

## **BVA OVERSEAS TRAVEL GRANT REPORT 2009 ALEX TORRANCE - TANZANIA**

### **An Investigation into the relationship between ectoparasites, alopecia and blood-borne pathogens in the Straw-coloured Fruit Bat and into the haematological and biochemical ranges in the same species**

#### **Introduction**

The Straw-coloured Fruit Bat, *Eidolon helvum* inhabits vast areas of sub-Saharan Africa in colonies of up to 1,000,000 individuals and yet we have a very limited understanding of its ecology and role in disease transmission. The migratory nature, expansive colonies and preference for urban and suburban roost sites of this species raises concerns as to its potential as reservoir for infectious disease and its spillover into human and domestic animal populations. The zoonotic potential of pathogens such as Henipavirus and Lagos Bat Virus, to which widespread seroprevalence exists in the species, is yet to be fully scrutinised and the use of the species as bush meat in Western African may potentiate its zoonotic capability.

Notable decreases in colony size have been documented, leading the species to be classified as Near Threatened on the IUCN Redlist. Furthermore, fruit bats of the genus *Eidolon* are known to play a critical role in pollination and seed dispersal of a variety of trees and a continued demise of such a widespread species may have significant environmental and economical effects. Taking into account all these aspects, *Eidolon helvum* is a species that demands careful investigation and serious conservation efforts.

#### **Aims and Hypothesis**

The aim of this project was to produce reference ranges for a variety of biochemical and haematological parameters that can serve as indicators of health status in individuals within a population. The results may indicate poor levels of nutritional status, disease or environmental stress within the colonies of Tanzania, which are not only hard to assess externally, but are of great importance to our understanding of the species.

It was also hypothesised that there may be some association between immune status characterised by haematological values, body condition and parasite load. *E. helvum* are commonly known to be parasitized by the nycteribiid batfly, *Cyclopodia greeffi* that feed on the bat's blood. Association between the burden of this ectoparasite and any haemoparasites found may be suggestive of an indirect or vector-borne route of transmission.

#### **Materials and Methods**

Colonies of *E. helvum* were located in Upanga, Dar es Salaam (S 06.80134; E 039.28249) and central Morogoro (S 06.82331; E 037.66622) and capture potential was assessed. The bats were caught in mesh mist nets (6-12m x 3m; 3.8mm mesh) as they returned to their roost in the morning. The bats were restrained manually by one handler wearing puncture resistant gloves, while the other handler unravelled the

bat and placed them in cloth bags prior to sampling. The bats were weighed in their bags and weight calculated by deducting the bag weight. A sample number was assigned to each bat and the sex, reproductive age (either sexually immature or mature) and breeding status was assessed. Morphometric measurements of the antebrachial length were recorded using callipers. Visual examination of the integument highlighted any alopecia and counts of ectoparasites were made. Hair and ectoparasites samples were taken, and skin scrapes were made from alopecic animals. A subjective assessment of body condition was ascertained by palpation of the pectoral muscles and scored on a scale of 1 (very poor) to 5 (very good). Occlusion of the brachial vein along the cranial border of the proximal wing allowed phlebotomy of 1ml of blood. Blood smears were made at the sampling site and then fixed with methanol. The remaining blood was placed in heparinised tubes, centrifuged and the plasma was separated for biochemical analysis. Values for total protein, albumin, alanine aminotransferase, creatinine and cholesterol were calculated by spectrometry techniques carried out at the veterinary department of the Sokoine University of Agriculture, Tanzania. Blood smears were stained with Giemsa stain and smear analysis was used to acquire an estimated white blood cell count and calculate the percentage of each white blood cell type. Any abnormalities in cell morphology or haemoparasites were noted.

## Results

Standard statistical analysis was carried out on the collected data and values for the mean, standard deviation (S.D.) and range were calculated for all biochemical and haematological parameters. Reference ranges were determined by excluding values outside two times S.D. from the mean, recalculating the mean and are expressed as the new mean  $\pm$  2 S.D.s.

All bats in the sample population (n=50) appeared healthy on physical examination and had a body condition score of between 2-3.5/5. From the release site all bats were seen returning to their roost and no individuals were recaptured. Haematology and plasma biochemistry results are reported in Table 1.

**Table 1. Plasma biochemistry and haematologic values for the Straw-coloured Fruit Bat (*Eidolon helvum*)**

Test	Mean $\pm$ S.D.	Range	Reference range
Alanine aminotransferase (U/L)	53.9 $\pm$ 16.6	12 - 82	24.3 - 85.5
Albumin (g/L)	32.6 $\pm$ 5.0	16.6 - 41.7	24.7 - 41.4
Cholesterol (mmol/L)	0.39 $\pm$ 0.43	0 - 1.98	0 - 1.17
Creatinine (mmol/L)	49.7 $\pm$ 8.5	36.1 - 66.6	32.7 - 66.7
Total Protein (g/L)	64.8 $\pm$ 9.1	48.5 - 95.3	48.7 - 78.7
Estimated white blood cell count ( $\times 10^3/L$ )	11.9 $\pm$ 6.0	5.2 - 25	1.6-19.6
Segmented neutrophils (%)	51.6 $\pm$ 14.9	34 - 85	25.6 - 71
Lymphocytes (%)	44.1 $\pm$ 15.2	13 - 61	13.7 - 74.5
Monocytes (%)	3.82 $\pm$ 2.26	0 - 8	0 - 8.3
Eosinophils (%)	0.09 $\pm$ 0.90	0 - 3	0 - 1.9
Basophils (%)	0.09 $\pm$ 0.29	0 - 1	0 - 0.7

Erythrocytes were normochromic, normocytic and showed normal mammalian discoid morphology. The haemoparasites that have previously been described were not identified in the blood samples collected from Tanzania. White blood cell morphology was unremarkable.

Preliminary analysis suggests that there are no significant differences in mean biochemistry and haematology values between different age groups, sexes or reproductive status.

No correlation was seen between incidence of alopecia and ectoparasite burden. Skin scrapes of alopecic individuals contained no parasites. Further investigation is required to assess correlation with white blood cell parameters so immune status involvement can be determined.

## **Conclusions**

Assessing the health status of prey species is often difficult task as evolutionary drive compels weaknesses to be concealed. Serum biochemistry and haematology are effective ways of providing valuable information of the health and immune status of individuals. These parameters are also useful for epidemiological investigations and to consider the pathogenesis of specific diseases. Data relating to *Eidolon helvum* are scarce and, taking into account their wide distribution, migratory nature and potential for disease transmission, information relating to blood parameters is essential.

This study looks at a representative sample of individuals from two geographically separated colonies in Tanzania that in reality are parts of the same metapopulation. Both sexes and a range of age, body condition and reproductive statuses are represented in the sample. Reference ranges calculated in this study attempt to discount any outliers so that future comparison can be made to assess normal health status for *E. helvum*. Field limitations restricted the ability to produce a full profile of biological and haematological parameters. In the absence of absolute white cell counts estimates were made based on the blood smear. The proportion of neutrophils and lymphocytes varied significantly in the individuals captured. This may be indicative of variable stress response to the capture process. It can be hypothesised that those that experienced higher levels of stress would have a stress leukogram, showing a relative neutrophilia and lymphopenia. Without absolute cell count it is hard to assess the relative lymphocytosis that is seen in some individuals.

The relatively low level of cholesterol compared with domestic species is the main variable to note from the biochemical profile obtained. The low levels that are seen in *E. helvum* are consistent with values in related species and are most likely associated with the relatively low protein and fat diet of frugivorous bats.

Investigation into the hypothesised relationship between parasite burden and the occurrence of alopecia revealed no correlation, however the sensitivity of skin scrapes is variable and the sample size was very small (n=2). Further investigation is required to identify the aetiology of the alopecia. *E. helvum* are quite unique in the high density roosting of many individuals on one or two trees and the lesions observed may be associated with squabbling at the roosting site. It is to be expected that weaker individuals would be more susceptible and that the stress associated with this will be represented in the haematological profile of these individuals.

This study contributes to our understanding of normal physiological parameters in a species that has been largely overlooked. Data collected allows future research to assess health status of individuals, however further research is required to fully understand the interactions between host and pathogen and the role that *E. helvum* plays in disease transmission.