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The gray whale (Eschrichtius robustus Lilljeborg, 1861) peripheral blood cells: the first morphological and cytochemical study

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Short Report

Keywords: hematology, marine mammals, mysticete, gray whale, cytochemistry, blood cell morphology

Posted Date: March 26th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9166832/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Veterinary Research Communications on April 23rd, 2026. See the published version at https://doi.org/10.1007/s11259-026-11223-2.

Abstract

This study provides the first morphological and cytochemical characterization of peripheral blood cells in the gray whale (*Eschrichtius robustus*). Blood samples were collected post-mortem from 30 wild whales (23 immature, 7 mature) harvested by Native people of Chukotka (Chukchi and Inuit) during traditional subsistence hunts under Russian-established quotas. Stomach fullness, blubber thickness, entanglement scars, and parasite load were recorded. Leukocyte differential counts showed wide individual variation: band neutrophils, 0–16%; segmented neutrophils, 19–70%; lymphocytes, 17–69%; immature eosinophils, 0–8%; and mature eosinophils, 0–18%. Nucleated red blood cells (nRBCs) were observed in some individuals (up to 15 per 100 leukocytes), as were Howell–Jolly bodies in red blood cells (RBCs) (1–167 per 100 leukocytes) and macrothrombocytes (up to 32 per 100 leukocytes). Cytochemical staining revealed strong glycogen (PAS) positivity in neutrophils, eosinophils, and platelets, and moderate positivity in monocytes. Phospholipid (SBB) staining was strong in eosinophils and variable in neutrophils and monocytes. LUNA and Luxol Fast Blue (LFB) staining were specific to eosinophil granules. Given post-mortem sampling, the absence of absolute cell counts, and the lack of live-animal reference data, these findings should be interpreted as a descriptive morphological baseline rather than indicators of health or disease. This study contributes fundamental data for future comparative research on mysticete hematology.

1. Introduction

Hematological data for baleen whales (Mysticeti) remain exceptionally scarce. While morphological and cytochemical studies have been conducted on several odontocetes and sirenians (Techangamsuwan et al. 2010; Woolford et al. 2015; Stacy and Nollens 2022; Cagle et al. 2023; Mischenko et al. 2023), no descriptions exist for any mysticete species. The few available reports are restricted to stranded sick individuals or a single captive calf (Zettner 1974; Gilmartin et al. 1974; Dailey et al. 2000; Reidarson et al. 2001), and the only published data on mysticete blood cells, RBC diameter measurements from a blue whale and a humpback whale, were reported over seven decades ago from samples collected ten or more hours post-mortem (Harboe and Schrumpf 1953). Thus, fundamental baseline data on blood cell morphology, differential leukocyte counts, and cytochemical characteristics for healthy, free-ranging mysticetes are completely lacking. The gray whale (*Eschrichtius robustus*) is a benthic-feeding mysticete of the North Pacific, closely related to balaenopteridae (Sasaki et al. 2005). The eastern North Pacific population undertakes one of the longest annual migrations of any mammal and is routinely exposed to distinct environmental pressures, making it a potential indicator of ecosystem health in Arctic and subarctic waters (Dailey et al. 2000; Raverty et al. 2024). Blood samples collected from gray whales harvested during traditional subsistence hunts in Chukotka provide a unique opportunity to obtain hematological material from freshly deceased animals. This study aims to provide the first morphological and cytochemical characterization of gray whale blood cells using light microscopy and cytochemical staining (PAS, SBB, LUNA, LFB). Given the post-mortem nature of sampling and the absence of absolute quantitative parameters, we present these findings as a descriptive morphological baseline without inferring health status. We acknowledge that observed features may reflect species

characteristics, individual variation, stress responses, and post-mortem changes. This foundational dataset is intended to serve as a reference for future comparative studies on mysticete hematology.

2. Materials and Methods

Sample Collection

Peripheral blood films were obtained from 30 wild gray whales (23 immature, 7 mature; 17 females, 13 males) from the Eastern North Pacific population. Samples were collected post-mortem in Mechigmensky Bay, Chukotka (Fig. 1a), from whales legally harvested by Native people under Russian quotas (Permits #053/2024, #P027-00113-87/01953076/2025). Whales were classified as immature (<11.5 m) or mature (≥11.5 m) based on body length (Agbayani et al. 2020). A veterinarian collected blood from an open vessel immediately upon the whale's arrival on shore. The post-mortem interval for 14 whales ranged from 28 min to 4 h 23 min and was estimated to be similar for the remaining 16 (Table 1). Blood was placed in EDTA tubes, and within one hour, 210 blood films were prepared, air-dried, and fixed. No animals were harmed intentionally for this research.

Ancillary Data

Body length, blubber thickness, stomach fullness, and the presence of ectoparasites (*Cyamus ceti*, *Cryptolepas rhachianecti*), endoparasites (*Priapocephalus eschrichtii*), and scars from killer whale bites or entanglement were recorded for each animal (Fig. 1 b–f). The indication of these particular parasites is based on data obtained in previous studies (Loginova et al. 2025a; Loginova et al. 2025b).

Staining and Microscopy

Films were stained with Romanovsky-Giemsa (Esipova et al. 2025) for morphology and differential counts.

Cytochemical staining was performed using the following methods:

PAS (Periodic Acid–Schiff) for glycogen detection: slides were fixed in an alcohol-formalin mixture for 10 min, then stained using a commercial PAS kit (DIAHIM-CITOSTEIN-PAS; Russia) following the manufacturer's protocol. Slides were oxidized in 1% periodic acid (10 min), incubated with Schiff's reagent (30 min in the dark), and counterstained with Mayer's Hematoxylin (20 min). PAS-positive substances appear pink or magenta.

SBB (Sudan Black B) for phospholipid detection: Films were fixed in formalin vapor (37°C, 10 min) and stained with a commercial SBB kit (DIAHIM-CITOSTEIN-SBB; Russia). Slides were incubated in SBB solution (60 min), rinsed in 70% ethanol, and counterstained with Romanovsky-Giemsa (20 min). Phospholipids appear black or dark brown.

LUNA staining for eosinophil granule basic protein: slides were fixed in 4% buffered formaldehyde (15 min) and stained according to Luna (1968). Slides were stained with hematoxylin-Biebrich Scarlet

solution (5 min), differentiated in 1% acid alcohol, rinsed, dipped in lithium carbonate solution, and counterstained with toluidine blue (3 min). Eosinophil granules stain bright red or orange.

LFB (Luxol Fast Blue) staining for eosinophil granule lipids: slides were fixed in 4% buffered formaldehyde (15 min) and stained with a commercial LFB kit (Servicebio; China) at 56°C for 24 h. Slides were differentiated in lithium carbonate and 70% ethanol, then counterstained with toluidine blue (3 min). Eosinophil granules appear bright blue-green.

All films were examined on a Carl Zeiss Axio Scope.A1 microscope. Leukocyte differential counts were based on 200 cells per slide. Cell morphology was described, and cell diameters were measured using ZEN 2.3 software. Cytochemical staining intensity was graded as negative, weak (+), moderate (++), or strong (+++) based on Cagle et al. (2023) classification. Horse blood films were used as positive controls for all staining procedures (Fig. S1).

3. Results

General animal information. Whale body length ranged from 8.0 to 12.6 m. Blubber thickness averaged 9.0 cm ± 2.1 cm. Ectoparasites were present on twenty-nine whales (18 with confluent infestation), and tapeworms were found in seven animals. Ten whales had killer whale bite marks, and five immature whales showed entanglement scars (Table 1).

Blood cell morphology.

Mature RBCs were circular with occasional central pallor, measuring 8.2 ± 0.9 (6.1–12.7) μm in diameter (Fig. 2 aa–ac). Moderate anisocytosis was observed. Howell-Jolly bodies (1.2 ± 0.5; 0.38–2.87 μm) were frequent, ranging from 27 to 167.5 per 100 leukocytes (Fig. 2 aa, ac). Polychromatophilic RBCs (presumed reticulocytes) were larger (10.1 ± 1.0; 8.4–15.6 μm) (Fig. 2 ab). nRBCs were seen in 12 whales, with counts up to 15 per 100 leukocytes and measured 9.0 ± 1.0 (6.2–12.0) μm (Fig. 2 ba–bc). RBCs were negative for all cytochemical stains (Fig. 2 ae–ag).

Granulocytes.Neutrophils (15.6 ± 0.9; 13.2–18.0 μm) were the most common leukocyte. Band neutrophils ranged from 0–16% and segmented from 19–70% (Fig. 2 ca, cb, cc; Table 1). Many neutrophils exhibited a peripheral basophilic, agranular zone, and occasional cytoplasmic vacuoles were noted (Fig. 2, ca, cb). A Barr body was observed in females (Fig. 2 cb). Neutrophils showed strong, diffuse cytoplasmic PAS positivity (Fig. 2 cd) and variable, weak to moderate focal SBB staining (Fig. 2 ce).

Eosinophils (14.8 ± 1.1; 12.2–17.8 μm) contained large, pink granules in blue cytoplasm (Fig. 2 da–dc). Mature eosinophils (0–18%) had predominantly bilobed nuclei. Immature forms (eosinophilic metamyelocytes up to 8%, band eosinophils 1–12%) were common (Fig. 2 da; Table 1). Strong diffuse cytoplasmic PAS positivity was observed (granules unstained) (Fig. 2 dd). Strong peripheral granular SBB

positivity confirmed phospholipids (Fig. 2 de). Eosinophil granules were specifically stained with LUNA (bright red) and LFB (bright blue-green) (Fig. 2 df, dg).

Basophils were not detected in any blood film. This may indicate that basophils are either present in very low numbers in gray whale peripheral blood or were absent from the sampled material.

Agranulocytes. Lymphocytes (11.3 ± 1.8; 8.1–18.8 μm) comprised 17–69% of leukocytes (Fig. 2 ea–ec; Table 1). Small lymphocytes had a thin rim of gray-blue cytoplasm; large lymphocytes were also present (Fig. 2 ec). PAS staining was negative to weakly positive (Fig. 2 ed). Staining for SBB, LUNA, and LFB was negative (Fig. 2 ee–eg). Occasional plasma cells were observed (Fig. 2 gb, gc). Monocytes (15.9 ± 1.9; 12.3–20.9 μm) comprised 0–7% of leukocytes. Nuclei varied in shape, and cytoplasm often contained vacuoles (Fig. 2 fa–fc). PAS staining was weak to moderate (diffuse or punctate) (Fig. 2 fd), and SBB staining was weakly positive and focal (Fig. 2 fe). They were negative for LUNA and LFB (Fig. 2 ff, fg).

Platelets (2.8 ± 0.8; 1.6–4.9 μm) had light blue cytoplasm with purple granules (Fig. 2 ga). Macrothrombocytes (7.3 ± 1.7; 5.4–13.6 μm) were common, with counts up to 31.5 per 100 leukocytes. Platelets showed diffuse, finely granular PAS positivity (Fig. 2 gd) but were negative for SBB, LUNA, and LFB (Fig. 2 ge–gg).

4. Discussion

This study presents the first morphological and cytochemical description of gray whale blood cells. The findings must be interpreted with caution due to inherent limitations: samples were collected post-mortem (up to 4.5 h after death) from animals subjected to the extreme stress of a hunt, and absolute cell counts were not obtainable. Studies on post-mortem human blood indicate that neutrophils, eosinophils, and monocytes retain normal morphology for up to 6 hours after death (Dokgöz et al. 2001), suggesting that our samples likely reflect antemortem characteristics, though agonal stress cannot be ruled out. Therefore, these data represent a descriptive baseline and cannot be used to infer health or disease in live populations.

The morphological features observed, such as the presence of nRBCs, Howell-Jolly bodies, and moderate anisocytosis, have been reported in other marine mammals, including beluga whales and dugongs (Woolford et al. 2015; Mischenko et al. 2023). Interestingly, a captive juvenile gray whale also exhibited occasional Howell-Jolly bodies and anisocytosis, though nRBCs were absent (Zettner 1974). The significance of these findings in our study is uncertain, as they may reflect normal species variation, a stress-related response, or early post-mortem change. The frequent observation of a peripheral basophilic zone in neutrophils is a feature associated with toxicity in veterinary hematology (Harvey 2012), but its presence in samples collected shortly after death suggests it could also be related to agonal stress or hypoxia. The eosinophil counts were highly variable (0–18%), with immature forms present in most whales. This contrasts with the absence or rarity of eosinophils in captive or rehabilitating gray whales (Zettner 1974; Reidarson et al. 2001) and even with absolute counts (0.92 × 10^9/L) reported for a stranded juvenile (Dailey et al. 2000), and likely reflects parasite exposure in free-

ranging individuals. Almost half of the whales in our study had total eosinophil counts exceeding 10%, and tapeworms were found in seven animals. However, without quantitative parasitological data from the same individuals, a direct correlation cannot be established, similar to observations in dugongs where parasite burden does not always correlate with eosinophil levels (Woolford et al. 2015).

The cytochemical staining patterns observed are broadly consistent with those reported for other marine mammals. The strong PAS positivity in neutrophils and eosinophils aligns with findings in manatees and dugongs (Woolford et al. 2015; Cagle et al. 2023). The strong granular SBB positivity in eosinophils confirms their high phospholipid content, a feature also noted in Irrawaddy dolphins (Techangamsuwan et al. 2010). The specificity of LUNA and LFB for eosinophil granules proved valuable for confirming eosinophil lineage across all maturation stages, as has been demonstrated in manatees (Cagle et al. 2023) and cats (Holmes et al. 2021). The presence of macrothrombocytes, also observed in beluga whales and various seal species, may be a common feature of marine mammal hematology, possibly related to their low total platelet counts compared to terrestrial mammals (Mischenko et al. 2023; Esipova et al. 2025).

In conclusion, this study provides foundational data on gray whale blood cell morphology and cytochemistry. The findings underscore the unique hematological characteristics of mysticetes and highlight the need for future studies incorporating absolute counts and samples from live animals to build upon this baseline. Collaborative opportunities with subsistence hunts remain a valuable, though inherently limited, source for such fundamental biological information.

Declarations

Acknowledgements

The research was carried out in collaboration with the Russian Federal Research Institute of Fisheries and Oceanography and the Center of Oceanography and Marine Biology "Moskvarium", based at the A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences – Primorsky Aquarium shared equipment facility (Vladivostok). The authors would like to express their gratitude to Nicole I. Stacy from the Department of Large Animal Clinical Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, USA, for consultation regarding blood cell morphology and specific staining of gray whale blood films. The authors also express their profound gratitude to the members of the territorial-neighborhood community “Lorino” and its chairperson A.A. Ottoi, as well as to A.P. Gynon, Senior State Inspector of the “Beringia” National Park, for their assistance in collecting materials.

Statement of Animal Ethics

The animal study protocol was approved by the Ethics Committee of the A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences (protocol code #10 dated May 29, 2025).

Funding

This work was supported by ongoing funding from the Russian Federal Research Institute of Fisheries and Oceanography and the A.V. Zhirmunsky National Scientific Center of Marine Biology, Far Eastern Branch, Russian Academy of Sciences. The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Conflict of Interest Statement

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Author Contributions

Conceptualization: [Polina Victorovna Esipova]; Methodology: [Polina Victorovna Esipova], ...; Formal analysis and investigation: [Polina Victorovna Esipova], [Ivan Fedorovich Belokobylskiy], [Irina Vyacheslavovna Suvorova], Writing - original draft preparation: [Polina Victorovna Esipova]; Writing - review and editing: [Polina Victorovna Esipova], [Ivan Fedorovich Belokobylskiy], [Irina Vyacheslavovna Suvorova]; Material preparation: [Polina Victorovna Esipova], [Ivan Fedorovich Belokobylskiy], [Irina Vyacheslavovna Suvorova]; Data collection: [Ivan Fedorovich Belokobylskiy]; Analysis: [Polina Victorovna Esipova], [Ivan Fedorovich Belokobylskiy], [Irina Vyacheslavovna Suvorova]. All authors read and approved the final manuscript.

Data Availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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Table

Table 1 is available in the supplementary files section

Figures



Figure 1

General information on gray whales. (a) Photograph of the whale on shore after harvesting. (b, c) Killer whale bite marks on gray whale bodies. (d) Old entanglement scars on the caudal peduncle (indicated by oval puncta). (e) Ectoparasites on the skin, presumably whale lice (*Cyamus ceti*) (1) and barnacles (*Cryptolepas rhachianecti*) (2). (f) Tapeworm (*P. eschrichtii*) found in the gastrointestinal tract.

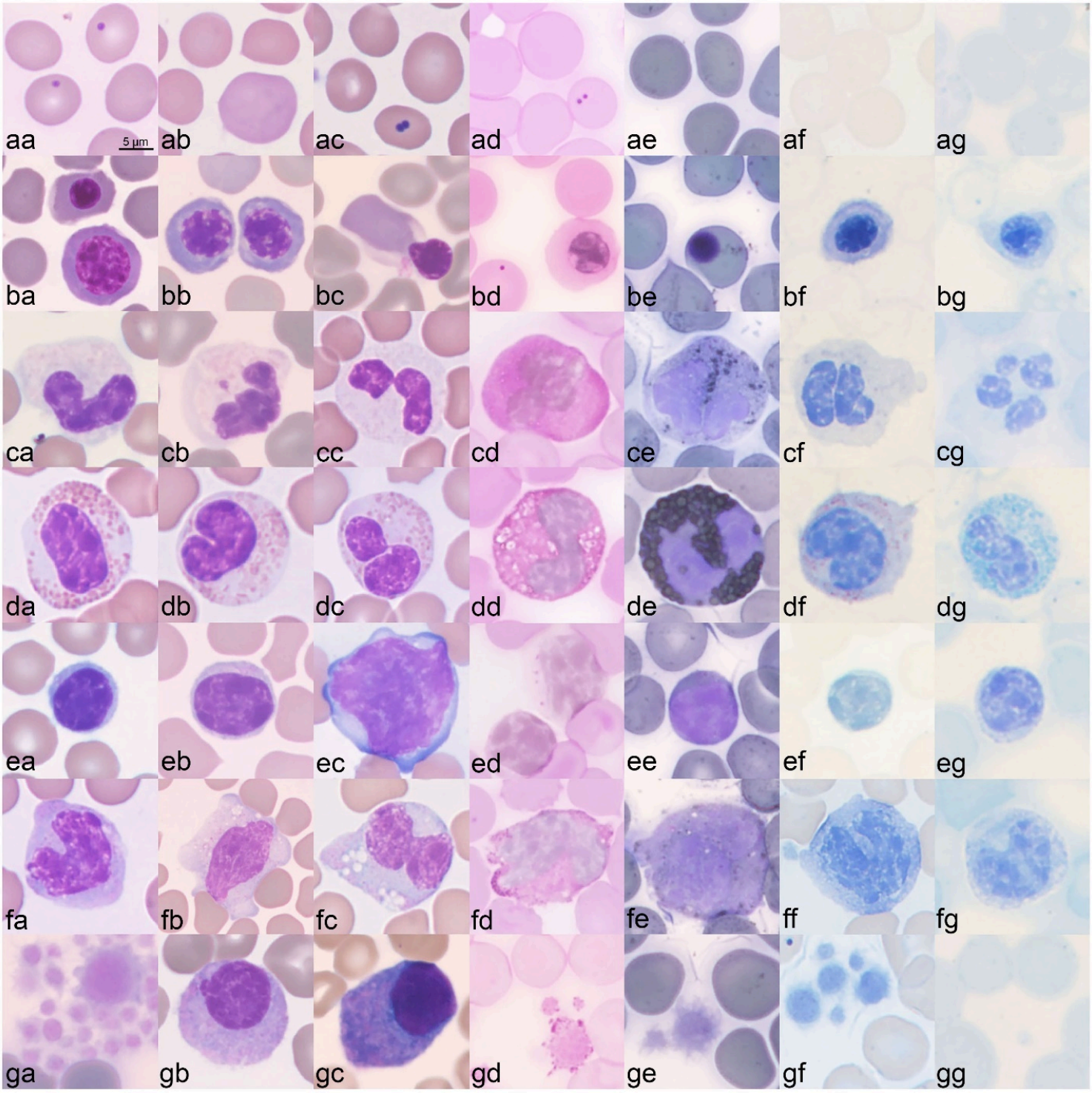


Figure 2

Morphology and cytochemistry of peripheral blood cells in gray whales. (aa) Mature RBCs with Howell–Jolly bodies. (ab) Polychromatophilic RBCs (presumably a reticulocyte). (ac) RBCs anisocytosis. (ad) Negative staining of RBCs for PAS, (ae) for SBB, (af) for LUNA, (ag) for LFB. (ba, bb) nRBCs at different maturation stages. (bc) Extrusion of the nucleus from a RBCs. (bd) Negative staining of nRBCs for PAS, (be) for SBB, (bf) for LUNA, (bg) for LFB. (ca) Band neutrophil with peripheral agranular basophilic cytoplasm. (cb) Segmented neutrophil with Barr body. (cc) Segmented neutrophil. (cd) Strong diffuse

granular cytoplasmic PAS staining in segmented neutrophils. (ce) Moderate focal SBB staining in a segmented neutrophil. (cf) Negative staining of segmented neutrophils for LUNA, and (cg) for LFB. (da) Eosinophilic metamyelocyte. (db) Band eosinophil. (dc) Mature segmented eosinophil with two nuclear lobes. (dd) Strong diffuse cytoplasmic PAS staining in eosinophils (granules unstained). (de) Strong granular peripheral SBB staining of eosinophil granules. (df) Strong granular LUNA positivity in eosinophils, and (dg) for LFB. (ea) Small lymphocyte. (eb) Small lymphocyte with granules. (ec) Large lymphocyte. (ed) Negative PAS staining in a small lymphocyte (left) and weakly positive granular PAS staining in a large lymphocyte (right). (ee) Negative staining for SBB, (ef) for LUNA, and (eg) for LFB in a small lymphocyte. (fa, fb, fc) Monocytes with and without vacuoles. (fd) Weakly positive granular peripheral PAS staining in monocytes. (fe) Weakly positive focal SBB staining in monocyte cytoplasm. (ff) Negative staining for LUNA, and (fg) for LFB in a monocyte. (ga) Platelets and a macrothrombocyte. (gb, gc) Plasma cells. (gd) Diffuse, finely granular cytoplasmic PAS staining in platelets and multifocal peripheral staining in a macrothrombocyte. (ge) Negative SBB staining in platelets and a macrothrombocyte. (gf) Negative staining for LUNA and (gg) for LFB in platelets.

Polychromatophilic RBCs (immature forms, presumably reticulocytes) were also present. These cells were generally larger than mature RBCs, measuring 10.1 ± 1.0 µm (8.4–15.6 µm) (Fig. 2 ab). nRBCs (metarubricytes) were observed in numbers ranging from 0 to 5 per 100 leukocytes (Fig. 2 ba–bc), with one immature female (#10) showing 15 nRBCs per 100 leukocytes. nRBCs measured 9.0 ± 1.0 µm (6.2–12.0 µm) and were often slightly larger than mature RBCs. Both oxyphilic metarubricytes (condensed chromatin, pink cytoplasm) and younger polychromatophilic rubricytes (condensed chromatin, central nucleus, wide gray-blue cytoplasm) were identified. Nuclear extrusion was also observed (Fig. 2bc). Both mature and immature RBCs stained negatively for PAS, SBB, LUNA, and LFB (Fig. 2 ae–ag).

Supplementary Files

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- SupplementaryFigS1Horsebloodfilmswereusedasapositivecontrol.docx
- Table1.docx