YOU CAN'T GET BLOOD FROM A STONE, BUT YOU NEED TO GET IT SOMEWHERE – VAMPIRE BAT (DESMODUS ROTUNDUS) FEEDING LOGISTICS

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Abstract

The beef blood collection procedures for three AZA zoological institutions to feed *Desmodus rotundus* are reviewed. Blood collected for the Philadelphia Zoo is done at slaughter and an anticoagulant is added. Blood collected for the Cincinnati Zoo is also done at slaughter but no anticoagulant is added and blood collected for the Brookfield Zoo is taken from live animals within a donor herd and an anticoagulant is added. All three Zoos have very successful programs and have held *Desmodus rotundus* for a more than a decade each using the included procedures. The purpose of this poster is to demonstrate that very different approaches can be used to address the same need.

Introduction

There are three species of true vampire bats, the common vampire bat (*Desmodus rotundus*), the hairy-legged vampire bat (*Diphylla ecaudata*), and the white-winged vampire bat (*Diaemus youngi*). Of the three only *Desmodus rotundus* are commonly kept in AZA facilities in the United States. As of May 2013 the captive population of *Desmodus rotundus* was 441, the captive population of *Diphylla ecaudata* was only one and there were no *Diaemus youngi* in AZA facilities (ZIMMS). This paper presents information from three AZA facilities: the Philadelphia Zoo, the Brookfield Zoo prior to 2005 and the Cincinnati Zoo & Botanical Garden, pertaining to securing and storing food for the common vampire bat (*Desmodus rotundus*).

Desmodus rotundus are mammal blood specialist; they are one of the few captive mammal species that must have the same food in captivity as they consume in the wild. Securing blood for a group of vampire bats is an important and sometimes daunting task; however, there is a variety of ways blood has been successfully secured and stored. The purpose of this paper is to provide information on three very different, yet successful protocols for securing and storing blood as well as feeding Desmodus rotundus in captivity.

Blood Collection and Storage

Blood for the Philadelphia Zoo vampire bats is secured through the Penn State University, Block and Bridle Club. Club members collect the blood from the Penn State Black Angus herd at the time of slaughter. Blood is collected into a seven gallon bucket; anticoagulant is added (Appendix 1) to the bucket prior to collection. The action of the blood dropping into the bucket causes enough agitation to distribute the anticoagulant through the blood. Blood is transferred to 1000 mL containers and frozen at $-20 \,^{\circ}\text{C}$. Blood is transferred to the Zoo frozen and stored for up to six months prior to feeding. Initially, a sample of each collection was sent to Midwest Laboratories (Midwest Laboratories, 13611 B Street, Omaha, NE 68144) for a pet food screen

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https://www.midwestlabs.com/pet-food-microbiology/. After multiple samples returned with no issues, the practice was discontinued; however, a sample from each steer is collected and held in storage in the event an issue arises which requires evaluation. See Appendix 2 for the full Philadelphia protocol.

Blood for the Cincinnati Zoo vampire bats is purchased from Stehlin's Meat Market (10134 Colerain Ave, Cincinnati, Ohio, 45251). Stehlin's Meat Market slaughters beef weekly and during that process collects blood sanitarily. The blood is collected by stunning the animal unconscientiously and slitting the throat. Once that happens, they peel back the skin and place a clean funnel against the neck. The funnel allows the blood to flow into a four gallon bucket. Once the bucket is full, they use the funnel to stir the blood until the clot or core is formed into a sponge type clump. This is all completed by visualization depending on the quantity of blood that comes from the animal. No anticoagulant is added to the blood. Once the blood is collected, it is stored in refrigeration until picked up by zoo personnel. Weekly the blood is transported on ice to the Zoo and either put into refrigeration until fed or frozen as back up.

In 2003, the Brookfield Zoo developed a partnership with Hema-Resource & Supply, Inc (28743 S. Meridian Rd., Aurora, OR, 97002) for the purpose of securing wholesome blood for their vampire bats. Blood from a donor herd of cattle was collected weekly from live animals via arterial draw. An anticoagulant was added to the blood. Blood was refrigerated at two-eight °C at Hema-Resource for up to 18 hours prior to shipment to the Zoo. During this time the blood was analyzed to ensure wholesomeness. Blood was shipped to the Zoo on ice. At the Zoo, the blood was refrigerated at 45 °F or frozen at -10 °F. Fresh blood for the first part of the week and thawed blood from the same shipment was offered towards the end of the week. For a complete description of the Brookfield blood handling read Lintzenich, B. & Ferris. W.H., 2005. Handling Blood for the Common Vampire Bat (*Desmondus rotundus*), Proc. NAG sixth Conference, Omaha, NE. Pp. 93 – 96.

Feeding

At the Philadelphia Zoo, the bat colony size is 19 animals. Frozen blood is thawed in the refrigerator at 8 °C for 48 – 72 hours. As the blood thaws, it is poured into shallow dishes. The dishes are placed on the floor of the exhibit. Multiple dishes providing 24 mL of blood with one drop of Enfamil Poly-Vi-Sol multivitamin supplement drops (Mead Johnson & Company, Evansville, IN 47721) per bat are distributed throughout the exhibit. Blood is offered once daily at 11:30 a.m. Colony intake is recorded for the first seven days of each month. Keepers report the steer number, the date the blood was collected, and colony intake. Since November 2011, the Zoo has had a stable population of adult bats; reproduction in this colony was terminated and males were castrated, thus providing a stable population for intake evaluation. Table 1 shows the intake per adult bat relative to the age of the blood.

At the Cincinnati Zoo, the current bat colony size is 100 animals. Three bowls of blood containing 14 oz each is offered in the morning; the bowls are refilled in the afternoon with an additional eight oz of blood per bowl. Weekly the Zoo purchases three gallons of freshly harvested blood. Of those three gallons, one gallon is frozen and held in reserve. A frozen blood reserve of three gallons is held as back up. The backup blood is rotated though. Every week the animal area will receive two fresh gallons of blood and one frozen gallon. The frozen blood is

thawed in the refrigerator over a two day period with two gallons fed out fresh. The thawed frozen blood is used as a stop gap until next week's order comes arrives. Each bowl is supplemented with one cc of poly-vita-drops (Major Pharmaceuticals 31778 Enterprise Dr, Livonia, MI 48150) in the morning. This colony is a breeding colony which has been successful. Cincinnati Zoo has acquired a group of bats twice since 2007 to reach the colony size of 100 and add genetics to the group.

Discussion

Vampire bats are sensitive to contaminants; analysis of rejected blood at the Philadelphia Zoo indicates that an Aerobic Plate Count in excess of 8,000 cfu/g will be rejected by the bats. Keepers report bats will consume water readily over blood when it is contaminated. Vampire bats are also very sensitive to seismic disturbances. On August 23, 2011, an earthquake, which measured 1.7 on the Richter magnitude scale, rocked Philadelphia. The vampire bats refused to leave their roost for the entire day and did not eat. The following day consumption and activity returned to normal. The blood was analyzed to ensure it was the quake, the aerobic plate count for seven submitted samples ranged from 10-150 cfu/g, well below the Philadelphia acceptable tolerance level of 5,000 cfu/g. The blood offered the bats at Cincinnati Zoo has never been tested.

Based on Philadelphia Zoo Data (Table 1), blood intake in a non-reproductive colony of bats will average 23 mL/bat/day. Intake is not affected by the age of the blood until it reaches six months (180 days). At this point intake drops. Blood analysis appear to indicate that the moisture content of blood rises and the protein value drops the longer blood is stored (Table 2); however, further evaluation of storage time is needed to prove this trend.

Conclusion

Blood for vampire bats can be obtained in various ways. Each institution must evaluate their need and obtain blood from a reliable source. It is incumbent on the holding institution to evaluate the blood and ensure it is wholesome. Observation of bat behavior is important in the evaluation of the blood and the health of the colony. Factors unrelated to food, such as seismic activity, disruptions in the colony, number of feeding sites and exhibit temperature and humidity all can affect blood intake and must be taken into consideration when evaluating blood intake.

Literature cited

- 1. Lintzenich, B.A., W.H. Ferris. 2005. Handling blood for the common vampire bat (*Desmodus rotundus*). Proc. Sixth Nutrition Advisory Group Conference, Omaha, NE, 16-19 October. Pp. 93-96.
- 2. Toddes, B. 2006. *Philadelphia Zoo, Anticoagulant Mixing Instructions*, Philadelphia Zoo Internal Document, Philadelphia, PA.
- 3. Toddes, B. 2006. *Philadelphia Zoo, Blood Collection Protocol for Vampire Bats Desmodus rotundus*), Philadelphia Zoo Internal Document, Philadelphia, PA.

Table 1: Sampling of intake data from the Philadelphia Zoo

Table 1: Sampling					Consuption
					per adult bat
Date	Adult Bats	Consumed (mL)	Steer#	Age of Blood (Days)	(mL)
01-Sep-12	20	475	4	144	23.75
02-Sep-12	20	450	4	145	22.5
03-Sep-12	20	480	4	146	24
04-Sep-12	20	475	4	147	23.75
05-Sep-12	20	450	4	148	22.5
06-Sep-12	20	480	4	149	24
07-Sep-12	20	480	4	150	24
26-Sep-12	20	275	2	197	13.75
27-Sep-12	20	375	2	198	18.75
28-Sep-12	20	350	3	198	17.5
29-Sep-12	20	325	3	199	16.25
30-Sep-12	20	325	2	201	16.25
01-Oct-12	20	350	1&2	202	17.5
02-Oct-12	20	400	1	203	20
03-Oct-12	20	350	4	8	17.5
04-Oct-12	20	425	4	9	21.25
07-Oct-12	20	375	4	12	18.75
01-Nov-12	20	430	2	38	21.5
02-Nov-12	20	420	2	39	21
03-Nov-12	20	425	2	40	21.25
04-Nov-12	20	450	2	40	22.5
05-Nov-12	20	450	2	41	22.5
06-Nov-12	20	425	2	42	21.25
07-Nov-12	20	440	2	43	22
01-Jan-13	20	450	1	98	22.5
03-Jan-13	20	450	1	99	22.5
04-Jan-13	20	425	1	100	21.25
05-Jan-13	20	475	2	67	23.75
06-Jan-13	20	450	2	68	22.5
07-Jan-13	20	450	1&2	69	22.5
01-Feb-13	19	400	1	94	21.05
02-Feb-13	19	450	1	95	23.68
03-Feb-13	19	425	1	96	22.37
04-Feb-13	19	400	1	97	21.05

Highlighted area indicates a reduction in intake when blood exceeds six months in storage at -20°C

Table 2: Protein and dry matter analysis of blood samples stored at -20°C for different lengths of time in days

	Crude		
Age of Blood	Protein	Dry Matter	
(days)	(Kjeldahl)%	%	
99	20.2	22.35	
105	18.9	20.8	
204	18.5	20.78	
212	17	19.54	
254	17	19.6	

Analysis were done for the Philadelphia Zoo by Midwest Laboratories (Midwest Laboratories, 13611 B Street, Omaha, NE 68144)

Note: Blood was not sampled to determine appropriate storage times. Samples were taken from different animals and at different times of year which could have influenced the analysis.

Appendix 1: Philadelphia Zoo anticoagulant mixing instructions

A combination of three chemicals is used to prevent blood collected at slaughter from coagulating. The blood is collected for use as animal food for vampire bats within the Philadelphia Zoo's animal collection.

Chemicals are combined dry. Manufacturer precautions listed on the MSDS sheets must be followed when handling each chemical.

Steers typically spill four – seven gallons of blood at slaughter; Table 1 lists the chemicals and the amount of each chemical needed by gallon amount of spilled blood. Chemicals are combined dry and stored in whirlpac bags until use. Whirlpac are labeled as follows:

Philadelphia Zoo
Anticoagulant
Amount for ____ gallons
Prepared: Date

Chemical	1 gallon	2 gallon	3 gallon	4 gallon	5 gallon
tri-Sodium Citrate Dihydrate C ₆ H ₅ Na ₃ O ₇ .2H ₂ O	11	22	33	44	55
Citric Acid Anhydrous, powder HOC(COOH)(CH ₂ COOH) ₂	4	8	12	16	20
Dextose Anhydrous CH ₂ OH(CHOH) ₄ CHO	12.5	25	37.5	50	62.5

All Chemical amounts in grams

Appendix 2: Philadelphia Zoo blood collection protocol for vampire bats

Blood is collected for the vampire bats at the Philadelphia Zoo. In nature, vampire bats feed on the blood from a variety of mammals. The bats land on the host, make a small slit over a vain, and lap the blood with their tongue. Vampire bats are blood specialists, unlike the food eaten by the majority of other captive animals; there is no substitute food for blood.

Vampire bats are very small; adults typically range in weight between 35 - 50 g. The animals have very fast metabolisms and in captivity, need to feed at least every 12 hours. Captive vampire bats are very sensitive to bacterial contaminates in the blood; bats will not eat contaminated blood. Unfortunately, because it is imperative the bats feed regularly; the zoo does not have much time to respond if there is a problem with a batch of blood.

Proper handling is critical. The protocol for blood collection is designed to prevent contamination of the blood. Please follow the protocol carefully.

Items for Collection

- Seven gallon buckets marked at four and five gallons (one for each animal blood is collected from)
- stirring paddles
- ladles
- Anticoagulant in four and one gallon packs (provided by the Philadelphia Zoo Staff)
- 250 ml (one cup) measuring cups
- 3000 ml measuring cup
- 1000 ml (32 oz) Nalgene storage containers
- coveralls or tyvek suits
- surgical gloves
- Collectors should wear clean coveralls or tyvek suits and surgical gloves
- All collection containers, paddles, ladles and measuring cups must be washed and sanitized prior to use, unless purchased sanitized.

Collection Protocol

- 1. In a 3000 ml measuring cup dissolve four gallon bag of anticoagulant in two cups of distilled water (Anticoagulant is mixed and pre-measured by the Philadelphia Zoo). Stir solution with a small paddle until completely dissolved. In a 2nd 3000 ml measuring cup dissolve one gallon bag of anticoagulant in ½ cup of distilled water (note: a one cup measure is provided).
- 2. Pour four gallons anticoagulant solution into the bottom of a seven gallon bucket, set one gallon anticoagulant solution aside.
- 3. Collect blood into the bucket with anticoagulant solution; do not collect more than 5 gallons of blood (buckets have four gallon and five gallon marks on the side).
- 4. Stir anticoagulant with blood using the large paddle. If more than 4 gallons of blood has been collected add some of the reserved (one gallon solution) anticoagulant up to the full amount for five gallons. Stir blood for one two minutes.
- 5. Skim off the foam (with the ladle) that accumulates at the top of the blood after mixing and discard.

- 6. Ladle blood into 1000 ml. storage containers. Allow two inches at the top of the containers for freeze expansion. Affix lids and wipe any blood on the outside of the container away with a clean paper towel and hot water.
- 7. On containers, mark the date the blood was collected and the steer number.
- 8. Ladle 125 ml into sample containers (two samples are needed per bucket). Mark the date the blood was collected and the bucket number on the sample container. Use packing tape to secure lids on the sample cups
- 9. Place blood into the freezer as quickly as possible.

Special Notes:

- 1) Use a different bucket for each animal blood is collected from.
- 2) The same stirring paddle may be used for all collected blood. The paddle must be washed with dish detergents and rinsed between buckets.
- 3) Surgical gloves must be worn. Gloves must be changed between buckets (animals). You cannot change your gloves too frequently, when in doubt change gloves.
- 4) Blood is an excellent medium for bacterial growth. Please wash your hands thoroughly before blood collection and again after blood collection.
- 5) All containers must be washed, sanitized and allowed to air dry prior to blood collection. Storage containers will be cleaned and sanitized by the zoo prior to return to the university.