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Case Report

Idiopathic Dermal Necrosis in Black-tailed Prairie Dogs (*Cynomys ludovicianus*)

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Because black-tailed prairie dogs (*Cynomys ludovicianus)* are used as a model for research on gallstones and bacterial infections, performing urinary evaluations can provide invaluable data. This case report involves 5 prairie dogs that developed moist necrotic skin lesions after urine collection by cystocentesis. The information presented here serves as a resource regarding a potential adverse event that may develop after cystocentesis in black-tailed prairie dogs.

Black-tailed prairie dogs (*Cynomys ludovicianus*) are a burrowing herbivorous species in the order Rodentia and the family Sciurdae.[1] It is considered a keystone species in the grasslands of North America and is frequently kept in zoological collections and as pets.[6] Due to their propensity to develop several biliary pathologies, prairie dogs are often used for gallstone research.[1] This species also serves as a research model for diseases caused by bacterial infections, including *Clostridium difficile*, *Yersinia pestis*, and *Fransciella tularensis*.[6,8,9,11] As in other species, performing urinary analyses can aid in the health evaluation of prairie dogs and provide invaluable data in their clinical and physiologic evaluations.

Urinalysis is useful in the diagnosis of metabolic disorders, mainly renal and liver diseases.[10] Furthermore, urinary pathology can be used to evaluate for bacterial agents that are often involved in urinary tract infections. In addition, studies use urine as a point of measurement for drug pharmacokinetic studies that involve renal excretion rate.[2] The results of these studies can depend greatly on the method of urine collection.[12] Methods of urine collection include free catch, urinary catheterization, and cystocentesis. Cystocentesis is often the preferred method for obtaining urine samples, especially for studies that require minimal environmental contamination of the samples or involve bacterial culture and those that aim for controlled timing of sample collection.[12]

To perform cystocentesis, an animal is typically placed in either lateral or dorsal recumbency; the bladder is then localized by palpation to help immobilize it during sampling.[12] A 25- to 22-gauge, 1-in. needle is preferred for the collection, with the needle angled caudally toward the pelvic inlet so that as the bladder empties during collection, the needle remains in the bladder lumen.[12]

Here we describe 5 captive prairie dogs that developed necrotizing dermatitis after cystocentesis procedures.

Case Report

Captive black-tailed prairie dogs (n = 17), originating from 2 separate zoologic collections (Kansas), underwent complete clinical evaluation. The median age of the animals was 6 mo (range, 6 to 54 mo), and the median weight was 791g (range, 582 to 1200 g). Ten of the animals were male and came from one zoologic collection, and the remaining 7 were female and from the second collection. The animals were allocated into groups of 3 or 4 and evaluated on different days. All animal care procedures conformed to guidelines established by the IACUC at Kansas State University (approval no. 3311). The prairie dogs were group-housed in a concrete-lined room that was bedded with hay and kept at 21 to 23 °C. Food and water were freely available. The provided diet consisted of a mix of vegetables and commercial rodent blocks (Rodent Breeder 6F, Mazuri Exotic Pet Food, Richmond, IN).

By using 5% isoflurane gas (IsoFlo, Abbott Laboratories, North Chicago, IL) in 2 L/min oxygen, anesthesia was chamber-induced in each prairie dog. Animals were maintained under general anesthesia by using a small facemask and nonrebreathing circuit with 2.5% isoflurane delivered in 1.5 L/min oxygen. Parameters monitored included body temperature, which was measured rectally by using a handheld digital thermometer and maintained by using a warm-water blanket and heating packs, and vital signs, by using a stethoscope, a Doppler system (model 811-B, Parks Medical Electronics, Aloha, OR 97078), and a pulse oximeter (Nellcor Handheld Pulse Oximeter N20PA, Covidien, Dublin, Ireland). After induction of anesthesia, each animal received a complete physical examination. Blood was collected from the femoral vein, and CBC, serum biochemistry, blood gas analysis, whole-body radiographic imaging, and echocardiography were performed. All animals were deemed healthy.

While under general anesthesia, each prairie dog was placed in dorsal recumbency, and 70% isopropyl alcohol (Barton Solvents, El Dorado, KS 66111) was used to aseptically prepare the caudal third of the abdomen. A 12-MHz ultrasound probe was used to visualize the urinary bladder, and ultrasound-guided cystocentesis was performed. Approximately 3 to 5 mL of urine was collected by using a 6.0-mL syringe with a 22-gauge, 1.5-in needle, and the urine sample was immediately submitted to be processed.[3] Urine dipstick testing (Multistix 10 SG; Siemens Healthcare Diagnostics, Tarrytown, NY) was performed according to the manufacturer's guidelines and read by using an automatic urinalysis system (Clinitek 100 kit, Bayer, Elkhart, IN). For urine that tested positive for glucose, the results were

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verified through additional testing according to manufacturer guidelines (Clinitest tablets, Bayer). The urine specific gravity of each sample was determined by using a handheld refractometer (model 10436 Leica Veterinary Refractometer; Kernco Instruments, El Paso, TX). A urine protein sulfosalicylic acid precipitation test was performed, and results were compared with Kingsbury–Clark standards (Cargille Scientific, Cedar Grove, NJ). The urine protein:creatinine and GGT:protein:creatinine ratios were calculated. The urine sediment was evaluated microscopically under both low-power (100×) and high-power (400×) magnification. Finally, 1 mL of urine was submitted for PCR testing for pathogenic leptospires (Kansas State Veterinary Diagnostic Laboratory, Manhattan, KS). All urinalysis results showed no abnormalities when compared with those from other healthy prairie dogs.[3] Urine pH was within the range of 8 to 8.5 for all animals. All urine samples were negative for pathogenic leptospires by PCR assay and were negative for the presence of bacteria according to microscopic examination. Prior to the conclusion of anesthesia, each animal received a bolus of 30 to 40 mL lactated Ringer solution subcutaneously. The entire anesthetic procedure lasted 45 to 60 min. All animals did well under anesthesia, remained normothermic (approximately 37 °C) throughout the procedure, and recovered uneventfully. Once fully recovered, the animals were returned to their group housing enclosure.

Within approximately 5 to 7 d after sample collection, animal care staff at the zoologic institution noted necrotic lesions on the caudal ventral abdomen of 5 of the 17 (29%) prairie dogs evaluated. One case involved a 6-mo-old intact male (weight, 662 g) from one collection. A discolored, necrotic, and moist lesion (diameter, 3 cm) was present on the ventral caudal abdomen. The affected prairie dog was examined under general anesthesia (isoflurane, as described earlier), and no other lesions were noted. The affected prairie dog was isoflurane-anesthetized daily and received treatments including wound cleaning with diluted 1:1 chlorhexidine solution (Mölnlycke Health Care US, Norcross, GA) and application of 1% silver sulfadiazine cream (Ascend Laboratories, Montvale, NJ) for a total of 7 d. In addition, the prairie dog received penicillin G procaine (50,000 IU/kg SC for 3 d; Agri-Cillin, AgriLabs, St Joseph, MO) for a total of 3 treatments. Recovery was complete within 12 d. All other prairie dogs in this collection were evaluated, and no lesions were found.

The other affected prairie dogs ($n = 4$) originated from the second collection and were 2- to 3-y-old, intact females (weight, 759 to 1073 g) that had been tested on 2 different days. The first case noted in this group showed severe dermal necrosis extending from the thoracic inlet to the pubis (Figure 1). Due to severity of the lesions, this animal was euthanized by an attending veterinarian, and a full necropsy was performed at the Kansas State Veterinary Diagnostic Laboratory. The results of this examination revealed no abnormalities other than the affected ventral area of skin, which showed coagulative necrosis of the epidermis, dermis, subcutaneous tissue, and skeletal muscle. In addition, the subcutaneous adipose tissue and panniculus muscle contained intersecting tracts of fibrous connective tissue (Figure 2) and organizing granulation tissue admixed with degenerate neutrophils and rare fibrin thrombi in deep vessels. Extensive epidermal ulceration was present, and the skin surface was covered by a serocellular crust composed of degenerate neutrophils and a mixed population of gram-positive and gram-negative coccobacilli. The final histologic diagnosis for the skin was full-thickness coagulative necrosis, with suppurative cellulitis, and subcutaneous fibrosis and granulation tissue formation. The histologic appearance of the skin lesions was similar to that seen in cases of thermal or chemical burns. All other animals in this collection were examined, and 3 others had similar blackened, leathery, and crusted lesions (diameter, 1 to 2 cm). All of these affected animals were treated similarly to that described earlier and similarly showed complete recovery within 12 d.

Figure 1. Necrotic dermatitis lesion on the ventrum of the severely affected prairie dog that was submitted for necropsy. The head of the specimen is to the right, and the tail is to the left. This lesion extends from the thoracic inlet to pubis. The area in the caudal abdomen is a raised lesion (*) and was the site of the cystocentesis.

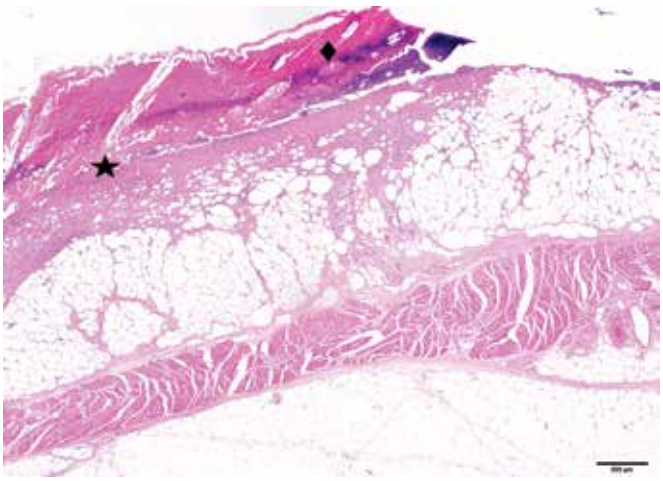

Figure 2. Histopathology of the severely affected prairie dog. This image demonstrates full-thickness ulceration of the epidermis (★) and coagulative necrosis, which extends into the underlying dermis and subcutaneous adipose tissue. The surface is covered by a thick serocellular crust (♦), and multifocal small bands of fibrosis and granulation tissue in the subcutaneous tissue contain frequent degenerate neutrophils.

Because the skin lesions on the affected prairie dogs appeared to originate in the same region as for cystocentesis, we therefore suspect that the lesions were associated with the urine sampling procedure.

Discussion

This report describes the relatively high incidence of a suspected necrotic dermatitis of the ventrum after cystocentesis

in prairie dogs. Cystocentesis procedures are often performed in many species during biochemical, nutritional, urologic, metabolic, toxicologic, and physiologic studies.[7,10,12,13] The most common adverse effects of the procedure include puncture of the adjacent bowel, laceration of the bladder, and laceration of the major vessels dorsal to the bladder. These potential issues can be avoided with direct ultrasonographic visualization of the sampling site, as was performed in these animals.

The exact cause of the adverse response observed in this case is unclear and reflect diverse many factors, including hypersensitivity to one or more of the multiple products used (for example, 70% alcohol), contact necrosis after prolonged heat source exposure, escaped urine left on the skin during the collection process, and idiopathic. All animal care procedures, including all of materials and methods used in this group of prairie dogs (including the cystocentesis protocol) are used without routinely any similar incidents in multiple other species. No new or untested products were used in these prairie dogs. The affected animals originated from 2 separate and unrelated groups. Each animal was examined once during the evaluation period. However, not all animals were evaluated on the same day, and thus the occurrence of the lesion cannot be related to a particular time. Furthermore, other animals (from other species) were tested between the various testing dates used during this evaluation. Each of the other animals were evaluated for various other reasons, yet similar testing methods were used, and none of those animals showed any similar lesions.

Because urine leaving the needle and collecting on the ventral abdomen during the cystocentesis procedure is a possible outcome, a hypersensitivity reaction to skin contact with the urine remains a viable explanation for necrotic dermatitis seen in the presented animals. Hypersensitivity reactions of the skin due to exposure to urine has been reported in other rodent-type species and is commonly referred to as urine scald.[7] Urine scald results from excessive exposure and contact of the ventrum to urine, as seen in cases of obesity, lumbrosacral fractures, or inappropriate husbandry.[4] The lesions are often malodorous, ulcerative, and moist,[7] similar to the lesions described in the prairie dogs. Therefore, urine scald resulting from urine leakage during the cystocentesis procedure remains a differential cause for the lesions. It is possible that the standard 22-gauge needle used for cystocentesis in these prairie dogs created a track through which urine continued to leak soon after collection, potentially affecting these animals during their recovery from the procedure in their cages, even though all animals in this clinical evaluation had a smooth and rapid recovery. Perhaps using smaller needles (25- to 23-gauge) is prudent when performing cystocentesis in prairie dogs. However, the effect of the leaked urine on the skin remains unclear, given that the urine properties of the affected animals showed no abnormalities (active sediment, pH, bacteria, and so forth) and no differences from other tested but lesion-free prairie dogs.

Additional reasonable causes for the lesion include thermal necrosis developing from unfitting exposure to the heat source under anesthesia. As stated in the IACUC anesthesia guidelines for rodents, it is imperative to maintain normal body temperatures, by providing an alternate heat source, throughout anesthetic procedures.[5] The prairie dogs we presented were provided heat through warm-water blankets and heating packs. Thermal dermatitis resulting from exposure to outside heat source is more common with electric heating pads but can result where other types of heat source are used. The lesions typically occur in the most dependent point of contact and can range from simple erythema to being vesicular, necrotic, and moist. When used in this case series, all heat sources were covered with cloth barriers, to prevent direct contact with the skin and, as previously mentioned, were routinely used in other animal species without eliciting similar lesions. Furthermore, the location of the lesions we described does not coincide with the location of the heat sources, which were placed all over the body but away from the cystocentesis site. Therefore, thermal contact burns seem unlikely in these cases.

We did not remove the hair over the sampling site and used 70% alcohol only for the aseptic preparation, as is frequent practice in other species.[13] At the time, we considered it best to leave the guarding hair intact and to prevent exposing the skin to irritation prior to this minor procedure. Perhaps clipping the hair, using a smaller needle (perhaps a 25-gauge), and performing a full-scrub protocol with a chlorhexidine-based solution might reduce the risk of urine becoming trapped within the fur and potentially leading to urine scald. However, the minimal protocol we used in these animals is similarly used across many species without any adverse effects in general or in regard to skin necrosis specifically.

In conclusion, prairie dogs might be sensitive to cystocentesis in general or specifically to one of the products used for the procedure. If so, urine sampling by cystocentesis should be carefully considered before it is performed in this species, and it would be imperative closely monitor subjects for signs of dermatides for several days after performing a cystocentesis procedure. The treatment protocol we used in the current evaluation was effective and sufficient to resolve all minor cases. However, the risk remains that a lesion might become severe enough that euthanasia becomes the most humane treatment for the animal. The undetermined cause, the relatively high-rate of occurrence, and the association with the urine sampling site suggest caution when performing cystocentesis in prairie dogs.

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