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Large scale systemic control short-circuits pathogen transmission by interrupting the sand rat (*Psammomys obesus*)-to-sand fly (*Phlebotomus papatasi*) *Leishmania major* transmission cycle

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Abstract

Systemic control uses the vertebrate hosts of zoonotic pathogens as "Trojan horses," killing blood-feeding female vectors and short-circuiting host-to-vector pathogen transmission. Previous studies focused only on the effect of systemic control on vector abundance at small spatial scales. None were conducted at a spatial scale relevant for vector control and none on the effect of systemic control on pathogen transmission rates. We tested the application of systemic control, using Fipronil-impregnated rodent baits, in reducing *Leishmania major* (*Kinetoplastida: Trypanosomatidae*; Yakimoff & Schokhor, 1914) infection levels within the vector, *Phlebotomus papatasi* (Diptera: Psychodidae; Scopoli, 1786) population, at the town-scale. We provided Fipronil-impregnated food-baits to all *Psammomys obesus* (Mammalia:Muridae; Cretzschmar, 1828), *the main L. major* reservoir, burrows along the southern perimeter of the town of Yeruham, Israel, and compared sand fly abundance and infection levels with a non-treated control area. We found a significant and substantial treatment effect on *L. major* infection levels in the female sand fly population. Sand fly abundance was not affected. Our results demonstrate, for the first time, the potential of systemic control in reducing pathogen transmission rates at a large, epidemiologically relevant, spatial scale.

KEYWORDS
cutaneous leishmaniasis, diseases, feed-through systemic control, Fipronil, parasite load, pathogen control

INTRODUCTION

The current, most effective, approach for mitigating cutaneous leishmaniasis infections in humans is to reduce exposure to sand fly bites (Antinori et al., 2012; Murray et al., 2005). This involves, among others, personal protection (using, e.g., repellents, insecticide-treated clothing or bed-nets), residual spraying with insecticides (Alexander & Maroli, 2003; Warburg & Faiman, 2011), and reservoir-rodent control (Ashford, 1996). However, the efficacy of these approaches is limited (Alexander et al., 2009; Denlinger et al., 2015; Dinesh et al., 2010; Hassan et al., 2012), and some may even have adverse environmental effects (Ashford, 1996; Pimentel, 1995). Source reduction is also not

practical due to the difficulty of detecting sand flies' breeding sites (Feliciangeli, 2004; Moncaz et al., 2014; Vivero et al., 2015). Hence, a more focused, targeted, and efficient control method is urgently needed (Tsurim et al., 2020b; Warburg & Faiman, 2011).

An alternative approach for the control of vector-borne zoonotic diseases is 'feed-through systemic control', which uses the vertebrate host as a 'Trojan horse', delivering the insecticide to the vector as it blood-feeds on the host. Recent experimental studies exhibited an effective reduction in the abundance of various pathogen vectors, such as ticks (Stafford & Williams, 2017), fleas (Borchert et al., 2009; Borchert et al., 2010; Poché et al., 2017; Poché et al., 2018), and sand flies (Derbali et al., 2014; Ingenloff et al., 2012; Mascari et al., 2007; Mascari et al., 2011; Mascari et al., 2012; Mascari & Foil, 2010), following systemic control application. During the past seven years, we have been studying the efficacy of using Fipronil-impregnated rodent baits for controlling the population of *Ph. papatasi*, the main vector of *L. major* in the Middle East. We recently demonstrated that *L. major* reservoir rodents, fed with such a bait, remained toxic to blood-feeding sand flies for at least two weeks (Tsurim et al., 2020a). We then applied it in the field by distributing impregnated baits next to active burrows of *Meriones crassus* (a local *L. major* reservoir) and demonstrated a significant reduction (86%) in female sand flies' emergence rate from treated burrows compared with control burrows (Tsurim et al., 2020b). In a similar study, Derbali et al. (2014) developed Fipronil-impregnated baits and demonstrated, using *Meriones shawi*, an up to six weeks residual effect of the treatment on sand fly adult and larval mortality.

However, all existing studies have evaluated the effect of feed-through systemic control only at the level of individual burrows or clusters of burrows. No study to date has evaluated the efficacy of this method at spatial scales relevant for a control programme (e.g., village or town scale). Furthermore, despite many studies claiming that this approach has the potential for 'breaking the pathogen's transmission cycle', no study to date has evaluated the effect of feed-through systemic control application on the reduction of the pathogen's transmission rate. In this study, we aimed to take the next step and evaluate these two issues. Specifically, the goal of the current study was to test the efficacy of feed-through systemic control in reducing *Ph. papatasi* abundance and *L. major* prevalence in the vector *Ph. papatasi* population on a large scale (town) by applying our Fipronil-impregnated baits to all reservoir burrows in the treated area.

As far as we know, our work demonstrates for the first time the efficacy of large-scale systemic control in reducing pathogen infection levels in the vector population.

METHODS

Study system

The experiment was conducted at an *L. major* endemic region in the northern Negev (Ben-Shimol et al., 2015; Jaffe et al., 2004), Israel, near the town of Yeruham (30°59′N 34°55′E; population size = ~10,000); with a history of periodic cutaneous leishmaniasis outbreaks (Biton et al., 1997). *The L. major* vector in this area is *Ph. papatasi* and the predominant mammalian reservoir is the Fat Sand Rat (*P. obesus*) (Berger et al., 2014; Jaffe et al., 2004; Wasserberg et al., 2002, 2003). The fat sand rat is a solitary, diurnal, and territorial rodent that occupies a home range of approximately 10 m radius around its burrow system, which is typically located underneath or next to chenopodiaceae bushes (Berger, 2009; Daly & Daly, 1974; Ilan & Yom-Tov, 1990; Wasserberg, 2002). We applied a feed-through systemic control treatment to all *P. obesus* burrows in a treatment plot along the southern perimeter of the town and compared vector sand fly abundance and *L. major* infection level within the vector population with a similar untreated control plot along the northern perimeter of the town (Figure 1).

Study design

Treatment and control areas were spatially segregated; each was ~230 hectares in area and located along two edges of the town (Figure 1). The southern plot was arbitrarily designated to receive the systemic insecticidal treatment, while the northern plot served as an untreated control. A 300–1200 m wide strip of the built urban environment, vacant of *P. obesus* burrows, separated the plots (Figure 1). In order to characterise the baseline conditions of the two study areas, in the second half of June 2017, we mapped all *P. obesus* burrows in the plots using the iPad3's built-in GPS and QGIS (QGIS Development Team, 2016). Mapping was done by slowly walking throughout the area along linear transects, ~20 m apart, actively searching for characteristic burrow entrances (Wasserberg et al., 2003) within at least 500 m of the town's margin, as sand fly dispersal does not normally exceed a few hundred metres (Orshan et al., 2016; Wasserberg et al., 2002, 2003). We found a total of 181 burrows in the southern plot and 313 in the northern plot (Figure 1).

Bait preparation and deployment

We used the baits developed and tested in previous studies (Tsurim et al., 2020a; Tsurim et al., 2020b), adapted for *P. obesus*. Bait pellets weighed 0.25 grams/pellet and contained 0.1 gr/kg (0.01%) Fipronil. Burrows were baited three times during the sand fly activity season, from the end of May until the beginning of November (Table 1). In each baiting session, we applied three bait-pellets (i.e., 0.75 gr) per burrow. Hence, each treated burrow received a total of $3 \times 0.75 = 2.25$ gr of baits, corresponding to 0.225 mg of Fipronil. Preliminary lab and field trials indicated that *P. obesus* readily took and consumed these baits. In the lab, three individuals readily consumed similar bait-pellets within 24 hr. In a preliminary field trial, we deployed similar bait-pellets, also impregnated with Fluorescein, a systemic fluorescent dye. Following the bait-pellet application, we found Fluorescein-marked *P. obesus* faeces around treated burrows (unpublished data).

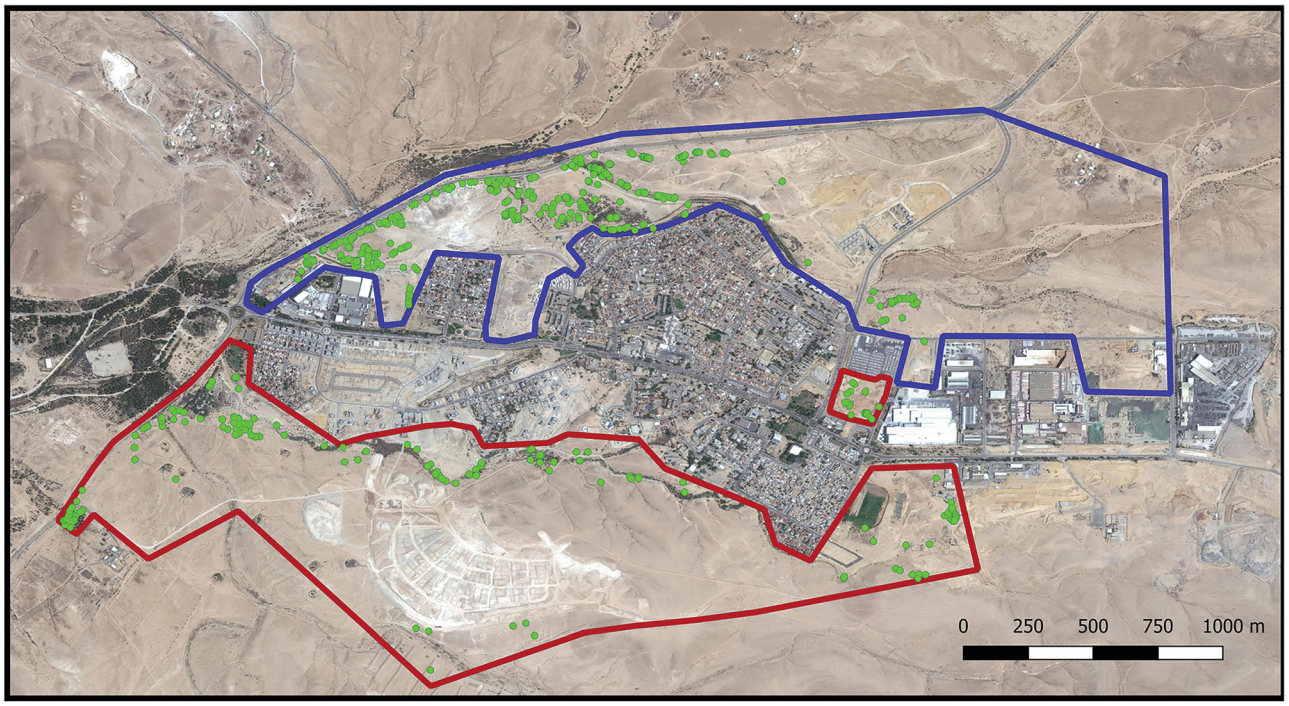

FIGURE 1 The study area: The control (north) plot (blue, dark, outline) and the treatment (south) plot (red, light, outline), separated by built neighbourhoods of the town of Yeruham. Green points mark sand rat (*P. obesus*) burrow systems mapped inside the study plots

Sand fly trapping and monitoring

We used CO_2[dry ice]-baited CDC traps without a light bulb, placed in an updraft setting, with the opening 10 cm above ground (Alexander, 2000; Orshan et al., 2016). We conducted nine sand fly trapping sessions (Table 1). Three occurred during the "baseline" phase, prior to bait-pellet application, and six during the "experimental" phase, after the onset of bait-pellet application (Table 1). In each sand fly trapping session, we placed 12–13 traps in each of the control and treatment plots. The traps were positioned an hour before sunset in a west–east array, closely following the houses line, >10 m away from the nearest sand rat burrow (Figure 1), and were collected an hour after sunrise. The mean distance between traps was 71 ± 37 (SD) m (range of 12–183 m). Trap locations varied between trapping sessions. Captured sand flies were sorted by sex. Baseline studies confirmed that *Ph. papatasi* was the only Phlebotomus species in the study area.

Quantification of *Leishmania* parasite-load in collected sand flies

All trapped female sand flies were kept at −20°C, in trap-specific pools of up to 20 specimens. Trap yields of >20 females/night were accordingly divided into more pools. We then used the Geneaid gSYNC™ DNA extraction kit to extract DNA of each sand fly pool separately. *Leishmania* kDNA marker was amplified by quantitative real-time kinetoplast-DNA PCR (qRT-kDNAPCR) (Abbasi et al., 2013). We quantified the total *L. major* parasite-load per pool and then divided this quantity by the number of females in each pool to get a measure of the average total *L. major* parasite-load per female for that pool. We then averaged across all the pools from that trap for that night to obtain the mean, per-capita *L. major* parasite-load per trap/night. This provides a direct measure of the mean infection level in the sand fly population because it is a composite measure of the proportion of infected females within a trap's collection pool and infection intensity per female (reflective of the amount of *L. major* parasite DNA within individual infected females), which is assumed to be strongly correlated to the female's infectiousness (Abbasi et al., 2013; Miller et al., 2014). This is in contrast with the standard MIR (Minimal Infection Rate; calculated as the number of positive female pools divided by the total number of females tested, assuming that a positive pool contains only a single infected female), which typically tends to underestimate infection levels (Chakraborty & Smith, 2019; Gu et al., 2003).

Leishmania-positive samples were examined using ITS-1 PCR (Abbasi et al., 2013; El Tai et al., 2000; El Tai et al., 2001) to verify the *Leismania*-species identity.

Data reduction, analysis, and predictions

Sand fly data

We lumped the data into two phases: the 'Baseline' phase that included the three sampling sessions prior to treatment application and the 'Experimental' phase that included the six sampling sessions after the onset of treatment application. Given that sand fly numbers

TABLE 1 Experiment schedule and summary results: Dates of trapping sessions and of bait application.

Action	Phase	Date	Plot (treatment)	Female abundance	Male abundance	Parasite load
trapping	Baseline	10/05/2017	N	16 ± 7.57 [3]	3.33 ± 0.88 [3]	0 ± 0 [3]
trapping		10/05/2017	S	7.69 ± 2.15 [13]	6.46 ± 2.34 [13]	4640.19 ± 4588.13 [9]
trapping		28/05/2017	N	3 ± 0.71 [13]	1.15 ± 0.56 [13]	0 ± 0 [10]
trapping		28/05/2017	S	11 ± 2.37 [10]	3.2 ± 1.02 [10]	0 ± 0 [10]
trapping		26/06/2017	N	0.75 ± 0.3 [12]	0.25 ± 0.13 [12]	0 ± 0 [5]
trapping		26/06/2017	S	11.67 ± 3.55 [12]	5.75 ± 2.26 [12]	8805.44 ± 8805.44 [11]
Bait application I	Experimental	*27/06/2017*				
trapping		13/07/2017	N	2.33 ± 0.72 [12]	0.67 ± 0.28 [12]	597.02 ± 345.33 [9]
trapping		13/07/2017	S	13 ± 3.23 [12]	6.08 ± 2.12 [12]	10.44 ± 9.92 [11]
Bait application II		*25/07/2017*				
trapping		31/07/2017	N	2.83 ± 0.81 [12]	1.42 ± 0.42 [12]	23374.12 ± 15387.84 [8]
trapping		31/07/2017	S	13.09 ± 4.23 [11]	6.18 ± 2.55 [11]	1287.68 ± 854.08 [10]
trapping		14/08/2017	N	5.67 ± 2.08 [12]	3.67 ± 1.46 [12]	8981.78 ± 7141.52 [7]
trapping		14/08/2017	S	7.75 ± 1.97 [12]	7.5 ± 3.1 [12]	904.88 ± 627.49 [11]
Bait application III		*10/09/2017*				
trapping		18/09/2017	N	7 ± 2.42 [10]	4.4 ± 1.85 [10]	7421.22 ± 3438.29 [9]
trapping		18/09/2017	S	10.08 ± 2.37 [12]	9.5 ± 3.11 [12]	625.63 ± 484.09 [9]
trapping		26/10/2017	N	0.58 ± 0.29 [12]	0.58 ± 0.23 [12]	1088.54 ± 1088.54 [4]
trapping		26/10/2017	S	1.17 ± 0.32 [12]	0.83 ± 0.27 [12]	18.09 ± 18.09 [8]
trapping		06/11/2017	N	0.08 ± 0.08 [12]	0 ± 0 [12]	0 ± 0 [2]
trapping		06/11/2017	S	0.25 ± 0.13 [12]	0 ± 0 [12]	0 ± 0 [2]

Note: For sand fly abundance summary results report per trap mean ± 1SE number of individuals caught. For parasite load, summary results report the mean ± 1SE per-capita parasite load per trap. Sample size are given in brackets [n] and denote the number of traps included in the analysis.

are overdispersed 'count' data, we analysed these data by fitting a negative binomial regression model (Generalized Linear Model with Negative Binomial errors distribution and log-link function) (Zuur et al., 2009). We used a two-way model, testing the effect of 'Plot' (Control vs. Treatment; coded 0 and 1, respectively), 'Phase' (Baseline vs. Experimental; coded 0 and 1, respectively), and their interaction on the number of flies per trap. A biologically meaningful effect of the treatment would be indicated by a significant 'Plot-by-Phase' interaction, predicting a constant or increasing temporal trend between the baseline and experimental phases for the control plot, but a decreasing temporal trend for the 'treatment' plot.

L. major parasite-load data

As in the sand fly data, we lumped data into two phases: "Baseline" and "Experimental". Parasite-load, the response variable, measured using qPCR, indicates the overall mean level of parasitemia per female, per sand fly pool of a given trap per night. Since we are interested in the load of attached promastigotes, we excluded data from blood-engorged females as the qPCR measure from those might reflect the *Leishmania* parasite-level in the ingested blood. *L. major* parasite-load data is a continuous variable, yet its distribution tended to be highly right skewed (data not shown). Therefore, we used a gamma regression model (a Generalized Linear Model with gamma distributed errors and an identity link function) to analyse these data (Bossio & Cuervo, 2015). As described above, we used a two-way model, testing the effect of 'Plot' and 'Phase' and their interaction on the per-capita *L. major* parasite-load. A biologically meaningful effect of the treatment would be indicated by a significant 'Plot-by-Phase' interaction, predicting an increasing temporal trend between the 'baseline' and the 'experimental' phases for the 'control' plot. This is due to the seasonal build-up of *L. major* prevalence in the reservoir host (Wasserberg et al., 2003). In contrast, in the treatment plot, we predicted that the treatment would short-circuit host-to-vector transmission because females feeding on a treated host would immediately die. Hence, parasite pick-up by the vector from an infected host will be intercepted. Therefore, we expected the infection level in the sand fly population to not increase or even decrease over the transmission season.

RESULTS

General

A total of 143 sand fly pools were collected throughout the study (79 in the south plot and 64 in the north plot). A total of 1873 sand flies were trapped throughout the study, of which 1186 (63.3%) were

TABLE 2 Analysis results with generalized linear model with negative binomial errors distribution, and log-link function, of the effect of plot (control vs. treatment), experimental phase (baseline vs experimental), and their interaction on the number of sand flies caught per trap.

	Estimate	Standard error	Z statistic	*p*
(a) Females				
Intercept	1.2321	0.2744	4.49	<0.0001
Plot	1.0704	0.3607	2.968	0.003
Experimental phase	−0.1431	0.3257	−0.439	0.660
Plot × Experimental phase	−0.1474	0.434	−0.34	0.734
(b) Males				
Intercept	0	0.3585	0	1
Plot	1.665	0.4563	3.649	0.0003
Experimental phase	0.539	0.4171	1.292	0.1963
Plot × Experimental phase	−0.5946	0.5414	−1.098	0.2721

Note: Total sample size (*n*) = baseline north (28) + baseline south (35) + experimental north (70) + experimental south (71) = 204.

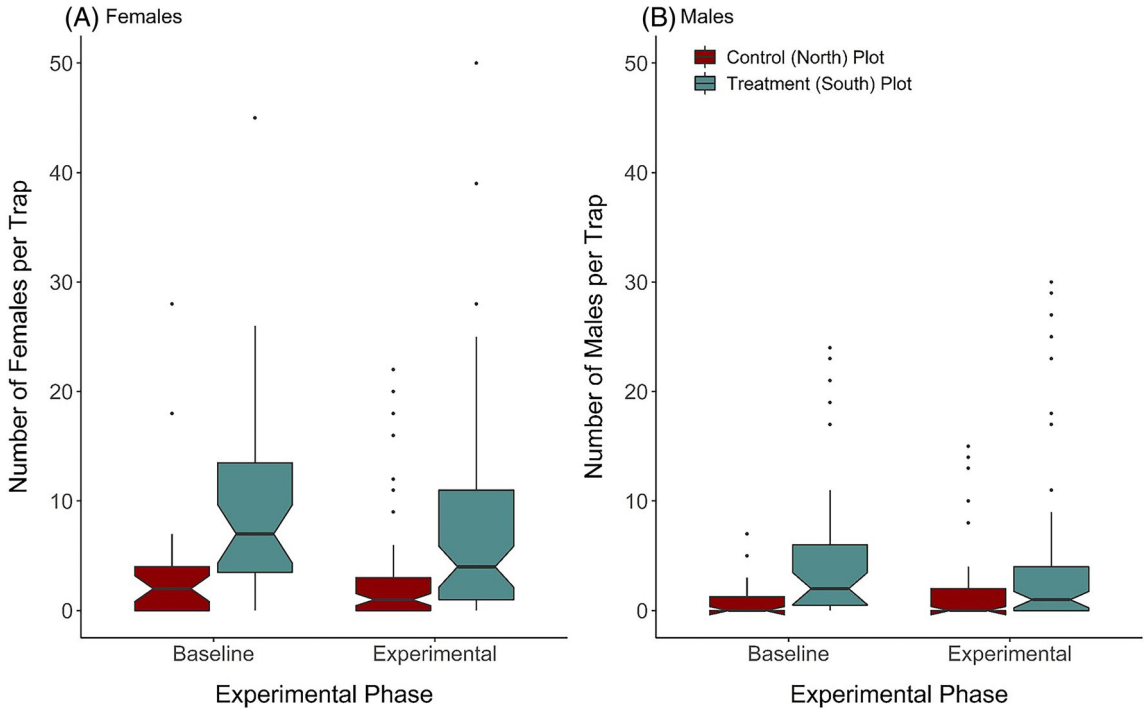

FIGURE 2 Comparison of (a) female (left) and (b) male (right) sand fly abundance (number of sand flies per trap) between the control (north) and treatment (south) plots, in the baseline versus experimental phase. Median, box = inter quartile range (IQR), notch = 95% confidence interval, whiskers = non-outlier zone (max whisker = 1.5 × IQR)

females. 42 (3.5%) of the females were blood engorged and therefore excluded from the parasite-load analysis. In all *Leishmania* positive pools, the pathogen was identified to be *L. major*.

The effect of feed-through systemic control on *Ph. papatasi* abundance

In terms of temporal dynamics, in both the southern and northern plots, sand fly numbers increased in late May, stayed high throughout the summer months until early September, and then dropped sharply in October and November. The southern plot was overall more productive than the northern plot, with 75.9% of the captured sand flies. (Table 1). Indeed, for both males and females, a significant 'Plot' effect indicated that mean abundance was higher in the southern compared with the northern plot (Table 2, Figure 2). That was true for both the 'baseline' and the 'experimental' phases (Figure 2). Most importantly, though, was the finding that, for both females and males, the 'Plot-by-Phase' interaction was not statistically significant (Table 2). For females, numbers slightly decrease between the 'baseline' and 'experimental' phases in both control and treatment plots, with numbers in the treatment plot decreasing at a slightly faster rate (Figure 2a). For males, numbers slightly increased between the baseline and the experimental phase in the 'control' plot and remained

TABLE 3 Results of a generalized linear model with gamma distributed errors and identity link function, analysing the effect of plot (control vs. treatment), experimental phase (baseline vs experimental), and their interaction on the average *L. major* parasite-load per female, per trap.

	Estimate	Standard error	t statistic	*p*
Intercept	0.003	0.0008	1.419	0.158
Plot	4621	2523	1.832	0.069
Experimental phase	8369	4007	2.088	0.0387
Plot × Experimental phase	−12,430	4741	−2.621	0.0098

Note: Total sample size (n = number of traps) = baseline north (18) + baseline south (30) + experimental north (39) + experimental south (51) = 138.

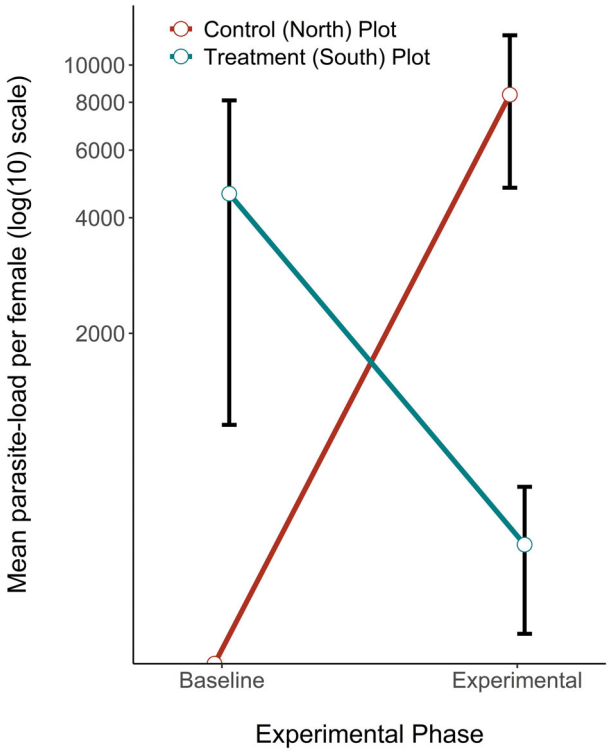

FIGURE 3 Comparison of sand fly female's infection level (mean per-capita parasite load) between the control (north) and the treatment (south) plots, in the baseline versus experimental phase. Error bars denote ±SE

fairly constant in the 'treatment' plot (Figure 2b). These results indicate that the feed-through treatment did not have a biologically meaningful effect on sand fly abundance.

The effect of feed-through systemic control on per-capita *L major* parasite-load

Parasite load slightly varied between the plots and a marginally significant effect of 'plot' was observed (Table 3), with a higher mean overall parasite load in the control (north) plot (5726 ± 2493; mean ± SE, n = 57) than in the treatment (south) plot (2066 ± 1297; mean ± SE, n = 81). The parasite load was highly variable over time (Table 1). A significant 'phase' effect (Table 3) was observed, with low mean parasite load during the 'baseline phase' (2888 ± 2177; mean ± SE, n = 48), then increasing later in the season, during the 'experimental phase' (3946 ± 1599; mean ± SE, n = 90).

However, most important was the detection of a significant 'Plot-by-Phase' statistical interaction (Table 3, Figure 3), reflecting opposite temporal trends in infection dynamics in the treatment and control plots. Parasite load substantially increased in the control (north) plot from 0 ± 0 (mean ± SE, n = 18) during the baseline phase to 8369 ± 3579 (mean ± SE, n = 39) during the experimental phase. In contrast, in the experimental (southern) plot, parasite load was fairly high early in the season, during the baseline phase (4621 ± 3466; mean ± SE, n = 30). But then, following the experimental intervention, it decreased and remained relatively low throughout the remainder of the transmission season (563 ± 233; mean ± SE, n = 51). This result strongly supports our prediction regarding the effect of the feed-through control treatment on the reduction of *L. major* transmission.

DISCUSSION

Studies evaluating the efficacy of feed-through systemic control using different control agents have all focused on the effect of this treatment on reducing the vector's viability and its emergence rates from treated sources (Borchert et al., 2009; Borchert et al., 2010; Mascari et al., 2013; Poché et al., 2017; Stafford & Williams, 2017). Specifically, with sand flies, lab studies mostly evaluated the survival of blood-feeding females and faecal-feeding larvae (Mascari et al., 2011; Mascari et al., 2012; Wasserberg et al., 2011), while small-scale field studies focused on the effect of burrow treatment on a female's emergence rate (Mascari et al., 2013; Poché et al., 2018; Tsurim et al., 2020a). To date, no studies have evaluated the effect of this control approach on mitigating pathogen transmission rates, and none have been done at a spatial scale applicable to a control campaign. In this study, we evaluated the effect of feed-through systemic control at a town scale on both sand fly regional abundance and on potential transmission risk to humans.

Bait palatability

In previous lab (Tsurim et al., 2020b) and field (Tsurim et al., 2020a) studies, we found that Jird species (*Meriones tristrami* and *M. crassus*) readily took our Fipronil-treated food pellets without exhibiting any

adverse effects. Earlier observations indicated that, in the lab, *P. obesus* likewise readily took and consumed our Fipronil-impregnated bait-pellets without exhibiting any adverse effects. In a preliminary field trial, we deployed similar bait-pellets that contained the systemic fluorescent dye Fluorescein to five *P. obesus* burrows. Following the bait-pellet application, we found Fluorescein-marked *P. obesus* faeces in the vicinity of the treated burrows (unpublished data). Furthermore, in a sub-sample of the treated burrows in the present study (that was scrutinised for that purpose), all bait-pellets disappeared the day following pellet application. Hence, evidence strongly indicates that the sand rats in the present study indeed took and consumed our Fipronil-impregnated bait-pellets.

Effect of Fipronil-treated bait application on sand fly abundance

With respect to sand fly population patterns in time and space, during the baseline phase, male and female sand fly abundance were higher in the southern (the to-be 'treatment' plot), compared with the northern 'control' plot. A likely cause of this difference may be higher soil moisture (Wasserberg et al., 2003) in the southern plot. This is consistent with the lusher vegetation observed there (data not shown). Yet, in contrast with the conventional expectation, following the treatment application, male and female sand fly abundance did not substantially decrease in the treatment plot. Furthermore, male and female sand fly abundance remained higher in the treatment (south) plot relative to the control (north) plot. Additionally, the population trends during the 'baseline' and 'experimental' phases did not differ between the two plots. Yet, the slightly steeper decline in female numbers in the treatment plot may suggest a stronger effect there. As expeted, with the males, no difference in temporal trends was observed.

This result is inconsistent with previous studies of other groups (Derbali et al., 2014; Mascari et al., 2013; Poché et al., 2018) or even of our own group (Tsurim et al., 2020a). The difference, however, is that those studies were conducted at a relatively small spatial scale of an individual or a small patch of burrows, hence evaluating the small-scale effect of the treatment on sand fly emergence rates. In contrast, the present study was conducted on a town-wide scale, in which all sand rat burrows within at least 500 m of the town's margin were treated. However, non-sand rat burrows were not treated. Hence, it is very likely that alternative blood-meal hosts and alternative breeding sites (e.g., burrows of non-reservoir animals) are still present in the area (Wasserberg et al., 2002) and capable of maintaining a viable sand fly population. Hence, even if effective in reducing the viability of sand fly numbers emerging from treated burrows, it might be somewhat naïve to expect that by treating only the sand rat burrows, one would achieve an area-wide effect on the entire sand fly population. Therefore, we are not surprised that, at this scale, we did not detect a significant population-level effect. In order to obtain such a population-level effect on sand fly abundance, a meticulous survey and treatment of all potential breeding sites and blood-meal sources must be undertaken, an effort which is probably impractical in the context of a vector control campaign.

Other factors, such as sand fly stage, state, and age, may affect the attraction of sand flies to their blood-meal hosts. More importantly, it is not known if blood-meal seeking females are differentially attracted to systemically treated rodents. These are all important questions for further investigation but were beyond the scope of this study. Nonetheless, as described below, the systemic control approach is expected to reduce the *L. major* parasite load and hence the transmission rate.

Effect of Fipronil-treated bait application on reducing sand flies' per-capita *L. major* parasite load

We found a significant Phase-by-Plot interaction effect on per-capita *L. major* parasite-load, reflecting the effect of the Fipronil-bait application on halting, and probably decreasing, Leishmania infection level in the sand flies of the treated plot. Specifically, in the control (north) plot, per-capita parasite load was zero during the 'baseline' phase, subsequently increasing precipitously to a mean of >8000 promastigotes/female during the 'experimental' phase. This increase probably reflects the seasonal build-up in *L. major* infection prevalence in the reservoir hosts, which results in an increase in the prevalence and infection intensity in the female sand fly population (Berger et al., 2014; Wasserberg et al., 2003). In contrast, mean *L. major* parasite-load in the treatment (south) plot tended to decrease between the 'baseline' and 'experimental' phases. This difference in the temporal trends of infection levels between the 'Treatment' and 'Control' plots can only be explained by the suppressive effect of the treatment on the survival of sand fly females that blood-fed on the systemically-affected sand rats.

During the 'baseline' phase, mean per-capita Leishmania parasite-load in the treatment (south) plot tended to be higher, compared with the control (north) plot. The reason for this is not clear, but is consistent with the higher sand fly abundance found there, which is often correlated with a higher prevalence of infection in the reservoir host (Wasserberg et al., 2003).

One limitation of the metric we used to measure the *Leishmania* infection level in the sand flies is that it does not allow differentiating between the fraction of infected females (prevalence) and the actual mean number of promastigotes per infected female. This measure was used, mainly, for logistical reasons, since measuring the infection load for each individual female sand fly was not practical. Nonetheless, we believe that it does provide a realistic measure of infection risk to humans because human contact rate is a direct outcome of the prevalence of infected females in the population, while transmissibility (probability of transmission given contact) is a direct outcome of the infection load in an infected female (Begon et al., 2002). In any case, the expectation is that during the sand fly activity season, both infection prevalence in the sand fly population and the parasite-load per infected fly should increase due to the buildup of infection in the *P. obesus* host population (Wasserberg et al., 2003). Indeed, separating these two factors warrants a different study. Similarly, evaluation of

the residual length of this treatment effect warrants further study. Both of these issues were beyond the scope of this study. However, overall, our study (as far as we know) is the first study to ever evaluate the effect of feed-through systemic control on pathogen infection levels within the vector population at a spatial scale relevant for a vector control campaign.

CONCLUSIONS

From a public health perspective, the most significant outcome of this study is that by implementing a large-scale systemic control intervention, we were able to suppress the buildup of *L. major* infection within the vector population. By rendering the source of the infection, the *P. obesus* sand rat, toxic to blood-feeding sand fly females, we short-circuited the vector-reservoir contact point, consequently halting the 'production' of new infected sand flies. This should have a double impact: first, it would depress the buildup and spread of the infection within the reservoir host population and, thereby, reduce its endemicity rates to a much lower level; second, it has the potential to reduce the incidence and level of infectivity of free-flying infected flies, the vehicles of spillover of the *L. major* infections to humans, thereby reducing enzootic and, more importantly, zoonotic transmission rates.

From an environmental health perspective, we argue that this approach has a relatively minimal adverse environmental effect. First, application is effectively targeted with baits, only affecting the within-burrow environment. Second, in terms of the amount of insecticide used, this approach is very conservative and requires extremely small amounts of the active chemical. In our study, we used only ~40 mg of Fipronil to achieve the yearly coverage of all 181 burrows in the ~230 ha treated (southern) plot. Treating the whole town perimeter would have required ~120 mg/year. In this study, we did not address the residual effect of a single bait application. A separate study focusing on the length of the residual effect would be needed. Based on other studies, such as Derbali et al. (2014) and our previous lab experiments (Tsurim et al., 2020b), one can expect a residual time period of 4 to 6 weeks. Considering a 4 to 6 months leishmania transmission season and the accumulating treatment effect on leishmania prevalence in the reservoir, 4–5 repeated bait applications will probably suffice to achieve sustainable protection of the human population. The costs of such an application programme are hard to assess at this early stage, and will also depend on local conditions, but a rough estimation would be 3–5 USD per 1000 m^2 per season. Hence, this highly focused approach, which is surgically targeting the vector-reservoir point of contact, has a relatively low environmental impact ('environment friendly'), but a high potential impact for reducing *L. major* entomological risk to humans and thereby mitigate cutaneous leishmaniasis burden to local, yet neglected, human populations.

AUTHOR CONTRIBUTIONS

Ido Tsurim, Zvika Abramsky, and Gideon Wasserberg conceived and designed the study. Ido Tsurim and Gil Ben Natan worked for the acquisition of data in the field. Alon Warburg and Ibrahim Abbasi conducted the molecular analyses and worked on laboratory data acquisition and analysis. Ido Tsurim, Gideon Wasserberg, Zvika Abramsky, and Alon Warburg worked on data analysis and interpretion. All authors contributed to manuscript preparation. All authors approved the submission of the final version.

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CONFLICT OF INTEREST

The authors declare that that there is no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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