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Laboratory Evaluation of Rubidium as a Long-Lasting Marker for Bloodfeeding Sand Flies (Diptera: Psychodidae)

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ABSTRACT The objective of this study was to evaluate the use of the trace element rubidium (Rb) as a long-lasting systemic biomarker for bloodfeeding females of the sand fly *Phlebotomus papatasi* Scopoli. Baits containing Rb chloride were found to be palatable to hamsters in this study. We were able to detect Rb using a portable X-ray fluorescence analyzer in all sand flies that fed on Rb-treated hamsters for at least 14 d postbloodmeal. We also detected Rb in sand flies that took a bloodmeal from hamsters up to 10 d after the hamsters were withdrawn from a Rb-treated diet. Results of this study constitute proof of concept for the incorporation of Rb chloride into rodent baits for marking bloodfeeding sand flies, and suggest that Rb marking could be used as a technique for evaluating rodent-targeted sand fly control methods and in ecological studies on sand flies.

KEY WORDS sand fly, biomarker, rubidium, self-marking, *Phlebotomus papatasi*

Phlebotomine sand flies are able to transmit *Leishmania* parasites through their bite, and human infection with certain species of this parasite can cause disfiguring or life-threatening disease. The World Health Organization (WHO) has described leishmaniasis as an emerging and uncontrolled disease that disproportionately affects poor populations around the world (WHO 2011).

With few exceptions, leishmaniasis is a zoonosis that affects man when he encroaches into an area of transmission. Rodents serve as the reservoirs for several *Leishmania* parasites including *Leishmania major*, *Leishmania mexicana*, and *Leishmania amazonensis* (Dedet et al. 1989, Disney 1968, Kerr et al. 1995, Saliba et al. 1994). The concept of breaking the cycle of transmission of *L. major* recently has been explored through targeting sand flies that feed on rodent reservoirs by incorporating systemic and feed-through insecticides into rodent baits (Mascari et al. 2011). While such a targeted intervention would have an epidemiological impact, it may not noticeably reduce the overall sand fly population because of females feeding on hosts other than targeted rodents. Furthermore, it would be valuable to have a benchmark indicator for the successful targeting of sand flies feeding on rodents in addition to the overall outcome of reduced *Leishmania* infection rates in sand flies, reservoirs, or man.

The fluorescent tracer technique has been developed as a control efficacy diagnostic tool and in ecological studies for sand flies. The fluorescent dye rhodamine B has been shown in the laboratory and field to temporarily mark sand flies that have taken a bloodmeal from orally treated rodents (Mascari and Foil 2009, Mascari et al. 2011). The objective of this study was to evaluate the use of the trace element rubidium (Rb) as a long-lasting systemic biomarker for bloodfeeding sand flies.

Materials and Methods

Sand Flies. The sand flies used in this study were from a laboratory colony of *Phlebotomus papatasi* Scopoli maintained at LSU AgCenter (Baton Rouge, LA). All stages were reared in darkness at 28°C and 90% RH. In the colony, larvae were fed dried rabbit feces, and adults were given 20% sucrose solution ad libitum. Adult female sand flies were allowed to take bloodmeals from hamsters (*Mesocricetus auratus*).

Rodents. Six hamsters were housed individually in micro-isolator cages ((Lab Products Inc., Seaford, DE). The maintenance of the hamsters and the experimental procedures of this research followed Animal Care and Use Protocols 08-056 and 11-035 that have been approved by the Institutional Animal Care and Use Committee at Louisiana State University, Baton Rouge, LA. All animal research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals.

Treatment of Rodents. Two hamster diets were prepared using a powdered laboratory rodent diet (5001 Rodent Diet, LabDiet, PMI Nutrition International, Brentwood, MO) as a base. One diet contained 100

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g/kg soybean oil as a palatability and sticking agent plus 5 g/kg Rb chloride (RbCl, Sigma, St. Louis, MO), and the other diet contained 100 g/kg soybean oil alone.

Three hamsters were assigned randomly to each diet group. The hamsters were provided with 15 g of their respective diets each day for 3 d. The amount of food consumed by each hamster was recorded daily and was compared between diet groups using analysis of variance (ANOVA) performed with the GLM procedure (SAS Institute 2001). The Tukey multiple comparison procedure was used to separate significantly different means. The dose of RbCl for each hamster was calculated (in milligrams per kilogram of body weight), and the values were converted to doses of Rb. After feeding on their respective diets for 3 d, all hamsters were fed an untreated pellet diet for the remainder of the experiment.

Bioassay. Immediately after being withdrawn from their Rb-treated or untreated powdered diets, the hamsters were chemically immobilized via intraperitoneal injection of ketamine HCl (100 mg/kg body weight) plus xylazine HCl (10 mg/kg body weight). Immobilized hamsters were placed individually into a 3.8 liter clear plastic cage with 25 female sand flies aged 3–5 d. Sand flies were allowed to feed on the hamsters for at least 30 min before the hamsters were removed and recovered. All unfed sand flies were removed from the cages and excluded from the study. Five bloodfed sand flies then were removed from each bioassay cage, killed by freezing, placed individually in 2 ml microcentrifuge tubes and desiccated over calcium sulfate until being examined for the presence of Rb. The remaining bloodfed sand flies were provided with 20% sucrose solution ad libitum and held at 28°C and 90% RH. Subsequently on 3, 7, 10, and 14 d after bloodfeeding, five sand flies were removed from the cages, killed, and desiccated.

Additional cohorts of 3–5 d old female sand flies were bloodfed on the hamsters 3, 7, and 14 d after the hamsters were removed from their Rb-treated or untreated powdered diets. All of these bloodfed sand flies were killed immediately after feeding and were desiccated.

Rb Detection. The concentration of Rb present in sand flies was determined using a portable x-ray fluorescence (XRF) analyzer (Delta model, Innov-X Systems, Woburn, MA). Individual sand flies were placed on a stage and oriented above the beams of the XRF analyzer. Three beams were used to analyze each sample, with readings of 30 s for each beam. Values for the concentration of Rb present in each specimen that were provided by the XRF analyzer were converted to parts per million. The concentration of Rb present in sand flies for each posttreatment or postbloodmeal time period was compared using ANOVA performed with the GLM procedure, and significantly different means were separated using Tukey's multiple comparison procedure (SAS Institute 2001).

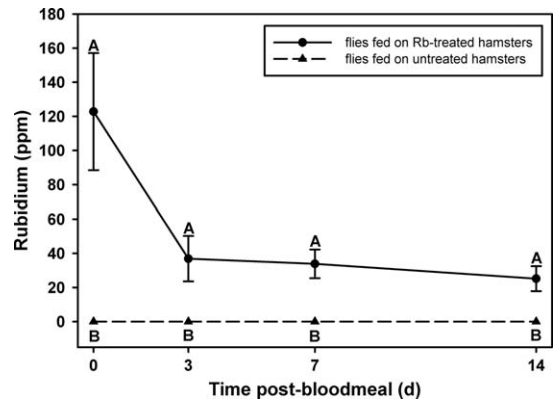


Fig. 1. Concentration of Rb detected in sand flies fed on Rb-treated hamsters at different intervals postbloodmeal. Values at a time period with the same letter are not significantly different ($P > 0.05$).

Results

Treatment of Rodents. The mean amount of food consumed daily by hamsters fed a diet containing 5,000 mg/kg RbCl (8.4 ± 0.7 g) was not significantly different from the amount of food consumed by hamsters fed an untreated diet (8.0 ± 1.2 g; $F = 0.58$, $df = 1$, $P = 0.4572$). The mean daily dose of Rb for hamsters was 303.9 ± 34.7 mg/kg body weight.

Bioassay. The XRF analyzer did not detect Rb in any of the sand flies that took a bloodmeal from an untreated hamster throughout the study. The concentration of Rb detected in sand flies immediately after bloodfeeding on Rb-treated hamsters was significantly higher than the concentration detected in sand flies 3, 7, and 10 d postbloodmeal (Fig. 1; $F = 65.97$, $df = 4$, $P < 0.0001$). However, the concentration of Rb remained constant in bloodfed sand flies between 3 and 14 d postbloodmeal, and was significantly different from 0 ppm.

We observed an inverse relationship between the concentration of Rb detected in sand flies and the posttreatment interval of the hamster that served as a bloodmeal host (Fig. 2). The concentration of Rb in sand flies that took a bloodmeal from Rb-treated hamsters was significantly different from 0 for up to 10 d posttreatment ($F = 56.58$; $df = 5$; $P < 0.0001$).

Discussion

Previous studies have demonstrated the use of Rb to mark bloodfeeding mosquitoes and to study post-bloodmeal behavior for certain species (Kimsey and Kimsey 1984, Liew and Curtis 2004, Wilkins et al. 2007). Marking of bloodfed phlebotomine sand flies has been achieved in the field through detection of radioactive carbon in sand flies that had been acquired through bloodfeeding on parenterally treated gerbils (Dergacheva et al. 1996). However, active capture and treatment of rodents with a biomarker would not be compatible with field studies on rodent-targeted sand fly control methods. The fluorescent tracer technique

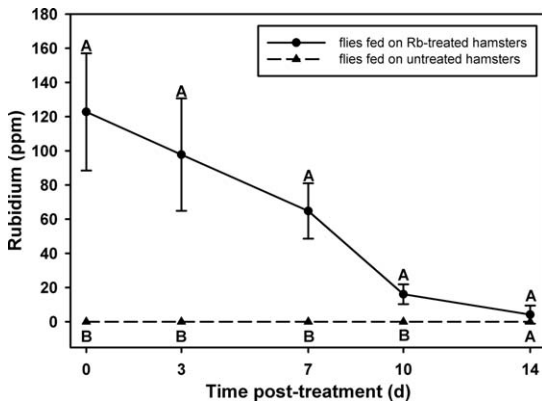


Fig. 2. Concentration of Rb detected in sand flies fed on Rb-treated hamsters at different intervals after hamsters had been withdrawn from treated diets. Values at a time period with the same letter are not significantly different ($P > 0.05$).

is a system through which rodents passively acquire a biomarker through treated bait. In field studies, rhodamine B has been incorporated into rodent baits for feed-through and systemic marking of sand flies, allowing the determination of adult sand flies that have fed on the feces of baited rodents as larvae, or that have taken bloodmeals from baited rodents (Mascari et al. 2011).

However, rhodamine B does not permanently mark bloodfeeding sand flies, and its use may underestimate the number of sand flies feeding on baited rodents. Incorporation of Rb (that persists for at least 14 d postbloodfeeding) rather than rhodamine B (that transiently marks bloodfed sand flies) into rodent baits could provide a more accurate estimation of the number of sand flies that are feeding on baited rodents.

Rhodamine B also can be used in conjunction with insecticide applications targeting rodents for quality control of their insecticidal activity against sand flies (Mascari et al. 2011). In these studies, the collection of bloodfed sand flies marked with rhodamine B is considered an indication of the failure of an insecticide application. However, it is possible that exposure to a sublethal dose of certain insecticides could lead to temporary akinesia after bloodfeeding and, as a consequence, exclusion from the population being sampled (Foil et al. 1991). Because rhodamine B only marks bloodfeeding sand flies for a short period of time, it is possible that the dye could be eliminated before sand flies are recovered and subsequently re-enter the population being sampled. Use of Rb, which the results of this study show marks sand flies for at least 14 d postbloodfeeding, could reduce the possibility of failing to detect sand flies that have survived bloodfeeding on insecticide-treated rodents but that have been temporarily sequestered from the sample population.

The XRF analyzer used in this study to detect Rb also may offer further advantages over current methods to detect sand flies that have fed on baited rodents

that must be performed in a laboratory. These results indicate that Rb can be detected in sand flies using a field-portable XRF analyzer, and therefore could provide real time results and allow a faster response to problems with performance of insecticide treatments in the field. Using the methods described in this study, analysis of an individual sample takes 90 s, and over 200 samples can be processed easily in one day. A handheld XRF analyzer can cost more than \$25,000, but they are often available for use in governmental and academic environmental science labs throughout the world. The results of this study are promising, and future field studies to further evaluate the use of Rb-marking of sand flies in control and ecological studies are planned.

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