institute of Pathology, Washington, D.C.
3. Heatley, J. J., Mauldin, G. E. and Cho, D. Y. 2005. A review of neoplasia in the captive African hedgehog (*Atelerix albiventris*). *Semin Avian Exot Pet Med* **14**: 182–192. [CrossRef]
4. Hsieh, P. C., Yu, J. F. and Wang, L. C. 2015. A retrospective study of the medical status on 63 African hedgehogs (*Atelerix albiventris*) at the Taipei Zoo from 2003 to 2011. *J. Exot. Pet Med.* **24**: 105–111. [CrossRef]
5. Ito, F. A., Jorge, J., Vargas, P. A. and Lopes, M. A. 2009. Histopathological findings of pleomorphic adenomas of the salivary glands. *Med. Oral Patol. Oral Cir. Bucal* **14**: E57–E61. [Medline]
6. Katabi, N., Gomez, D., Klimstra, D. S., Carlson, D. L., Lee, N. and Ghossein, R. 2010. Prognostic factors of recurrence in salivary carcinoma ex pleomorphic adenoma, with emphasis on the carcinoma histologic subtype: a clinicopathologic study of 43 cases. *Hum. Pathol.* **41**: 927–934. [Medline] [CrossRef]
7. Kuramochi, M., Izawa, T., Nishimura, S., Shimada, T., Kuwamura, M. and Yamate, J. 2017. Pleomorphic adenoma of the labial gland, characterized by reticular pattern of myoepithelial cells in a dog. *J. Vet. Med. Sci.* **79**: 1163–1166. [Medline] [CrossRef]
8. Lopes, M. L. D. S., Barroso, K. M. A., Henriques, Á. C. G., Dos Santos, J. N., Martins, M. D. and de Souza, L. B. 2017. Pleomorphic adenomas of the salivary glands: retrospective multicentric study of 130 cases with emphasis on histopathological features. *Eur. Arch. Otorhinolaryngol.* **274**: 543–551. [Medline] [CrossRef]
9. Munday, J. S., Löhr, C. V. and Kiupel, M. 2017. Tumors of the alimentary tract, pp. 544–548. *In*: Tumors in Domestic Animals, 5th ed. (Meuten, D. J. ed.), Wiley Blackwell, Ames.
10. Okada, K., Kondo, H., Sumi, A. and Kagawa, Y. 2018. A retrospective study of disease incidence in African pygmy hedgehogs (*Atelerix albiventris*). *J. Vet. Med. Sci.* **80**: 1504–1510. [Medline] [CrossRef]
11. Raymond, J. T. and Gerner, M. 2000. Mammary gland tumors in captive African hedgehogs. *J. Wildl. Dis.* **36**: 405–408. [Medline] [CrossRef]
12. Raymond, J. T. and Garner, M. M. 2001. Spontaneous tumours in captive African hedgehogs (*Atelerix albiventris*): a retrospective study. *J. Comp. Pathol.* **124**: 128–133. [Medline] [CrossRef]
13. Raymond, J. T. and White, M. R. 1999. Necropsy and histopathologic findings in 14 African hedgehogs (*Atelerix albiventris*): a retrospective study. *J. Zoo Wildl. Med.* **30**: 273–277. [Medline]
14. Smrkovski, O. A., LeBlanc, A. K., Smith, S. H., LeBlanc, C. J., Adams, W. H. and Tobias, K. M. 2006. Carcinoma ex pleomorphic adenoma with sebaceous differentiation in the mandibular salivary gland of a dog. *Vet. Pathol.* **43**: 374–377. [Medline] [CrossRef]
15. Speight, P. M. and Barrett, A. W. 2002. Salivary gland tumours. *Oral Dis.* **8**: 229–240. [Medline] [CrossRef]
16. Stennert, E., Guntinas-Lichius, O., Klussmann, J. P. and Arnold, G. 2001. Histopathology of pleomorphic adenoma in the parotid gland: a prospective unselected series of 100 cases. *Laryngoscope* **111**: 2195–2200. [Medline] [CrossRef]
17. Turner, P. V., Brash, M. L. and Smith, D. A. 2018. Hedgehogs. pp. 387, 388, 395. *In*: Pathology of Small Mammal Pets, Wiley Blackwell, Hoboken.
18. Wells, G. A. and Robinson, M. 1975. Mixed tumour of salivary gland showing histological evidence of malignancy in a cat. *J. Comp. Pathol.* **85**: 77–85. [Medline] [CrossRef]
19. Zbären, P. and Stauffer, E. 2007. Pleomorphic adenoma of the parotid gland: histopathologic analysis of the capsular characteristics of 218 tumors. *Head Neck* **29**: 751–757. [Medline] [CrossRef]