

Techniques for Nonterminal Blood Sampling in Black-Tailed Prairie Dogs (*Cynomys ludovicianus*)

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Black-tailed prairie dogs (*Cynomys ludovicianus*) are used as an animal model for research on gallbladder stones and several infectious diseases. A comprehensive, instructive resource regarding the appropriate techniques for venipuncture and collection of nonterminal blood samples in this species has not yet been published. Blood samples (1 mL or larger) were readily obtained from the jugular vein, femoral vein, or cranial vena cava, whereas peripheral sites, such as the cephalic vein, saphenous vein, and tarsal vein, mainly were useful for obtaining smaller volumes. The detailed and illustrated information presented here can aid clinicians and researchers in performing venipuncture, anesthesia, and handling of this species.

Black-tailed prairie dogs are ground-dwelling squirrel-like rodents (Rodentia: *Sciuridae*).² In addition to squirrels, known members of this family include chipmunks and woodchucks (groundhogs, marmots). Of the 5 prairie dog species, black-tailed prairie dogs are the most common, both in the wild and in captivity. Ecologically, prairie dogs are considered a keystone species in prairie ecology.³ Prairie dogs are a useful animal model for human gallbladder disease and infectious diseases caused by *Clostridium difficile*, *Yersinia pestis*, *Francisella tularensis*, and *Orthopoxvirus*.⁵ Because blood collection is required for the study and health monitoring of this species,⁵ we here describe several nonterminal methods useful in black-tailed prairie dogs.

All animals were handled in a humane and appropriate manner in accordance with Kansas State University humane care and use policies. The College of Veterinary Medicine of Kansas State University endorses and abides by the American Veterinary Medical Association policy on Animal Welfare as formulated by the AVMA Animal Welfare Committee.⁴

Blood Sample Volume

No studies are available currently that report the total blood volume of prairie dogs. A taxonomically related rodent species, an active (nonhibernating) ground squirrel (*Citellus parryi ab-lusis*), has a total blood volume of 59 mL (approximately 7% to 8% of body weight),¹⁰ similar to that of other rodent species.^{6,8} Ideally, only healthy and mature animals are sampled. Black-tailed prairie dogs older than 6 mo typically have reached their adult size (female, approximately 850 g; male, 850 to 1200 g).³ In general, the recommended maximal proportions of the total blood volume that can be collected with a minimal likelihood of adverse side effects are 0.5% daily, 5% weekly, 7.5% biweekly, and 10% monthly.¹

Collecting more than 15% of the total blood volume as a single sample may result in hypovolemic shock, whereas dividing the total collection volume into multiple, smaller samples over time may prevent such acute effects.¹ Therefore, the maximal

volume of blood that can be withdrawn safely and appropriate recovery times between collections should be considered before beginning the procedure.

Blood samples of 1 mL or larger can be obtained from the jugular vein, femoral vein, or the cranial vena cava of black-tailed prairie dogs. In addition, peripheral sites such as the cephalic vein, saphenous vein, and tarsal vein, are useful for smaller blood samples, but collection from these sites is dependent on local perfusion, which can be influenced greatly by hydration status, body temperature, blood pressure, peripheral vascular resistance secondary to anesthetic drugs, and ambient temperatures.

Anesthesia and Handling

General anesthesia is commonly required in prairie dogs to facilitate collection of the blood sample as well as to perform other procedures, such as thorough physical examination.^{7,9} Because this species is refractory to handling and can bite, anesthesia reduces the risk of handling-associated stress, which can affect physiologic blood parameters.⁷ In addition, anesthesia increases the safety of both animals and handlers. Handlers should take appropriate measures when working with wild prairie dogs and be aware of the potential for the transmission of zoonotic pathogens, including *Yersinia pestis*, *Francisella tularensis*, *Bartonella* spp., and *Rickettsia* spp.⁵ Chamber induction with isoflurane is recommended for prairie dogs; and anesthesia can then be maintained by using a tight-fitting mask connected to a nonbreathing system.^{2,11}

Tips Regarding Blood Collection

Blood samples should be collected immediately after anesthetic induction to avoid decreases in body temperature, isoflurane-mediated hypotension (both of which increase the difficulty of blood collection from peripheral venipuncture sites), and isoflurane-associated effects on the results. To facilitate the visualization of landmarks and vessels, blood collection sites should be clipped and free of fur and prepared aseptically by using a routine protocol. Peripheral veins can be preheated and locally occluded with a tourniquet—a rubber band clamped with a hemostat works well—to enhance their visualization. Preheating typically is unnecessary if ambient temperatures are maintained and the animal is not hypo-

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thermic; however, when needed, a single-use heat pack (for example, Hood Thermopad, Summerland, British Columbia, Canada) can be placed over the peripheral vein for 2 to 5 min. When consistent with the intended use of the sample, syringes for peripheral venipuncture of prairie dogs can be heparinized, to decrease clot formation.

Sites for Blood Collection

Jugular vein. Blood samples as large as 6 mL can be collected from the jugular vein of prairie dogs by using a 23- to 25-gauge needle on a 1- to 6-mL syringe. This method of blood collection requires 2 people: one to restrain the prairie dog and the other to obtain the sample. The animal can either be placed in dorsal recumbency, with its forelimbs extended caudally (Figure 1), or positioned upright over the edge of a table, with forelimbs pointed toward the floor (Figure 2), in a similar manner as for domestic cats. During the procedure, use finger pressure to occlude the jugular vein at the level of the shoulder. Because the vein may not be visible, the venipuncture site can be imagined to lie along a line drawn from the angle of the mandible to the thoracic inlet. Apply digital pressure at the needle exit site for hemostasis.

Femoral (medial saphenous) vein. Blood samples (1 to 3 mL) can be obtained from the femoral vein of prairie dogs by using a 25- to 27-gauge needle on a 1- to 3-mL syringe. This collection technique requires local occlusion through the use of either a tourniquet or another handler to apply pressure in the inguinal region. Place the prairie dog in either lateral or dorsal recumbency (Figure 3). The femoral vein is located centrally on the medial aspect of the thigh, and the medial saphenous vein is a minor branch of the femoral vein that lies on the medial side of the distal limb. Apply digital pressure at the needle exit site for hemostasis, and remove the tourniquet.

Cranial vena cava. Blood samples as large as 6 mL can be collected from the vena cava, bilaterally from the thoracic inlet, by using a 25- to 27-gauge needle on a 1- to 6-mL syringe. Sample collection from this site requires one person to collect the sample and 1 or 2 handlers to stabilize the prairie dog in dorsal recumbency, with the head extended cranially and the forelimbs extended distally. Palpate the manubrium and clavicle and introduce the needle just lateral to the manubrium and into the clavicular notch at a 45° angle (Figure 4). Minimize lateral movement of the needle once the thoracic cavity has been entered, to prevent laceration of the cranial vena cava. Apply gentle traction on the syringe plunger after entering the skin, because both the jugular vein and cranial vena cava can be encountered here.

Cephalic vein. Small blood samples (1 mL or less) can be obtained from the cephalic vein of prairie dogs by using a 25- to 27-gauge needle and a 1-mL syringe. This peripheral vein should be preheated and occluded with a tourniquet. The collection technique is similar to that for domestic animals, and the cephalic vein may accommodate an intravenous catheter in prairie dogs older than 6 mo or weighing more than 700 g (Figure 5). After sample collection, apply digital pressure at the needle exit site for hemostasis, and remove the tourniquet.

Lateral saphenous vein. The lateral saphenous vein of prairie dogs accommodates blood samples of no more than 1 mL, which are collected by using a 25- to 27-gauge needle on a 1-mL syringe. Place the subject in lateral recumbency, apply a tourniquet, and preheat the vein (Figure 6). The lateral saphenous vein courses diagonally over the tarsus on the lateral aspect of the



Figure 1. The prairie dog is restrained in extended dorsal recumbency for blood collection from the jugular vein (red dotted line) as the collector occludes the vein at the level of the shoulder.



Figure 2. For upright jugular-vein blood collection, the prairie dog restrained over the edge of a table, with the vein (red dotted line) occluded at the level of the shoulder.

distal limb. Once the sample has been obtained, apply digital pressure for hemostasis and remove the tourniquet.

Tarsal vein. Minimal-volume blood samples (100 µL or less) can be obtained from the tarsal vein of prairie dogs by using a 27-gauge needle and an insulin syringe or a capillary tube (Figure 7). Place the prairie dog in lateral recumbency, preheat the tarsal vein, and occlude it by using tourniquet. After the needle is introduced, blood can be collected into a syringe; alternatively, a capillary tube and associated blood-collection tube can be used to collect blood from the skin surface. After sample collection, apply digital pressure at the needle exit site for hemostasis, and remove the tourniquet.



Figure 3. Using a tourniquet (a rubber band and hemostat) and preheating the vein facilitate blood collection from the femoral vein of a prairie dog. The animal's distal limb is to the left and the proximal limb and body are to the right, with the tail at the bottom of the figure and the head at the top.



Figure 4. The cranial vena cava is accessed by introducing the needle at an approximate 45° angle lateral to the manubrium in the clavicular notch (sternum, black line; clavicles, green line). Take care to avoid lateral movement of the needle.



Figure 5. The cephalic vein of prairie dogs provides blood samples smaller than 1 mL and is a useful site for intravenous catheterization.

Recovery after Blood Collection

Because prairie dogs tend to develop acidosis and azotemia under isoflurane anesthesia,² we recommend that each animal receives a 40-mL subcutaneous bolus of lactated Ringer solution



Figure 6. Blood samples of less than 1 mL can be obtained from the saphenous vein of prairie dogs after preheating the vein and applying a tourniquet.



Figure 7. Minimal-volume blood samples can be collected from the tarsal vein (dotted red line) of prairie dogs by using a hematocrit tube at the needle exit site after applying a tourniquet and preheating the vein.

at the end of the anesthetic procedure. In addition, this treatment provides fluid replacement in cases where large-volume blood samples were collected.⁸ Allow prairie dogs to recover from anesthesia in a quiet, secure environment until they regain the righting reflex. Animals should then be placed in a carrier or observation cage and monitored visually until full recovery and return to regular behavior. Side effects of severe hemorrhage are rare,¹² but blood sampling is not without risk.

References

1. Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, Vorstenbosch C, European Federation of Pharmaceutical Industries Association and European Centre for the Validation of Alternative Methods. 2001. A good-practice guide to the administration of substances and removal of blood, including routes and volumes. *J Appl Toxicol* 21:15–23.
2. Gardhouse SM, Eshar D, Bello N, Mason D. 2015. Venous blood gas analytes during isoflurane anesthesia in black-tailed prairie dogs (*Cynomys ludovicianus*). *J Am Vet Med Assoc* 247:404–408.
3. Hoogland JL. 1996. *Cynomys ludovicianus*. Mammalian species 535:1–10.
4. Kansas State University, College of Veterinary Medicine. [Internet]. 2016. Animal use policy: statement on animals used in research, testing, and teaching. [Cited 10 September 2016]. Available at: <http://www.vet.k-state.edu/research/support/animal-use-policy/>.

5. **Keckler MS, Gallardo-Romero NF, Langham GL, Damon IK, Karem KL, Carroll DS.** 2010. Physiologic reference ranges for captive black-tailed prairie dogs (*Cynomys ludovicianus*). *J Am Assoc Lab Anim Sci* **49**:274–281.
6. **Lindstrom NM, Moore DM, Zimmerman K, Smith SA.** 2015. Hematologic assessment in pet rats, mice, hamsters, and gerbils: blood sample collection and blood cell identification. *Clin Lab Med* **35**:629–640.
7. **Ott Joslin J.** 2009. Blood collection techniques in exotic small mammals. *J Exot Pet Med*:117–139.
8. **Parasuraman S, Raveendran R, Kesavan R.** 2010. Blood sample collection in small laboratory animals. *J Pharmacol Pharmacother* **1**:87–93.
9. **Pilny AA, Hess L.** 2004. Prairie dog care and husbandry. *Vet Clin North Am Exot Anim Pract* **7**:269–282.
10. **Svihla A, Bowman HC.** 1952. Oxygen carrying capacity of the blood of dormant ground squirrels. *Am J Physiol* **171**:479–481.
11. **Wenger S.** 2012. Anesthesia and analgesia in rabbits and rodents. *J Exot Pet Med* **21**:7–16.
12. **Williams WR, Kendall LV.** 2015. Blood collection in the guinea pig (*Cavia porcellus*). *Lab Anim (NY)* **44**:207–208.