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Sampling small quantities of blood from microbats

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INTRODUCTION

Sampling blood from bats can be valuable for a range of studies including antibody detection for disease surveillance (Young *et al.*, 1996; Johara *et al.*, 2001; Li *et al.*, 2005), analysis of blood biochemistry (McLaughlin *et al.*, 2007) and populations genetics (Cardinal and Christidis, 2000; Appleton *et al.*, 2004). However, sampling sufficient volumes of blood, plasma or serum to satisfy a study's requirements from microbats can be challenging.

In the past, a range of techniques have been used including cardiac puncture (La Motte, 1958), bleeding from the orbital sinus (Baer, 1966), nicking a brachial or jugular vein with a scalpel (Baer and McLean, 1972) and puncture of the propatagial or uropatagial vein (Gustafson and Damassa, 1985; Entwistle *et al.*, 1994; Wimsatt *et al.*, 2005; Ellison *et al.*, 2006). Cardiac puncture yields good quantities of blood, however considerable mortality is often experienced (La Motte, 1958; Baer, 1966). Bleeding from the orbital sinus has commonly been used to sample bats, however yielding sufficient volumes of blood can sometimes be difficult (Baer and McLean, 1972) and Swann (1997) identified that the technique may have an adverse affect on the survival of some species of rodents. As such, cardiac puncture and orbital bleeding are no longer recommended as appropriate techniques for bleeding animals that are intended for release, however, cardiac puncture is still appropriate when exsanguination under anaesthesia is required (Morton *et al.*, 1993). Morton (1993) also recommended that a scalpel blade should not be used as it was imprecise and may lead to accidental mutilation of the animal, or operator if the animal was not adequately restrained. Several studies have described the sampling of blood via venipuncture using a heparinised haematocrit tube or glass micropipette and were able to yield sufficient volumes of blood (10–200 µl) to satisfy the study's requirements. They also identified that neither bleeding nor the use of anaesthesia had an effect on survival (Baer and McLean, 1972; Gustafson and Damassa, 1985; Wimsatt *et al.*, 2005; Ellison *et al.*, 2006). It is important that bleeding techniques are continually refined (Morton *et al.*, 1993) and so we describe a technique for sampling small quantities of blood from microbats and report the volumes taken from 1,129 bats.

MATERIALS AND METHODS

Bats were caught between 2006 and 2009 using a handnet or harptrap and placed individually into light-weight cloth bags (10 cm × 15 cm) secured with a drawstring (Hall, 1979). These cloth bags were then suspended from plastic tubing inside a polythene cooler using plastic clothes pegs (Hall, 1979). A thermometer and hygrometer were used to monitor the internal environment of the cooler so that it could be maintained at a temperature and humidity similar to that of the bats roost. The coolers' lid was left slightly ajar to allow adequate ventilation and to prevent excess humidity.

Morphometric measurements were taken from the bats before being bled. The bats' mass was measured to the nearest 0.5 g using a spring balance and its forearm length was measured to the nearest 0.1 mm using callipers. For bleeding, bats were manually restrained between the thumb and palm of the non-preferred hand. The bats' wing was extended until its fore and upper arm formed a 90° angle and then restrained between the fore and middle finger (Fig. 1A). The venipuncture site was prepared with a 70% ethanol swab and a sterile 25 g needle was used to puncture either the brachial (Fig. 1B) or the propatagial vein. Venous blood would then bead on the surface of the skin (Fig. 1C) and could be collected in 12 µl aliquots using a 20 µl micropipette and sterile tip (Fig. 1D). The first aliquot of blood

was added directly to 108 µl of phosphate buffered saline (PBS). Additional aliquots of blood were sampled and added to the same PBS until the maximum recommended blood volume was collected (less than 10% of the circulating blood volume or 6 µl/g of an animals mass, (Morton *et al.*, 1993). A clean cotton wool ball and pressure from the thumb were applied to the venipuncture site until bleeding ceased. Additional 108 µl aliquots of PBS were immediately added to the sampled blood to achieve a final dilution of 1:10 and mixed briefly using the pipette. Blood was centrifuged or allowed to settle overnight at 4ºC and the diluted plasma fraction removed for storage at -20ºC and later analysis. A volume of PBS equivalent to the plasma fraction was added to the remaining blood cells to maintain a 1:10 dilution and provide a haemostatic buffer. Alternatively, the sampled blood could be applied directly to filter paper (Ruangturakit *et al.*, 1994). A subset (n = 89) of the 1,129 bats that we bled had their blood sample observed for any evidence of clotting.

Field work was conducted with approval from: Animal Ethics, Queensland Primary Industries and Fisheries (QPIF), Department of Employment, Economic Development and Innovation (DEEDI); Environmental Protection Agency, Queensland Parks and Wildlife Service and the Northern Territory Parks and Wildlife Commission (NTPWC).

Results

We bled 1,129 individuals representing eight species of microbats (Table 1). On average we collected 4 µl of blood/g of the bats' mass (SD = 1.6, min–max = 0.1–12.0). Experienced operators could sample a bat in less than six minutes and for each 12 µl of blood sampled we were able to retrieve 100 µl of plasma diluted 1:10 in PBS. Partial clotting was observed in approximately 2% of samples (n = 2). All bats were released at their site of capture and observed flying back to or around the entrance of the roost; no deaths were recorded whilst bats were in our care.

Discussion

We have described a technique to sample up to 6 µl of blood/g from microbats. When removing this volume of blood from rats, K. J. Nahas, P. Provost, C. Sobry and Y. Rabemampianina

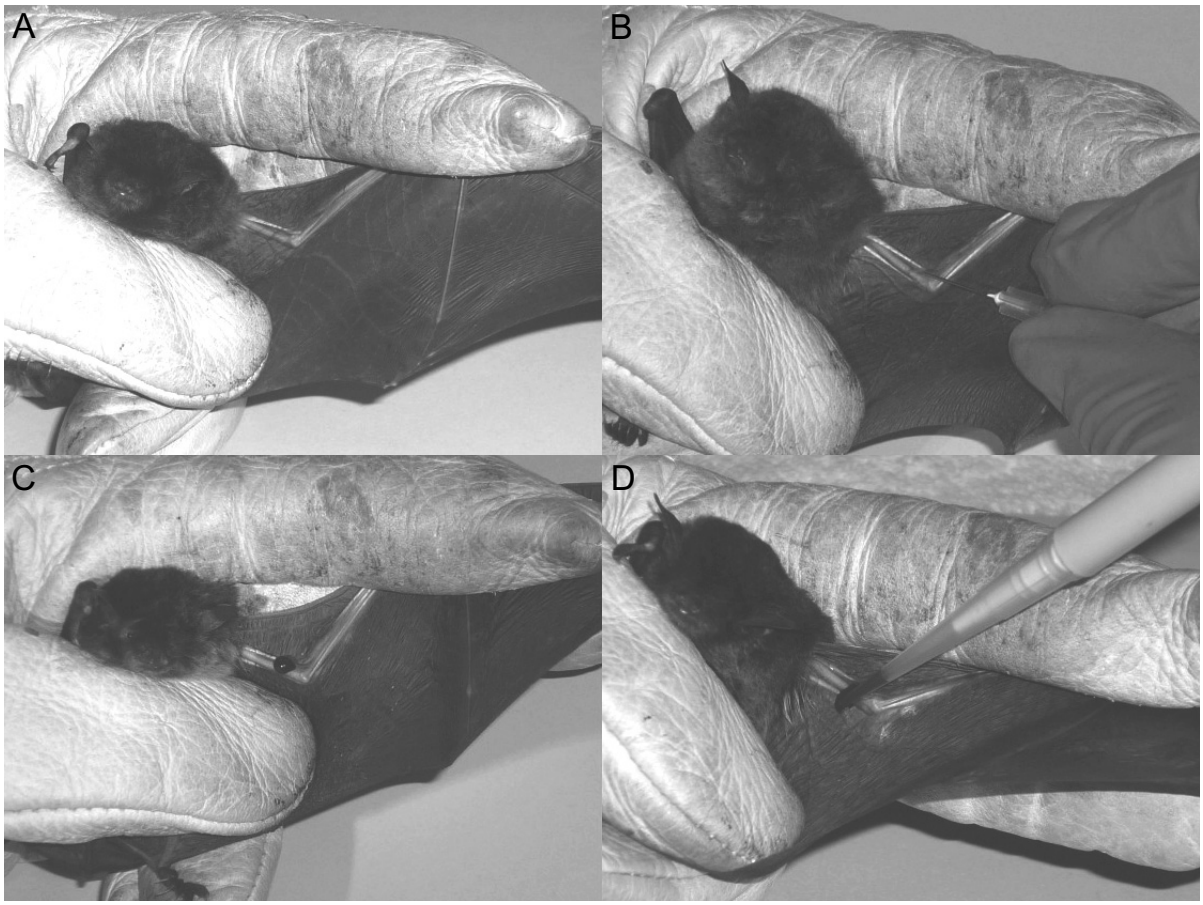

Fig. 1. Bats were manually restrained between the thumb and palm of the non-preferred hand and their wing extend until its fore and upper arm formed a 90° angle (A). The bleed site was prepared with a 70% ethanol swab and a 25 g needle was used to puncture either the brachial (B) or the propatagial vein. Venous blood would then bead on the surface of the skin (C) and could be sampled using a micropipette and sterile tip (D)

TABLE 1. Mean volume of blood/g of the bats' mass sampled from 1,129 bats representing eight species of microbats; $\bar{x}$ ± SD (min–max)

Species	*n*	Blood volume (µl)	Mass (g)	Blood volume/Mass (µl/g)
Hipposideros ater	27	33 ± 9 (12–48)	6.1 ± 0.6 (5.0–7.0)	5.4 ± 1.5 (2.4–8.7)
Macroderma gigas	38	43 ± 18 (12–60)	104.6	0.4 ± 0.2 (0.1–0.6)
Miniopterus australis	180	37 ± 11 (12–60)	7.5 ± 0.8 (5.5–10.5)	5.0 ± 1.5 (1.1–9.2)
M. schreibersii	273	49 ± 14 (12–84)	14.2 ± 1.6 (10.0–18.0)	3.5 ± 1.0 (0.7–6.3)
Myotis adversus	31	51 ± 13 (12–60)	10.4 ± 1.2 (8.0–12.5)	4.9 ± 1.4 (1.0–7.5)
Rhinolophus megaphyllus	471	44 ± 12 (12–72)	11.2 ± 1.5 (8.0–15.5)	4.0 ± 1.2 (1.1–7.6)
Rhinonycteris aurantius	78	27 ± 10 (12–48)	8.2 ± 0.8 (6.5–10.5)	3.3 ± 1.2 (1.3–6.0)
Vespadelus troughtoni	31	33 ± 12 (12–72)	5.3 ± 0.6 (4.0–6.5)	6.1 ± 2.3 (2.0–12.0)

(unpublished data) identified that haematological parameters including red blood cell count, haemoglobin level, haematocrit, mean corpuscular volume and red cell distribution width all returned to normal within 14 days. We found that a 25 g needle was suitable for puncturing the brachial or propatagial vein of the insectivorous bats that we bled, however, a smaller 27 g needle may be preferred by the operator for puncturing other veins, including the interfemoral (Wimsatt *et al.*, 2005) or the brachial or propatagial vein of smaller insectivorous bats. On the rare occasion when the brachial artery, which lies adjacent, was accidently punctured instead of the vein, extraneous bleeding occurred (9.2 µl/g collected from a *M. australis* and 12 µl/g collected from a *V. troughtoni*). When this occurred, the beaded blood was immediately collected using a larger micropipette and a clean cotton wool ball and pressure from the thumb were applied to the puncture site until bleeding ceased. In these cases, with the immediate response to a punctured artery and even sometimes with a punctured vein, extraneous blood was lost onto the cotton wool. This loss was neither quantified nor included in the analysis. However, given that the mean volume of blood/g of the bats' mass sampled did not exceed 6 µl for this study there was often still a volume of blood available to be lost to the cotton wool. It is for this reason and for the benefit of the bats being sampled that we recommend aiming to collect less than 6 µl of blood/g of the bats' body mass.

We observed that experienced operators could sample a bat within six minutes. This included taking morphometric measurements, sampling blood, ensuring that bleeding had ceased, recording details and preparing equipment for the next bat to be sampled. Manual restraint and bleeding without anaesthesia simplifies fieldwork and does not effect the survival of bats (Entwistle *et al.*, 1994; Wimsatt *et al.*, 2005; Ellison *et al.*, 2006) and most small rodents (Swann *et al.*, 1997), since the associated stress of anaesthesia would probably be greater than the discomfort of venipuncture (Morton *et al.*, 1993). Also, by wearing leather and nitrile gloves, and by discarding used needles directly into a biohazard container after venipuncture, we found it a simple task to manually restrain bats without the need for anaesthesia whilst decreasing the risk of a bat bite or needle stick injury, as was also found by Ellison (2006).

Our technique of immediately diluting blood 1:10 in PBS allowed the retrieval of plasma without the need for anti-coagulants. For each 12 µl of blood sampled we were able to retrieve 100 µl of diluted plasma. This diluted plasma fraction was removed for storage at -20ºC where IgE antibodies are stable for at least 37 years (Henderson *et al.*, 1998). Alternatively, sampled blood could be applied to filter paper, where IgG antibodies are stable for at least five months (Ruangturakit *et al.*, 1994). Partial clotting was observed in approximately 2% of blood samples, but even with these clotted samples we were able to retrieve sufficient volumes of serum to satisfy the study's requirements. Antibody detection tests, such as an enzyme-linked immunosorbent assay (ELISA) require only a small volume of undiluted serum or plasma, approximately 2 µl, which is usually diluted 1:50 during the test methodology. To perform an ELISA using our diluted plasma we modified the ELISA methodology to account for the existing dilution.

No deaths were recorded whilst bats were in our care and upon release bats were observed flying back to or around the entrance of the roost. Whilst we are unable to comment on the long-term survival of these released bats, Entwistle (1994), Wimsatt (2005) and Ellison (2006) all reported that sampling blood from bats did not decrease their survival rate when compared to control groups that were also captured and handled but not bled. In an unrelated mark-recapture study in which we used our blood sampling technique (C. S., Smith, C. E. de Jong,

G. Crameri, J. MaEachern, M. Yu *et al*., unpublished data), we recaptured 42 of 52 *Myotis macropus*. This study did not have a control group and calculating survival rates was not possible, however, it was encouraging to observe the short-term (three months) survival of recaptured bats which we had sampled.

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