

Mystery Case: Thoracic effusion in a horse

Evelyn Kuhlmeier¹, Manon Straub², Barbara Riond¹, Hannah Junge², Franco Guscelli³, Marilisa Novacco¹

¹ Clinical Laboratory, Department of Clinical Diagnostics and Services, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

² Equine Internal Medicine, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

³ Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Zurich, Switzerland

Evelyn Kuhlmeier - evelyn.kuhlmeier@uzh.ch

Specimen: Thoracic effusion

Signalment: 14-year-old grey Shagya Arabian horse

History: The horse presented with signs of colic. Multiple cutaneous nodules were noted on the neck, trunk, and prepuce.

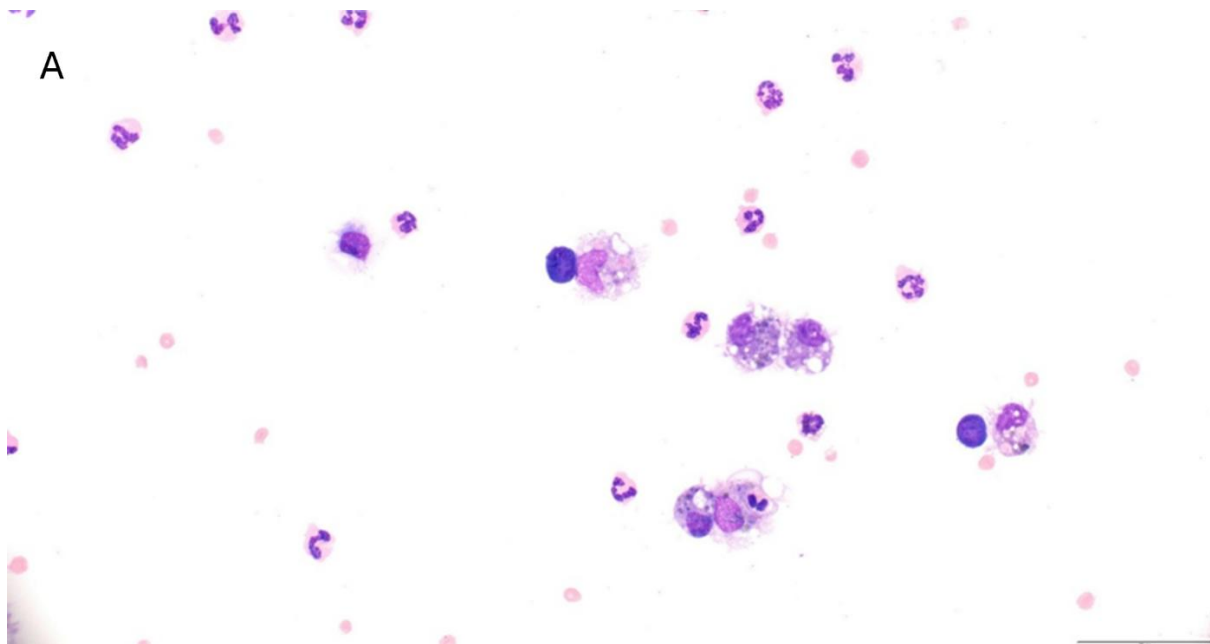
Clinical findings: Hematology, performed using the Sysmex-XN1000V analyzer with a manual differential count, revealed moderate anemia and mild leukocytosis, accompanied by markedly increased serum amyloid A and fibrinogen concentrations. A mild hyperbilirubinemia was also present (Table 1).

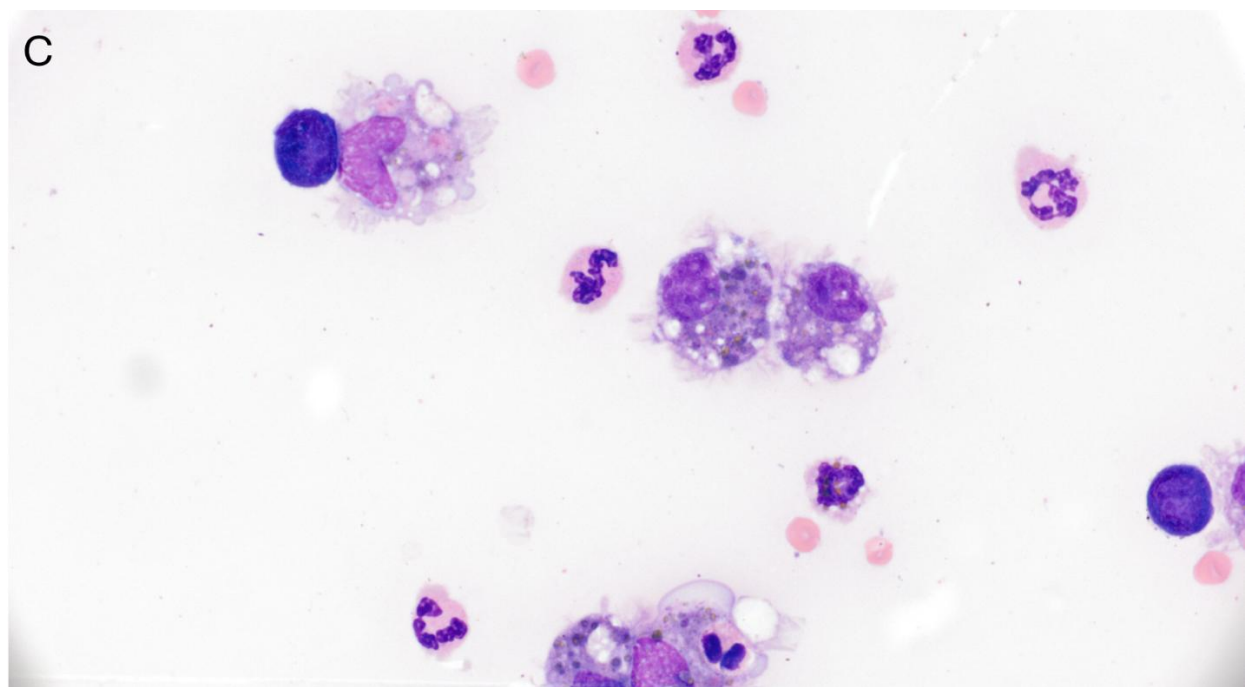
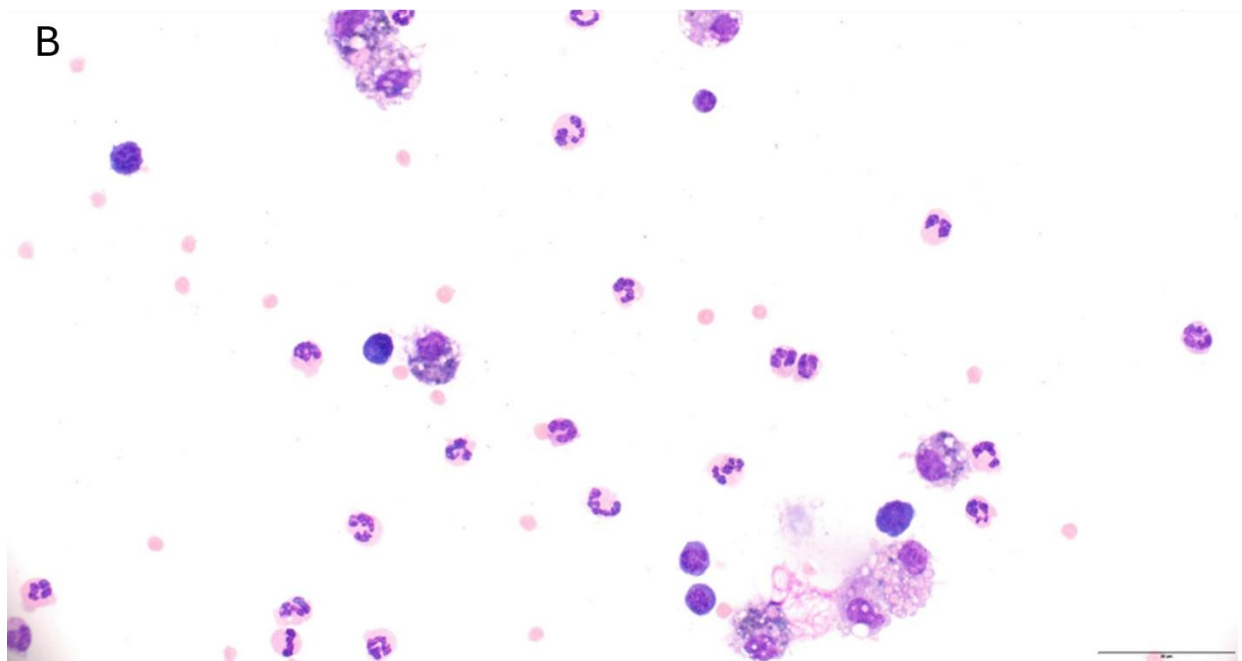
Parameter	Result	Reference interval
Hematology		
Hematocrit	25 %	30 – 42 %
Hemoglobin	9.3 g/dL	10.8 – 14.9 g/dL
Erythrocytes	$5.59 \times 10^6/\mu\text{L}$	$6.2 - 9.0 \times 10^6/\mu\text{L}$
Thrombocytes	$193 \times 10^3/\mu\text{L}$	$87 - 168 \times 10^3/\mu\text{L}$
Leukocytes	$11 \times 10^3/\mu\text{L}$	$4.7 - 8.2 \times 10^3/\mu\text{L}$
Band neutrophils	$0.55 \times 10^3/\mu\text{L}$	$0.00 - 0.08 \times 10^3/\mu\text{L}$
Neutrophils	$8.66 \times 10^3/\mu\text{L}$	$3.02 - 5.78 \times 10^3/\mu\text{L}$
Eosinophils	0	$0.00 - 0.22 \times 10^3/\mu\text{L}$
Basophils	$0.11 \times 10^3/\mu\text{L}$	$0.00 - 0.07 \times 10^3/\mu\text{L}$
Monocytes	$0.27 \times 10^3/\mu\text{L}$	$0.00 - 0.18 \times 10^3/\mu\text{L}$
Lymphocytes	$1.37 \times 10^3/\mu\text{L}$	$1.02 - 3.47 \times 10^3/\mu\text{L}$

Parameter	Result	Reference interval
Chemistry		
Total Bilirubin	54.2 umol/L	17.4 – 35.2 umol/L
Acute Phase Proteins		
Serum amyloid A	2'726 mg/L	0.5–1.2 mg/L
Fibrinogen	7.1 g/L	1.25–2.85 g/L

Table 1. Relevant hematologic, biochemical, and acute-phase protein parameters in the affected horse.

A large colon impaction was diagnosed and successfully treated. Thoracic ultrasonography demonstrated bilateral pleural effusion, pulmonary consolidation, and a suspected pericardial mass. Radiographs raised concern for a caudal ventral alveolar pattern and/or pleural free fluid, which could represent pleuropneumonia, hemorrhage or atelectasis. A soft tissue mass caudodorsal to the guttural pouch was also observed. Endoscopic examination identified dorsal displacement of the soft palate and multifocal lesions within both guttural pouches, most prominent in the left medial compartment. Tracheobronchial secretions were collected for cytological evaluation and revealed a moderate increase in mucus production and moderate neutrophilic inflammation. Thoracocentesis was performed for cytological analysis of the pleural effusion. A total of 2 mL of turbid, orange pleural fluid was collected. The evaluation revealed a total nucleated cell count of 29'800 cells/ μ L, using the Sysmex XN-1000V, and a protein concentration of 40 g/L measured by refractometry. Manual differential cell count showed a predominance of neutrophils (77%), with macrophages accounting for 13% and lymphocytes for 10% of nucleated cells.





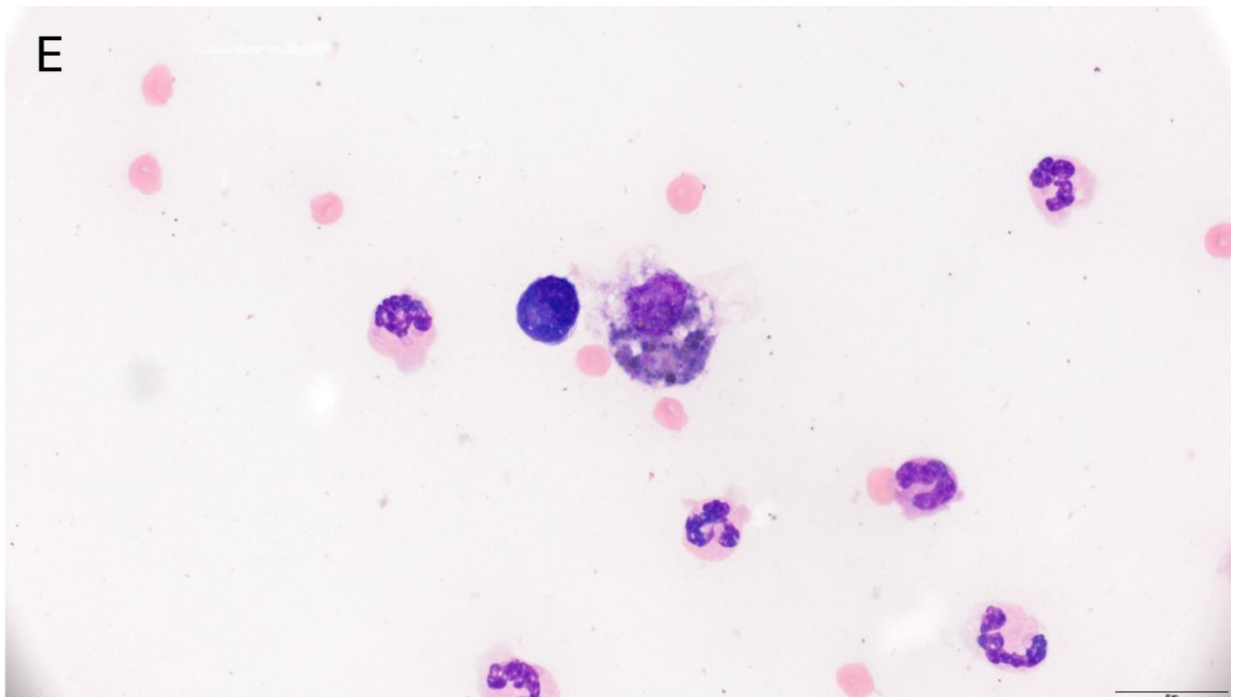
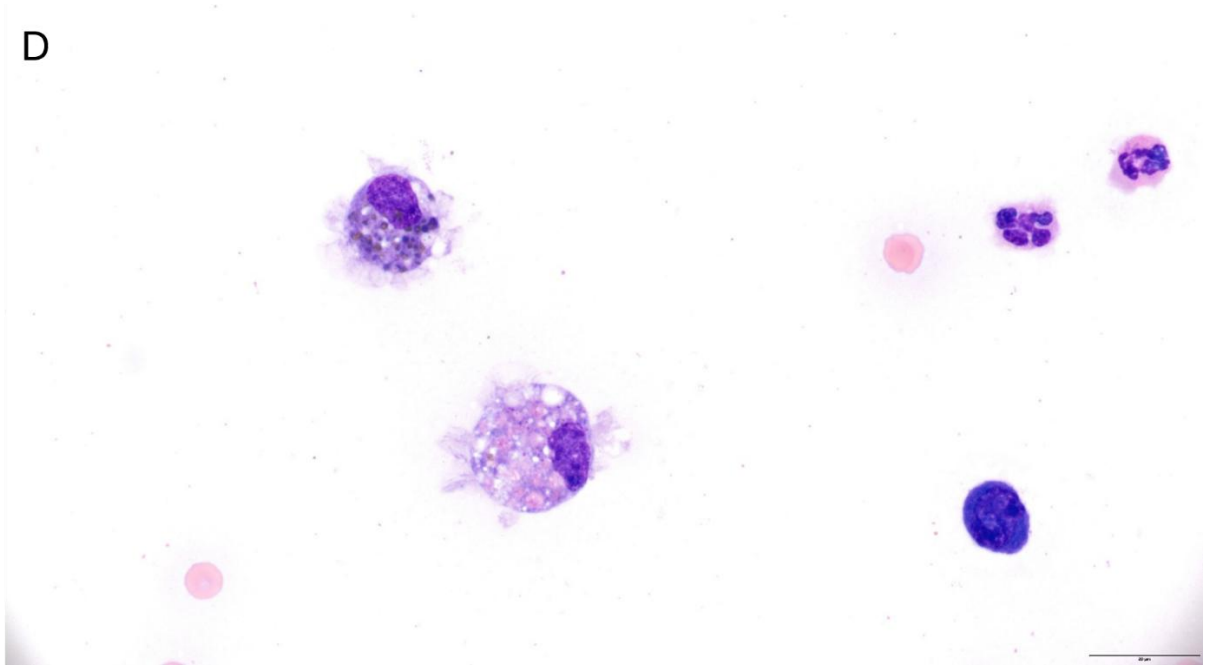


Figure 1. A-B (50x) and C-E (100x). May-Grünwald Giemsa stained direct smear of the thoracic effusion.

Questions:

- 1. What is the cytological description of the thoracic effusion, and what is your interpretation?**
 - 2. What are your main differential diagnoses for the black-greenish pigment seen in the macrophages of the thoracic effusion? What kind of further diagnostic tests would you consider performing?**
 - 3. What additional clinical findings and sampling sites should be investigated to clarify the diagnosis?**
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1. What is the cytological description of the thoracic effusion, and what is your interpretation?

May-Grünwald Giemsa stained direct smears of the thoracic effusion were available for microscopic evaluation. The stained smears were of moderate cellularity and moderate quality. On a slightly eosinophilic background some erythrocytes were identified. The nucleated cell population consisted of some non-degenerate/shrunken neutrophils, occasional macrophages and few small lymphocytes. Frequently the macrophages showed leukophagia, erythrophagocytosis and/or intracytoplasmic black-greenish pigment (possibly hemosiderin). Occasional medium to large sized lymphocytes, few plasma cells and rare Mott cells were noted. Additionally, few round large basophilic cells showing small to moderate amounts of deeply basophilic cytoplasm, a round to oval paracentrally to eccentrically located nucleus with coarsely clumped chromatin pattern and 1–2 distinct nucleoli were seen (Figure 1). Few mitotic figures and rare cells showing signet-ring appearance were evident. No infectious agents were identified.

The increased nucleated cell count and protein concentration classified the pleural effusion as an exudate. In addition, the presence of erythrocytes, erythrophagocytosis and macrophages containing black material were suggestive for a concurrent mild acute and previous or chronic hemorrhage. No infectious agents were identified; however further diagnostics, such as bacterial culture and sensitivity testing, were suggested, since their presence could not be totally excluded.

Since the intracytoplasmic black-greenish pigment found in the macrophages may represent hemosiderin, suggesting previous or chronic hemorrhage, Prussian blue staining was recommended for confirmation. In addition, the occasionally noted round large basophilic cells may representing reactive mesothelial cells or large lymphocytes as part of an inflammatory or reactive process. However, an underlying neoplastic process could not be excluded, and further diagnostics, such as diagnostic imaging to investigate possible detectable masses, were recommended.

2. What are your main differential diagnoses for the black-greenish pigment seen in the macrophages of the thoracic effusion? What kind of further diagnostic tests would you consider performing?

The primary differential diagnosis for the intracytoplasmic black pigment is hemosiderin accumulation secondary to chronic hemorrhage. However, other pigments such as melanin or less commonly lipofuscin should also be considered.

Additional special stains may be performed to further characterize the pigment. A Prussian Blue stain can be used to confirm the presence of hemosiderin, whereas a Fontana-Masson stain can be applied to identify melanin pigment. In this case

Prussian Blue staining was negative (Figure 2), excluding hemosiderin accumulation. In contrast, Fontana-Masson staining gave a positive reaction (Figure 3), confirming the presence of melanin pigment. This stain is not performed routinely in diagnostic service at the pathology department of the University of Zurich (Switzerland) and was therefore specifically requested to further characterize the lesion.

Additional immunocytochemical markers for melanocytic differentiation (e.g., PNL2) could potentially also be considered to help distinguish melanophages from melanocytes but were not performed in this case.

Lipofuscin should also be considered as a differential diagnosis for pigmented cytoplasmic granules. This pigment typically appears as fine yellow-brown to golden-brown intracytoplasmic granules and accumulates in long-lived cells as a result of oxidative damage and lysosomal degradation. Histochemical stains such as Sudan Black B may aid in its identification¹. These investigations were not performed in the present case.

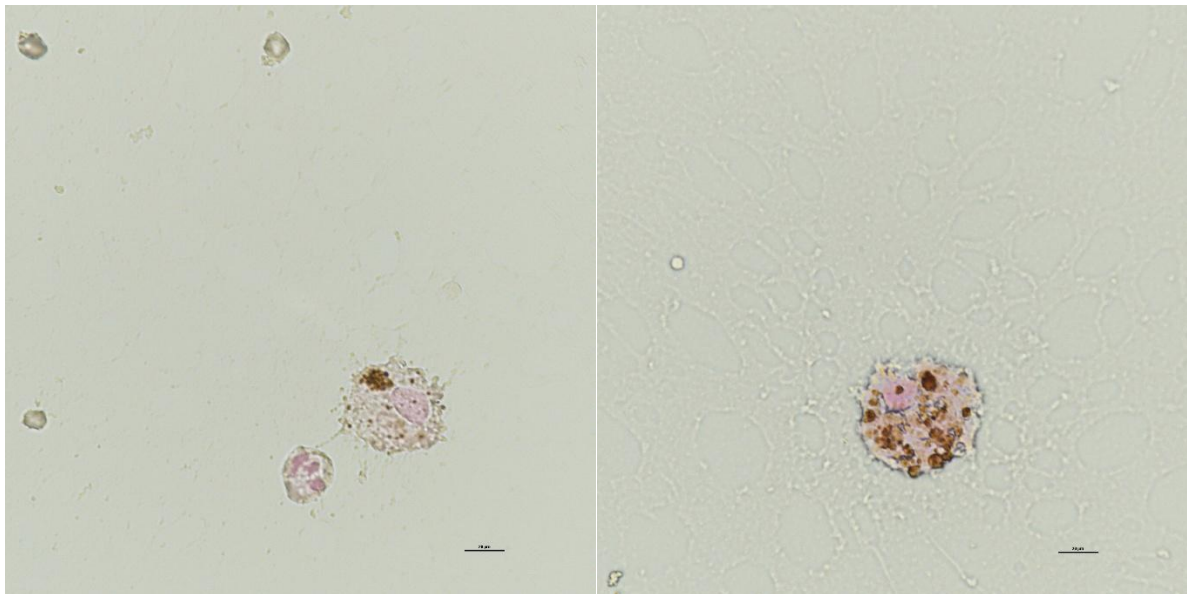


Figure 2. Pleural effusion demonstrating negative Prussian blue staining. The absence of blue staining within the intracytoplasmic brown pigment indicates that the pigment is not hemosiderin (40×).

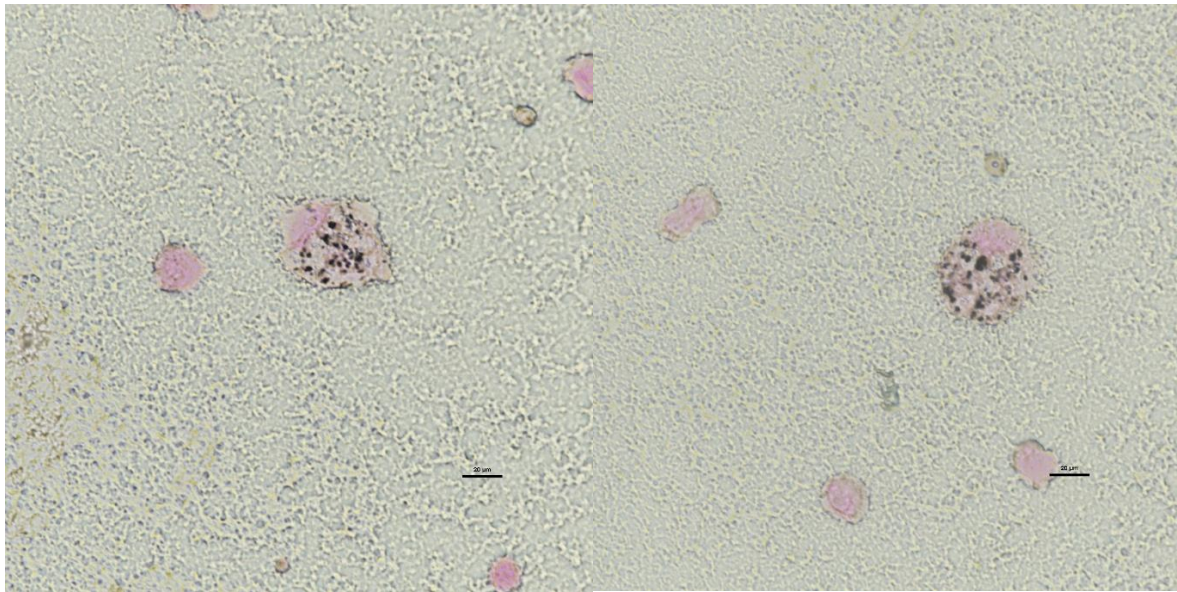


Figure 3. Pleural effusion demonstrating positive intracytoplasmic Fontana–Masson staining, with black-staining pigment within the cells consistent with melanin (40×)

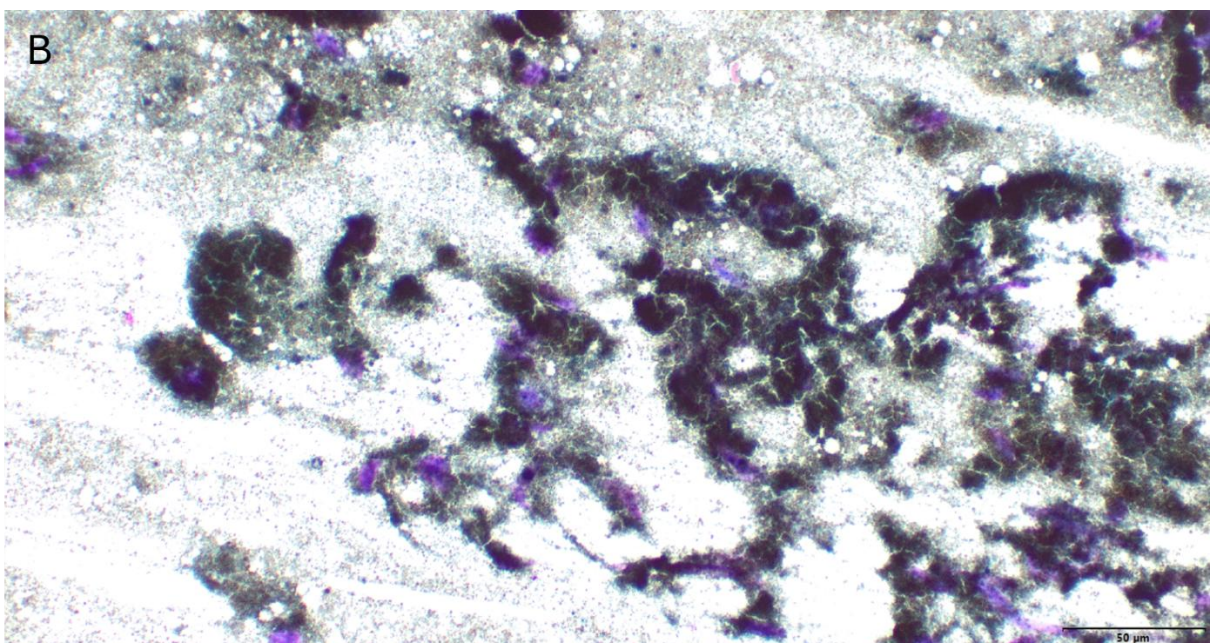
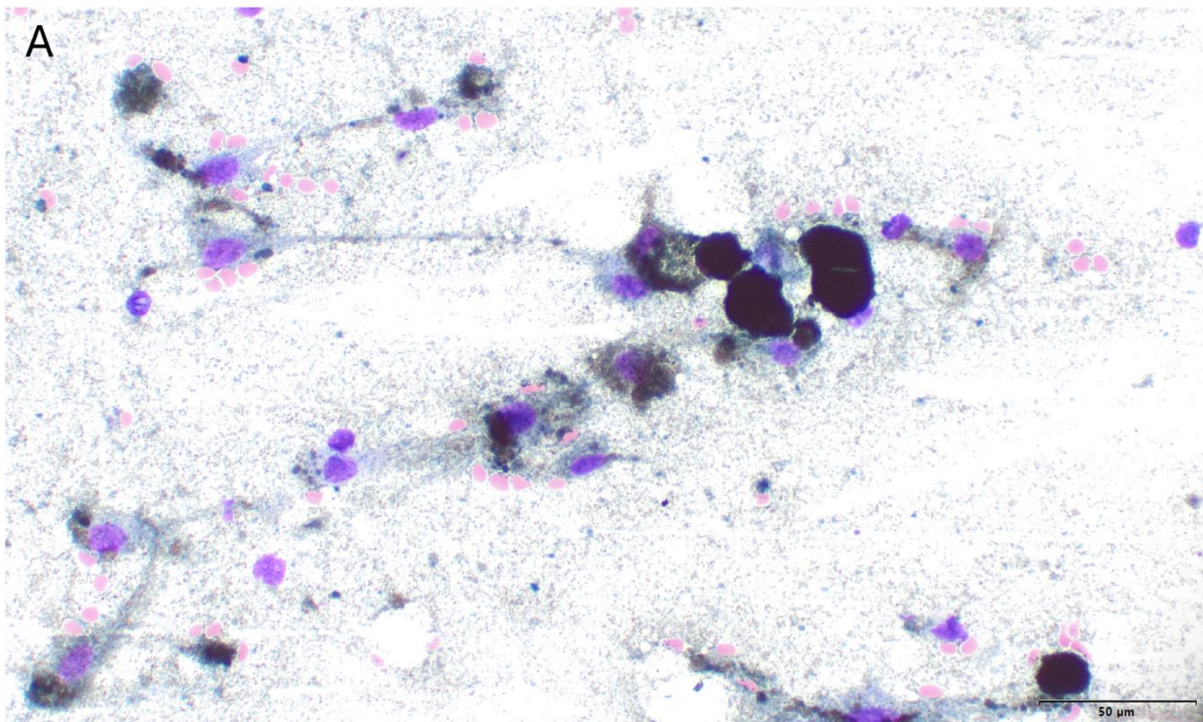
3. What additional clinical findings and sampling sites should be investigated to clarify the diagnosis?

The identification of melanin within pigmented cells in the pleural effusion raised suspicion for an underlying melanocytic tumor. The pigmented cells were morphologically more consistent with melanin-laden macrophages (melanophages) than with melanocytes. Although melanophages are not diagnostic of melanocytic tumors and may be encountered in reactive or normal lymph nodes in dogs without evidence of melanocytic neoplasia² (a finding that has not been reported yet in horses) the presence of melanin within these cells warranted further investigation to identify a potential primary melanocytic tumor and assess possible metastatic spread. Therefore, investigation within the thoracic cavity or other organs to assess correlated lesions was considered mandatory.

In the present case, clinical examination revealed multiple cutaneous masses. Fine-needle aspirates obtained from a cutaneous shoulder mass and the mandibular lymph node (not clinically enlarged) were submitted for cytological evaluation. The mass observed in the thoracic cavity and the masses present in both guttural pouches were not further investigated.

From the mass of the shoulder three May–Grünwald Giemsa stained direct smears of moderate cellularity and moderate quality were available (Figure 4). On a markedly pigmented background with occasional erythrocytes, some small aggregates of spindleoid to oval cells were noted. These cells showed moderate amounts of basophilic and dust-like black to dark brown pigmented cytoplasm, a wispy

cytoplasmic border, an oval to elongated and occasionally indented paracentrally located nucleus with fine chromatin pattern and indistinct nucleoli. These cells showed moderate anisocytosis and anisokaryosis. The pigment granules on the background and within the cells were small, dust-like oval to rod-shaped and black to dark brown in color. Occasional round large heavily pigmented cells with obscured cellular detail were also noted. No infectious agents were seen. Overall, the findings were characterized by the presence of moderately atypical melanocytes and indicated a melanocytic neoplasm. Given the moderate degree of atypia and the history of the patient, a melanoma was the primary concern. Histopathology was recommended for a definitive diagnosis.



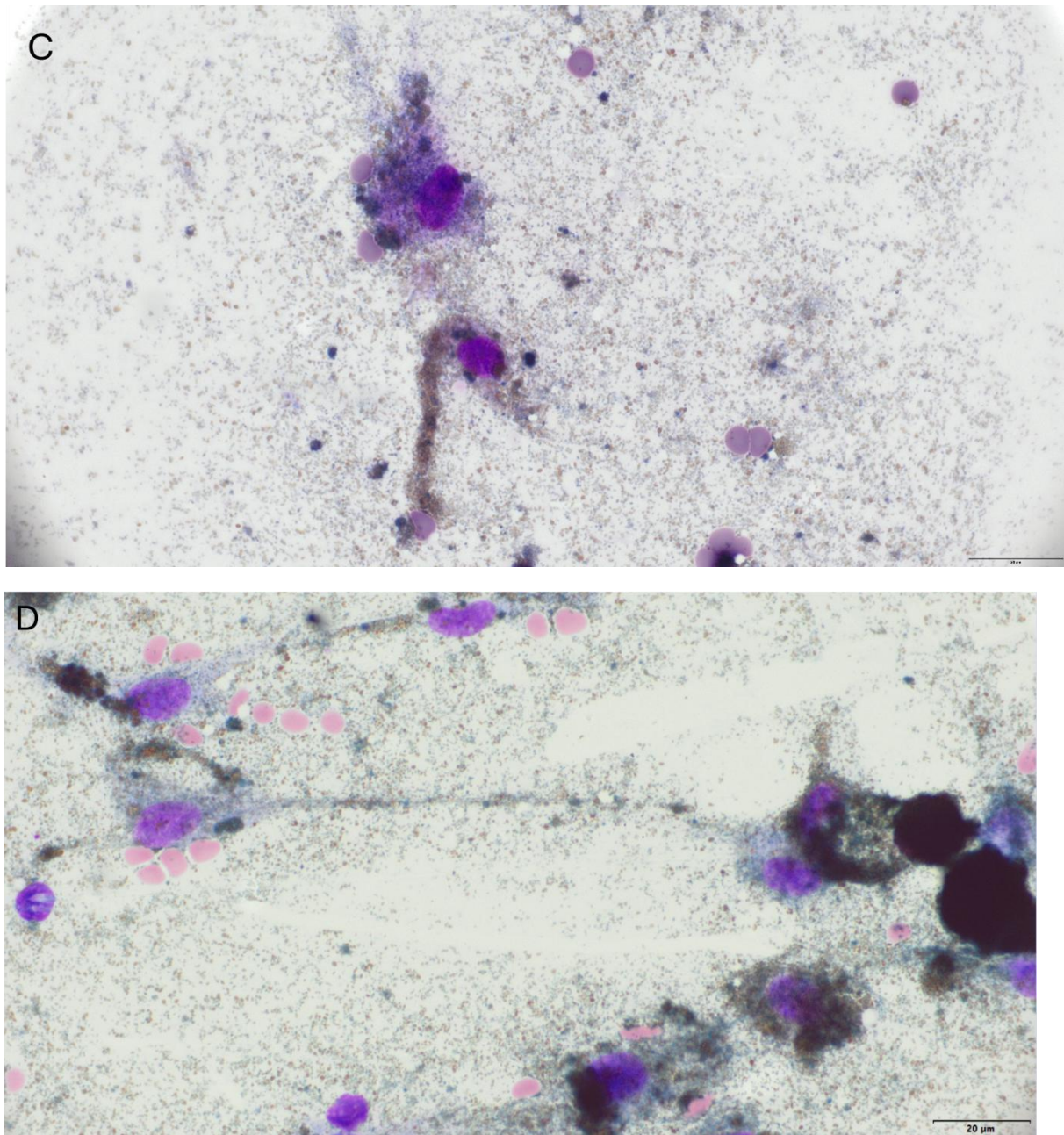
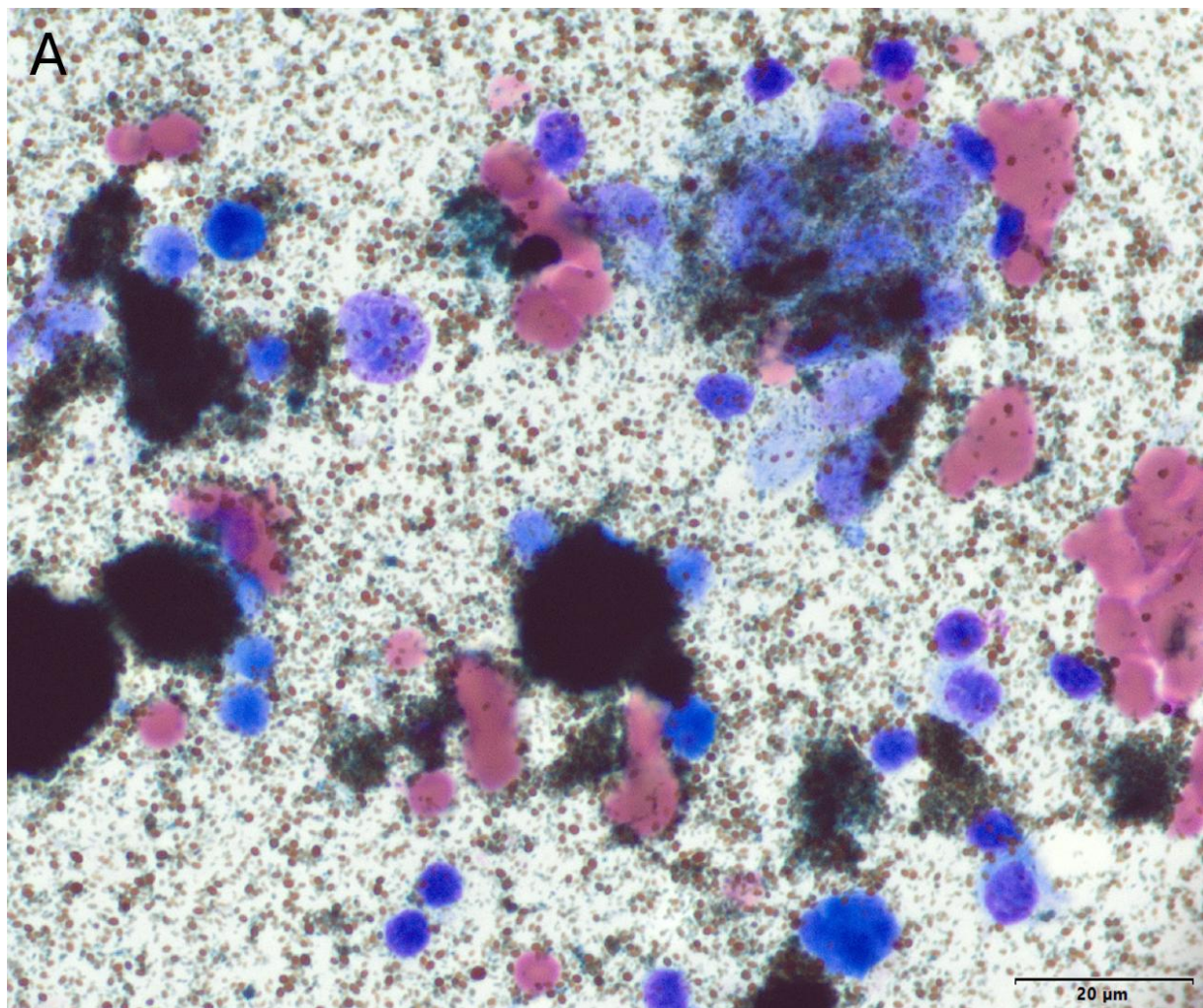


Figure 4. A-B (50x) and C-D (100x) May-Grünwald Giemsa stained direct smear of the mass on the shoulder.

From the right mandibular lymph node three May-Grünwald Giemsa stained direct smears of moderate cellularity and moderate quality were available for cytological examination (Figure 5). On a dark highly pigmented background, a heterogeneous population of lymphocytes was noted. There were some small and mature lymphocytes, few medium sized lymphocytes and rare large lymphocytes. Few plasma cells were also seen. In addition, occasional heavily pigmented cells (possible melanophages) with obscured cellular detail were noted. Few small aggregates of spindeloid to oval cells with dust-like intracytoplasmic black to brown, fine and round

to rod-shaped pigment granules, an oval to elongate paracentrally located nucleus with fine chromatin pattern and indistinct nucleoli were noted. These cells showed mild to moderate anisocytosis and anisokaryosis. Rare small clusters of well-differentiated salivary epithelial cells were seen. Overall, the findings were characterized by the presence of heavily pigmented cells and mild reactive lymphoid hyperplasia. The high amount of melanin granules obscured cellular detail of the pigmented cells. Rare pigmented spindle cells, interpreted as melanocytes, were present and were suggestive for a metastatic melanocytic tumor. However, histopathology was recommended for a definitive diagnosis. In addition, the mildly increased number of plasma cells indicated a concurrent reactive lymphoid hyperplasia.



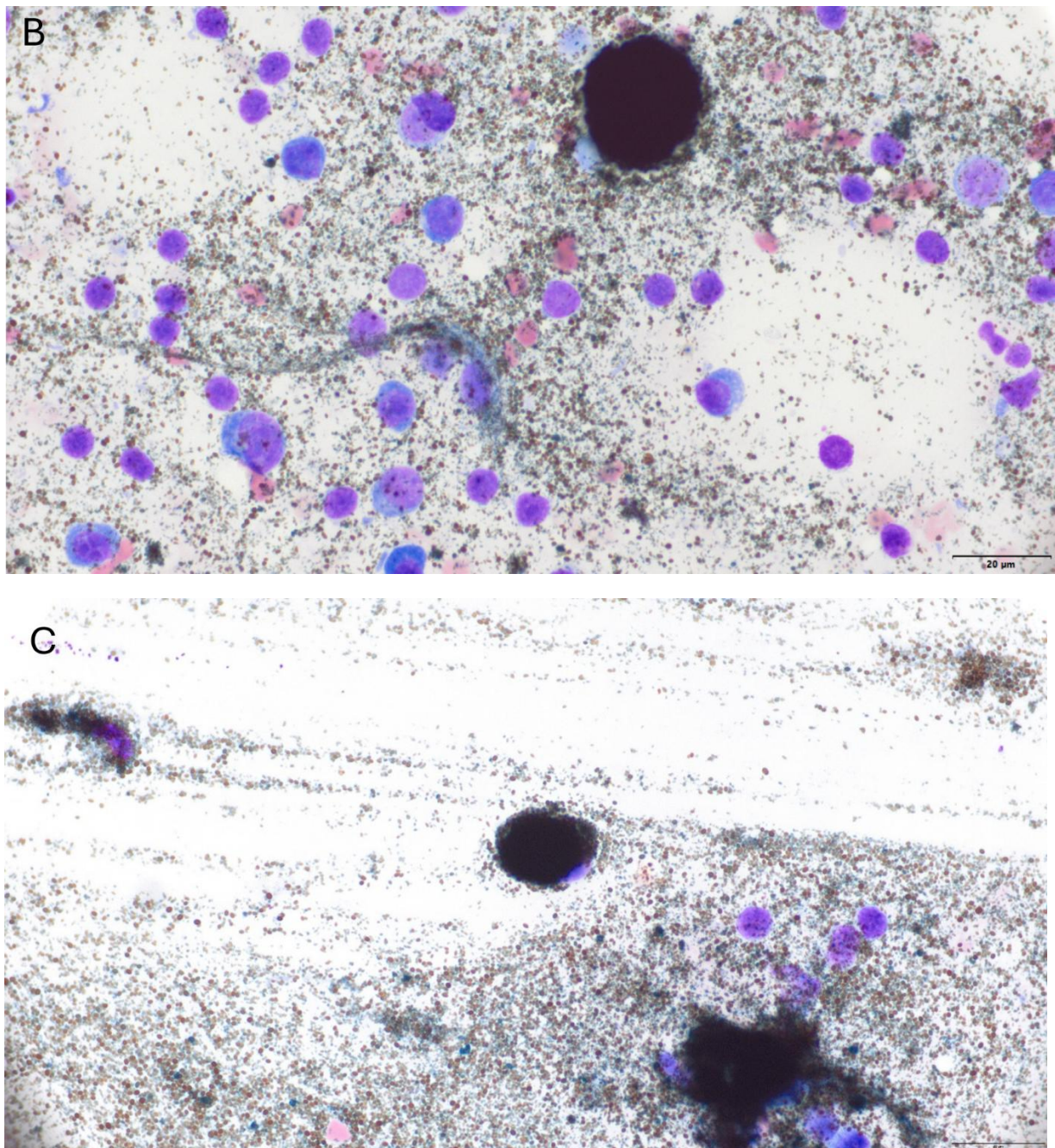


Figure 5. A, B and C May-Grünwald Giemsa stained smear of the right mandibular lymph node (100x)

Conclusion:

In conclusion, cytological examination identified the cutaneous lesion of this horse as a possible primary melanocytic neoplasm and the mandibular lymph node as well as the thoracic cavity as suspicious for metastatic involvement. Several additional masses were however also noted especially within the thoracic cavity and both guttural pouches, which were not further evaluated and potentially represent primary or metastatic lesions. Direct involvement of the thoracic cavity by melanocytes could not

however be demonstrated based on the absence of unequivocal melanocytes in the thoracic effusion.

The intracytoplasmic black-brown coarse granulation observed in macrophages within the pleural effusion was initially suggestive of hemosiderin, a common finding in body cavity effusions associated with previous or chronic hemorrhage in horses. However, negative Prussian Blue staining excluded hemosiderin, while positive Fontana–Masson staining confirmed the presence of melanin in the present case. The pigmented cells were morphologically consistent with macrophages rather than melanocytes, indicating melanophages. This case highlights that dark intracytoplasmic pigment in macrophages is not always attributable to hemosiderin and that additional diagnostic testing to determine pigment identity should be considered, even in the presence of concurrent evidence of hemorrhage (e.g. concurrent presence of erythrocytes and erythrophagocytosis).

Follow-up:

During hospitalization, the horse experienced recurrent febrile episodes accompanied by tachycardia and tachypnea and also developed localized swelling of the left side of the head and neck, clinical signs of Horner's syndrome, facial nerve paresis, and coughing. Two days later thoracic effusion was evaluated again and the nucleated cell count was 97'580/ μ L with a total protein concentration of 52 g/L. Occasional large round cells (Figure 6) similar to the ones noted in the first effusion sample (Figure 1) were found. These cells are most likely mesothelial cells/reactive lymphocytes, however a neoplastic origin could not be completely excluded.. The horse was euthanized. No necropsy was performed.

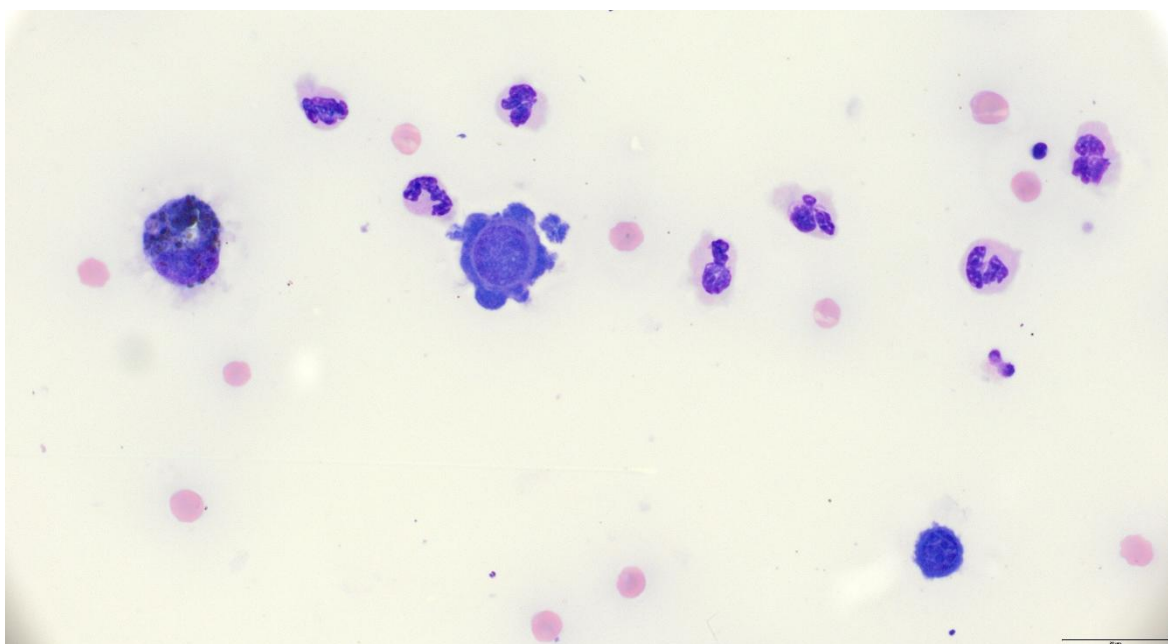


Figure 6. Follow-up pleural effusion sample collected two days later.

Discussion:

Melanocytic tumors are among the most common cutaneous neoplasms in horses, particularly in grey horses. Up to 80% of grey horses develop melanocytic lesions during their lifetime, likely due to a genetic predisposition associated with the grey coat color^{3,4}. Arabian Horses are overrepresented⁵, however the predisposition in this breed might be a consequence of the high prevalence of grey color³. The depigmentation process in this breed starts around the eyes and in the perianal region, and melanomas are most often found in these locations⁶. Equine melanocytic tumors are generally classified into four main categories: melanocytic naevi, dermal melanomas, anaplastic (malignant) melanomas, and disseminated melanomatosis. While dermal melanomas represent the most frequent form and often remain biologically indolent, progression to invasive or disseminated disease has been well documented^{3,7-10} with reports indicating that up to two thirds of cases progress to malignancy⁵. The phenomenon that these tumors however can remain quiescent for many years before progression is also described as “dormancy”¹¹. Metastasis of melanocytic tumors occurs via blood or lymphatic vessels, with cutaneous melanomas generally metastasizing first to the regional lymph nodes. Associated pleural effusion as in this case has only been rarely described^{12,13}.

The current case highlights that the interpretation of pigmented cytoplasmic material in body cavity effusion as well as in other fluids (as bronchoalveolar lavage and transtracheal wash specimens) can represent a significant diagnostic challenge in clinical pathology. The intracytoplasmic black-green pigment identified within macrophages initially raised suspicion for hemosiderin accumulation secondary to previous or chronic hemorrhage, particularly given the concurrent presence of erythrocytes and erythrophagocytosis (the latter may occur with previous hemorrhage or as an *in vitro* artefact). However, differentiation between hemosiderin and melanin may be difficult on routine May-Grünwald Giemsa or Wright-Giemsa stained preparations. Macrophages containing phagocytized melanin pigment (melanophages) exhibit coarse granular intracytoplasmic pigment. Similarly, macrophages containing hemosiderin (hemosiderophages) contain intracytoplasmic granules that range in color from black to golden brown and are often aggregated¹⁴.

In the present case, no clearly identifiable neoplastic melanocytes were observed within the thoracic effusion by cytological examination. Instead, the pigmented cells morphologically resembled macrophages containing phagocytized melanin. Although neoplastic melanocytes were not identified in the present case, their presence in pleural effusions has been documented previously in horses¹³ as well as in humans¹⁵ with melanocytic tumors. Similarly melanophages have been reported in the thoracic cavity associated with melanocytic tumors in horses¹⁶⁻¹⁸.

The cytologic findings in the mandibular lymph node aspirate and the cutaneous shoulder mass supported the diagnosis of a melanocytic tumor. In particular, the

presence of moderately atypical melanocytes in the shoulder mass was indicative of a melanocytic tumor. In the lymph node aspirate, abundant melanin granules, rare spindloid pigmented cells, and numerous melanophages were observed. Although melanophages may also be present in lymph nodes of horses, following phagocytosis of melanin draining from a melanocytic tumor and may mimic metastatic melanoma¹⁴ they should not be considered indicative of metastatic involvement in the absence of neoplastic melanocytic cells. However, in the present case the combination with rare spindloid pigmented cells and the cytologic features of the primary cutaneous lesion raised suspicion for metastatic spread to the lymph node.

Furthermore, the identification of Fontana–Masson-positive pigment within macrophages in the pleural effusion provided additional support for the interpretation that the thoracic cavity was involved in the disease process. However, definitive confirmation of both the melanocytic nature of the tumor and metastatic involvement of the lymph node and thoracic cavity would have required histopathologic examination, which was not performed in this case. As immunocytochemistry has, to the authors' knowledge, not been well described for equine melanocytic neoplasia, immunohistochemistry could have also provided additional diagnostic support, as melanocytes typically express PNL2, whereas macrophages do not¹⁴. Conversely, Iba1 could have been used to identify macrophages. However, immunohistochemical analysis was not performed in the present case. Interestingly, across the equine literature, Melan A has repeatedly shown limited utility for detecting melanocytic neoplasia in horses^{6,19}. This case further illustrates the importance of appropriate follow-up diagnostics in specimens containing pigmented macrophages. While Prussian Blue staining remains useful to confirm hemosiderin in cases of suspected chronic hemorrhage, this interpretation should be carefully correlated with clinical findings. This is of particular importance when concurrent cutaneous lesions (particularly pigmented or black nodules) are present. In such cases, fine-needle aspiration of cutaneous or other accessible masses can provide crucial information for determining the origin and significance of the pigment.

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