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Research article

Spectacular manifestations of systemic diseases of the snake: a histopathological description of four cases

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Abstract
This paper reports histopathological findings in the spectacles of four snakes diagnosed with systemic gout, inclusion body disease, disseminated lymphoma and myeloproliferative disease, respectively. Gout was characterised by urate ghost tophi in the stroma and outer epithelium of the spectacle. Inclusion body disease affected all layers of the spectacle with intracytoplasmic eosinophilic inclusions. Two cases of neoplasia, lymphoma and myeloproliferative disease, affected the ocular adnexa and the spectacular transition zone. These cases provide novel insight into how the spectacle may respond to four different systemic diseases with world-wide distribution.

Introduction

The eyelids of snakes fuse and become transparent during the embryonic state, creating the structure known as the spectacle. The spectacle consists of three layers: the outer epithelium, stroma and inner epithelium, and is connected to the periocular scales through a transition zone (Da Silva et al. 2014). Despite being transparent, the spectacle has nerves and blood vessels in the central collagenous layer (Da Silva et al. 2014). As part of the integument (Schwartz-Karsten 1933; Duke-Elder 1958) the spectacle is commonly involved in conditions affecting the skin (Da Silva et al. in press) and is expected to be affected in systemic disease. The most common disease findings of the spectacle are inflammation, spectaculitis, and problems with the shedding of the outermost keratin layers, spectacular dysecdysis (Da Silva et al. in press). However, other pathological findings are also seen in the spectacle. This case-series provides a histological description of the changes in the snake spectacle as seen in four systemic conditions: gout, inclusion body disease, disseminated lymphoma and myeloproliferative disease. Images are provided for reference.

Case reports

The specimens examined were all retrieved from the archives of Northwest ZooPath (Washington, USA), a private pathology consultation service receiving submissions from zoological institutions, as part of a larger study on diseases of the spectacle. From all four cases, tissues were collected at necropsy and fixed in 10% neutral buffered formalin before being submitted from zoos in the USA.

Upon receipt, the heads were decalcified with Rapid cal (BBC Scientific, Mt. Vernon, WA, USA) and embedded in paraffin, after which they were sectioned transversely at 5 µm, mounted on glass slides, stained with hematoxylin and eosin (HE) and examined by light microscopy.

Case 1

Generalised renal, visceral and articular gout was diagnosed in a 15-year-old female eastern fox snake (*Pantherophis vulpinus*). Necropsy revealed bilaterally swollen kidneys with multiple cysts and uric acid deposits. Histological examination showed several uric acid tophi in multiple organs, including lung,

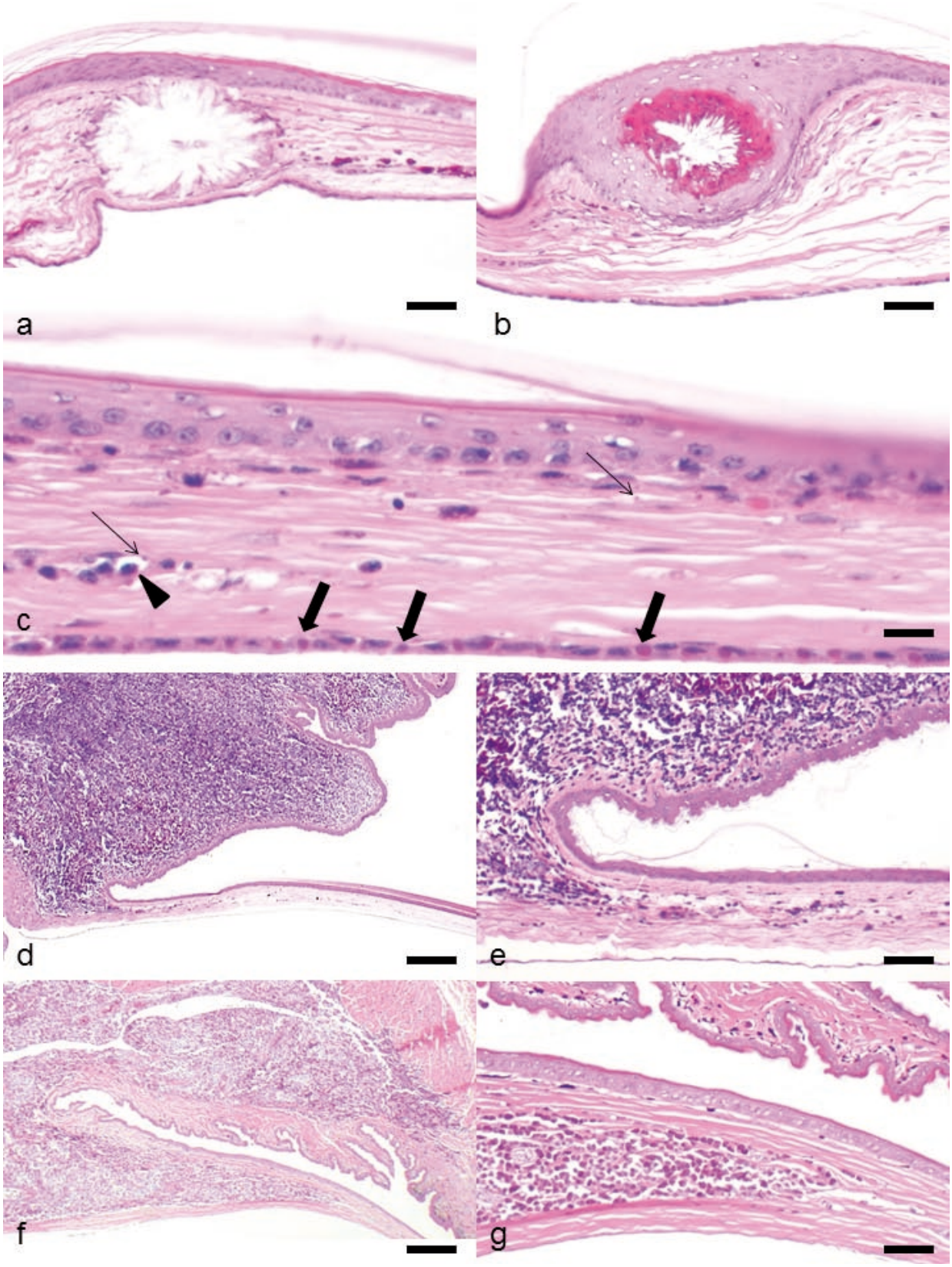

Figure 1. (a) Urate tophus in the stroma of the spectacle proper (arrow) surrounded by a mild inflammatory reaction in an eastern fox snake (*Pantherophis vulpinus*) with gout. Bar = 40 µm. Hematoxylin and eosin. (b) Urate tophus (arrow) undermined (to be extruded) by the germinal layer of the spectacle in an eastern fox snake (*Pantherophis vulpinus*) diagnosed with gout. Bar = 40 µm. Hematoxylin and eosin. (c) Eosinophilic intracytoplasmic inclusion bodies in the cells of the inner epithelium (thick arrow), and also in an endothelial cell (thin arrow) and a lymphocyte (arrow head) in a red-tailed boa constrictor (*Boa constrictor constrictor*) with inclusion body disease. Bar = 30 µm. Hematoxylin and eosin. (d) Infiltration of the dermis of the periocular scale and transition zone of the spectacle by neoplastic small, round, lymphoid tumour cells in a northern water snake (*Nerodia sipedon)*. Note that the remaining components of the spectacle are spared. Bar = 150 µm. Hematoxylin and eosin. (e) Higher magnification of Figure 1d. Neoplastic small, round lymphoid tumour cells in the middle stromal layers of the transition zone in a northern water snake (*Nerodia sipedon)*. The tumour cells have eosinophilic cytoplasm and vesicular nuclei with multiple nucleoli consistent with lymphoma. Bar = 45 µm. Hematoxylin and eosin. (f) Infiltration of neoplastic cells into the dermis of the periocular scale of a carpet python (*Morelia spilota*) with myeloproliferative disease. Bar = 500 µm. Hematoxylin and eosin. (g) Higher magnification of Figure 1f. Neoplastic myeloid cells in the stroma of the transition zone of the spectacle of a carpet python (*Morelia spilota*) diagnosed with myeloproliferative disease. Bar = 100 µm. Hematoxylin and eosin.

liver, oral mucous membranes and skin, secondary to the renal manifestation of gout. Tophi were also observed in the stroma (Fig. 1a) and outer epithelium of the spectacle (Fig. 1b). In the specimen examined, two tophi, one 80 x 120 µm, the other 40 µm in diameter, were visible at opposite peripheral sites of the same spectacle; the dorsal one was located in the stroma and the ventral tophus was found in the outer epithelium. The tophus in the outer epithelium was lined with a serocellular crust and the hyperplastic outer epithelium had expanded into the stroma. In the otherwise normal-looking stroma surrounding the dorsal tophus, there was a mild lymphocytic infiltrate. In addition to the tophi, the spectacle also had a retained layer of outer keratin with normal appearance. The inner epithelium was unremarkable.

Case 2

Inclusion body disease (IBD) was seen in a 1-year-old male red tailed boa constrictor (*Boa constrictor constrictor*) (Fig. 1c). Microscopic examination demonstrated multifocal intracytoplasmic eosinophilic inclusions of variable size primarily in the inner epithelium, but also in the stroma of the spectacle. The stroma also displayed some mild neovascularisation and granulocytic and lymphocytic inflammation. Inclusions were also seen in the endothelial cells and lymphocytes within the stroma. Aside from the inclusions and the mild inflammatory reaction, the spectacle maintained an overall normal appearance. Within the eye, inclusions were found in the cornea, choroid and retina. Throughout the rest of the body, inclusions of variable size were seen in the skin, adrenal gland, tongue, liver, trachea, oesophagus, kidney, stomach, intestine, heart and testicle, as well as in in circulating lymphocytes and lymphoid cells of the oesophagus. Inclusions were also present throughout the brain.

Case 3

Disseminated lymphoma was diagnosed in an 8-year-old female northern watersnake (*Nerodia sipedon*) that had previously been diagnosed with oral lymphoma by biopsy. The lymphoid malignancy was present throughout the facial soft tissues, where tumour cells extended through the dermis of the periocular scales to the transition zone of the spectacle (Fig. 1d and e). The outer and inner epithelium of the spectacle were not affected, nor was the stroma of the spectacle proper. The tumour was made up of sheets of homogenous neoplastic small, round lymphoid cells with eosinophilic cytoplasm and vesicular nuclei with multiple nucleoli. Throughout the body, the dental arcades, tongue, musculoskeletal system, skin, trachea, intestine, stomach, pituitary, spleen, thymus, lung, oesophagus, great vessels, liver, bone marrow, skeletal muscle and ovary were all affected.

Case 4

Myeloproliferative disease was observed in a 15-year-old female carpet python (*Morelia spilota*). In this snake, homogenous neoplastic myeloid cells had infiltrated all of the facial soft tissue examined. The cells resembled monocytic or granulocytic cell line precursors with vague to discrete, cytoplasmic granule differentiation. Occasional mitotic figures and bi-nucleated cells were also observed. The cells infiltrated the stroma of the transition zone of the spectacle, creating a wedge-like appearance (Fig. 1f and g). The subspectacular space and the spectacular outer or inner epithelium were not affected, nor was the stroma of the spectacle proper. The cells were also present multifocally throughout the lungs, kidney, liver, intestine, heart, fat bodies, spleen, trachea, pancreas, peripheral blood, thyroid and mesentery.

Discussion

Four cases are described in which systemic diseases had manifested themselves in the spectacle of snakes. In each case, disease had affected multiple organ systems and was widely disseminated throughout the body.

In the case of gout, the tophi in the section examined were deposited at the periphery of the spectacle proper, opposite each other, one dorsally and one ventrally. One tophus was clearly in the stroma whereas the other was in the outer epithelium. Snakes are uricotelic, and uric acid is excreted through the kidneys (Zwart 2006). Renal dysfunction therefore usually precedes uric acid deposits in crystalline form (Zwart 2006). Urate deposition is usually the result of persistently elevated levels of uric acid in the blood, and one important cause of this state in captive snakes is dehydration (Selleri and Hernandez-Divers 2006). The crystals are dissolved by formalin and histological specimens are left with a "ghost" silhouette of the urate crystals, referred to as a tophus. As the crystals are transported via the blood, the stromal location of a tophus deposition in the spectacle, which is vascularised, is self-explanatory. The appearance of a tophus in the outer epithelium is likely to be a result of re-epithelisation of the outer epithelium, undermining the tophus in order to eventually eliminate it, as occurs with skin and shell lesions in reptiles (Garner et al. 1997).

Inclusion body disease is an infectious viral disease seen mainly in boid snakes (Chang and Jacobson 2010), but other species may also be affected (Raymond et al. 2001). The inclusions were observed in all layers of the spectacle and in different cell types. IBD has been reported to affect multiple organs and various cell types (Schumacher et al. 1994) so it was not surprising to find that the cells of the spectacle also could be affected.

Lymphoma and myeloproliferative disease infiltrated the vascular transition zone of the spectacle but did not extend into the remaining components of the spectacle. The cells appeared to have spread in the connective tissue along the path of least resistance, where vasculature was rich and connective tissue was loose, creating a triangular appearance from the hinge region into the transition zone. It is not known whether the conditions were unable to continue into the very organised stroma of spectacle, or whether it was a coincidence that both cases were found at the same time, just before they entered the spectacle. Neoplasia is common in snakes (Schilliger et al. 2011) with the highest prevalence in colubrids (Garner et al. 2004). Hematopoietic tumours, especially lymphoid tumours, are particularly prevalent (Garner et al. 2004).

To conclude, the spectacle can be an indicator of systemic disease as seen in these four cases. The vascular network of the spectacle provides access to both infectious agents as well as inflammatory reaction. Whether vision was disturbed in these cases is unknown; however, common to all four cases was the widespread dissemination of the conditions, indicating that once having reached the spectacle, the conditions were severe.

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