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MELANOCYTIC NEOPLASMS IN A BLACK RHINOCEROS (*DICEROS BICORNIS*) AND AN INDIAN RHINOCEROS (*RHINOCEROS UNICORNIS*)

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Abstract: Melanocytic neoplasms were diagnosed in a captive black rhinoceros (*Diceros bicornis*) and a captive Indian rhinoceros (*Rhinoceros unicornis*) from different facilities. The first case was a 10-yr-old, captive-born male black rhinoceros that presented with a small firm cutaneous mass on the dorsal midline. Aspirate cytology results were suggestive of a melanocytic neoplasm, and histologic examination of the excised mass confirmed a well-differentiated neoplasm with much pigment production, minimal anaplasia, and no mitotic figures. Several months after mass removal, a similar mass with identical histologic features was excised from the right medial thigh. The second case was a 28-yr-old, wild-born female Indian rhinoceros that presented with a draining wound at the coronary band of a rear digit. Histologic examination of a biopsy from this lesion revealed a melanocytic neoplasm with moderate cellular anaplasia, frequent mitoses, and scant pigment production. At necropsy, the tumor was found to ablate P3 and most deep tissues of the toe. No evidence of vascular invasion or metastasis was found. These two cases represent the only melanocytic neoplasms in Rhinoceridae reported in detail in the literature.

Key words: rhinoceros, *Diceros bicornis*, black rhinoceros, *Rhinoceros unicornis*, Indian rhinoceros, melanocytic neoplasm.

INTRODUCTION

The Rhinoceridae family is composed of five species in four genera that range across two continents. Black rhinoceroses (*Diceros bicornis*) and white rhinoceroses (*Ceratotherium simum*) inhabit areas of southern and eastern Africa, whereas the Asian species are found in locations in accordance with their common names: Indian rhinoceroses (*Rhinoceros unicornis*) in India, Javan rhinoceroses (*Rhinoceros sondaicus*) in Java and Vietnam, and Sumatran rhinoceroses (*Dicermocerus sumatrensis*) in Sumatra, Borneo, and Malaysia. Five to 10% each of the worldwide population of white, black, and Indian rhinoceroses are held in captivity, whereas only five Sumatran and no Javan rhinoceroses are present in captivity.[13] All, except the southern subspecies of white rhinoceros, are listed as endangered or critically endangered by the International Union for Conservation of Nature and Natural Resources Red List of endangered species. The main threats to most of these species in the wild are poaching and habitat destruction.[9]

Although uncommon in Rhinoceridae, neoplasms have been reported and include uterine leiomyomas and a horn base squamous cell carcinoma in Indian rhinoceroses; pharyngeal squamous cell carcinoma, thyroid carcinoma, hepatocellular carcinoma, and lymphoblastic leukemia in black rhinoceroses; and uterine and cutaneous squamous cell carcinoma and seminoma in white rhinoceroses.[6,13,14,16,17] The interdigital melanocytic neoplasm described in the Indian rhinoceros of this report was likely the same case included in a textbook listing of tumors found in Rhinoceridae, but case details were not reported.[13] This report describes the clinical and pathologic features of two different cases of melanocytic neoplasm in a black and an Indian rhinoceros.

CASE REPORTS

Case 1

A 10-yr-old male, 1,100-kg, captive-bred southern black rhinoceros (*Diceros bicornis minor*) presented in August 2006 with a several-day history of a raised, firm, 7.5 × 5 mm, darkly discolored cutaneous mass on the dorsal midline, just caudal to the withers (Fig. 1). This animal was housed singly in a complex supporting five

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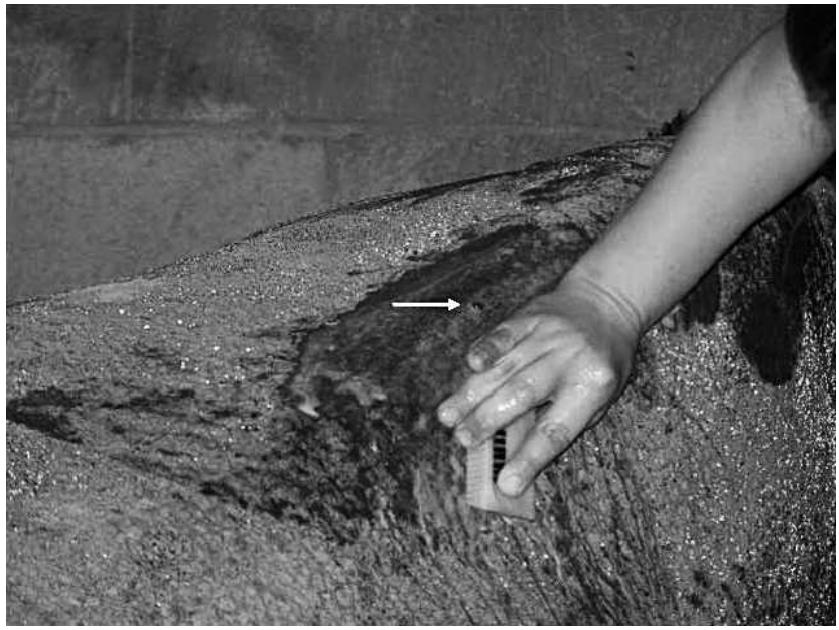

Figure 1. Black rhinoceros, case 1, first melanocytic neoplasm on dorsum (arrow) at time of surgical excision.

black rhinoceroses, each with an approximately 1 acre outdoor dirt yard with minimal natural shade, a shaded concrete patio area, and an indoor stall to which there was access at all times except during daily cleaning. The facility was located in central Texas, and weather was such that outdoor access was provided and used for the majority of the year.

Shortly after initial presentation, a small amount of clear, thin, gray fluid was obtained via aspirate of the mass with a 22-ga needle on a 3-ml syringe. Cytologic evaluation revealed a specimen comprised predominantly of epithelioid cells filled with dark pigment interpreted as melanin, indicating a probable melanocytic neoplasm (Fig. 2, inset).

Approximately 1 mo after initial presentation, the rhinoceros was immobilized for mass resection with a combination of etorphine hydrochloride and azaperone (M99, 1 mg/ml, ZooPharm, Fort Collins, Colorado 80522, USA; 1 μg/kg i.m.; Stresnil®, Schering-Plough, Kenilworth, New Jersey 07033, USA; 0.11 mg/kg i.m.) via hand injection with an 18-ga, 38-mm long needle in the midcervical region. Right lateral recumbency was achieved in 17 min, after which the animal was rolled more sternally. Heart rate, respiratory rate, temperature, pulse oximetry, and I-STAT (Heska Corporation, Fort Collins, Colorado 80525, USA) venous blood gas values were monitored throughout the procedure. Oxygenation and ventilation were excellent despite a slower respiratory rate (2–4 breaths/min). Values for complete blood cell count and serum chemistry revealed a mild leukopenia characterized by a lymphopenia (white blood cell count 4,800 cells/μl, reference range 8,338 ± 2,102; 1,104 lymphocytes/μl, reference range 3,228 ± 1,068), which was replicated in multiple previous samples from this animal and presumed to be normal individual variation. Reference values were established by the International Species Information System (ISIS Physiological Data Reference Values, 2001 ed., Apple Valley, Minnesota 55124, USA).

The surgical area was prepared with chlorhexidine scrub and alcohol. A 5-cm circular area with the mass at its center was removed via sharp dissection. The thin epidermis incised easily, whereas the much tougher dermis required more pressure. An 8-mm-deep partial-thickness concave defect in the dermis was excavated for removal of the mass and was allowed to heal by second intention, as primary closure of this area was not possible. Small bleeding vessels were cauterized (Change-A-Tip, Bovie Aaron Medical Corp., St. Petersburg, Florida 33710, USA) and the wound was packed with an equine pyrethrin ointment (SWAT, Farnum, Phoenix, Arizona 85013, USA) to deter insects. This was reapplied as necessary throughout the healing process, which took approximately 1.5 mo for complete re-epithelialization. Anesthesia was reversed with

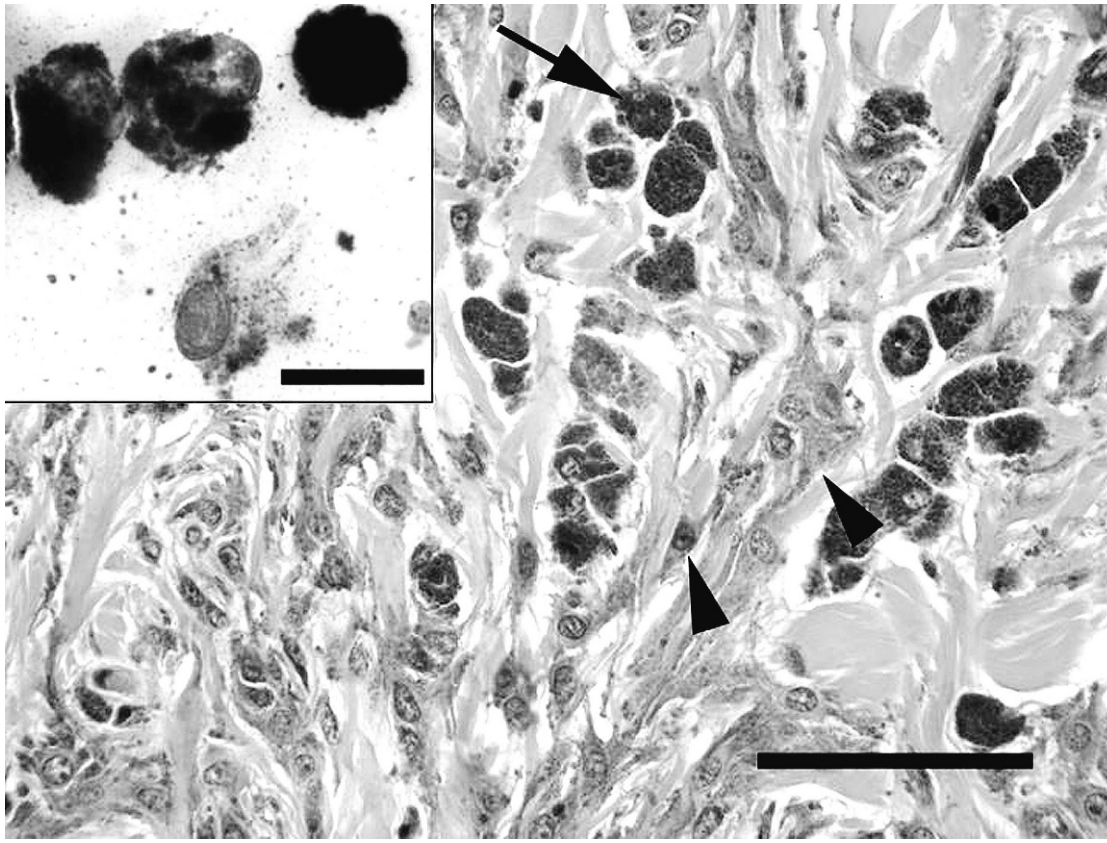

Figure 2. Black rhinoceros, case 1, melanocytic neoplasm. Note well-differentiated epithelioid (arrow) and spindle or stellate cell components (arrowheads), with minimal anaplasia and much cytoplasmic melanin. HE, bar = 40 μm. Inset: Cytologic appearance of melanocytic neoplasm, clustered epithelioid cells with intracytoplasmic melanin, and a single spindle cell with scant melanin. Modified Wright-Giemsa, bar = 130 μm.

naltrexone hydrochloride (ZooPharm; 50 μg/kg i.m., 50 μg/kg i.v.), and the rhinoceros stood within 1 min of administration. The animal was treated empirically with flunixin meglumine (Schering-Plough Animal Health, Kenilworth, New Jersey 07033, USA: 1.25 mg/kg i.v. once), trimethoprim/sulfamethoxazole (Interpharm, Inc., Hauppauge, New York 11788, USA; 15 mg/kg p.o. b.i.d. × 7 days), and tetanus toxoid (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa 50501, USA; 1 ml i.m.).

On gross examination, the mass was raised, circumscribed, and dark brown to black on cut surface. Histologically, the mass was comprised of interlacing streams of neoplastic spindle cells admixed with clusters of epithelioid cells, the majority of which contained large amounts of cytoplasmic melanin. The cells had mild anisokaryosis, single small nucleoli, and no mitotic figures (Fig. 2). The neoplasm was minimally invasive, did not extend to examined margins and no vascular invasion was noted. These results were considered consistent with a well-differentiated melanocytic neoplasm, based on melanin production by the tumor and minimal anaplasia.

Five months later, an additional approximately 1 cm^3 mass of similar appearance was noted on the skin of the right medial thigh. Aspirates obtained were cytologically similar to the initial mass. Five weeks after initial presentation of the mass, it had not changed or increased in size. The rhinoceros was anesthetized again with the use of the previously described protocol, and the mass was removed via a 3-cm-diameter ovoid full-thickness skin incision. The skin of the medial thigh was much more easily incised than the skin over the dorsum. A two-layer primary closure was achieved with a subcuticular layer of 3.0 vicryl and skin sutures of 0 PDS (polyglactin 910 and polydioxanone; Ethicon, Piscataway, New Jersey 08855, USA). Reversal of anesthesia was smooth and uneventful. Ten days later, the

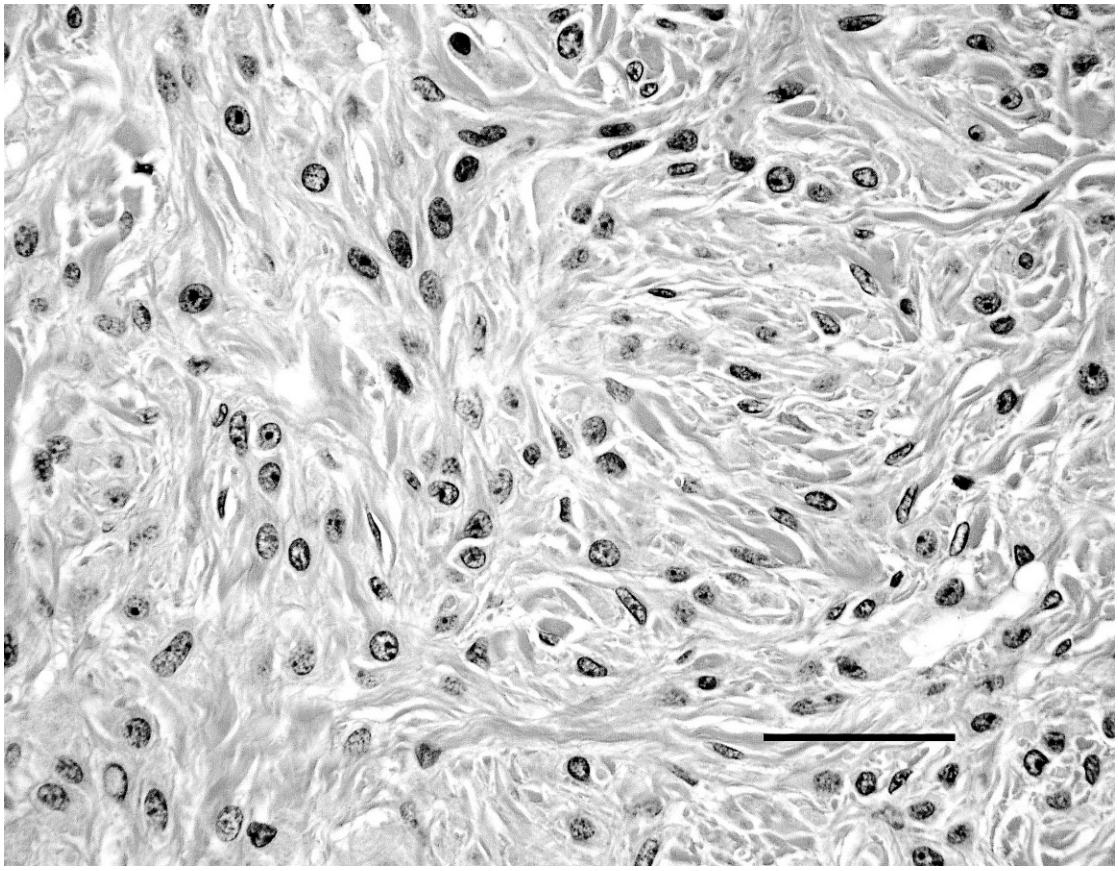

Figure 3. Black rhinoceros, case 1, melanocytic neoplasm. In this bleached section, note bland appearance of spindle and stellate cell components, with minimal anaplasia, relatively small nucleoli, and absence of mitotic figures. HE, bleached, bar = 170 μm.

ventral two-thirds of the incision had dehisced and continued to exude mild serous discharge. Sutures were removed at this time, and a 1-cm defect in the skin was present. This defect healed completely and without further complications within 3 wk of the surgery.

On gross examination of the second lesion, the dermis contained a 1-cm^3 raised circumscribed mass that on the cut surface was dark brown to black. Histologic evaluation found it to be comprised of loosely aggregated clusters of epithelioid cells packed with melanin, distributed throughout the superficial dermis and associated with mild focal junctional activity in the overlying epidermis. Cells were widely separated by thick bundles of collagen and few streams of scantly pigmented stellate or spindle cells. A bleached histologic section revealed that the neoplastic cells had mild anisokaryosis and small nucleoli, and no mitotic figures were observed (Fig. 3). The lesion did not extend to the examined margins and no vascular invasion was noted. The tumor was characterized as benign or a low-grade malignant melanocytic neoplasm.

Two additional approximately 5-mm^3 nodules were discovered in close proximity to one another on the skin of the right medial thigh approximately 1 yr after the first mass was removed. Cytologic evaluation of aspirates was again suggestive of melanocytic neoplasm; however, it was decided to leave these two nodules in situ because of the low-grade histologic features of the previously diagnosed melanocytic neoplasms. One lesion was notably smaller after aspiration, and within several months had disappeared completely. No gross change has been noted in the second mass in the past 3 yr and no new masses have been observed.

Case 2

A 28-yr-old female, approximately 1,620-kg Indian rhinoceros (*Rhinoceros unicornis*) presented in March 1998 with a swollen draining

fistulous wound at the median coronary band of the third digit of the left rear foot, which corresponded to a deep crack running centrally up the nail from the ground surface. This animal was housed singly in a complex supporting three Indian rhinoceroses, each with daytime access to an approximately 1/3-acre dirt and grass yard with a concrete pool, and an open-air roofed stall with a concrete floor at night. The wound intermittently drained serosanguinous, slightly mucoid material, and the nail crack grew out and reformed multiple times between initial appearance and euthanasia 5 yr later. The animal was intermittently lame on this limb and was treated symptomatically as needed with trimethoprim/sulfamethoxazole (Lannett Company, Inc., Philadelphia, Pennsylvania, 19136; 20 mg/kg p.o. b.i.d. × 5–30 days) and phenylbutazone (Phoenix Pharmaceuticals, Inc., St. Joseph, Missouri, 64507; 1.2–1.9 mg/kg p.o. b.i.d.).

A culture taken from the defect 3 yr after presentation grew *Proteus mirabilis*, beta *Streptococcus* group G, and methicillin-resistant *Staphylococcus aureus*. At this time, exuberant tissue began to bulge out of the fistulous defect. Radiographs showed mostly soft tissue damage and swelling, with mild focal fragmentation of the dorsal surface of the third phalange (P3) of the affected toe in the region of the cracked nail. No evidence of osteomyelitis was observed radiographically.

The rhinoceros was anesthetized 4 yr after presentation for further evaluation of the foot. Carfentanil citrate (ZooPharm; 0.66 μg/kg i.m.) and detomidine hydrochloride (Dormosedan, Pfizer Animal Health, Exton, Pennsylvania 19341, USA; 3.7 μg/kg i.m.) were hand injected with the use of an 18-g, 38-mm needle. Depth of anesthesia was adequate for physical examination, but movement of the rear limb was noted when the mass was manipulated. Supplemental ketamine (Ketaset, Fort Dodge Animal Health; 100–250 mg i.v.) was administered several times in attempts to achieve a surgical plane of anesthesia, but anesthetic depth did not change with multiple supplementations. Reversal with naltrexone (0.15 mg/kg s.c.) was quick and smooth, with standing at 5 min after injection. During this procedure, the toe crack and the associated proliferative tissue were debrided. The tissue was very vascular and presumed to be granulation in origin. Deep swabs taken for culture and sensitivity yielded a heavy growth of *Enterococcus* sp. and no anaerobic growth. Debrided material was submitted for biopsy. The defect was packed with compounded dimethyl sulfoxide-metronidazole paste (Wickliffe Veterinary Pharmacy, Lexington, Kentucky 40508, USA) and the foot was bandaged.

Histologic examination of the biopsy specimen revealed an ulcerated and poorly differentiated melanocytic neoplasm. The tumor was comprised of interlacing streams of neoplastic epithelioid cells, some of which contained scant cytoplasmic melanin. The cells had moderate amounts of eosinophilic cytoplasm, moderate to marked anisokaryosis, single large nucleoli, and up to four mitotic figures per high-power field. It was considered a poorly differentiated malignant melanocytic neoplasm due to its loss of differentiation based on scant to no pigment production, considerable cellular anaplasia, and high mitotic index. Local tissue invasion was expected, although no vascular invasion was noted in the examined sections.

Within 7 mo, the proliferative tissue had increased in size to 15–20-cm diameter with irregular margins. The tissue was soft but solid with a few areas of black pigmentation, the rest being gray or pink and irregularly fibrous with an extensive blood supply. Radiographs showed a smooth rounded hollow of missing bone from the central distal edge of P3, interpreted as likely atrophy from pressure or poor blood supply, rather than direct invasion by a neoplasm. Nine months after the previous procedure, another debulking under anesthesia was performed to slow growth of the mass with CO_2 laser ablation (25-watt diode laser, CeramOptic, Inc., East Longmeadow, Massachusetts 01028, USA), followed by topical application of liquid nitrogen.

The specimen submitted from the second debulking was comprised of interlacing streams and sheets of haphazardly arranged, neoplastic spindle to polygonal cells. The cells had moderate amounts of eosinophilic cytoplasm with occasional cytoplasmic melanin granules. The cells had moderate to marked anisokaryosis with large vesicular nuclei containing one or two large nucleoli and up to eight mitotic figures per high power field. The tumor interfaced with the overlying epidermis, but junctional activity was not distinct. No vascular invasion was noted in the examined sections. The histologic diagnosis was high-grade melanocytic neoplasm. Because the tumor had considerable cellular anaplasia and a high mitotic index at this stage of development, it was expected to have aggressive biological behavior with considerable potential for local tissue invasion and metastasis.

Figure 4. Indian rhinoceros, case 2, melanocytic neoplasm of the third digit of the left rear foot at time of euthanasia.

In the year following the last debulking, the rhinoceros was intermittently lame on the affected rear limb, although the mass did not attain the previous size. Lameness became more frequent and the rhinoceros was euthanized 5 yr after initial presentation with sodium pentobarbital and sodium phentoin (Euthasol, Virbac AH, Inc, Fort Worth, Texas 76161, USA; 155 ml i.v.) after being anesthetized with 1.2-mg carfentanil and 10 mg detomidine i.m. by hand injection. At necropsy, a 20-cm-diameter soft, abraded, and raw gray mass protruded through the hoof wall of the third digit of the left hind foot (Fig. 4). The mass extended from beneath the hoof wall proximally toward the coronary region externally. There was a large hollowed defect in the hoof wall on the plantar aspect where it had failed to grow from beneath the mass. On section through the center of the digit, the mass was soft and irregularly black and ablated the bone of P3 and most deep tissues of the toe. Additionally, it bulged into but did not cross the distal interphalangeal joint. Other bones and tissues proximal to the mass were grossly normal. No gross or histologic evidence of metastases was found. Histologically the tumor had features as described in the most recent antemortem biopsies (Fig. 5), was clearly infiltrating and obliterating digital bone, and also had one focus of cartilaginous metaplasia. Additional findings of note at necropsy included uterine leiomyomas and an endometrial adenocarcinoma.

DISCUSSION

To the authors' knowledge, these are the first detailed reports of melanocytic neoplasms in Rhinoceridae and are disparate in clinical presentation and histologic morphology. This is also the first case of endometrial adenocarcinoma reported in Rhinoceridae.

Melanocytes are dendritic cells of neuroectodermal origin that are found primarily in the epidermis and dermis.[22] They produce pigment that can act as redox buffers, cation binders, and radiation sinks, and are considered genoprotective.[18] Neoplastic transformation of melanocytes or melanoblasts has been linked to mutation of tumor suppressor genes (e.g., p53, retinoblastoma protein), mutation of proto-oncogenes, altered expression of epithelial cadherins, and upregulation of angiogenic factors, but overall it is poorly understood.[24]

Although sun exposure is considered a high risk for melanocytic neoplasm formation in humans, similar initiation of oncogenesis by UVA and UVB radiation has only been documented in Angora goats, the South American opossum, *Monodelphis domestica*, and the fish, *Xiphophorus*.[11,21,26] Initiators are less well known for other animal species, but a genetically linked increased frequency of spontaneously mutated cells has been hypothesized previously.[2,7] Of the cases documented here, only the initial nodule located on the dorsal midline in the black rhinoceros was in an area of high sun exposure.

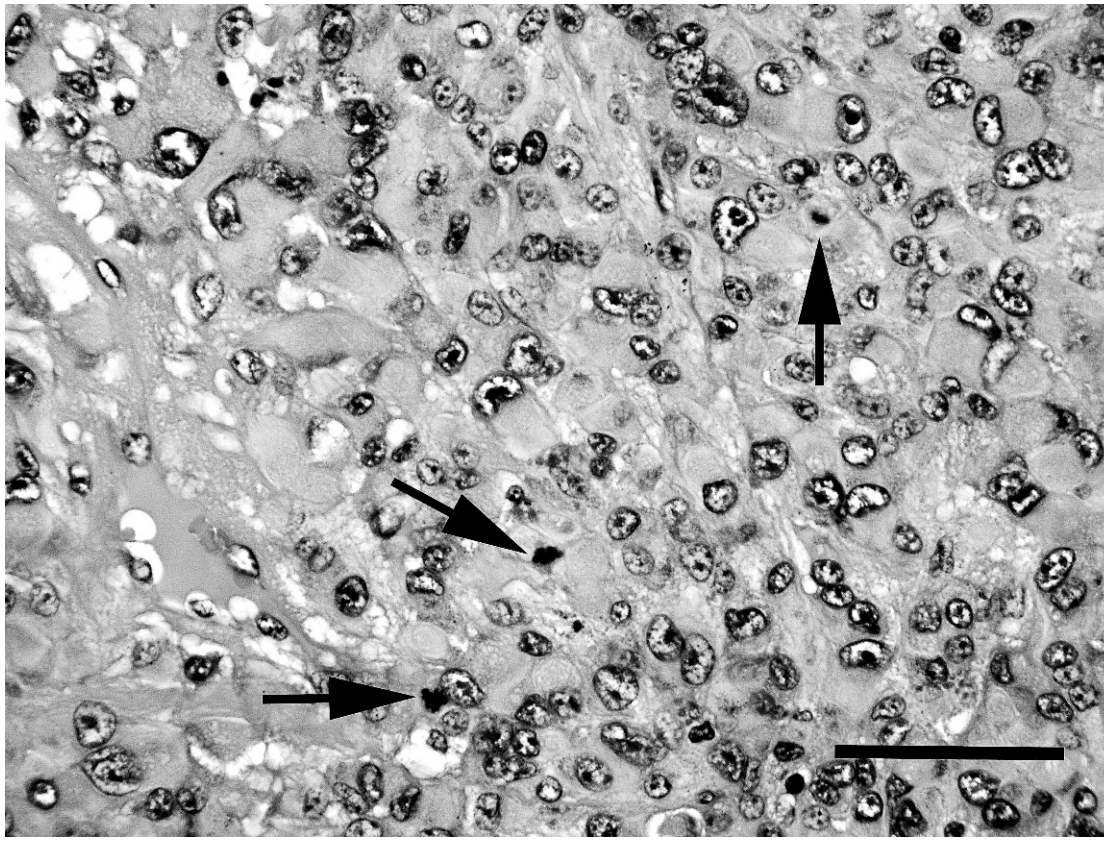

Figure 5. Indian rhinoceros, case 2, melanocytic neoplasm. Note high degree of cellular anaplasia, lack of intracellular melanin, and high mitotic index (arrows). HE, bar = 130 μm.

It also seems unlikely that a minimally haired animal native to open grasslands would be prone to UV-induced tumor formation. Only rhinoceros 1 successfully reproduced, and no melanocytic neoplasms have been documented in the 4-yr-old offspring thus far; however, a possible genetic linkage is an important consideration in such closely managed breeding populations.

Cutaneous melanocytic tumors have been documented in all domestic animal species, but are most significant in the dog and the horse, accounting for up to 10% of all skin tumors in dogs and up to 15% in horses.[5,10] Aged gray horses are particularly prone to melanocytic tumors, with 80% of gray horses developing melanocytic neoplasms by 15 yr of age.[25] The gray horse melanocytic neoplasm is hypothesized to be associated with a disturbance in melanin metabolism associated with graying that stimulates formation of new melanoblasts and melanoblast mitosis, predisposing the cells to neoplastic transformation.[4] Initially, 90% of melanocytic neoplasms in gray horses are diagnosed as benign, but ultimately 67% undergo malignant transformation.[22]

In humans, the pigment pheomelanin found in yellow and red hair can become carcinogenic after exposure to UV radiation, whereas the eumelanin found in black hair is thought to be photoprotective.[18] In cattle, melanocytic tumors account for approximately 6% of all tumors, most of which arise from the skin. These are predominantly found in cattle with red, gray, or black coats, and reports of malignancy are rare.[12,20]

Digital melanocytic neoplasms typically carry a poor prognosis in domestic animals, because of difficulty of resection in large species and propensity for aggressive growth and metastasis. In humans and in horses, a link between prior trauma to the nail or hoof and development of melanocytic neoplasm has been proposed.[8,15] In the Indian rhinoceros case, it is unknown whether the tumor was a precipitating cause of the cracked and infected nail, or if this was initially some sort of traumatic insult that might have led

to formation of the neoplasm. Foot trauma is not uncommon in rhinoceroses in captivity, however, and this is the first case of melanocytic neoplasm documented in this location.

Although in these cases the clinical presentation and histologic characteristics of the neoplasm seem to correspond with one another closely, the histopathology of melanocytic neoplasms often does not correlate well with biological behavior.[22] Predicting malignancy of such tumors in domestic species based on histopathology must rely instead upon a thorough understanding of tumor biology in the affected species, location of the neoplasm, invasiveness, frequency of mitotic figures, and degree of cellular anaplasia.[22] Even so, these histopathologic characteristics have been thought to correlate with actual clinical staging only moderately.

Recent research in dogs has continued to provide variable results. In one canine study, the 1-yr survival rate for histologically malignant melanocytic tumors was 69% for lip, 46% for haired skin, and 43% for nail bed. Of the haired skin tumors, neither clean surgical margins or individual histologic features managed to predict outcome of the tumors. Histologic features of the nail bed tumors also did not predict survival time but, as almost half of the dogs were still alive at 1 yr, surgical removal was still recommended.[19] Another study found that dogs with histologically well-differentiated melanocytic neoplasms of the mucous membranes of the lips and oral cavity had prolonged survival after only local excision of these lesions, thus again supporting surgical excision of such masses.[3] In a separate study, models utilizing mitotic index and nuclear atypia were useful in behavioral categorization.[23] If a similar model was employed to evaluate these cases, the melanocytic neoplasm of the black rhinoceros would be expected to be benign behaviorally, whereas that of the Indian rhinoceros would likely be malignant and aggressive, based on histology. However, the applicability of this model in species other than dogs is questionable, given the marked interspecific variation in melanoma pathogenesis and presentation.

Melanocytic neoplasia is thought to be one of the few tumor types in domestic species in which location of the tumor is an important prognostic indicator.[22] In dogs, melanocytic neoplasms involving the oral cavity, digit, and mucocutaneous junctions are typically considered malignant, regardless of other features.[1] Based on the cases reported here, these criteria may apply to Rhinoceridae, although evaluation of more cases in rhinoceroses would be needed for confirmation.

CONCLUSIONS

Melanocytic neoplasms have variable prevalence and pathologic features, depending on the species and location affected. The cases above emphasize these points, as the black rhinoceros had a benign form in clinical presentation, as well as in gross and histologic appearance, whereas the Indian rhinoceros had a much more aggressive form that ablated bone and soft tissue, was intractable in terms of treatment, and eventually contributed to the decision to euthanize the animal. Clinical signs of the individuals corresponded closely to the aggressiveness of the tumor. Diagnosis of melanocytic neoplasia should be considered in cases of dermal nodules or digital masses in rhinoceros species. Based on the two cases above, resection may either be curative or be palliative but with the possibility of leading to more aggressive regrowth. Further investigations into potential causes and treatments would be worthwhile in future cases.

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