A multicentric intermediate-size B-cell lymphoma with epitheliotropism in a Freiberger mare

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Short running title: B-cell lymphoma with epitheliotropism in a Freiberger mare

Conflicts of interest: No conflicts of interest have been declared.

Sources of funding: This study was self-funded.

Acknowledgments
The authors would like to thank Hans Gantenbein, Manuela Bozzo, Erika Bürgi, and Bettina de Breuyn, Institute of Animal Pathology, Vetsuisse Faculty, University of Bern, and the team of Pathologie Länggasse, Ittigen, for their assistance in necropsy and in preparation of the histopathological and immunohistochemical specimens, respectively.

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Abstract

This report describes a multicentric intermediate-size B-cell lymphoma with epitheliotropism in a Freiberger mare affecting multiple mucous membranes, skin, and internal organs. The clonal neoplastic B-cell population is accompanied by numerous reactive polyclonal small T-cells. Differential diagnoses for these unusual findings are discussed, and a MALT lymphoma was considered.

Keywords: Lymphoma, B-cell, Intermediate-size, Epitheliotropism, Horses

Introduction

Equine cutaneous lymphomas are typically T-cell-rich B-cell lymphomas (TCRBCL), while cutaneous epitheliotropic T-cell lymphomas (ETCL) are uncommon.[1-2] Progression to Sezary syndrome of the latter is extremely rare.[2] T-cell rich B-cell lymphomas are composed of sheets of numerous reactive small T-lymphocytes intermingled with histiocytic cells, occasionally eosinophils, and typically small numbers (usually less than 10%) of neoplastic large B-cells that do not display epitheliotropism.[2] Cutaneous and subcutaneous nodules and masses often occur on the abdomen, thorax, and limbs, but may develop anywhere on the body. In general, TCRBCL lesions wax and wane over several years and potentially progress to disseminated large B-cell lymphoma (DLBCL).[2]

Other reported types of lymphoma in horses include peripheral T-cell lymphomas, DLBCLs, enteropathy-associated T-cell lymphomas of small cell type (EATL 2),[2] and low-grade B-cell lymphomas such as follicular lymphomas.[1] In addition to affecting skin, lymphoma has been described in the pancreas, thyroid gland, spinal cord, mediastinal lymph nodes, liver, spleen, and the gastrointestinal tract.[2]

Epitheliotropism is typically observed with T-cells lymphomas and has been described in the skin and intestines in animals and humans.[2] Low grade B-cell lymphomas such as extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue (MALT) lymphomas, follicular lymphomas, and mantle cell lymphomas can exhibit epitheliotropism in humans and need to be differentiated from non-neoplastic lesions such as lymphoepithelial sialadenitis, HIV-associated salivary gland disease, and rheumatoid lung disease.[3-7]

Case Report

A 15-year-old Freiberger mare suddenly developed bilateral suppurative keratoconjunctivitis and upper eyelid swelling, ulcerative stomatitis, and extensive muzzle crusting. While eye and muzzle lesions slightly improved following therapy with systemic antibiotics (Rota-TS® Oraldoser, 6 ml/100kg) and nonsteroidal anti-inflammatory drugs (Finadyne®, 1,1 mg/kg), the stomatitis progressed within 2 weeks. In addition, the mare showed inspiratory stridor, tachycardia (60 beats per minute), tachypnoea (20/min respiratory rate), submandibular lymphadenomegaly, and weight loss despite good appetite. Hemogram (laser scatter analysis) revealed mild anemia (5.6 Teras per Liter; reference range 6.0 - 12.0) and leukocytosis (64%, reference range 20 - 45). Absolute differential blood count revealed a pronounced

lymphocytosis (19.3 Gigas per Liter (G/L); reference range 1.5 - 4.0); mild neutrophilia (9.3 G/L; reference range 3.0 - 7.0), and mild monocytosis (1.2 G/L); reference range 0.04 - 0.3). The mare was then euthanized due to progressive inappetence and overall poor condition and submitted for diagnostic post-mortem examination.

Necropsy revealed multiple firm, well demarcated white masses in both upper eyelids, measuring up to 3cm x 3cm x 2cm (**Figure 1a**). Extensive crusting covered the muzzle and nostrils (**Figure 1b**) and reddening and multifocal ulcerations were noted in the nasal cavity, oral cavity and tongue (**Figure 1c**). The tongue and esophagus were covered by multifocal diphtheroid membranes. Additional masses, measuring up to 15cm x 15cm x 10cm were observed in the mediastinum, between the pulmonary artery and the aorta, in the substernal and perirenal fat, and in the subserosal tissue of the large colon.

Samples of all affected organs were fixed in 10% buffered formalin and embedded in paraffin. Histologic features were assessed on 5 µm H&E-stained paraffin sections. All internal masses were comprised of sheets of intermediate-sized lymphocytes, with nuclei measuring approximately 1.5 times the size of a red blood cell, with 0-2 mitoses per 400x HPF. These cells were intermingled with large numbers of small lymphocytes. A similar heteromorphic lymphocytic infiltrate was accompanied by epitheliotropism to the epidermis of the muzzle (**Figures 2a and b**) and mucosal epithelia of nasal and oral cavity, conjunctiva (**Figures 2c and d**), tongue, and esophagus. The intraepithelial lymphocytes formed small aggregates in the epidermis of the muzzle (**Figure 2b**). The stratum corneum of the muzzle sample contained superficial pigmented fungal hyphae, while massive numbers of yeast organisms, interpreted as *Candida* sp. colonized the lingual epithelium (not shown). No fungal organisms or other microorganisms were identified in the conjunctival mucosa.

Immunohistochemical analyses (IHC) were performed on 4µm paraffin sections from several masses as well as skin, conjunctiva and tongue following protocols previously described.[8] Information about antibodies and reagents are listed in **Table 1**. The masses were composed of 20-30% CD3 positive small T-cells and 50-70% small to intermediate-sized cells expressing CD20, Pax-5, and CD79a. In the muzzle, tongue, and conjunctiva, the intra- and subepithelial infiltrate consisted of equal numbers of CD3-positive T- cells and of CD79a and CD20 positive B cells, 10-15% of which expressed PAX5 (**Figures 3a-e**).

DNA extracted from formalin-fixed paraffin-embedded tissues of the upper eyelid mass, muzzle, and nasal mucosa was collected for clonality testing by PCR, evaluating equine antigen receptor gene rearrangements (PARR) as previously described.[9] Identical clonal immunoglobulin heavy chain (IgH) rearrangement (**Supplementary Figure 1**) and clonal kappa deleting element (KDE) rearrangement confirmed B-cell origin of the neoplastic cell population. The lesional T-cell population revealed a polyclonal T-cell receptor gamma (TRG) gene rearrangement (not shown).

Histological features, immunophenotype of neoplastic cells and clonality results support the diagnosis of an intermediate-size B-cell lymphoma presenting with multicentric neoplastic masses and epitheliotropism in skin and mucosal epithelia.

The oral candidiasis and the superficial muzzle fungal colonization were considered a consequence of compromised epithelial barrier function due to immunosuppression or antibiotic therapy.

Discussion

In this case report, we describe the occurrence of a multicentric intermediate-size B-cell lymphoma in a Freiberger mare with epitheliotropism, a feature that is usually associated with T-cell lymphomas in animals.[2] In addition to masses in several internal organs, heteromorphic neoplastic lymphocytes infiltrated the epidermis of the muzzle and conjunctival, lingual, nasal, oral, and esophageal mucosae. The presence of intraepithelial intermediate-size neoplastic B-cells in the muzzle, the lingual, and conjunctival mucosae was confirmed by immunophenotype, expressing CD20, PAX5, and CD79a, and clonal B-cell receptor rearrangement. Both immunophenotype and clonality testing confirmed the presence of accompanying reactive T-cells. However, these findings are discordant with a TCRBCL, the most described lymphoma type in the horse, as that one is composed of large centroblast-like B-cells and lacks epithelitropism.[1,2]

Epitheliotropism has been well documented in human B-cell lymphoma with typically more indolent course including follicular lymphoma, MALT lymphoma, and mantle cell lymphoma, as well as reactive nonneoplastic lesions.[2-7] The diagnosis of MALT and mantle cell lymphoma in humans requires the observation of a primary involvement of the marginal or mantle zone of reactive lymphoid tissues, and MALT lymphomas are usually a consequence of chronic inflammation.[3,6] These reactive lesions were not observed in this mare. Moreover, in contrast to the indolent course in people, this horse presented with fast clinical progression and severe lesions. However, morphologically similar lymphomas can present with markedly different clinical behavior in different species, as has been well documented in cutaneous ETCL in humans versus dogs.[10-12] MALT and mantle cell lymphomas can affect several organs simultaneously in people,[3,6] and early potential reactive lesions could have been less obvious clinically in this mare with a rapid transition to aggressive lymphoma.

In conclusion, this case report describes the occurrence of a multicentric intermediate B-cell lymphoma with epitheliotropism in a Freiberger mare. A MALT lymphoma must be considered despite some differences in clinical behavior and histologic features when compared with the disease in humans.

Figure legends

Figure 1. Postmortem macroscopic findings in the upper eyelid, muzzle, and tongue a) Sagittal section of the upper eyelid with an approximately 3 x 2 x 2 cm, poorly demarcated, firm white masses (arrowhead), and a severe thickening of the upper conjunctiva (arrow). (b) Haired skin from the muzzle with severe crusting and fissuring. (c) Hyperemic lingual mucosa with multifocal ulcerations, and diphtheroid plaques.

Figure 2. Histopathologic features of lesions of the muzzle (a and b) and conjunctiva (c and d). A dense lichenoid infiltrate (a; H&E 100x) is composed of lymphocytes obscuring the dermo-epidermal junction and forming intraepidermal aggregates (b;

H&E400x) in skin of the muzzle. A similar lichenoid infiltrate obscures the junction between conjunctival epithelium and lamina propria and reaches up to the adjacent sclera (c; H&E 100x), composed of lymphocytes (d; H&E 400x).

Figure 3. The intraepithelial infiltrates are characterized by intermediate-sized neoplastic lymphocytes with admixed small lymphocytes (a; arrowhead). The intermediate neoplastic cells express CD79a (b; arrowhead), CD20 (c; arrowhead) and PAX5 (d; arrowhead) confirming B-cell origin. The small lymphocytes expressed CD3 (e; arrowhead) indicating presence of reactive T-cells. 800x

Figure 4. Clonality testing (PARR): Evaluation of IgH Framework 3 rearrangement reveals identical clones in sample from upper eyelid (S1), muzzle (S2) and nasal mucosa (S3). Samples were run in triplicates. P: polyclonal control; C: clonal control.

Table legends

Table 1. Information and source of antibodies, pretreatment, and detection system used for immunophenotyping of lesional cells.

Primary Antibody	Source	Pretreatment	Dilution	Detection System	Source
CD3 (Clone LN10 anti-CD3, mouse monoclonal)	Leica Biosystems, Germany	Tris EDTA based pH9, 20 min, 100°C	1:100	Bond Polymer Refine Detection System, including peroxide block, post primary reagent (anti-mouse), polymer reagent (anti-rabbit), 3,3'-diaminobenzidine (DAB) chromogen and HE counterstain	Leica Biosystems, Germany
CD20 (rabbit polyclonal)	Lab Vision™ Thermo Fisher Scientific, USA	Citrate based buffer pH6, 20 min, 100°C	1:200		
CD79a (Clone HM57, mouse monoclonal)	Santa Cruz Biotechnology, USA	Tris EDTA based pH9, 20 min, 95°C	1:200		
PAX5 (NCL-L-PAX-5, mouse monoclonal)	Leica Biosystems, Germany	Tris EDTA based pH9, 20 min, 95°C	1:40		

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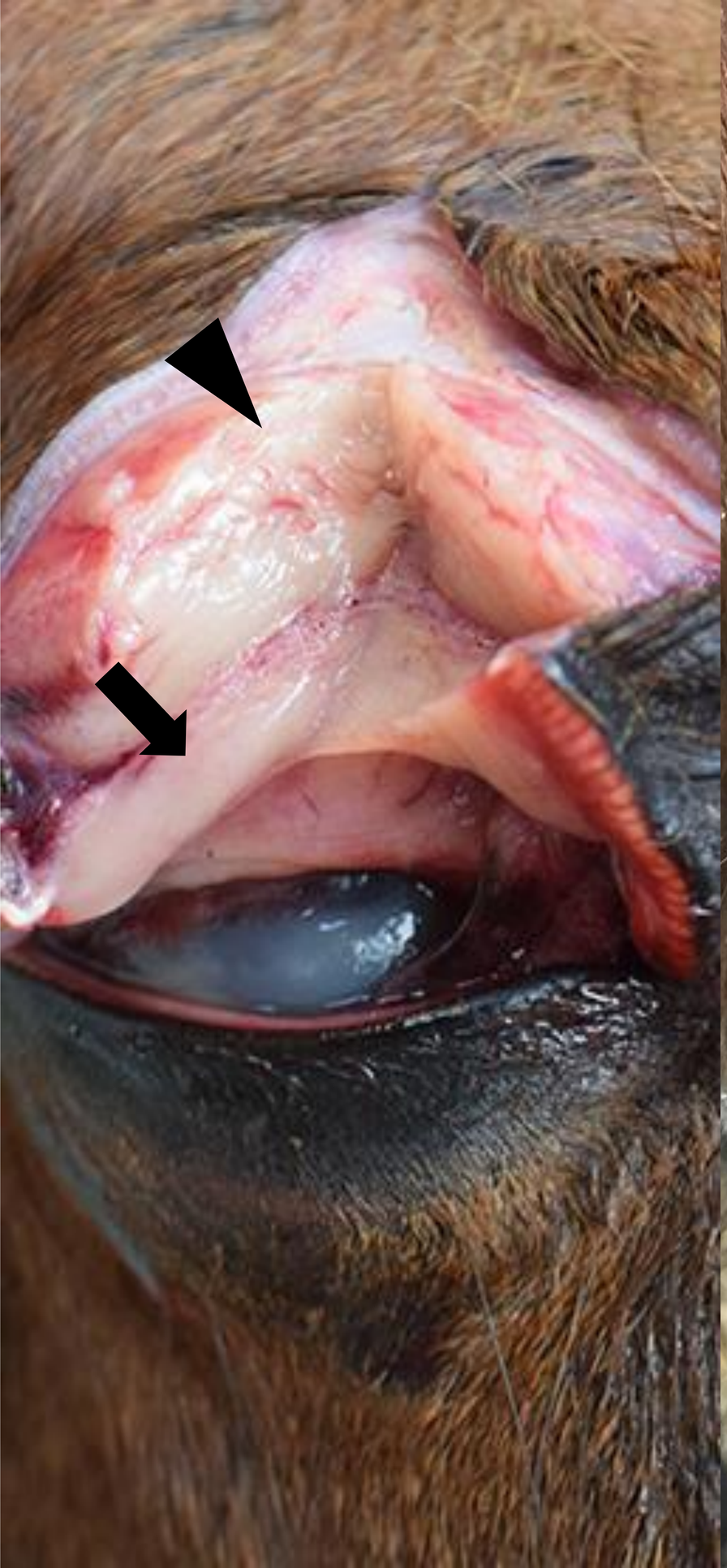

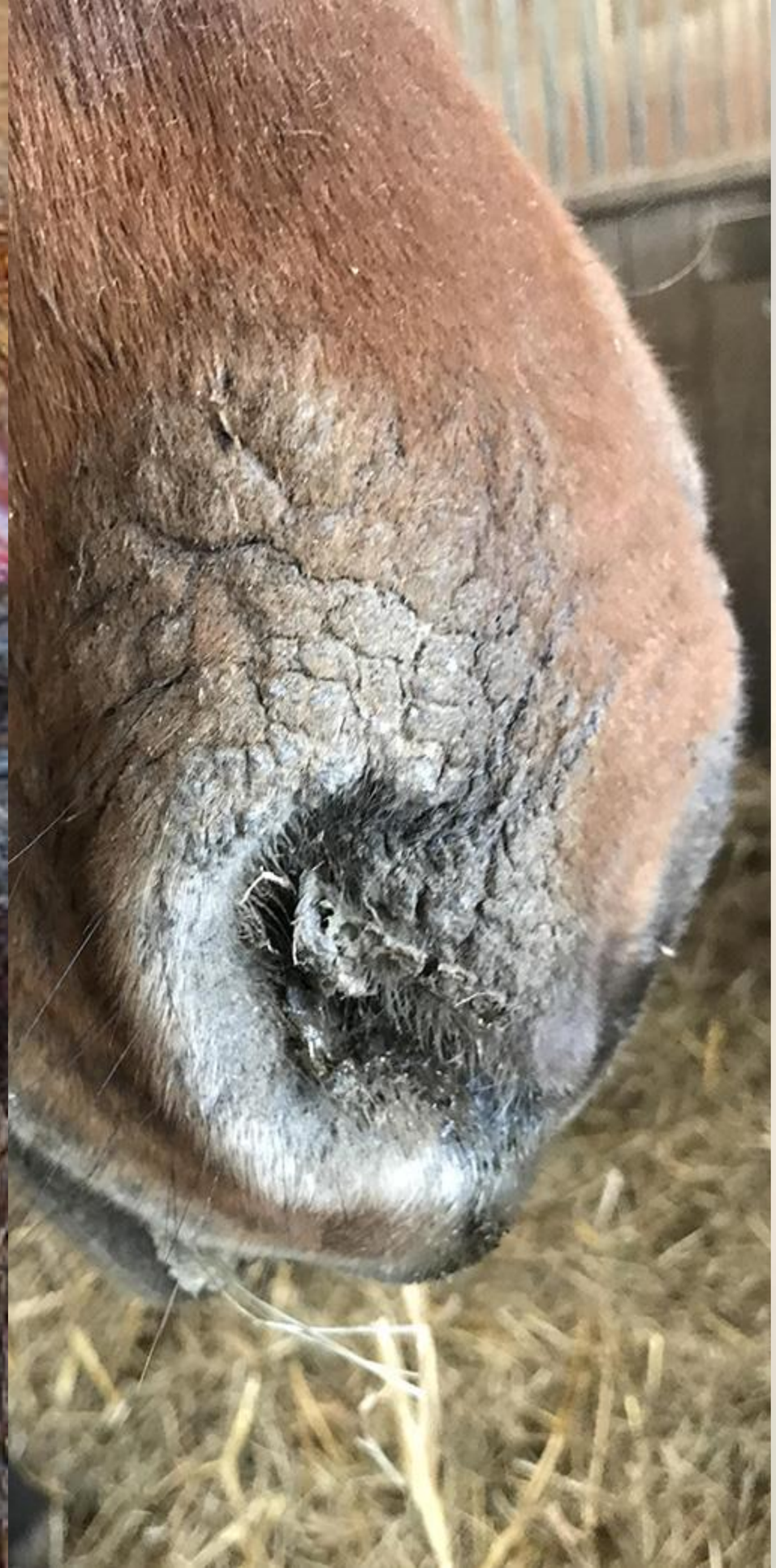

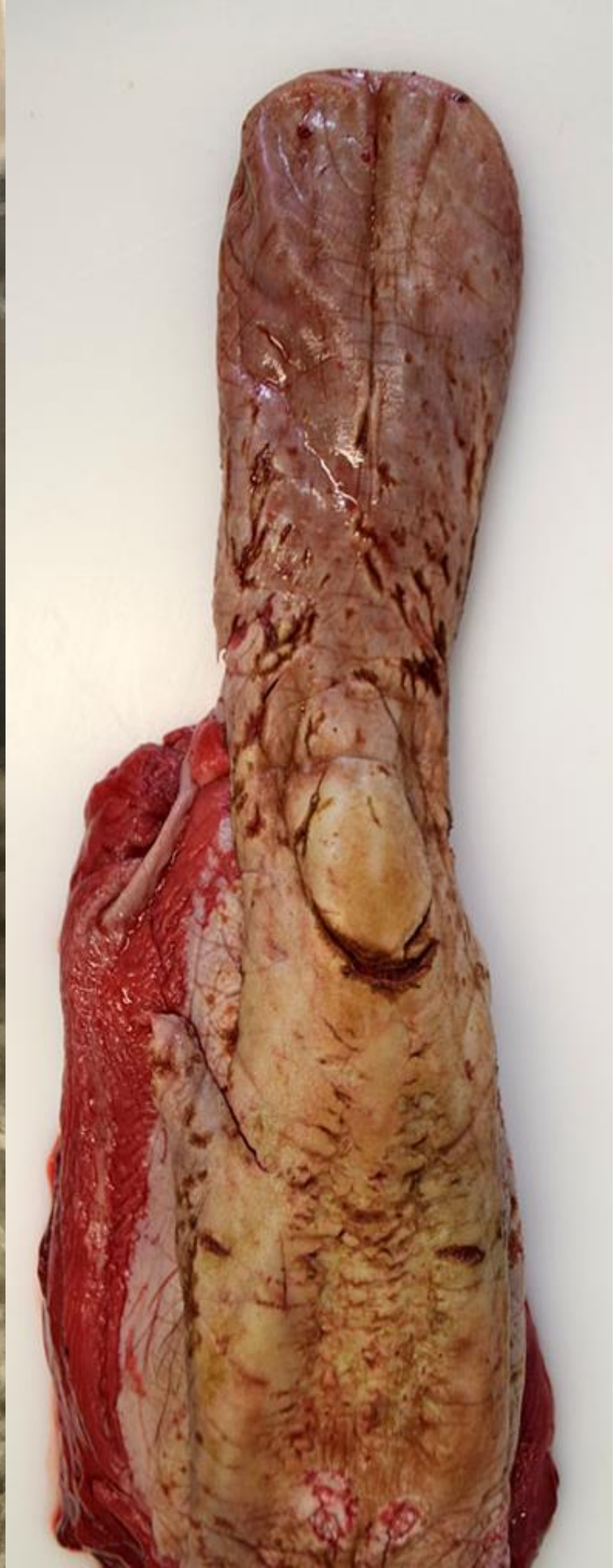

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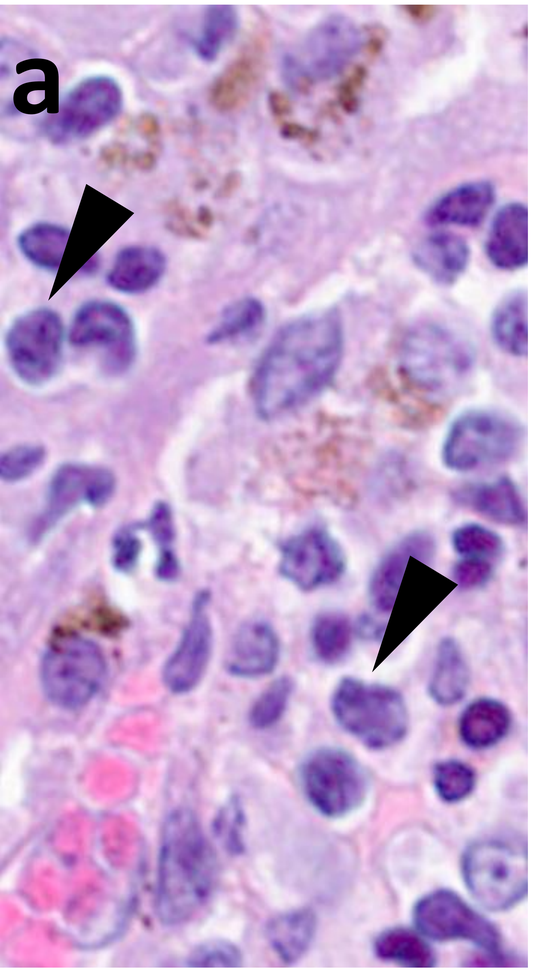
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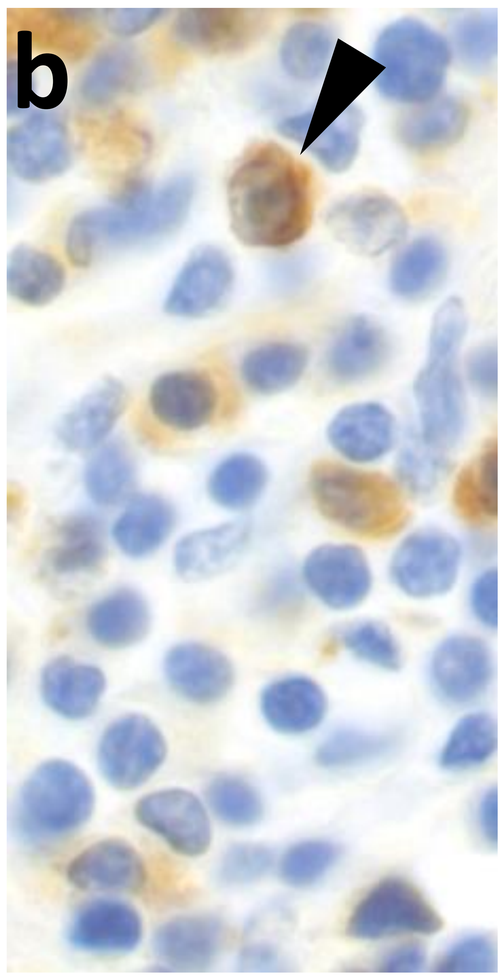
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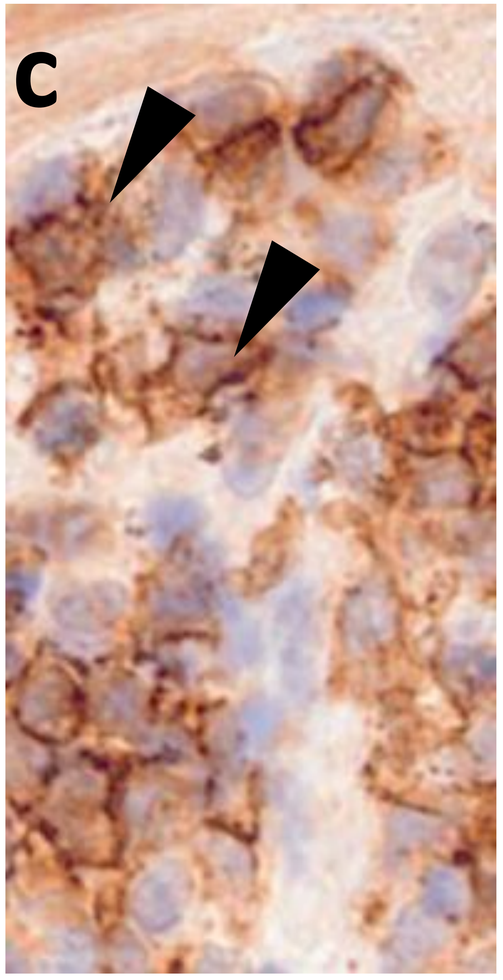
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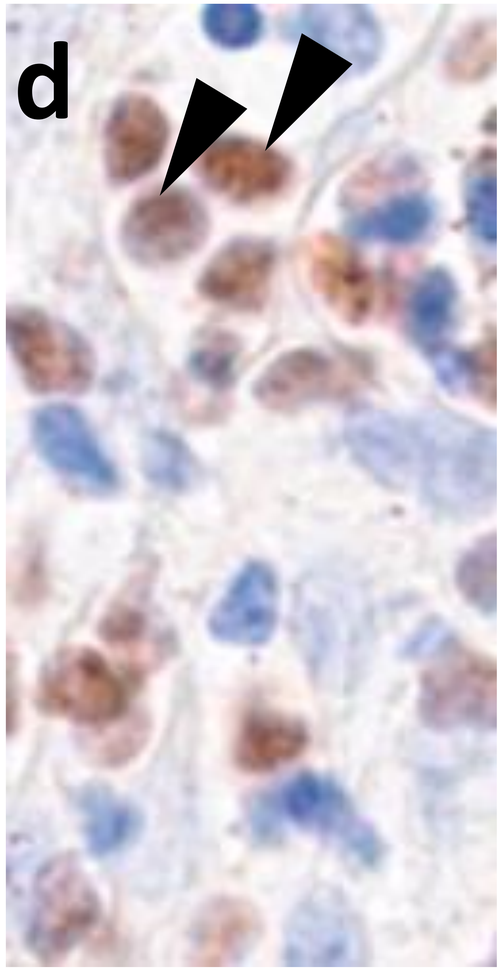
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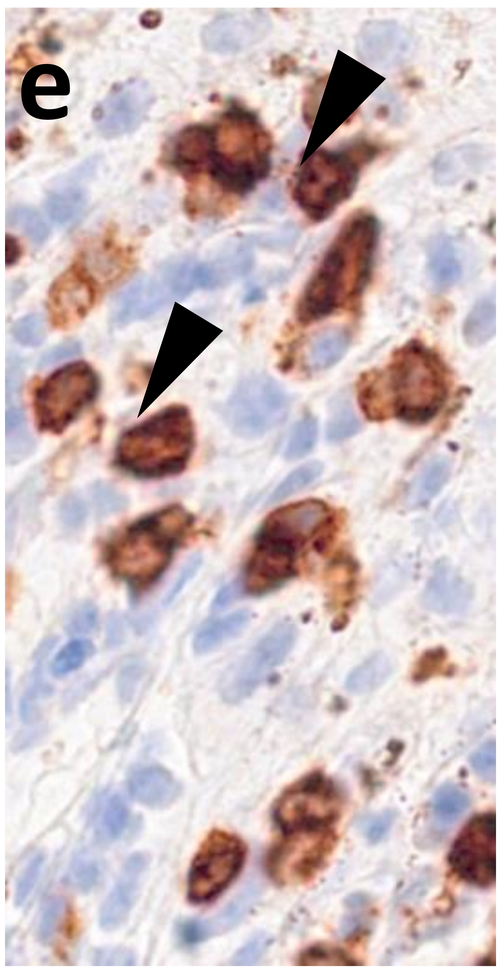
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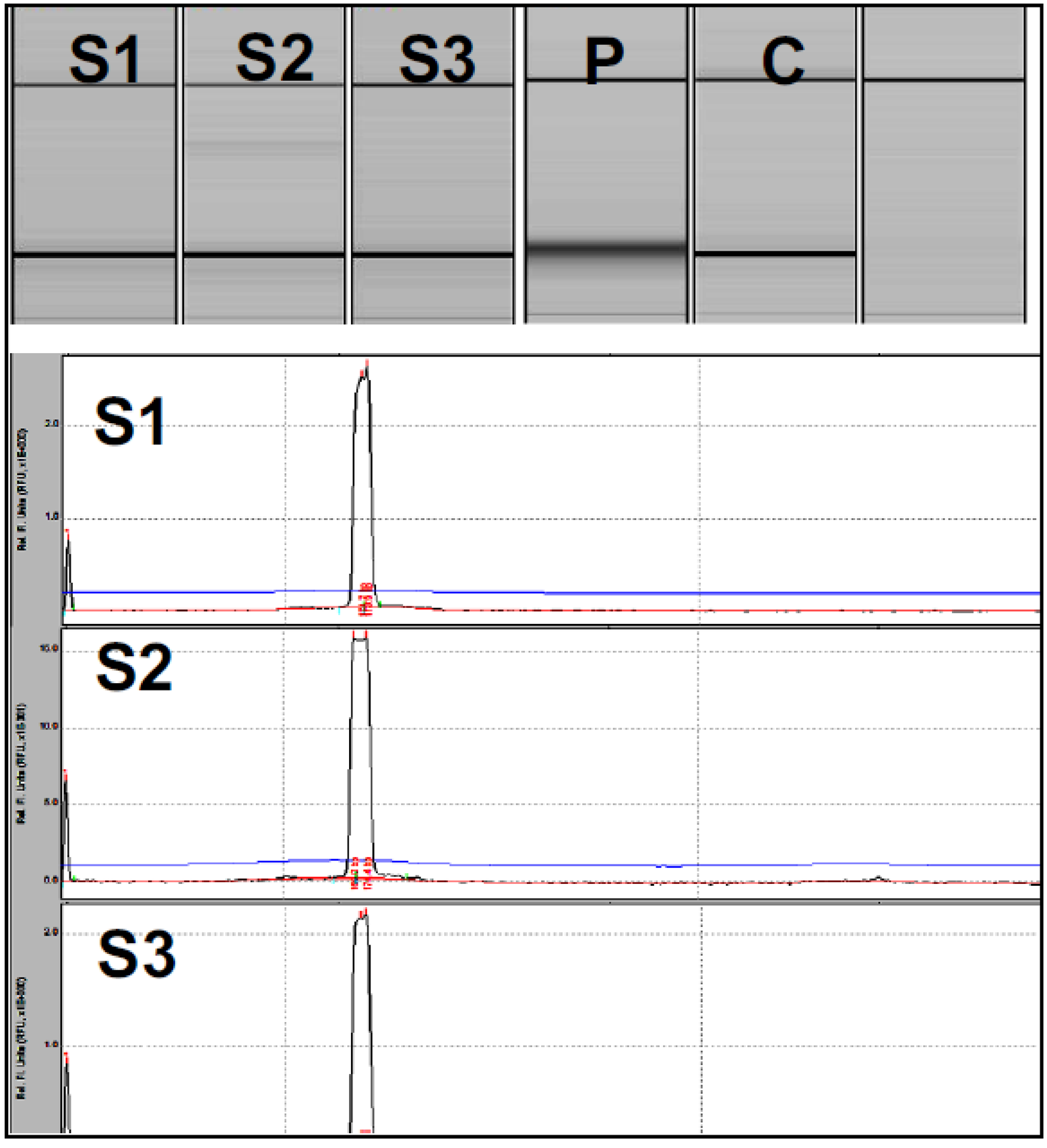
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