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Laboratory and field evaluation of rodent bait treated with fipronil for feed through and systemic control of *Phlebotomus papatasi*

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ABSTRACT

The sand fly *Phlebotomus papatasi* is the main vector of *Leishmania major*, etiologic agent of zoonotic cutaneous leishmaniasis (ZCL), which is endemic in North Africa, the Middle East, and Asia. In North Africa, *Meriones shawi* is one of the two main reservoir hosts of *L. major*. *P. papatasi* populations are maintained in borrowing rodents such as *M. shawi*. Three fipronil-treated rodent baits were evaluated for systemic and feed through insecticidal activity against *P. papatasi* feeding on *M. shawi*. Through blood feeding bioassays, mortality rates of females *P. papatasi* increased with the concentration of fipronil in the rodent bait varying from 0.001% to 0.005%. In the laboratory, more than 90.0% of *P. papatasi* were killed within 48 h after blood feeding on the desert's jirds, *M. shawi*, treated up to 29 days prior with a single application of fipronil at a concentration of 0.001%, 0.0025% and 0.005%. Through larval bioassays, mortality rates of larvae that have fed on faeces of treated bait for *M. shawi* increase with the concentrations of fipronil. Faeces of orally-treated *Meriones* were significantly toxic to larvae for 5 weeks with a concentration of 0.005%. In the field, application of treated bait resulted in 80.0% reduction in the populations of *P. papatasi* up to 6 weeks after a single application of fipronil at a concentration of 0.005%. This is the first study to demonstrate field efficacy of fipronil-treated rodent baits for *P. papatasi* control and the first study to evaluate this approach in *M. shawi*, a principal ZCL reservoir host. These results suggest that fipronil-treated rodent baits can be used to effectively reduce the populations of *P. papatasi* associated with *M. shawi* in ZCL endemic areas.

1. Introduction

Zoonotic cutaneous leishmaniasis (ZCL) is a neglected tropical disease that affects thousands of people annually in the endemic areas of Central and Southern Tunisia (Ben Ismail, 1993; Chelbi et al., 2007, 2009). This disease is caused by the parasite *Leishmania major*, and is transmitted by the sand fly *Phlebotomus papatasi* (Ben Ismail et al., 1987a). In this region, the fat sand rat, *Psammomys obesus*, and the desert's jird *Meriones shawi*, are the principal reservoir hosts of *L. major* (Rioux et al., 1986, 1992; Ben Ismail et al., 1987b; Fichet-Calvet et al., 2003; Ghawar et al., 2011). There is no ZCL vaccine available, so vector and reservoir host control are currently the only methods for prevention of this disease.

During the last 20 years, agricultural development has led to the destruction of the *P. obesus* habitat, resulting in the disappearance of this rodent species around human settlements in many endemic parts of Tunisia. *M. shawi* has consequently become the principal reservoir host of *L. major* in many of these areas (Ben Salah et al., 2007). *M. shawi* is nocturnal, feeds mostly on grain and fruit, and inhabits complex burrows systems associated with one of its main food resources, the jujube tree, *Ziziphus zizyphus*. These burrows have moderate, stable temperatures and elevated humidity which creates a suitable microclimate for the immature and adult stages of *P. papatasi*. Adult female *P. papatasi* also utilize *M. shawi* as their primary blood meal source, and the rodent faeces and plant debris that accumulate in these burrows are the main food source for sand fly larvae (Zhioua, unpublished). Therefore, the closely linked relationship between *P. papatasi* and *M. shawi*, make insecticide application targeted at *M. shawi* an effective approach for controlling populations of *P. papatasi* associated with this reservoir host species.

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Identification of larval sand fly habitat is problematic, and even if identified many habitats such as rodent burrows are not easily accessible, so current *P. papatasi* control measures only target adult sand flies (Killick-Kendrick, 1987; 1999). Indoor residual spraying (IRS) (Benzerroug et al., 1992; Morsy et al., 1993) and insecticide treated curtains or bed nets (Elnaiem et al., 1999; Yaghoobi-Ershadi et al., 2006; Jalouk et al., 2007) have been somewhat successful at reducing the incidence of ZCL. Zooprophylaxis has also been explored as a method to reduce the indoor abundance of *P. papatasi* (Chelbi et al., 2008), but the effectiveness of this method at managing ZCL has not been established.

Other ZCL control strategies involve reduction of the rodent reservoir host population. Destruction of rodent burrows and poisoning ZCL rodent reservoirs has reduced the incidence of ZCL in some areas (Yaghoobi-Ershadi et al., 2000, 2005; Veysi et al., 2012), but this approach has not been effective in all locations. For instance, in the town of Sidi Bouzi, located in a ZCL endemic region of Central Tunisia, destruction of *P. obesus* rodent burrows around this town did not reduce the incidence of ZCL (Ben Salah et al., 2007). This intervention may not have been effective in this location due to the presence of another competent ZCL reservoir in this region, *M. shawi*, which was not targeted by the habitat modification (Ben Salah et al., 2007). Additionally, reduction of the rodent population by poisoning or habitat destruction is costly, labour-intensive, and has high impact on the environment.

In recent years, insecticide treated rodent baits have been explored as a method to control insect vectors that feed on rodent hosts (Borchert and Poché, 2003; Borchert et al., 2009). These baits have systemic and feed through insecticidal activity, which kills the blood feeding female, and the immature stages that feed on rodent faeces (Poché et al., 2013). Thus, linking adult sand flies with their larval diet is of major epidemiological importance (Mascari et al., 2013b). Various insecticides such as imidacloprid (Borchert et al., 2009; Wasserberg et al., 2011) and fipronil, (Ingenloff et al., 2012) have been evaluated for use in rodent bait. It is important to point out that all these aforementioned studies were performed in laboratory bioassays. In a previous study, we showed the efficacy of imidacloprid-treated bait for controlling populations of *P. papatasi* feeding on *M. shawi* in the laboratory and in the field (Derbali et al., 2013).

More recently Poché et al. (2013) showed that systemic use of fipronil can be used in larger animals, such as cattle, to control populations of *Phlebotomus argentipes*, vector of *Leishmania donovani*, etiologic agent of visceral leishmaniais (VL) in the Indian subcontinent. Using an oral dose of the insecticide can reduce sand fly populations, but the impact of this approach on reducing the incidence of ZCL or VL remains to be determined. In the present study fipronil-treated rodent baits was evaluated in the laboratory and in the field for palatability to *M. shawi* and for efficacy as a systemic and feed through insecticide against *P. papatasi*.

2. Materials and methods

2.1. Bait preparation

Baits for this project were formulated and manufactured at Genesis Laboratories, Inc., Wellington, Colorado, USA. Gain-based rodent bait formulations containing fipronil were mixed in a Hobart Mixer. Paraffin was added to the mix and the products extruded with an industrial Bonnet Extruder to form paraffin-based blocks of approximately 35 g each. Fipronil was added to the bait at three concentrations: 0.001, 0.0025, and 0.005%.

Fipronil concentration in three baits was validated using high performance liquid chromatography (HPLC) with ultraviolet (UV) absorbance detection at Genesis Laboratories Inc. (Wellington, Colorado, USA). A complete validation of the analytical procedures for the extraction and quantification of fipronil was performed at Genesis Laboratories, Inc. (Wellington, Colorado, USA) before the analysis of the experimental bait samples to ensure the accuracy of the fipronil concentrations in bait.

2.2. Sand flies

The sand flies used in this study were from a colony maintained at the Vector Ecology laboratory at Pasteur Institute of Tunis since 2003 as described by Chelbi and Zhioua (2007). Sand fly larvae were fed with a mixture of composted rabbit faeces and crushed rabbit chow, and adult female sand flies were allowed to take blood-meal from *M. shawi* from a colony established at the animal facility of Pasteur Institute of Tunis.

2.3. Meriones shawi

Rodents were housed individually in micro-isolator cages. The maintenance of *M. shawi* and the experimental procedures in this research followed Animal Care and Use Protocol which was approved by the Institutional Animal Care and Use Committee at Pasteur Institute of Tunis, Tunisia. Research was conducted in compliance with the Animal Welfare Act and other Federal statutes and regulations relating to animals and experiments involving animals. Three *M. shawi* diets were prepared containing 0.001%, 0.0025%, and 0.005% of fipronil. Seven *M. shawi* were assigned randomly into each diet group. Fipronil-treated bait were provided to each group for 48 h and then replaced by untreated rodent pellet chow. The amount of food consumed during 48 h was calculated for each *M. shawi* and compared statistically using an analysis of variance (ANOVA) performed using the statistical program SPSS 19.0 for Windows.

2.4. Feed-through larval bioassays

Bioassays were conducted in polystyrene vials (5 cm in diameter × 7 cm deep) with a 1-cm-thick basal layer of plaster of Paris extending through a hole drilled in the bottom. The walls were plastered in addition to the bottom of the bioassay pot and a layer of sand (2 mm thick) was added on top of the plaster-lined bottom to reduce fungal contamination of larvae and water condensation (Chelbi and Zhioua, 2007). Bioassay pots were placed in a humidity chamber at 90% RH which consists of a plastic box (15 cm wide, 27 cm long, 12 cm height) with a tightly fitting lid over a substrate of wet sand to maintain adequate and uniform moisture inside the pots (Chelbi and Zhioua, 2007). Humidity chamber containing bioassays pots were then placed in the growth chamber at 29–30 °C with a photoperiod of 17:7 (Chelbi and Zhioua, 2007). Each pot was closed with a polyethylene cap with a hole and a cloth mesh to allow air-flow.

At the fifth day post-treatment, faeces that were voided by each *M. shawi* were collected. The faeces were pooled by diet group, air dried, crushed using a glass mortar and pestle. Thirty third-instars larvae were placed in each bioassays pot and then 0.5 g of treated faeces were sprinkled evenly over the plaster and sand at the bottom of the bioassay pot. Control bioassay pots were sprinkled with 0.5 g of untreated larval food. There were three replications (90 larvae total) for each larval diet group. We used third-instars larvae for this study because first and second-instars are very sensitive (Chelbi and Zhioua, 2007). Larvae were examined daily for abnormal behavioural and morphological characteristics. The presence of frass at the bottom of the bioassay pot and dark material in the guts of larvae was monitored to ensure that mortality was not induced by starvation. Larval mortality was evaluated by monitoring the