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Original Article

Effectiveness of Attractive Toxic Sugar Baits (ATSB) in Reducing Cutaneous Leishmaniasis among Military Personnel in Isfahan Province, Central Part of Iran

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Abstract

Background: We aimed to evaluate the effectiveness of Attractive Toxic Sugar Baits (ATSB) in reducing the incidence of Cutaneous leishmaniasis (CL) among military personnel in Isfahan Province, Iran.

Methods: The ATSB solution was prepared and applied to vegetation located approximately 500 meters from residential areas to target and eliminate sand fly vectors. Clinical samples were subsequently collected from individuals with suspected CL. The number of CL new cases among military personnel in 2022 was compared with collected historical data from 2012 to 2021, including the number of CL new cases, demographic characteristics, and seasonal incidence patterns. Statistical analysis was performed using ANOVA to assess whether the intervention led to a significant reduction in disease occurrence.

Results: Following the application of ATSB, the new cases of CL among military personnel decreased from an average of 196 cases per year (2012–2021) to 55 cases in 2022. Seasonal analysis revealed a decline across all seasons, with the most notable reduction observed in autumn. Demographic analysis showed reductions in both male and female groups. However, statistical analysis using ANOVA indicated that the observed differences before and after the intervention were not statistically significant (P=0.087).

Conclusion: Despite a decline in CL case numbers in the studied areas, the reduction following ATSB application was not statistically significant. Nevertheless, ATSB may serve as a promising complementary approach for controlling CL in endemic regions. Further studies with larger sample sizes and extended follow-up periods are recommended to validate its effectiveness.

Introduction

Leishmaniasis is a group of diseases caused by protozoan parasites from the genus *Leishmania*, transmitted to humans through the bites of infected female phlebotomine sand-flies (1, 2). It is recognized as an important yet neglected tropical disease (NTD), with an estimated 350 million people at risk (3, 4). Cutaneous leishmaniasis (CL) is one of the major vector-borne diseases in Iran, with *L. major* being the causative agent of zoonotic cutaneous leishmaniasis (ZCL) and *L. tropica* being the causative agent of anthroponotic cutaneous leishmaniasis (ACL) (5, 6). In Isfahan, CL is primarily caused by *L. major*, transmitted zoonotically through rodent reservoirs. Over the past decade, periodic outbreaks have been reported, particularly among populations with high outdoor exposure, such as military personnel (7, 8). Despite ongoing control measures, the persistence of vector habitats and limited access to effective vector control tools continue to challenge the reduction of disease incidence in the region (9-12).

Control strategies primarily include environmental management and chemical interventions; however, their effectiveness has been limited by insecticide resistance, environmental constraints, and logistical challenges (13-15). One of the emerging methods in recent years is the use of Attractive Toxic Sugar Baits (ATSB), which target sand flies through their natural sugar-feeding behavior (16).

The primary chemical control methods have included indoor residual spraying of organochlorines, carbamates, organophosphates, and synthetic pyrethroids. However, these chemical control measures have not been entirely successful due to the development of insecticide resistance, environmental concerns, and budget constraints that limit the scope and sustainability of control efforts in at-risk areas (17-20). ATSB method has been successfully used to control mosquitoes and sand flies in various regions, including the Middle East, Africa, and the Americas (21-23). Field evaluations have demonstrated that ATSB effectively controls sand fly populations when applied to patches of vegetation and barrier fences in areas with or without natural plant coverage (16, 24). Several studies conducted in Iran have demonstrated the efficacy of ATSB in reducing vector densities in CL endemic regions. Yaghoobi-Ershadi et al. (25) demonstrated significant reductions in sand fly densities in endemic regions of central Iran using sugar baits containing boric acid. Similarly, Saghafipour et al. (26) and Ramazani et al. (9) promising outcomes following ATSB application to vegetation and artificial surfaces in desert and semi-arid zones. These findings support the potential role of ATSB as a complementary component in integrated vector management (IVM) programs, especially in high-risk areas such as Isfahan Province.

Therefore, we aimed to further evaluate the efficacy of ATSB in reducing the incidence of CL among military personnel in Isfahan, Iran.

Method and Materials

Study area

This cross-sectional study was conducted during August and September of 2021 and 2022 among military personnel in the Isfahan Province, Iran. In this area, migration rates are meager, and the indigenous population has access to piped water, with ZCL being highly prevalent. Rodent nests are commonly found near villages, where *P. papatasi* is the primary vector and *Meriones libycus* serves as a significant reservoir for ZCL. Many individuals in this region are consistently exposed to *L. major*. The local economy relies primarily on handicrafts, agriculture, and animal husbandry, while Isfahan Province is also one of the most industrialized areas in Iran. The soil in the region is predominantly clay and sand, with oak shrubs (*Tamarix aphylla*) being the dominant vegetation. Other plant species in the area include the Beneh tree and Acacia. The climate is dry, with an annual rainfall of 117 mm. Average temperatures range from -10.6 °C in January to 40.6 °C in July (Fig. 1).

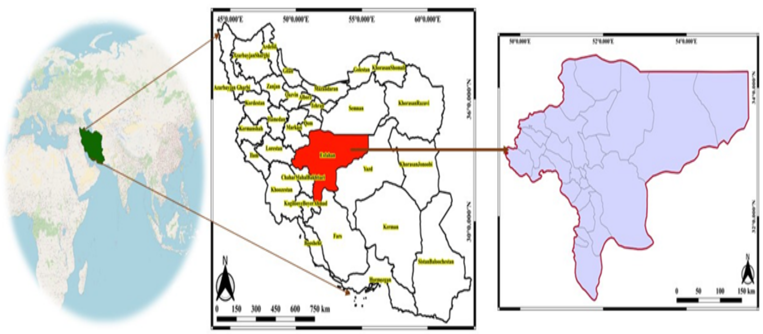

Fig. 1: The study area in Isfahan Province, Iran

Preparation of ATSB Solutions

ATSB was prepared in tanks by combining 0.1 kg of boric acid (1%), 1 kg of brown sugar (10%), and 9 liters of water. This mixture was then sprayed onto the selected field plants using steel hand pumps equipped with pressure regulators. The spraying was carried out at one-month intervals, coinciding with the peak biting seasons of the sand fly vector in Isfahan, specifically in June, July, August, and September. The concentration and frequency of applications were determined based on the active ingredient's lifespan and the biting season of the vector, aiming to maintain a continuous presence of the bait throughout the period.

Fig. 2: Spraying ATSB in spots on the vegetation

Field Plant Selection Criteria

Field plants chosen for the application of ATSB were strategically located around five hundred meters away from residential houses within the base. The selection criteria included proximity to human habitation, accessibility for spraying, and the presence of vegetation that would retain moisture, which is favorable for sand fly breeding. Plants were selected based on their ability to absorb and retain the ATSB mixture, ensuring sustained exposure to the bait (Fig. 2).

ATSB sprayed on vegetation

ATSB spraying was applied to vegetation in military units of Isfahan Province to evaluate its effectiveness in reducing CL, compared to data from the past ten years. The spraying was carried out on a 50-hectare plot of land for a total of 4 sessions from early June to late September.

Sample collection

Samples were prepared from suspected skin lesions of suspected CL cases among military personnel in Isfahan province, Iran. The number of CL new cases, demographic characteristics (including sex and age), and CL seasonal incidence numbers were recorded using structured questionnaires. Patients were diagnosed using a parasitological method based on microscopic examination. For this, suspected CL skin lesions were scraped with a sterile scalpel, and the exudate material fluids were fixed with absolute methanol, air-dried, and then stained with Giemsa 10%. Diagnosis of CL was confirmed by the presence of amastigote forms of *Leishmania* parasite observed under a light microscope at 1000× magnification (27).

Monitoring

To monitor the incidence of CL among military personnel, patient records and surveillance reports were reviewed from medical facilities within the base. These sources included data on the number of confirmed CL cases, as well as demographic and epidemiologic information, and seasonal incidence trends from 2012 to 2022. This comparative analysis enabled the identification of incidence trends and assessment of the effectiveness of the ATSB method in reducing CL cases

Statistical analysis

To evaluate the effectiveness of the intervention, the annual mean number of cutaneous leishmaniasis (CL) cases in 2022 (post-intervention period) was compared with the corresponding data from the years 2012 to 2021 (pre-intervention period). A two-way ANOVA was employed to assess statistical differences, considering the aggregated multi-year baseline and a single intervention year. This method was selected to account for the interaction between year and seasonal trends across the study period. All analyses were conducted using GraphPad Prism 10.4, and a significance level of $P < 0.05$ was considered statistically meaningful.

Ethics approval

The Ethics Committee of Aja University of Medical Sciences approved the study (Ethics no: IR.AJAUMS.REC.1402.006).

Results

Data collection

In the first step, the number of CL new cases, demographic characteristics (including sex and age), and CL seasonal incidence number of confirmed CL from 2012-2022 were determined in Table 1.

Table 1: Data on the number of CL new cases, demographic characteristics (including sex and age), and CL seasonal incidence numbers were analyzed between 2012 to 2022 among military personnel in the Isfahan province, Iran

Year	Number of CL new cases (yr)	Sex (Male/Female)	Mean age (years)	Incidence of seasonal CL number			
				Spring	Summer	Fall	Winter
2012	283	207/76	27.7	1	33	218	31
2013	181	127/54	28.1	0	36	139	6
2014	160	139/21	23.9	1	22	126	11
2015	206	152/54	24.8	1	24	156	25
2016	102	77/25	24.1	0	10	78	14
2017	123	111/12	27.4	1	4	108	10
2018	114	86/28	25.4	1	5	94	14
2019	221	148/73	26.3	0	8	187	26
2020	467	338/129	22.6	5	92	344	26
2021	82	59/23	25.3	1	11	64	6
2022	55	33/22	25.4	0	11	38	6

Number of CL new cases/year

The number of CL new cases varied over the study period, with 283 cases reported in 2012, 181 in 2013, 160 in 2014, 206 in 2015, 102 in 2016, 123 in 2017, 114 in 2018, 221 in 2019, and a peak of 467 cases in 2020. Subsequently, a decline was observed, with 82 cases in 2021 and 55 cases in 2022 among military personnel in Isfahan, Iran. Following the implementation of ATSB spraying, the number of CL cases decreased markedly to 55 in 2022. The results indicated a decrease in CL incidence in 2022; however, the p-value obtained from the ANOVA test was 0.087, which did not reach statistical significance (Fig. 3).

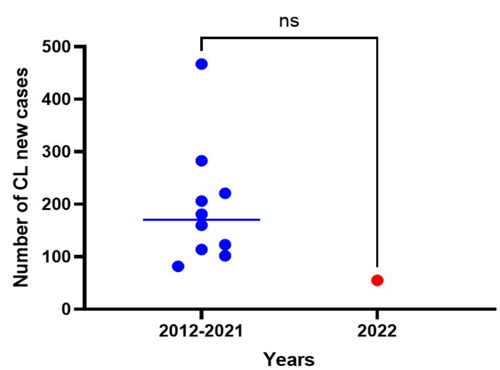

Fig. 3: Annual number of CL new cases/year among military personnel in the northwest region of Isfahan Province, Iran

Incidence of seasonal CL number

The average seasonal incidence of CL among military personnel in the northwest region of Isfahan Province, Iran, from 2012 to 2021 is shown in Fig. 1. However, statistical analysis indicated that this reduction was not statistically significant.

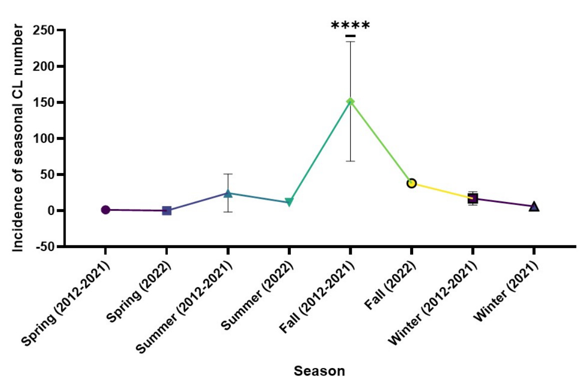

Fig. 4: Annual incidence of seasonal CL cases among military personnel in the northwest region of Isfahan Province, Iran

Discussion

This report is the first to document the application of ATSB among military personnel in Isfahan province, Iran (28, 29). While the decline in CL incidence following ATSB implementation was not statistically significant, the observed downward trend, when contextualized within a decade-long historical baseline, suggests potential effectiveness of the CL strategy. A key limitation of the study was the inability to perform entomological monitoring of the sand fly vector (*P. papatasi*). Vector surveillance, including pre- and post-intervention abundance assessments, is vital for verifying direct impacts on sand fly populations and ensuring reproducibility. However, due to logistical and institutional constraints within the military setting, such as limited temporal access for field operations and a lack of entomological personnel, such data could not be collected. It is important to emphasize that this was a preliminary field trial, primarily aimed at assessing operational feasibility and potential trends in CL reduction. Future studies will integrate systematic vector monitoring, including light trap collections and sugar bait station counts, to better evaluate vector population dynamics for ATSB application (17, 26, 30, 31). Several prior studies support the potential utility of ATSB. Saghafipour et al. demonstrated a significant reduction in sand fly abundance and CL incidence using boric acid-based sugar baits in similar arid regions of Iran (26). Similarly, Müller et al. reported over 90% suppression of *P. papatasi* populations using ATSB-treated vegetation in Israel and the Jordan Valley. These findings reinforce the rationale for deploying ATSB in endemic desert environments, where natural sugar sources are limited and vectors heavily rely on exogenous sugar for metabolic functions (24).

Sugar-based baits exploit the natural sugar feeding behavior of sand flies, especially in xeric environments. Glucose, sucrose, and fructose are primary energy sources for these vectors, and the addition of boric acid, a stomach poison, interferes with their nervous system without environmental accumulation. In this study, we used brown sugar (10%) combined with boric acid (1%) and applied the solution monthly during peak transmission seasons. Vegetation was carefully selected for its capacity to absorb and retain moisture, ensuring prolonged bait efficacy (32-35). Despite the lack of entomological confirmation, the epidemiological data indicate a promising downward trend. The post-intervention period (2022) recorded the lowest annual case count in over a decade, with marked reductions during the autumn peak transmission season. While the reduction was not statistically significant, this likely reflects the limited sample size and one-year intervention period, rather than ineffectiveness of the ATSB method. The findings thus provide a strong rationale for scaled-up trials incorporating extended monitoring timelines and expanded geographic coverage. Studies have shown that fruits like mango, banana, apple, and grape attract sand flies, and ATSB formulations using fruit extracts and insecticides have achieved control rates between 36% and 100% in mosquito studies (21, 36-38). In CL control, nectarine extract combined with oral insecticides has effectively reduced sand fly populations. In Iran, brown sugar with boric acid sprayed on plants and treated fences has proven effective against *P. papatasi* (16, 26, 39). The use of ATSB as a vector control method can have significant public health benefits. By targeting sand fly populations, which are responsible for transmitting diseases such as CL, ATSB can indirectly reduce the incidence of these diseases, thereby improving public health in endemic regions. Since ATSB specifically targets vectors without harming humans, it minimizes the negative side effects associated with chemical insecticides. This approach offers a sustainable and effective solution for vector control, particularly in high-risk areas, by reducing reliance on harmful pesticides and contributing to more environmentally friendly and long-term disease management strategies.

The number of participants was relatively small, and the research was conducted in a limited geographic area. Because of that, the results may not represent other regions with different environmental or epidemiological conditions. Also, since the ATSB method was only studied over a short period, a longer monitoring time across more years is needed to confirm its effectiveness more reliably.

Conclusion

ATSB presents a promising, eco-friendly approach for reducing CL incidence. Although the observed decline was not statistically significant, the downward trend suggests potential effectiveness. These findings support further refinement and integration of ATSB into vector control programs, particularly in endemic areas where conventional methods remain insufficient.

Conflict of Interest

The authors declare that there is no conflict of interests.

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