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# HEALTH ASSESSMENT OF THE CHRISTMAS ISLAND FLYING FOX (*PTEROPUS MELANOTUS NATALIS*)

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ABSTRACT: During July–August 2010, 28 Christmas Island flying foxes (*Pteropus melanotus natalis*) were captured and anesthetized for examination, sample collection, and release to determine the potential role of disease in recent population declines. Measurements and samples were taken for morphologic, hematologic, biochemical, and parasitologic analysis. These are the first blood reference ranges reported for this species. These data are being used to inform investigations into conservation status and population management strategies for the Christmas Island flying fox.

*Key words:* Christmas Island flying fox, hematology, morphologic, plasma biochemistry, *Pteropus melanotus natalis*.

## INTRODUCTION

Christmas Island (135 km$^2$) is a remote Australian Territory located in the Indian Ocean, 360 km south of Jakarta, Indonesia. The permanent population of Christmas Island is approximately 2,000 (Australian Bureau of Statistics 2011). Although 63% of the island is a national park, the island has been heavily mined for phosphate since settlement in 1888. The current mining lease covers 14% of the island with additional land being sought for mining (Phosphate Resources Limited 2012).

Since human settlement, Christmas Island's native species have been subject to a range of threatening processes, including introduction of non-native species. Invasive species introduced to Christmas Island include the barking gecko (*Hemidactylus frenatus*), Asian wolf snake (*Lycodon capucinus*), black rat (*Rattus rattus*), feral cat (*Felis catus*), yellow crazy ant (*Anoplolepis gracilipes*), giant centipede (*Sclolopendra morsitans*), giant African snail (*Achatina fulica*), and numerous other reptile, insect, and plant species. Each of these species has the potential to impact native populations through competition, predation, ecosystem alteration, and introduction of infectious disease.

Human activities, such as hunting and habitat destruction and alteration, also threaten Christmas Island's native wildlife. Multispecies declines on Christmas Island have been highlighted as an indicator of deteriorating ecosystem health (Schultz and Barker 2008; Hall et al. 2011). The most severe example is the documented decline of the island's unique mammals.

Christmas Island's native rodents, the Maclear's rat (*Rattus macleari*) and bulldog rat (*Rattus nativatatis*), were thought to be extinct as early as 1904 (Lamoreux 2009). The extinction of Maclear's rat has been temporally associated with the introduction of the black rat and its blood parasite, *Trypanosoma lewisi* (Wyatt et al. 2008). The Christmas Island shrew (*Crocidura trichura*) has not been sighted since 1985 (Lumsden and Schulz 2008). The Christmas Island pipistrelle (*Pipistrellus*

*murrayi*) has not been detected since 2009 (Lumsden et al. 2010) and is considered extinct. The only remaining native mammal, the Christmas Island flying fox (*Pteropus melanotus natalis*), also appears to have undergone recent precipitous population declines since first being studied by Tidemann in 1984 (Beeton et al. 2010). If these declines continue at their current rate, extinction in the near future is likely.

The Christmas Island flying fox is a unique and poorly studied species. It is a small flying fox that roosts in large camps of up to several hundred or in smaller groups of 1–3 (Churchill 2008). Unlike many other Pteropid species, the Christmas Island flying fox is diurnal and active throughout the day and use coastal updrafts to traverse the island to foraging sites. Historical surveys indicate a skewed sex ratio with females outnumbering males 3:1 (Tidemann 1985). The Christmas Island flying fox has been described as a keystone species due to its integral role in seed and pollen dispersal, and extirpation of the species could cause important ecologic changes to the island (Beeton et al. 2010).

The most comprehensive study of the ecology and biology of the Christmas Island flying fox was carried out by Tidemann (1985). During that study, 147 flying fox were shot at foraging sites and in camps across the island. Weight and forearm length were measured, and stomach contents examined for *Toxocara* spp. (Nematoda); however, complete morphologic, biochemical, hematologic, and parasitologic data were not collected.

In 2010, the Christmas Island Expert Working Group recommended a list of urgent actions to address the potential threats to the population. Recommendations included active demographic monitoring, screening for toxins and diseases, and assessing the potential for captive breeding programs to protect the species from extinction (Beeton et al. 2010). With respect to disease, recommendations to establish baseline prevalence levels for pathogens, disease, and parasites were given the highest priorities by the working group (Beeton et al. 2010).

Our study was developed to determine a set of baseline health and pathogen data for the Christmas Island flying fox and investigate disease as a potential cause of continued population decline. Documenting the presence or absence of potential pathogens will help us understand their role in the ecosystem and protect endemic biodiversity at both the macroscopic and microscopic level. Results will form baseline population data for free-living Christmas Island flying foxes and may be used in ongoing conservation and population management by highlighting future variations from these findings in association with potential disease-driven events and provide baseline health data that can be used to assess animals for use in a captive breeding program should that become necessary.

## MATERIALS AND METHODS

### Capture and handling

The atypical, solitary, roosting habits, diurnal activity, and unusual flight behavior of the Christmas Island flying fox and the typically tall, dense nature of vegetation and rocky terrain of Christmas Island dictated the use of canopy mist netting at feeding sites as the most appropriate method of capture. A suitable foraging site was identified on the grounds of the Christmas Island Golf Course in the northeast corner of the island, close to the coast (latitude 10°25′36.59″S, longitude 105°42′3.92″E) from which all animals were captured.

The flying foxes were opportunistically captured at the site over several hours before and after dusk for 7 days. A mist net (18×3 m) was raised to the top of a gap between two 30-m-high trees on guidelines that had been preplaced by catapulting the line over suitable branches of an identified feed tree and an adjacent tree using a slingshot and lead weights. The net was monitored continuously, and flying foxes were caught approaching or leaving the feed tree. When a flying fox encountered the net, operators immediately lowered the net until the flying fox was approximately 1 m from the ground, where one person physically restrained the animal

and another disentangled it. Once disentangled, flying foxes were placed individually into a cotton pillowcase secured with a tie. This procedure typically took <1 min. Animals were transported to a field laboratory where they were anesthetized with isoflurane (Abbott Australasia, Botany, New South Wales, Australia) in oxygen (Jonsson et al. 2004). Once anesthetized, each flying fox was described by sex, age (juvenile=<1 yr; subadult=1–3 yr; adult=>3 yr), and condition (score based on relative pectoral muscle mass; McLaughlin et al. 2007). Age class was determined using morphologic measurements and secondary sex characteristics, including tooth staining and tooth wear and in females, teat elongation.

Each animal was marked with an implantable microchip in accordance with the Guidelines for Transponder Placement and Recording (Stalis et al. 2010) so that if it was caught again it could be immediately released. Flying foxes were allowed to recover from anesthesia and released within 4 hr of capture and following oral hydration with fruit juice at the location they were captured. Personnel wore nitrile gloves while handling anesthetized animals and nitrile gloves covered with welding gloves when handling alert flying foxes. All personnel handling flying foxes had recent rabies titers determined to confirm immunity to the related Australian bat lyssavirus (Department of Health and Ageing 2013).

### Morphologic measurements

Standard morphologic measurements for each animal included weight, forearm length, ear length from notch to tip, hind foot length from heel to toe tip, excluding claws, length of metacarpal in third digit of wing, length of metacarpal in fifth digit of wing, and maximum skull length (Churchill 2008).

### Hematology

Approximately 0.5–1-mL of blood was collected from the propatagial vein into a lithium-heparin tube (Plowright et al. 2008) using a 23- or 25-gauge needle and 1-mL syringe. Multiple blood films were prepared, allowed to air dry, and fixed in 70% methanol prior to staining with Quick Dip™ (Thermo Fisher, Scoresby, Victoria, Australia). Manual differential white blood cell counts were performed using a 100-cell count technique at 400× magnification. Blood cell morphology and assessment of the slide for blood parasites was undertaken at 1,000× using oil immersion.

Remaining whole blood was stored at 4 C overnight (<8 hr) before being assessed. After resuspension, a manual white blood cell count using an Improved Neubauer Hemocytometer (Thermo Fisher) was calculated and a hematocrit reading measured for each sample.

### Plasma biochemistry

After hematology testing, blood samples were centrifuged, and plasma was poured into a sterile cryovial, frozen in liquid nitrogen, and transported to the mainland for further analysis. Plasma biochemical analysis was done on thawed samples using a Thermo Fisher™ Konelab 20XTi clinical chemistry analyzer and standardized Thermo Fisher™ reagents (REM Systems, Sydney, New South Wales, Australia).

### Statistical analysis

Blood parameters were compared between males and females, and adults, subadults, and juveniles using a two-tailed, unequal variance *t*-test in Microsoft Excel (Microsoft Australia, Sydney, New South Wales, Australia). The Bonferroni correction for multiple testing was applied to manage type 1 errors (Abdi 2007). Statistical significance was accepted at $P<0.05$. Reference ranges for adults of six *Pteropus* species aged between 4.5 and 5.5 yr were extracted from the International Species Information System and compared with those for adult and subadult Christmas Island flying foxes.

### Bloodborne parasites

Blood was analyzed using PCR for the detection of *Trypanosoma* spp., *Babesia* spp., *Theilera* spp., and *Plasmodium* spp., which may be difficult to detect on blood films from healthy animals. Drops of blood from each animal were deposited onto Whatman™ filter paper (Interpath Services, Heidelberg West, Victoria, Australia) and allowed to air dry before being placed into sterile cryovials or sterile Whirl-pak™ bags (Thermo Fisher). Samples were stored at room temperature for transportation. We extracted DNA from the filter paper using the method of Rani et al. (2010), except that the papers were cut on clean filter paper rather than on microscope slides.

A nested PCR protocol, targeting a variable region of the trypanosome 18S rDNA, was performed for initial sample screening, as described by Austen et al. (2009). The PCR amplification was performed in a 25-mL volume with the final mix containing 10–50 ng of Trypanosoma DNA, 12.5 pmol of primer (external primers SLF forward and S-762 reverse were used in the first PCR round

and two internal primers S-825 forward and SLIR reverse were used in the second PCR round), 1 unit TAQ DNA polymerase, 250 mM of each dNTP, 1.5 mM MgCl2, 2.5 units of 10× reaction buffer, and Baxter Ultra-Pure H2O (all from Fisher Biotech, Perth, Western Australia, Australia). The initial denaturing temperature was 94 C for 5 min with additional steps of 50 C for 2 min and 72 C for 4 min followed by 35 cycles of 94 C for 30 sec, 55 C for 30 sec, and 72 C for 60 sec. The program was completed with a final extension of 7 min at 72 C. A sample of 1 μL of the PCR mixture from the first PCR reaction was used as the template for the second PCR reaction.

To amplify *Theileria* spp. and *Babesia* spp., a nested set of universal piroplasm primers were used to amplify an 850-base pair (bp) fragment of the 18S rDNA gene, as described by Jefferies et al. (2007). The PCR amplification was performed in a 25-mL volume with the final mix containing 10–50 ng of piroplasm DNA, 12.5 pmol of primer (external primers BTF1 forward and BTR1 reverse were used in the first PCR round, and internal primers BTF2 forward and BTR2 reverse were used in the second round), 1 unit TAQ DNA polymerase, 200 mM of each dNTP, 1.5 mM MgCl2, 2.5 units of 10× reaction buffer and Baxter Ultra-Pure H2O (all from Fisher Biotech). The initial denaturing temperature of 94 C for 3 min, 58 C for 1 min, and 72 C for 2 min was followed by 45 cycles of amplification (94 C for 30 sec, 58 C for 20 sec, and 72 C for 30 sec) and a final extension of 72 C for 7 min for 25-mL reactions. The same conditions were followed for the secondary round of amplification, except that the annealing temperature was increased to 62 C, using 1 mL of DNA template from the primary reaction.

All PCR amplifications included a negative control of sterile molecular-grade water. The positive controls for *Trypanosoma* and piroplasm were genomic DNA preparations of *Trypanosoma copemani* and *Theileria* spp. from infected quokkas (*Setonix brachyurus*) identified (and sequenced) during previous analyses. The PCR amplification products were electrophoresed on a 1% agarose gel and products detected using SYBR Safe DNA stain (Invitrogen, Life Technologies Australia Pty Ltd, Mulgrave, Victoria, Australia) on an ultraviolet transilluminator (Fisher Biotec).

#### Ectoparasite identification

The face, wings, and fur were thoroughly examined for ectoparasites, which were collected and fixed in a solution of glycerine ethanol (5% glycerol, 70% ethanol, and 25% distilled water). Parasite identification was based on morphologic and mensuration comparison with original descriptions and other literature.

#### Fecal parasite analysis

Feces dropped while animals were in transport were collected and placed into a sterile cryovial. If none were produced, a rectal swab was taken, and any fresh feces produced as a result of rectal swabbing were also collected. Within 8 hr of collection, fecal samples were examined by wet preparation by agitating a sample in physiological saline and examining the suspension under a coverslip at 400× magnification for larvae, ova, and flagellates. Fecal flotation was carried out with the Ovitector© system (BGS Medical products Inc., Venice, Florida, USA) according to the manufacturers recommendations. The entire slide was scanned for parasites at 400× magnification.

## RESULTS

#### Capture and handling

Twenty-eight Christmas Island flying foxes were captured, anesthetized, examined, and released over 7-days. No animals were recaptured. The majority of captures were males ($n=18$). No adult females were captured. All animals captured appeared healthy, with no apparent signs of disease, and exhibited fair to excellent body condition based on pectoral muscle mass.

Captured bats had several unusual behavioral differences when compared with mainland flying fox species. Bats were passive and nonvocal when handled and did not exhibit stressful reactions to any aspect of the procedure. Released bats typically paused and rested at the release point for 5–10 min before climbing higher and flying away. This disinclination to fly immediately on release required postrelease supervision to avoid predation by feral cats or robber crabs (*Birgus latro*).

#### Morphologic measurements

Morphologic data are presented in Table 1. Comparisons with other *Pteropus* spp. are presented in Table 2.

TABLE 1. Mean (±SD) weight (g) and standard measurements (mm) of Christmas Island flying fox (*Pteropus melanotus natalis*) captured during August 2010.

| Sex | Age | *n* | Weight | Forearm | Ear length | Foot length | Metcarpal length (3)[a] | Metacarpal length (5)[a] | Skull maximum length |
|---|---|---|---|---|---|---|---|---|---|
| Male | Adult | 12 | 395.6±52.2 | 130.4±2.7 | 28.7±2.5 | 36.9±2.2 | 81.3±3.5 | 86.9±2.6 | 55.6±1.5 |
| Male | Juvenile | 6 | 243.3±35.4 | 117.5±4.3 | 28.4±0.6 | 35.2±2.0 | 71.9±3.8 | 77.5±4.4 | 51.8±1.5 |
| Female | Subadult | 5 | 315.8±65.3 | 125.4±6.1 | 28.6±2.6 | 35.6±1.7 | 78.4±4.1 | 83.6±5.6 | 53.8±2.4 |
| Female | Juvenile | 5 | 200±29.9 | 112.7±4.4 | 27.8±1.3 | 33.6±4.3 | 70.5±2.7 | 74.3±3.5 | 50.2±2.9 |

[a] Metacarpal length (3) = length of metacarpal in third digit of wing; metacarpal length (5) = length of metacarpal in fifth digit of wing.

#### Hematology

Blood cell morphology appeared normal, with some animals exhibiting very mild polychromasia or anisocytosis. Hemoparasites were not observed in blood smears. There was a significant difference in percentage of monocytes between male and female flying foxes but no significant differences in hematologic values between age classes (Table 3). One bat had mild to moderate leucopenia caused by low concentrations of lymphocytes and neutrophils; another had a moderately elevated white blood cell count with an increase in heterophils and lymphocytes. Neither of these animals was anemic, both were in good physical condition, and the values were within the ranges for other flying fox species. The significance of these results is uncertain. Comparison of hematologic results with other *Pteropus* spp. (Table 4) shows no significant differences between these species and the Christmas Island flying fox.

#### Plasma biochemistry

There were significant differences in the mean alkaline phosphatase (ALP) between male and female, and adult/subadult and juvenile Christmas Island flying foxes (Table 5); however, this significance was only maintained between adult/subadults and juveniles after a Bonferroni correction was applied. There were also significant differences in albumen, calcium, and phosphate concentrations in the plasma of adult/subadult compared with juvenile Christmas Island flying foxes (Table 4). Only phosphate remained significantly different after the application of the Bonferroni correction. The mean plasma ALP and phosphate concentrations were higher in the juvenile flying foxes.

No flying foxes appeared to be compromised on the basis of plasma biochemistry. There were significant differences between Christmas Island flying foxes and other *Pteropus* spp. in regard to ALP, amylase, and lipase, with the Christmas Island flying fox having higher plasma concentrations in each of these parameters (Table 4). Phosphate was also significant until the Bonferroni correction was applied.

#### Bloodborne parasites

Amplification of the 18S rRNA gene for piroplasms (*Theileria* spp. and *Babesia* spp.) produced a PCR product of approximately 850 bp in two of the 28 flying foxes. This PCR product could not be identified because attempts to sequence the amplified DNA were unsuccessful. There was no evidence of *Trypanosoma* spp. or *Plasmodium* spp. based on PCR analysis of blood.

#### Ectoparasite identification

Mites found in the pelage of 3/28 flying foxes were identified as *Meristaspis calcaratus*. No other ectoparasites were found.

#### Fecal parasites

Coccidia were identified by fecal floatation from 4/16 fecal samples analyzed.

TABLE 2. Mean (range) for comparative weight (g) and standard morphologic measurements (mm) for several *Pteropus* spp., including the Christmas Island flying fox (*Pteropus melanotus natalis*) in boldface.

| | Large-eared flying fox (*Pteropus macrotis*)[a] | **Christmas Island flying fox (*P. m. natalis*)** | Little red flying fox (*Pteropus scapulatus*)[a] | Spectacled flying fox (*Pteropus conspicillatus*)[a] | Black flying fox (*Pteropus alecto*)[a] | Grey-headed flying fox (*Pteropus poliocephalus*)[a] |
|---|---|---|---|---|---|---|
| Weight | 297 (214–450) | **313.79 (156–546)** | 384 (258–500) | 639 (380–950) | 674 (590–880) | 781.5 (410–1270) |
| Forearm | 133 (126–141) | **123.59 (106.8–133.6)** | 123.6 (116.3–140.0) | 166.3 (150–183) | 171 (153–191) | 163.5 (151.6–177) |
| Ear length | 34.5 (34–35) | **28.44 (24.3–32.5)** | 31.9 (28.3–39.6) | 31.3 (25.0–36.0) | 34.4 (29.0–37.0) | 29.7 (19.3–39.2) |
| Foot length | 51.5 (49.0–54.0) | **35.75 (27.3–40.0)** | 34.4 (30.0–43.5) | — | — | 38.1 (34.6–43.6) |
| Metacarpal length (3)[b] | — | **76.88 (66.4–84.6)** | 82.3 (77.8–91.6) | — | — | 112.1 (105.1–120.5) |
| Metacarpal length (5)[b] | — | **82.06 (68.7–90.3)** | — | — | — | — |
| Skull maximum length | — | **53.47 (45.7–58.0)** | — | 76 (64–92) | 68.8 (67.2–70.2) | — |

[a] Adapted from Churchill (2008).

[b] Metacarpal length (3) = length of metacarpal in third digit of wing; metacarpal length (5) = length of metacarpal in fifth digit of wing.

Coccidia were identified in adult and juvenile flying foxes in low to moderate numbers. One ascarid-like ova was found on floatation, but identification was not possible. Mite ova were identified in 4/16 fecal samples. The individuals with mite ova in feces were different from those found with *M. calcaratus* in the pelage. Because identification of the fecal mite ova was not possible, we could not discern whether these were *M. calcaratus*, other parasitic mites, or free-living mites ingested incidentally during feeding or grooming.

## DISCUSSION

We believe this is the first time that the Christmas Island flying fox has been live captured, sampled, and released. The golf course site presented the only practical capture opportunity, but no adult females were captured. The female-to-male ratio of 1:2 contrasted with the 3:1 ratio reported by Tidemann (1985), whose sampling covered multiple sites throughout the eastern and central regions of the island. This discrepancy could reflect a change in the female to male ratio in the population or a bias created by the site of collection.

Adult females have been reported to congregate in maternity roosts at Hosnies Spring, Dolly Beach, and Greta Beach on the eastern side of the island. It is possible that adult females are not typically found outside of these maternity roosts or were in low numbers at the closest large camp site (Greta Beach), thus limiting our chances of capturing them. Further investigation is needed to determine the structure of the population and ascertain if the low number of females is driving the island-wide population decline.

This is the first report of hematologic and biochemical values in this species (Table 4). Although physical restraint and inhalant anesthesia have been associated with biochemical alterations in flying foxes (Heard and Huft 1998), physical restraint is required to catch the animals, and

TABLE 3. (a) Comparison of hematologic values between male and female and (b) adult/subadult and juvenile Christmas Island flying foxes (*Pteropus melanotus natalis*).

| Test[a] | Male ($n$=18) Mean±SD | Male ($n$=18) Range | Female ($n$=10) Mean±SD | Female ($n$=10) Range | $P$ value[b] |
|---|---|---|---|---|---|
| (a) | | | | | |
| HCT (%) | 41.7±2.2 | 28–46 | 41±3.8 | 36–48 | 0.313 |
| WBC ($10^9$/L) | 8.12±2.95 | 2.6–14.7 | 9.15±4.32 | 6.4–13.2 | 0.602 |
| Neutrophils (%)* | 45.1±10.5 | 28–66 | 37.7±4.8 | 29–44 | 0.020 |
| Neutrophils ($10^9$/L) | 3.78±1.59 | 1.69–6.91 | 3.40±1.02 | 2.37–5.15 | 0.465 |
| Lymphocytes (%)* | 53.8±9.1 | 35–69 | 60.1±5.0 | 54–69 | 0.034 |
| Lymphocytes ($10^9$/L) | 4.25±1.63 | 0.91–7.35 | 5.11±1.38 | 3.24–7.66 | 0.161 |
| Monocytes (%)* | 1.2±0.9 | 0–3 | 0.4±0.5 | 0–1 | 0.003 |
| Monocytes ($10^9$/L) * | 0.10±0.08 | 0–0.31 | 0.04±0.05 | 0–0.13 | 0.024 |
| Eosinophils (%) | 1.0±0.8 | 0–2 | 1.4±1.2 | 0–3 | 0.385 |
| Eosinophils ($10^9$/L) | 0.09±0.08 | 0–0.24 | 0.13±0.13 | 0–0.33 | 0.372 |
| Basophils (%) | 0 | 0 | 0.1±0.3 | 0–1 | 0.343 |
| Basophils ($10^9$/L) | 0 | 0 | 0 | 0 | N/A |
| | Adult/subadult ($n$=17) | | Juvenile ($n$=11) | | |
| (b) | | | | | |
| HCT (%) | 40.7±2.5 | 36–44 | 41.4±3.1 | 38–46 | 0.717 |
| WBC ($10^9$/L) | 8.39±2.81 | 2.6–13.2 | 8.06±1.83 | 5.8–11.1 | 0.515 |
| Neutrophils (%) | 40.8±6.7 | 28–55 | 40.9±8.5 | 29–58 | 0.975 |
| Neutrophils ($10^9$/L) | 3.74±1.37 | 1.69–5.71 | 3.88±1.91 | 2.2–7.56 | 0.828 |
| Lymphocytes (%) | 55.5±8.5 | 35–69 | 56.8±8.6 | 40–69 | 0.712 |
| Lymphocytes ($10^9$/L) | 4.58±1.75 | 0.91–7.66 | 4.27±1.04 | 3.24–5.99 | 0.572 |
| Monocytes (%) | 0.9±0.6 | 0–2 | 1.0±1.1 | 0–3 | 0.882 |
| Monocytes ($10^9$/L) | 0.08±0.05 | 0–0.18 | 0.06±0.08 | 0–0.22 | 0.605 |
| Eosinophils (%) | 1.1±0.9 | 0–3 | 1.1±1.1 | 0–3 | 0.838 |
| Eosinophils ($10^9$/L) | 0.10±0.09 | 0–0.26 | 0.09±0.10 | 0–0.26 | 0.945 |
| Basophils (%) | 0 | 0 | 0 | 0 | N/A |
| Basophils ($10^9$/L) | 0 | 0 | 0 | 0 | N/A |

[a] HCT = hematocrit; WBC = white blood cell count.

* The difference between males and females with regard to neutrophils (%), lymphocytes (%), and monocytes (% and $10^9$/L) were significantly different ($P<0.05$); however, only monocytes (%) appear significant after the Bonferroni correction for multiple comparisons.

[b] NA = not applicable.

inhalant anesthesia during sample collection is accepted as best practice to protect animal welfare and human health. Restraint times were minimized to reduce any potential effects on hematologic and biochemical profiles.

There was little evidence, by blood analysis, that any individuals were suffering infectious disease. The significant difference in percentage of monocytes between male and female Christmas Island flying foxes (Table 3) may be due to the lack of adult females in this study; however, there were no significant differences on comparison of adult/subadult to juvenile flying fox hematologic values. All of the hematologic values of the Christmas Island flying fox fell within the normal range for all other comparable *Pteropus* spp. (Table 4).

Because ALP and phosphate are typically associated with bone growth and metabolism, we feel it is not unusual that growing, juvenile flying foxes had higher concentrations of circulating ALP and phosphate (Heard and Whittier 1997). The high ALP, amylase, and lipase plasma concentrations (and to some extent phosphate) compared with other *Pteropus* species may be due to differences in sampling and analysis, dietary differences, the higher proportion of

TABLE 4. Comparative hematologic and biochemical values for *Pteropus* spp. Adapted from International Species Information System. All results are for adults aged 4.5–5.5 yr and Christmas Island flying fox (boldface) classified as subadults and adults.

| Parameter[a] | Little golden-mantled flying fox (*Pteropus pumilus*) | Variable flying fox (*Pteropus hypomelanus*) | **Christmas Island flying fox (*Pteropus melanotus natalis*)** | Rodriguez flying fox (*Pteropus rodricensis*) | Pemba flying fox (*Pteropus voeltzkowi*) | Indian flying fox (*Pteropus giganteus*) | Large flying fox (*Pteropus vampyrus*) |
|---|---|---|---|---|---|---|---|
| Weight (g) | 145–200 | 425–450 | **217–546** | 225–605 | 430–610 | 364–1,000 | 984–1,330 |
| ALP (U/L)* | 898 (184–1,910) | 528 (218–884) | **1,478.56 (248.3–2,708.82)** | 647 (146–1,790) | 566 (211–1,505) | 795 (93–3,102) | 889 (276–3,896) |
| ALT (U/L) | 12 (3–60) | 10 (3–20) | **9.94 (2.74–17.14)** | 24 (6–49) | 14 (4–34) | 20 (3–71) | 14 (3–47) |
| AST (U/L) | 66 (44–90) | 29 (20–42) | **34.06 (17.88–50.24)** | 66 (28–144) | 68 (35–136) | 78 (28–171) | 70 (23–249) |
| Albumin (g/L) | 38 (33–40) | 37 (33–40) | **40.19 (36.63–43.75)** | 39 (27–63) | 43 (32–52) | 41 (28–57) | 38 (32–53) |
| Amylase (U/L)* | — | 163.2 | **1701.92 (1,219.36–2,184.48)** | 112.7 (68.82–195.4) | 422.5 (280.1–550.4) | 240.9 (186.5–322.5) | — |
| T-Bil (μmol/L) | 2 (2–2) | 2 (2–3) | **2.89 (1.77–4.01)** | 7 (0–17) | 5 (3–9) | 3 (0–12) | 2 (2–2) |
| CK (U/L) | — | 165 (96–288) | **393.89 (0–836.53)** | 325 (52–1152) | 801 (309–3,066) | 974 (1–9408) | 1,555 (198–3,965) |
| Calcium (mmol/L) | 2.1 (1.78–2.4) | 2.18 (1.3–2.4) | **2.08 (1.98–2.18)** | 2.15 (1.58–2.63) | 2.3 (1.88–2.55) | 2.35 (1.73–2.93) | 2.08 (1.85–2.85) |
| Cholesterol (mmol/L) | 1.74 (0.18–3.37) | 0.80 (0.08–4.14) | **0.43 (0.15–0.71)** | 0.36 (0–0.86) | 0.67 (0.29–1.24) | 0.96 (0.26–2.36) | 0.44 (0.08–2.62) |
| Creatinine (μmol/L) | 62 (53–71) | 62 (18–133) | **46.11 (33.35–58.87)** | 53 (27–97) | 88 (71–115) | 53 (18–124) | 71 (53–106) |
| GGT (U/L) | — | — | **7.31 (4.77–9.85)** | 18 (8–23) | 2 (0–5) | 83 (11–269) | — |
| Globulins (g/L) | 30 (20–35) | 40 (22–60) | **25.29 (21.91–28.67)** | 28 (15–37) | 36 (19–52) | 29 (13–40) | 34 (22–45) |
| Glucose (mmol/L) | 7.49 (2.33–13.32) | 8.71 (3.83–15.1) | **3.36 (2.55–4.98)** | 5.77 (0.61–12.82) | 4.27 (0.06–9.71) | 3.83 (0–11.38) | 8.55 (1.61–5.83) |
| LDH-P (U/L) | — | 116 | **219.09 (70.33–367.85)** | 227 (71–825) | 228 (136–346) | 434 (48–1236) | — |
| Lipase (U/L)* | — | — | **13.47 (0–43.87)** | 0 | 1.11 (0.28–1.95) | 1.95 (0.83–4.17) | — |
| Mg (mmol/L) | — | — | **0.69 (0.61–0.77)** | 0.85 (0.33–1.03) | — | — | — |
| Phosphate (mmol/L)* | 1.29 (0.58–1.97) | 1.62 (0.97–3.49) | **2.43 (1.39–3.47)** | 1.55 (0.68–2.62) | 1.84 (1.10–2.97) | 1.74 (0.71–3.71) | 1.16 (0.55–2.29) |
| Protein total (g/L) | 68 (57–73) | 76 (60–99) | **65.21 (58.93–71.49)** | 67 (51–91) | 78 (64–89) | 76 (51–108) | 73 (59–90) |
| Triglycerides (mmol/L) | — | — | **0.51 (0.13–0.89)** | 0.45 (0.26–0.69) | 0.67 (0.34–1.04) | 0.57 (0–1.68) | — |
| Urea (mmol/L) | 3.93 (2.14–6.43) | 2.14 (0.71–3.2) | **0.98 (0–2.58)** | 4.99 (1.43–9.28) | 6.78 (3.57–11.07) | 5.36 (1.07–24.28) | 2.49 (1.43–4.99) |
| Uric acid (mmol/L) | — | — | **0.01 (0–0.03)** | 0.012 | 0 | 0.018 (0–0.077) | — |
| WBC ($10^9$/L) | 17.72 (7.5–24) | 7.54 (2.8–14) | **8.39 (2.6–13.2)** | 7.43 (2–20.2) | 8.71 (4.6–17.9) | 7.69 (3–24.1) | 8.23 (3.9–16.4) |

TABLE 4. Continued.

| Parameter[a] | Little golden-mantled flying fox (*Pteropus pumilus*) | Variable flying fox (*Pteropus hypomelanus*) | **Christmas Island flying fox (*Pteropus melanotus natalis*)** | Rodriguez flying fox (*Pteropus rodricensis*) | Pemba flying fox (*Pteropus voeltzkowi*) | Indian flying fox (*Pteropus giganteus*) | Large flying fox (*Pteropus vampyrus*) |
|---|---|---|---|---|---|---|---|
| HCT (%)* | 45.8 (42.3–52.9) | 45.8 (37–50.4) | **40.7 (36–44)** | 47.7 (29.9–65.5) | 49.5 (37–59) | 48.2 (34–62) | 45.2 (39–57) |
| Neutrophils ($10^9$/L) | 7.90 (2.32–12.9) | 3.26 (1.26–6.3) | **3.74 (1.69–5.71)** | 4.42 (1.01–13.2) | 5.57 (2.73–11.1) | 4.39 (0.39–12) | 5.57 (2.54–14.4) |
| Lymphocytes ($10^9$/L) | 8.76 (1.93–20.0) | 3.88 (1.34–8.06) | **4.58 (0.91–7.66)** | 2.3 (0.22–12.0) | 2.74 (0.61–6.81) | 2.25 (0.16–14.0) | 1.97 (0.42–8.06) |
| Monocytes ($10^9$/L)* | 0.56 (0.3–0.72) | 0.20 (0.06–0.7) | **0.08 (0.00–0.18)** | 0.47 (0.04–3.22) | 0.55 (0.05–1.49) | 037 (0.03–2.83) | 0.36 (0.05–1.66) |
| Eosinophils ($10^9$/L) | 0.45 (0.11–0.96) | 0.20 (0.06–0.38) | **0.10 (0.00–0.26)** | 0.36 (0.03–2.97) | 0.14 (0.05–0.20) | 0.33 (0–1.76) | 0.42 (0.07–1.52) |
| Basophils ($10^9$/L) | 0.00 | 0.00 | **0.00** | 0.07 (0.04–0.12) | 0.00 | 0.07 (0–0.21) | 0.00 |

[a] ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate aminotransferase; T-Bil = total bilirubin; CK = creatinine kinase; Ca = calcium; GGT = gamma-glutamyl transferase; LDH-P = lactate dehydrogenase; Mg = magnesium; WBC = white blood cell count; HCT = hematocrit.

* The average and range for ALP, amylase, and lipase is significantly higher ($P<0.05$) in the Christmas Island flying fox, while phosphate, hematocrit, and monocytes ($10^9$/L) also appear significantly different until the application of the Bonferroni correction for multiple comparisons.

young animals we sampled, or physiologic differences between species (Heard and Whittier 1997; Herd and Huff 1998; McLaughlin et al. 2007).

The Christmas Island flying fox is small compared with other members of the *Pteropidae* family (Table 2). The Christmas Island flying foxes captured during this study were smaller in almost every respect to those reported by Churchill (2008). Although findings may be attributed to discrepancies in measuring technique, with the comparatively small number of individuals surveyed during this study and the propensity for capture of juvenile/subadult animals, there may be true morphologic population changes as a response to decreased resources (Rode et al. 2010; Sheridan and Bickford 2011), and further study is needed.

Given the positive PCR findings for piroplasms in two individuals, we recommend that whole blood be collected for further investigation and identification of the organism.

*Meristaspis calcaratus* was recorded from one Christmas Island flying fox by Tidemann (1985); we also recovered the mite at very low densities. This family of mites (Spinturnicidae) is common in flying foxes, infesting the skin, wing membrane, and fur. They have been recorded from several species of *Pteropus* from Papua New Guinea, Malaysia, Madagascar, Vanuatu, Guam, and Nuie Island (Baker and Delfinado 1964; Prociv 1987). The significance of these mites within the population is unknown.

This is the first report of coccidian parasites in the Christmas Island flying fox. There are few reports of coccidiosis in *Pteropus* spp., with only 15 species of described coccidians in bats worldwide; all are of the genus *Eimeria* (Duszynski 1997). There is one report of suspected *Sarcocystis* sp. in the grey-headed flying fox (*Pteropus poliocephalus*) and published reports from four non-Australian bat species (unknown genus; Ladds 2009).

TABLE 5. (a) Comparison of plasma biochemical values between male and female, and (b) adult/subadult and juvenile Christmas Island flying foxes (*Pteropus melanotus natalis*).

| | Mean±SD | Range | Mean±SD | Range | |
|---|---|---|---|---|---|
| Test[a] | Male (*n*=18) | | Female (*n*=10) | | *P* Value |
| (a) | | | | | |
| ALP (U/L)* | 1,569.61±670.59 | 228.43–291.79 | 2611.28±1221.64 | 168–5,054.56 | 0.038 |
| ALT (U/L) | 10.84±5.81 | 0–22.46 | 8.61±2.86 | 2.86–14.33 | 0.242 |
| AST (U/L) | 34.81±7.76 | 19–29–50.33 | 37.63±13.49 | 10.65–64.61 | 0.647 |
| Albumin (g/L) | 39.30±1.71 | 35.88–42.72 | 39.04±2.29 | 34.46–43.62 | 0.788 |
| Amylase (U/L) | 1,697.08±203.22 | 1,290.64–2,103.52 | 1,625.78±181.77 | 1,262.24–1,989.32 | 0.415 |
| T-Bil (umol/L) | 2.72±1.15 | 0.42–5.02 | 3.06±0.91 | 1.24–4.88 | 0.442 |
| CK (U/L) | 374.30±231.40 | 0–837.1 | 302.78±117.29 | 68.2–537.36 | 0.348 |
| Ca (mmol/L) | 2.08±0.06 | 1.96–2.2 | 2.19±0.14 | 1.91–2.47 | 0.075 |
| Cholesterol (mmol/L) | 0.45±0.19 | 0.07–0.83 | 0.45±0.08 | 0.29–0.61 | 0.919 |
| Creatinine (umol/L) | 43.49±6.43 | 30.63–56.35 | 46.35±8.06 | 30.23–62.47 | 0.427 |
| GGT (U/L) | 7.11±2.29 | 2.53–11.69 | 7.58±0.79 | 6–9.16 | 0.515 |
| Globulins (g/L) | 24.57±1.41 | 21.75–27.39 | 25.82±2.89 | 20.04–31.6 | 0.278 |
| Glucose (mmol/L) | 2.87±1.05 | 0.77–4.97 | 3.44±1.00 | 1.44–5.44 | 0.225 |
| LDH-P (U/L) | 231.28±82.01 | 67.26–395.3 | 186.14±36.64 | 112.86–259.42 | 0.090 |
| Lipase (U/L) | 18.21±25.56 | 0–69.33 | 12.00±6.78 | 0–25.56 | 0.427 |
| Mg (mmol/L) | 0.69±0.03 | 0.63–0.75 | 0.70±0.03 | 0.64–0.76 | 0.516 |
| Phosphate (mmol/L) | 2.61±0.60 | 1.41–3.81 | 2.85±0.63 | 1.59–4.11 | 0.352 |
| Protein (g/L) | 64.30±2.86 | 58.58–70.02 | 64.65±3.81 | 57.03–72.27 | 0.815 |
| Triglycerides (mmol/L) | 0.52±0.22 | 0.08–0.96 | 0.52±0.26 | 0–1.04 | 0.966 |
| Urea (mmol/L) | 1.27±1.07 | 0–3.41 | 1.05±0.51 | 0.03–2.07 | 0.508 |
| Uric acid (mmol/L) | 0.01±0.01 | 0–0.03 | 0.01±0.02 | 0–0.05 | 0.345 |
| | Adult/subadult (*n*=17) | | Juvenile (*n*=11) | | |
| (b) | | | | | |
| ALP (U/L)* | 1478.56±615.13 | 248.3–2,708.82 | 3,054.81±422.58 | 2,209.85–3,899.77 | 0.001 |
| ALT (U/L) | 9.94±3.60 | 2.74–17.14 | 8.76±6.79 | 0–22.34 | 0.700 |
| AST (U/L) | 34.06±8.09 | 17.88–50.24 | 39.23±10.49 | 18.25–60.21 | 0.355 |
| Albumin (g/L)* | 40.19±1.78 | 36.63–43.75 | 38.10±2.46 | 33.18–43.02 | 0.044 |
| Amylase (U/L) | 1,701.92±241.28 | 1,219.36–2,184.48 | 1,698.00±212.98 | 1,272.04–2,123.96 | 0.967 |
| T-Bil (µmol/L) | 2.89±0.56 | 1.77–4.01 | 2.43±1.85 | 0–6.13 | 0.483 |
| CK (U/L) | 393.89±221.54 | 0–836.53 | 260.79±118.97 | 24.03–497.55 | 0.083 |
| Ca (mmol/L)* | 2.08±0.05 | 1.98–2.18 | 2.19±0.13 | 1.93–2.45 | 0.046 |
| Cholesterol (mmol/L) | 0.43±0.14 | 0.15–0.71 | 0.44±0.16 | 0.12–0.76 | 0.877 |
| Creatinine (µmol/L) | 46.11±6.38 | 33.35–58.87 | 40.36±6.82 | 26.72–54 | 0.085 |
| GGT (U/L) | 7.31±1.27 | 4.77–9.85 | 9.5±4.01 | 1.48–17.52 | 0.243 |
| Globulins (g/L) | 25.29±1.69 | 21.91–28.67 | 24.30±2.60 | 19.1–29.5 | 0.348 |
| Glucose (mmol/L) | 3.36±0.81 | 2.55–4.98 | 2.82±1.14 | 0.54–5.1 | 0.298 |
| LDH-P (U/L) | 219.09±74.38 | 70.33–367.85 | 186.59±36.22 | 114.15–259.03 | 0.197 |
| Lipase (U/L) | 13.47±15.20 | 0–43.87 | 34.16±37.26 | 0–108.68 | 0.287 |
| Mg (mmol/L) | 0.69±0.04 | 0.61–0.77 | 0.68±0.02 | 0.64–0.72 | 0.276 |
| Phosphate (mmol/L)* | 2.43±0.52 | 1.39–3.47 | 3.15±0.34 | 2.47–3.83 | 0.000 |
| Protein (g/L) | 65.21±3.14 | 58.93–71.49 | 62.93±2.73 | 57.47–68.39 | 0.071 |
| Triglycerides (mmol/L) | 0.51±0.19 | 0.13–0.89 | 0.58±0.38 | 0–1.34 | 0.622 |
| Urea (mmol/L) | 0.98±0.80 | 0–2.58 | 1.42±0.94 | 0–3.3 | 0.314 |
| Uric acid (mmol/L) | 0.01±0.01 | 0–0.03 | 0.01±0.02 | 0–0.05 | 0.271 |

[a] ALP = alkaline phosphatase; ALT = alanine transaminase; AST = aspartate aminotransferase; T-Bil = total bilirubin; CK = creatinine kinase; Ca = calcium; GGT = gamma-glutamyl transferase; LDH-P = lactate dehydrogenase; Mg = magnesium.

* ALP was significantly different between males and females and between adult/subadults and juveniles ($P<0.05$); however, significance was only maintained between adult/subadults and juveniles after the Bonferroni correction for multiple comparisons. Albumen, calcium, and phosphate also appear significantly different between adult/subadults and juveniles; however, only phosphate remained significantly different after the Bonferroni correction for multiple comparisons.

In all cases, infection was considered nonpathogenic.

The ascarid, *Toxocara pteropidis*, has been recorded in many *Pteropid* species, with upper airway obstruction, ill-thrift demeanor, and morbidity recorded in grey-headed flying fox (*P. poliocephalus*) and variable flying fox pups (*Pteropus hypomelanus*; Heard et al. 1995; Olsson and Woods 2008). Because we found only one ascarid-like ova, we recommend that further surveys for *Toxocara* sp. be undertaken in the Christmas Island flying fox population. Definitive identification of both coccidia and this ascarid-like ova is needed, and ongoing monitoring is required to determine if they are associated with disease.

Understanding and preserving long-standing ecologic and host-parasite relationships is an important element in retaining species fitness and fostering resilience to introduced organisms (Thompson et al. 2010). The biologic data collected in this study expands current knowledge of the ecology and pathobiology of this species and provides baseline hematologic and biochemical data for ongoing health assessment and conservation of this population. To truly understand the potential for disease as a key threatening process in the survival of the Christmas Island flying fox, we recommend that the population structure, dietary requirements, resource utilization, parasitologic relationships, and adult female health status be further investigated.

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