DOI: 10.1638/2021-0129 *Journal of Zoo and Wildlife Medicine* 53(2): 485–491, 2022

THE USE OF INTRADERMAL SKIN TESTING AND HYPOSENSITIZATION INJECTIONS TO CONTROL SEASONAL DERMATITIS IN GREATER ONE-HORNED RHINOCEROSES (*RHINOCEROS UNICORNIS*)

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Abstract: Allergic dermatitis was diagnosed in a 25-yr-old female greater one-horned rhinoceros (*Rhinoceros unicornis*) and her 6-yr-old female offspring by skin biopsy, intradermal skin testing (IDST), and allergen-specific serum IgE testing. Dam and offspring presented with seasonal, erosive, and ulcerative dermatitis affecting the face, legs, and trunk starting at 6 and 2 yr of age, respectively. IDST was performed at the caudal pinnal base using 61 regionally specific allergens. Specific serum allergen responses were detected using Heska's Equine ALLERCEPT® Allergen Panel. Histopathology of the lesions was consistent with an allergic etiology. Injectable allergen-specific immunotherapy was initiated in both animals and within 6 to 18 mon after commencing hyposensitization clinical improvement was noted. This report documents a repeatable methodology for IDST and serological allergen testing for use in rhinoceroses. The hyposensitization protocol detailed here can help guide future treatment protocols.

CLINICAL BRIEF

Dermatopathies described in rhinoceroses are primarily limited to black and white rhinoceroses (*Diceros bicornis* and *Ceratotherium simum*) with idiopathic or poorly understood causes.[2,3,7,12,13,15] There are no known published reports of allergic dermatitis in rhinoceroses to date, but this may reflect the difficulty in confirming allergic etiologies. Large animal allergic dermatitis is best characterized in equines, but this field still lags behind domestic small animals.[9–11,19] The methodologies to diagnose allergic dermatitis, case management, and results of hyposensitization therapy in two related greater one-horned rhinoceroses (*Rhinoceros unicornis*) for seasonal ulcerative dermatitis are presented here. Both rhinoceroses were housed individually with indoor–outdoor access dependent on weather conditions. There were a total of one male and five female rhinoceroses housed in the building on concrete flooring and the outdoor enclosure contains a wallow. The rhinoceroses were fed pelleted feed grain (ADF), hay (mixed, including Timothy and supplemental alfalfa, rarely), variable fruits, vegetables, and browse. Dense forest surrounds the outdoor exhibit space.

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Rhinoceros 1

In 2018, a 24-yr-old female greater one-horned rhinoceros was evaluated for worsening seasonal erosive and ulcerative dermatitis on the lateral aspect of all four limbs, skin folds of the limbs, aural base, and pinnal margins. Review of her medical history revealed a seasonality to the dermatitis with similar lesions dating back to 2001 and lesions lasting from spring to fall (approximately March to November). Lesions started with erythema and depigmentation of the skin primarily focused on the regularly spaced intertriginous areas. Initial lesions progressed to multifocal to coalescing exudative erosions and ulcerations with serosanguinous exudate (Fig. 1) and were pruritic. Monotherapies or combinations of treatment and management strategies were employed over the years, including zinc oxide skin protectant (Rugby, Livonia, MI 48152, USA), fly-control strategies (topical Repel-x fly spray [Farnam, Phoenix, AZ 85067, USA]), flytraps, fans, etc.), chlorhexidine topical cleaning (VetOne, Boise, ID 83705, USA), antibiotic anti-inflammatory topical ointment (Animax®; Fougera, Melville, NY 11747, USA), silver sulfadiazine topical ointment (Crown Laboratories, Johnson City, TN 37604, USA), prednisolone (300 mg tablets, Wedgewood Pharmacy,

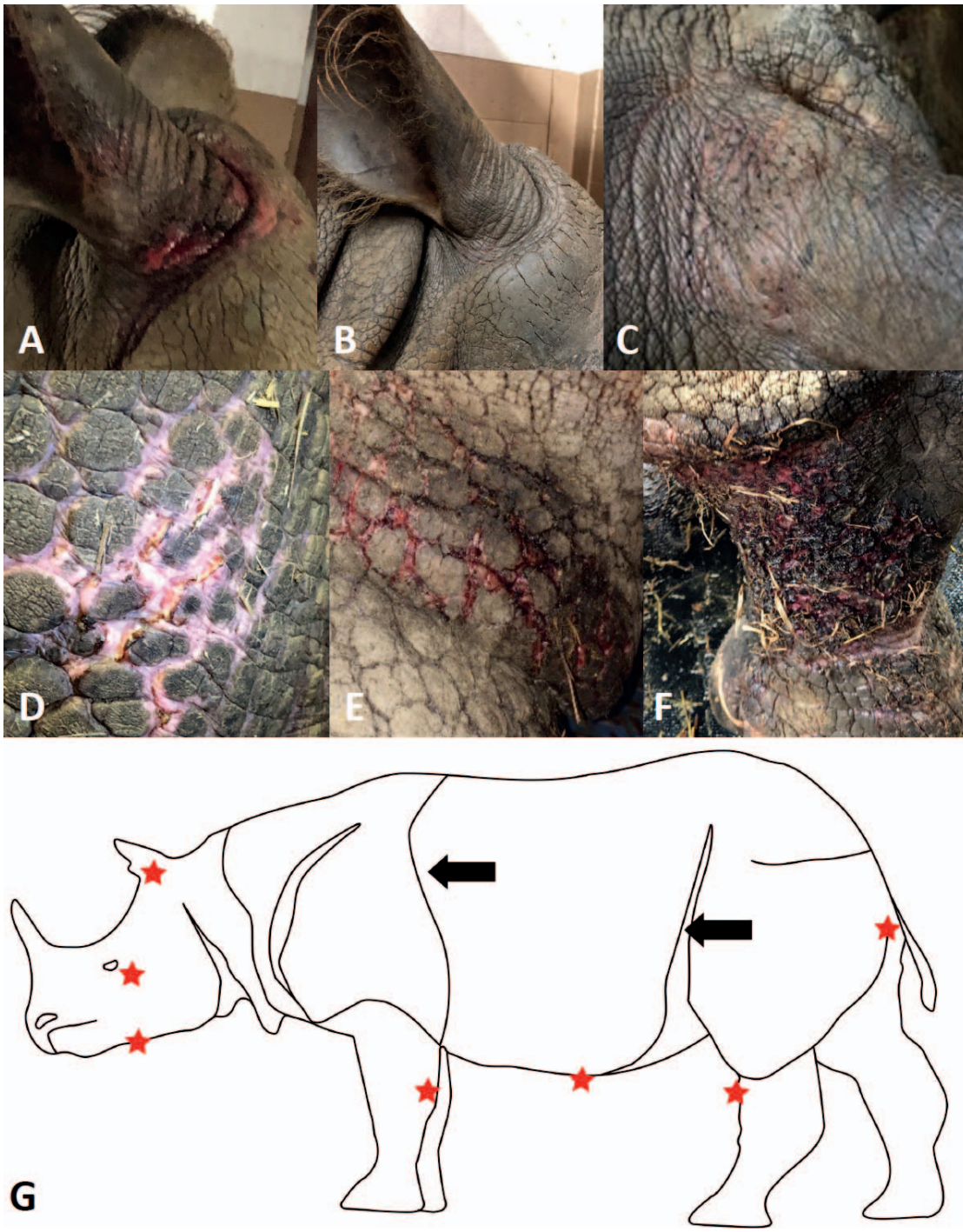

Figure 1. Allergic dermatitis skin lesion progression and intradermal skin testing in two greater one-horned rhinoceroses (*Rhinoceros unicornis*). (**A**) Typical ear-base dermatitis in rhinoceros 2; (**B**) rhinoceros 2, approximately 1 yr post hyposensitization treatment, demonstrating resolved ear-base dermatitis; **C**. Location of the intradermal skin testing, caudal ear base, pinnae. A grid is marked out in black permanent marker in this location. Ear is folded forward for imaging; (**D**) rhinoceros skin, lateral aspect of a forelimb with skin-fold depigmentation and erythema, representative of the initial clinical signs of allergic dermatitis; (**E**) rhinoceros skin, lateral forelimb, progression of clinical signs to skin-fold fissures with ulceration and bleeding; (**F**) rhinoceros skin, lateral hindlimb, progression of clinical signs to regionally extensive ulceration with bleeding; (**G**) administration sites of hyposensitization injections are indicated by arrows in the large cranial and caudal skin folds; red stars indicate the location of affected skin in both rhinoceros 1 and rhinoceros 2.

Table 1. Allergens eliciting a positive result from intradermal skin and serological testing and included in hyposensitization treatment of two greater one-horned rhinoceroses (*Rhinoceros unicornis*).

	Hyposensitization therapy allergen[a]
Rhinoceros 1	
Timothy	I
Sheep sorrel (red sorrel)	I, 1
Mixed feathers	I
White birch	I, 1
Red maple	I, 1
Walnut pollen, black	I
Red mulberry	I, 1
Lepidoglyphys destructor	S
Rhinoceroses 1 and 2	
Kentucky blue grass (June grass)	I, 1, 2a
Bermuda–Johnson grass mix	I, 1, 2a
Mosquito	I, 1, 2a
Culicoides variipennis	I, 1, 2a
Housefly	I, 1, 2a
Horsefly	I, S, 1; S, 2a
Moth	I, 1, 2a
American elm	I
Cladosporium sphaerospermum	I
Dermatophagoides farinae	S, 1; I, 2a
Rhinoceros 2	
Black or carpenter ant	I, 2a
Deer fly	I, 2a
Tyrophagus putrescentiae	I, S, 2a
Mouse	I, 2a
American sycamore	I, 2a
White pine	I
Red cedar	I, 2a
Privet	I, 2a
Aspergillus fumigatus	I, 2b
Penicillium	I, 2b
Mucor mix	I, 2b
Drechslera spicifera	I, 2b
Pyrethrum (*Chrysanthemum*)	I

[a] I, intradermal skin testing positive reaction; 1, Rhinoceros 1 hyposensitization therapy allergen; S, serum IgE positive; 2a or 2b, Rhinoceros 2 hyposensitization therapy allergen with antigens separated into vial a and vial b.

Scottsdale, AZ 85251, USA; tapering, 0.4 mg/kg PO, q12h for 3 d, then q24h for 3 d, then q48h for three doses), hydroxyzine (50 mg tablets, Epic Pharma, Laurelton, NY 11413, USA; 0.5 to 1 mg/kg PO, q12h), diphenhydramine (50 mg tablets, Major, Livonia, MI 48152, USA; 0.25 mg/kg PO, q24h), antibiotic anti-inflammatory topical spray (GenOne®; 0.85 mg/ml, VetOne; topical q12h to q24h). The lesion severity and duration of each episode became progressively worse with each year—characterized by larger areas affected within the above-described anatomic locations or deeper ulceration and less response to topical and systemic treatments. In 2018 nearly year-round treatment was needed to control clinical signs. Hematology and serum biochemistry analysis were unremarkable.

In February 2019, cytology of the ulcerative skin lesions revealed cocci, yeast, and short rods with numerous eosinophils supporting a hypersensitivity reaction with mixed secondary infection. Based on culture and sensitivity results from a skin wound swab culture, this animal was treated with trimethoprim sulfamethoxazole (960 mg tablets, Amneal Pharmaceuticals, Ahmedabad 382213, India; 24 mg/kg PO, q24h for 14 d). Wounds were cleaned with Malaseb (Bayer, Shawnee Mission, KS 66201, USA; topical q12h) and treated with GentOne Spray (topical, q12h). Treatment with pentoxifylline orally (3 gm/25 cc scoop powder; Wedgewood Pharmacy; 8 mg/kg PO, q12h) was started as this has been used in equine allergic dermatitis treatment regimens.[9,19] Pentoxifylline is a methyl-xanthine derivative used for its hemorheologic and anti-inflammatory properties. Dermatological uses have been studied in canines and shown to reduce mast cell degranulation and recruitment of cutaneous inflammatory cells, notably eosinophils.[14]

In August 2019, this rhinoceros was sedated with detomidine (10 mg/ml, Zoetis Inc, Kalamazoo, MI 49007, USA; 0.02 mg/kg IM). Blood was collected for hematology, serum biochemistry, and IgE-specific serum allergen testing (Heska Allercept, Loveland, CO 80538, USA). Biopsy (6-mm punch biopsies) of the affected skin confirmed eosinophilic dermatitis consistent with hypersensitivity reaction. The caudal base of the right ear was used to perform intradermal skin testing using 61 regionally specific allergens (Supplemental Table 1). Allergen concentrates from Stallergenes-Greer Laboratories (Lenoir, NC 28645, USA) were diluted to 1,000 protein nitrogen units (pnu)/ml unless otherwise stated in Supplemental Table 1. Saline and histamine (1:1,000,000 w/v) served as negative and positive controls, respectively. Each allergen and control were injected intradermally (0.1 ml) and reactions assessed at approximately 15 min after injection and again 24 h after injection for delayed reactions. The reactions were compared with controls and scored from 0 to 4 based on approximate turgidity, size, and erythema of wheals that formed at the site of injection. The negative control and positive control were graded 0 and 4, respectively. All reactions graded 2 or above were considered positive responses to the injected allergen and are detailed in Table 1. The strongest

Table 2. Subcutaneous hyposensitization injection dosing protocol used in two greater one-horned rhinoceroses (*Rhinoceros unicornis*).

Day[a]	Dose	
	ml	pnu[b]
Starter vial 1 (200 pnu/ml)		
0	0.1	20
3	0.2	40
6	0.4	80
9	0.8	160
12	1.0	200
Starter vial 2 (2,000 pnu/ml)		
15	0.1	200
18	0.2	400
21	0.4	800
24	0.8	1,600
27	1.0	2,000
Maintenance vial 3 (20,000 pnu/ml)		
30	0.1	2,000
33	0.2	4,000
36	0.4	8,000
39	0.8	16,000
42	1.0	20,000[c]

[a] Dosing interval goal was every 3 d but was adjusted to be within every 3 to 4 d to accommodate staffing, pandemic safety restraints, and animal compliance.
[b] pnu, protein nitrogen units.
[c] Maintenance doses continued every 10 d (1ml, 20,000 pnu).

reactions (grade 4) were against Kentucky blue (June) grass, mosquitos, and horsefly. Heska allergen-specific IgE serum testing revealed three allergen-specific IgE positive titers: *Dermatophagoides farinae* (dust mite), *Lepidoglyphys destructor* (storage mite), and horsefly.

Allergens chosen for hyposensitization were selected based on these test results, known exposures experienced by this animal, clinical sign seasonality, and availability (Table 1). Hyposensitization by subcutaneous injection was started in September 2019. Based on equine protocols, induction injections were to be delivered every 3 d (Table 2). Due to feasibility of treatment and constraints (accommodation of staffing schedules, including pandemic safety restraints [SARS-CoV-2] and animal compliance to injections), injections were administered every 3 to 4 d until the maintenance concentration was reached. Subcutaneous hyposensitization injections were started at 0.1 ml of 200 pnu/ml (20 pnu) and subsequent doses were doubled until the maintenance dose was reached (1 ml of 20,000 pnu/ml) (Table 2). The animal reached full concentration of allergen injection (1 ml; 20,000 pnu) in October 2019 and has continued to be dosed every 10 d since. Pentoxifylline was discontinued approximately 1 yr after commencing hyposensitization treatment due to near clinical resolution of dermatitis and trial weaning did not result in significant recurrence of clinical signs. Fly repellant continued to be used as needed. From October 2019 to the time of writing, this rhinoceros has only had five mild episodes of dermatitis, which lasted approximately 2 wk or less and were well controlled with topical treatments alone (Malaseb cleaning and/or GentOne spray topically for 2 wk or less). The severity of ulceration and overall amount of body surface area affected have been markedly less severe and resolved more rapidly after commencing topical treatments in the 1-1/2 yr since starting hyposensitization (limited to depigmentation, erythema, and superficial erosion variably in previously described areas). On visual assessment in January 2021, all skin lesions were resolved.

Rhinoceros 2

Rhinoceros 2 (6-yr-old female greater one-horned rhinoceros and an offspring of rhinoceros 1) presented in 2018 for erosive and ulcerative dermatitis on the lateral aspect of all four limbs and intertriginous areas of the limbs, ventral chin, and trunk with periocular depigmentation and crusting. Review of her medical history revealed a seasonality to the clinical signs with similar lesions dating back to 2015 (early spring to fall, approximately late February to October). Lesions started and progressed in a similar pattern as that described in rhinoceros 1; management and treatment followed a similar course. The lesion severity and duration of each episode became progressively worse with each year. In 2018 nearly year-round treatment was needed to control clinical signs. Hematology and serum biochemistry analysis were unremarkable.

In 2019, cytology of the skin lesions revealed cocci, yeast, and neutrophilic and eosinophilic infiltrates supporting a hypersensitivity reaction with mixed secondary infection. Empirically selected treatment with trimethoprim sulfamethoxazole (30 mg/kg PO, q24h for 14 d) was started. Wounds were cleaned with Malaseb and treated with GentOne Spray (q24h to q12h, as indicated). To control clinical signs, treatment with a tapering course of prednisolone (0.3 mg/kg PO, q12h for 3 d, then q24h for 3 d, then q48h for 3 doses), hydroxyzine (1 mg/kg PO, q12h for 14 d), and pentoxifylline (8 mg/kg PO, q12h) was initiated.

In September 2019, after weaning off prednisolone, rhinoceros 2 was sedated with oromucosal detomidine gel (7.6 mg/ml, Zoetis Inc.; 0.04 mg/kg transmucosal) followed by intramuscular administration of detomidine (0.018 mg/kg IM) at 40 min after mucosal gel administration to permit manipulation of the ears. The diagnostic testing performed was similar to that detailed above for rhinoceros 1. Individual allergens with positive intradermal reactions and serological identification are detailed in Table 1. The strongest reactions to IDST (grade 4) were against moth, mouse, red cedar, privet, and *Drechslera spicifera* (mold). Heska allergen-specific IgE serum testing revealed one allergen-specific IgE titer: *Tyrophagus* (storage mite). Allergens selected for hyposensitization required separation of the allergens intended for delivery into two separate vials. Protease activities against selected allergens in the mold allergen cocktail can degrade the pollen allergens in the other vial. This meant that two separate injections were planned, following the same protocol for frequency of delivery and increasing concentrations as detailed for rhinoceros 1 (Table 2) for each of the two allergen cocktails.

Hyposensitization via subcutaneous injections were initially started in January 2020. However, due to poor patient compliance to two injections and staffing limitations associated with onset of the COVID-19 pandemic, treatment was delayed until July 2020. Compliance was improved by mixing the two separate allergen cocktails into a single syringe immediately prior to delivery. Mixing immediately before delivery reduced the risk of the mold proteases degrading other allergens. The dosing schedule followed that as described for rhinoceros 1 (Table 2). The animal reached maintenance concentration of both sets of allergens in August 2020. Since this time, the animal has remained on every 10-d dosing for maintenance injections.

Rhinoceros 2 has remained on pentoxifylline treatment. Fly repellant continues to be used as needed. From August 2020 to present day this rhinoceros has had three mild episodes of dermatitis well controlled with topical treatments (Malaseb and GentOne spray). The severity of affected areas was limited to erythema and superficial erosion and the overall size of lesions in the previously detailed areas were reduced (stopped serosanguinous exudation and pruritus, and reduced number of areas affected). Lesions were also quicker to respond to topical treatment alone (requiring less than 2 wk of treatment to close erosive lesions) in the 13 mon since starting hyposensitization.

DISCUSSION

Diagnosis of allergic dermatitis can be challenging due to a lack of confirmatory testing.[10,17] Therefore, allergic dermatitis is a diagnosis of exclusion that is complicated by frequent secondary infections. Once diagnosed through exclusion, IDST is considered the gold standard through which to determine allergens for desensitization.

The diagnosis of allergic dermatitis in these two cases is supported by seasonal clinical signs, initial improvement with antihistamines and fly control, no signs of ectoparasitism, no recent change in husbandry, unaffected animals present in the same environment, and a positive response to desensitization. Equine allergic dermatitides (i.e. atopic dermatitis, insect bite hypersensitivity) are characterized by pruritus and urticaria. The rhinoceroses presented here displayed intermittent clinical descriptions of suspected pruritus, but initial skin lesions began as erythema and fissuring between the skin folds or creases and progressed to coalescing areas of exudative and ulcerative dermatitis. The distribution of lesions is reminiscent of other allergic dermatopathies in small and large animal species (i.e. ventral body, periocular or aural skin, and skin folds).[4–6] Lesion severity was likely compounded by both insect-bite hypersensitivity and environmental allergen hypersensitivities in these animals.

IDST reactions support antibody formation to allergens but do not necessarily positively correlate or confirm clinical significance of these allergens.[1,8,10] IgE serum titer (SAT) testing performed in these rhinoceroses was not validated in this species and should be interpreted cautiously. SAT detects allergen-specific IgE antibodies. Because other mechanisms may exist to elicit an allergic response, use of both tests may strengthen the list of possible offending allergens that can be selected for hyposensitization.[10,20] False positive reactions due to cross-reaction is possible in SAT testing but overall few allergens were identified in the cases presented here.[1,18] The positive clinical outcome from hyposensitization to selected allergens indicates that, although not diagnostic, IDST may aid in selecting appropriate antigens for treatment of suspected allergic dermatopathies in rhinoceroses.

The immunotherapy protocol selected here is used commonly in domestic species. Immunotherapy in equines can improve clinical signs in as little as 2–3 mon after the initiation of treatment,

similar to results seen in rhinoceros 2.[16] The goal of immunotherapy is not necessarily to achieve clinical resolution but to lessen the severity, duration, and dependence on treatment for flare-ups. Both rhinoceroses now have dermatological flare-ups managed with topical treatments. Rhinoceros 1 was able to be weaned off pentoxifylline treatment but at the time of writing, rhinoceros 2 remains under treatment with pentoxifylline. This is in part due to the delay in starting rhinoceros 2 on hyposensitization treatment and that weaning had not yet been attempted. The cases presented here have both decreased dependence on oral antihistamine treatments and decreased length of topical treatments, and require less overall animal department care and veterinary oversight.

The protocol for IDST and serum IgE testing presented here can be easily employed in a safe, single, short sedation. The information gained from these diagnostics should be interpreted with consideration of the specific animal's clinical history, clinical signs, and known allergens specific to the geographical region and season. Injectable desensitization has also shown to be feasible in these cases with keeper-delivered maintenance injections every 10 d. In the future, the frequency of injections may be decreased slowly and cautiously as the animals are monitored for recurrence of clinical signs.

Acknowledgments: The authors thank Wildlife Conservation Society Bronx Zoo staff Michelle Medina, Mary Gentile, and Phil Reiser (Department of Mammalogy) for ongoing animal care and treatment and Zoological Health Program veterinary and histology technicians for assistance with sample processing and handling.

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Accepted for publication 6 March 2022