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ADENOMA OF THE CLOACAL SCENT GLAND
IN A CALIFORNIA KINGSNAKE
(LAMPROPTELTIS GETULUS CALIFORNIAE)

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A multiple simple adenoma causing severe distortion of the tail base was identified in the cloacal scent gland of a female California Kingsnake (Lampropeltis getulus californiae). In addition to the normal epithelial layer of the gland and the skin, the tumour cells in the glandular epithelium also showed cross immunoreactivity with humanised anti-cytokeratin antibody. This is the first description of an adenoma in the scent gland of a reptile species. Neither epithelial nor mesenchymal tumours arising from the scent gland of reptiles have been reported previously. This report also highlights the possible use of humanised antibodies on reptile species for the fast, reliable and specific differential diagnosis of tumours.

Key words: Cloacal scent gland, multiple simple adenoma, California Kingsnake, immunohistochemistry, humanised antibody

The cloacal scent gland of snakes is a well-developed, paired, glandular organ located between the muscles of the tail root. The size of the gland varies according to species. The snakes use the holocrine excretion of the gland, which is a viscous, smelly substance to deter any aggressor or by other observations to mark the environment and to communicate between sex partners (Kissner et al., 2000; Sykes and Trupkiewicz, 2006).

The normal scent gland is lined by 8–10 cell layers where the deepest layer, attached to the basement membrane, is cuboidal with basal location of the nuclei (Young et al., 1999). The nuclei of the cells adjacent to the lumen of the gland degenerate. The gland is surrounded by connective tissue and muscle layers (Wood et al., 1995; Gál et al., 2010).

The chemical composition of the glandular secretion differs from species to species and, according to some research, between the two genders. Some volatile components of the secretion are used as signals (Wood et al., 1995; Kissner et al., 2000).

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Subacute and chronic inflammatory lesions of the cloacal scent gland, leading to notable destruction of the tail root, have been described (Gál et al., 2010). In these cases sometimes creamy congested substance accumulates in the lumen of the gland and triggers the inflammation. First heterophil granulocytes infiltrate the surrounding soft tissue area, later angiofibroblast and connective tissues proliferate, causing distortion of the tail root (Gál et al., 2010).

Adenocarcinoma has been described in the cloaca of reptiles. Similar tumours were found in two colubrid snakes and in a lizard. Adenomatous polyp was reported in green iguanas (Iguana iguana) (Garner et al., 2004). Another article reported an adenocarcinoma in the cloaca of a male Everglades ratsnake (Elaphe obsoleta rosalleni) (Sykes and Trupkiewicz, 2006). Neither epithelial nor mesenchymal tumours arising from the scent gland have been observed yet according to the available literature.

Case history

The carcass of a mature 13-year-old female California Kingsnake was presented for diagnostic examination to the Division of Exotic and Wild Animal Medicine, Department of Pathology and Forensic Veterinary Medicine, Faculty of Veterinary Science, Szent István University, Budapest, Hungary in October 2010. The snake was kept in a terrarium measuring 85 × 45 × 50 cm. The bedding was bark and fresh drinking water was continuously available in the terrarium. The animal was fed with 2–3 adult mice per week. Half a year prior to death the snake refused to eat. According to the owner, at the same time the tail root of the snake showed asymmetric enlargement behind the cloaca.

Following external examination the body cavity was opened by cutting the ventral scales to expose the internal organs. Following the regular process of reptile dissection the incision was continued to the end of the tail to examine the muscles and the scent gland.

 Samples from the enlarged perianal gland were taken and fixed in neutral, buffered 8% formaldehyde solution, routinely processed, and embedded in paraffin. Five-micrometer sections were cut with a Reichert sled-type microtome (Vienna, Austria) and were stained with haematoxylin and eosin according to standard methods, for histological examination. For the immunohistochemical (IHC) reaction 3–4 μm slides were prepared, deparaffinised, treated in microwave and soaked in hydrogen peroxide. The reactions were carried out as previously described by Szabára and Jakab (2010). For each immunohistochemical reaction, a negative control with omission of the primary antibody was included.

Routine bacteriological examination was also performed from the perianal gland filled with creamy content. The cultures were incubated overnight at 37 °C on blood agar and Drigalski plates under aerobic conditions.
Fig. 1. A. Normal (1) and neoplastic (2) cloacal scent gland in a California Kingsnake (*Lampropeltis getulus californiae*). B: Cut surface of the normal (1) and neoplastic (2) cloacal scent gland after formaldehyde fixation. C: Epithelial layer of the neoplastic scent gland. Haematoxylin and eosin (HE) staining, ×120. D: Positive cytokeratin reaction of the neoplastic epithelial layer in the scent gland. Immunohistochemical (IHC) staining with cytokeratin (CK), ×100
The dissection of the snake’s carcass did not reveal any lesions on the skin, on the scales, in the eyes or in the oropharyngeal cavity. The tail root showed asymmetric bulging on the left side of the tail behind the cloaca. The area was soft and undulating on palpation. After the removal of the skin, a lobulated multinodular structure was visible that modified the structure of the scent gland. The left-sided scent gland was 2.5 cm long and a sac-like object (Fig. 1A). The scent gland on the right side contained a small amount of brownish-yellow, creamy, fetid discharge, from the tissue growth on the left side no content could be expressed. Small, lentil-sized (around 3–4 mm in diameter), brownish-red fat bodies were observed in the body cavity. Other organs did not show any pathological changes.

Following fixation, the cut surface of the scent glands differed. The right gland had a normal structure, but the left gland was distorted by an encapsulated, soft, multinodular tissue mass. The nodules of the tissue proliferation were filled with creamy content and were connected by the connective tissue capsule surrounding them (Fig. 1B).

The haematoxylin and eosin stained section of the right scent gland showed normal wall structure. The gland was encapsulated in connective tissue, with a basal cell layer where the nuclei were positioned either in the central part or at the base of the cells close to the basement membrane. The overlying cell layers were composed of cells with irregularly shaped, sometimes pycnotic nuclei and foamy cytoplasm. Following immunohistochemical staining with cytokeratin (CK), the complete layer of the glandular epithelium showed positive reaction in the cytoplasm, pale mahogany-brown colouring was observed both in the basal layer and in the 9–10 cell layers facing the lumen. Mild homogeneous brownish staining was seen in the lumen. The skin of the snake was used as a positive control for the cytokeratin reaction where intensive mahogany-brown granular staining was demonstrated.

When evaluating the histological sections from the distorted left scent gland the connective tissue capsule was found to be of normal thickness around the nest-like nodular structures consisting of epithelium, up to 20 cell layers thickness. The epithelium was homogeneously stained by haematoxylin and eosin and showed lamellar structure. With higher magnification the nuclei of the cells in this area were different in size and shape, with non-homogeneous staining and signs of perichromasia. The position of the nuclei remained central in the cell layers adjacent to the lumen of the gland. Few heterophil granulocytes infiltrated the epithelial layer (Fig. 1C). With CK IHC reaction the complete epithelial layer appeared positive, with increased intensity to the lumen. In the cytoplasm of the cells mahogany brown granulation was detected (Fig. 1D). Pale but real brownish pigmentation was visible in the laminated layers adjacent to the lumen.

This case describes a multiple simple adenoma of the cloacal scent gland causing significant enlargement and distortion of the gland in an adult California Kingsnake (*Lampropeltis getulus californiae*). The epithelial cells of the normal...
and neoplastic glands were positive for cytokeratin marker as was the internal positive control, the skin of the snake. The staining identified the keratohyaline granules in the epithelial cells. The right normal scent gland was made up of regular holocrine secretory cells, while in the left neoplastic gland abnormal cell layers were found. We assume that the continuous growth of the enlarged scent gland partially due to the tumour growth and also due to the accumulation of the secretory product of the glands increased pressure on the cloacal region, causing discomfort to the animal. This condition led to refusal to feed and then to emaciation, as indicated by the emptied fat depots, and eventually to the death of the snake.

Humanised antibodies have been proven to show cross immune-reactivity on animal tissues, too. Until now, the immune reactions of mammalian tissue samples were checked with some of these antibodies (Szabára and Jakab, 2010). According to our data, the humanised anti-cytokeratin antibodies can also be used in reptiles to prove the epithelial origin of the examined tissue types, which is an important finding in relation to the diagnostic challenges arising from the increased number of exotic species kept as pets and their numerous neoplastic lesions. This report also highlights the possible use of humanised antibodies on reptile species for fast, reliable, specific and differential diagnosis.

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