CASE REPORT

Conjunctival habronemiasis in a square-lipped rhinoceros (*Ceratotherium simum*)

Igal H. Horowitz,* Richard R. Dubielzig,† Ana-Maria Botero-Anug,‡ Araceli Lucio-Forster,§ Dwight D. Bowman,§ Ariella B. Rosenzweig,* Shahar Frenkel¶ and Ron Ofri**

**Tel-Aviv Ramat-Gan Zoological Center (Safari), Ramat-Gan 52109, Israel; †Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin, Madison, WI 53706, USA; ‡PathoVet, Rehovot 76391, Israel; §Department of Microbiology and Immunology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, USA; ¶Department of Ophthalmology, Hadassah-Hebrew University Medical Center, Jerusalem 91120, Israel; and **Koret School of Veterinary Medicine, Hebrew University of Jerusalem, Rehovot 761001, Israel*

Address communications to:
R. Ofri
Tel.: ++972 54 8820523
Fax: ++972 8 9467940
e-mail: ron.ofri@mail.huji.ac.il

Abstract

A captive female square-lipped rhinoceros born in 1993 had been showing intermittent signs of bilateral conjunctivitis and conjunctival proliferation since 1998. Periodic improvement was noted, especially in winter, but overall the condition had deteriorated over the years. Treatment with various topical, intralesional, and systemic antibiotics and glucocorticosteroids was largely ineffective, as were repeated dewormings. No primary cause for these lesions was found in biopsies taken in 2000 and 2006, although a severe infiltrate of numerous eosinophils was observed in the latter. As the condition worsened, secondary corneal changes were noted, and eventually vision was lost due to proliferative conjunctival tissue. Aggressive resection of the proliferating tissue in 2013 restored vision and submitted biopsies yielded a diagnosis of severe allergic conjunctivitis, eosinophilic granuloma, and habronematid (*Habronema* or *Draschia*) larval infection. As no other rhinoceros in the herd was affected, including two calves born to the patient who were in close contact with their mother, it was concluded the presentation was most likely due to a hypersensitivity reaction to the dead or dying larvae. Fly repellent is now regularly applied around the eye of this rhinoceros, and a protective face mask has been fitted. Ongoing periodic relapses are treated with oral ivermectin, topical antibiotics, and steroids.

Key Words: conjunctiva, *Draschia*, *Habronema*, hypersensitivity, ivermectin, rhinoceros

INTRODUCTION

The white rhinoceros (*Ceratotherium simum*), also known as the square-lipped rhinoceros, is the largest member of the Rhinocerotidae family. The species, which is native to Africa, consists of two subspecies, the southern white rhinoceros, which is the most common rhinoceros subspecies in existence, and the northern white rhinoceros which has dwindled to only a few remaining individuals.[1]

Reports of ocular disease in rhinoceroses are infrequent and include two cases of corneal ulcers that were treated surgically[2,3] and a case of enucleation following proptosis.[4] The paucity of reports may stem from the rarity of this species, as well as the paucity of published baseline ocular data, which to date includes only determination of visual acuity based on ganglion cell density,[5] as well as the refractive error,[6] intraocular pressure, and Schirmer tear test values.[7] The aim of this paper was to describe a case of conjunctival habronemiasis in a southern white rhinoceros.

CASE REPORT

A female southern white rhinoceros born in 1993 in South Africa's Unfoluzi National Park was transferred in September 1998 to The Tisch Family Zoological Gardens, Jerusalem, Israel. The animal was transferred again in September 2003 to the Zoological Center Tel-Aviv Ramat-Gan (Safari), Israel. The rhinoceros herd of the Safari includes 10 animals living in an open area, about

200 acres in size, together with about 650 other herbivorous animals of various species including antelopes, zebras, and hippopotami. The animals are free ranging in this area, and come in regular contact with each other. Their diet includes grain or oat hay, alfalfa, and bovine concentrate pellets (14% protein). Licking stones providing mineral supplementation (Solsel, European Salt Company, Hannover, Germany) are available and water *ad libitum*. Many of the herbivorous animals in the area share the same food lines. All of the Safari's herbivores are regularly dewormed with 0.1% fenbendazole (Vetmarket, Segula, Israel) incorporated into the bovine concentrate pellets. This drug is administered in a 26 day cycle (consisting of 6 days of treatment, 14 days without treatment and six more days of treatment) that is repeated every 6 months.

In September 2007, the animal gave birth to a healthy male calf. She miscarried in May 2009, but an autopsy on the 61 kg fetus did not reveal any infectious causes known to cause abortion. In June 2012, she gave birth to a second healthy male calf. To date, this animal is the only fertile female in the Safari's rhinoceros herd. Other than the abortion and the adnexal disease, her medical history is unremarkable.

In December 1998, keepers at The Tisch Family Zoological Gardens first noticed signs of intermittent, bilateral conjunctivitis. These included serous discharge, chemosis, and congestion of conjunctival vessels, which were more severe in the right eye. The animal was trained to accept topical medications, and treatments with various antibiotics and anti-inflammatory drugs, including ciprofloxacin, tetracycline, gentamycin, and dexamethasone, were attempted. Oral trimethoprim sulfa (TMS Pure substance 100%, Teva, Kfar Saba, Israel) were also prescribed on several occasions. Regardless of the drug administered, treatment would usually result in temporary relief, but the signs would recur soon after. In September 2000, the animal was anesthetized for a complete ocular examination. A proliferative mass was removed from the third eyelid of the left eye, and although detailed cytological and/or histopathological reports are not available, no parasites were seen. No signs of ocular disease, other than the conjunctivitis and the single proliferative mass, were noted. The corneal examination was unremarkable, and no foreign bodies were found. *Moraxella bovis* was cultured at the time, and both eyes were treated with topical 5% chloramphenicol ointment (Synthomycine 5% Rekah Pharmaceutical Ind. LTD. Holon, Israel).

Following the animal's transfer to the Safari, the problem in the left eye resolved, but deteriorated slowly in the right eye, where significant proliferation of the conjunctiva of both eyelids could be observed (Fig. 1). The globe itself seemed to be unaffected. In September 2006, the animal was anesthetized again for a comprehensive examination, using an intramuscular injection of 12 mg detomidine (Orion Pharma, Espoo, Finland) and 12 mg butorphanol (Fort Dodge Animal Health, Fort Dodge, IA, USA) followed by an intramuscular injection of 0.7 mL Large Animal Immobilon (containing 2.45 mg/mL etorphine HCl and 10 mg/mL acepromazine maleate; Novartis Animal Health, Litlington, UK). No abnormalities were noted in the left eye. In the right eye, severe proliferation of the palpebral conjunctiva of both the upper and lower eyelids was noted, with several foci of ulceration and hemorrhage. The bulbar conjunctiva and third eyelid were not affected by the proliferative process. Mild corneal pigmentation and vascularization were observed in the right eye, and these findings were deemed to be secondary to irritation by the proliferating conjunctival lesion. Four long-term slow-release dexamethasone implants (BRAVO polymer loaded with 1 mg of dexamethasone; Nicast Ltd., Lod, Israel) were implanted in the palpebral conjunctiva of both eyelids of the affected eye. Anesthesia was reversed with an intravenous injection of 20 mg atipamezole (Veterinary Antisedan; Orion Pharma) and 200 mg naltrexone (Kyron Laboratories, Benrose, South Africa). A blood count performed at the time was within normal limits, except for eosinophilia. A biopsy of the palpebral conjunctiva from the right eye revealed severe and diffuse infiltrate of numerous eosinophils, aggregations of lymphocytes and reactive fibrosis.

Figure 1. Severe proliferative reaction of the conjunctiva of the right eye of a square-lipped rhinoceros in 2006. The globe, including the bulbar conjunctiva, is unaffected.

Since then, the condition of the right eye has fluctuated, with repeated improvements without resolution, and subsequent deteriorations. The fluctuations were seasonal, with deterioration usually beginning in April and peaking in June–August, annually, before improving once more in autumn and winter. In May 2012, for the first time since the animal's arrival from Jerusalem, signs of mild inflammation were also noted in the left eye. However, the male calf (born in 2007) was never affected, even though it was

in constant contact with its mother, nor was any other rhinoceros in the herd affected.

Long-term topical treatment with tetracycline ointment, dexamethasone solution, and fly repellent, administered during the spring and summer outbreaks, had no noticeable effect. The animal was regularly dewormed with fenbendazole twice a year, together with the rest of the Safari's herbivores, as previously described. In addition, as parasitic infection was suspected, it also received oral ivermectin (1% oral solution, 1 mL/50 kg [200 μg/kg], IL Medi Market, Netanya, Israel) that was mixed with her food weekly over 6 weeks once or twice a year. Treatment with triamcinolone acetonide cream was attempted once, but was suspended because it caused a severe local reaction.

Severe conjunctival proliferation eventually covered both corneas, causing loss of vision in both eyes (Fig. 2). In June 2013, the animal was anesthetized for a third time to attempt excision of the lesion. Anesthesia was induced with an intramuscular injection of 25 mg detomidine (Orion Pharma) and 25 mg butorphanol (Fort Dodge Animal Health) and maintained with 100 mg of intravenous ketamine (Vetoquinol, Lure, France). Conjunctival findings in both eyes were similar to those seen in the right eye in 2006. Once again, the bulbar conjunctiva was unaffected, although this time the process also involved both third eyelids. Following aggressive debulking of the palpebral conjunctiva of both eyes and excision of the third eyelid of the left eye, we were able to see signs of bilateral chronic keratitis, including corneal fibrosis, pigmentation, and superficial vascularization. However, in both eyes the cornea was fluorescein negative, and the rest of the globe seemed to be unaffected. Four periorbital injections, 1 mL each, that included diphenhydramine (Diphenhydramine HCl, 100 mg/mL; Tamar Marketing, Rishon LeZion, Israel), amoxicillin (Amoxy LA Veterinary 150 mg/mL, Norbrook, Ireland), enrofloxacin (Baytril 50 mg/mL; Bayer HealthCare, Leverkusen, Germany), and methylprednisolone (Depo-Medrone V, 40 mg/mL; Pharmacia, Puurs, Belgium) were administered in both eyes. No attempt was made to suture the site, and tamponade was used to achieve hemostasis. Anesthesia was reversed with an intravenous injection of 50 mg atipamezole (Veterinary Antisedan; Orion Pharma), 200 mg naltrexone (Kyron Laboratories), and 25 mg of subcutaneous atipemazole.

Figure 2. Advanced proliferative process in the right eye of a square-lipped rhinoceros in 2013. The globe is no longer visible, and ulceration and hemorrhage of the lesion are evident.

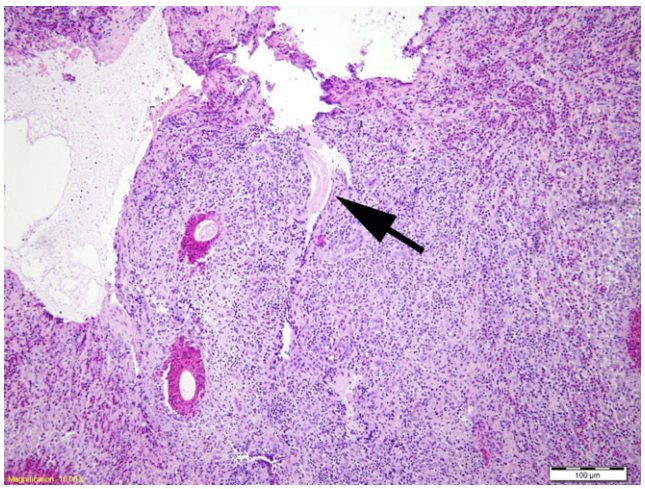

Figure 3. Histological section of biopsied material from the eye of a square-lipped rhinoceros suffering from chronic adnexal disease. Note the heavy diffuse eosinophilic infiltrate throughout and the two degenerate larval fragments (one transverse (left) and one longitudinal (arrow)), characteristic of habronemiasis.

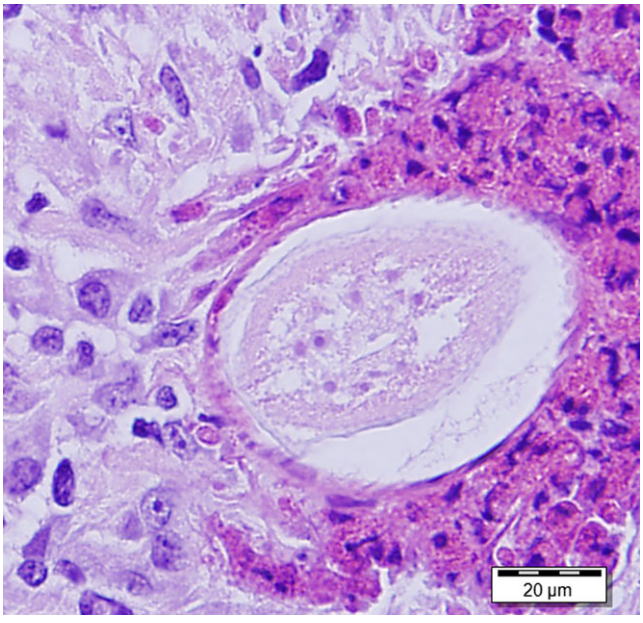

Figure 4. Transverse section through a degenerate habronematid larva recovered from biopsied material from the eye of a square-lipped rhinoceros suffering from chronic adnexal disease.

Figure 5. The patient fitted with a homemade face mask to minimize contact of flies with the eyes.

Histological evaluation of the excised conjunctiva showed reactive fibrosis and diffuse eosinophilic infiltration throughout the substantia propria. Larger eosinophil-rich granulomas surrounding transverse and vermiform longitudinal sections of parasites were also observed. These were identified as larvae due to their small size (approximately 60 μm in diameter) and the lack of reproductive organs. Seventeen to twenty small ridges were apparent on the cuticle on each lateral side of the larva. (Figs 3 and 4).

Following the procedure, significant improvement was noted in both eyes, and the animal became visual again, but two relapses have been documented since. These were treated with oral ivermectin, topical antibiotics, and steroids. Additionally, fly repellent is now regularly applied around the eyes, which are further protected by a custom made face mask (Fig. 5).

DISCUSSION

Although adnexal habronemiasis is typically diagnosed in horses, there are case reports of cutaneous and periocular *Habronema* infections in additional species including donkeys, mules and zebras,[8] a dromedary camel[9], and a dog.[10] The authors are not aware of any reports of (ocular or nonocular) habronemiasis in rhinoceroses and have been able to find just a single case report of a rhinoceros infected with *Parabronema roundi*, an intestinal parasite member of the *Habronematidae* family.[11,12]

In the present case, because of the severe proliferative reaction, and as no other rhinoceros (including neither calf) was affected, infectious conjunctivitis was considered unlikely. The three leading differentials for the presentation were neoplasia, a foreign body reaction or a hypersensitivity reaction. The first two differentials were suggested because the patient was the only affected animal in the Safari's rhinoceros herd, but these diagnoses were less probable due to the long-standing, seasonal, and occasionally bilateral presentation. Instead, in view of the seasonal manifestation and the pathology report from 2006, a hypersensitivity reaction was considered the most likely diagnosis. Based on the clinical presentation and the exuberant conjunctival proliferation, habronemiasis was strongly suspected, yet several counter-arguments were offered against parasitic infection: The patient had received regular dewormings with nematocidal drugs, it was the only affected rhinoceros in the Safari's herd, and no larvae had been detected in biopsies taken in 2000 and 2006. Habronemiasis, however, is regarded to be at least in part a hypersensitivity reaction to dead or dying larvae,[13–15] so it is possible that the disease was actually triggered by the nematocidal treatments, or the patient's innate immunity. On the other hand, as fenbendazole is not the treatment of choice for nematodes involved in habronemiasis, such treatments may not have been fully efficacious. Furthermore, it is frequently observed that individual horses in a herd are predisposed to yearly recurrences of habronemiasis,[14,16] perhaps associated with the presence of adult worms in the stomach, or due to a genetic predisposition;[8] thus, differential individual susceptibility seems the rule, rather than the exception. Lastly, with respect to larval detection, larvae are typically found in only 50% of specimens examined for habronemiasis[13,17] For example, examination of biopsies from 25 horses revealed nematodes in only 11 specimens.[14]

While a presumptive diagnosis of habronemiasis is strongly suggested by a history of seasonal, nonhealing, and recurring granulomatous skin or adnexal lesions, definitive diagnosis relies on finding habronematid larvae associated with these reactions.[8,16] The nematodes we observed in histologic sections from the resected tissue in 2013, though fairly degenerate, could be identified as larvae with cuticular ornamentation and morphological characteristics consistent with those of the Habronematinae subfamily of spirurid nematodes, such as *Habronema* or *Draschia*, as described and depicted by Gardiner and Poynton.[18] Other nematodes potentially associated with similar dermal or adnexal lesions might include *Pelodera* or *Strongyloides* larvae, *Halicephalobus* larvae and adults, and the developing stages of the filariids, *Onchocerca* and *Parafilaria* (which is present in Africa). As noted above, the nematodes observed in the present case were larvae. The larvae of *Pelodera* and *Strongyloides*, which might be found in tissue, would be smaller in size and have marked double lateral alae.[18] *Halicephalobus* larvae can also be ruled out as they too would be significantly smaller; adult worms of this genus are only 20 μm in diameter.[19] Lastly, filariid nematodes such as *Onchocerca* and *Parafilaria* lack longitudinal ridges.[20] Therefore, the size, cuticular ornamentation, location of these larvae, and tissue reaction elicited, allow their identification as habronematid larvae,

most likely *Habronema* or *Draschia*. Obviously, further confirmation of the diagnosis could be made through PCR testing. However, obtaining fresh samples from the animal would entail the potential risk of another general anesthesia. Extraction of DNA from the paraffin sections was not attempted as the samples had been fixed in formalin for a long time. Also, we expected to find few larvae or larval pieces in the block or individual sections, and all previously observed larvae were in advanced stages of decomposition. Furthermore, GenBank has sequences for only two *Habronema* species and none for *Draschia*, making even a generic identification unlikely. We propose that successful molecular characterization of recovered larvae, which we suspect to be unlikely, is not a prerequisite for a definitive diagnosis of habronemiasis in a case where larvae are observed in biopsies of lesions typical of this infection, would provide no other information than a verification of the morphological identification at best, and would not have any significant effects on the management of this case and the treatment of adnexal habronemiasis.

Habronemiasis is a common cause of ulcerative cutaneous and adnexal disease in horses in temperate and tropical areas worldwide.[8] The disease is the result of infection with larvae of the habronematid nematodes *Habronema muscae*, *H majus*, or *Draschia megastoma*.[13] The adult stages of these parasites inhabit the stomach, producing eggs and larvae which are passed with the feces. Larvae must then be ingested by the maggots of flies. These insects serve as intermediate hosts for the nematode, where larvae develop into the infective third larval stage.[13,21] Patent infection in horses is generally acquired through inadvertent ingestion of dead flies in watering troughs or from larvae deposited on the lips by feeding flies. Infected flies feeding on the conjunctiva or abraded periocular skin, however, may deposit infective larvae at these sites resulting in adnexal disease[13,21] due to a granulomatous inflammatory reaction to the larvae in these tissues.[17] The disease may present as solitary or multiple lesions characterized by ulceration, exudation, intermittent hemorrhage, exuberant granulation, and pruritus.[14] Lesions tend to be nonhealing and occur seasonally in the months of highest fly activity, typically subsiding in colder months.[8] These lesions often include small yellow plaques containing necrotic, caseous, or calcified material surrounding nematode larvae.[13,14] These plaques were not evident in our patient.

As larvae were only detected in 2013, it is possible that the animal had been suffering from conjunctivitis of unknown etiology since 1998; we cannot rule out the possibility that a parasitic infection occurred only at a later stage, perhaps when the animal was moved from the Tisch Family Zoological Gardens in Jerusalem to the Safari in Ramat Gan, or even later, as flies would be attracted to the inflamed conjunctiva. Such infection would have been facilitated by the intermittent, long-term topical steroid treatment that had been administered over the years.

The recommended treatment for conjunctival habronemiasis is a combination of surgical debulking, possibly with cryotherapy, and topical, intralesional or systemic anti-inflammatory drugs to control the inflammatory responses to the larvae.[13,14,17] Ivermectin (or moxidectin) is the parasiticide of choice for these infections.[8] Although the patient received various modalities of anti-inflammatory treatments over the years, we suspect that the biopsies collected in 2000 and 2006 did not constitute sufficient surgical debulking, and therefore were not curative. Alternatively, multiple instances of infection may have taken place in an individual that is hypersensitive to this parasite. We are hopeful that the radical debulking performed in 2013, combined with aggressive anti-parasitic and anti-inflammatory treatment, will prove to be curative, and that fly control and the protective face mask will prevent reinfestation. However, should relapses occur, transportation of the animal to a country with colder climate and lower prevalence of habronemiasis will be considered.

Our findings indicate that habronemiasis should be added to the list of differential diagnosis for cutaneous and adnexal disease in rhinoceros.

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