An Alternative, Less Invasive Blood Sample Collection Technique for Serologic Studies Utilizing Triatomine Bugs (Heteroptera; Insecta)

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Source: Journal of Wildlife Diseases, 42(2) : 466-469

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-42.2.466

Journal of Wildlife Diseases, 42(2), 2006, pp. 466–469

An Alternative, Less Invasive Blood Sample Collection Technique for Serologic Studies Utilizing Triatomine Bugs (Heteroptera; Insecta)

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ABSTRACT: The collection of blood samples for serological studies is often stressful for the focus animal. Recently, the use of blood-sucking bugs, such as *Dipetalogaster maximus* or *Triatoma infestans* (Reduviidae; Triatominae; Heteroptera), has been suggested as a new and less invasive method for blood collection. To evaluate this technique, we collected paired blood samples from 20 domestic rabbits (*Oryctolagus cuniculus*) during a study of rabbit hemorrhagic disease virus (RHDV). For each rabbit, blood samples were collected by the conventional method (needle and syringe from the vena auricularis) and through feeding by *D. maximus*. Samples were tested for RHDV antibodies using standard test kits at three different dilutions. Antibody titers were identical for 56 paired samples and differed in only four cases. The simple matching indices were 1 for the 1:10 dilution and 0.9 for the 1:100 and 1:1000 dilutions. The major advantages of the new technique are 1) the possibility to obtain blood from animals where veins are inaccessible and 2) the fact that anesthesia of focus animals may not be necessary.

Key words: *Dipetalogaster maximus*, noninvasive blood sampling, serology, stress.

The acquisition of blood from captive or free-ranging mammals or birds is often hampered or made impossible because of the inaccessibility of veins or by the necessity to immobilize the focus animal. However, blood parameters, such as antibody titers, are fundamental for diagnostic purposes. Recently, a new blood sampling technique has been developed that could potentially facilitate the acquisition of blood in cases when conventional methods are not applicable. This innovative technique involves the use of blood-sucking bugs: *Triatoma infestans, Rhodnius prolixus*, or *Dipetalogaster maximus* (Reduviidae; Triatominae; Heteroptera) (von Helversen et al., 1986).

Von Helversen and Reyer (1984) and Voigt et al. (2003) used this technique to acquire 50–100 µl blood from bats of 10 g body mass. Blood collection is especially difficult in these small-sized mammals because veins are too small for a conventional needle. Voigt et al. (2003, 2005) validated this technique for use in doubly labeled water experiments. They reported a potential for contamination of the blood sample with bug hemolymph or intestinal liquids. In a third validation study, the suitability of bugs was tested for use in endocrinological studies. Voigt et al. (2004) compared levels of steroid hormones, (progesterone, testosterone, and cortisol) in blood samples that were taken from the same individual with the conventional technique (a 0.60 × 30–mm needle and syringe) and through bug feeding (fourth larval instar of *D. maximus*). In contrast to Voigt et al. (2003, 2005), the authors took blood from the crop of the animals, thus avoiding contamination with hemolymph. Pair-wise comparisons revealed no significant differences in hormone concentrations related to blood sampling method. Blood hormone concentrations remained unbiased even after 8 hr within the bugs' intestinal tract, and based on hydrocorticosterone levels, the authors determined that blood collection through bug feeding caused less stress to the focus animal than the conventional needle and syringe method (Voigt et al., 2004).

Most recently, bugs have been used in free-ranging common terns (*Sterna hirundo*) (Becker et al., 2006). During these experiments, a triatomine bug was kept in an artificial hollowed egg. The starving bug then pierced its proboscis through a small window in the egg and collected a blood sample (median 187 µl blood) from the breeding parent bird. The focus

animals neither took notice of the blood collection nor abandoned their clutch. In a second study, triatomine bugs were used for blood collection from various primates in captivity without restraining the focus animals (Thomsen and Voigt, 2006). Both case studies emphasize that triatomine bugs may collect blood noninvasively from wild or captive animals.

In this study, we validate the use of bugs for serologic studies. We hypothesized that antibodies are prone to chemical degeneration and enzymatic digestion. Therefore, we expected that concentrations of antibodies measured in blood obtained with the conventional method and the "bug method" should differ significantly.

To test this hypothesis, we performed an experiment with 20 domestic rabbits (*Oryctolagus cuniculus*) from a breeding stock of 50 animals at the Tierpark Friedrichsfelde, Berlin, Germany. Both blood sampling methods were applied to each animal. The conventional technique included blood acquisition from the auricular vein (vena auricularis) of one ear using a 0.60 × 30–mm needle (Braun, Melsungen, Germany). For the bug method, we used fifth larval instars of the triatomine bug *D. maximus* that were bred in captivity at the Leibniz-Institute for Zoo and Wildlife Research (at least two generations in captivity). A single bug was placed onto the ear of the rabbit opposite from that stuck with the needle. The bug punctured the rabbit's skin within several minutes or even seconds, and after 5 to 15 min, bugs completed their blood meal. Within a few seconds afterwards, we obtained approximately 1.5 ml blood from the crop of the insects using a conventional needle and syringe (Voigt et al., 2004). The blood samples were centrifuged at 1,200 × G for 30 min. The serum was separated and kept frozen at −20 C until further analysis.

For antibody detection, we used rabbit hemorrhagic disease virus (RHDV) antibody-blocking enzyme-linked immunosorbent assay test kits according to Frölich et al. (1996), following the directions of the manufacturer (Danish Veterinary Institute for Virus Research, Lindholm, Denmark). Positive (Bundesforschungsanstalt für Viruskrankheiten, Tübingen, Germany) and negative control sera (Institut für Versuchstierkunde, Berlin, Germany) for RHDV were included. Sera that deviated >3 standard deviations from the mean optical density of negative control sera in a dilution of ≥1:10 were considered antibody positive. To test for agreement between the results, we calculated the simple matching index for each of the dilution titers and then performed a binomial test. We used Excel (Microsoft Inc. Version 97, Redmond, Washington, USA) for data analysis and SPSS (SPSS Inc. 1998, Chicago, Illinois, USA) for statistical analysis.

Antibody titers of the blood samples taken by the two different blood sampling techniques yielded the same results in 56 cases. At a dilution step of 1:10 we found a perfect match of results. At both the 1:100 and 1:1000 titers, two of the matched samples differed. For both dilution steps we encountered one case for which the result was positive when the sample was taken conventionally and negative when the sample was taken with the bug method and one case for which a conventional sample was negative but the paired sample obtained with the bug tested positive. The simple matching indices are 1, 0.9, and 0.9. The results matched significantly for all three dilutions (Binomial test against 0.5, $P<0.001$ in all three cases).

We could not find a significant effect of intestinal liquids on the presence or absence of antibodies in blood ingested by bugs. Thus, we conclude that antibodies were not degenerated or digested in the bug after obtaining the blood meal. In conclusion, triatomine bugs are a suitable tool for use in serological studies on RHDV in rabbits. Other serological studies may also be possible with blood-

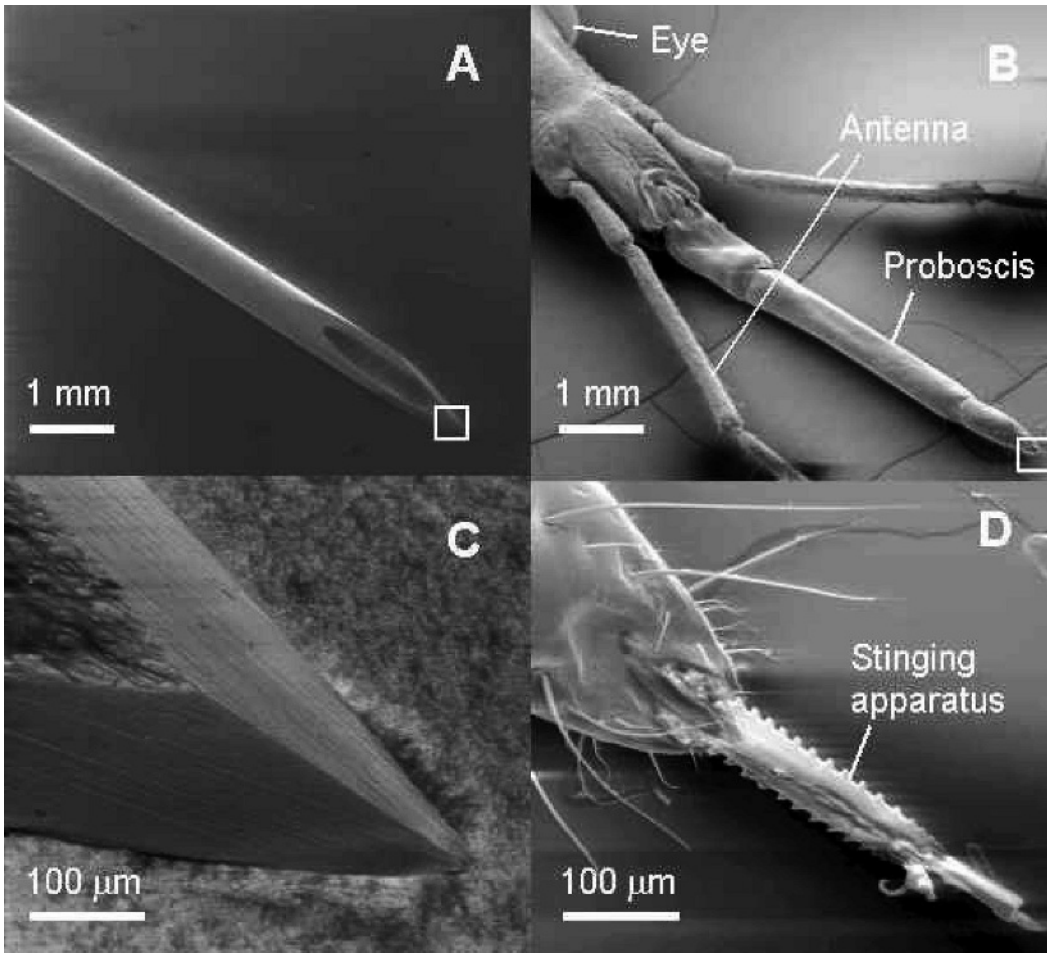

FIGURE 1. Electron microscope image of a 0.60 × 30–mm needle (A, C), the head and stinging apparatus (B), and the tip of the proboscis (D) of a *Dipetalogaster maximus*. Images A and B were taken with a magnification factor of 12 and C and D with a factor of 200. The boxes in image A and B indicate the tip of the needle (A) and proboscis (B) that are shown at a larger magnification below. The bug punctures the skin only with the saw-blade–like tip of the proboscis shown in image D, whereas the whole diameter of the needle as seen in image A is inserted into the skin when using the conventional blood sampling method.

sucking bugs; however, we recommend validating the bug method when testing for antibodies against viruses other than RHDV. We also recommend using bugs only once for a given animal to avoid cross-infection with pathogens; the bug colony should be tested for relevant pathogens before application to any animal. This innovative technique may provide new opportunities for disease research in both in wild and captive animals; this approach may provide a means to collect blood samples from species that have proven difficult to sample by conventional approaches. The advantages of the bug method are as follows: 1) small animals with cryptic veins can be included in serological studies because triatomine bugs find a suitable vein even though the capillary may not be visible to humans; 2) because of the small size of the stinging apparatus (Fig. 1), focus animals are most likely less stressed when bugs are used; 3) under certain circumstances focus animals may even not notice that they are exposed to a blood-sucking bug; 4) focus

animals may not have to be immobilized because bugs inflict less stress on them, thus reducing the dangers and costs associated with narcosis; 5) bugs do not cause hematomas as occasionally occurs in routine veterinary procedures; and 6) sampled blood volumes can be predetermined by using larval instars of different sizes.

We would like to thank Doris Fichte, Dagmar Viertel, Barbara Caspers, Mirja Faßbender, and Martin Dehnhard for help during the experiment. Ruth Thomsen gave helpful comments on an earlier draft of this manuscript.

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Received for publication 24 June 2005.