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# Erythrocyte size as one of potential causes of host preferences in cimicids (Heteroptera: Cimicidae: *Cimex*)

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**Abstract**. Cimicids are haematophagous insects whose life cycle, reproduction and survival rate depends on the blood of its hosts. Blood ingredients play a crucial role there. Two lineages have been identified in *Cimex lectularius* – bat- and human-associated bed bugs. Also bat bugs *C. pipistrelli* differ in particular bat hosts. We found some differences between the two lineages of bed bugs in the speed of moulting, length of life and reproduction success in cross-host experiments. It was considered that the bug proboscis could be very narrow and that red blood cells might not be able to pass through it. Therefore the first aim of this study was to find out whether the red blood cell (RBC) size has an impact on the occurrence of cimicids in bat and human host. Except one observation on *Plecotus auritus*, bat bugs never occurred in certain bat species i.e. *Barbastella barbastellus*, *Rhinolophus hipposideros* and *Plecotus austriacus*. We classified them as non-specific bug hosts, while the other bat species as specific hosts. The second aim of this study was to compare RBC size in specific and non-specific bat hosts. We collected blood samples from seven bat genera represented by 12 vespertilionid species and one rhinolophid species. Diameters of red cells were measured. We found some differences between the bat species, however, there was no clear correlation in erythrocyte size between specific and non-specific bat hosts and humans. Therefore RBC size is probably not the reason why some bat species are not parasitized by cimicids.

**Bat and bed bugs, RBC, hematocrit, bats, human**

## Introduction

Two *Cimex* species connected with bats, viz. *Cimex lectularius* Linnaeus, 1758 (bed bug) and *Cimex pipistrelli* Jenyns, 1839 (bat bug), posses numerous adaptations to ecology and anatomy of their hosts. Nevertheless they have never been found together in the same roost (Balvín et al. in prep.). Moreover, two different host lineages – bat- and human–associated – seem to exist within *Cimex lectularius* (Wawrocka & Bartonička 2013) which, according to Balvín et al. (2012a), never met even in evolutionary line. They differ at the genetic and morphological scale, which is a clear adaptation to a specific host. Similarly, existence of many ecotypes associated with different bat species was shown in *Cimex pipistrelli* (Balvín et al. 2013).

Cimicids have never been found in certain bat species i.e. *Plecotus auritus*, *P. austriacus*, *Barbastella barbastellus* or *Rhinolophus hipposideros* and *R. ferrumequinum*, except one newly described finding from Ukraine (Balvín et al. 2012b). One of the explanations may be their, a bit different than in the other species, roosting strategy that plays an important role and affects bat behaviour, occurrence or diversity (Kunz 1982, Findley 1993). Immune response, hormonal status (Jones 1996), roosting strategy and also blood components influence not only the sucking ability but also digestion, survival and development rate of blood sucking parasites (Krasnov

2008). Zedníková (2010) showed that *C. pipistrelli* is able to feed on non-specific species under laboratory conditions. However we do not know how sucking on non–specific (non-hosted in natural habitats) species affects ontogeny, survival in F1 and F2 generations, fertility or oviposition. RBCs, as the main blood components, seem to be the hardest items to digest due to their high protein amount (Gooding 1972). Therefore size parameters of erythrocytes may determine the success of sucking. For example chicken erythrocytes are 11.2 µm in diameter, while human erythrocytes only 6–8 µm (Reinhardt & Siva-Jothy 2007, Benoit 2011). This fact makes humans a better host than chicken for *Cimex lectularius* whose proboscis canal is 8–12 µm in diameter (Hase 1926, Tawfik 1968). The aim of this study was to find out whether the size of RBC could be a reason why some bat species are commonly parasitized by cimicids while others not. Therefore we measured RBC size, hematocrit and also the amount of red cells in specific and non-specific bat hosts of bed and bat bugs. We did the same for human commercial blood.

## Material and Methods

Blood samples were taken from the median vein of bat wings with scalpel and bleeding was stopped immediately by antiseptic liquid (Betadine). A small drop of blood was situated on a glass slide and coloured with May-Grundwald and Giemsa colours. The smallest (width) and largest (length) dimensions of red blood cells were measured using program QuickPHOTO MICRO 2.3. We measured 20 red cells for each microscope slide at 100× magnification using immerse oil (Fig. 1). With syringe (Hamilton, Chromoservis) we took 4 µl of blood and diluted it 200 times in physiological salt. Bürker glass was used to count and compare RBC in specific and non-specific hosts. The total RBC number was counted manually; mean from 10 visual fields (1 $mm^2$) was taken. Counting was carried out at 200× magnification. This sample was compared with a slide made in the same way from human commercial blood (laboratory commercial blood, Japan Medical Supply, B+). RBC size was counted for each species, sex (male/female) and status (adult/juvenile). To compare red cell size among species, the Kruskal – Wallis test was conducted. Statistical comparison of parameters in sex and age was made using the Mann-Whitney U test where possible.

Blood from 12 bat species from seven different genera. Therefore we caught females and males, adults and juveniles of these species: *Rhinolophus hipposideros* (Borkhausen, 1797) (n=1), *Myotis myotis* (Borkhausen, 1797) (n=2), *M. bechsteinii* (Kuhl, 1817) (n=1), *M. nattereri* (n=1), *M. mystacinus* (Kuhl, 1817) (n=1), *M. brandtii* (Eversmann, 1845) (n=1), *M. daubentonii* (Kuhl, 1817) (n=1), *Vespertilio murinus* Linnaeus, 1758 (n=1), *Eptesicus nilssonii* (Keyserling et Blasius, 1839) (n=2), *Pipistrellus pygmaeus* (Leach, 1825) (n=2), *Barbastella barbastellus* (Schreber, 1774) (n=1), and *Plecotus auritus* (Linnaeus, 1758) (n=5).

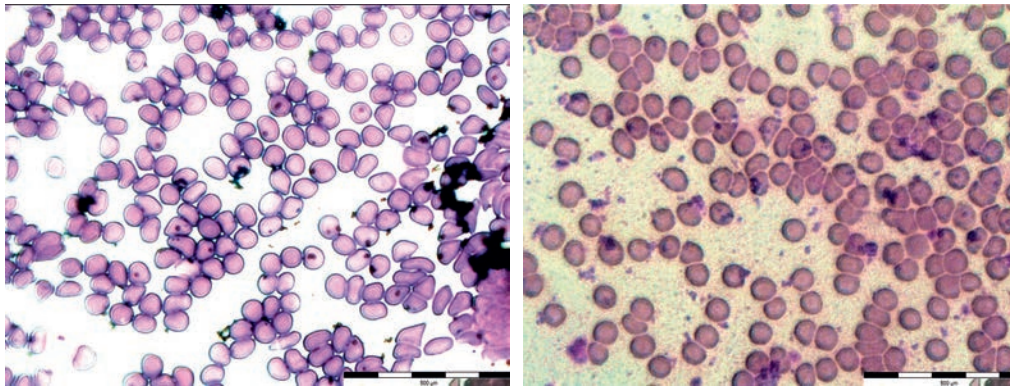

Fig. 1. Red blood cells of *Myotis myotis*, a specific host of the bed bug (left) and *Plecotus auritus*, a non-specific host (right), as viewed in the QuickPHOTO MICRO 2.3.

Table 1. Size of red blood cells in selected bat species and man (min size = the smallest dimension, width; max size = the largest dimension, length)

| species | mean min size±SD (μm) | mean max size±SD (μm) |
|---|---|---|
| *Rhinolophus hipposideros* | 4.3±0.42 | 4.6±0.43 |
| *Myotis myotis* | 5.6±0.55 | 5.9±0.45 |
| *Myotis daubentonii* | 6.6±0.41 | 6.9±0.51 |
| *Myotis brandtii* | 4.5±0.82 | 4.8±0.86 |
| *Myotis bechsteinii* | 6.5±0.51 | 6.6±0.49 |
| *Myotis nattereri* | 6.2±0.38 | 6.4±0.40 |
| *Myotis mystacinus* | 5.3±0.26 | 5.7±0.32 |
| *Pipistrellus pygmaeus* | 5.2±0.39 | 5.6±0.40 |
| *Eptesicus nilssonii* | 6.1±0.26 | 6.4±0.37 |
| *Vespertilio murinus* | 5.6±0.39 | 5.8±0.46 |
| *Barbastella barbastellus* | 5.8±0.74 | 6.2±0.61 |
| *Plecotus auritus* | 5.4±0.66 | 5.7±0.66 |
| human | 6.4±0.31 | 6.6±0.43 |

## Results

Comparison between sexes within the same species did not show any significant differences in RBC size (U test, $p>0.05$, $n_1=3$, $n_2=3$) and neither did the comparison between adults and juveniles within the species (U test, $p>0.05$, $n_1=4$, $n_2=4$). The Kruskal-Wallis test did not reveal statistically significant differences between particular bat species ($H=15$, $df=11$, $p=0.18$). In all tested bats, both specific and non-specific hosts, the size of erythrocytes ranged between 4.5–6.9 μm. Nevertheless, five of 12 species showed a significantly larger RBC size (Table 1) in comparison with the mean RBC size for all studied bat species (5.7 μm), i.e. *Eptesicus nilssonii* (10% higher), *Barbastella barbastellus* (5%), *Myotis daubentonii* (18%), *M. bechsteinii* (15%), and *M. nattereri* (10%). Out of these species, only *Barbastella barbastellus* was not infested by cimicids, however, the other species have larger erythrocytes. The size of RBC in human blood (B+) was estimated at 6.5 μm, which is a higher value than in most bat species.

Finally, we compared the number of RBCs in bats and humans. Hematocrit level as well as the number of RBCs is higher in bats than in humans (156% higher in *Myotis nattereri*, 141% in *M. daubentonii*, 192% in *Pipistrellus pygmaeus*, and 79% in *Vespertilio murinus*) (Table 2). The highest hematocrit level as well as RBC number was found in *Pipistrellus pygmaeus*.

Table 2. Comparison between human and bat blood structure. Hematocrit (percentage of volume of red blood cells per unit blood) and amount of RBC in μl.

| species | hematocrit (%) | RBC ($10^6$/μl) |
|---|---|---|
| *Myotis myotis* | 41 | 9.1 |
| *Myotis nattereri* | 58 | 12.3 |
| *Myotis daubentonii* | 52 | 11.6 |
| *Pipistrellus pygmaeus* | 61 | 14.0 |
| *Plecotus auritus* | 49 | 12.3 |
| *Vespertilio murinus* | 46 | 8.6 |
| human | 42 | 4.8 |

## Discussion

### Specific and non-specific bat hosts

Feeding success of blood-sucking insects depends on physiological and nutritional conditions of their host. Differences in blood chemistry and physiology may be one of the mechanisms determining host preferences in bats. Poulin (2007) described two main filters that determine host choice by parasites, one of them being a compatibility filter that excludes all host individuals on which parasites cannot feed for morphological, physiological and immunological reasons. That is why haematological data on bats could be important to understand host choice in cimicids and their species specificity. Previous research has shown that many components and aspects of host blood (e.g. T-lymphocytes, antibodies, mast cells or granulocytes) influence not only the sucking ability but also digestion, survival and development rate of parasites (Krasnov 2008). Size of red cells is definitely one of the important factors (cf. Reinhadt & Siva-Jothy 2007). Specific host seems to be a good reservoir of blood meal and bugs, in order to avoid risk, prefer to feed less often but more intensively. In other hosts (non-specific), after taking blood meal, bugs did not continue till repletion (Barbarin et al. 2013, personal observation). We did not find significant differences in the amount of red cells between the studied bat species (specific: *Myotis myotis*, *M. nattereri*, *M. daubentonii*, *Pipistrellus pipistrellus*, and non-specific: *Plecotus auritus*), which means that we cannot find explanation for host preferences in RBC density (Table 1). Nevertheless, according to a blood analysis carried out in the Egyptian fruit bat (*Rousettus aegyptiacus*) (Korine et al. 1999), blood profile changes between seasons and also depends on activity of the animal and reflects animal fitness.

It has been shown that in the wild, *Cimex pipistrelli* does not occur or only very rarely in certain bat hosts (*Barbastella barbastellus*, *Plecotus auritus*, *P. austriacus*, and *Rhinolophus hipposideros*), but it seems to be able to feed on them under laboratory conditions (Zednikova 2010). The easiest explanation for the absence of bugs in these bats would be that specific bug hosts do not meet non-specific hosts in the roosts, where the transmission of ectoparasites is most likely. However, in the case of roosts in attics, there are quite often more species of bats roosting together. During monitoring of bat populations in the Czech Republic, about 140 roosts of nursery colonies of *Myotis myotis* are checked annually and over 20 of them are shared with some of the species ranking among non-specific hosts (such as *Plecotus* spp. or *Rhinolophus hipposideros*) (Czech Bat Conservation Trust database, unpubl.). Despite that, it seems that the transfer to a new host is very rare. *B. barbastellus* prefers to roost in crevices of dead beech trees, shows frequent roost switching behaviour and forms quite small colonies (Russo et al. 2004), which makes it not suitable for cimicids. Moreover, this species never shares its roosts with other bat species, especially the bug specific hosts. *Rhinolophus* bats, originally cave-dwelling species (Jepsen 1970), shows night roosting activity with frequent switches and their day roosts are found in barns, stables, garages as well as in caves or underground tunnels and cellars (Knight & Jones 2009). Caves, which usually offer a wide thermal range (Tuttle & Stevenson 1978), are very cold for bugs in the temperate zone (Balvín et al. 2012b).

Nevertheless, many studies indicate that blood composition and chemical components are an important issue in the case of bed bugs. In *Cimex lectularius*, the proboscis canal is a simple tube and blood is stored in midgut. Erythrocyte diameter can influence sucking ability of the bugs, especially when it is larger than the internal diameter of the proboscis tube. Feeding experiments using rabbit, chicken, cavia and human blood showed that human blood was most convenient for the bugs and kept their survival at the highest level (Barbarin et al. 2013). These results suggest that technical and chemical blood components which differ in the above mentioned hosts

may affect blood intake in the bed bug, however, the authors did not measure RBC diameter. In our study we did not find significant differences between humans and bats in RBC size (range 4.5–6.9 μm in bats, 6.2–6.8 μm in human), though the size of human RBC is at the upper limit of the bat range.

**Bat and human lineage of *Cimex lectularius***

Bats are quite atypical hosts in terms of the short time bugs have to feed on them, and that is probably why evolution favoured maximization of the diameter of the proboscis canal. Percentage of hematocrit in the host blood has an impact on host choice and feeding time. Decrease of hematocrit would shorten the time needed to take full meal (Daniel & Kingsolver 1983). *Myotis myotis* has the lowest level of hematoctrit (41%) of all tested bat species. This fact might explain why it is the most common host of *Cimex lectularius*. Similarly as Neuweiler (2000), we confirmed that bats possess a higher number of erythrocytes than humans, with the highest number in *Pipistrellus pipistrellus* (14.0). High concentration of hemoglobine in bat RBC gives them quite high oxygen capacity at the level of 30% which is almost twice more than in the case of ground dwelling mammals (Neuweiler 2000). We also found that red cell size is smaller in bats than in humans and this together with their higher numbers helps bats to manage oxygen exchange during such energy consuming activity as flight is.

All blood-sucking insects had to develop special mechanisms for blood intake. Occurrence of bugs in certain host species is determined by many factors such as temperature, host availability but also morphology of mouthparts and saliva components (Guarneri et al. 2000, Sant'Anna et al. 2001). Marcus & Safier (1993) proved that bed bug saliva contains special molecules that neutralize haemostatic answers of host body. This is mostly apyrase, an inhibitor of Factor Xa, responsible for the coagulation cascade (Valenzuela et al. 1996), and nitrophorin that enables nitric oxide (NO) transports (Valenzuela et al. 1995). These molecules thus halt immune system response and help to avoid blood coagulation. One of the reasons why some bat species are not infested by cimicids in the wild may be a stronger immune defence and the higher number of white blood cells (eozynofiles or neutrofiles). Therefore more studies on bat blood components are needed to find out which factors determine certain species as specific or non-specific hosts.

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