*Simple method for locating a suitable venipuncture site on the tail of the Virginia opossum (*Didelphis virginiana*)*

Amanda Jo Williams-Newkirk, Johanna S. Salzer, Darin S. Carroll, Thomas R. Gillespie & Gregory A. Dasch

European Journal of Wildlife Research

ISSN 1612-4642
Volume 59
Number 3

Eur J Wildl Res (2013) 59:455-457
DOI 10.1007/s10344-013-0706-y

Springer

Eur J Wildl Res (2013) 59:455–457
DOI 10.1007/s10344-013-0706-y

TECHNICAL NOTES

Simple method for locating a suitable venipuncture site on the tail of the Virginia opossum (*Didelphis virginiana*)

Amanda Jo Williams-Newkirk · Johanna S. Salzer · Darin S. Carroll · Thomas R. Gillespie · Gregory A. Dasch

Received: 7 December 2012 / Revised: 12 February 2013 / Accepted: 14 February 2013 / Published online: 13 March 2013

Abstract We identified a site suitable for venipuncture on the tail of the Virginia opossum (*Didelphis virginiana*) that is reliably and easily located. The prominent hemal arch associated with the ventral surface of caudal vertebra 5 serves as an easily palpated anatomical landmark for locating the ventral caudal vein for blood collection. Because this venipuncture site is only thinly covered by fur and visualization of the vein is not necessary for its location, site preparation and total animal handling time for routine venipuncture are minimal. Blood may be collected from immature and adult male and female animals, and the technique is easily taught to new technicians with minimal danger of injury to the animal.

Communicated by C. Gortazar

Amanda Jo Williams-Newkirk and Johanna S. Salzer contributed equally to this research.

Electronic supplementary material The online version of this article (doi:10.1007/s10344-013-0706-y) contains supplementary material, which is available to authorized users.

A. J. Williams-Newkirk · J. S. Salzer · T. R. Gillespie
Program in Population Biology, Ecology and Evolution, Department of Environmental Studies, Emory University, 400 Dowman Drive, Math and Science Center 5th Floor E510, Atlanta, GA 30322, USA

A. J. Williams-Newkirk (✉) · G. A. Dasch
Rickettsial Zoonoses Branch, Division of Vector-borne Diseases, Centers for Disease Control and Prevention, 1600 Clifton Road NE, MS-G13, Atlanta, GA 30333, USA
e-mail: igy7@cdc.gov

J. S. Salzer · D. S. Carroll
Poxvirus Branch, Division of High Consequence Pathogens, Centers for Disease Control and Prevention, 1600 Clifton Road NE, MS-G06, Atlanta, GA 30333, USA

Keywords Blood collection · *Didelphis virginiana* · Opossum · Tail vein · Venipuncture · Wildlife management

The Virginia opossum, *Didelphis virginiana*, is ubiquitous in both urban and rural habitats east of the Rocky Mountains and along the west coast of the USA, as well as throughout Central America (McManus 1974). Their generally high population densities and ease of trapping make them a popular subject of wildlife studies. Use of the species as a laboratory model has also increased since the 1970s when Jurgelski (1974) developed protocols for maintaining populations in captivity.

Venipuncture for blood sample collection is a routine procedure performed on both wild and captive opossums, but locating an easily accessible vein is often difficult. Many sites have been recommended in the literature, but all are associated with some problems. The medial saphenous vein (Roellig et al. 2009) is a common venipuncture site on many mammals, but shaving the site is often required to visualize the vein on opossums. Once accessed, this vein also has a tendency to collapse or form hematomas, and the rapid clotting of opossum blood makes obtaining a sufficient sample difficult. Moore (1984) advocated collection from the brachiocephalic vein near the midline of the clavicle on the ventral surface. However, this vessel cannot be visualized and there is the possibility of accidentally puncturing nontarget structures when the procedure is performed by less experienced personnel. Similarly, the jugular vein is a popular target site among experienced researchers, but the thick, muscular neck of opossums and the possibility of inadvertently puncturing other structures in the neck make it a difficult technique to master. Cardiac puncture has been used for blood sampling (Durden et al. 1993; Ruiz-Pina and Cruz-Reyes 2002), but it requires deep anesthesia and is

increasingly discouraged during survival procedures due to pain in all mammals (American Association for Laboratory Animal Science 2005). Venipuncture of the lateral caudal vein (Krupp and Quillin 1964; Jurgelski 1974) avoids the problems of excessive pain and the possibility of severe injury to the animal, but the vessel is small, difficult to locate, and tends to collapse. Several other veins in the pouch of females have been recommended by Jurgelski (1974), but they are not applicable to males and juveniles and also do not appear to have been widely adopted by researchers.

The ventral caudal vein is an appealing alternative to the previously described venipuncture sites. Much like the lateral caudal vein, it is located in the tail and is unlikely to cause severe pain or injury during venipuncture, but because it is larger, it is less likely to collapse than the lateral caudal vein. Blood collection from this site is well described for Australian marsupials by Holz (2002) and Clark et al. (2004) and is frequently used for many new world opossums, including *D. virginiana* (Jurgelski 1974; Austad 1993; Horta et al. 2009, 2010; Rejmanek et al. 2009). However, many researchers abandon the technique due to difficulty in locating the vein in *D. virginiana* because there is no way to visualize the vein, necessitating a "blind" puncture. We have found no published descriptions of the technique that provide specific instructions for locating the optimal site for blood collection from the ventral caudal tail vein. During a field study on ectoparasites of mammals in Georgia, we discovered that a reliable anatomical landmark exists for locating the ventral caudal vein in all stages and sexes of *D. virginiana*. The technique is described below and a video demonstration is provided online (Online Resource 1).

We trapped 18 opossums (2 adult males, 12 adult females, 2 subadult males, and 2 subadult females) at Panola Mountain and Sweetwater Creek State Parks near Atlanta, GA, USA using 81.3×25.4×30.5-cm Tomahawk Live Traps (Hazelhurst, WI, USA) each baited with 155.9 g of commercial canned cat food. Traps were placed in mixed deciduous and pine forests in deep shade. Each day, traps were opened in late afternoon and checked early the following morning (269 total trap nights). Captured animals were processed immediately and released the same day. All animal handling procedures were approved by the Emory University (#2000954) and Centers for Disease Control and Prevention (#2092DASMULX) Institutional Animal Care and Use Committees. Permission for trapping was obtained from the Georgia Department of Natural Resources (permit #102012).

Each opossum was confined in the rear of the trap by inserting stakes between the bars of the trap and then anesthetized with a combination of 30 mg/kg ketamine and 6 mg/kg xylazine injected IM (Stoskopf et al. 1999; Holz 2002). Dosages were based on estimated weights. Once animals were fully anesthetized, they were removed from the trap and placed on a portable table. An assistant held the animal in dorsal recumbency so that it rested on its back with the tail hanging freely over the side of the table. Holding the opossum in this position enabled the venipuncturist to easily visualize the tail base and locate the ventral midline of the caudal vertebra. Beginning at the base of the tail and proceeding distally, the venipuncturist gently flexed the tail dorsoventrally while palpating for the hemal arches on the ventral surface of the caudal vertebrae. These arches are easily identified as the only palpable boney processes on the tail's ventral midline (Fig. 1). The second palpable hemal arch distal to the tail base is also the largest and identifies caudal vertebra 5. The hemal arch of caudal vertebra 5 is markedly farther from the hemal arch on vertebra 6 than from the hemal arch on vertebra 4 (Fig. 1), such that when palpated, the ventral surface of the tail between the arches on vertebrae 5 and 6 feels like a large, fleshy depression (approximately 2 cm in length). In preparation for venipuncture, the skin in the region of this depression was thoroughly cleaned and disinfected with 91 % isopropyl alcohol to prevent the introduction of fecal material into the tissue during blood collection. A 25-gauge × 5/8-in. needle was inserted perpendicular to the tail in the center of the fleshy depression at the midline until the needle gently contacted the bone. The needle was then slowly backed out while applying gentle negative pressure with the syringe (both 1- and 3-mL syringes were effective) until blood entered the hub of the needle. The vein is located very close to the ventral surface of the caudal vertebrae, and we routinely obtained 1 mL of blood at this site, which was all that was required for our study. We taught the technique to colleagues that later reported easily collecting 3 mL from the site. Pressure was applied with the thumb to the venipuncture site after the removal of the needle to reduce the risk of hematoma formation; no hematoma was visualized on any of the animals sampled.

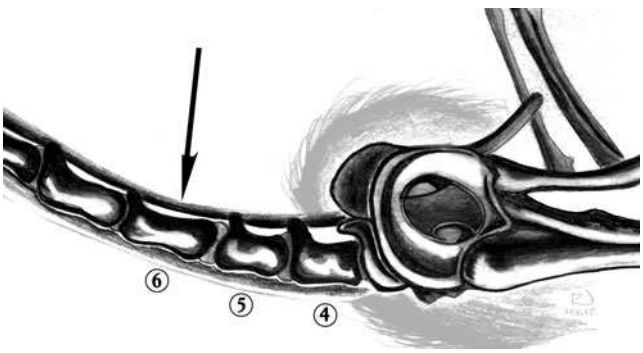

Fig. 1 Anatomical sketch of venipuncture site on the tail of the Virginia opossum (*D. virginiana*). Specimen is resting on its dorsum with the ventral surface up. The pelvis and a subset of the caudal vertebrae are shown. Caudal vertebrae are numbered as given in Ellsworth (1976). The *arrow* indicates the location of the described venipuncture site

Blood was successfully collected using this technique from all 18 opossums captured, including juveniles weighing as little as 0.5 kg. Whether smaller individuals could be sampled in the same manner was not evaluated. On the few occasions when several attempts were required to access the vein, it was always due to difficulty identifying the midline of the tail because the animal was not positioned evenly on its dorsum.

This technique is suitable for blood collection in field, laboratory, and clinical settings. It can be rapidly taught to inexperienced technicians without risk of injury to the opossum, and it is useful for drawing 1–3 mL of blood. Larger blood volume collections may be possible but were not attempted in this study. The reduced handling time is particularly valuable in situations where multiple procedures need to be performed during extended anesthesia and may reduce anesthetic dosage requirements. A short video demonstrating blood collection from the ventral caudal vein in the Virginia opossum under field conditions is provided in the online supplementary material (Online Resource 1).

Acknowledgments We would like to thank Jessica Harmon, Ryan Lash, Robert Newkirk, Tim Rose, Karen Wu, and Audrey Zeis for their invaluable assistance in the field. We are also grateful to Byron Freeman at the Georgia Museum of Natural History for providing access to opossum specimens for reference for Fig. 1, to Robert Newkirk for providing the illustration, and to Carl Brown at Emory University for his helpful suggestions. Images and video footage in the training video were kindly provided by Tim Rose, Jimmie Williams, and Kim Williams. This research was supported in part by the appointment of Salzer and Williams-Newkirk to the Research Participation Program at the Centers for Disease Control and Prevention administered by the Oak Ridge Institute for Science and Education and by the Department of Environmental Studies at Emory University.

References

American Association for Laboratory Animal Science (2005) Cardiac puncture. AALAS Learning Library: Animal Care and Use in Research and Education. https://www.aalaslearninglibrary.org/demo/References.asp?intReferenceID=26595. Accessed 10 Sep 2012

Austad SN (1993) Retarded senescence in an insular population of Virginia opossums (*Didelphis virginiana*). J Zool 229:695–708

Clark P, Holz P, Duignan PJ (2004) Collection and handling of blood samples. In: Clark P (ed) Haematology of Australian mammals. CSIRO Publishing, Collingwood, pp 1–20

Durden LA, Klompen JSH, Keirans JE (1993) Parasitic arthropods of sympatric opossums, cotton rats, and cotton mice from Merritt Island, Florida. J Parasitol 79:283–286

Ellsworth AHF (1976) The North American opossum: an anatomical atlas. R. E. Krieger Pub. Co., Huntington

Holz P (2002) Restraint and anesthesia of possums (Diprotodontia: Burramyidae, Pseudocheiridae, Petauridae, Tarsipedidae, Acrobatidae). In: Zoological restraint and anesthesia. International Veterinary Information Service, Ithaca, pp 1–5

Horta MC, Moraes-Filho J, Casagrande RA, Saito TB, Rosa SC, Ogrzewalska M, Matushima ER, Labruna MB (2009) Experimental infection of opossums *Didelphis aurita* by *Rickettsia rickettsii* and evaluation of the transmission of the infection to ticks *Amblyomma cajennense*. Vector Borne Zoonotic Dis 9:109–118

Horta MC, Sabatini GS, Moraes-Filho J, Ogrzewalska M, Canal RB, Pacheco RC, Martins TF, Matushima ER, Labruna MB (2010) Experimental infection of the opossum *Didelphis aurita* by *Rickettsia felis, Rickettsia bellii*, and *Rickettsia parkeri* and evaluation of the transmission of the infection to ticks *Amblyomma cajennense* and *Amblyomma dubitatum*. Vector-Borne and Zoonotic Dis 10:959–967

Jurgelski W Jr (1974) The opposum (*Didelphis virginiana* Kerr) as a biomedical model. I. Research perspective, husbandry, and laboratory technics. Lab Anim Sci 24:376–403

Krupp JH, Quillin R (1964) A review of the use of the opossum for research—husbandry, experimental techniques and routine health measures. Lab Anim Care 14:189–194

McManus JJ (1974) *Didelphis virginiana*. Mamm Species 40:1–6. doi:10.2307/3503783

Moore DM (1984) A simple technique for blood collection in the opossum (*Didelphis virginiana*). Lab Anim 18:52–54

Rejmanek D, Vanwormer E, Miller MA, Mazet JAK, Nichelason AE, Melli AC, Packham AE, Jessup DA, Conrad PA (2009) Prevalence and risk factors associated with *Sarcocystis neurona* infections in opossums (*Didelphis virginiana*) from central California. Vet Parasitol 166:8–14

Roellig DM, Ellis AE, Yabsley MJ (2009) Genetically different isolates of *Trypanosoma cruzi* elicit different infection dynamics in raccoons (*Procyon lotor*) and Virginia opossums (*Didelphis virginiana*). Int J Parasitol 39:1603–1610

Ruiz-Pina HA, Cruz-Reyes A (2002) The opossum *Didelphis virginiana* as a synanthropic reservoir of *Trypanosoma cruzi* in Dzidzilché, Yucatán, México. Mem Inst Oswaldo Cruz 97:613–620

Stoskopf MK, Meyer RE, Jones M, Baumbarger DO (1999) Field immobilization and euthanasia of American opossum. J Wildl Dis 35:145–149