Multiple cutaneous mast cell tumours in a Boa imperator

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SUMMARY

Mast cell tumours (MCTs) are common in dogs and cats, but are only rarely reported in reptiles. This case documents the clinical and pathomorphological results from a Boa imperator with multiple cutaneous nodules, diagnosed as MCT based on histopathology, immunohistochemistry and electron microscopy approaches. Grossly, there were multifocal, poorly demarcated, mostly ulcerated nodules ~3 cm in diameter on the skin. Histologically, the dermis and the subcutaneous tissue were infiltrated by round cell populations with eosinophilic granules, Toluidine blue and Giemsa stain revealed metachromatic granules. Using immunohistochemistry, some cells exhibited cytoplasmic immunostaining positive for tryptase. Ultrastructurally, variable quantities of intracytoplasmic, spherical and electron-dense granules were also detected. The MCT literature on snakes is scarce, especially for the family Boidae, but MCTs should be considered a differential diagnosis for nodular skin lesions in reptiles.

BACKGROUND

Mast cell tumours (MCTs) are common cutaneous tumours in domestic animals, especially dogs, where they present as the most common cutaneous tumour. However, MCTs are rarely described in reptiles.1-3 The current literature lists one malignant mastocytoma in the genus Boa, but no further information or descriptions are provided. Herein, we describe MCT in a Boa imperator, using a combinatorial approach comprising clinical and detailed histological analyses, immunohistochemical characterisation and an ultrastructural approach.

CASE PRESENTATION

A 4-year-old, anerythristic (type 2), male Boa imperator originally from Ecuador was presented to veterinary practice because of elevated masses on the skin. These masses where first noted 3 months prior to presentation. The general condition of the snake was good. One mass was surgically removed for pathological examination.

INVESTIGATIONS

The sample sent for histopathology was 9 mm×7 mm×5 mm, covered in partly brownish skin and had a mottled brown-white cut surface. Routine processing included 10 per cent formalin fixation, and tissue embedding in paraffin and cutting into 5-µm sections. Sections were stained with H&E for light microscopic examination.

On histopathological examination, 70 per cent of the section was infiltrated with roundish cells, separating and surrounding collagen bundles. The dermal mass was non-encapsulated, poorly circumscripted, moderately cellular and composed of loosely arranged sheets of round tumour cells, with single cells extending to the cut borders. These round cells were ~10 µm in diameter, had distinct cell borders, abundant eosinophilic cytoplasm with small eosinophilic granules, and a round central nucleus with coarsely stippled chromatin, and an indistinct basophilic nucleolus. There were up to eight mitotic figures present per 10 high-power fields. Few binucleated cells and cells ~20 µm in diameter were also noted. The neoplasm was slightly vascularised with few haemorrhages, and the epidermis was extensively ulcerated, with superficial purulent inflammation. Within the mass and interspersed between tumour cells, moderate numbers of granulocytes were present (figure 1).

Granules in most neoplastic cells exhibited a metachromatic appearance when stained with Toluidine blue, whereas granulocytes between tumour cells presented a slightly bottle-green appearance (figure 2). Using Giemsa stain, tumour cells showed a positive reaction, characterised by small, fine blue granules, whereas the granulocytes stained red.

For immunohistochemical examination, formalin-fixed, paraffin-embedded, 5-µm sections were labelled with primary antibodies and stained with ABC-method with 3,39-diaminobenzidin-tetrahydrochloride (DAB, Sigma Aldrich, St. Louis) and papanicolaou counterstain for light microscopic examination.8 Tumour cells were negative for the epithelial cell marker, cytokeratin (pan ab-3, clone Lu-5; Thermo Fisher Scientific) and the mesenchymal cell marker, vimentin (clone V9; DakoCytomation Denmark A/S).9 In tissue surrounding the tumour cells, a positive reaction for cytokeratin and vimentin was observed in the epidermis and in the muscles, respectively, and thereby used as internal positive controls. Thus, these antibodies exhibited cross-reactivity to snake tissue. However, tumour cells did not demonstrate positive immunostaining with the vimentin antibody, suggesting no cross-reactivity with snake mast cells. Using an antibody against tryptase (clone AA1; DakoCytomation Denmark A/S), some cells showed a cytoplasmic staining (figure 3). C-Kit (polyclonal; DakoCytomation Denmark A/S) staining patterns are well established in canine MCTs as a prognostic marker,9 but this is not yet established in snakes. However, there...
was no reproducible positive staining of tumour cells, therefore c-kit does not reliably cross-react with snake mast cells. Hence, in light of our accumulated data, a MCT was diagnosed.

**DIFFERENTIAL DIAGNOSIS**

In reptiles, various gross differential diagnoses exist for skin masses. It is important for a clinician to decide whether a grossly visible mass has an underlying neoplastic, non-infectious or infectious pathology to reliably direct an owner for further treatment. In most cases, further diagnostics are required and must be carefully balanced to reduce unnecessary suffering and/or antimicrobial treatments. A fine-needle aspirate is helpful in aiding the diagnostic process.

Detailed anamnesis, including clinical history and husbandry can help narrow down non-infectious aetiologies. Non-infectious lesions can occur anywhere on the body of a snake, but usually they do not present as elevated nodules. These could manifest as traumatic lesions, such as rodent bites, with subsequent dysecdysis, in live-fed animals, or from inappropriate heating and housing. Nonetheless, secondary infections and inflammation can obscure underlying non-infectious or even neoplastic processes.

Infectious lesions include mycotic, bacterial and parasitic origins. Cytology and microbiological examinations help identify infectious agents, but a secondary infected lesion or a false-negative result can impact an outcome. A positive test for fungi in cases of epidermal lesions with subsequent dysecdysis can suggest mycotic dermatitis. Cutaneous mycoses are commonly caused by fungi such as *Aspergillus*, *Candida albicans*, *Fusarium*, *Trichophyton mentagrophytes* or *Ophidiomyces ophiodiicola* which is an emerging infectious agent in wild and captive species.

Bacterial dermal infections that resemble an MCT should be included on the differential diagnosis list, to include dermatophilosis and blister disease (ventral dermal necrosis, scale rot or vesicular dermatitis). Occasionally, secondary bacterial infections can occur, for example, *Pseudomonas* species, *Aeromonas* species or *Proteus* species infections. Further differential diagnoses for nodules and non-healing wounds also include granulomas of mycobacterial aetiology. Abscesses can also resemble neoplasms, and can occur focally or in groups.

Equally, snake skin parasites, especially in wild-caught individuals, for example, sparganosis caused by *Spirometra* species, *Macdonaldius oschert* or pentastomids, can induce differential MCT diagnosis in snake skin.

In general, suspicion for cutaneous neoplasm arises if cytological preparations, like fine-needle aspirates or smears, reveal abnormal cell morphologies in the absence of inflammation and/or an infectious agent. For MCTs, this approach may provide a diagnosis without surgical intervention. Cutaneous neoplasms in snakes occur as single or multiple lesions, mostly on the dorsal and lateral body, but in principle they can occur anywhere, from the head to the cloaca. Viscera metastasis is rare, but cases have been published. Epithelial neoplasms including papillomatosis and squamous cell carcinoma have been described. Mesenchymal neoplasms like lipoma, chromatophoroma or soft-tissue sarcoma, including fibrosarcoma, malignant peripheral nerve
sheath tumour or neurofibroma, have been reported in snakes, and have to be adequately distinguished by cytology or histology approaches.\textsuperscript{12,17,18}

OUTCOME AND FOLLOW-UP
After MCT diagnosis, the snake was euthanised. Postmortem examination revealed multifocal randomly distributed dermal nodules, of $-3\text{cm}$ in diameter which were elevated and mostly ulcerated. These white/red nodules showed a clear border and were dense to elastic (figure 4). Besides a diffuse macrovesicular hepatic lipidosis, no other pathological changes were found in the other organs.

Histologically, all skin tumours resembled the biopsy sample, which partially extended into the subcutis. Using electron microscopy, semi-thin and ultra-thin sections were prepared to demonstrate cytoplasmic granules in tumour cells.

Ultrastructurally, tumour cells showed a clear cytoplasmic membrane and round nuclei, with moderate heterochromatin levels. Mild anisocytosis and anisokaryosis were also confirmed. Intracytoplasmic granule numbers were variable, homogeneously electron dense, and spherical with a $0.2$ to $1.1\,\mu\text{m}$ diameter, corresponding with mast cell morphology in reptiles.\textsuperscript{19,20}

In summary, nodule histopathology and electron microscopy findings supported the diagnosis of a poorly-differentiated multicentric cutaneous MCT. Histological examination and appearance with multifocal distribution point to a malignant process as known for MCTs in mammals.

Usually, canine MCTs are graded into two\textsuperscript{21} or three\textsuperscript{22} grades; however, in this case the tumour was characterised as grade III,\textsuperscript{22} high grade.\textsuperscript{21} Because the grading system is validated for dogs only, it is misleading to use this grading system for the prognostic assessment of reptile patients.

DISCUSSION
MCT reports in reptiles are scant. Only two cases in snakes are reported in the literature: an eastern kingsnake (\textit{Lampropeltis getula getula}, formerly \textit{L. getulus getulus}), with tumour spread to the inner organs, and mastocytoma listing in a \textit{Boa constrictor} in a case compilation.\textsuperscript{1,2} In contrast to this case, both aforementioned cases do not describe any multifocal appearance of the skin. In addition to cases in snakes, descriptions of a MCT in a common green Iguana (\textit{Iguana iguana}) and two cases in tortoises are also reported.\textsuperscript{3,4,4} A tumour in a desert tortoise (\textit{Gopherus agassizii}, formerly \textit{Xerobates agassizii}) was part of the case compilation but without descriptions,\textsuperscript{3} but the MCT in a giant Galápagos tortoise (\textit{Chelonoidis nigra}, formerly \textit{Geochelone nigra vicina}) described focal occurrence. Death of this animal was not reported\textsuperscript{4} and the MCT presented with granulocytes between tumour cells, as described here.\textsuperscript{5} In another report, an African Fat-Tail Gecko (\textit{Hemitheconyx caudicinctus}) was described with a systemic mastocytoma, with neoplastic cells in organs.\textsuperscript{5}

For reptilian granulocytes, histological differentiation between eosinophils and heterophils is unreliable by H&E staining. Eosinophils occur rarely in snakes, when compared with chelonioids and crocodilians, and cytoplasmic granules of eosinophils are cytologically eosinophilic to blue green.\textsuperscript{23} Cytochemically, the eosinophils of most reptiles are positive for benzidine peroxidase,\textsuperscript{24} but one study has suggested that in some reptile species, there is no real difference between heterophils and eosinophils, as they are combined in one cell type.\textsuperscript{25} Furthermore, eosinophil function in non-mammalian vertebrates is not fully understood.\textsuperscript{23} Thus, the granulocytes present in this MCT were most likely eosinophils; however, it cannot be ruled out that in snakes, these cells are heterophils.

Snake basophils and mast cells are relatively similar, but granules in mast cells are smaller.\textsuperscript{26} In some reptile species, basophils also show metachromatic staining with Toluidine blue, and with Giemsa, large blue granules are visible.\textsuperscript{23,24,25} Because of basophil and mast cell similarities in reptiles, their precise differentiation is not possible yet. Our investigations comprising small granule observations by electron microscopy, histological staining and tumour location and distribution in the skin, support an MCT diagnosis. Furthermore, the tumour growth pattern was similar to those in mammals and other reptiles.

Mast cells occur in all vertebrate species; however, there are limited data on their characterisation for snakes. In snake tissue, mast cells occur in high numbers in the mesentery, tongue, underneath the serosa in the gastrointestinal tract, the epidermidium and between muscle fibres and rarely at the adventitia of vessels and around nerve fibres.\textsuperscript{27} They are morphologically distinct between different animal species in terms of size, content and granule composition. In snakes, these cells are relatively small (7 to 11 \(\mu\text{m}\)), roundish or spindle shaped, and their granules have an amphoteric character, unlike mast cells in dog and rats.\textsuperscript{27} In addition, snake granules can be stained with Toluidine blue and Giemsa as in mammals\textsuperscript{27}; however, they are extremely water soluble, so they can easily be lost during histological

Learning points

- Clinical and gross differential diagnoses for skin masses in reptiles should include neoplastic, non-infectious and infectious skin lesions.
- Mast cell tumours occur in the \textit{Boidae} family and should be considered as rare differential diagnosis for cutaneous masses in snakes.
- The histological appearance of a mast cell tumour (MCT) in a \textit{Boa constrictor} resembles a canine MCT, but further immunohistochemical and ultrastructural investigations must be performed to confirm diagnosis in challenging cases.
- The current literature does not provide reliable clinical prognosis of MCTs in reptiles. A prognosis must be evidence based and guarded.
- Fine needle aspirations can be a useful tool in diagnosing skin lesions.
In conclusion, for exotic animal medicine, full necropsy or histopathological examination of excised tissue and follow-up should be promoted, as such cases expand our knowledge base for tumour behaviour. In doing so, more reliable prognostic evaluation can be generated. For snake skin nodules, MCTs occur in the family Boidae and should be considered as a rare differential diagnosis for cutaneous masses in snakes.

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