

# Vesicular stomatitis virus in two species of rhinoceros at a California zoological park

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## OBJECTIVE

To describe an outbreak of vesicular stomatitis virus (VSV) in southern white rhinoceros (SWR; *Ceratotherium simum simum*) and greater one-horned rhinoceros (GOHR; *Rhinoceros unicornis*) at a safari park in San Diego, CA, from May to September 2023.

## ANIMALS

21 SWR and 5 GOHR in professionally managed care.

## METHODS

Rhinoceros of both species presented with a range of clinical signs and severities. Lesion locations were categorized as cutaneous (coronary bands, heels and soles, limbs, ventrum, neck folds, and ears) and mucocutaneous (lips, nostrils, mucous membranes of the oral cavity, and vulva). Clinical signs included lethargy, lameness, difficulty with prehension, hyporexia to anorexia, and hypersalivation. Severely affected rhinoceros had clinical pathology findings consistent with systemic inflammation.

## RESULTS

Vesicular stomatitis New Jersey virus was confirmed via PCR from swabs of lesions in 10/26 (38%) rhinoceros. Of these 10 confirmed cases, 9 (90%) were SWR and 1 (10%) was a GOHR. A further 6/26 (24%) were considered probable cases, and 10/26 (38%) were considered suspect cases based on clinical signs, but the inability to appropriately sample due to the housing environment precluded confirmation. Histopathology samples from 3 rhinoceros were consistent with VSV, and viral RNA was localized in histologic lesions via RNA in situ hybridization for 1 case. All rhinoceros survived infection despite severe systemic illness in 2 animals.

## CLINICAL RELEVANCE

This case series describes the clinical appearance and progression of VSV in 2 rhinoceros species. To the authors' knowledge, this is the first report of VSV in a rhinoceros.

**Keywords:** southern white rhinoceros (*Ceratotherium simum simum*), greater one-horned rhinoceros (*Rhinoceros unicornis*), vesicular stomatitis virus, vesicular disease, disease outbreak

Vesicular stomatitis virus (VSV) is the causative agent of vesicular stomatitis, a disease most commonly reported in equids but documented in many domestic livestock species including cattle, sheep, goats, camelids, and swine.<sup>1</sup> The virus is a negative single-stranded RNA virus in the family Rhabdoviridae, genus *Vesiculovirus*. The USDA considers vesicular stomatitis a “notifiable disease”<sup>2</sup> because of the similarity of VSV gross lesions to foot-and-mouth disease

virus (FMDV) gross lesions and the potential for economic loss. Transmission is not completely elucidated but is suspected to be largely vector driven. *Aedes* mosquitoes, *Lutzomyia* sandflies, *Simulium* black flies, and *Culicoides* biting midges are the most common invertebrates implicated in outbreaks.<sup>3</sup> In addition to vector-mediated spread, the virus can be transmitted via direct contact with vesicular lesions, nasal secretions, or saliva.<sup>1</sup> Contaminated feed, water, or tools can be indirect means of transmission as the virus can survive on hay, milking equipment, and mangers for 1 to 3 days.<sup>2</sup> Livestock do not maintain VSV long term and an animal reservoir is not currently known. VSV is a zoonotic pathogen and can

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cause influenza-like symptoms in humans who come into contact with lesions, saliva, or nasal secretions from affected animals.<sup>1,4</sup>

In domestic livestock, VSV causes vesicles, papules, erosions, and ulcers on the mouth, feet, prepuce, and mammary glands.<sup>1</sup> The anatomical sites predominantly affected in each outbreak likely depend on the type of insect vector responsible, the vector's feeding strategy, and the host species and density.<sup>1</sup> In the absence of complicating factors such as secondary bacterial infection, vulnerable age, previously underlying medical condition, or severe oral lesions resulting in anorexia, infections typically resolve within 2 to 3 weeks without intervention.<sup>5</sup> Antibodies to VSV have been identified in many nondomestic species without reported evidence of disease, including ruminants (deer, pronghorn, and bighorn sheep), carnivores (bears, wild canids, and bobcats), rodents, nonhuman primates, rabbits, birds (turkey and ducks), and others (taman-*dua*, raccoons, opossums, and fruit bats).<sup>6-8</sup> There have been no previous reports of VSV in a nonequid *Perissodactyla* (rhinoceros and tapirs) species.

Two serotypes of VSV are endemic in southern Mexico, central America, and northern South America: the New Jersey virus (VSNJV) and the Indiana virus (VSIV).<sup>5</sup> Genetic analysis of VSV from recent outbreaks in 2019 and 2020 indicates that the virus arises from Mexico and in years with favorable environmental conditions, it moves northward into the southern US, including Arizona, New Mexico, and Texas.<sup>5</sup> The outbreaks generally occur during warmer months when vector activity is high and coincides with increased movement of livestock.<sup>5</sup> Over the last decade there have been 5 documented VSV outbreaks in the US in 2014, 2015, 2019, 2020, and 2023.<sup>2</sup> VSV has not been reported in California since outbreaks in 1984 to 1985.

The recent California VSV outbreak began on May 17, 2023, with a positive equine case in San Diego County, CA.<sup>2</sup> Between May and September, more than 200 VSV-affected premises were identified in California, Nevada, and Texas.<sup>2</sup> The index rhinoceros case at the San Diego Zoo Safari Park presented less than 2 weeks after the index San Diego County equine case. This report is a descriptive case series detailing the subsequent outbreak of VSV in both southern white rhinoceros (SWR; *Ceratotherium simum simum*) and greater one-horned rhinoceros (GOHR; *Rhinoceros unicornis*) housed at a safari park in southern California.

## Methods

### Study population

All affected rhinoceros lived under professional care at the San Diego Zoo Safari Park in Escondido, CA, San Diego County. There are 2 species housed at this facility: GOHR and SWR. The GOHR population consisted of 5 individuals housed within a large mixed-species field habitat with ungulates from the Asian continent. One group of 10 SWR was housed in a nearby mixed species field habitat containing

ungulates from the African continent. A second group of eleven SWR lived in a separate single-species habitat consisting of an indoor barn and several outdoor yards, with separate care staff.

### Case evaluation

Rhinoceros were evaluated daily by wildlife care specialists and any rhinoceros with cutaneous lesions or abnormal behavior was evaluated by a veterinarian. Cutaneous or oral lesions that could be safely evaluated with behavioral restraint or opportunistic examination were sampled using a sterile dacron or polyester swab. The swab was then placed into a sterile vial containing 1 mL of tris-buffered tryptose broth supplied by the USDA. The sample was then submitted to the National Veterinary Services Laboratories Foreign Animal Disease Diagnostic Laboratory for vesicular disease testing. When possible, blood samples were obtained via behavioral restraint or during anesthetized procedures for treatment. Samples underwent rule-out and confirmatory testing for FMDV and VSV. ELISA was performed on blood samples. Real-time reverse transcription PCR (qRT-PCR) was performed on swabs from lesions to detect and characterize virus isolates, including qRT-PCR for FMDV<sup>9</sup> and a multiplex qRT-PCR for VSV<sup>10</sup> on the extracted RNA using the TaqMan Fast Virus 1-Step Master Mix (Applied Biosystems) at a 1X concentration. Additionally, virus isolation was performed on all swabs submitted. All tests were performed in accordance with the International Organization for Standardization 17025 accredited procedures at the Foreign Animal Disease Diagnostic Laboratory. CBC, chemistries, and fibrinogen were concurrently evaluated at the San Diego Zoo Wildlife Alliance in-house diagnostic laboratory. Three rhinoceros had lesions biopsied and evaluated histologically. Many of the rhinoceros, especially those in large, mixed species field habitats, were inaccessible for diagnostic sampling. Additionally, in many instances where sampling was possible, the technique was less than ideal due to the inability to clean the lesion of debris before sampling, or the animal stationed for only brief swabbing with short contact time or inadequate pressure on the lesion.

In addition to the testing performed at National Veterinary Services Laboratories, conventional PCR was performed in-house to generate a sequence that would serve as a template for designing RNA in situ hybridization (ISH) probes. RNA was extracted from nasal swabs of affected SWR utilizing the QiAamp viral mini kit (catalog No. 52906) following manufacturer guidelines. Then, 1 mL of 1X PBS was added to the swab and vortexed for a 20-second sample before extraction. The New England Biolabs OneTaq One-Step RT-PCR Kit (catalog No. E5315S) was utilized. Each PCR reaction contained the following components: 9.1  $\mu$ L of nuclease-free water, 0.2  $\mu$ L of each primer (0.4  $\mu$ M for each), 12.5  $\mu$ L of 1 Taq Reaction Mix, and 1  $\mu$ L of 1 Taq 1 Step Enzyme Mix. Then, 2  $\mu$ L of RNA was added per 23  $\mu$ L Master Mix for a total of 25  $\mu$ L each PCR reaction. The RT-PCR conditions used were as follows: 48 °C for 30 minutes; 94 °C for

1 minute [94 °C for 90 seconds, 50 °C for 2 minutes, and 68 °C for 1 minute] X 40; and 68 °C for 5 min. An Eppendorf Master Cycler Pro S was used for the thermocycling process. The primers used targeted VSV NJ (phosphoprotein gene)<sup>11</sup>: primers VSV NJ102P-5'-GAGAGGATAAATATCTCC-3' and VSV NJP831R-5'-AGACGATAYTTCATTCTTGCT-3' generate an amplicon with an expected size of 729 bp. The amplicons were run on a 0.8% agarose gel with 2.5% ethidium bromide. The gel was visualized via the Alpha imager. The PCR products were gel extracted using the Millipore Ultrafree-DA Centrifugal Filter Unit (catalog No. 42600). The gel-extracted DNA was sent in for Sanger sequencing at Eton Bioscience. The sequences were analyzed using Geneious Prime, version 2023.1.2. An RNA ISH probe named V-VSV-O2-PP was designed to target nucleotides 2 to 706 of the generated sequence using custom software as previously described.<sup>12</sup> RNA ISH was performed using the RNAscope 2.5 HD Red Chromogenic Reagent Kit according to the manufacturer's instructions (Advanced Cell Diagnostics). Briefly, 5-µm sections of formalin-fixed, paraffin-embedded tissue were mounted on AutoFrost charged adhesion slides (Cancer Diagnostics Inc), baked at 60 °C in a dry oven, and deparaffinized. The sections were treated with an endogenous peroxidase blocker for 10 minutes at room temperature before boiling in a target retrieval solution for 15 minutes. Protease Plus was then applied for 30 minutes at 40 °C. Target probes were hybridized for 2 hours at 40 °C, followed by a series of signal amplification and washing steps. Hybridization signals were detected by chromogenic reactions using Fast Red. Slides were counterstained in 50% hematoxylin for 2 minutes and decolorized with 0.2% ammonium hydroxide. After being rinsed in deionized water, they were dried in a 60 °C oven, dipped in xylene, and coverslipped using xylene-based SHUR/Mount medium (Triangle Biomedical Sciences).

## Case definition

Rhinoceros were considered “confirmed cases” of VSV infection when they had gross lesions that were PCR positive for VSV. Animals were considered “probable cases” if they had gross lesions and/or clinical signs consistent with VSV infection but were unable to be swabbed due to behavioral or habitat challenges limiting safe sampling access. Animals were also considered “probable cases” if they had a negative PCR result but had gross lesions and/or clinical signs consistent with the confirmed case presentations. Animals were considered “suspect cases” when they had mild clinical signs of a short duration (24 to 48 hours) that were temporally correlated with the outbreak and/or cutaneous lesions that could have been consistent with mild trauma or concurrent disease (ie, chronic allergic dermatitis in 1 case) rather than true VSV lesions.

## Results

### Outbreak description

The index case, a female SWR from the nonfield group, presented on May 26, 2023, with lethargy

and increased frequency of defecation, which progressed the following day to bilateral inguinal swellings and a slow, stiff gait. This animal was started on an oral nonsteroidal anti-inflammatory. Five days later, an adult female SWR from the field group presented with ulcerative lesions of the nares and lips. Due to concern about recent reports of VSV in the local equine population, regulatory agencies were contacted, and VSV testing commenced. Over the following 2 weeks, additional rhinoceros developed clinical signs, and PCR and serologic testing were performed. The last rhinoceros to begin showing clinical signs presented 16 days after the index case. Of the 26 rhinoceros on the premises, 10 (38%) were confirmed cases with PCR-positive results for VSNJV. Of the 10 confirmed cases, 9 (90%) were SWR and 1 (10%) was a GOHR. Of the remaining animals, 7 (27%) were considered probable cases and 9 (35%) were considered suspect cases (**Table 1**). Details for all 26 cases, including signalment, clinical signs, lesion locations, and diagnostic testing are provided (**Supplementary Table S1**).

**Table 1**—Signalment of confirmed, probable, and suspect cases of vesicular stomatitis virus in rhinoceros at a safari park in Escondido, CA, in June 2023.

Characteristic	Confirmed case* No.	Probable case* No.	Low suspect case* No.
Species			
SWR (n = 21)	9	6	6
GOHR (n = 5)	1	1	3
Age			
< 5 years old (n = 8)	2	2	4
> 5 years old (n = 18)	8	5	5
Sex			
Male (n = 7)	3	1	3
Female (n = 19)	7	6	6

GOHR = Greater one-horned rhinoceros. SWR = Southern white rhinoceros.

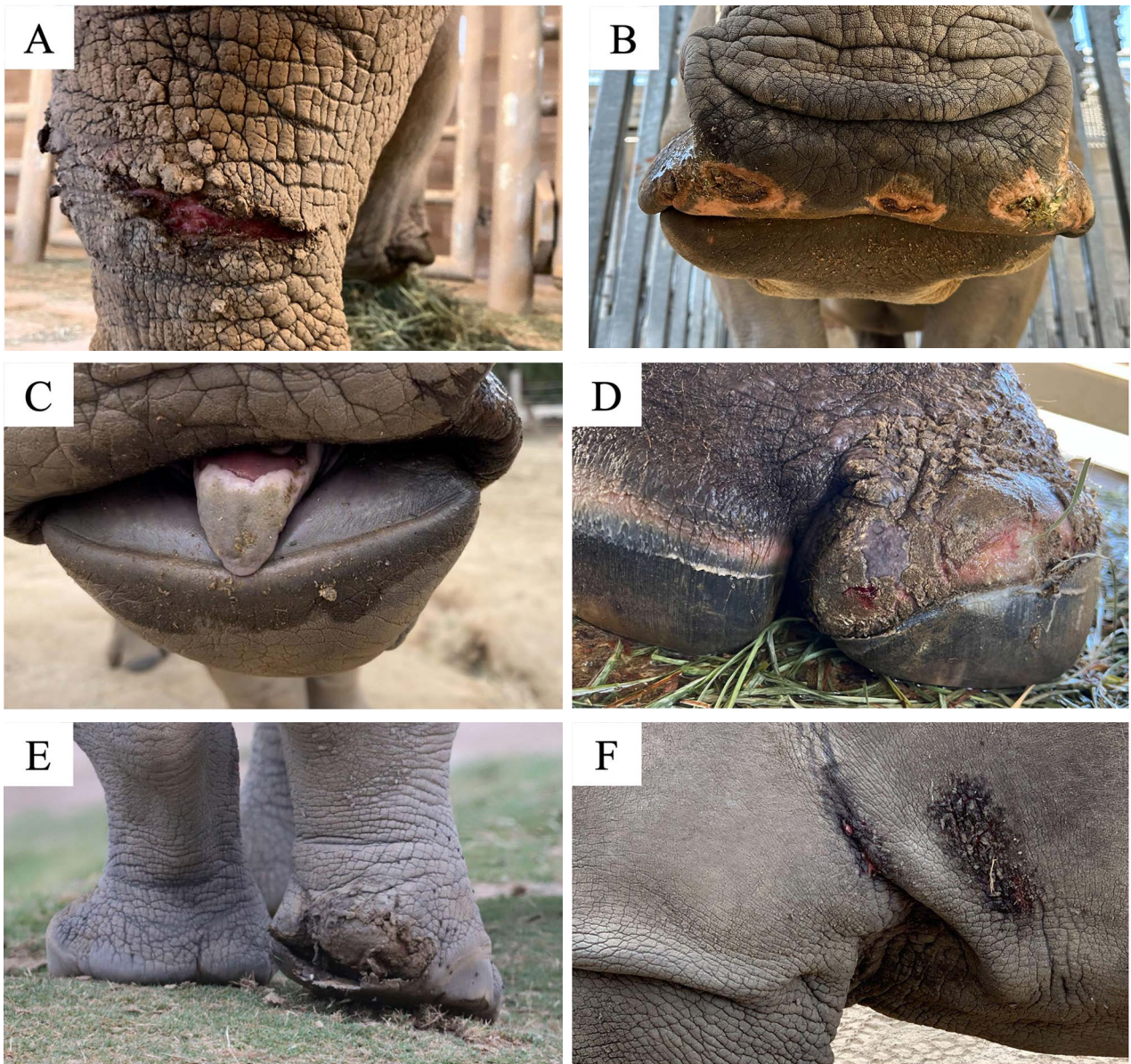
\*Confirmed cases, n = 10; probable cases, 7; low suspect cases, 9.

### Location and severity of gross lesions

Lesions were in 2 primary locations (**Figure 1**): cutaneous (coronary bands, heels and soles, limbs, ventrum, neck folds, and ears) and mucocutaneous (lips, nostrils, mucous membranes of the oral cavity, and vulva).

Several cutaneous lesions started as focal swellings (vesicles) and progressed to partial or full-thickness ulcerations of the skin (**Figure 2**). The presentation of the ulceration was dependent on the anatomic location; on the limbs and ventrum, the skin ulcerated in a linear pattern and then widened or coalesced. Several animals had lesions in the axillary region or between the neck folds that presented similarly to fly bite dermatitis with multifocal to coalescing skin ulcerations and erosions. These lesions had intermittent clear, serous discharge and





**Figure 1**—Vesicular stomatitis virus lesions in southern white rhinoceros. Representative photos of lesions found on the distal limbs (A), the nares and lips (B), the tongue (C), the coronary band (D), the heel (E), and the axillary region (F).

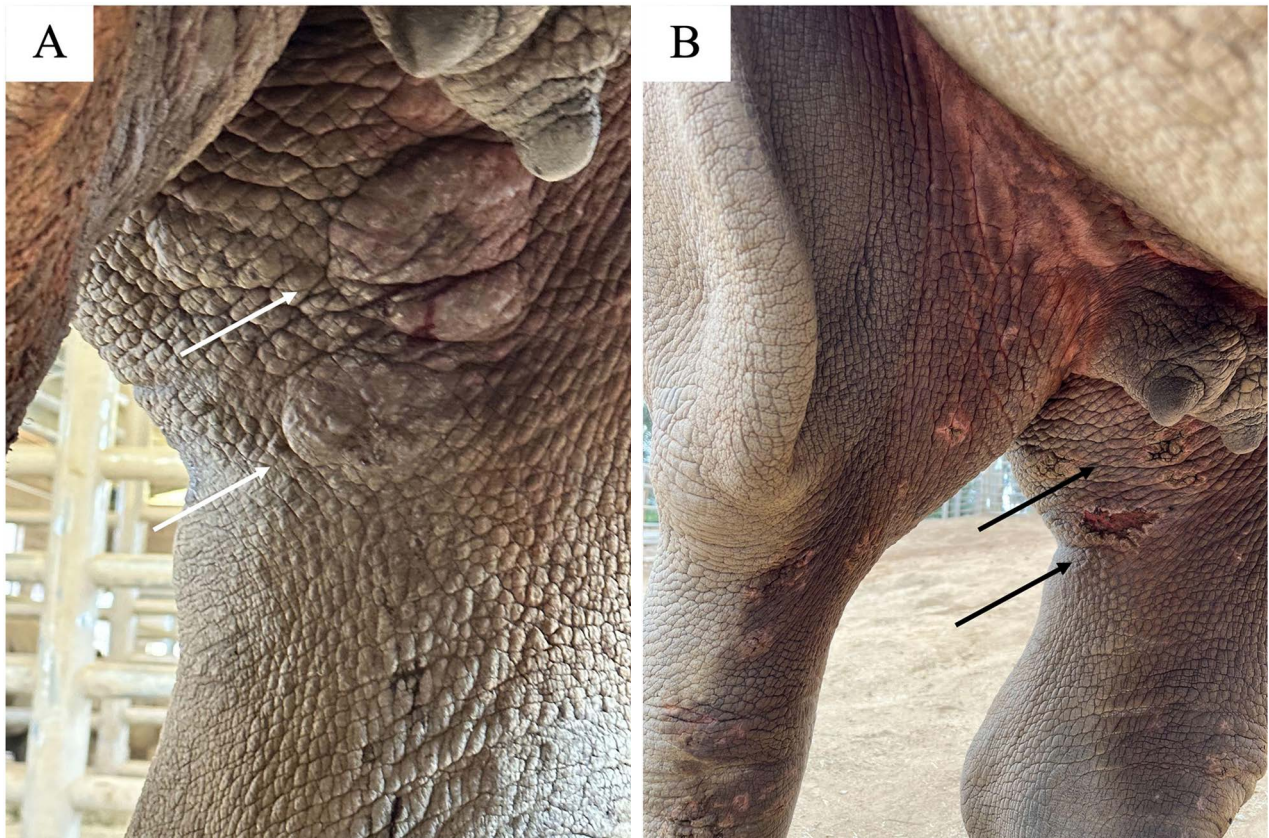
were usually more superficial than the distal limb or ventrum lesions. No purulent material or significant secondary abscessation was appreciated from the cutaneous lesions.

Foot lesions varied in location and included the coronary band, interdigital spaces, heel, and sole. Lesions at the coronary band led to the separation of the nail from the skin and usually extended laterally and medially into one or more of the interdigital spaces. These lesions were either moist with clear discharge or hemorrhagic for a few days and then progressed to partial nail separation from the underlying corium. The most severe lesions presented on the plantar aspect of the foot and progressed to sloughing the soft tissues of the heel, sole, and interdigital space. In 1 case, both forelimbs were affected, and the entirety of the foot pads sloughed

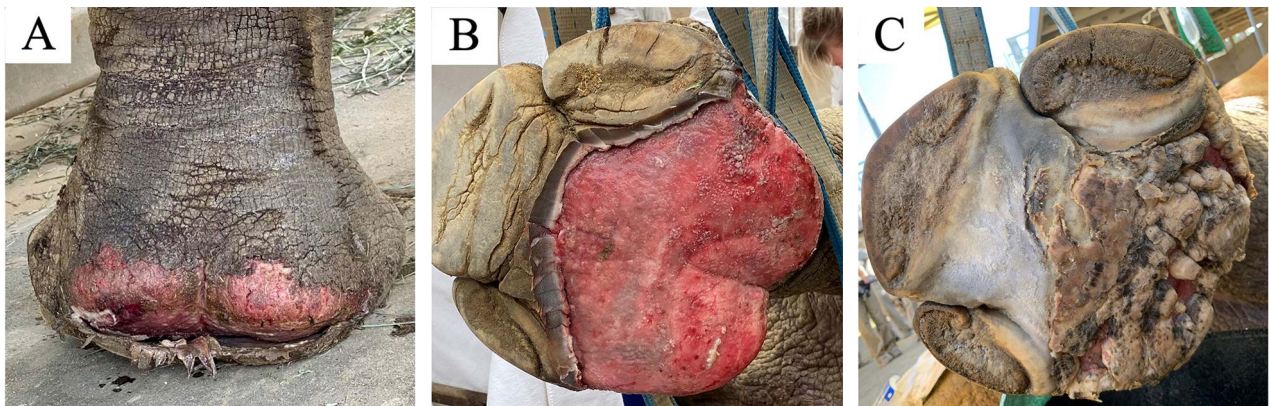
(**Figure 3**). In the other severe case, only 1 hindlimb was affected, and about 50% of the sole sloughed. Purulent discharge was documented in multiple cases affecting the limbs.

Lesions of the oral or nasal cavities presented as ulcerations of the mucous membranes or on the skin of the outer lips or nares, often preceded by hyporexia or hypersalivation (**Figure 4**). No vesicles were appreciated within the oral cavity before seeing ulcerative lesions. Lesions typically became less erythematous over time and then contracted. Animals that presented with lip lesions usually started with ulcerations that progressed to sloughing of surrounding skin. Many of the nares or lip lesions healed with persistent areas of depigmentation. Three young, male rhinoceros ( $\leq 4$  years) presented similarly; 1 of these 3 animals was still nursing during





**Figure 2**—Vesicle before and after rupture in a southern white rhinoceros secondary to vesicular stomatitis virus. Photos showing (white arrows; A) vesicles on the medial proximal limb and inguinal space before (black arrows; B) rupture of the vesicles leading to ulcerative lesions.



**Figure 3**—Progression and resolution of a severe sole sloughing lesion secondary to vesicular stomatitis virus in a southern white rhinoceros. Photos showing lesions on day 5 (A), day 7 (B), and day 57 (C) from initial clinical signs.

the time of the outbreak. These lesions presented as diffuse ulcerations of the tongue, soft palate, and mucous membranes of the maxillary and mandibular gums and took approximately 2 months to resolve. Purulent discharge was observed in multiple cases. A single rhinoceros had 1 lesion on her vulva, a focal ulceration that healed within 2 weeks. The lesion left a small region of depigmentation when healed.

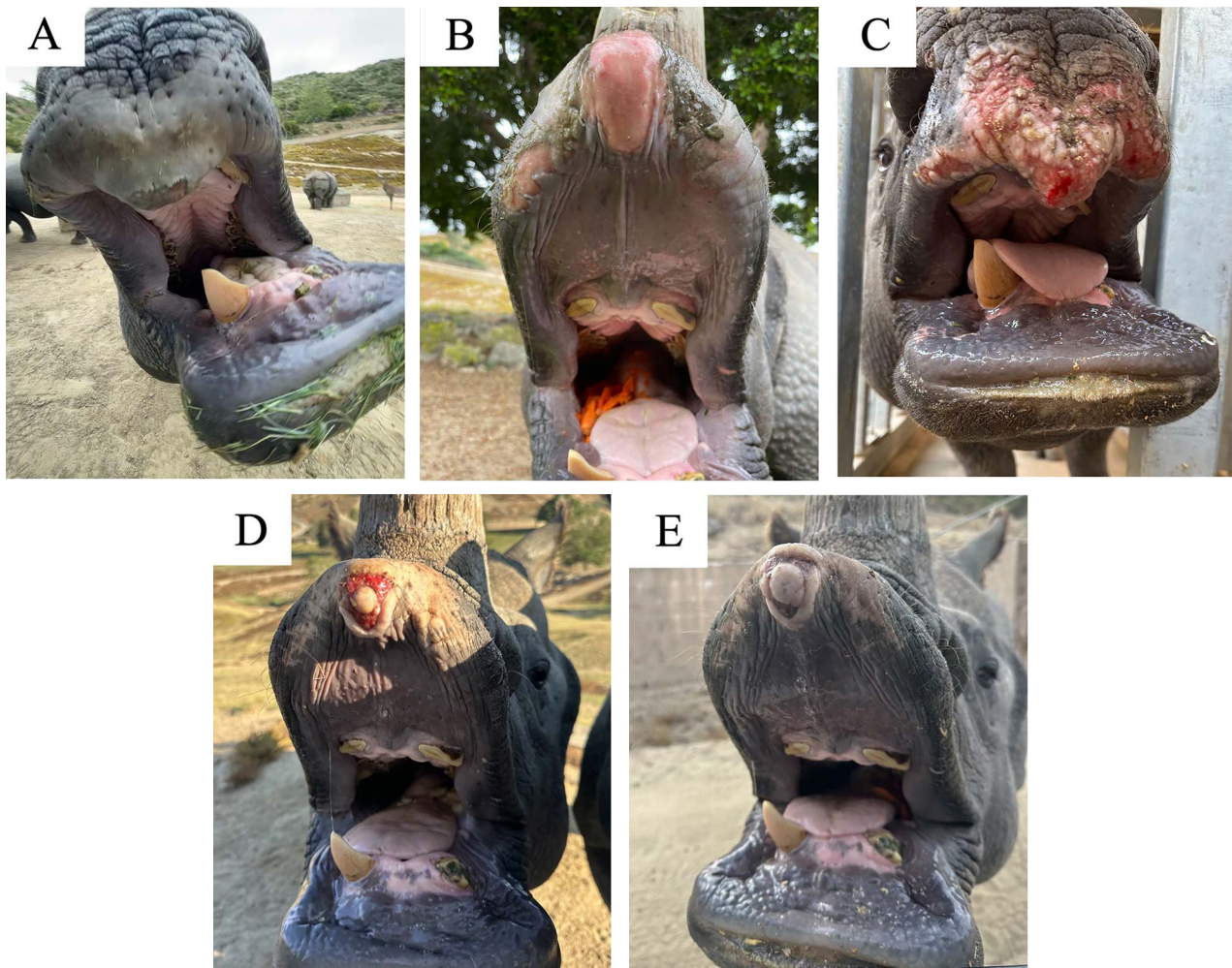
Eight rhinoceros had lesions in only 1 anatomic location, while eleven rhinoceros had lesions in at

least 2 locations. There were no consistent patterns of primary and secondary lesion locations. Healing time was fastest for cutaneous axillary lesions and most prolonged for the sole and heel lesions.

### Clinical signs

Clinical signs included lethargy (n = 11), lameness (8), hyporexia to anorexia with several showing difficulty in food prehension (8), ptyalism (5), and head shaking secondary to an ear lesion (1).





**Figure 4**—Progression and resolution of a severe lip ulceration secondary to vesicular stomatitis virus in a greater one-horned rhinoceros. Photos taken from day 1 (A), day 2 (B), day 14 (C), day 27 (D), and day 45 (E) from initial clinical signs.

Lameness associated with the severe heel and sole lesions took the longest to resolve. Mild lethargy, decreased appetite, and eating gingerly improved within 1 to 2 days in 5 animals. In many instances, nonspecific clinical signs such as lethargy, hyporexia, isolation from the herd, or lameness were observed shortly before the observation of ulcerative lesions.

### Diagnosics

Twenty animals were sampled for VSV PCR from swabs of lesions, 10 of which were positive on their initial PCR for VSNJV. All PCR-tested animals were negative for VSIV and FMDV. Of the 10 animals that were PCR positive, 3 were positive on the corresponding virus isolation. Five animals also had blood samples submitted for viral serology (ELISA). All 5 animals were seropositive for VSNJV and negative for VSIV and FMDV on ELISA. Samples with a percent inhibition  $\geq 50\%$  were considered positive, and samples  $< 50\%$  were considered negative. One animal had evidence of seroconversion on day 11 following the onset of clinical signs. Of the 5 seropositive

rhinoceros, 3 (60%) were documented positive for VSNJV on PCR from swabs of lesions. Of the animals that tested positive on PCR, 9/10 (90%) were sampled again at various time points throughout their clinical disease course depending on accessibility and safety of sample collection. The time to a negative PCR ranged from 9 to 25 days. One animal was persistently positive at day 46 on swabs of her sole lesions despite healing of the lesions and resolved systemic disease.

### Clinical pathology and histopathology

CBCs, chemistry profiles, and fibrinogen were assessed in 6 animals during the outbreak. These samples were collected from 2 animals that were severely affected and sampled under anesthesia and 4 animals that were mild to moderately affected and accessible for sampling under behavioral restraint. The major clinicopathologic findings were hyperfibrinogenemia ( $> 300$  mg/dL) in 5/6 (83%) animals and severe neutrophilic left shift with relative percentages of band neutrophils reaching up to 19% in 2/6 (33%) animals. Histopathology was performed

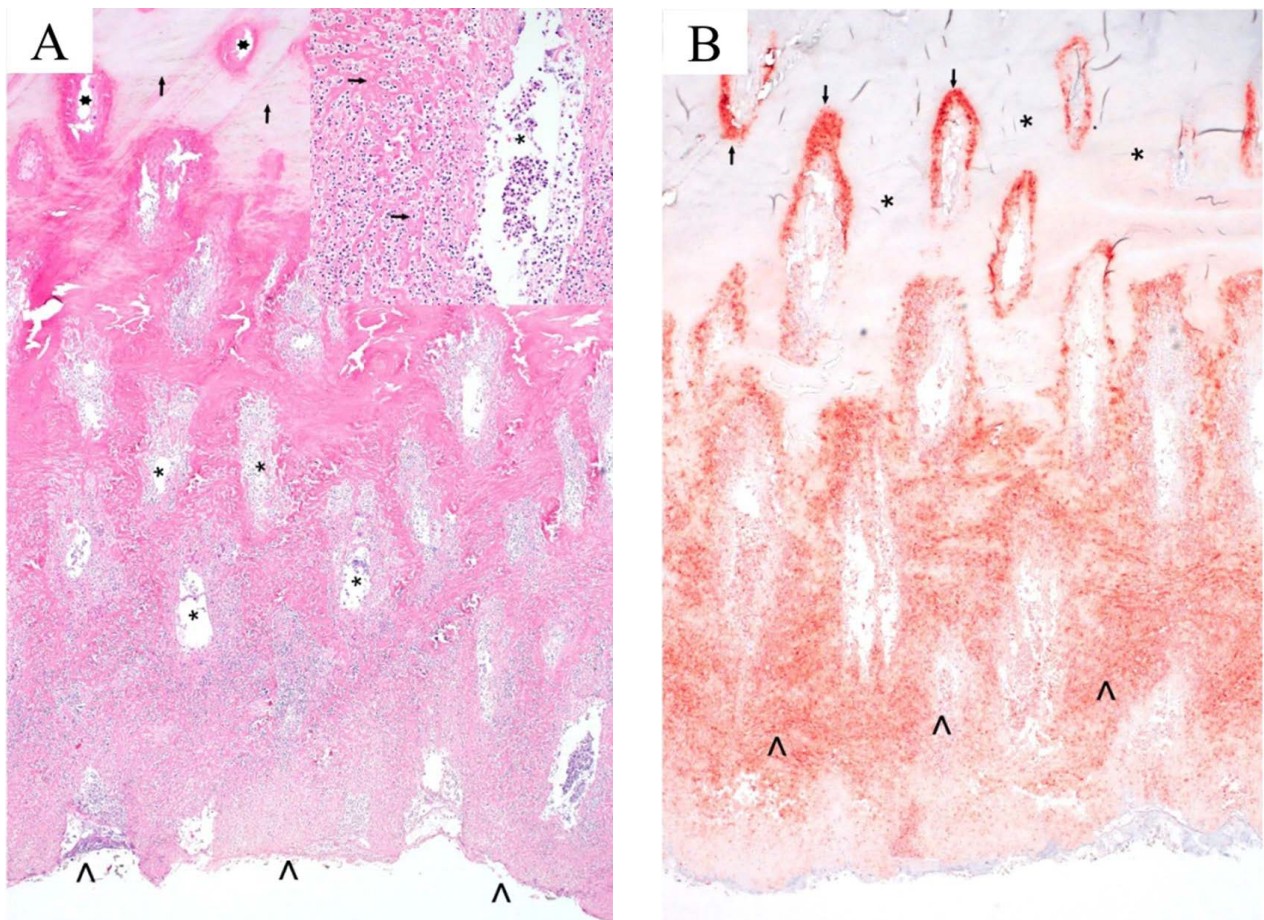


on samples from 2 cases with sole lesions and 1 animal with a cutaneous crust on the distal limb. Histologically, biopsied skin and foot pad lesions were similar in all examined sections from all animals. Changes included extensive areas of acute epidermal necrosis and suppurative inflammation with numerous sometimes coalescing vesicles separating cords of necrotic cells in the suprabasilar regions. The deep margins frequently contained dense colonies of mixed bacteria mixed with foreign debris adhered to the sloughed epidermis (**Figure 5**). Sections from 1 foot pad biopsy were probed with RNA ISH showing intense positive signal throughout the sections, specific to epidermal cells and necrotic and inflammatory debris, while lacking any signal or staining of unaffected regions.

### Treatment

Seventeen animals required treatment during the VSV outbreak. Analgesics were prescribed for

14/26 cases and included firocoxib (n = 14; 0.1 mg/kg, PO, once a day), meloxicam (1; 0.1 mg/kg PO, once a day), flunixin meglumine (3, 0.8 to 1 mg/kg, PO, once a day; 2, 1 mg/kg, IM; 2, 1 mg/kg, IV; and 1, 1 mg/kg, topically, once a day), and/or gabapentin (6; 5.6 mg/kg, PO). Topical wound care included betadine scrub (BD-EZ Scrub Povidone-Iodine Brush/Scrub; Becton Dickinson Co; n = 8), silver sulfadiazine cream (8), Silver Honey Rapid Wound Repair Spray Gel (Absorbine; 8), HoneyHeel honey-based barrier cream (Red Horse Products Ltd; 8), and/or mouthwash solution containing diphenhydramine, aluminum hydroxide, magnesium hydroxide, simethicone liquid, and lidocaine (4). Topical insecticide was used opportunistically (SWAT; Farnam Companies). Two SWR required systemic antibiotic therapy with trimethoprim sulfamethoxazole (n = 2; 30 mg/kg, PO, once a day), 1 also received cefazolin (1; 2.5 mg/kg, IV, once a day) and ceftiofur crystalline free acid (1; 7 mg/kg, SC, q 1, per week). This



**Figure 5**—A—Hematoxylin and eosin foot pad biopsy from a southern white rhinoceros with vesicular stomatitis virus. Section showing diffuse necrotizing dermatitis with numerous pockets of necrotic and inflammatory debris throughout the section (presumed vesicles, asterisks) and including tubular horn (stars). Arrowheads mark the basal aspect of the section. Keratinocytes of the intertubular horn (vertical arrows) are intact. Hematoxylin and Eosin, 4X magnification. Inset showing higher magnification of dis cohesive cords of epidermal cells (horizontal arrows) separated by acute inflammatory cells and debris that coalesce into large pockets (asterisk). Hematoxylin and eosin; 20X. B—In situ hybridization foot pad biopsy. Serial section from (A) shows extensive punctate red signal throughout the diffusely necrotic layers (arrowheads), with intense staining of the necrotic cortex of tubular horn (arrows) and sparing of signal in the intact intertubular horn (asterisks). V-VSV-O2-PP probe; hematoxylin counterstain, 4X magnification.

animal and another SWR required multiple anesthetic procedures to provide intensive wound care, including the application of custom-made boots.

## Discussion

Of the 26 rhinoceros housed at this facility, all were considered at least suspect VSV cases. Of these cases, 10 were PCR positive for VSNJV for a confirmed prevalence of 38%. However, 6 additional animals were considered probable cases (based on clinical signs, gross lesions, and serology) for a probable prevalence of 62%. The authors suspect that confirmed disease prevalence in this outbreak is falsely decreased due to inherent sampling difficulties. Several animals with gross lesions identical to those seen in positive cases tested negative on PCR, likely from insufficient swab contact with the lesion. In most cases, samples were obtained opportunistically in the field where contact time and pressure on the surface of the lesions was limited, and the lesions were often partially covered by debris. Sampling of oral lesions may have been complicated by chewing motion and the presence of food in the oral cavity. Several animals were unapproachable without sedation or anesthesia and therefore were not sampled despite the presence of gross lesions and clinical signs consistent with rhinoceros that tested positive for VSV using PCR.

This VSV outbreak had a high morbidity and no mortality. In domestic species, the VSV morbidity rate is variable and can range from 5% to 90% within a single herd.<sup>13</sup> Mortality is low in outbreaks involving cattle and horses,<sup>10</sup> with higher rates of mortality in pigs.<sup>3</sup> Rhinoceros are taxonomically similar to equids, both are within the order Perissodactyla. During the outbreak at this facility, no other species, including several equid species, were confirmed positive for VSV despite a proximity of less than 5 miles to the nearest VSV-positive domestic equid premise and adjacent habitat housing to VSV-positive rhinoceros. While oral and foot lesions, particularly coronary band and interdigital areas, are common across species, in rhinoceros the foot lesions also affect the heel and sole.<sup>2</sup> Multifocal distal limb and ventrum swellings, which progressed to linear cracks and ulcerations, were observed in the rhinoceros. Rhinoceros have an extensive thick vascular dermis with an overlying thin epidermis, which may predispose them to developing this unique pattern of lesions.<sup>14</sup> Dissimilar to cattle outbreaks, no mammary gland involvement was noted in the rhinoceros.<sup>1</sup>

For rhinoceros with lesions in more than 1 anatomical location, it is unknown whether the secondary site was from the same episode of viremia, or if there was a separate inoculation event by an insect vector or other form of transmission.

Clinical signs were similar to those reported in domestic species and were correlated with lesion location. Disease course was similar to that reported in domestic species, with oral lesions healing faster than foot lesions. However, overall time to resolution was longer in the rhinoceros species, an average of

48 days, compared to 2 to 3 weeks in most domestic species.<sup>1</sup> In many domestic species, hyperthermia is an initial clinical sign typically presenting on day 1 and resolving within 1 to 3 days of onset.<sup>1</sup> Five of the rhinoceros in this cohort had a comparatively shorter clinical sign duration than other clinical rhinoceros, with lethargic behavior, hyporexia, or eating gingerly presenting and resolving within 24 to 48 hours. It is possible that these animals were experiencing brief viremia but ultimately did not develop gross lesions. In experimentally infected horses, viral shedding stopped and was no longer detectable via PCR by day 10.<sup>15</sup> The rhinoceros in this outbreak had significantly longer periods of PCR-detectable VSV shedding, with animals testing positive 8 to 46 days after the initial positive PCR result. Follow-up testing to confirm eventual negative PCR status was opportunistic and sporadic and was a significant limitation in identifying a clear endpoint of viral shedding. Most of the affected animals required some level of medical treatment, with oral nonsteroidal anti-inflammatories being the most common. Firocoxib was most frequently prescribed due to its ease of administration. Flunixin meglumine, however, was anecdotally more effective at managing the perceived pain associated with VSV lesions. Antibiotics were only prescribed for 2 animals based on the severity of their lesions and marked left shift. The combination of a topical betadine scrub and silver honey spray was the most effective topical treatment for accessible cutaneous lesions and subjectively improved healing time. One rhinoceros with poor oral medication compliance was treated with transdermal flunixin solution with no adverse effects noted, but efficacy was difficult to evaluate and drug absorption was not assessed.

Recommendations for VSV prevention and control in domestic species mostly involve vector control and exclusion by housing vulnerable animals in vector-proof shelters.<sup>16,17</sup> Access to pasture or water increases the odds of developing clinical disease during an outbreak.<sup>17</sup> About half of the rhinoceros in this outbreak had variable access to an indoor barn, while the field habitat rhinoceros did not. Despite this, clinical disease was seen in rhinoceros in both habitat locations at this facility. In domestic species outbreaks, areas where owners report an increase in insect populations have a greater risk of developing disease.<sup>17</sup> The strategies for reducing VSV risk are of variable efficacy; examples include frequently removing manure, keeping vegetation low to the ground, using topical insecticide, decreasing surrounding standing water, and making various husbandry changes to indoor barns such as the use of fans, lower lighting, and keeping doors closed.<sup>5,18-19</sup> Understanding the local insect species is important for reducing the severity of a VSV outbreak, as the efficacy of mitigation techniques depends on the type of biting insect responsible for the outbreak.<sup>20-21</sup> Vector ecologists at the County of San Diego's Vector Control Program anecdotally noticed higher *Simulium* spp and *Culicoides* spp counts in both carbon dioxide-baited encephalitis virus surveillance



traps and gravid traps in 2023 than in recent years (Nathan McConnell, PhD, Vector Ecologist, County of San Diego, unpublished data, January 13, 2024). As VSV transmission is incompletely understood but likely involves direct and indirect methods, separation and isolation of affected animals is recommended.<sup>5</sup> Individual animal quarantine was not possible in this rhinoceros group due to the number of animals affected and the lack of vector-proof housing. The safari park facility was placed under state-mandated quarantine with no outgoing mammal movement until all VSV lesions had resolved.

VSV should be included as a differential diagnosis for skin or oral lesions in rhinoceros presenting in areas of VSV risk. This outbreak emphasizes the importance of knowing the disease status of the local animal population surrounding a zoological institution and maintaining good communication with local, state, and federal veterinarians. VSV is a notifiable disease due to its close resemblance to FMDV. The rhinoceros population in this series had high morbidity and no mortality associated with VSV infection. In addition to the direct health impact on these animals, VSV had a broader impact on this animal institution by limiting rhinoceros and other mammal movement within and outside of the premises, all of which can be an impediment to population management of critical species.

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## **Supplementary Materials**

Supplementary materials are posted online at the journal website: [avmajournals.avma.org](http://avmajournals.avma.org)



**Supplemental table 1** -Signalment, clinical signs, lesion location, and diagnostic results in an outbreak of vesicular stomatitis virus in 21 Southern white rhinoceros (SWR; *Ceratotherium simum simum*) and 5 greater one-horned rhinoceros (GOHR; *Rhinoceros unicornis*).

<u>Species</u>	<u>Age (v)</u>	<u>Sex</u>	<u>Case type</u>	<u>Clinical signs</u>	<u>Clinical sign severity</u>	<u>Lesion location(s)</u>	<u>Lesion severity</u>	<u>PCR results</u>	<u>Virus isolation results</u>	<u>Serology results</u>	<u>Other results or notes</u>
SWR	3	M	C	Dysphagia, hyporexia, ptyalism, lesions	Mod	Oral cavity, forelimbs	Severe	Positive	Negative	NA	*
SWR	4	M	C	Dysphagia, hyporexia, ptyalism, lesions	Mod	Oral cavity	Severe	Positive	Positive	NA	Normal CBC/chemistry; insufficient sample for serology
SWR	6	F	C	Lethargy, anorexia, ptyalism, lesions	Severe	Lips and oral cavity	Severe	Positive	Negative	NA	*
SWR	8	F	C	Lethargy, hyporexia, ptyalism, lesions	Mod	Oral, lips, nostrils	Mod	Positive	Negative	Positive	HF (500)
SWR	13	F	C	Lameness, lethargy, otitis, lesions	Mod	Limbs, inguinal and ventrum, ears	Severe	Positive	Negative	NA	HF (500); HP (cutaneous crust from inguinal region): epidermal necrosis and serocellular crusting
SWR	14	F	C	Lameness, lethargy, lesions	Severe	Coronary bands, heels, multifocal skin, vulva	Severe	Positive	Positive	Positive	HF (600); LS (19%); HP (sole): vesicular pododermatitis; positive ISH (solebiopsy)

SWR	16	F	C	Lameness, lesions	Mod	Coronary bands, multifocal cutaneous	Severe	Positive	Negative	NA	HF (500); LS (8%)
SWR	25	F	C	Hyporexia, dysphagia, lesions	Mild	Multifocal cutaneous (lateral thorax, axillary)	Mild	Positive	Negative	NA	*
SWR	28	M	C	Lameness, lesions	Mod	Inguinal limbs, coronary band	Mod	Positive	Negative	Positive	HF (600)
SWR	13	F	P	Lesion only	NA	Chin, limbs	Mild	Negative	Negative	NA	*
SWR	1y	F	P	Hyporexia, ptyalism, lesions	Mod	Oral cavity, nostril	Severe	Negative	Negative	NA	*
SWR	2	F	P	Lesion only	NA	Nostril	Mild	Negative	Negative	NA	*
SWR	8	F	P	Lameness, lesions	Severe	Sole and heel	Severe	Negative	Negative	Positive	HP (skin): vesicular epidermitis and dermatitis
SWR	15	F	P	Lesions only	NA	Limbs	Mild	Negative	Negative	Positive	*
SWR	16	F	P	Lameness, lesions	Mild	Lip, limbs	Mild	NA	NA	NA	*
SWR	3	M	S	Hyporexia (1d)	Mild	Neck and chest	Mild	Negative	Negative	NA	Lesions suspected to be seasonal fly bite hypersensitivity; seen 6 weeks after initial outbreak presentation
SWR	3	F	S	Lethargy, hyporexia (1d)	Mild	None observed	NA	NA	NA	NA	*



SWR	4	F	S	Lesion only	NA	Horn base	Mild	Negative	Negative	NA	Lesion suspected to be trauma
SWR	8	F	S	Lesion only	NA	Neck	Mild	NA	NA	NA	*
SWR	17	F	S	Lethargy, dysphagia (1d)	NA	None observed	NA	NA	NA	NA	*
SWR	26	F	S	Lethargy, dysphagia (1d)	Mild	None observed	NA	NA	NA	NA	*
GOHR	17	F	C	Lameness, lethargy, dysphagia, hyporexia, lesions	Mod	Oral, lip, heel and sole	Mod to Severe	Positive	Positive	NA	*
GOHR	7	F	P	Lameness, lethargy, lesions	Mod	Oral, coronary bands	Mod	Negative	Negative	NA	*
GOHR	2	M	S	Lethargy (1d); possible lesion	Mild	Oral	Mild	Negative	Negative	NA	Lesion suspect trauma from browse; chronic pathology for this rhinoceros
GOHR	8	M	S	Lesions only	NA	Multifocal skin	Mild	Negative	Negative	NA	Concurrent suspected chronic allergic dermatitis
GOHR	19	F	S	Lethargy (1d)	Mild	None observed	NA	NA	NA	NA	*

Case classification: C= confirmed, P = probable, S=suspect

Severity: Mild; Mod=moderate; Severe

HF: Hyperfibrinogenemia (Fibrinogen > 300 mg/dL)

NLS: Neutrophilic left shift (relative % of band neutrophils > 2%)

HP: Histopathology

ISH: *in situ* hybridization

\*= indicates that no further diagnostics were performed.