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Doutorado em Ecologia

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MOVEMENT, INFESTATION AND MANAGEMENT of *Rattus norvegicus* IN URBAN SLUMS

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Thesis submitted to the Post-Graduate Program in Ecology: Theory, Application and Values, as part of the requirements for obtaining the title of Doctor in Ecology.

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To the memory of my father, my loving mother, dearest wife and kids

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DISCLOSURE TEXT

MOVIMENTO, INFESTAÇÃO E MANEJO de *Rattus norvegicus* em FAVELAS URBANOS

Adedayo Michael Awoniyi

Os ratos sinantrópicos são pragas amplamente disseminadas em todos os continentes, exceto na Antártica. Os ratos constituem sérias ameaças às propriedades familiares, à produção agrícola e à saúde pública, e são difíceis de controlar, especialmente nos ambientes urbanos de baixa renda de países subdesenvolvidos e em desenvolvimento. Previsivelmente, o atual aumento global da população de residentes urbanos de baixa renda afetou o aumento da população de ratos urbanos, proporcionando aos ratos condições (como superlotação, má gestão de resíduos, esgoto a céu aberto, entre outras) que favorecem sua proliferação, que geralmente resultar em alta interação humano-roedor e provável disseminação de infecções transmitidas por roedores. O corolário da proliferação da população de roedores na saúde pública e nas perdas agrícolas sustenta consideravelmente a necessidade de desenvolver programas de manejo eficazes. No entanto, conforme demonstrado por estudos anteriores, vários aspectos da biologia de ratos, como dispersão ou taxa de movimento, potencial alta reprodutivo e resistência aos métodos de controle ou intervenção comuns (aplicação aleatória de rodenticida), entre outros, contribuíram muito para o fracasso de várias iniciativas de controle de roedores . Portanto, o gerenciamento bem-sucedido das perdas associadas à expansão da população de ratos depende de forma confiável de controle da população de ratos ou técnicas de redução, o que continua sendo um gargalo para os cientistas envolvidos em estudos com roedores. Portanto, este estudo idealizou um método fácil de usar e com boa relação custo-benefício, ou seja, o uso da Rodamina B para avaliar a distância percorrida por ratos dentro e fora das residências. O método oferece uma vantagem sobre os métodos convencionais (por exemplo; captura-marca-recaptura e rastreamento de rádio) que são caros e difíceis de aplicar em assentamentos urbanos devido ao medo de introduzir ratos infectados no ambiente, uma vez que os métodos convencionais envolvem a captura, marcação e recaptura de animais sem garantia de 100% de que todos os animais serão recapturados após sua liberação para o meio ambiente. Além disso, o estudo caracterizou o comportamento de roedores de curto e longo prazo a uma combinação de intervenções (Manejo Integrado de Pragas - IPM) em terrenos urbanos complexos, como New Providence - Bahamas e Salvador - Brasil. O estudo relata que ratos são capazes de se mover até 90m em ambientes urbanos e que o RB é um método eficaz e eficiente para investigar o movimento de ratos em ambientes urbanos de baixa renda. Da mesma forma, relatamos que a combinação de intervenções químicas (aplicação de rodenticida) e não químicas (sanitárias / infraestruturais) diminuiria substancialmente a taxa de infestações de ratos por pelo menos 5-6 meses em ambientes urbanos. Os resultados deste estudo devem ser úteis para orientar os legisladores e outros envolvidos em programas de manejo de roedores para formular uma abordagem mais abrangente para o manejo da população de roedores, mesmo em um ambiente urbano complexo. Por último, o estudo deve ajudar a diminuir a alta interação ou conflito humano-roedor, ao mesmo tempo que diminui os riscos de infecções transmitidas por roedores que estão associadas a pragas de roedores.

DISCLOSURE TEXT

MOVEMENT, INFESTATION AND MANAGEMENT of *Rattus norvegicus* IN URBAN SLUMS

Adedayo Michael Awoniyi

Synanthropic rats are widely spread pests all over the continents, except in Antarctica. Rats constitute serious threats to household properties, agricultural production and public health, and they are difficult to control, especially in the low-income urban environments of underdeveloped and developing countries. Predictably, the current global increase in the population of low-income urban residents has affected the upsurge of the urban rat population by affording rats with conditions (such as overcrowding, poor waste management, open sewer among others) that favour their proliferation, which usually result into high human-rodent interaction and probable dissemination of rodent-borne infections. The corollary of rodent population proliferation on public health and agricultural loss considerably supports the need to develop effective management programs. However, as demonstrated by previous studies, various aspects of rats biology such as dispersal or movement rate, mammoth reproductive potential and resistance to the common control or intervention methods (haphazard rodenticide application) among others have greatly contributed to the failure of several rodent control initiatives. Therefore, the successful management of losses associated with rat population expansion dependably rests on well-organized rat population control or reduction techniques, which remains a bottleneck to scientists involved in rodent studies. Hence, this study devised an easy to use and cost-effective method, that is, the use of Rhodamine B to assess the distance travelled by rats within and around households. The method offers an edge over the conventional methods (for example; capture-mark-recapture and radio tracking) that are expensive and difficult to apply in urban settlements due to the fear of introducing infected rats into the environment since the conventional methods involve capturing, marking and recapturing of animals without 100% guarantee that all animals will be recaptured after their release into the environment. Additionally, the study characterized both short and long-term behaviour of rodents to a combination of interventions (Integrated Pest Management - IPM) in complex urban terrains such as the New Providence – the Bahamas and Salvador -

Brazil. The study reports that rats are capable of moving up to 90m in urban settings and that RB is an effective and efficient method for investigating the movement of rats in low-income urban environments. Likewise, we report that the combination of chemical (rodenticide application) and non-chemical (sanitary/infrastructural) interventions would substantially decrease the rate of rat infestations for at least 5-6 months in urban environments. Results from this study should be useful in guiding the policymakers and others involved in rodent management programs to formulate a more comprehensive approach for managing the rodent population even in a complex urban environment. Lastly, the study should help to lessen the high human-rodent interaction or conflict, while also abating the risks of rodent-borne infections that are associated with rodent pests.

Resumo

Ratos sinantrópicos são uma praga invasiva prevalente em ambientes urbanos de baixa renda; são difíceis de controlar e constituem uma ameaça à saúde pública e à agricultura para a raça humana. Em comunidades urbanas de baixa renda, a expansão e migração de roedores entre famílias ou bairros é um problema persistente, exigindo esforços de controle. No entanto, o avanço de programas eficazes de controle de roedores requer uma compreensão profunda de seu movimento e comportamento (curto e longo prazo) para intervenções químicas e sanitárias/infraestruturais. Portanto, no primero capitulo, usou-se Rodamina B (RB) um corante flourescente não tóxico, para avaliar a provável distância percorrida por ratos dentro de domicílios em um ambiente de favela na cidade de Salvador (BA-Brasil); ja no segundo capitulo, examinou-se o resultado de curto prazo de intervenções químicas e sanitárias em avistamentos de ratos em sete comunidades urbanas de baixa renda de New Providence, nas Bahamas; e no terceiro capitulo, caracterizou-se o resultado de longo prazo de intervenções químicas e de infraestrutura no nível de infestação de ratos em três vales de uma comunidade de baixa renda na cidade de Salvador. No premeiro capitulo, prendemos ratos a uma distância máxima de 90m por quatro dias consecutivos e relatamos que os ratos viajam até 90m de distância, mesmo em uma comunidade urbana complexa. Também estabelecemos que o RB é um método alternativo confiável que pode ser usado para investigar o movimento de pequenos mamíferos, mesmo em ambientes urbanos. No segundo capitulo, registramos sistematicamente os avistamentos de ratos antes e três meses após as intervenções (aplicação de rodenticida e melhoria do sistema de saneamento) e observamos uma ligeira diminuição nos avistamentos de ratos após a intervenção, embora com eficácia variada entre os locais de amostragem. Por fim, no terceiro capitulo, registramos sistematicamente a infestação de ratos antes e três anos após as intervenções (aplicações de rodenticidas e infraestruturais). O estudo ocorreu em três vales, com os vales 1 e 4 servindo como vales de tratamento (receberam ambos os tipos de intervenções), enquanto o vale 2 serviu como controle (não recebeu intervenção química). Nós relatamos que as intervenções diminuíram substancialmente as infestações de ratos por 5-6 meses, nos vales de tratamento com um nível de infestação aleatório registrado no vale de controle. Portanto, concluímos que a rodamina B é um método viável, mais barato e seguro para rastrear o movimento de roedores em ambientes urbanos e também que, embora a intervenção química por si só ofereça um método barato e fácil de controlar ratos, no entanto, sua eficácia geral é de curta duração. . Como resultado, defendemos que um sistema de abordagem integrado específico do local que incorpora métodos químicos e não químicos de controle de roedores deve ser considerado ao planejar programas de manejo de roedores para obter um resultado sustentável a longo prazo.

Palavras-chave: *Rattus norvegicus*; Rodamina B; Roedor; Rodenticida; Manejo de roedores; Comunidade urbana; Zoonoses

Abstract

Synanthropic rats are worldwide invasive and prevalent pest in low-income urban environments; they are difficult to control and constitute a public health and agricultural threat to the human race. In low-income urban communities, rodent expansion and migration between households or neighbourhoods is a persistent problem, demanding control efforts. Therefore, advancing effective rodent control programs requires an in-depth understanding of their movement and behaviour (short and long-term) to chemical and sanitary/infrastructural intervention. Thus, this thesis is divided into three chapters, with each representing an article. The first chapter uses Rhodamine B (RB) a non-toxic dye to assess the distance travelled by rats within or areas around households in a slum environment in the city of Salvador (BA-Brazil); the second chapter examined the short-term outcome of chemical and sanitary interventions on rat sightings in seven low-income urban communities of New Providence, in the Bahamas; and the third chapter characterized the long-term outcome of chemical and infrastructural interventions on rat infestation level in three valleys of a low-income community of Salvador. In chapter one, we trapped rats to a maximum distance of 90m for four consecutive days and reported that rats do travel up to 90m distance even in a complex urban community. We also established that RB is a reliable alternative method that can be used to investigate the movement of small mammals even in urban settings. In chapter two, we systematically recorded rat sightings before and three months after interventions (rodenticide application & improvement of sanitation system), and observed a slight decrease in rat sightings after the intervention, although with varied effectiveness across the sampling locations. Lastly, in chapter three, we systematically recorded rat infestation before and three years after interventions (rodenticide applications and infrastructural). The study occurred in three valleys, with valleys 1 and 4 serving as the treatment valleys (received both types of interventions), while valley 2 served as the control (received no chemical intervention). We reported that the interventions substantially decreased rat infestations for 5-6 months, at the treatment valleys with a haphazard infestation level recorded at the control valley. Therefore, we concluded that Rhodamine B is a viable, cheaper and safer method for tracking rodent movement in urban settings, and also that, although chemical intervention alone offers a cheap and easy method of controlling rats, however, its overall effectiveness is short-lived. As a result, we advocate that a site-specific integrated approach system that incorporates both chemical and non-chemical methods of rodent control should be considered when planning rodent management programs to obtain an effective long-term result.

Keywords: *Rattus norvegicus*; Rhodamine B; Rodent; Rodenticide; Rodent management; Urban community; Zoonoses

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Thesis Structure

A presente tese está estruturada em três capítulos como segue:

Chapter I – ***Using Rhodamine B to Assess the Movement of Small Mammals in Urban Slum***

The first article of the chapter: ***Using Rhodamine B to Assess the Movement of Small Mammals in Urban Slum*** evaluates the travel distance of rats in an urban slum community of Salvador (Pau da Lima), while also evaluating the efficacy of using Rhodamine B (RB) as an alternative method for tracking the movement of small mammals even in a complex urban slum environment such as Pau da Lima, Salvador.

Chapter II – ***Effect of Chemical and Sanitary Intervention on Rat Sightings in Urban Communities of New Providence, the Bahamas***. This chapter characterizes rodent population richness in low income communities of New Providence, the Bahamas, and how their population reacts (short term – three months post intervention) to chemical and sanitary intervention in the same communities.

Chapter III – ***Population Dynamics of Synanthropic Rodents after a Chemical and Infrastructural Intervention in an Urban Low-income Community***. This chapter evaluates the dynamics of rat population that is, their long term (three years) response to interventions (chemical and infrastructural interventions) in an urban low-income community of Salvador, Brazil.

General Introduction

Synanthropic rodents such as brown or Norway rat (*Rattus norvegicus* - Berkenhout, 1769), black rat (*Rattus rattus* – Linnaeus, 1758) and the house mouse (*Mus musculus* - Linnaeus, 1758) are highly successful rodents that inhabit urban and rural areas in all continents but Antarctica (Morand et al., 2015; Battersby et al., 2008). The brown rats are believed to have been introduced into the Americas around 1750s through trans-Atlantic navigation during the European colonization (Puckett et al., 2016). They are considered "highly prosperous" because of their ability to live in any human/human-altered environment likewise their capability to feed on any available human food and even more, including paper, soap, furniture, candy, meat, vegetables, grain, seeds, nuts, clothes, electric cables, carrion, refuse, pets' faeces and fruits whenever accessible (IDoH, 2020; Battersby et al., 2008).

Rats populations proliferate successfully where there is abundant food, water and harbourage (Panti-May et al., 2016; Costa et al., 2014). For this reason, rodents are omnipresent in most low-income urban communities where conditions that favour their existence for example, poor trash collection, open sewers, standing water and vacant or dilapidated buildings are usually available (Himsworth et al., 2013; Riley et al., 2007). Increase in rodent occurrence in low-income urban communities of Brazil is a common feature as reported by Masi et al., (2009). Given the expected global increase in the number of urban dwellers from the 751 million reported in 1950, to about 6.7 billion that is projected for 2050 (United Nations, 2018), the already poor sanitation systems and infrastructure in many low-income urban environments is likely to further deteriorate, thereby leading to higher rodent propagation and rat-human interactions.

Rats travel several meters on average in search of food, water and shelter, and their movement patterns differ between urban and natural environments (Byers et al., 2019), with resource availability and physical barriers seemingly influencing their movement between households in urban environments (Feng and Himsworth, 2014). For example, Meehan (1984) reported that rats only travel between 3-10m in urban environments especially when conditions that favour their existence are available, while Kajdacsi et al., (2014) reported high genetic diversity among trapped rats within sampling sites separated by few hundred meters.

Rat infestations present a range of challenges to human and society. For example, juvenile rats can enter buildings through very small openings, (a factor that is critical when planning rodent control initiative (IDoH, 2020)). Rats may also initiate fires when gnawing on electric cables and many of reported fire outbreaks of an unknown origin are associated with rats. Rats may also create passage/opening through or beside electric cables and containers in the course of their relentless gnawing, thereby causing damage to water pipes, household materials and contaminate food (Battersby et al., 2008). Most importantly, rats are menace to human health (especially the less privileged/populations that reside in low-income urban communities) and agriculture. Indeed, rats serve as reservoir of important zoonotic diseases such as: plague, leptospirosis, hantavirus, lassa fever etc. (Battersby 2015; Costa et al., 2015; Himsworth et al., 2013; Meerburg et al., 2009, Singleton et al., 2003), while causing economic damage worth billions of dollars to farmlands, industries and household properties (Pimentel et al., 2005; Childs et al., 1991). For example, in 2021, Australia recorded an episode of mice plague that spread across its states, with some farmers losing as much as $300,000 in ruined crops as the mice chewed through anything they could get their teeth in (Australia mice plague - BBC News 2021)

Despite the social and economic importance of rodents (Panti-May et al., 2016; Battersby 2015; Costa et al., 2015; Morand et al., 2015; Pimentel et al., 2005; Childs et al., 1991), efforts targeted towards their control have yielded mixed results (Oyedele et al., 2015). Residual rat populations can rapidly recover following a period of decline via either reproduction or immigration from a nearby population (Hansen et al., 2020). In the past decades, some insights about space use by small mammals were obtained by capture-mark-recapture methods (Prevedello et al., 2008), radio-tracking (Millspaugh and Marzluff, 2001) and the spool and line device (Boonstra and Craine, 1986). These methods are less effective in urban environments due to their high cost and environmental heterogeneity in urban areas (Prevedello et al., 2008).

To control rat population in agricultural settings, chemical i.e. application of rodenticides (Buckle & Smith, 2015), and non chemical & non-lethal chemical e.g. physical removal of population (Pascal et al., 2005), prevention and reduction of immigration (Sullivan, 2002) and biological control (Smith & Meyer 2015; Lenton, 1980) have been generally used. However, most of these methods are not effective/appropriate especially when applied alone in urban settings owing to the:

heterogeneity of the urban settings; high cost of application; high technical know-how demand; ethical issues among others (Murray & Sanchez, 2021; Parsons et al., 2017; Oyedele et al., 2015). Therefore, rodenticides application plus or a combination of these methods (integrated pest management-IPM) is the most common or advisable method for rodent population management in urban environments (Buckle and Eason, 2015; de Masi et al., 2009).

The majority of previous studies on the urban ecology of rats have been limited to distribution (Traweger et al., 2006) and factors that control distributions (Himsworth et al., 2013, Traweger et al., 2006). Conversely, little research has been done on the movement, that is rats' travel distance within and around households, likewise on how rat populations react (short and long-term) to interventions (chemical and sanitary/infrastructural), especially in low-income urban communities. The lack of in-depth understanding of these two aspects of rodent ecology has consequently hindered effective rodent control programs, especially the development of suitable rodenticide spacing and intensity, timing and extent of targeted areas of intervention. Here, this project studied the distance travelled by rats between and around households using method that has not been previously used in urban areas, and the impact of chemical and environmental (infrastructural) interventions on the propagation of rodents in low-income urban communities. Specifically, the study used Rhodamine B -RB (a non-toxic biomarker that has been validated for measuring the distribution and movement of small mammals (Fisher 1999; Mohr et al., 2007; Monadjem et al., 2011)), to trap rats up to 90m distance within households and areas around households in an attempt to formulate an effective protocol that is suitable for controlling rat population in urban environments. Similarly, we characterized the short and long-term responses of rats to chemical and sanitary/infrastructural interventions in low-income urban communities using slightly modified protocols that have been previously validated by CDC (2006), Eyre et al. (2020), Hacker et al. (2016), Promkerd et al. (2008) and Walsh (2014). Results from these findings will help to develop an informed and effective rat control program that is suitable for use in urban environments, while lowering the probability of transmission of zoonotic diseases that are associated with the high human-rat interactions, especially in poor urban communities.

Objectives

General objective

To characterize the distance travelled by rats between households in a heterogeneous environment, and the impact of chemical plus environmental interventions on rat populations in urban slum communities

Specific objectives

- Characterize the distance travelled by rats between households and areas around households in an urban environment of Salvador, Brazil
- Evaluate the short-term effect of chemical and sanitary intervention on the abundance/sighting of rats in low-income communities of New Providence, the Bahamas
- Evaluate the short- and long-term effect of chemical intervention on the activity and abundance of rats in a slum environment of Salvador, Brazil

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Chapter I

Using Rhodamine B to Assess the Movement of Small Mammals in Urban Slum

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Using Rhodamine B to Assess the Movement of Small Mammals in an Urban Slum

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Abstract

1. The small mammals, especially rats are pest species that are present in cities worldwide. The rat moves around and into residences and other anthropogenic structures. It is especially ubiquitous in urban slums and a threat to infrastructure and public health due to the pathogens it carries and transmits. Effective control of rat populations in most urban areas has been unsuccessful, despite several rodent control efforts. Limited information about rat movement distance has hindered identification of control units and effective scales at which to enact control during interventions.

2. We evaluated the suitability of Rhodamine-B, a non-toxic biomarker, for assessing the distance travelled by rats in urban slums. We tracked rats over two campaigns between 2019 and 2020.

3. Overall, 27.9% of trapped rats showed signs of Rhodamine-B in their whiskers under fluorescence microscope. This shows that our method provides a viable alternative for investigating the movement of small mammals in this area. We found that rats move up to 90m distance in urban slums, with smaller rats travelling more actively than bigger rats.

4. Information obtained from this study should be useful in guiding efficient rodent control initiatives to reduce the risk of household rodent infestation and rodent-borne disease in urban slums.

Keywords: Rat*;* Rhodamine-B; Rodent; Snap-trap; Slum; Zoonoses

Introduction

Many species of small mammals, especially rats: the brown or Norway rat (*Rattus norvegicus*), the roof rat (*Rattus rattus*) and the house mouse (*Mus musculus*) are considered to be the most serious pest species in residential, industrial, and agricultural contexts accounting for losses worth billions of dollars (Morand et al., 2015; Costa et al., 2014a; Pimentel et al., 2005; Battersby et al., 2008; Childs et al., 1991). Likewise, rats serve as reservoirs of important zoonotic diseases, that is, infectious diseases that are transmitted between species from animals to humans or vice versa, for example leptospirosis, Seoul hantavirus, toxoplasmosis and capillariasis (Panti-May et al., 2016; Battersby 2015; Costa et al., 2014a; Himsworth et al., 2013; Singleton et al., 2003).

Rat sightings or populations can reach high densities where shelter, food and water are available (Awoniyi et al., 2021; Panti-May et al., 2016), and rats are ubiquitous in urban slums where conditions, including poor trash collection, open sewers, and standing water provide resources and harborage (Himsworth et al., 2013; Riley et al., 2007). Therefore, given the expected projection in global urbanization from the 751 million urban dwellers recorded in 1950 to about 6.7 billion by 2050 (United Nations, 2018), it is probable that the inadequate environmental conditions in urban slums will worsen over time thereby resulting in rats' proliferation, with increased risk of their dispersal among households and peridomicilliary areas . Additionally, it is estimated that about 3 billion people will either reside in slums or informal settlements by 2030 (United Nations SDG, 2018), thereby increasing human-rat contact (Himsworth et al., 2013; Riley et al., 2007).

Rats show different movement patterns between urban and rural or natural areas (Byers et al. 2019). For example, studies have shown that rodents in urban areas travel shorter distances than their non-urban counterparts (Byers et al., 2019; Himsworth et al., 2013). Several factors such as resource availability, predators, social structure, habitat and human disturbances have been shown to influence rat population density, migration pattern, and home range size (Costa et al., 2014b; Nathan et al., 2008). Furthermore, there are several studies on the movement of rat and its home range in non-urban settings (Monadjem et al., 2011; Traweger et al., 2006; Brown et al., 2001; Lindsey et al., 1999; Taylor & Quy 1973), but little is known about rat movement in urban areas (Himsworth et al., 2013; Mohr et al., 2007; Meehan 1984). For example, rats have been reported to travel from tens of meters in the course of a single night and on rare occasions up to a kilometer in rural or sylvan areas (Taylor & Quy 1973). However, in urban settings, movement seems to be restricted. Meehan (1984) reported that rats only travel between 3-10m in an urban setting when food, water and harborage are available. The abundance and/or density of resources, as well as physical barriers such as streets and other open areas, are some of the main factors known to affect area fidelity and extension of the movement of small mammals (Combs et al, 2018; Feng and Himsworth, 2014).

A wide array of techniques have been used to study the movement of rats in urban settings, employing both direct (e.g., capture-mark-recapture using tags or passive integrated transponders (PIT), GPS, telemetry) and indirect measurement (e.g., track marks, genetic distance, bait consumption) methods (Byers et al., 2019), each presenting different trade-offs between precision, information gathered and intensity of work. Most studies on rat movement in urban areas have been conducted in developed countries in North America (e.g., Parsons et al., 2015), Europe (e.g., Traweger and Slotta-Bachmayr, 2005) and Japan (Byers et al., 2019; Tanaka and Kawashima, 1951), with little attention to the southern hemisphere, and one single study making multiple comparisons between northern and southern hemisphere sites (Combs et al., 2018). For example Brazil, despite accounting for most of the studies performed in the southern hemisphere, has used only indirect methods i.e. track plates and genetic microsatellites (Hacker et al., 2016; Richardson et al., 2017), of which only microsatellite techniques allows a proxy for distances to be estimated through genetic structure and spatialized captures. Using the microsatellite techniques, Richardson et al., (2017) reported a strong pattern of genetic divergence among rats captured in small areas separated by <50m in Brazil.

Therefore, the need for adequate information on the dispersal distances, home range and other rat movement patterns common in urban slums of the developing tropical nations is urgent, as a part of the ecological data necessary for efficient pest control plans (Zeppelini et al., 2020), allowing effective delimitation of priority areas that require treatment or intervention of migrant recolonization. However, most of the previous techniques applied to estimate rodent dispersal are unsuitable in urban slums due to the lack of regular city blocks in slums, which can hinder the ability to install an effective

PIT sensor network as well as the difficulty in tracking animals either by following marks or telemetry, while GPS techniques might suffer from signal issues due to terrain and constructions (Byers et al., 2019). The high costs of remote sensing equipment, high labor demand and the presence of social barriers to the movement of teams in following the devices' signal due to crime and/or suspicion from local inhabitants necessitate the need for a cheap, easy to deploy and reliable technique to make studies viable in these settings.

Here, we examined the suitability of using Rhodamine B (RB) as an alternative to the aforementioned methods in tracking the movement of rats within and among households in an urban slum over two campaigns. RB is a non-toxic dye (biomarker) that has already been tested and shown to be effective in measuring the spatial distribution and movement of small mammals and other mammals like the wild pig (Monadjem et al., 2011; Mohr et al., 2007). Once ingested in non-lethal quantities, it is incorporated into the keratinous structures of animals (bones, hair and teeth) where it will be detectable as a fluorescent band for about four and half months after ingestion under fluorescence microscopy (Jacob et al., 2002; Fichet-Calvet 1999). We hypothesized that; (i) rats will accept RB plus conventional bait mixture after two days pre-baiting (non-dyed), (ii) RB will offer an effective alternative for evaluating rat movement even in complex urban terrains, and (iii) numbers of rats with signs of RB will decrease away from the bait station (buffer zone).

Materials and methods

Study Area

The study was performed in Pau da Lima, (13°32′53·47″ S; 38°43′51·10″ W) a slum community located in the city of Salvador (BA, Brazil), with an estimated area of 0.17km^2 and a population of about 128,997 (IBGE, 2010). The area is characterized by its strong altitudinal gradient, low socioeconomic status of the residents (mean per capita daily household income of less than or equal to USD 1.30 (at 2014 exchange rates) (Costa et al., 2014b), and inadequate sanitation and trash collection (Reis et al. 2008). Pau da Lima was chosen for this study because of previous research and its history of high rat abundance all year round (Panti-May et al., 2016).

Study design

Rhodamine B (RB) preparation

A concentration of 0.2% (2g) of RB (Sigma-Aldrich) was mixed into 1kg of conventional bait (Peanut butter, hotdog and Maize bran) as previously described by Monadjem et al., (2011) and Mohr et al., (2007). This concentration has been reported to be non-lethal and detectable (fluorescence) for several weeks in mouse whiskers under UV microscopy (Willekens 2003).

Pre-baiting

To avoid neophobia and increase trapping success, a non-dyed pre-baiting was carried out to familiarize the rats with the conventional bait (Gurnell 1980). We placed non-dyed bait in the central bait station in addition to four other identified points with active rat signs within a selected buffer zone of 10m circumference (Fig. 1) for two successive days that is, Campaign 1 (C1) October 01 – 02, 2019 and Campaign 2 (C2) October 13 – 14, 2020 respectively. This two days period has been shown to be sufficient in acclimatizing rats to new baits (Weerakoon et al., 2013). Each bait station was re-baited every day and checked for signs of consumption.

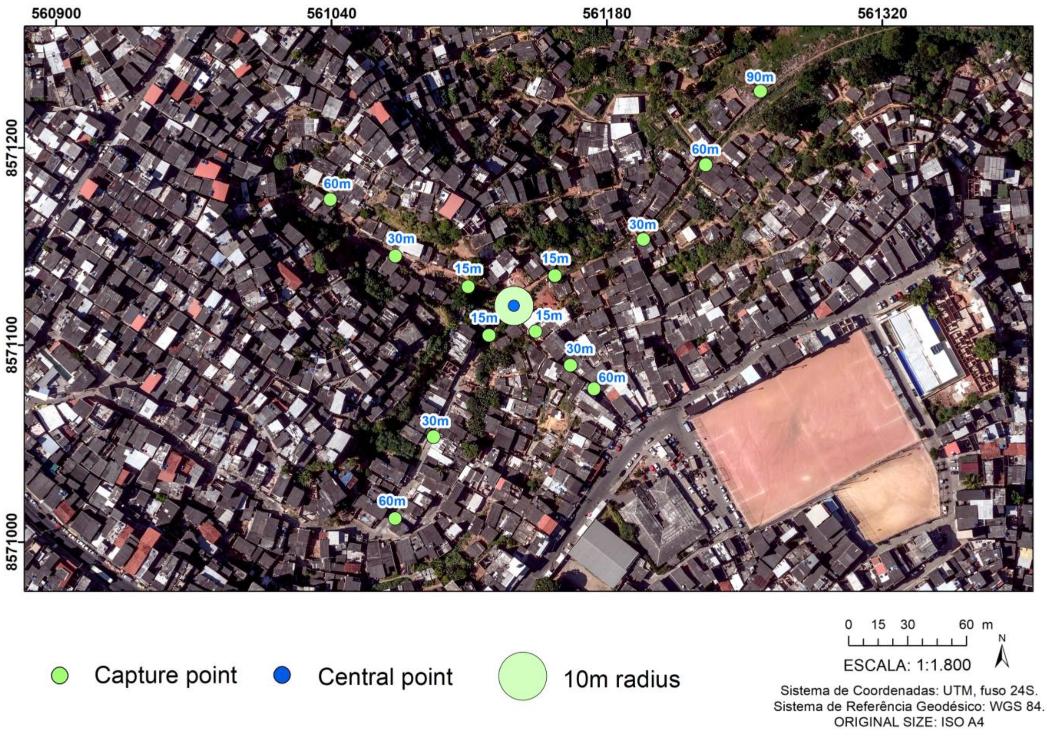

Fig. 1. Distribution pattern of the snap traps at distance 15, 30, 60 and 90m away from the central bait station within the valley

RB baiting

Following the period of non-dyed pre-baiting, the dyed baits were placed for three consecutive days i.e. (October 03 – 05, 2019 for C1 and October 15 – 17, 2020 for C2) at the same bait stations used for the non-dyed pre-baiting, and rebaited daily. We did not measure the daily bait consumption, since lizards and dogs can access the bait stations. Also, we placed a total surplus bait mixture of 2.4kg in small portions at the bait stations (buffer zone) to offset any effect of bait consumption by the non-target species.

Animal Trapping and sample collection

As shown in figure 2, given that RB dye has been reported to be more detectable in the whiskers of rats between four to twenty-two weeks post RB consumption (Tolkachev 2019; Fichet-Calvet 1999), five weeks after the RB baiting, we slightly modified the method previously described by Woodman et al., (1996), to trap rats using Victor® mouse snap trap over the two campaigns. Briefly, depending on size of the peri-domestic space (size of the backyard), we placed between 3-5 sausage baited (mainly peanut butter bait, plus locally purchased hotdog) Victor snap traps at each sampling point per day. Traps were positioned at points 15, 30, 60, and 90m **(**Fig. 1**)** from the central bait station for four consecutive nights, with no trapping done within the buffer zone because we were interested in the distance travelled by rats away from the buffer zone. Rodent trapping in C1 occurred between November 13 - 16, 2019, and C2 between November 24 - 27, 2020. We trapped rats up to 60m distant from the bait station for the first campaign (C1) and up to 90m for the second campaign (C2). Snap traps were activated at dusk and checked the following morning. In addition to residents' recommendation of suitable trapping sites, where possible, traps were placed along rodent runs or burrows, close to walls, near rodent droppings, open sewer or garbage points within or outside households. Captured animals were weighed, sexed, labelled and transported to the laboratory in plastic bags for whisker sample collection. All animal handling procedures and methods were observed according to the protocol described by Mills et al., (1995).

We estimated trap success using a method previously described by Cavia et al., (2012). Briefly, we multiplied the total number of trapped rats by 100 and divided it by the total number of trapping efforts minus half the total number of traps with blood/fur or non-target species. Stolen and damaged traps were excluded from the calculation. Although we were limited in statistical inference due to our small sample size, however, we used Chi–Square test to evaluate the relationship between RB positive and negative group, and Fishers test for small samples for the association between other population characteristics. All analyses were performed in R 4.0.0 version (R Core Team, 2019). At least six whiskers were collected from each animal for microscopic examination, and were stored separately in an Eppendorf tube and kept in a refrigerator until further analysis.

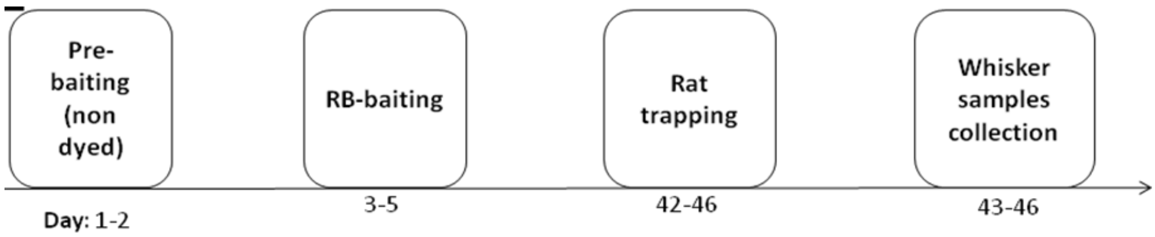

Fig. 2. Timeline of the field protocol for animal trapping and sampling collection

Examination of Whiskers

We used the protocol previously validated by Fisher (1999) to prepare the whiskers for microscopic examination. Briefly, we rinsed whiskers in a beaker containing 70% ethanol for at least 2mins. Immediately after this, we placed the whiskers in distilled water for 3mins and replaced/washed beakers between samples. Rinsed whiskers were air-dried at room temperature for at least 24hrs. After this, we placed at least two whiskers on a glass slide with a drop of fluoromount and covered it with a cover slide. Mounted hairs were allowed to dry at room temperature for at least 3 days before examination under a fluorescence microscope (Nikon Ds-Q12) with low magnification (40x) for RB fluorescent signs.

Ethical statement

This study was conducted in accordance with the Brazilian laws regarding ethics in research. The Ethics Committee on the Use of Animals in Research (CEUA) of the Institute of Biology, Federal University of Bahia, Salvador, Brazil gave the approval and permission to conduct research on rats with Project Number: 05/2018.

Results

The total trapping effort was 438 trap nights over the course of the two campaigns (220 for C1 and 218 for C2). Out of this, 43 traps (29 for C1, and 14 for C2) were either lost or damaged, thus leaving us with a total effort of 395 (191 for C1 and 204 for C2) effective trap nights (Supplemental material). In total, we captured 43 rats (25 for C1 and 18 for C2). Among the captured animals, Norway rats (*R. norvegicus*) were the principal species (40 individuals, 93%), followed by *Mus musculus* (2 individuals, 4.7%), while one individual (2.3%) was badly eaten by ants beyond identification (data not shown). Overall, we recorded a trapping success of 11.2%.

Table 1: Demographic characterization of RB positive and negative Norway rat population in an urban slum of Salvador, Brazil

		Rhodamine B positive	**Rhodamine B negative**	***p-value***
Number of rats		12 (28%)	31(72%)	0.004[a]
Sex				1.000
	Male	7 (30%)	16 (70%)	
	Female	5 (26%)	14 (74%)	
Mean (SE) Body Mass		309.16 (141.14)	240.48 (159.17)	0.827
Age categories				0.477
	Young (< 200g)	3 (18%)	14(82%)	
	Sub-adult (200-399g)	5(36%)	9(64%)	
	Adult (≥400g)	4(33%)	8(67%)	
Distance in meters (Sex:F;M)				0.901
15		3 (33%) [2-F;1-M]	6(67%) [2-F;4-M]	
30		2 (22%) [1-F;1-M]	7(78%) [2-F;5-M]	
60		4 (24%) [1-F;3-M]	13 (76%) [8-F;5-M]	
90		3 (38%) [1-F;2-M]	5 (62%) [3-F;1-M]	

SE, standard error; Fisher's exact test and [a]Chi-Square test

Note: A rat captured at 90m distance had missing sex information (badly eaten by ants)

Rats were captured at all trapping distances. Among the captured rats, 12 of 43 (27.9%) showed signs of RB fluorescent marking in their whiskers under the fluorescence microscope (Table 1). However, none of the rats showed any external signs of RB in their fur. All the 12 positive rats showed at least one distinctive glowing orange-red fluorescent band (Fig. 3) in at least two of the examined whiskers. Contrary to our hypothesis, there was no reduction in the number of positive rats away from the central bait station (buffer zone), with 90m distance unexpectedly recording the maximum percentage of rats with signs of RB, that is, 3 out of the total 8 captured rats showed signs of RB in their whiskers (38%), followed by 15m distance 3/9 (33%), while at 30 and 60m, 2/9 (22%) & 4/17 (24%) showed signs of RB in their whiskers, respectively.

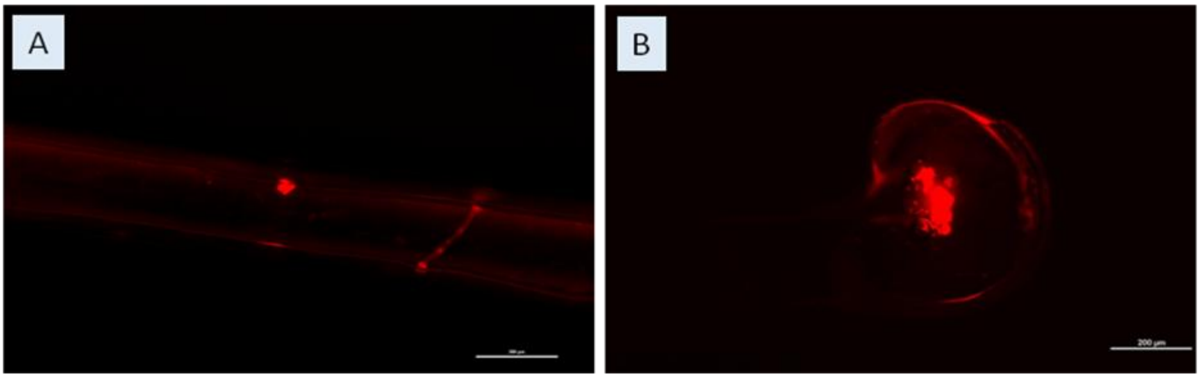

Fig. 3**.** Positive whiskers as seen under the microscope at 200µm scale (a) RB fluorescent marks in the stem of the whisker (b) RB fluorescent marks at the base of the whisker

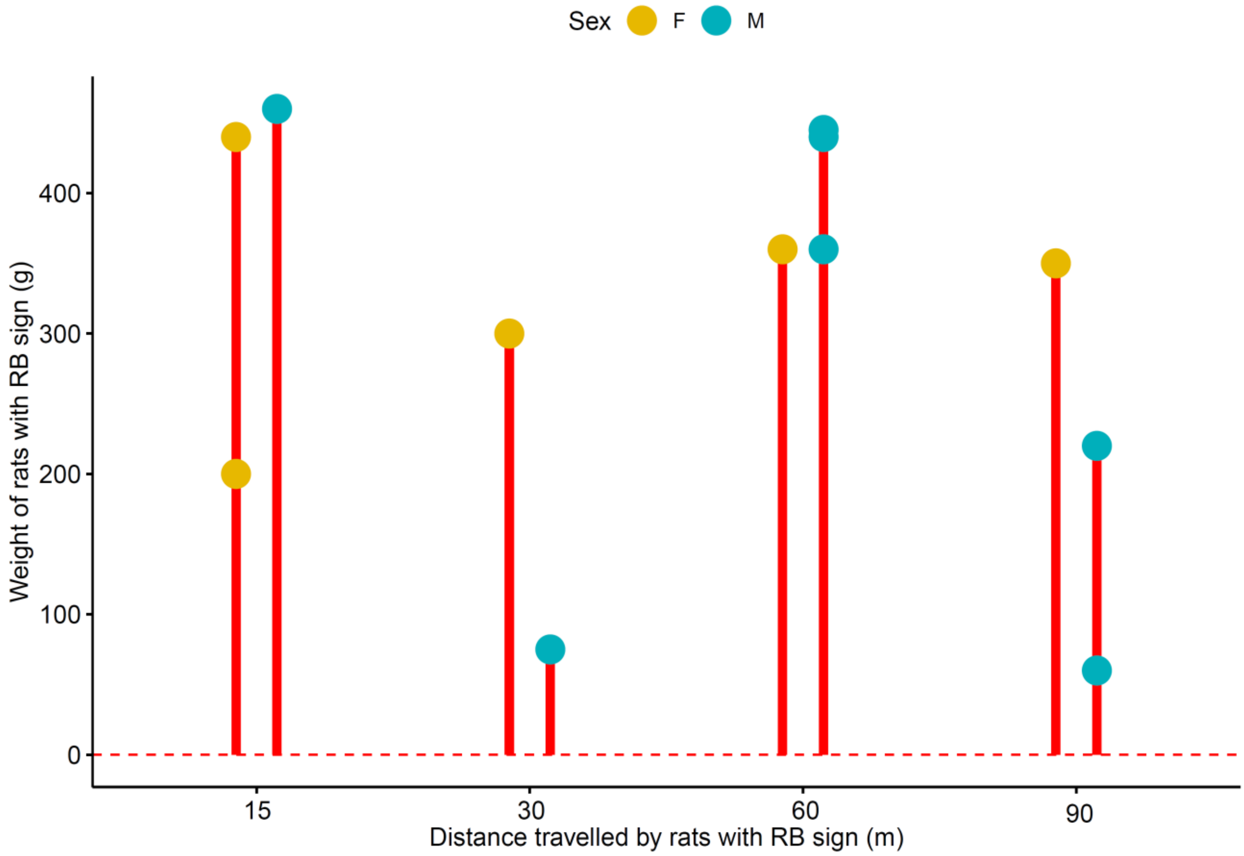

Fig. 4. Distance of RB positive Norway rats by individual weight and sex across two trapping campaigns

We recorded a non-significant higher percentage of male rats (58.3%) with signs of RB in their whiskers than female rats (41.7%) (Fig. 4). Nevertheless, the weight of rats was similar at 15m and 60m distances, although a higher number of male rats were captured at 60m distance (3 of 4, 75%). Even though we could not statistically make inference due to our small sample size, it does appear that smaller male rats ($\leq$ 200g) travel more actively from the central bait station than bigger male rats.

Discussion

We have evaluated for the first time according to our knowledge, the movement of rats in an urban slum using Rhodamine B. The 27.9% overall RB positive individuals recorded in this study corroborate our first two hypotheses and indicate the acceptability of RB-dyed bait mixture by rats after at least two days of non-dyed pre-baiting. This also shows that our method provides a potential effective, cheap and safe alternative for investigating the movement of small mammals in complex slum environments. This alternative is important because it does not require methods that raise the ethical quandary of reintroducing potentially infected rats into the environment without a guarantee of recapture. It is also free of signal problems that GPS techniques used by other methods might suffer in slum settings due to terrain or constructions difficulties (Byers et al., 2019). Further, this method is also applicable in urban non-slum, rural and natural environments, where understanding of small mammal movement ecology is desirable to aid effective control or eradication in an attempt to limit the economic and health costs associated with rodents.

Although we hypothesized that the number of rats with signs of RB would decrease away from the central bait station, the distribution and distance of RB positive rats were similar at all distances (Fig. 4). The 11.2% overall trapping success substantiates previous findings of high rat population abundance obtained by Panti-May et al., (2016) in the same slum environment of Salvador, Brazil. The overall 27.9% RB positive rats in our study is higher than the 12.9% recorded by Mohr et al., (2007) in Tanzania around grain stores and meat markets. Similarly, the pattern of distance travelled by rats within or around households in this study (Fig. 4) is different from results observed from previous studies in open farmland, poultry and rural environment respectively, where most RB positive animals were captured near the baiting station <25m (Marien et al., 2018; Monadjem et al., 2011; Gómez Villafañe et al., 2008). Here, most of the RB positive rats captured in our study were at the 90 & 15-meter mark. This contrasts with the findings from Meehan (1984) who reports that rats only travel between 3-10m in an urban environment. Though we could not statistically make inference due to our small sample size, our results show that rats can travel up to at least 90m even in complex urban terrain such as Pau da Lima, Salvador, Brazil. Similarly, even though the lack of sampling at the buffer zone somewhat limits the assessment of trends in rat movement by age and sex, we still report that smaller male rats wander more actively than larger males, as indicated by the 100% of RB positive male rats at 90m. Given that smaller rats are often younger, our finding suggests that younger rats may travel further than their counterparts

While we could not statistically confirm the dissimilarity of travel distance between sex and age, the higher number of RB positive smaller male rats observed at 90m distance may be due to the non-dominant male rats trying to avoid or seeking to establish their territory away from the dominant male rats (Macdonald et al., 1999). Our finding is also in agreement with the results of studies with other small mammals such as *Rattus exulans* in Hawaiian rainforest (Lindsey et al., 1999) and *Rattus argentiventer* in a lowland rice farm in West Java Indonesia (Brown et al., 2001) where male rats were reported to travel further distance than female rats. Nonetheless, information obtained from this research will be useful in the definition of effective bait spacing during rodent pest management initiatives.

Although this study is limited to 90m distance due to the difficult terrain of our study site, further investigation on the travel distance of rats in urban slums up to the distances of say 150-200m using the same RB techniques may reveal rat movement distances further than the ones shown here. Another limitation of our experimental setup is the inability of the type of trap used (Victor mouse snap trap) to capture other small mammals like Guinea pig (*Cavia porcellus*), Big-eared Opossum (*Didelphis aurita*), Big-eared Opossum (*D. albiventris*) e.t.c. due to its size. However, the consumption of RB bait by non-target species like lizards, dogs and cats shows that this method is still viable for characterizing the movement of other small mammals in either urban or rural environment up to 200m distance provided the right trapping device is used. The period and site of our sampling collection might also somewhat limit the generalizability of our result because we only trapped rats during one season of the year. Additionally, given that C1 and C2 were separated by a period of one year, we do not think that C1 might have affected the trapping success recorded in C2 since rat residual population usually recover through reproduction or immigration from a nearby population following a brief period (6 months) of population decline “boomerang effect” (Hansen et al., 2020; Lambropoulos et al., 1999), nevertheless there is a slight probability that C1 might have lowered the trapping success of C2. However, our results show that RB dyed bait is acceptable by small mammals and effective for tracking rodents’ movement in both rural and urban settings. Moreover, while this method is only effective for detecting RB-positive rats for at most four and half months after ingestion of RB dyed bait (Tolkachev 2019; Jacob et al., 2002; Fichet-Calvet 1999), our results provide both new empirical information about rat movement in tropical urban settlements and a proof of efficiency of the method under tropical slum conditions.

Conclusion

We have used RB to illustrate the movement of rats, while showing its efficacy in a difficult urban slum terrain for the first time. Results from our study perhaps showed that this new method: is easy to use, cost effective, produces fast result, is devoid of practices that raise ethical uncertainty of reintroducing potentially infected rats into the study site, free of GPS-signal difficulties associated with conventional methods, and provides a viable alternative to previous conventional methods used in investigating the movement of small mammals. Additionally, the new ecological information provided from this study would be useful when developing future rodent control programs, such as the definition of control units and bait spacing with possible application in pest management and indirect zoonoses control.

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Author’s contributions

AMA, FC & HK conceived the project and all authors participated in its design; AMA, FNS, BIAX, AMB & DCCS performed the field experiments; JOS produced the map; FC provided field equipment and reagents; AMA carried out fluorescence microscopy examination of the samples and drafted the manuscript; FNS, CGZ, BIAX, AMB, DCCS, FC, MB & HK discussed the results and commented on the manuscript at all stages; CGZ, EMS, FC, MB & HK revised the manuscript and EMS, FC, MB & HK supervised the project. All authors read and approved the final manuscript.

Data availability statement

All data and code used in this study are available in Zenodo under Creative Commons 4.0 license, accessible through https://doi.org/10.5281/zenodo.5027554 (Awoniyi, Zeppelini, et al., 2021).

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Supplemental material: Total trapping efforts and trapped Norway rats with signs of RB by distance and campaign

		Campaign one (C1)		
Distance (m)	**Trapping effort**	**No of rats captured**	**No of rats with RB signs**	**% of rats with RB signs**
15	69	6	3	50 %
30	71	5	1	20 %
60	51	14	4	29 %
Total	**191**	**25**	**8**	**32 %**
		Campaign two (C2)		
15	53	3	0	0 %
30	66	4	1	25 %
60	65	3	0	0 %
90	20	8	3	37.5%
Total	**204**	**18**	**4**	**22.2 %**
Grand Total	**395**	**43**	**12**	**27.9%**

Chapter II

Effect of Chemical and Sanitary Intervention on Rat Sightings in Urban Communities of New Providence, the Bahamas

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Effect of Chemical and Sanitary Intervention on Rat Sightings in Urban Communities of New Providence, the Bahamas

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Abstract

Rats are invasive pest species that commonly infest low-income urban environments. Their association with humans constitutes a threat of rodent-borne disease transmission. We evaluated the outcome of a chemical and sanitary intervention on rat sightings in seven low-income urban settlements of New Providence, the Bahamas. The intervention consisted of rodenticide application, education about environmental sanitation, and improvement in waste disposal. Rat sightings were systematically recorded by trained staff before and three months after the intervention. The intervention slightly decreased rat sightings, with an average of 2.7-fold with varied effectiveness across locations. Four out of seven locations (57%) registered a decrease in rat sightings. Our results suggest that social and environmental differences among communities may be responsible for the mixed efficacy observed in the current rodent management practice in urban communities of the Bahamas. However, a new set of control measures needs to be developed for areas where rodent decline was not observed. This study provides novel data on how rat population behaves post-intervention in a unique ecological setting like the Bahamas, presenting an informed judgment for their management especially in the event of a natural disaster.

Keywords: Rat, Rat sightings, Rodenticide, Rodent control, Urban community, Zoonoses

Introduction

Rats (genus *Rattus*) are widely distributed and successful invasive species that are present in all continents except Antarctica [1]. They were likely introduced into North America in the 1750s through trans-Atlantic navigation during the European colonization [2]. They adapt well to human-altered environments and are regarded as notorious agricultural and urban pests [3]. In urban settings, their infestation is often associated with low socioeconomic status, inefficient waste management, open sewers, overcrowding, and other infrastructure characteristics that can be exploited for food and harborage [4].

Frequent rat sightings have been reported as an indicator of poor sanitation and diseases [5]. Rats are famous reservoirs of several infectious diseases (e.g. leptospirosis, toxoplasmosis, lassa fever and other viral hemorrhagic fevers) and play important role in their transmission to humans [6-8]. Besides their health implication, their presence in the islands has led to the destabilization of the ecosystem, competition, and extinction of local fauna [9], in conjunction with the destruction of agricultural produce and household properties worth billions of dollars [10].

The main strategies used for controlling rat infestations have been; application of chemical-rodenticides [6], environmental modification [11] and physical population removal [12]. However, most of these control efforts have yielded limited effectiveness [13], with the added possibility of negative impacts on zoonoses prevalence [14]. Advances are hindered due to the trade-off between techniques. Physical removal is labour and resource-intensive, environmental management is labour-intensive and depends on ecological know-how. Chemical control has an easy application but allows population rebound if resources are available and can act as a selective pressure for neophobia and poison resistance among the remaining population [6,3]. Thus, an effective rodent control program demands evidence-based planning and a multi-scale approach.

Despite the increasing labour force in the Bahamas, the national unemployment rate remains somewhat high (16%) and even higher among the youth (30%), thereby forcing residents to live below the poverty line [15]. In urban communities of New Providence, most of the population live in overcrowded apartments with poorly constructed privies, improper sewage and waste management system [16], thereby providing conditions that encourage human-rat cohabitation [4].

Therefore, given the ecological and geographical composition of the Bahamas and its projected sea-level rise and floodings [17], there are growing concerns about possible rat proliferation and probable rodent-borne disease outbreak in the country. This is probable considering the likelihood of a rodent-borne disease outbreaks following a natural disaster [18]. Previous studies have shown that natural disasters may change the mortality and spatial distribution patterns of rodents, and consequent increase in pathogen prevalence [19]. Therefore, it is critical to evaluate if the ongoing integrated rat management program is effective against probable rat population explosion, especially in the event of natural disaster in the Bahamas. It is based on this that we report our observations of the short-term effect (11 weeks, approximately three months) of a chemical and sanitary intervention hereinafter referred to as "intervention" on rat sightings in selected locations of New Providence, the Bahamas.

Materials and Methods

Study site

The study was carried out in low-income settlements of New Providence Island, the Bahamas. New Providence is located between Andros Island (West) and Eleuthera Island (East) with Latitude N25 04' 00" and Longitude W 77 21' 00". It is a principal island in the Bahamas, majorly of woody vegetation, consisting of shrubs and low trees, with an area of 80 sq mi and a population of 246,329 [20]. Seven settlements tagged locations "A, B, C, D, E, F & G" (figure 1) with a record of regular rat sightings were used. Locations were selected as part of a local program to improve those communities including a transformation and restoration of the area performed in phases. Rodent control described here was one of the initial phases of the program. Each location contains a minimum of 10 blocks, with 5 premises (mostly wooden houses) per block. The locations are interspersed with small businesses and possess environmental/sanitation conditions that could impact rodent intervention efforts e.g. derelict vehicles, garbage accumulation (both regular and discarded materials like furniture), abandoned or unfinished constructions and overgrown properties. Refuse collection in some of the locations is partially satisfactory with limited bulk waste collection in most of the sites.

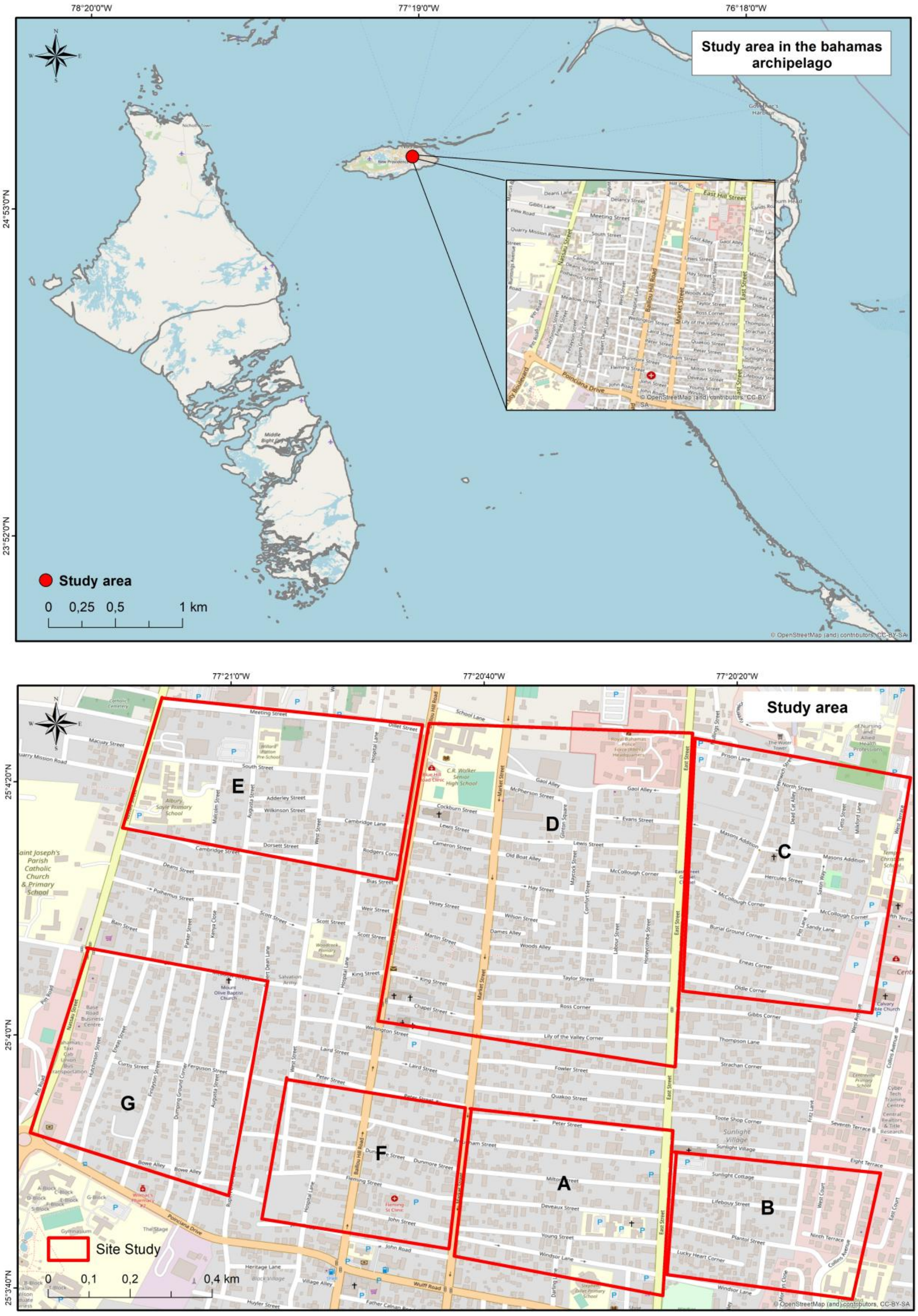

Figure 1 Map of the study locations in low-income communities of New Providence, the Bahamas

<u>Study design</u>

<u>Intervention</u>

We applied an anticoagulant rodenticide (brodifacoum) to infested households, conducted education about environmental sanitation (i.e. enlightened the heads of households, and shop owners about the importance of appropriate waste disposal; putting waste out at collection point on the morning/evening of collection instead of days before collection; keeping pets' food out of reach of rats, and the need to reduce or obstruct other sanitation-related means by which rats could gain access to food and or water) and where applicable damaged/missing trash cans were replaced by the Bahamas Ministry of Environment and Housing. The intervention was performed from 3rd May 2019 to 23rd May 2019. Before the commencement of baiting, we clearly explained the purpose of the intervention to the residents and enlightened them about the importance of practising appropriate waste disposal. Using the United States' Center for Disease Control and Prevention (CDC) [21] guidelines for interior and exterior rodent inspection form, we identified spots suitable for rat baiting at infested properties and areas around the properties in the study locations.

Baiting

We applied brodifacoum-tracking powder to either identified infested properties or areas around the properties following the manufacturer's instructions of placing bait stations in areas with active rodent signs, accessible only to rodents and not to other non-target species. Two bait stations of full holding capacity were placed at each block, visited and replaced once a week for three consecutive weeks.

Evaluation

We slightly modified the method previously used by Promkerd et al. [22] and Walsh [23] to evaluate rat sightings before and three months after the intervention. Rat sightings were recorded in a survey form (supplementary material 1) over the period of 4 hours (3hrs 20mins stationary observation, and 40mins slow-movement observation through previously defined circuits) per day by 2 to 3 trained personnel stationed at each location for three consecutive days. Recordings were done from 7 pm to 11 pm - being the period when rats are most active, except in situations where; there is minimal disturbance during the day, subordinate rats trying to avoid dominant individuals or, rats trying to avoid predation [24, 21]. The pre-intervention evaluation was done between 30th April 2019 to 2nd May 2019, and post-intervention between 17th July 2019 to 19th July 2019 respectively.

Statistical analysis

Comparisons of rat sightings among locations before and after intervention were statistically analyzed using a paired samples Wilcoxon Test with a significance level of 0.05. Also, we ran a Generalized Linear Mixed Models (GLMMs) with Poisson error distribution (log link function) using the Lme4 package to investigate the effect of the intervention with the location as a random factor. All analyses were performed in R 3.6.1 version [25].

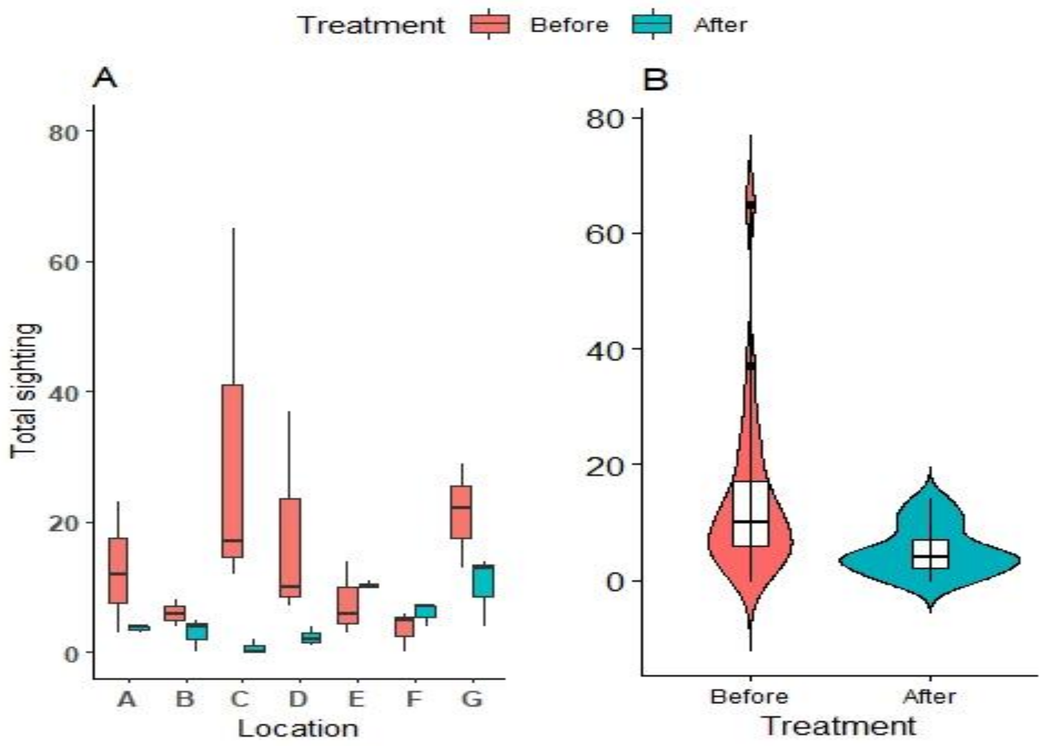

Figure 2 Frequency distribution of rat sightings before and after intervention in low-income communities of New Providence Island, the Bahamas

A- Rat sightings before and after intervention by locations in New Providence

B- Overall distribution of rat sightings before and after intervention in New Providence

Results

With the surveys, we found a noticeable rodent activity across all locations before the intervention, with locations C and D having the highest rat sightings and locations B and F with the lowest sightings (figure 2a). There was an apparent disparity in rat sightings before and after the intervention (figure 2b), with a mean reduction ratio ranging from 0.61 to 46.76 (table 1). At locations A, B, C, D, and G, the mean rat sightings decreased after intervention but increased at locations E and F (figure 2a). Overall, the intervention had a negative effect on the number of rat sightings after intervention as compared to before intervention, with an average reduction of 9 rats in the number of rat sightings (table 2) and a varied level of effectiveness noticed across the sampling locations (table 1). The paired samples Wilcoxon Test illustrated a significant overall mean reduction ratio (2.77:1, P = 0.003), with a non-significant reduction in the mean values of rat sightings at location A, B, C, D and G after the intervention respectively (table 1). On the other hand, at locations E and F, a non-significant increase in the mean values of rat sightings was reported after the intervention (table 1).

Table 1 Variations in rat sightings before and after intervention in urban communities of New Providence Island, the Bahamas

	Before intervention		**After intervention**			
Location	**Mean (Std)**	**Median**	**Mean (Std)**	**Median**	**Mean Reduction ratio**	***P-value***
A	12.67 ± 10.01	12.00	3.66 ± 0.57	4.00	3 .46 : 1	0.142
B	6.00 ± 2.00	6.00	3.00 ± 2.64	4.00	2.00 : 1	0.054
C	31.33 ± 29.26	17.00	0.67 ± 1.15	0.00	46.76 : 1	0.054
D	18.00 ± 16.52	10.00	2.33 ± 1.52	2.00	7.73 : 1	0.054
E	7.66 ± 5.68	6.00	10.33 ± 0.57	10.00	0.74 : 1	0.857
F	3.66 ± 3.21	5.00	6.00 ± 1.73	7.00	0.61 : 1	0.945
G	21.33 ± 8.02	22.00	10.33 ± 5.50	13.00	2.06 : 1	0.089
Total	14.38 ± 14.87	10.00	5.19 ± 4.22	4.00	2.77 : 1	**0.003**

Table 2 Summary of the generalized linear mixed effects model showing rat sightings before and after intervention in urban communities of New Providence Island, the Bahamas

	Rat Sightings		
Predictors	*Estimates*	*CI*	*p*
(Intercept)	14.38	9.61 – 19.15	**<0.001**
Before intervention	*Reference*		
After intervention	-9.19	-15.75 – -2.63	**0.006**

Discussion

The intervention caused on average a 2.8-fold overall significant decrease in rat sightings with a mean reduction ranging from 0.61 to 46.71 amid variations in effectiveness among sampling locations. The high rat sightings before intervention are not surprising, given the availability of factors that support the proliferation of rats in the study locations. Therefore, these high rat sightings may be associated with the residents' socioeconomic conditions, with most of them living in impoverished conditions [26]. The differences in the effectiveness of the intervention across locations and the broad margin in the mean reduction ratio in rat sightings may be due to differences in features and management practices among these locations.

The reduction in rat sightings (although non-significant) noticed after intervention at four out of the seven locations suggests that socio-environmental attributes (specifically education level and fair waste management) in these locations are suitable for the type of intervention currently employed in the communities. However, the lower number of rat sightings recorded at locations E and F before the intervention may account for the non-significant increase noticed after the intervention. Likewise, the non-significant decrease of rodent sightings at location A might be due to the lower socio-environmental conditions of the residents i.e. poor standard of living, waste management and education level (data not shown), which may provide extra nourishments, thus limiting the effectiveness of the intervention [27].

Rat population may peak shortly after a brief decline immediately after natural disasters [28]. As a result, given the ecological setting of New Providence, an island with shallow and warm water that is prone to hurricanes and flooding, it would be impracticable to clean up waste in the event of natural disaster, thus bringing about conditions that are favourable to rodent proliferation and probable pathogen prevalence [29-30]. Hence, it is critical to evaluate local strategies used for regular rodent control that can potentially be used to deal with the potential rat population increase in the event of natural disasters.

Therefore, although the effectiveness of the intervention differs across the study locations, our result suggests that it provides an immediate means of controlling the rat population in most of the communities, especially those with modest socio-economic status. This outcome will potentially be helpful in this unique ecological setting like the Caribbean during natural disasters. However, it would be necessary to develop a new set of control measures for areas where rodent decline was not observed. Additionally, if a long-term management plan is to be guaranteed, it would be indispensable to conduct a longer-span study to better understand how rodent populations behave at longer timespans (six months to one year after intervention).

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Declarations

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Conflict of interest

The authors declare that they have no competing interests

Ethical approval

NA

Consent to participate

NA

Availability of data and Code

The datasets and codes used during the current study are available from the corresponding authors on reasonable request

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Chapter III

Population Dynamics of Synanthropic Rodents after a Chemical and Infrastructural Intervention in an Urban Low-income Community

Article submitted to Scientific Reports

Population dynamics of synanthropic rodents after a chemical and infrastructural intervention in an urban low-income community

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Abstract

Synanthropic rodents are ubiquitous in low-income communities and pose risks for human health, as they are generally resistant to control programs. However, few or no studies have evaluated the long-term effect of chemical and infrastructural interventions on rodent population dynamics, especially in urban low-income communities, or evaluated the potential recovery of their population following interventions. We conducted a longitudinal study in a low-income community in the city of Salvador (BA, Brazil) to characterize the effect of interventions (chemical and infrastructural) on the dynamics of rodent population, and documented the post-intervention recovery of their population. We evaluated the degree of rodent infestation in 117 households/sampling points over three years (2014 – 2017), using tracking plates, a proxy for rodent abundance/activity. We reported a significant lower rodent activity/abundance ten months after the chemical and infrastructural interventions ($Z=-4.691$ ($p<0.001$)), with track plate positivity decreasing to 28% from 70% after and before interventions respectively. Therefore, the combination of chemical and infrastructural interventions significantly decreased the degree of rodent infestation in the study area. In addition, no rodent population rebound was recorded until almost a year post-intervention, and the post-intervention infestation level did not attain the pre-intervention level all through the study area. Moreover, among pre-treatment conditions, access to sewer rather than the availability of food was the variable most closely associated with household rodent infestation. Our study indicates that Integrated Pest Management (IPM)-approaches are more effective in reducing rodent infestation than the use of a single method. Our findings will be useful in providing guidance for long-term rodent control programs, especially in urban low-income communities.

Keywords: Integrated Pest Management (IPM), Low-income communities, Rat infestation, Rodent control, Rodenticides

Introduction

Rodents are highly adaptable animals, capable of colonizing human-altered environments, and can rapidly establish large populations where abundant resources are available, e.g. in urban low-income communities[1]. Synanthropic rodents are considered pests given their negative impacts on human health and economy[2-4]. Specifically, rats are reservoirs for several important viral, bacterial and parasitic diseases, which have likely caused more human casualties than wars[5-9]. Likewise, rodents destroy and contaminate agricultural products and infrastructure worth billions of dollars per year, while their sightings affect residents' mental health[9].

Urban low-income communities typically provide the environmental conditions that promote the proliferation of rodents[10-12], which in turn complicates the management of their populations in these environments[13]. For instance, inadequate housing and sanitation (e.g. open sewers) and lack of adequate garbage collection services promote rodent presence[12], and these are expected to worsen globally given the expected increase in the population of low-income community dwellers from the 751 million recorded in 1950 to more than 3 billion by 2050[14]. Also, it is estimated that 1 in every 4 persons will either reside in informal settlements or require adequate and affordable housing by 2030[15]. Therefore, urban population increase will likely stress the local infrastructure further, especially in developing and under-developed countries, thereby leading to higher rodent proliferation and thus, more frequent human-rodent interactions.

Rodent control is one of the main public health measures implemented in urban poor communities, mainly to reduce the risk of disease transmission[3]. The methods applied are mainly chemical, using rodenticides with immediate short-term effects in the population[3,5,16]. However, several other interventions can be implemented simultaneously for longer-lasting effects. Non-chemical methods include closing of open sewer canals and regular solid waste collection[17]; hunting, trapping, capturing and other physical removal methods[18,19]; movement barriers and environmental modifications (e.g. electric fencing, pavement) [20,21]; likewise biological control using pathogens or predatory animals[22-26] . The paradigm of Integrated Pest Management (IPM) that recommends the use of several methods together has been adopted in several urban centers, with mixed results[27].

Rodent infestations are difficult to control and/or manage, since the surviving population can rapidly recover through reproduction[28] or immigration[6]. Following a period of population decline, rats can travel up to at least 90 meters even in a heterogeneous urban environment[23, 29, 30], and repopulate the area. Rodent control is especially difficult in urban environments due to the heterogeneity of the urban environment and because most control methods have been developed for rural or agricultural settings. Non-chemical methods also tend to have practical setbacks; for example, physical removal of rodents is time and labour-intensive, environmental modifications are expensive and labour-intensive, while electric fencing and inhibition of reproduction are non-species specific and could affect humans and other non-target species alike. As a result, lethal chemical agents remain the main method employed during rodent control programs in urban areas[31,32]. Rodenticides are somewhat effective in controlling populations three months post-intervention[10,33]. However, the long-term efficacy of a combination of chemical and infrastructural interventions on the dynamics of rodent populations, as well as the time lag between control deployment and the onset of rodent repopulation, are not clear.

Here, a case-control study was performed in Pau da Lima, an urban poor community in the periphery of Salvador, Brazil[34]. Pau da Lima consists of four valleys with similar environmental conditions that support relatively large rodent populations[11]. Three valleys (1, 2 and 4) with apparent signs of rodent infestation out of the four valleys were used for this study. The three valleys had concurrent infrastructural and chemical interventions. However, while the infrastructural intervention took place across all valleys, chemical intervention occurred only at valleys 1 and 4 (hereby referred to as the treatment valleys), while valley 2 received no chemical intervention and served as the control valley.

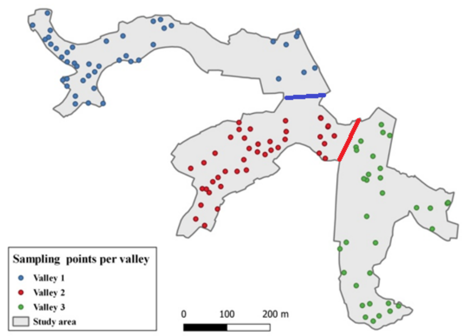
For the first time, we followed up with the trends in rodent infestation before and after interventions over a long-term period even in a tricky urban terrain such as Salvador, and evaluated 1) whether the combination of chemical and non-chemical interventions is indeed effective in the long-term control of rodent populations; 2) the timeline potentially required by the rodent population to return to their initial population abundance after interventions; and 3) the environmental and peri-domestic factors that are associated with rodent population rebound in the three valleys. Therefore, unlike previous studies[32,33] that only evaluated rodent infestation before and six months after intervention, this study offers a more comprehensive longitudinal study on how rodent populations respond to chemical and non-chemical interventions in a complex urban environment such as Salvador.

Methods

Study area

Pau da Lima (13°32′53·47″ S; 38°43′51·10″ W), is a low-income urban community (area. 0.17 km^2) with about 128,997 inhabitants[35]. The community is characterized by inadequate housing facilities, poor basic social amenities, unsatisfactory sanitation system i.e. open sewers, improper garbage disposal and deficient garbage collection services with a history of household rodent infestation[11]. We conducted our study in three valleys (valleys 1, 2 & 4) that have been previously described by Panti-May et al. [1] (Fig. 1A).

A



B

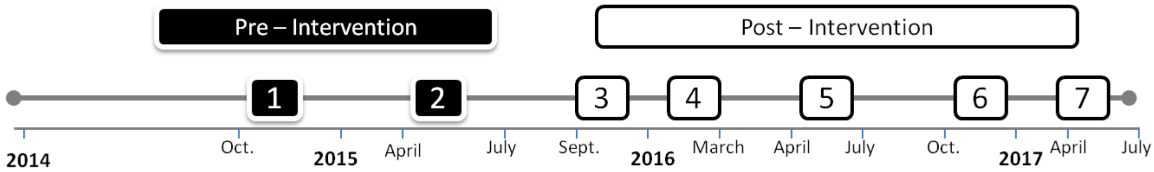


Figure 1: A) The distribution of the sampling points in the valleys (1, 2 & 4) used in Pau da Lima. B) Breakdown of the study timeline for rodent infestation surveys from campaign 1 to 7.

Sample size and frequency

One-hundred and twenty sampling points were randomly selected from the longitudinal study monitoring the urban rodents in Salvador, Brazil. However, we excluded 3 sampling points due to inaccessibility. These 117 points were randomly selected from the three valleys (Fig. 1A), with 46 sampling points from valley 1, 38 from valley 2 and 33 from valley 4, respectively.

The longitudinal sampling occurred over three years (2014 – 2017), and the surveys were divided into a total of 7 campaigns ("C1" through "C7") in all valleys, with each campaign lasting 3-4 months as shown in figure 1B. Also, the surveys were divided into two phases, pre-intervention (C1 & C2) and chemical & infrastructural - post-intervention (C3 – C7) (see Fig. 1B).

Interventions

Chemical intervention Valleys 1 and 4 served as the treatment and received chemical intervention (rodenticides), while valley 2 was considered as the control and received no chemical intervention. Using the United States' Center for Disease Control and Prevention, CDC[36] guidelines for interior and exterior rodent inspection, we identified, through rodent signs such as burrows, trails, or feces, active rodent spots at sampling points in both valleys 1 and 4. At each sampling point, trained staff of the Center for Control of Zoonoses (Centro de Controle de Zoonoses - CCZ) applied anticoagulant rodenticides (paraffin blocks and contact powder) within households and in peri-domestic areas in early September 2015 (i.e. first week of September), shortly before the commencement of the first post-intervention campaign (C3) which started in the second week of September through November 2015 respectively. Before the initiation of the intervention, residents were duly informed of the purpose of the study and the necessary procedures required in dealing with rodents during the study period.

Also, all residents of the three valleys were educated about the importance of using appropriate waste disposal; putting waste out at collection points on the morning/evening of collection instead of a few days before collection; keeping pets' food out of rodents' reach, and the need to reduce or obstruct other sanitation-related means by which rodents could gain access to food and/or water.

The formulations used for the chemical intervention were: paraffin block (brodifacoum 0.005%) and contact powder (coumatetralyl 0.75%) respectively. Brodifacoum is a second-generation rodenticide that requires just a single dose while coumatetralyl is a first-generation rodenticide that requires multiple exposures. The paraffin blocks were applied chiefly in humid places, for example, sewers and leakages around houses, while contact powder was used in identified rodent trails and burrows that are chiefly accessible only to rodents and not non-target species.

Environmental/ infrastructural intervention All valleys had infrastructural interventions accomplished by the city administration, that is, modification of open sewers (Fig. 2A, B & C) and the construction of public squares (common paved spaces that are free of garbage and water puddles) and pavements, both roadways and sidewalks (Fig. 2C & D), between early 2014 to the middle of 2015 which reduces rodents' sources of food (debris from standing water) and harbourage (burrow).

Figure 2: A & B) An open sewer between households in the study area, C & D) The study area undergoing environmental modification i.e. channelling of the drainage into a major junction, and the construction of community square, roadway and sidewalk.

Evaluation

Track plates (TPs) This method has been previously validated as a proxy for rodent infestation and comprehensively described by Hacker et al.[37] and Eyre et al.[38]. Weather-resistant lampblack was applied to 0.2x0.2m acetate sheet plates using a paint roller. The acetate plates dry off quickly (< 5 minutes) and allow detection of different types of marks left by rodents such as paw prints, tail marks and scratches. All TPs with any of these marks were recorded positive for rodent infestation. A total of 5 TPs were placed in a cross pattern for 2 nights at each sampling location throughout the campaigns.

The TPs were checked the morning after placement, identified by sampling location and photographed. Upon assigning 5 x 5-cell grids over each photograph, making 25 data points per plate, TPs were examined and scored by two independent trained examiners for rodent marks. Score discordances of ≥3 cells between examiners were flagged and reviewed to achieve a consensus.

Environmental evaluation Rodents' access to water and food sources and harbourage for rodents were recorded at each sampling point by trained CCZ personnel using a modified US' CDC guidelines for interior and exterior rodent inspection forms[36]. Likewise, other information such as house ownership status and the presence of pets were also recorded at each sampling point.

Statistical analysis

To evaluate the expected decline and potential rebound in rodent population after the interventions, we used generalized linear mixed effects mode (GLMMs) with logistic link and binomially distributed error structure. Our response variable at each location and campaign was coded as 1 if at least one of the TPs at a given location was positive for rodent signs, or else coded as 0. We treated valley and campaign as fixed effect factors and location as a random effect.

Before testing for a change in probability of rodent infestation (using TP positivity as a proxy) after interventions, we controlled for environmental variables that influence rodent infestation. We first used separate GLMMs to test for the relationships between the response variable and each of the following explanatory variables: presence of mud; soil & vegetation; number of domestic animals; access to garbage, water & food; proximity to sewer; and harbourage access i.e. presence of construction materials. Variables with p-values of ≤ 0.15 from the single factor models were included in a provisional multi-factor model, since opting for the more conventional level of 0.05 at this stage could fail to identify all the important variables[39]. The provisional model also included the interaction between campaign and valley to allow for any effects of interventions to vary across valleys. A mixed forward and backward stepwise model selection approach was used to determine the final model using the Akaike Information Criterion (AIC). We chose the most parsimonious model with $\Delta AIC < 2$ compared to the minimum as the final model[40].

Within the final model, we tested the overall significance of any intervention using the Z-statistic $C/SE(C)$, where C is the contrast between pre-intervention and post-intervention campaigns, i.e. average of the estimated main effects for campaigns 1 and 2 minus the average of the estimated main effects for campaigns 3 to 7, and $SE(C)$ is the standard error of *C*. Similarly, we used a "difference of differences" contrast to test whether the size of the difference between pre-intervention and post-intervention campaigns differed significantly between the chemically treated valleys, 1 and 4, and the control valley, 2.

All analyses were performed in R version 4.1.1[41], using the lme4 (nAGQ = 9) [42] and MuMIn (v1.43.17)[43], ggpubr (v0.4.0)[44], packages.

Statement of ethical approval

While there was no direct dealing with any live animal throughout this study, as rat activity/infestation level was obtained through indirect method using track plate. However, the Ethics Committee on the Use of Animals in Research (CEUA-CPqGM) of the Fundacao Oswaldo Cruz, Centro de Pesquisas Goncalo Moniz, Salvador- Bahia, Brazil gave the approval and permission to conduct research on rats with Project Numbers: 003/2012 and 019/2016. All participating residents (interviewees) involved in the environmental aspect of the study gave their informed consent before participation, and there was no interviewee below the age of 18.
The entire study was conducted in accordance with the Brazilian laws regarding ethics in research.

Results

Population fluctuations across campaign per valley

We recorded rodent activity across both treatments pre and post-intervention (Fig. 3). The Z-statistic to test the overall significance of any intervention was Z=-4.691 ($p<0.001$) corresponding to significantly lower rat activity post-intervention. The Z-statistic to test the difference between the effects of the interventions with and without chemical treatment was Z=-2.649 ($p=0.008$), corresponding to a significantly smaller pre-intervention vs post-intervention difference in the valleys that were chemically treated. All three valleys, but especially the chemically treated valleys (1 &4) had higher signs of rodent infestation before intervention than after intervention, with mean track plate positivity decreasing from 70% to 28% before and after interventions respectively. The overall peak infestation levels were recorded during the pre-intervention campaigns (Supplemental material 1), with a varied infestation patterns in C1 and C2 followed by a broad decline from C3 to C5 in valley 1 and 4, and a similar decline from C4 to C5 in valley 2 and then a somewhat increases from C5 to C7 across all valleys. The broad decline observed in the infestation level from C3 to C5 lasted for 10 months (Fig. 3). Also, the valley that received no chemical intervention showed the highest positivity level throughout the span of the study (Fig. 3 and Table 1), while the treatment valleys (valleys that received chemical intervention) rarely exceeded 40% positivity at any given time, especially after the intervention. A GLMM with the interaction between valley and campaign indicated that C5 (lowest infestation level) and C6 (onset of rodent repopulation, especially for valley 2) presented statistically different infestation level as compared to the remaining campaigns (Table 1). Also, campaigns 2, 3 and 5 in valley 2, and campaigns 3, 5 through 7 in valley 4 had a statistically different rodent infestations level from the other observations (Table 1).

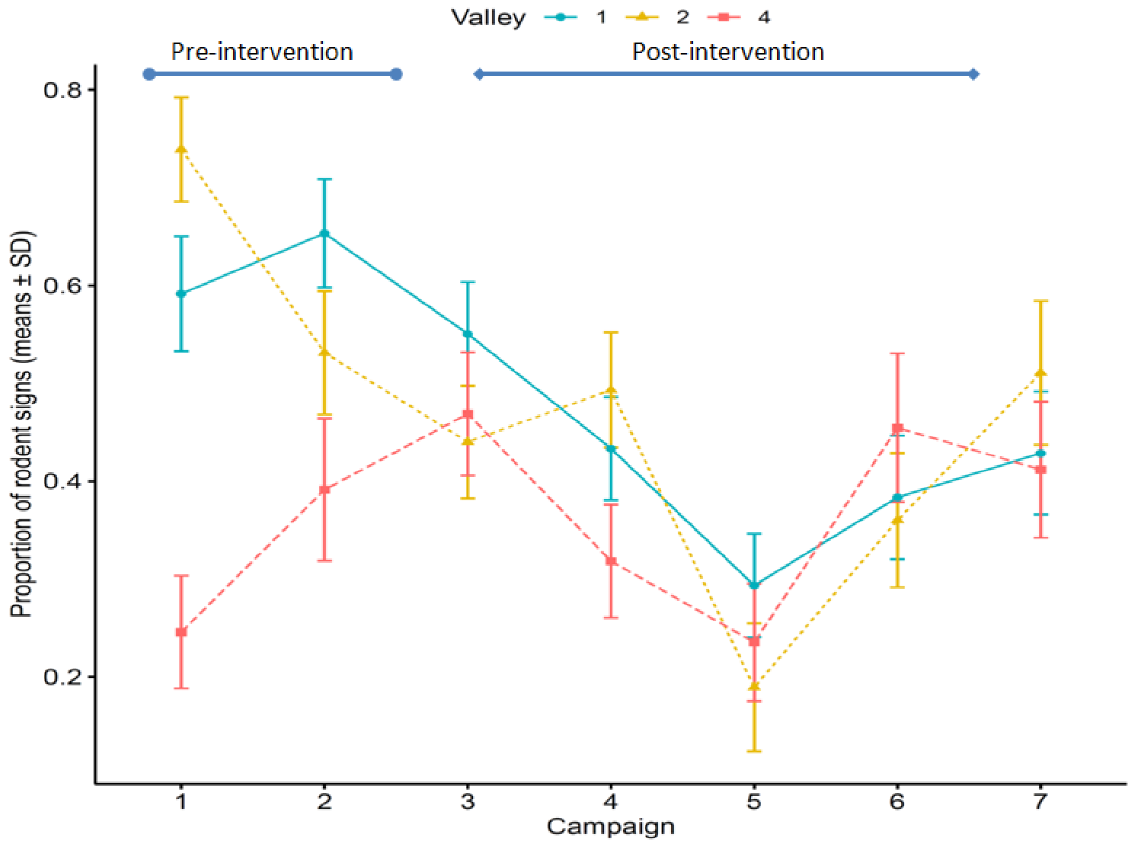


Figure 3: Mean with standard deviation of rodent infestation by campaign and valley (valley 1 – blue, valley 2 – yellow & valley 4 –red colour respectively)

Table 1: Summary of the generalized linear mixed-effects model with rodent infestation associated variables, and the interaction between valley and campaign to determine the recovery rate of rodents after the interventions.

	Plate positivity		
Predictors	*Odds Ratios*	*CI*	*P*
(Intercept)	0.65	0.30 – 1.42	0.278
Valley [2]	1.83	0.76 – 4.39	0.176
Valley [4]	0.18	0.07 – 0.45	**<0.001**
Campaign [2]	1.31	0.63 – 2.74	0.473
Campaign [3]	0.81	0.40 – 1.65	0.564
Campaign [4]	0.43	0.21 – 0.89	**0.023**
Campaign [5]	0.21	0.10 – 0.46	**<0.001**
Campaign [6]	0.28	0.13 – 0.63	**0.002**
Campaign [7]	0.36	0.17 – 0.79	**0.011**
Access to Sewer [Yes]	1.43	1.02 – 1.99	**0.036**
Presence of Vegetation [Yes]	1.90	1.09 – 3.28	**0.022**
Presence of Mud [Yes]	1.16	0.83 – 1.62	0.387
Access to Garbage [Yes]	1.45	1.05 – 1.99	**0.023**
Valley [2] * Campaign [2]	0.27	0.09 – 0.80	**0.019**
Valley [4] * Campaign [2]	1.60	0.48 – 5.34	0.442
Valley [2] * Campaign [3]	0.28	0.10 – 0.80	**0.017**
Valley [4] * Campaign [3]	3.47	1.15 – 10.45	**0.027**

Valley [2] * Campaign [4]	0.65	0.23 – 1.85	0.420
Valley [4] * Campaign [4]	2.96	0.97 – 9.07	0.057
Valley [2] * Campaign [5]	0.22	0.06 – 0.85	**0.028**
Valley [4] * Campaign [5]	3.85	1.12 – 13.24	**0.033**
Valley [2] * Campaign [6]	0.44	0.13 – 1.44	0.174
Valley [4] * Campaign [6]	9.80	2.82 – 34.05	**<0.001**
Valley [2] * Campaign [7]	0.86	0.27 – 2.76	0.799
Valley [4] * Campaign [7]	5.91	1.80 – 19.46	**0.003**
Random Effects			
σ^2	3.29		
$\tau_{00\ Location}$	0.74		
ICC	0.18		
$N_{Location}$	117		
Observations	1317		

Factors associated with plate positivity

Seven variables (valley, campaign, treatment, presence of mud, & vegetation, access to garbage and sewers) with p-values of ≤ 0.15 from the initial analysis were considered for the generalized linear mixed-effects model (Supplemental material 2).

The final model retained six of these seven variables (valley, Campaign, mud, vegetation, sewers and access to garbage) with an AIC of 1661.8. The next best model had the same □AIC, but retained the entire seven variables. Presence of vegetation, access to garbage and sewers were positively associated with plate positivity-rodent infestation (Table 1). Additionally, from the table 1 above, the fully treated valley-valley 4 showed an overall significant reduction in infestation level after interventions

(OR: 0.18 [0.07 - 0.45], p=0.001). Likewise, there was dissimilarity in infestation level between the treatment and control valleys before and after intervention, with valley 2 (control) having a significant lower infestation level during C2 (OR: 0.27 [0.09 - 0.80], p=0.019), and valley 4 recording a non-significant p=0.057 (C4) and significant p=0.033 (C5) sustained reduction in infestation level (Table 1) which also conforms with Fig. 3 above.

Discussion and Conclusion:

We reported a long-term decline in rodent infestation after interventions, which is different to previous studies[32,33] that only evaluated the rodent infestation level before and after intervention via a two sampling campaigns. Here, for the first time, we were able to systematically track the continuous dynamics in urban rodent infestation for a period of three years (the most comprehensive longitudinal study to our knowledge) and then describe any associated rodent repopulation even in a complex heterogeneous urban terrain like Salvador. We reported the onset of the population rebound around 10 months post-intervention, specifically commencing after C5 across the three valleys. Also, the fully treated valleys did not return to the pre-intervention infestation levels throughout the span of the study. Therefore, the effectiveness of the interventions and the time required for the onset of rodent repopulation in our study is longer than those previously reported by de Masi et al.[32] in São Paulo, Brazil, and Lambropoulos et al.[33] in Baltimore, USA, who both report that rodent repopulation take around 6 months to return to pre-intervention levels, using just two campaign regimes (before and after interventions) to evaluate rodent infestation levels.

We reported high rodent infestation before intervention in the three valleys, although with a sharp pre-intervention decline in valley 2 (that is C1 – C2) as against the other valleys which could be due to natural fluctuation in population or differences in micro-environmental attributes of the valleys[2]. The high rodent infestation noticed pre-intervention might be due to the impoverished socioenvironmental conditions of the study area such as open sewage, improper garbage disposal and deficient garbage collection services and even overcrowded apartments, particularly before the interventions. These conditions have been reported to provide sufficient water, food and harbourage sources that encourage the onward proliferation of rodents in any given environment[1,11,32,45,46].

Over the years, the main strategy used for household rodent infestation management has been the application of chemical rodenticides. Although this method is relatively easy to execute, it requires consistent investment and has a limited success-rate, with the added risk of inadvertently increasing rodents' chances of contracting zoonoses[47], while allowing population rebound and selective neophobia resistance among the residual population[3,48]. However, the overall significant reduction seen in rat infestation post-intervention (Z-statistics), and reduction in the degree of infestation recorded in C4 and C5 across all valleys suggests that chemical and infrastructural interventions evaluated here may be critical in reducing rodent infestation. Nevertheless, this might not be an absolute solution to the problem, considering there was a significantly smaller reduction in plate positivity post-intervention in the chemical treated valleys, than the chemical control valley (Z-statistic for testing the difference in the effect of intervention across valleys), which might be due to varying degree of environmental confoundings across the valleys. However, the inability of the valleys to completely attain the pre-intervention infestation level after the chemical and infrastructural interventions substantiates the fact that IPM-approaches are more reliable in the long-term management of rodent infestation than the application of a single control method[49-51].

Looking more closely at the combination of chemical and environmental control, a considerable reduction in rodent infestation was observed after the chemical intervention (C3 and beyond); similarly, we observed a substantial U-shaped recovery from the lowest infestation point (C5) across all the study valleys (Table 1, Fig. 3). This seems to indicate that chemical intervention alone may not be sufficient for controlling rodent infestation especially in a highly rodent infested environments. This contrasts with previous findings in the Bahamas[10], where a reduction was observed in the rodent infestation proxy (sightings reported by locals). However, the difference in the nature of the response variables used in the studies might affect and hinder comparisons, particularly given the limitations of using rodent sightings to estimate infestation level. In contrast, the significant reduction in rodent infestation observed in valley 2 before intervention and during infrastructural intervention C3 could indicate environmental modification as an important component in rodent infestation management. The eventual population recovery reported in valley 2 also indicates that environmental intervention alone is aswell not sufficient for controlling rodent populations in urban settings[52]. Nevertheless, it reduces the carrying capacity of the environment by denying rodents the access to resources[13].

Based on our model, in addition to the pre-treatment conditions, household accessibility to sewer had the highest association with rodent infestation level which may be as a result of the rodents being able to obtain their major source of nourishment from the sewer. Additionally, households with close proximity to sewers had higher percentage of rodent infestation (Supplemental material 3a). Brooks[53] puts that rodents flourish in this type of environment since it provides them with water and garbage that are daily thrown into the sewer.

Likewise, other factors reported as independent risk factors for rodent infestation were the presence of mud, vegetation and access to garbage (Supplemental material 3b-d) as these provide an alternative food source for rodents[46]. These conditions are similar to those earlier reported to be positively associated with rodent infestation by Costa et al.[49] except for the mud. Nevertheless, the pre-treatment condition of valley 4 being the lowest independent risk factor associated with rodent infestation in this study might signify that the valley has a relatively better socioeconomic condition or practice better hygiene than the other valleys, thereby lowering rodents' source of food, water or harbourage.

Although we were able to collate practical data on rodent infestation in a longitudinal study divided over 7 campaigns for almost three years in a difficult urban environment, we were unable to survey a complete control valley (a valley without chemical and infrastructural intervention). Our original plan was to compare three independent valleys with two valleys having both chemical and infrastructural intervention and the other without any intervention. However, our original research plan was altered since the city administration decided to implement an infrastructural intervention in all the three valleys. While our result is useful in shaping the holistic impact of chemical and structural interventions both as a separate entity and a whole, further investigations using a completely negative valley (without any form of intervention) should yield a clearer result.

In conclusion, our result shows that the combination of a chemical and infrastructural intervention is a more reliable and lasting way of solving rodent problems than just the application of a single method. These findings should be useful in guiding the policymakers, non-governmental organizations, ecologists, rodent pest control organizations and others interested in rodent control on effective long-term rodent control programs. Lastly, the incorporation of this approach should assist in combating the proliferation of rodents in the urban low-income communities, while also aiding the indirect control of vector-borne zoonoses in these urban low-income environments.

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Data availability statement: All datasets and codes used during this study are available in Zenodo under Creative Commons 4.0 license, accessible through https://doi.org/10.5281/zenodo.5796022 (Awoniyi et al., 2021).

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Formal analysis: A.M.A., F.N.S., C.G.Z., P.J.D., F.C. & H.K.

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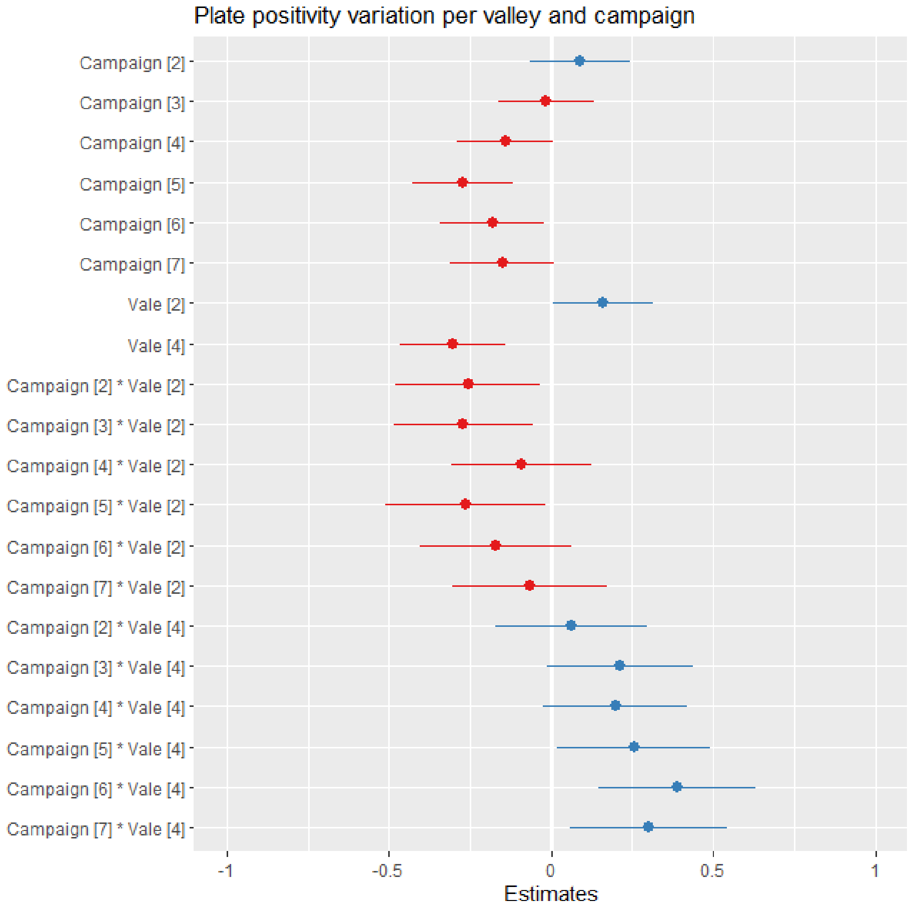
Supplemental materials

Supplemental material 1: The variation of rodent infestation (plate positivity) per valley and campaign

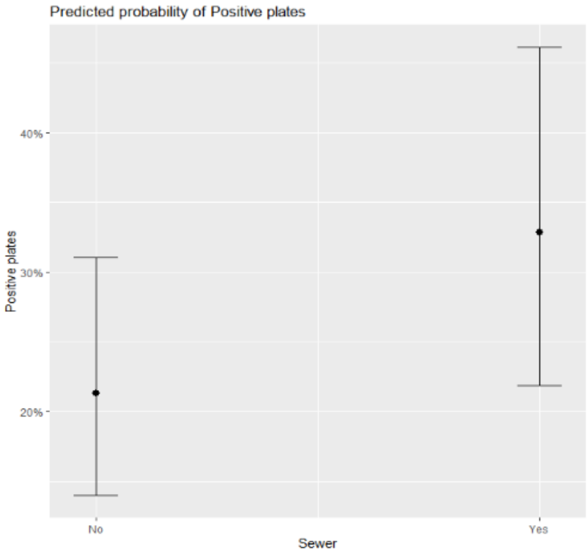


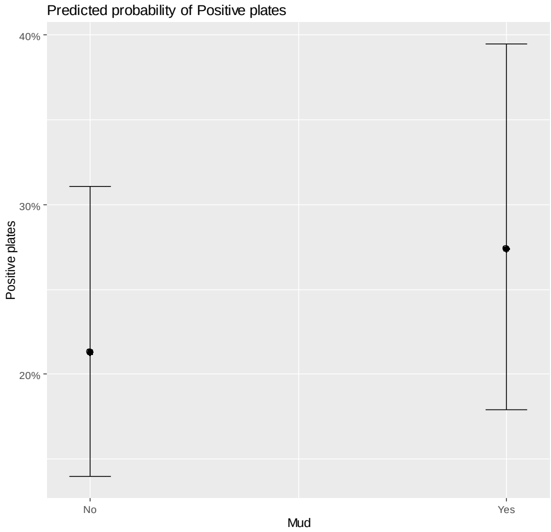
Supplemental material 2: Summary of the bivariate analysis for variables selection in the general model with the variables description and *p*-values

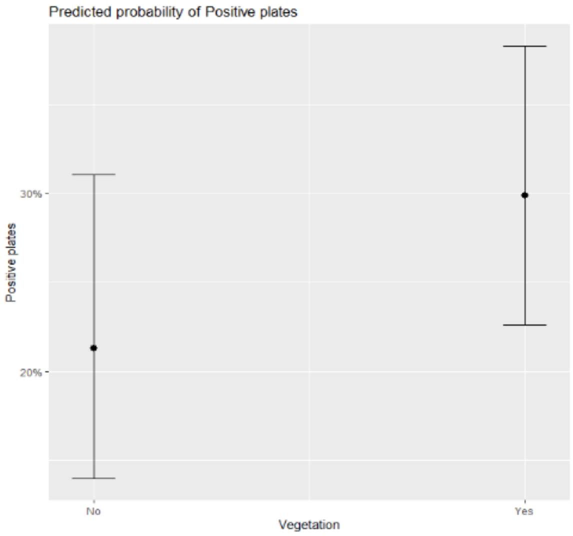
S/N	Variable	Description of variable	p-value
1.	Valley	Categorical, one of the valleys where the experiment was conducted	***0.0279***
2.	Campaign/Treatment	Categorical (pre & post-intervention). Period the campaign was conducted	***8.23e-07***
3.	Mud	Presence or absence (1 or 0). Whether there was mud ≤ 20m of the sampling point	***0.009222***
4.	Soil	Presence or absence (1 or 0). Whether there was exposed soil ≤ 20m of the sampling point	*0.4473*
5.	Vegetation	Presence or absence (1 or 0). Whether there was vegetation ≤ 20m of the sampling point	***0.001757***

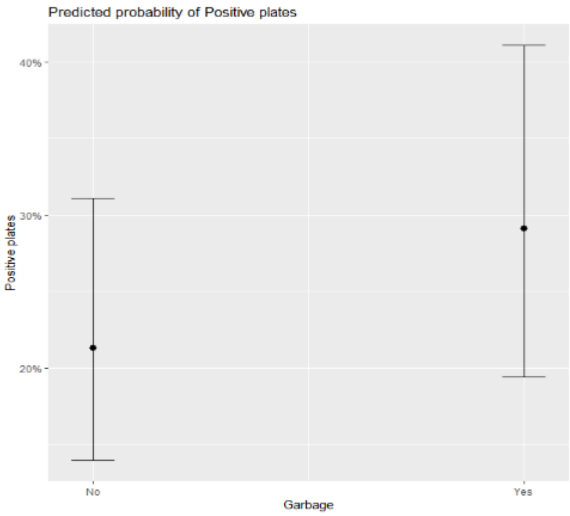
6.	Dogs	Presence or absence and counts. Whether the sampling point (household) has dogs and the number	*0.253*
7.	Chicken	Presence or absence and counts. Whether the sampling point (household) has chicken and the number	*0.878*
8.	Garbage	Categorical (Yes or No). Whether there was exposed garbage ≤ 20m of the sampling point	***1.67e-05***
9.	Water	Presence or absence (1 or 0). Presence of water access ≤ 20m of the sampling point	*0.413*
10.	Food	Presence or absence (1 or 0). Presence of food access ≤ 20m of the sampling point	*0.9370*
11.	Sewer	Presence or absence (1 or 0). Presence of sewer access ≤ 20m of the sampling point	***0.002351***
12.	Construction materials	Presence or absence (1 or 0). Presence of construction materials ≤ 20m of the sampling point	*0.4771*
13.	Debris	Categorical (Yes or No). Presence of debris ≤ 20m of the sampling point	*0.21949*

Supplemental material 3: Prediction of rodent dynamics by the significantly correlated variables









General conclusions

Understanding rat movement patterns within or/and around households in low-income urban communities is a step in planning efficient interventions. This study adapted and tested an effective and reliable method (using Rhodamine B - RB) in low-income urban areas. Using Rhodamine B – RB proved suitable for evaluating the movement of rodents in urban communities without the concern of releasing or re-introducing infected rodents into the community, and we here found that habitat complexity/heterogeneity did not impede rat movement. Indeed, despite the differences in availability of food, water, and harbourage, rats travelled at least 90 m between trapping sessions.

Further, we described another component of rat ecology that is highly relevant for effective control, namely, how rat populations respond, short - and long- term, to interventions (chemical, sanitary and infrastructural). Using systematic observation of rodent sightings before and three months after chemical and sanitary interventions (short-term reaction), the study presented a small decrease in the number of rodent sightings after the intervention, with a variation in effectiveness across sampling locations. In Salvador, Brazil, we found that rodent infestation decreased after chemical and infrastructural interventions and remained substantially lower three years later (long-term reaction). Specifically, after an initial substantial decrease in rodent infestations, rat population rebounded but never attained the pre-intervention levels across all sampling locations.

Therefore, we conclude that RB is a viable, cheaper and safer method for tracking rodent movement even in complex urban environments, and also that, although chemical intervention alone offers a cheap and easy method of controlling rodents, however, its overall effectiveness is short-lived. As a result, we advocate that a site-specific integrated approach system that incorporates both chemical and non-chemical methods of rodent control should be considered when planning rodent management programs to obtain a sustainable long-term result. Lastly, results from this project will be useful in guiding the policymakers and others involved in rodent management to formulate a more comprehensive approach for managing the rodent population even in low-income urban environments, while also abating the risks of rodent-borne infections that are associated with the rodent pest.

ANNEXES

Annex one

Co-authored Articles (1)

Article published in Vector borne and zoonotic diseases

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Increased rat sightings in urban slums during the COVID-19 pandemic and the risk for rat-borne zoonoses

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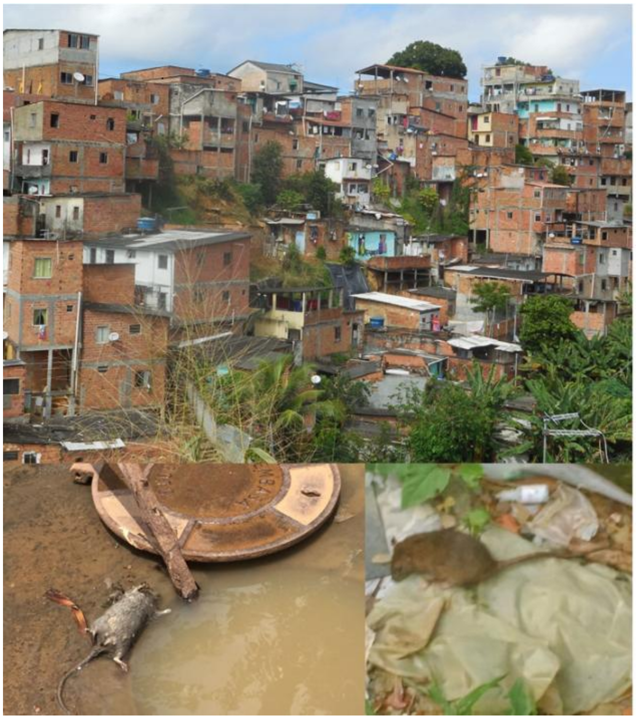
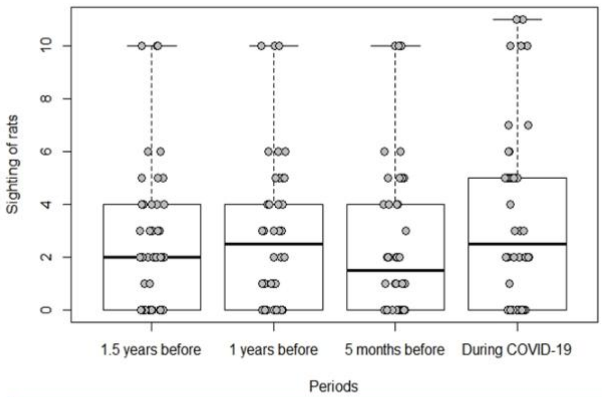
Keywords: COVID-19 pandemic; urban slums; rats

Manuscript

Social isolation has been implemented in many countries around the world to reduce the impact of COVID-19 during the 2020 pandemic. Although, this measure is critical in preventing the spread of the virus, the initiative has lead to rodent-human behavioural changes, resulting in health, economic and social impacts (Corburn et al., 2020; Heymann & Shindo, 2020; Battersby et al., 2008). Globally, an increase in rodent related problems has been reported in various media outlets by experts during the pandemic. In low and middle income countries (LMIC) such as Brazil, where a large proportion of urban residents live in slum communities, with substandard water, sanitation and hygiene coverage, social isolation seems to have provided an ecological opportunity for rodents' expansion, and any increase in the population of rodents during the social isolation could aggravate the risk of zoonoses among vulnerable residents, since rodents are reservoirs of several zoonotic diseases (Battersby 2015).





	Sighting of rats during the Covid-19 pandemic		
Predictors	*Odds Ratios*	*CI*	*p*
(Intercept)	1.95	1.43 – 2.66	**<0.001**
1.5 years before (Ref.)	-	-	-
1 years before	1.04	0.82 – 1.33	0.749
5 months before	0.95	0.74 – 1.22	0.685
During COVID-19	**1.27**	**1.00 – 1.60**	**0.045**
Observations	184		

Figure 1. Distribution of the number of sighted rats among residents of an urban community in Brazil (Sanitary District of Pau da Lima, Salvador-Brazil) and the effect of social isolation during the COVID-19 pandemic on the sighting of rats inside urban slum spaces.

We collected data on rat sightings from consenting participants during home visits in a slum community-based longitudinal study on urban leptospirosis in Salvador, Brazil. Participants were interviewed over more than two years in three biannual follow-ups. Then, out of the 287 residents participating in the cohort during 2018-2019, 46 answered the same questionnaire via an instant messaging application three months after the commencement of social isolation (June 2020) (Fig 1). Their responses were included in a mixed effects model to compare the number of rat sightings at different time periods. These periods were grouped as 18 months, 12 months and 5 months before COVID-19, plus the COVID-19 sample, which was temporally equivalent to the first pre-COVID sample. Rat sightings were 123, 128, 114 (all pre-COVID) and 156, respectively. The perceived increase in the number of rats seen by residents during the social isolation, representing 1.27 (CI: 1.00-1.60) in the number of rats sightings, the most among the different time periods (before and during COVID-19) in Brazil (Fig. 1). There was, however, no difference in the proportion of people who saw rats across the periods.

The closing of trade, reduced trash collection (that was already precarious) and an increase in the number of residents living in small spaces may all have contributed to the increase in the accumulation of garbage, a source of food for rodents. The increase in rat activity may in turn increase residents' exposure and susceptibility to diseases, for example toxoplasmosis, leptospirosis, salmonellosis, diarrhea and viruses like haemorrhagic fever with pulmonary syndrome, haemorrhagic fever with renal syndrome, Lassa fever among others through elevated rodent-human contact and subsequent disease transmission. In a similar vein, social isolation is coinciding with arbovirus epidemics in the same city, suggesting environmental changes and human behavioural changes that may influence both vectors and reservoirs of disease (Ribeiro et al., 2020),

The COVID-19 pandemic has brought about changes in human behaviour likewise increase in inequality and vulnerability of residents in poor urban communities. Above all, the health authorities have to target multiple efforts that go beyond control actions for the prevention of COVID-19, since these populations live in a scenario of increased exposure and risk to multiple diseases in this pandemic.

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Co-authored Articles (2)

Article submitted to Infection ecology and epidemiology

Prevalence of pathogenic *Leptospira* spp. in non-volant small mammals of Hutan Lipur Sekayu, Terengganu, Malaysia

Nur Juliani Shafie[1*], Najma Syahmin Abdul Halim[1], Mohamed Nor Zalipah[1], Shukor Md-Nor[2], **Adedayo Michael Awoniyi**[3] and Federico Costa[3,4,5,6,7]

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*Corresponding author: E-mail: nur.shafie@umt.edu.my (NJS)

Abstract

Leptospirosis is an important zoonotic disease that is transmitted worldwide through infected small mammals such as rodents. In Malaysia, there is paucity of information on the animal reservoirs that are responsible for leptospirosis transmission, with only few studies focusing on leptospirosis risk in recreational areas. Therefore, in this study we characterized the species composition and the prevalence of pathogenic *Leptospira* spp. in non-volant small mammals of Hutan Lipur Sekayu, Terengganu. We performed ten trapping sessions totaling 3,000 trapping efforts between September 2019 and October 2020. Kidney samples from captured individuals were extracted for the PCR detection of pathogenic *Leptospira* spp. Overall, we captured 45 individuals from 8 species (1.56% successful trapping effort), with 9 individuals testing positive for pathogenic *Leptospira,* that is 20% (n = 9/45) prevalence rate. Also, we recorded a 100% pathogenic *Leptospira* infestation rate among the captured *Maxomys whiteheadi***,** 50% in *Sundamys muelleri,* while *Maxomys rajah, T. glis* and *R. tiomanicus* had 25%, 20% and 18.2% infestation rate respectively. These findings demonstrate a low density of non-volant small mammals in Hutan Lipur Sekayu, but with modest potential to maintain pathogenic *Leptospira* transmission cycle thereby rendering the recreational area a potential infestation ground for leptospirosis.

Keywords: Leptospirosis; prevalence; small mammals; recreational area; rodents; Malaysia

Annex two

Published articles

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RESEARCH ARTICLE

Methods in Ecology and Evolution BRITISH ECOLOGICAL SOCIETY

Using Rhodamine B to assess the movement of small mammals in an urban slum

Adedayo Michael Awoniyi[1] | Fábio Neves Souza[1] | Caio Graco Zeppelini[1] | Bárbara Inês A. Xavier[1] | Ana Maria Barreto[1] | Diogo César C. Santiago[1] | Juliet Oliveira Santana[2] | Eduardo Mendes da Silva[1,3] | Federico Costa[1,2,4,5,6] | Michael Begon[7] | Hussein Khalil[8]

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Handling Editor: Robert B. O'Hara

Abstract

1. The small mammals, especially rats are pest species that are present in cities world-wide. The rat moves around and into residences and other anthropogenic structures. It is especially ubiquitous in urban slums and a threat to infrastructure and public health due to the pathogens it carries and transmits. Effective control of rat populations in most urban areas has been unsuccessful, despite several rodent control efforts. Limited information about rat movement distance has hindered identification of control units and effective scales at which to enact control during interventions.
2. We evaluated the suitability of Rhodamine B, a non-toxic biomarker, for assessing the distance travelled by rats in urban slums. We tracked rats over two campaigns between 2019 and 2020.
3. Overall, 27.9% of trapped rats showed signs of Rhodamine B in their whiskers under fluorescence microscope. This shows that our method provides a viable alternative for investigating the movement of small mammals in this area. We found that rats move up to 90 m distance in urban slums, with smaller rats travelling more actively than bigger rats.
4. Information obtained from this study should be useful in guiding efficient rodent control initiatives to reduce the risk of household rodent infestation and rodent-borne disease in urban slums.

KEYWORDS

rat, rhodamine B, rodent, slum, snap trap, zoonoses

Short Communication

Effect of chemical and sanitary intervention on rat sightings in urban communities of New Providence, the Bahamas

Adedayo M. Awoniyi[1] · Andrew Thompson[2] · Luther Ferguson[2] · Melony Mckenzie[2] · Fabio N. Souza[1] · Caio G. Zeppelini[1] · Federico Costa[1,3,4,5,6]

Received: 26 October 2020 / Accepted: 1 March 2021

Abstract
Rats are invasive pest species that commonly infest low-income urban environments. Their association with humans constitutes a threat of rodent-borne disease transmission. We evaluated the outcome of a chemical and sanitary intervention on rat sightings in seven low-income urban settlements of New Providence, the Bahamas. The intervention consisted of rodenticide application, education about environmental sanitation, and improvement in waste disposal. Rat sightings were systematically recorded by trained staff before and three months after the intervention. The intervention slightly decreased rat sightings, with an average of 2.7-fold with varied effectiveness across locations. Four out of seven locations (57%) registered a decrease in rat sightings. Our results suggest that social and environmental differences among communities may be responsible for the mixed efficacy observed in the current rodent management practice in urban communities of the Bahamas. However, a new set of control measures needs to be developed for areas where rodent decline was not observed. This study provides novel data on how rat population behaves post-intervention in a unique ecological setting like the Bahamas, presenting an informed judgment for their management, especially in the event of a natural disaster.

Keywords Rat · Rat sightings · Rodenticide · Rodent control · Urban community · Zoonoses

1 Introduction

Rats (genus *Rattus*) are widely distributed and successful invasive species that are present in all continents except Antarctica [1]. They were likely introduced into North America in the 1750s through trans-Atlantic navigation during the European colonization [2]. They adapt well to human-altered environments and are regarded as notorious agricultural and urban pests [3]. In urban settings, their infestation is often associated with low socioeconomic status, inefficient waste management, open sewers, overcrowding, and other infrastructure characteristics that can be exploited for food and harbourage [4].

Frequent rat sightings have been reported as an indicator of poor sanitation and diseases [5]. Rats are famous reservoirs of several infectious diseases (e.g.

Adedayo M. Awoniyi and Andrew Thompson have contributed equally to this work.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s42452-021-04459-x.

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Annex three

Co-authored published articles

VECTOR-BORNE AND ZOONOTIC DISEASES
Volume XX, Number XX, 2020

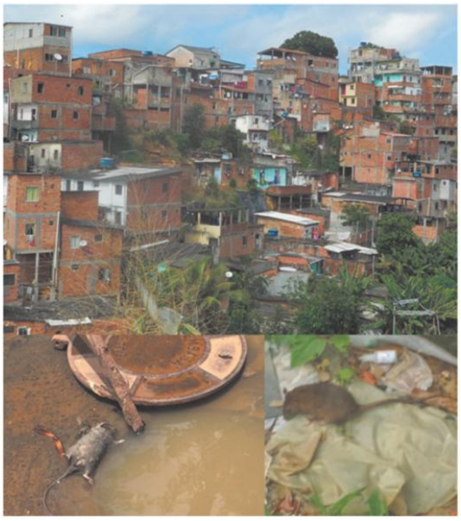
DOI: 10.1089/vbz.2020.2686

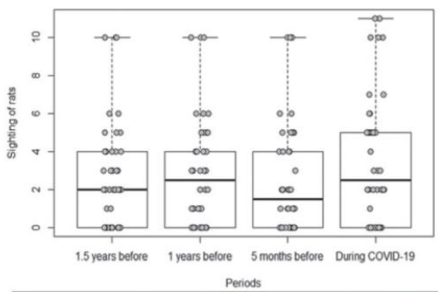
EDITORIAL

Increased Rat Sightings in Urban Slums During the COVID-19 Pandemic and the Risk for Rat-Borne Zoonoses

Fábio Neves Souza,[1] Adedayo Michael Awoniyi,[1] Fabiana Almerinda G. Palma,[1] Mike Begon,[2] and Federico Costa[1,2]

Social isolation has been implemented in many countries around the world to reduce the impact of COVID-19 during the 2020 pandemic. Although, this measure is critical in preventing the spread of the virus, the initiative has lead to rodent-human behavioral changes, resulting in health, economic and social impacts (Corburn et al., 2020; Heymann & Shindo, 2020; Battersby et al., 2008). Globally, an increase in rodent-related problems has been reported in various media outlets by experts during the pandemic. In low and middle income countries (LMIC) such as Brazil, where a large proportion of urban residents live in slum communities, with substandard water, sanitation, and hygiene coverage, social isolation seems to have provided an ecological opportunity for rodents' expansion, and any increase in the population of rodents during the social isolation could aggravate the risk of zoonoses among vulnerable residents, since rodents are reservoirs of several zoonotic diseases (Battersby 2015).





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