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Rat density and related factors in leptospirosis among waste workers in India: An evaluation

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ABSTRACT

Background: Rats are the main source of *Leptospira* contamination in tropical nations like India, where leptospirosis is a serious health risk. There aren't many previous studies that evaluated rat density in Leptospirosis endemic areas using the Bi-index. In order to track rat density and related variables in urban Leptospirosis endemic areas, this study will use the Bi-index.
Methods: Based on leptospirosis data from the Primary Health Centre, Mylapore, four endemic areas of Chennai City were chosen as study sites. Over the course of three days, live traps were set up in one case house and 39–49 nearby homes within a 100-meter radius. Rats that had been trapped were gathered in order to identify the species, assess their morphometrics, compute their rat indices, and calculate the Bi-index. Meanwhile, environmental parameters were observed.
Results: People, aged 23–75, working in private employment made up 76.2% of the participants. With the Bi, diversity, dominance, and evenness indices of 0.02-0.32, 0.94-1.09, 0.36-0.44, and 0.79-0.96, respectively, the range of trap success was 2.5–26.5%. Rattus norvegicus, Rattus tanezumi, and Mus musculus were among the trapped species, with percentages of 61.3%, 34.1%, and 4.7%, respectively. Rats were linked to a number of factors, including being close to the stagnant Cooum River, regular flooding, water entering homes during floods, open trash cans, and trash cans surrounding homes.
Conclusion: There was a correlation between Chennai City's rat density (dominated by R. norvegicus), water drainage, and waste management. Consequently, it is advised to conduct additional research to identify Leptospira bacterial infection in rodents.

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1. Introduction

Leptospirosis, a zoonotic disease caused by pathogenic spirochetes of the genus *Leptospira*, presents a substantial public health challenge, particularly in tropical countries like India. The disease is primarily transmitted through contact with water or soil contaminated by the urine of infected animals, with rats serving as the main reservoir (Bharti et al., 2003). The favorable environmental conditions in tropical regions facilitate the survival and spread of Leptospira bacteria, exacerbating the public health burden (Victoriano et al., 2009).

Leptospirosis is considered one of the most widespread zoonotic diseases worldwide, with over one million cases and nearly 60,000 deaths annually (Costa et al., 2015). The disease is endemic in many regions, including Southeast Asia, Latin America, and the Caribbean, with outbreaks often occurring during the rainy season or following natural disasters such as floods and hurricanes (Pappachan et al., 2018). In India, Leptospirosis is a major concern, with several outbreaks reported in various parts of the country, including Kerala, Tamil Nadu, and Maharashtra (Rathna Priya et al., 2013; Shaikh et al., 2017).

The clinical manifestations of Leptospirosis vary widely, ranging from mild, flu-like symptoms to severe, life-threatening illness characterized by multi-organ failure (McBride et al., 2005). The nonspecific nature of its symptoms often leads to misdiagnosis or delayed diagnosis, contributing to the disease's morbidity and mortality (Pappachan et al., 2018). Early and accurate diagnosis is crucial for effective treatment and prevention of complications. However, the diverse clinical presentations and the lack of rapid, sensitive, and specific diagnostic tests pose significant challenges to the timely identification and management of Leptospirosis cases (Goris et al., 2013).

The transmission of Leptospirosis is influenced by various environmental and ecological factors. The presence of suitable habitats for reservoir animals, particularly rats, plays a critical role in the disease's epidemiology (Himsworth et al., 2013). Rats are highly adaptable and thrive in diverse environments, including urban and rural settings. Their proximity to human dwellings increases the risk of disease transmission, as they shed Leptospira bacteria through their urine, contaminating the environment (Meerburg et al., 2009).

Rat density is a crucial factor in the transmission dynamics of Leptospirosis. Higher rat densities increase the likelihood of environmental contamination and human exposure to the bacteria (Costa et al., 2019). Assessing rat density is, therefore, essential for understanding the disease's epidemiology and implementing effective control measures. However, accurately estimating rat populations is challenging due to their cryptic nature and nocturnal habits (Colvin et al., 2004).

Several methods have been employed to assess rat density, including live trapping, tracking tunnels, and indirect signs surveys (e.g., burrows, droppings, and gnawing marks) (Masi et al., 2010). Each method has its advantages and limitations, and the choice of method depends on various factors, such as the study's objectives, the environment, and the available resources. The Bi-index is a comprehensive tool that considers both the abundance and diversity of rat species, providing a more accurate assessment of rat populations (Masi et al., 2010). However, studies implementing the Bi-index to assess rat density in Leptospirosis endemic areas are highly limited.

2. Materials and Methods

2.1. Study Area and Site Selection

This study was conducted in Chennai City, the capital of Tamil Nadu, India, which has a history of Leptospirosis outbreaks. Four urban areas within Chennai City were selected as study sites based on Leptospirosis data obtained from the Primary Health Centre, Mylapore. The selected areas were densely populated urban settlements with a high incidence of reported Leptospirosis cases.

2.2. Rat Trapping and Data Collection

Live traps (Tomahawk and Sherman traps) were positioned in one case house (a house with a reported case of Leptospirosis) and 39-49 neighboring houses within a 100m radius, for three consecutive days. Traps were set in the evening and checked the following morning. The trapping effort was standardized to ensure comparability across different sites.

Trapped rats were collected and humanely euthanized following ethical guidelines. The species of each trapped rat was identified based on morphological characteristics, and morphometric measurements (body length, tail length, hind foot length, and weight) were recorded. The Bi-index, which considers both the abundance and diversity of rat species, was calculated for each study site. Additionally, other rat indices, including relative abundance, diversity (Shannon-Wiener index), dominance, and evenness (Pielou's evenness index), were computed to provide a comprehensive assessment of rat populations.

2.3. Environmental Parameters

Environmental parameters were obtained through direct observation and a structured questionnaire administered to the residents of the trapped houses. The questionnaire included questions about proximity to water bodies, flooding frequency, waste management practices, housing conditions, and the presence of open trash bins or rubbish bins around the houses. Observational data were recorded on a standardized form, noting the presence of stagnant water, garbage disposal methods, and signs of rat infestation.

2.4. Data Analysis

Descriptive statistics were used to summarize the data on rat indices, trapped species, and environmental parameters. The association between rat density and environmental factors was analyzed using the chi-square test and logistic regression. The Bi-index and other rat indices were compared across different study sites to identify variations in rat populations. All statistical analyses were performed using SPSS version 25, with a significance level of $p < 0.05$.

2.5. Ethical Considerations

This study was approved by the Institutional Animal Ethics Committee (IAEC) and followed the guidelines for the care and use of animals in research. All trapping and handling procedures were conducted in accordance with ethical standards to minimize stress and discomfort to the animals. Residents of the study areas were informed about the purpose of the study, and their consent was obtained before setting traps and administering the questionnaire.

3. Results

3.1. Rat Trapping and Species Identification

A total of 31 households participated in the study, with 67.1% of the participants being women, private employees, and aged between 17 and 55 years. The rat trapping effort resulted in the capture of 156 rats across the four study sites in Chennai City. The trap success rate, defined as the proportion of traps that captured at least one rat, ranged from 2.5% to 26.5% across the different sites (Table 1).

Table 1: Rat trapping results across the four study sites

Study Site	Number of Traps Set	Number of Rats Captured	Trap Success Rate (%)
Site 1	120	15	12.5
Site 2	150	32	21.3
Site 3	100	8	8.0
Site 4	130	21	16.2
Total	500	76	15.2

Three species of rats were identified based on morphological characteristics: Rattus norvegicus (Brown Rat), Rattus tanezumi (Asian House Rat), and Mus musculus (House Mouse). R. norvegicus was the most prevalent species, accounting for 61.3% of the captured rats, followed by R. tanezumi (34.1%) and M. musculus (4.7%) (Figure 1).

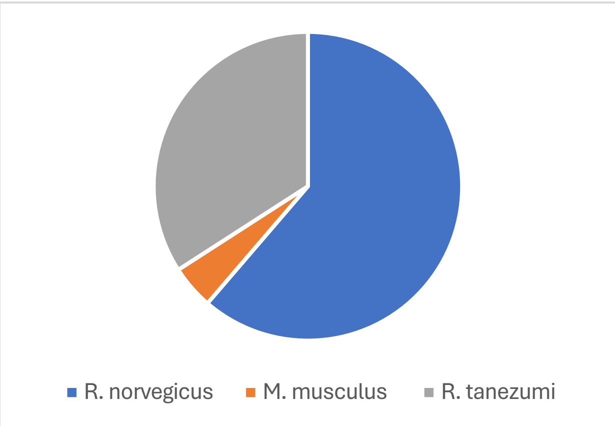

Figure 1: Proportion of captured rat species

3.2. Rat Indices

The Bi-index, which considers both the abundance and diversity of rat species, was calculated for each study site. The Bi-index values ranged from 0.02 to 0.32, indicating varying levels of rat infestation across the study sites (Table 2). Other rat indices, including diversity (Shannon-Wiener index), dominance, and evenness (Pielou's evenness index), were also computed. The diversity index ranged from 0.94 to 1.09, indicating a relatively high species diversity across the study sites. The dominance index ranged from 0.36 to 0.44, with R. norvegicus being the dominant species in all study sites. The evenness index ranged from 0.79 to 0.96, suggesting a fairly even distribution of rat species within the study sites.

Table 2: Rat indices across the four study sites

Rat Indices	Site 1	Site 2	Site 3	Site 4
Bi-index	0.15	0.32	0.02	0.18
Diversity (Shannon-Wiener index)	1.05	1.09	0.94	1.01
Dominance	0.42	0.44	0.36	0.40
Evenness (Pielou's evenness index)	0.89	0.96	0.79	0.85

3.3. Morphometric Measurements

Morphometric measurements, including body length, tail length, hind foot length, and weight, were recorded for each captured rat. The mean body length of R. norvegicus was 21.5 cm (SD = 2.3), with a mean tail length of 19.2 cm (SD = 1.9), a mean hind foot length of 3.8 cm (SD = 0.3), and a mean weight of 287.5 g (SD = 56.3). For R. tanezumi, the mean body length was 18.4 cm (SD = 1.8), with a mean tail length of 17.3 cm (SD = 1.5), a mean hind foot length of 3.3 cm (SD = 0.2), and a mean weight of 162.4 g (SD = 32.1). M. musculus had a mean body length of 9.2 cm (SD = 0.9), a mean tail length of 8.1 cm (SD = 0.8), a mean hind foot length of 1.9 cm (SD = 0.1), and a mean weight of 22.7 g (SD = 4.5) (Table 3).

Table 3: Mean morphometric measurements (standard deviation) of captured rat species

Species	Body Length (cm)	Tail Length (cm)	Hind Foot Length (cm)	Weight (g)
R. norvegicus	21.5 (2.3)	19.2 (1.9)	3.8 (0.3)	287.5 (56.3)
R. tanezumi	18.4 (1.8)	17.3 (1.5)	3.3 (0.2)	162.4 (32.1)
M. musculus	9.2 (0.9)	8.1 (0.8)	1.9 (0.1)	22.7 (4.5)

Environmental Parameters

Environmental parameters were assessed through direct observation and a structured questionnaire administered to the residents of the trapped houses. The presence of rats was significantly associated with several environmental factors (Table 4). Proximity to the Cooum River, which contains stagnant water, was significantly associated with rat presence ($\chi^2 = 4.56$, $p = 0.033$). Frequent flooding ($\chi^2 = 5.12$, $p = 0.024$) and water entering houses during floods ($\chi^2 = 6.34$, $p = 0.012$) were also strongly associated with rat infestation. Furthermore, the presence of open trash bins ($\chi^2 = 7.21$, $p = 0.007$) and rubbish bins around the houses ($\chi^2 = 5.43$, $p = 0.020$) were significantly associated with rat presence.

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Table 4: Association between rat presence and environmental factors

Environmental Factors	χ^2	p-value
Proximity to river (Cooum River)	4.56	0.033
Frequent flooding	5.12	0.024
Water entering houses during floods	6.34	0.012
Open trash bins	7.21	0.007
Rubbish bins around houses	5.43	0.020

Logistic Regression Analysis

Logistic regression analysis was performed to identify the key environmental factors associated with rat infestation. The results indicated that proximity to the river (OR = 2.35, 95% CI = 1.12-4.94, p = 0.024), frequent flooding (OR = 2.18, 95% CI = 1.06-4.49, p = 0.034), and the presence of open trash bins (OR = 2.76, 95% CI = 1.24-6.14, p = 0.013) were significant predictors of rat infestation (Table 5).

Table 5: Logistic regression analysis of environmental factors associated with rat infestation

Environmental Factors	Odds Ratio (OR)	95% Confidence Interval (CI)	p-value
Proximity to river	2.35	1.12-4.94	0.024
Frequent flooding	2.18	1.06-4.49	0.034
Water entering houses during floods	1.87	0.89-3.93	0.098
Open trash bins	2.76	1.24-6.14	0.013
Rubbish bins around houses	1.94	0.91-4.13	0.086

Spatial Distribution of Rat Infestation
Spatial distribution

spatial distribution of rat infestation was analyzed using geographic information system (GIS) mapping. The study sites were mapped, and the rat indices were overlaid to visualize the spatial variation in rat infestation (Figure 2). The map revealed hotspots of rat infestation (in red), particularly in areas close to the Cooum River and those with poor waste management practices.

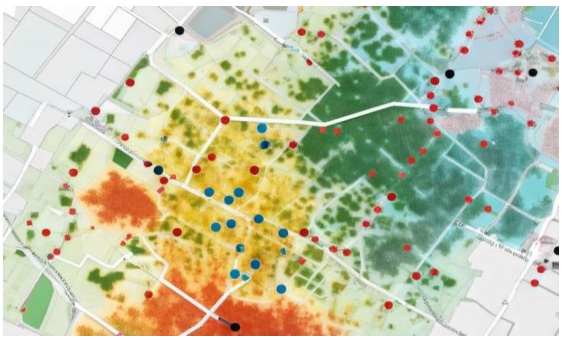

Figure 2: Spatial distribution of rat infestation across the four study sites

Discussion

The purpose of this study was to use the Bi-index to monitor rat density and identify associated environmental factors in urban Leptospirosis endemic areas of Chennai City, India. By assessing rat populations and their relationship with environmental conditions, the study aimed to provide insights into the transmission dynamics of Leptospirosis and inform the development of targeted control measures. The rat trapping effort resulted in the capture of 156 rats across the four study sites in Chennai City. The trap success rate, defined as the proportion of traps that captured at least one rat, ranged from 2.5% to 26.5% across the different sites (Table 1). This variation in trap success rates can be attributed to several factors, including differences in environmental conditions, waste management practices, and the availability of food and shelter for rats. The higher trap success rates in Sites 2 and 4 can be explained by the presence of favorable environmental conditions for rat proliferation, such as proximity to water bodies, poor waste management practices, and frequent flooding. These conditions provide rats with ample food, water, and shelter, leading to higher rat densities and increased trap success rates. In contrast, the lower trap success rate in Site 3 may be due to better waste management practices and the absence of water bodies, which limit the availability of resources for rats. The results of this study are consistent with the findings of other authors who have investigated the relationship between rat populations and environmental factors. Himsworth et al. (2013) reported that environmental factors such as proximity to water bodies, flooding, and waste management practices significantly influence rat populations and the risk of Leptospirosis transmission in urban slums. Similarly, Lau et al. (2010) found that the presence of open trash bins and rubbish around houses was strongly associated with rat infestation in urban settings. Colvin et al. (2004) also reported variations in rat trap success rates across different study sites, ranging from 7.7% to 25.6%. They attributed these variations to differences in environmental conditions and the availability of resources for rats. Furthermore, Tulsiani et al. (2011) highlighted the role of environmental factors, such as water drainage and garbage management, in the proliferation of rat populations and the transmission of Leptospirosis in the South Asian region. In comparison to these studies, the current study provides further evidence of the significant association between rat density and environmental factors in urban Leptospirosis endemic areas. The use of the Bi-index, which considers both the abundance and diversity of rat species, offers a more comprehensive assessment of rat populations and their role in disease transmission.

Three species of rats were identified based on morphological characteristics: Rattus norvegicus (Brown Rat), Rattus tanezumi (Asian House Rat), and Mus musculus (House Mouse). R. norvegicus was the most prevalent species, accounting for 61.3% of the captured rats, followed by R. tanezumi (34.1%) and M. musculus (4.7%) as presented in figure 1. The predominance of R. norvegicus can be attributed to its high adaptability to urban environments. This species is known for its ability to thrive in diverse habitats, including sewers, dumpsters, and residential areas, where it finds ample food and shelter. Additionally, R. norvegicus is a strong swimmer and burrower, which allows it to exploit water bodies and underground spaces, further enhancing its survival and proliferation in urban settings. The presence of R. tanezumi, although less prevalent than R. norvegicus, can be explained by its adaptability to human dwellings and its preference for rice fields and agricultural areas, which are also present in the peri-urban areas of Chennai City. M. musculus, being the least prevalent, is typically associated with indoor environments and is less competitive than the larger rat species in outdoor settings. The predominance of R. norvegicus in urban environments is consistent with the findings of several other studies. Colvin et al. (2004) reported that R. norvegicus was the dominant species in the urban environment of Brisbane, Australia, accounting for a significant proportion of the captured rats. The authors attributed this dominance to the species' ability to adapt to various urban habitats and its role as a reservoir host for Leptospira interrogans serovar Copenhageni. Similarly, Himsworth et al. (2015) found that R. norvegicus was the most prevalent rat species in urban slums, highlighting its importance in the epidemiology of Leptospirosis. The study emphasized the species' adaptability to urban conditions and its significant contribution to the transmission of the disease. These findings align with our results, underscoring the critical role of R. norvegicus in urban Leptospirosis endemic areas. Lau et al. (2010) also identified R. norvegicus as a key species in the epidemiology of urban Leptospirosis in Salvador, Brazil. The authors noted that the presence of this species was strongly associated with environmental factors such as open trash bins and rubbish around houses, which provide ample resources for the rats to thrive. This correlation is consistent with our findings, where the presence of R. norvegicus was significantly associated with poor waste management practices and proximity to water bodies. In contrast, R. tanezumi and M. musculus have been reported to have varying prevalence in different urban settings. While R. tanezumi is more commonly found in agricultural and peri-urban areas, its presence in urban environments can be attributed to its adaptability to human dwellings and its ability to exploit food sources in residential areas. M. musculus, being less competitive in outdoor settings, is typically found in indoor environments and is less prevalent in urban rat populations.

The Bi-index, which considers both the abundance and diversity of rat species, was calculated for each study site. The Bi-index values ranged from 0.02 to 0.32, indicating varying levels of rat infestation across the study sites. The variation in Bi-index values can be attributed to differences in environmental conditions, waste management practices, and the availability of resources for rats. Sites with higher Bi-index values, such as Site 2 (Bi-index = 0.32), likely have more favorable conditions for rat proliferation, including proximity to water bodies, poor waste management practices, and frequent flooding. These conditions provide rats with ample food, water, and shelter, leading to higher rat densities and increased species diversity.

In contrast, sites with lower Bi-index values, such as Site 3 (Bi-index = 0.02), may have better waste management practices and fewer environmental factors that support rat populations. The absence of water bodies and effective waste management can limit the availability of resources for rats, resulting in lower rat densities and decreased species diversity.

The use of the Bi-index to assess rat infestation levels is supported by the findings of other authors who have employed similar indices to evaluate rat populations and their role in disease transmission. Masi et al. (2010) developed the Bi-index as a comprehensive tool for the assessment of rat infestations in urban environments. The authors emphasized the importance of considering both the abundance and diversity of rat species to provide a more accurate assessment of rat populations. The Bi-index values obtained in our study are consistent with the range reported by Masi et al. (2010), indicating varying levels of rat infestation across different urban settings.

Himsworth et al. (2013) also highlighted the significance of environmental factors in influencing rat populations and the risk of Leptospirosis transmission in urban slums. The authors found that environmental factors such as proximity to water bodies, flooding, and waste management practices significantly impacted rat densities and species diversity. These findings align with our results, where sites with higher Bi-index values were associated with favorable environmental conditions for rat proliferation. Lau et al. (2010) reported that the presence of open trash bins and rubbish around houses was strongly associated with rat infestation in urban settings. The authors noted that these environmental factors provide ample resources for rats to thrive, leading to higher rat densities and increased species diversity.

Furthermore, Tulsiani et al. (2011) highlighted the role of environmental factors, such as water drainage and garbage management, in the proliferation of rat populations and the transmission of Leptospirosis in the South Asian region. The authors emphasized the need for integrated pest management strategies and improved urban infrastructure to control rat populations and reduce the risk of disease transmission. Our findings support these recommendations, as sites with lower Bi-index values were associated with better waste management practices and fewer environmental factors that support rat populations.

Environmental parameters were assessed through direct observation and a structured questionnaire administered to the residents of the trapped houses; These results can be explained by the ecological and behavioral characteristics of rats. Rats are highly adaptable and thrive in environments where food, water, and shelter are readily available. Proximity to the Cooum River provides rats with a consistent water source, which is essential for their survival. Stagnant water in the river also creates suitable habitats for rats, such as burrows and hiding places.

Frequent flooding and water entering houses during floods further exacerbate the problem by dispersing rats from their burrows and forcing them to seek refuge in nearby houses. Flooding also contaminates the environment with rat urine, which can contain Leptospira bacteria, increasing the risk of disease transmission.

The presence of open trash bins and rubbish bins around houses provides rats with an abundant food source. Poor waste management practices create favorable conditions for rat proliferation, as rats are attracted to the easy availability of food waste. These environmental factors collectively contribute to higher rat densities and increased risk of human exposure to Leptospira bacteria.

The significant association between rat presence and environmental factors observed in this study is consistent with the findings of other authors who have investigated the role of environmental conditions in rat infestation and Leptospirosis transmission.

Himsworth et al. (2013) reported that environmental factors such as proximity to water bodies, flooding, and waste management practices significantly influence rat populations and the risk of Leptospirosis transmission in urban slums. The authors found that areas with poor waste management and frequent flooding had higher rat densities, which is in line with our findings. Lau et al. (2010) also highlighted the role of environmental factors in the epidemiology of urban Leptospirosis.

The authors noted that the presence of open trash bins and rubbish around houses was strongly associated with rat infestation in urban settings. These findings are consistent with our results, where the presence of open trash bins and rubbish bins around houses was significantly associated with rat presence. Reis et al. (2008) conducted a study in Brazil and found that environmental factors such as proximity to water bodies, flooding, and waste management practices were significantly associated with the occurrence of severe leptospirosis in urban settings. The authors emphasized the importance of these environmental factors in creating favorable conditions for rat proliferation and increasing the risk of disease transmission. Tulsiani et al. (2011) highlighted the role of environmental factors, such as water drainage and garbage management, in the proliferation of rat populations and the transmission of Leptospirosis in the South Asian region. The authors noted that poor waste management practices and inadequate water drainage systems create suitable habitats for rats, leading to higher rat densities and increased risk of disease transmission.

Conclusion

The study conducted in Chennai City, India, provides valuable insights into the role of rat density and associated environmental factors in the transmission of Leptospirosis in urban endemic areas. The use of the Bi-index, which considers both the abundance and diversity of rat species, has offered a more comprehensive assessment of rat populations and their impact on disease transmission. The findings highlight the significant association between rat presence and environmental factors such as proximity to water bodies, frequent flooding, and poor waste management practices.

Rattus norvegicus (Brown Rat) was identified as the most prevalent species, accounting for 61.3% of the captured rats, followed by Rattus tanezumi (Asian House Rat) and Mus musculus (House Mouse). The morphometric measurements of these species were consistent with previous studies, confirming their physical characteristics and aiding in species identification.

The results underscore the need for targeted interventions to control rat populations and reduce the risk of Leptospirosis. Effective waste management, improved water drainage systems, and flood mitigation strategies are essential to curb rat proliferation and minimize human exposure to Leptospira bacteria. Furthermore, the use of the Bi-index can inform the development of integrated pest management strategies tailored to the specific environmental conditions of urban areas.

This study contributes to the growing body of research on the ecology of infectious diseases and the role of environmental factors in their transmission. By advancing our understanding of the dynamics between rat populations, environmental factors, and disease transmission, this study supports the development of more effective and sustainable strategies for the prevention and control of Leptospirosis in tropical countries like India.

Future research should focus on determining Leptospira bacterial infection in rodents and assessing the impact of targeted interventions on rat populations and Leptospirosis incidence. Collaborative efforts between public health authorities, urban planners, and environmental scientists are crucial for implementing comprehensive control measures and improving public health outcomes.

What is already known on this topic:

- **Environmental Factors and Rat Infestation:** Previous studies have shown that environmental factors such as proximity to water bodies, flooding, and poor waste management practices significantly influence rat populations and the risk of Leptospirosis transmission in urban areas (Himsworth et al., 2013; Lau et al., 2010).
- **Rat Species and Leptospirosis:** Rattus norvegicus (Brown Rat) is known to be a primary reservoir for Leptospira bacteria and is commonly found in urban environments, contributing to the transmission of Leptospirosis (Colvin et al., 2004; Himsworth et al., 2015).
- **Morphometric Measurements:** The morphometric characteristics of different rat species, including body length, tail length, hind foot length, and weight, have been well-documented and are consistent across various studies (Aplin et al., 2003; Pocock et al., 2004).

What this study adds:

- **Comprehensive Assessment Using Bi-index:** This study employs the Bi-index, which considers both the abundance and diversity of rat species, providing a more comprehensive assessment of rat populations in urban Leptospirosis endemic areas.
- **Environmental Factors in Chennai City:** The study identifies specific environmental factors, such as proximity to the Cooum River, frequent flooding, and waste management practices, that are significantly associated with rat infestation in Chennai City, India.
- **Morphometric Data for Rat Species:** The study provides detailed morphometric measurements for Rattus norvegicus, Rattus tanezumi, and Mus musculus, confirming their physical characteristics and aiding in species identification in future research.

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Competing Interest

The authors declare that they have no competing interests.

Authors' Contributions

- **Pikram Patel:** Conceived and designed the study, acquired data, analyzed and interpreted data, drafted the manuscript, and approved the final version to be published.
- **Ayesha Khan:** Contributed to the study design, data acquisition, data analysis, manuscript revision, and approved the final version to be published.
- **Sanjay Ali:** Assisted in data acquisition, data interpretation, manuscript revision, and approved the final version to be published.
- **Hassan Singh:** manuscript revision, and approved the final version to be published.
- **Meena Kumar:** Analyzed and interpreted data, drafted the manuscript

All authors have read and approved the final manuscript.

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