

SEPTICEMIA AND CHRONIC ABSCESES IN IGUANAS (*CYCLURA CORNUTA* AND *IGUANA IGUANA*) ASSOCIATED WITH A *NEISSERIA* SPECIES

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Abstract: A *Neisseria* species was isolated from septicemic, abscessed, and healthy iguanid lizards at the National Zoological Park in Washington, D.C., between August 1984 and November 1985. *Neisseria* septicemia caused the death of one rhinoceros iguana in 1984. Of seven common iguanas, four had chronic, recurrent tail abscesses from which a *Neisseria* species was isolated in 3 cases. Microscopically, all four iguanas with tail lesions had colonies of gram-negative diplococci surrounded by a Splendore-Hoeppli reaction. A *Clostridium* species was cultured from the tail abscess of the fourth iguana and recovered initially from another affected iguana that later yielded *Neisseria*; *Clostridium* was considered to be a wound contaminant. The mouths of the living rhinoceros iguanas and of both healthy and abscessed common iguanas were cultured, and 50% were found to be oral carriers of *Neisseria*. All isolates of *Neisseria* were the same species, but did not correspond to any currently described species. Common iguanas were successfully treated by tail amputation, but debridement followed by local and parenteral antibiotic therapy was associated with delayed healing and incomplete resolution of the lesions.

INTRODUCTION

Bacterial infection is a major disease problem among reptiles, with the gram-negative bacteria causing the highest morbidity and mortality, and commonly causing septicemias and abscesses.⁶ Specific organisms typically found in reptiles are *Aeromonas*, *Pseudomonas*,¹¹ *Citrobacter*,¹⁰ *Proteus*,¹⁴ *Salmonella*, *Arizona*,¹⁰ *Serratia*,⁵ and *Edwardsiella* (National Zoological Park clinical laboratory records, unpublished). The purpose of this report is to describe an un-

usual bacterial invader, a *Neisseria* species (family Neisseriaceae), which was isolated from healthy, abscessed, and septicemic iguanid lizards at the National Zoological Park (NZP) in Washington, D.C., between August 1984 and November 1985.

Apparently, the only prior report of *Neisseria* in reptiles was presented in a study of herpetofauna in Germany.¹² Bacteriological cultures were taken from abscesses and organs of 148 sick or dead animals kept by zoos and private owners. A mixture of *Neisseria catarrhalis* and *Pasteurella* sp. was isolated from an abscess in one turtle. *Neisseria catarrhalis* has since been reclassified as *Branhamella catarrhalis* (family Neisseriaceae) based on its biochemical, physiological, and genetic properties.³ Thus, it appears that the genus *Neisseria* has not been reported previously in reptiles.

MATERIALS AND METHODS

History of animals studied: In 1984, the iguana collection at NZP included five rhinoceros iguanas (*Cyclura cornuta*) and seven common iguanas (*Iguana iguana*) obtained from several sources, including private donors, over the previous 5 yr. Each of the lizard species was exhibited in a separate cage.

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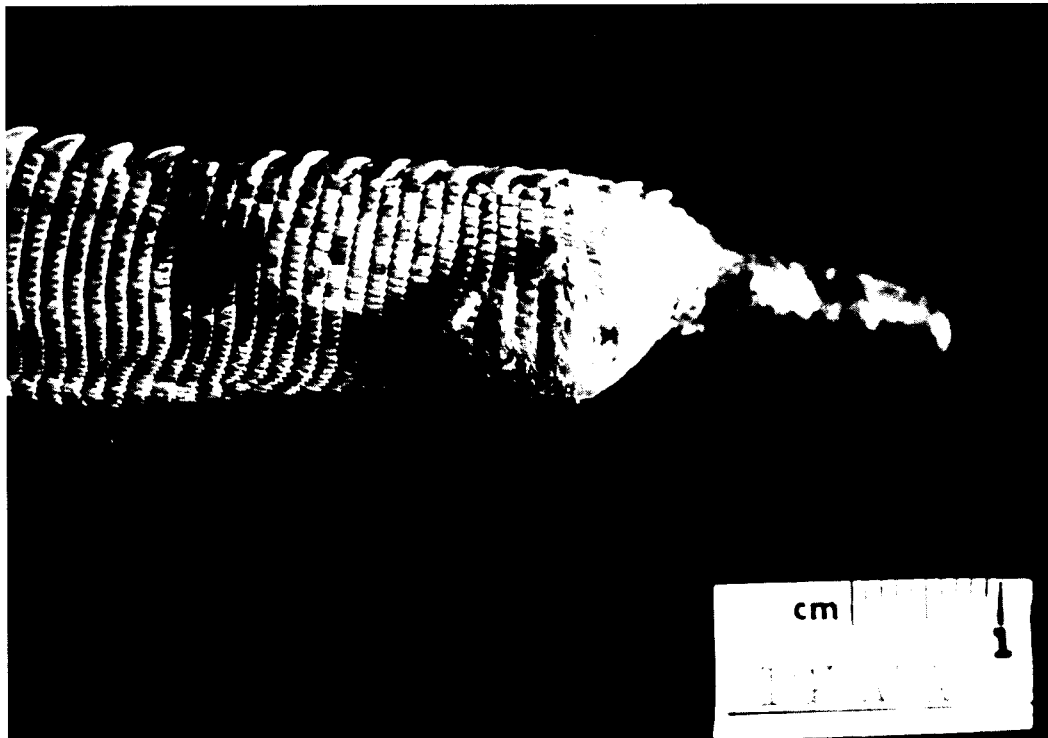


Figure 1. Gross appearance of tail from an iguana with *Neisseria*-induced lesion.

In August 1984, an adult male rhinoceros iguana was presented for necropsy with no history of health problems.

Between February and November 1985, four of the seven common iguanas had multiple chronic tail lesions (Fig. 1). These were either fluctuant or firm abscesses up to 1 cm in diameter, which displayed necrosis and ulceration of the overlying skin (two cases) or lysis of underlying vertebrae (two cases). The masses contained either fluid or caseous material. Each of these animals had lived in the exhibit cage for at least 7 mo before the first case occurred.

Pathology: A complete necropsy was performed on the rhinoceros iguana. The tail lesions on the affected common iguanas were usually removed for histopathologic examination. In two instances in which radiographs demonstrated lytic changes in the caudal vertebrae underlying the abscess sites, chronic infection necessitated surgical amputation of the tail proximal to the abscesses. In two other instances, therapeutic amputation was performed by manually removing the abscessed segment along natural fracture planes between vertebrae.

Samples of all organs from the rhinoceros iguana and the amputated tails or excisional biopsies of the masses were fixed in 10% buffered formalin and processed routinely for paraffin embedding and sectioning

at 6 μ m. Amputated tails were decalcified prior to embedding and sectioning. All tissues were stained with hematoxylin and eosin (H&E) and selected sections were stained by Ziehl-Nielsen, gram (Brown and Brenn and Brown-Hopps), and periodic acid-Schiff methods.

Microbiology: A sample of liver from the rhinoceros iguana was taken aseptically at postmortem examination and cultured on heart infusion agar with 5% sheep blood. Tail masses on the common iguanas were swabbed with culturettes and inoculated onto the same type of medium. The mouths of all common iguanas and of two of the four rhinoceros iguanas in the lizard collection were cultured similarly. All cultures were incubated at 37°C and examined for bacterial growth after 24 hr in NZP's clinical pathology lab.

Subcultures of a gram-negative diplococcus isolated from the liver of the rhinoceros lizard, the tail lesions from two affected common iguanas, and the mouths of three common and one rhinoceros iguana were submitted to the National Veterinary Services Laboratories (NVSL), Ames, Iowa, for confirmation of identification and special testing, including antibiotic susceptibility.

Culture characterization: All isolates submitted to the NVSL (NZP accession numbers 303010, 303689, 303688, 303690, 303221, 303283, 303284) were inoculated onto heart infusion agar with 5% bovine blood

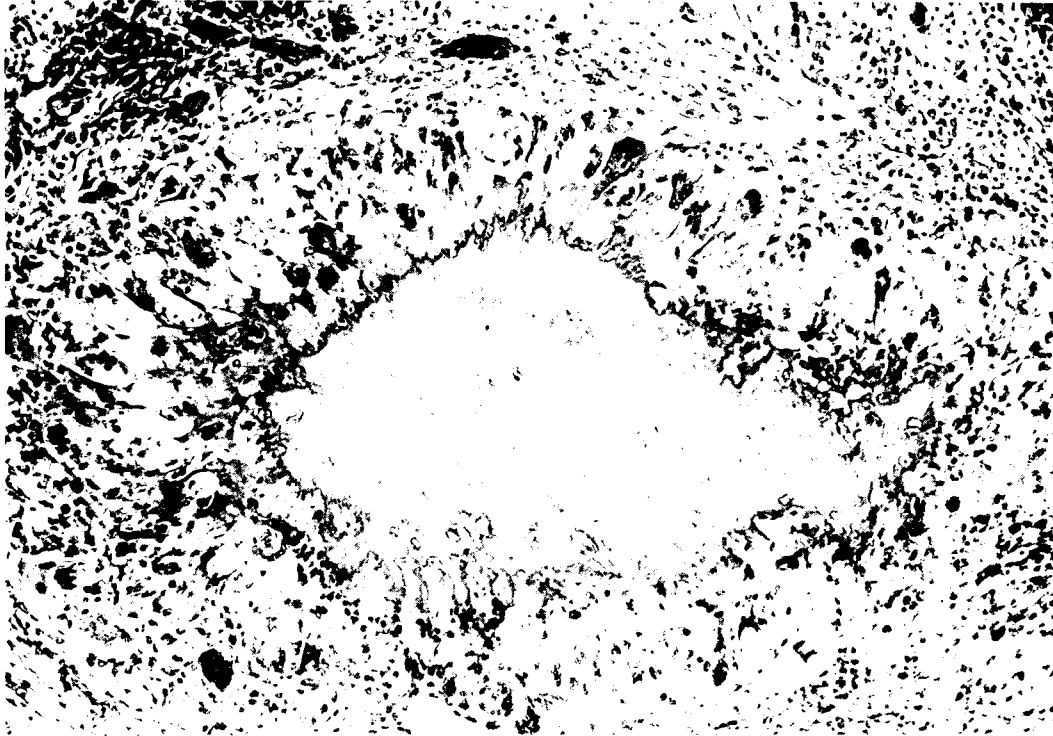


Figure 2. Photomicrograph of large dermal pyogranuloma from iguana with *Neisseria*-associated lesion in the tail. H&E, $\times 432$.

(blood agar) and incubated at 37°C for 48 hr. Isolated colonies were subcultured to blood agar slants and incubated at 37°C for 48 hr and at room temperature for an additional 24 hr. Growth from each of these slants was inoculated onto three additional blood agar slants in order to provide sufficient growth for inoculation of the biochemical test media.

A modified cystine-tryptic digest agar (CTA) medium consisting of CTA agar (1.5%) slants with 2% carbohydrate was used to test for utilization of the following carbohydrates: fructose, glucose, lactose, maltose, mannitol, mannose, and sucrose.¹³ Motility was determined by examining wet-mount preparations of growth from blood agar slants incubated at 25°C. Tests for catalase, deoxyribonuclease, oxidase, urease, aesculin hydrolysis, nitrate and nitrite reduction, utilization of sodium citrate and sodium acetate, and growth on MacConkey agar were performed according to standard techniques.⁹

Antibiotic susceptibility was determined by a standard disk diffusion method.¹ It was necessary to supplement the Mueller-Hinton agar with 5% defibrinated sheep blood and 1% IsoVitaleX^a to obtain sufficient growth for reliable results. The antibiotic disks^b used included ampicillin, 10 μg ; carbenicillin, 100 μg ; chloramphenicol, 30 μg ; gentamicin, 10 μg ; kanamycin, 30 μg ; novobiocin, 5 μg ; penicillin, 10 units;

streptomycin, 10 μg ; sulfadiazine, 300 μg ; tetracycline, 30 μg ; tobramycin, 10 μg ; and trimethoprim, 5 μg .

Postsurgical management: Tail stumps were sutured and excisional biopsy sites were debrided and treated for 2–8 wk with a series of antibiotic or antiseptic agents, including copolymer gel, providone iodine, nitrofurazone ointment, mafenide acetate cream, and proteolytic enzymes. Tails or tail stumps were also bandaged. All affected iguanas were given parenteral antibiotic therapy consisting of 400 mg of carbenicillin per kg of body weight injected subcutaneously for 7–12 days. Iguanas were removed from the exhibit and kept in isolation during these treatments, and were only returned to the exhibit cage if their lesions healed.

RESULTS

Lesions: The carcass of the rhinoceros iguana was found to be in poor nutritional condition, with no subcutaneous or abdominal fat stores. Gross findings included multiple 2–3-mm white foci in the anterior chamber of both eyes. Similar foci were found in the parenchyma of the intestinal organs, kidneys, spleen, liver, and lungs. The

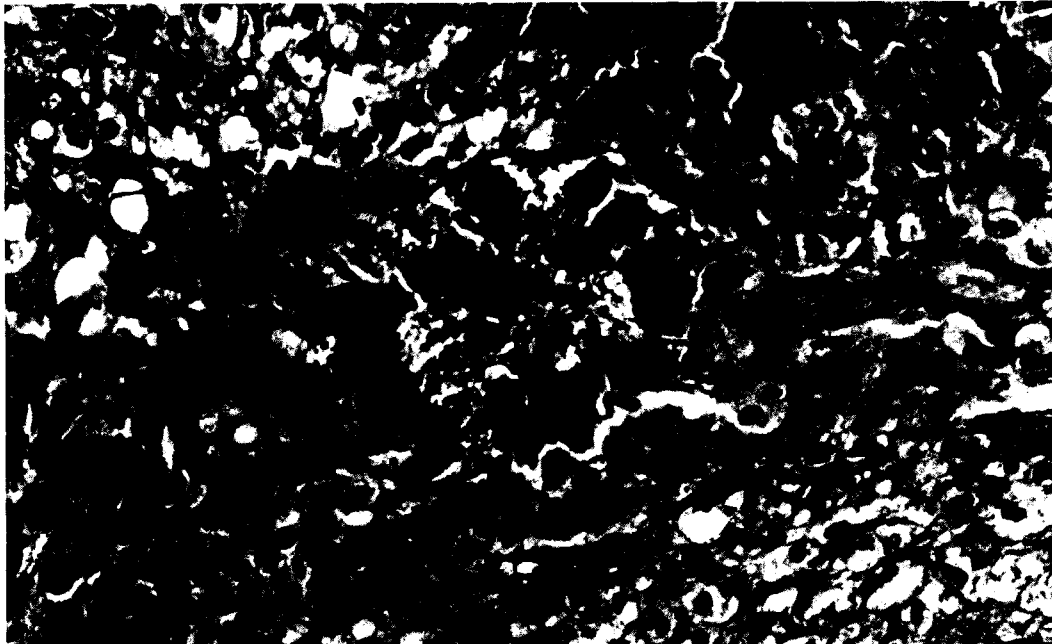


Figure 3. Higher magnification of *Neisseria*-associated tail lesion from an iguana. Note clubbed structures around bacterial colonies indicative of Splendore-Hoeppli reaction. H&E, ×128.

lungs were filled with a foamy exudate (pneumonia), and the mucosal surface of the trachea was hemorrhagic. Blood was also found in the mouth, esophagus, and stomach, suggesting that hemoptysis and ingestion of the blood had occurred. Histologic studies revealed multiple granulomas in the eyes, heart, liver, lung, kidney, and brain. In some cases, these lesions contained colonies of gram-negative diplococci that were

surrounded by dense, eosinophilic, amorphous material radiating from the periphery. These appeared similar to the “sulfur granules” described in actinomycotic lesions in mammals⁸ and reported in humans as Splendore-Hoeppli reactions to certain fungal, parasitic, and bacterial organisms.⁷ The heart also had necrotizing myocarditis, and the spleen was moderately hyperplastic. Each tail abscess biopsy revealed a gran-

Table 1. Culture results from iguanas with abscesses or granulomas.

NZP accession number	Species*	Date	Organism cultured	Location and type of lesion
303010	R	11-20-84	<i>Neisseria</i> sp.	Liver, granulomas
303689	C	2-11-85	<i>Neisseria</i> sp.	Tail, abscess
		5-31-85	<i>Neisseria</i> sp.	Tail, abscess
		11-9-85	<i>Neisseria</i> sp.	Tail, abscess
303688	C	2-25-85	<i>Clostridium</i> sp.	Tail, abscess
		8-16-85	<i>Neisseria</i> sp.	Tail, abscess
302518	C	5-7-85	<i>Clostridium</i> sp.	Tail, abscess
303686	C	6-6-85	<i>Neisseria</i> sp.	Tail, abscess

* R, rhinoceros iguana; C, common iguana.

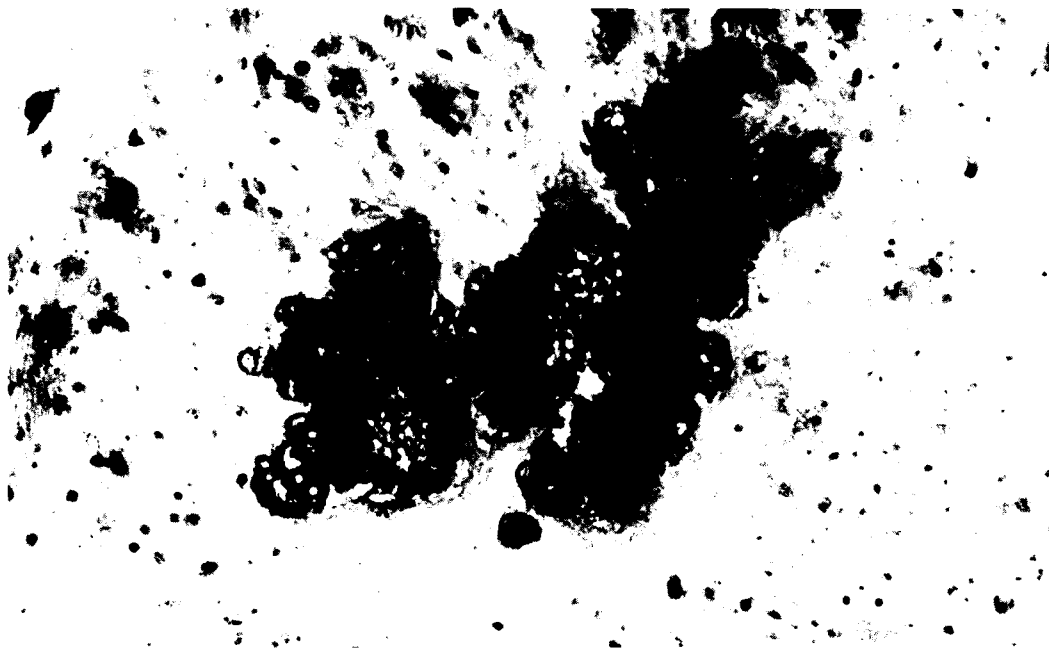


Figure 4. Colonies of gram-negative cocci in a tail lesion stain densely with Brown-Hopps tissue gram stain, $\times 128$.

ulomatous response involving coalescing aggregates of necrotic heterophils surrounded by fibrous connective tissue, macrophages, and numerous multinucleated giant cells (Fig. 2). Within the centers of the heterophil accumulations were colonies of small (1–2- μm) gram-negative diplococci surrounded by Splendore-Hoeppli reaction (Figs. 3, 4). Pyogranulomatous osteomyelitis was also observed in a coccygeal vertebra beneath an abscess site in one iguana.

Culture results: A gram-negative diplococcus identified as *Neisseria* sp. was recovered in pure culture from liver lesions in the rhinoceros iguana, from six tail abscesses in the common iguanas (Table 1), and from mouths of three of seven common iguanas, one of two rhinoceros iguanas, and another common iguana recently obtained from a private donor and not in the exhibit (Table 2).

Clostridium sp. was isolated from two other tail abscesses. One was from an iguana that twice developed abscesses (NZIP accession number 303688). When abscesses first

appeared on this animal in February, *Clostridium* was cultured. In August, it developed new abscesses from which *Neisseria* was cultured. *Clostridium* was also cultured from number 302518, which had abscesses

Table 2. Results of oral cultures from iguanas in a collection with infections caused by a *Neisseria* species.

NZIP accession number	Species*	<i>Neisseria</i>	Weight (kg)
303689	C	–	0.125
303688	C	–	0.146
302518	C	–	1.500
303686	C	–	0.065
303690	C	+	0.620
303221	C	+	0.834
303283	C	+	1.190
Not accessioned†	C	+	NR‡
303284	R	+	NR
303285	R	–	NR

* C, common iguana (there were eight in the collection at time of culture); R, rhinoceros iguana (there were four in the collection at time of culture).

† Not a member of the exhibit collection.

‡ NR, not recorded.

Table 3. List of *Neisseria* isolates examined at NVSL.*

NZP accession number	Species†	Site cultured
303010	R	Liver
303689	C	Tail abscess
303688	C	Tail abscess
303690	C	Mouth
303221	C	Mouth
303283	C	Mouth
303284	R	Mouth

* All isolates had the same biochemical and antibiotic susceptibility test results.

† R, rhinoceros iguana; C, common iguana.

that were clinically and histologically similar to lesions that contained diplococci.

The seven gram-negative diplococcus isolates submitted to the NVSL had identical biochemical test results (Table 3). The isolates were positive for catalase, oxidase, and nitrate reduction. Negative results were observed for motility, aesculin hydrolysis, urease, deoxyribonuclease, nitrite reduction, utilization of acetate and citrate, and growth on MacConkey agar. Acid was not produced from any of the carbohydrates tested. Based on these test results, the isolate was confirmed to be a *Neisseria* species. The organism does not appear to belong to any of the currently described species in that genus.

Antibiotic susceptibility results were also the same for the seven isolates. Each was susceptible to ampicillin, carbenicillin, chloramphenicol, gentamicin, kanamycin, penicillin, streptomycin, sulfadiazine, tetracycline, and tobramycin. The isolates were resistant to novobiocin and trimethoprim.

Clinical outcome: Surgical coccygeal amputation sites healed within 11 days. Iguanas with manually fractured tails healed more slowly: 303689 took 3 wk for the stump to heal and 303688 exhibited even slower healing, since the tail broke through the abscess at amputation. After 2 wk of treatment including appropriate parenteral antibiotics, the remaining portion of the abscessed site was still moist and swollen, and it was

believed that persistent inflammation was slowing healing.

Tail lesions treated with debridement alone also healed poorly. Iguana 303689 was treated with antibiotic- and/or antiseptic-impregnated dressings for 2 wk, during which time the lesions became contracted and appeared improved, but then exacerbated. The iguana remained untreated for 1 mo but abscesses recurred, so debridement and 2 more weeks of bandage therapy were instituted. After this the wounds remained clean but were not resolving. After 7 wk with no further treatment the abscesses recurred and were debrided and treated with dressings for 2 more months. By this time, wounds appeared healed but a small fluctuant, raised area remained. Five months later abscesses recurred and the tail was amputated.

The tail lesions on 302518 were debrided and treated for 4 wk as previously noted, after which all wounds healed well.

DISCUSSION

Since the rhinoceros iguana had no history of health problems, the underlying cause of his *Neisseria* septicemia is not known. His poor nutritional status probably predisposed him to the disease. At least one of his cagemates was found to be carrying *Neisseria* orally, so the bacteria could have been transmitted by a bite wound inflicted at some earlier time. Alternatively, they could have entered through an open wound in his own oral cavity if he was himself a carrier of *Neisseria*. It is common for oral wounds to occur as reptiles pursue prey through coarse cage substrates, inadvertently causing foreign bodies to lodge in the soft tissues of their mouths.¹⁵ Neither puncture wounds or abscesses were observed in the integument or the oral cavity of the rhinoceros iguana to indicate the true route of infection.

Although other forms of trauma cannot be ruled out as initiating factors, the tail abscesses of the common iguanas are believed to be the result of bite wounds in-

flicted by oral carriers of *Neisseria*. None of the iguanas with abscesses were themselves carrying *Neisseria* orally, so they could not have induced their own abscesses by licking and contaminating preexisting wounds. *Neisseria* species often parasitize the mucous membranes of mammals.³ They are generally transmitted by direct contact and rarely occur as airborne, waterborne, or soil contaminants.⁴ Bite wounds are the suspected vector of transmission because aggression is typical in the species, and such bite wounds have been observed numerous times in the past at NZP. Furthermore, iguanas that carried *Neisseria* orally tended to be larger and more likely to bite the smaller ones, all of which had abscesses (Table 2).

A retrospective study of NZP's pathology records since 1970 revealed that chronic tail abscesses occurred in a common iguana in 1973, but cultures were negative. Records indicated that cagemates of this iguana had also developed similar abscesses prior to that date, but apparently organisms were not isolated from those lesions either. The former iguana was culled from the reptile collection in 1973 because of the tail abscesses and a missing front limb. In 1982 and 1983, abscesses occurred in the head (two cases), tail, and oral cavity of common iguanas. *Pseudomonas maltophilia* was cultured from the first head abscess, but the second produced no growth aerobically or anaerobically. The tail and oral abscesses were not cultured. In 1983, a common iguana developed multiple tail abscesses, which were treated for 9 days until the animal eventually died of gout. Cultures of heart blood were noncontributory, and the abscesses were never cultured. Thus, chronic tail abscesses have indeed occurred in the past of NZP, but no consistent trends were observed among these cases nor were pathogens regularly identified.

The outbreak of tail abscesses in iguanas over the previous year, on the other hand, has produced consistent gross and histologic lesions, suggesting that the etiologic agent in all cases was *Neisseria*. Though two cul-

tures yielded *Clostridium*, no gram-positive rods were seen histologically in lesions. Instead, gram-negative cocci surrounded by Splendore-Hoeppli reactions were found consistently in tissue sections of the lesions. Thus, it is likely that *Clostridium* was merely a wound contaminant.

The chronic nature of these abscesses and their lack of response to appropriate systemic antimicrobial therapy and localized treatment indicate that *Neisseria* is a highly pathogenic organism in iguanas. Debridement is an unsatisfactory method of treatment because it was associated with delayed healing and incomplete resolution of the infection. In some instances abscesses were recurrent even though iguanas were isolated during treatment and healing periods and had not suffered new bite wounds. This suggests that the organisms may be hematogenously disseminated. In contrast, reports of iguana abscesses caused by both *Serratia marcescens* (one case) and *Micrococcus* sp. (one case) claim that the lesions healed rapidly following debridement.² In the former case, the floor of the wound measured 1.5 cm in diameter immediately following excision of pus. Within 18 hr this diameter was reduced by half, and within a week the wound was 4 mm in diameter and covered by a scab. *Neisseria* abscesses, on the other hand, failed to dry out and did not scab over quickly. It is claimed that a better alternative to merely shelling the pus from an abscess is to surgically remove the entire lesion and its capsule, and then suture the site.⁶ This method has not yet been employed at NZP on abscesses caused by *Neisseria*.

Of all the treatments administered to the affected iguanas, amputation appears to be the best method of treating these tail abscesses. The disadvantage of this method is that it makes the iguanas undesirable as representative exhibit animals.

Neisseria may be a difficult pathogen to eradicate in spite of its apparent susceptibility to several antimicrobial agents. Since it can be carried in the mouths of otherwise healthy iguanas, apparently as part of the

normal flora, it can be inoculated into other iguanas via bite wounds. Management procedures to prevent abscesses should take into account causes of aggression in iguanas. Aggression may stem from confining spaces, improper male-to-female ratios, and inadequate numbers of feeding stations.

It is unknown why this *Neisseria* species has only recently surfaced as a pathogen in the iguana collection at NZP. No new iguanas have been added since 1983, and past oral cultures have never yielded this organism. It is possible that abscesses noted in earlier cases could have been due to *Neisseria*, but that the organism, being somewhat fastidious, could not be cultured at that time. However, the pattern of inflammation seen in the current cases with the formation of the Splendore-Hoeppli reaction has not been seen previously in any reptiles from the NZP collection. This suggests that *Neisseria* is a newly introduced organism.

The pathogenesis of *Neisseria* infection in reptiles should be studied further. Animals with resident oral *Neisseria* can serve as a reservoir for continuation of epizootics, and attempts to eliminate this bacterium from the collection have been unsuccessful thus far.

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PRODUCTS MENTIONED IN TEXT

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