DIAGNOSIS AND TREATMENT OF VITILIGO IN A SUB-ADULT EASTERN BLACK RHINOCEROS (*DICEROS BICORNIS MICHAELI*)

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Abstract: A captive-born female sub-adult Eastern black rhinoceros (*Diceros bicornis michaeli*) developed areas of non-ulcerated, non-pruritic depigmentation around the nares at 2 yr of age. Over the subsequent 18 mo, the symmetrical multifocal depigmented macules increased in size and distribution to include facial fold crypts, interdigital skin, lips, nares, palmar carpi, ventral abdomen, ventral mandible, axillae, lateral brachium and antebrachium, lateral thighs, ventral tail, and perineal region with an estimated 15% of the skin affected. Facial fold skin biopsies revealed multifocal hypopigmentation with melanin incontinence and mild perivascular lymphohistiocytic dermatitis. The gross appearance and histologic lesions were consistent with vitiligo. Treatment with UV-B narrowband phototherapy was performed on the lateral thighs, lateral elbows, palmar carpi, and rostral maxilla for a period of 12 mo. Significant repigmentation of the treatment areas was achieved.

Key words: Black rhinoceros, depigmentation, Diceros bicornis, phototherapy, vitiligo.

BRIEF COMMUNICATION

Vitiligo is a common integumentary disease of people, affecting 1–2% of the world's population.⁷ The acquired dermatopathy has an inherited multifactorial genetic pattern and is characterized by depigmentation of the integument resulting from a loss of functioning epidermal melanocytes.⁵ While vitiligo appears less commonly in the veterinary literature, it has been documented in several species including cattle (*Bos taurus*),¹³ water buffalo (*Bubalus bubalis*),³ dogs (*Canis familiaris*),¹ horses (*Equus caballus*),¹² cats (*Felis domesticus*),¹ and chickens (*Gallus gallus*).¹

Dermatologic disease is a common cause of morbidity in the captive black rhinoceros, *Diceros bicornis*, in the United States, affecting nearly half of the captive adult population.¹⁰ This report describes the clinical presentation, diagnosis, and treatment of vitiligo in a captive Eastern black rhinoceros, *Diceros bicornis michaeli*.

A 2-yr-old captive-born female Eastern black rhinoceros, weighing approximately 850 kg, presented with focal, non-ulcerated, non-pruritic, macular depigmentation of the integument, measuring less than 0.5×1 cm, immediately ventral to the right and left nares. A physical examination was performed, and no other abnormalities were detected. Using operant conditioning, blood was collected from the radial vein for a complete blood count and select serum chemistries. Results were considered normal when compared with reference ranges.⁸ Over the subsequent 18 mo, the depigmented macules increased to patches, and the number and distribution increased to include maxillary and mandibular lips, nares, ventral mandible, facial fold crypts, axillae, inguinal areas, lateral thighs, lateral brachium and antebrachium, palmar carpi, perineum, ventral tail, interdigital integument, and ventral abdomen (Fig. 1). The areas of depigmentation varied in size with the largest lesion occurring over the left lateral thigh and measuring approximately 48×22 cm. The multifocal, symmetrical, depigmented patches involved approximately 15% of the body surface. Multiple physical, ophthalmic, and complete fecal examinations did not detect any additional abnormalities.

Serum was submitted for select endocrine testing including total thyroxine (T₄), total triiodothyronine (T₃), and cortisol, as well as antinuclear antibody testing (ANA). Results of T₄ (1.07 µg/dl), T₃ (39 ng/dl), and cortisol (0.4 µg/dl) were comparable to a conspecific housed at the same barn (1.66 µg/dl, <20 ng/dl, and 0.3 µg/dl, respectfully) and were considered normal. Repeated ANA titers were considered negative at <1:16. Using operant conditioning, three sets of skin biopsies from grossly affected and unaffected facial fold crypts were obtained in December 2006. The biopsies were collected using a local anesthetic infusion (lidocaine hydrochloride 2%,

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Figure 1. Gross appearance of right lateral elbow of an Eastern black rhinoceros (*Diceros bicornis michaeli*) with vitiligo. **a.** Appearance of skin prior to treatment with large area of depigmentation visible. **b.** Appearance of skin after 1 yr of UV-B narrowband phototherapy treatment with an estimated 90% repigmentation.

Phoenix Pharmaceutical, Inc., St. Joseph, Missouri 64507, USA; 0.5 ml, s.c.), a 5-mm cupped biopsy forceps, and a No. 10 scalpel blade. Topical antiseptic ointment (chlorhexidine ointment 1%, Phoenix Pharmaceutical, Inc.) was applied to the resulting defect. Samples were placed in 10% neutral buffered formalin for histologic examination and in 0.9% sterile sodium chloride for microbial analysis. Facial fold crypt integument biopsies (two to three per animal) were also obtained from the clinically normal maternal and paternal adult, female sibling, and an unrelated male conspecific and submitted for histologic examination and bacterial culture as previously described. All biopsy specimens were processed routinely, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E). Sections were stained for melanin using the Fontana-Masson technique.

Histologic examination of the clinically affected integument revealed multifocal segmental reduction and loss of pigment especially in the basal epithelial cells of the epidermal layer with some sections demonstrating mild epidermal hyperplasia, hyperkeratosis, and epidermal edema. Some melanin incontinence and mild perivascular lymphohistiocytic dermatitis were also noted in the biopsy samples (Fig. 2). The clinically normal integument from this animal also had mild multifocal hypopigmentation within the basal epithelial cells. The samples from conspecifics were judged to be histologically within normal limits (Fig. 2) except for the paternal adult, whose histologic findings included loss of pigment within the basal epithelial cells, mild perivascular dermatitis with melanin incontinence, and perivascular inflammation in the superficial dermis. Corynebacterium sp. and Staphylococcus epidermidis were cultured from the ground skin samples and were similar to conspecifics.

The gross appearance, postnatal onset, and histologic lesions in this case were consistent with vitiligo.^{5,6,9,11} Due to the potential risk of integument damage from solar exposure, treatment of the depigmented lesions was initiated. Treatment sites were selected based upon the amount of solar exposure that the skin would receive in a normal standing position. Therapy consisted of UV-B narrowband 311 nm phototherapy per-



Figure 2. Skin, black rhinoceros. **a.** Normal skin. H&E. Bar = $263 \mu m$. **b.** Vitiligo, perivascular infiltrates in superficial dermis (arrow). H&E. Bar = $233 \mu m$. Inset: perivascular infiltrates represent melanomacrophages and lymphocytes. H&E. Bar = $72 \mu m$. **c.** Normal skin; note density of melanin granules in the epidermis, especially the basal cell layer (arrowheads), and absence of melanomacrophages around blood vessels (arrows) in the dermis. Fontana-Masson stain. Bar = $180 \mu m$. **d.** Vitiligo; note reduced density of melanin granules in the epidermis, especially the basal cell layers (arrowheads) and melanomacrophages around dermal blood vessel. Fontana-Masson stain. Bar = $190 \mu m$.

formed under operant conditioning three times per wk on nonconsecutive days using two Dermalight 80 psoracombs (National Biological Corporation, Twinsburg, Ohio 44087, USA). This unit produced a radiation outlet treatment field of 4×11 cm per treatment site.

Treatment was initiated at 20 sec per site at a distance of 3 cm from the skin surface resulting in a dose of 70 mJ/cm² per treatment location. Treatment was increased incrementally by 70 mJ/cm² (20 sec) each treatment session. Once a dose of 980 mJ/cm² (4 min and 40 sec) per site was achieved, subsequent treatments were performed for the same time duration. On treatment days, the patient was restricted to an indoor stall until 1600 hours Central Standard Time (CST), at which time outside solar exposure was considered to be insignificant.⁴ On nontreatment days, the

patient was allowed outside access if the depigmented areas were painted with a mud/water mixture, or there was complete cloud cover. A total of nine areas on the rhinoceros were treated, including the proximal and distal lateral thighs, lateral elbows, palmar carpi, and rostral maxilla. The patient underwent a total of 156 treatment sessions at the maximum dose of 980 mJ/cm² per treatment site over the treatment period of 12 mo. This resulted in a cumulative dose of 152.9 J/cm² per treatment site. During phototherapy of the rostral maxilla, the patient's eyes were shielded. Staff present were required to wear UV safety glasses, gloves, and long sleeves to protect against UV exposure.

Within 2 wk of phototherapy initiation, treated integument underwent a color change from light-pink to orange. Over 2–3 mo, the skin returned to

its pretreatment color, and multifocal pinpoint areas of gray began to appear. Over the next several months, the pigmented foci enlarged and coalesced to give the skin the typical gray appearance. Overall, significant repigmentation was achieved. Site-specific repigmentation was an estimated 90% on the lateral elbows (Fig. 1), 70% on the lateral thighs, 60% on the palmar carpi, and 20% on the rostral maxilla. No side effects were noted during the treatment period. Posttreatment, no erythema or solar damage has been noted on any of the repigmented skin. The repigmentation is still present 20 mo following cessation of treatment.

Vitiligo is characterized by the loss of melanin pigmentation in the skin resulting from loss of epidermal melanocytes.^{5,12} While the exact cause remains unclear, multiple mechanisms have been proposed including autoimmune, autocytotoxic, and neural.^{1,6} In people, the predisposition to vitiligo is inherited with approximately 12-35% of pediatric vitiligo patients having family members with the disease.^{6,7} Vitiligo is transmitted as an autosomal dominant trait in people, whereas in animal models, such as the delayed amelanotic (DAM) chicken, the trait is autosomal recessive.¹ The pathogenesis of vitiligo development and potential mode of inheritance in this animal and species is unknown; however, the histologic changes present in the paternal adult do suggest a possible genetic connection.

In this case, the clinically normal skin also had decreased amounts of melanin present in the basal epithelial cells. This is consistent with human vitiligo patients in which there is decreased epidermal basal pigmentation in normal appearing skin.¹¹ The rhinoceros's pattern of depigmentation, initially starting at the nares and lip, is consistent with other reported cases of vitiligo in animals in which the patient develops achromic macules spreading progressively and symmetrically, with depigmentation most commonly occurring at the facial mucocutaneous junctions.¹

Although there are no reported effective treatments in animals, several treatment options have been utilized in human medicine for patients with vitiligo, including psoralen photochemotherapy (PUVA); topical immunomodulators; narrowband UV-B phototherapy; systemic steroids; carbon dioxide laser ablation; excimer laser therapy; surgical therapy; synthetic vitamin D3 analogs; and permanent tattooing.^{5,7,12} In people with moderate to severe vitiligo, narrowband UV-B phototherapy is the initial treatment of

choice due to the efficacy of the therapy and lack of systemic adverse effects.⁵ UV-B phototherapy in humans promotes repigmentation of the skin by inducing local immunosuppression, stimulating the production of melanocyte-stimulating hormone, and increasing melanocyte proliferation and melanogenesis.⁵ Posttreatment biopsies were not obtained in this case, therefore the histologic appearance of repigmentation in this animal is unknown.

Treatment was well tolerated by the patient. Repigmentation of the treatment areas is similar to results observed in people with extensive vitiligo, in which the repigmentation initially appears as follicular with a freckled appearance coalescing into larger pigmented macules over time.² Significant repigmentation was seen in all treated areas, with the lateral elbows having the highest percentage of repigmentation. The limited repigmentation of the rhinoceros's maxillary lip may be due to the constant motion and subsequent variation in the skin to wand distance, skin thickness, or other unknown factors. The long-term prognosis for this patient is favorable as the location and amount of repigmentation has substantially increased the level of protection from solar-induced dermatitis.

Based on the substantial amount of repigmentation, lack of observable side effects, and ease of administration, the use of UV-B narrowband phototherapy was an effective treatment modality for vitiligo in this case.

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