By Emma Gulliver and Heenal Mehta

Skin deep

The case of a dog with a rare autoimmune condition.

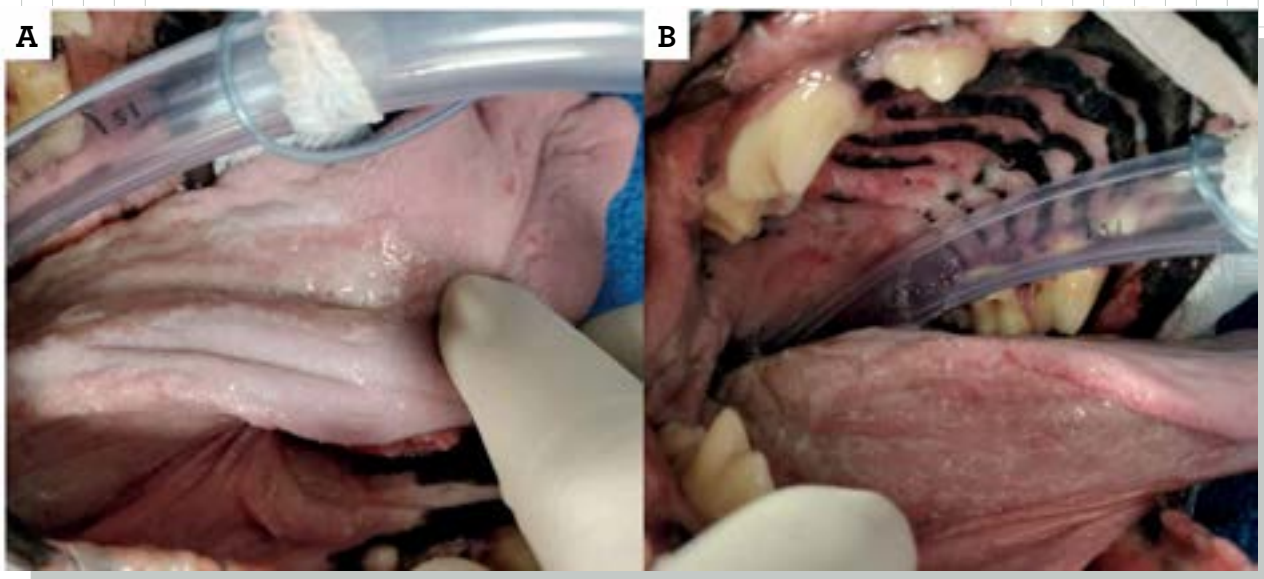

FIGURE 1: Gross lesions included ulcerations on the tongue (A,B) and palate (B), with several ruptured vesicles on the top of the tongue (A, right of image).

Introduction and case presentation

A seven-year-old male neutered German Shepherd x Husky was presented to his veterinarian for lethargy, excessive paw licking, salivation and halitosis. Oral examination revealed two areas of erosion and ulceration over the maxillary gingiva adjacent to the upper premolar four, and blood-tinged saliva. The dog was trialled on amoxycillin-clavulanate and meloxicam, and one week later the initial ulcers appeared to have healed. However, additional areas of ulceration had developed on the lower gingiva and buccal frenulum.

Biopsy was elected for further investigation. At the time of biopsy the following week, ulceration was present over the tongue, hard palate and upper and lower gingival margins, and there were intact vesicles along the lips and mucocutaneous junction (figure 1). The submandibular lymph nodes were palpably enlarged. There were small areas of crusting and ulceration at the base of the whiskers and junction of the pad, and haired skin of the left metacarpal pad. A complete blood count and serum biochemistry were unremarkable.

Histopathology

Biopsies obtained from the lesions in the oral cavity revealed regions of full-thickness epithelial loss with a thick fibrinonecrotic pseudomembrane overlying the exposed submucosa. Towards the edges of the ulcerations, there were areas of suprabasilar clefting (supra = above; basilar = basal layer) overlaid with occasional acantholytic cells (figure 2). The residual basal cells appeared raised, forming a 'tombstoned' layer. Areas of intact mucosal epithelium revealed mild cytoplasmic swelling and prominent intercellular bridging (spongiosis). There were scattered lymphocytes, neutrophils and eosinophils in the superficial submucosa.

Diagnosis

The combination of clinical presentation, vesicular to ulcerative lesions and histopathologic evidence of suprabasilar clefting with acantholysis was suggestive of pemphigus vulgaris (PV).

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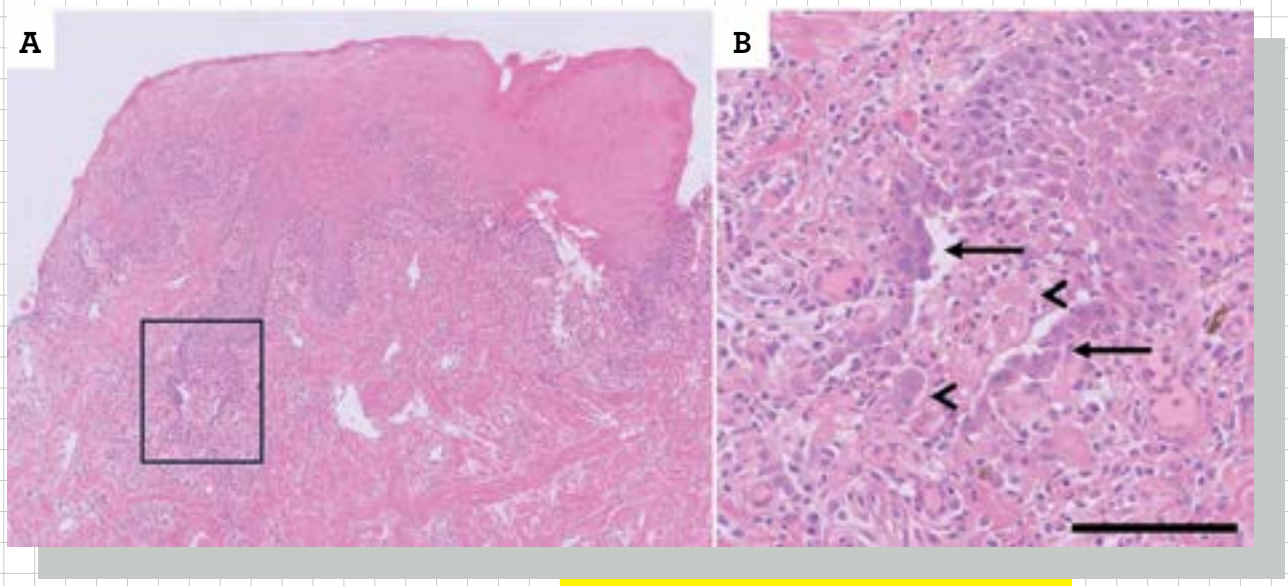

FIGURE 2: Histologic lesions suggestive of pemphigus vulgaris. (A) Low-power view of hyperplastic oral mucosa, with inset (B) showing a suprabasilar clefting with 'tombstoned' basal epithelial cells (arrow) and several rounded acantholytic cells (arrowhead). Scale bar = 100um.

Discussion

The 'pemphigus complex' refers to a group of immune-mediated diseases of the integument, characterised by vesiculobullous, pustular and erosive or ulcerative lesions. This complex encompasses several conditions of varied pathophysiology.

The histologic arrangement of the integument (including epidermis and mucus membranes) is of stratified squamous epithelium. A collagenous basement membrane anchors the epithelium to the underlying dermal or submucosal collagen and is covered with a layer of mitotically active basal cells (the 'stratum basale'). These cells undergo orderly differentiation through to the strata spinosum, granulosum (keratinising epithelium only) and finally corneum (keratinising epidermis) or superficiale (nonkeratinizing mucosa) (figure 3). Cell-cell and cell-basement membrane adhesion involves different molecules, and disruption of these by various mechanisms leads to a loss of adhesion and reduced strength and resistance to shearing forces. This can occur secondary to various genetic, infectious and immune-mediated diseases, and in the case of the pemphigus complex is thought to result from the development of autoantibodies that target particular adhesion molecules (Mauldin & Peters-Kennedy, 2016).

Pemphigus vulgaris is a very rare disorder in dogs, and results from a loss of adhesion between the basal epithelial layer and the overlying stratum spinosum. Animals with PV will have blister-like vesicles and ulceration due to suprabasilar clefting and acantholysis. Lesions most often affect the mucus membranes or mucocutaneous junction of the oral cavity, genitalia or perianal region, but may also affect the haired skin, nail and pad (Tham et al., 2020).

In contrast, the loss of adhesion that characterises the much more common disorder of pemphigus foliaceous (PF) occurs when autoantibodies target adhesion molecules in the stratum spinosum or granulosum. Animals with PF have a more superficial pustular dermatosis with intracorneal (intra = within; corneal = pertaining to the stratum corneum) acantholysis, crusting and ulceration, usually affecting the ears, periocular skin and face and the area around the nipples (Mauldin and Peters-Kennedy, 2016).

The presence of acantholysis is a key diagnostic feature for some (but not all) pemphigus diseases and refers to a squame that has lost its adhesion to the surrounding cells; it therefore appears rounded with discrete borders. While its presence is very useful, it is non-specific and may also be seen with bacterial and fungal dermatitis and in some squamous cell carcinomas. The case signalment, lesion appearance, distribution and microbiological testing help to rule out these possibilities when acantholysis is present.

Lesions mimicking immune-mediated pemphigus disease may very rarely be associated with neoplasia or adverse drug reactions. This is an important distinction to make, as the approach to treatment

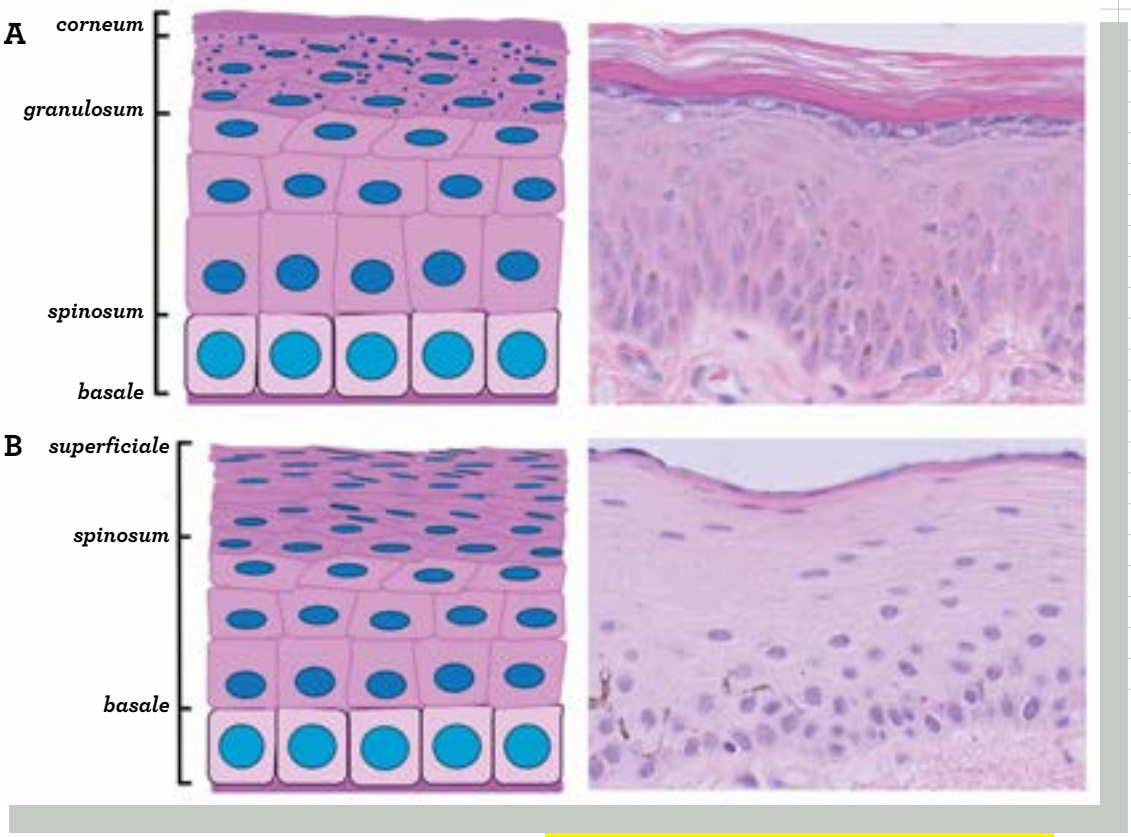

FIGURE 3: Microstructure of the integument, including the haired skin (A) and mucosa (B).

and prognosis varies considerably. Paraneoplastic pemphigus has some histologic overlap between PV and erythema multiforme and has been reported in dogs with thymic and splenic neoplasia. Correlation with evidence of disease in another body system is required to rule this out.

Adverse drug reactions can vary vastly in their clinical presentation and lesion character, and include the development of urticaria, perivascular dermatitis, vasculitis and vesiculobullous or pustular lesions. In these cases the diagnosis is one of exclusion and relies on a close evaluation of the clinical history, timing and improvement upon withdrawal of the drug (Mauldin and Peters-Kennedy, 2016).

What other considerations are there for the clinical signs?

The suspicion for immune-mediated disease was high based on the presence of progressive vesicular and ulcerative lesions in the oral cavity, haired skin and mucocutaneous junction. However, differential diagnoses need to be considered depending on the age of the patient and the distribution and appearance of lesions, and can be more difficult when intact vesicles or pustules are not present.

For dogs with cutaneous lesions, important rule-outs include infectious dermatitis such as bacterial pyoderma (bullous impetigo), dermatophytosis and demodicosis, particularly in young or immunosuppressed dogs. Epitheliotropic lymphoma may be considered in older dogs with crusting or ulcerative lesions of the skin or mucocutaneous junctions. There are other immune-mediated integumentary diseases that vary in their pathophysiology, lesion character and potential clinical outcomes, including mucus membrane pemphigoid, epidermolysis bullosa acquisita, erythema multiforme, vasculitis, the lupus complex and canine chronic gingivostomatitis. Biopsy is often required to differentiate these.

Optimising diagnoses in dogs with suspected immune-mediated skin or mucosal disease

There are some limitations to achieving a definitive diagnosis in veterinary species, as specific confirmatory immunologic tests are either unavailable or cost-prohibitive for some diseases. However, there are several key points that will help to optimise a diagnosis:

- Case signalment.
- Clinical history, including lesion duration, distribution, progression, comorbidities and treatment history.

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- Photographs of the lesions.
- Cytology can be useful in identifying the presence of acantholysis, but it is not specific to immune-mediated diseases.
- Biopsies of affected tissue, including intact pustules or vesicles where possible, and the junction between normal tissue and areas of erosion or ulceration. Placing biopsies in containers labelled according to the biopsy site is recommended.
- Microbiological testing, including bacterial and fungal culture, as indicated.

Case outcome

Following biopsy, the dog was trialled on prednisolone 2mg/kg PO SID (per mouth, once daily). After two weeks the lesions had improved significantly, but he had developed side effects warranting a dose reduction and was tapered to 0.5mg/kg PO every other day. Once on the reduced dose, he developed an area of erythema along the mandibular gingiva, and was started on cyclosporine 4mg/kg PO SID. At the time of writing, the oral ulcerative and vesicular lesions had resolved, and the dog was tolerating treatment. (vs)

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REFERENCES:

Mauldin EA, Peters-Kennedy J. 'Integumentary System', in *Jubb, Kennedy & Palmer's Pathology of Domestic Animals: Volume 1.* 6th edition. Eds: G Maxie. Elsevier Health Sciences. 2016

Tham HL, Linder KE, Olivry T. Deep pemphigus (pemphigus vulgaris, pemphigus vegetans and paraneoplastic pemphigus) in dogs, cats and horses: a comprehensive review. *BMC Veterinary Research*, 16, 457, 2020

Acknowledgements:
Thanks to CareVets Hobsonville for this interesting case.

Be our eyes and ears

Veterinary professionals play a key role in looking out for emerging and exotic pests and diseases

Test your knowledge with this case study

A 3-year-old Pitbull terrier cross presents with reduced appetite and lethargy (no vomiting or diarrhoea). It's up to date on vaccinations, but a few months behind on flea/tick and worming medication. The owners have no other dogs.

Physical exam and laboratory results:

- Mucus membranes pale and tacky
- Temperature 39.5°C
- A tick found on ear pinnae
- PCV = 17
- Thrombocytopenia

What are your top exotic differential diagnoses?
Turn the page upside down to see the answer

If you see something unusual, report it to the Biosecurity New Zealand exotic pest and disease hotline: **0800 80 99 66**

Answer: Lyme disease, Ehrlichiosis, Anaplamosis, Rocky Mountain Spotted Fever, Babesiosis, Bartonellosis, and Hepatozoonosis.

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