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Leptospirosis: An insight into community structure of small mammal's host in urban environment

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Abstract. Leptospirosis is a zoonotic disease caused by bacteria of the genus *Leptospira* and most often acquired through contact with environments contaminated with leptospires shed in the urine of infected mammals. In urban environment, rodents are well-known as the main carriers of this bacteria, however there were no intensive study on the population structure of these animals, and how it associated with this disease. Hence, we use a case study from an outbreak in a residential area in Selangor, Malaysia, to investigate how community structure of small mammals, associated with the prevalence of *Leptospira*. One hundred cage traps were placed randomly in and around these houses in five phases with two months interval for a year. Community structures (species, sex, and age) were assigned for each individual, prior to screening for pathogenic *Leptospira*, using a partial lipL32 gene from the kidney samples. 185 small mammals from four species were captured, *Rattus norvegicus* (74.5%, N=138), *R. rattus* (20%, N=37), *Tupaia glis* (5%, N=9), and *Suncus murinus* (0.5%, N=1). From this number, 29 individuals were found PCR positive for pathogenic *Leptospira* (*R. norvegicus*, N=20; *R. rattus*, N=6; *T. glis*, N=2; *S. murinus*, N=1). The study shows that *Leptospira* occurrence in the small mammals were significantly correlated to age category and sampling phases, with Spearman Correlation (r_s) p=0.02 and p=0.04 respectively. Adult individuals were significantly more prevalent with *Leptospira* infection, whereby March and June were found to associate with higher *Leptospira* prevalent among the small mammals, potentially coincide with low rainfall and relative humidity level. This information is important in designing a specific control method for rodents in *Leptospira* outbreak areas. In addition, intensive sampling and regular cleaning effort were found to significantly reduce the small mammal *Leptospira* reservoir, thus should be implemented in intervention strategies in the urban environment.

INTRODUCTION

Leptospirosis is a bacterial zoonoses that was once a neglected tropical disease, are now re-emerging as a significant burden to public health especially in Southeast Asia, and Malaysia is one of the endemic region (Kit, 2002; Victoriano *et al.*, 2009; Costa *et al.*, 2015). A systemic study had revealed Oceania region that include: Australia, New Zealand and pacific island countries and territories to be the most affected by leptospirosis with morbidity (150.68 cases/ 100,000 populations per year) and mortality (9.61 deaths/ 100,000 populations per year) (Costa *et al.*, 2015). The tropical weather and high humidity of the environment allows the bacteria to remain alive for long periods in the environment (Sulong *et al.*, 2011; Levett, 2015). Furthermore, increase rainfall and flooding had also aggravated the disease distribution with the most notable outbreak

occurred in: Nicaragua (1995), Peru and Ecuador (1998), Orissa (1999), Malaysia (2000), Jakarta (2002), Mumbai (2000 and 2005), and The Philippines (2009) (Schneider *et al*., 2013).

Leptospirosis is caused by pathogenic species of *Leptospira* bacteria specifically *Leptospira interrogans* and to date there are more than 23 species of *Leptospira* bacteria discovered worldwide (Bourhy *et al*., 2014; Azali *et al*., 2016). Inada *et al*. (1916) were the first to describe the epidemiology of leptospirosis and concluded the role of rats (rodents) as a major reservoir for *Leptospira* transmission to human. Rodents, including rats and mice, are recognized as an asymptomatic reservoir, while humans are considered incidental hosts and infection occurs predominantly by contact of abraded skin or mucous membranes with water or moist soil contaminated with urine of infected animals (Ko *et al*., 2009; Mori *et al*., 2017). Despite rodents especially rats were recognized as the maintenance host, domestic animals such as cats and dogs; livestock such as cattle, pigs and goats; and wildlife can also harbour pathogenic *Leptospira* (Smith *et al*., 1961).

Previously, the epidemiology of leptospirosis is commonly attributed to occupational exposure, after floods, hurricanes or disaster and has recently related to recreational exposures (Nardone *et al*., 2004; Baranton & Postic, 2006; Monahan *et al*., 2009). However, in recent years, the outbreaks of leptospirosis are frequently reported to associate with urban problems in developing countries (Johnson *et al*., 2004; LaRocque *et al*., 2005). The abundance of commensal rats (*R. rattus* and *R. norvegicus*) in urban area place humans in close microenvironments with the reservoir host, thus clearly involved in the transmission cycle (Al Kattan *et al*., 2017; Pui *et al*., 2017). In Malaysia, the abundance of reservoir animal species, especially rodents in the urban area are reported mainly due to poor environmental hygiene and poor garbage management which attracts high number of rats to breed and forage (Benacer *et al*., 2013; Yusof *et al*., 2019).

In Malaysia, leptospirosis is considered as an endemic disease and caused significant impact on the wellbeing and livelihood of the individuals in this country (Kit, 2002). Besides, Malaysia became the epicentre of this disease due to favourable weather and climate conditions that promotes the growth and survival of the bacteria *Leptospira* (Trueba *et al*., 2004). Rodents are well known as a carrier and maintenance hosts of this bacteria in both urban and wild environment (Cosson *et al*., 2014; Azhari *et al*., 2018). Transmission of this diseases is related to the environmental factors such as improper waste management and poor sanitation which provide suitable conditions for high population growth of rodents and thus increase the risk of this disease to human (Dias *et al*., 2007; Ries *et al*., 2008; Benacer *et al*., 2018). Two commensal rats' species (*R. norvegicus* and *R. rattus*) were found to be the highly prevalent rat species infected with *Leptospira* in Kuala Lumpur (Benacer *et al*., 2016), Selangor (Yusof *et al.*, 2019) and Sarawak (Pui *et al*., 2017).

The investigation on leptospirosis in Malaysia previously focused on humans and domestic animals in the vicinity of outbreak sites that are mostly associated with occupational risk and recreational activities (Bahaman *et al*., 1988). However, there were no intensive study on the population structure of *Leptospira* carrying small mammals, and environmental risk factors associated with the disease in urban environment. Understanding the population structure of reservoir host could potentially help in the disease management. Hence, the present study was undertaken to investigate how community structure of small mammals' hosts, associated with the prevalence of *Leptospira*, a case study from an outbreak in a residential area in Kajang district of Selangor state in Malaysia. This study could help in designing a specific control method for rodents in *Leptospira* outbreak areas and will guide health professionals in leptospirosis intervention strategies especially in urban environment.

MATERIALS AND METHODS

Study site

This study was conducted in Sungai Ramal Baru (02°59'17.9" N, 101°45'56.0" E), an urban site located in the residential areas of Kajang town in Selangor state (2,684 m^2). This site has been chosen as case study following recent outbreak among the residents in 2015. It also located a shelter home for orphan with indecent hygiene and sanitation, improper garbage management and infrastructure facilities which became the major factors that contribute to the leptospirosis outbreak in this area.

Trapping and identification of host

One hundred cage traps (20x20x30 cm) were placed randomly in and around the houses in five phases with two months interval for a year (2016-2017) to capture non-volant small mammals. It was conducted within 6 days 5 nights for each phase. Baits used include oil-palm fruits, ripe bananas, sweet potatoes covered with peanut butter and dried salted fish (Ishak *et al*., 2018). Cage traps were checked once in the morning and damaged or stolen baits were replaced with the new one. The captured animals were placed in the sampling box and brought back to the research station for host identification and samples collection. Each individual host was identified according to Francis (2008) based on morphological measurements such as body and fur colours, length and colour pattern of the tail, body weight, head and body length, hind foot, size and shape of footpads. The captured animals were then euthanized with carbon dioxide (CO_2) chamber by inhalation to asphyxiate the rodents (Research Animals Department RSPCA, 2011). All animal work was conducted with ethical approval from Animals Ethics Committee, Faculty of Medicine, Universiti Kebangsaan Malaysia (FST/2016/SHUKOR/18-MAY/750-MAY-2016-SEPT.-2018-AR-CAT2). This study did not involve any endangered or protected species.

Detection of *Leptospira* from host's kidney

After the animals were euthanized, kidney tissues were removed, cut into small pieces (1 cm x 1 cm) and transported back to laboratory for DNA extraction. Before extraction, kidney samples were rinsed with sterile phosphate buffered saline solution to remove any environmental debris and possible contaminants (Azhari *et al*., 2018). DNA was extracted using the QIAamp DNeasy Blood and Tissue Kit (Qiagen, USA) according to the manufacturer's protocols. A conventional PCR method was performed to target a 242 bp region of *Leptospira* lipL32 gene (commonly present in pathogenic species) using the primers (lipL32-45F 52-AAG CAT TAC CGC TTG TGG TG-32 and lipL32-286R 52-GAA CTC CCA TTT CAG CGA TT-32) as previously described by Stoddard *et al*. (2009). PCR reaction was conducted in a final volume of 25µl containing of 12.5µl ready-to-used Mastermix, Econotaq® PLUS GREEN 2X Mastermix (Lucigen, USA) on a Biorad MyCycler™ machine, 0.5µl forward and reverse primer at a concentration of 10µM, and adjustable amount of distilled water and template DNA. Amplification was carried out under the following conditions: an initial 2-minute denaturation at 94°, followed by 35 cycles of 94° for 30 s, 48° for 30 s, 72° for 1 minute and final extension at 72° for 10 min. Subsequently, the reaction was stopped at 4°. The duration of the final PCR amplification was approximately 1 hour 50 minutes. All amplifications were performed with a negative control (water) and a positive control (*Leptospira interrogans* serovar *javanica*). The amplified products were analyzed on a 1.5% agarose gel stained with Gel Red Health View Nucleic Acid Gel Stain 1×. Samples were interpreted as positive for pathogenic *Leptospira* sp. if band of 242 bp were obtained. Samples were negative if no band was detected.

Environmental parameter

Environmental parameters such as rainfall, relative humidity (RH) and temperature were obtained from the Meteorological Department based on the nearest meteorological station to the study site, on monthly basis for a year.

Statistical analysis

The Spearman Correlation (r_s) analysis was performed to determine correlation of community structure (species, age category, gender, physical condition and pregnant status) and sampling phases to the positive *Leptospira* animals. Next, correlations were also performed to determine the correlation between positive *Leptospira* animals with environmental parameters (rainfall, relative humidity and temperature) for each sampling cycle. Significant correlations were shown if p-value is less than 0.05 ($p<0.05$). Similar analysis was performed on two dominant species; *R. norvegicus and R. rattus*.

RESULTS

185 individuals of small mammals from four different species have been captured within a duration of 25 trap-nights. The most dominant species were Norway rats, *Rattus norvegicus* (74.5%, N=138), followed by house rats, *R. rattus* (20%, N=37), common treeshrew, *Tupaia glis* (5%, N=9), whereby the house shrew, *Suncus murinus* (0.5%, N=1) was the least species captured as indicated in Table 1. Based on gender, females (53%) were slightly more abundant than males whereas age category, showed adult hosts (75%) were the more captured compared to juveniles. Besides that, majority of the captured female individuals were found not pregnant (95%) and only eight individuals were pregnant.

From the total 185 individuals, 29 individuals (15.7%) were found PCR positive for pathogenic *Leptospira*. Figure 1 showed the representative of gel image of lipL32 after

Table 1. Summary of community structure of small mammals and number of positive pathogenic PCR *Leptospira*

Community structure	Number of individuals recorded	Number of PCR Positive *Leptospira* (pathogenic)
Total individuals	185	29
Sampling phases		
1st (January)	95	1
2nd (March)	27	11
3rd (June)	23	13
4th (September)	29	2
5th (November)	11	2
Gender		
Male	86	11
Female	99	18
Age category		
Adult	138	26
Juvenile	47	3
Physical condition		
Good	133	21
Fair	28	6
Poor	24	2
Pregnant status		
No	177	29
Yes	8	0

Figure 1. Representative gel image of lipL32 PCR amplification in 1.4% agarose. Lanes M, 100 bp DNA ladder; positive and negative controls; bands showing positive PCR amplicons for pathogenic *Leptospira* at the expected size of 242bp.

PCR amplification. The species of pathogenic *Leptospira* in this study have been described previously (Azhari *et al.*, 2018). Positive *Leptospira* were detected in *R. norvegicus* (N=20), *R. rattus* (N=6), *T. glis* (N=2) and *S. murinus* (N=1) as shown in Table 2. The study showed that *Leptospira* occurrence in the small mammals were significantly correlated to age category, with Spearman Correlation (r_s= -0.23; p=0.02). Adults were found to be more prevalent compared to juvenile hosts (Table 4). In addition, sampling phase was also significantly correlated to the positive *Leptospira* hosts, (r_s= 0.293; p<0.000), with March and June month of sampling contribute to high prevalence of *Leptospira* among the hosts. However, hosts species, gender, physical condition and pregnancy status did not correlate to positive *Leptospira* occurrence among the hosts (p>0.05). *Leptospira* incidence among the small mammals shows significant correlation with rainfall and relative humidity (r_s=-0.327, p<0.000), but there was not significant correlation with temperature (r_s= 0.014; p=0.852) (Table 3). Positive *Leptospira* animals were more prevalent during low rainfall (266.27±15.42mm), compared to high rainfall (374.05±7.57mm). Similarly, positive *Leptospira* animals were also more prevalent at lower relative humidity (72.07±0.49%) compared to higher relative humidity (72.956±0.18%).

Table 2. Prevalence of pathogenic *Leptospira* among small mammal hosts

Host species	Number of individuals captured	Number of positive *Leptospira*	Prevalence (%)
Rattus norvegicus	138	20	14.5
Rattus rattus	37	6	16.2
Tupaia glis	9	2	22.2
Suncus murinus	1	1	100
Total	185	29	15.67

Table 3. Environmental parameters at the study site based on sampling phases

Sampling phase (month)	Rainfall (mm)	Relative humidity (%)	Temperature (°C)
1st (January)	435.6	73.2	29.1
2nd (March)	186.2	70.4	30.a0
3rd (June)	288.4	72.2	29.0
4th (September)	269.0	71.4	28.9
5th (November)	475.4	81.0	27.5

Table 4. Bivariate analysis of small mammals on community characteristics using Spearman correlation

Variables	Spearman's correlation	P-value
Sampling phases	0.293	0.000*
Age category	-0.230	0.002*
Host species	0.620	0.401
Gender	0.740	0.317
Physical condition	-0.250	0.736
Pregnant status	0.002	0.976
Rainfall	-0.392	0.000*
Relative humidity	-0.327	0.000*
Temperature	0.14	0.852

* Characteristics that showed significant differences (P<0.05 by Spearman Correlation method).

R. norvegicus species (N=138), also showed significant correlation between positive *Leptospira* individuals with age and sampling phases (N=20, r_s=0.228; p=0.007 and r_s=227; p=0.007) as shown in Table 5. Prevalent was still higher among adults, whereby March and June were also correlated to high incidence of *Leptospira*. *Leptospira* incidence was significantly correlated to rainfall (r_s=-0.319; p=0.000), with higher prevalent in lower rainfall level (271.64±21,17mm) compared to higher rainfall level (364.68±9.07mm). In contrast, *Leptospira* incidence among *R. rattus* (N=37) did not show significant correlation to any community structure variables except sampling phases (r_s=571; p=0.000)

Table 5. Bivariate analysis of *Rattus norvegicus* and *Rattus rattus* on population characteristics using Spearman correlation

Host species	Variables	Spearman's correlation	P-value
Rattus norvegicus	Sampling phase	0.227	0.007*
	Age category	-0.228	0.007*
	Gender	0.082	0.337
	Physical condition	-0.010	0.903
	Pregnant status	-0.080	0.352
	Rainfall	-0.319	0.000*
	Relative humidity	-0.088	0.306
	Temperature	0.112	0.190
Rattus rattus	Sampling phase	0.571	0.000*
	Age category	0.246	0.142
	Gender	-0.041	0.810
	Physical condition	-0.029	0.865
	Pregnant status	-0.131	0.441
	Rainfall	-0.750	0.000*
	Relative humidity	-0.4	0.014*
	Temperature	0.297	0.075

* Characteristics that showed significant differences (P<0.05 by Spearman Correlation method).

which also coincide with rainfall (N=6, r_s=-0.750; p=0.000) and relative humidity (N=31, r_s=-0.4; p=0.014) which falls during March and June sampling phase (Table 5). Similar to above, *Leptospira* prevalent among this species were also higher during lower rainfall (254.33±21.55mm) and lower relative humidity (71.57±0.37%).

DISCUSSION

From this study, the overall prevalence of *Leptospira* infection among small mammal hosts in urban environment (15.7%) was fairly similar to finding from Loan *et al.* (2015) in Mekong Delta, Vietnam with 18.3%. However, study by Benacer *et al.* (2013) in the city of Kuala Lumpur, Malaysia revealed that the infection rate of *Leptospira* through PCR using *Leptospira* cultures was low among urban rats (6.7%, N=20/300). Ironically, the prevalence in these study were low as compared to other parts of the world such as Denmark, 48-89% (Krojgaard *et al.*, 2009), and Canada, 79% (Minter *et al.*, 2019).

In this study, the most dominant rat species captured was *R. norvegicus* where its distribution and abundance have been reported previously by Zain *et al.* (2012) in Kuala Lumpur, Pulau Pinang (Oyedele *et al.*, 2015) and Selangor (Azhari *et al.*, 2018; Yusof *et al.*, 2019). This rat species is commonly known as Norway rat, one of the invasive species and adapted well in urban ecosystems (Feng *et al.*, 2014). This species can be found living near to human habitations and depends on human resources and wastes for survival (Feng *et al.*, 2014). From our study, *R. norvegicus* was found to be the third most prevalent in *Leptospira* infection, after *T. glis* and *R. rattus*. This species has been reported as a reservoir of leptospires bacteria in both tropical and temperate cities (Desvars *et al.*, 2011; Costa *et al.*, 2014; Pui *et al.*, 2017). A study conducted in Salvador, Brazil, found that the different locations of capture, sex, and age of the *R. norvegicus* population, influenced the amount of leptospiral infections, maintenance and spirochete loads shed in their urine, which polluted the environment eventually leading to human infection (Costa *et al.*, 2015). In addition, this species was also shown as the most commonly infected rat species in the Mekong Delta of Vietnam (Loan *et al.*, 2015), Thailand (Doungchawee *et al.*, 2005), and Cambodia (Ivanova *et al.*, 2012). Faria *et al.* (2008) described that higher population density of this species was the main factors that facilitates the transmission of leptospirosis disease in Brazil.

Similarly, *R. rattus*, the next most dominant species in urban areas were also known to be reservoir hosts of leptospires transmitted to humans in urban locations (Koizumi *et al.*, 2009). A study in urban areas in Sarawak, Malaysia by Pui *et al.* (2017) also reported the occurrence of pathogenic *Leptospira* in *R. rattus*. This rodent species can adapt very well to various climatic and environmental conditions, and were among the major domestic rats found in urban areas, open fields, and residential areas of Malaysia. In fact, a study of the distribution of urban rodents in Kuala Lumpur revealed that 91.8% (89/97) of rodents trapped were *R. rattus* (Paramasvaran *et al.*, 2013). This case study is in line with previous research works conducted by (Benacer *et al.*, 2013; Benacer *et al.*, 2016) in Kuala Lumpur and Sarawak (Pui *et al.*, 2017) which identified house rat, *R. rattus* as the most prevalent *Leptospira* host that dominated urban cities. This black rat's species was found in various habitat types in Malaysia including forest reserve (Zakaria *et al.*, 2001), recreational forest (Ishak *et al.*, 2018) and largely in urban and village areas that are close to human habitation (Nur Syazana *et al.*, 2013; Yusof *et al.*, 2019). This species can adapt very well to the climatic and environmental conditions as hosts of leptospires of human health importance and can be found throughout the world from their source populations in SE Asia and China respectively (Aplin *et al.*, 2011). The ability of these commensal rats to utilize garbage for food and rubbish piles for shelters made them a perfect reservoir hosts for the transmission of various infectious diseases that associated with human activities (Nkogwe *et al.*, 2011).

On another note, this study shed very important finding that the common treeshrew (*T. glis*) was also shown to carry *Leptospira* with 22.2% prevalent. This species is active during the day (diurnal) and can be found on the ground searching for foods (Francis, 2008). The foraging behaviour of this species on the ground would increase the chances of acquired *Leptospira* infection. In addition, Mariana *et al.* (2010) had demonstrated that this common treeshrew as potential carriers of ticks from wild into the houses. There were few studies on the prevalence of *Leptospira* in this species as previously recorded in urban settlement area in Kajang, Selangor by Azhari *et al.* (2018). Next, *Suncus murinus* is shown as a potential reservoir of leptospirosis (Kundin *et al.*, 1970). Although their life patterns and living habits have not been studied in any great detail, they appear to occupy an intermediate ecological niche between the essentially interior house mouse and the exterior commensal rats (Kundin *et al.*, 1970). They are found sometimes inside the house but nesting and generally foraging outside the village and farmyard (Lim, 2015). In addition, this species has shown to be the carrier for *Leptospira* recorded in Indonesia (Widiastuti *et al.*, 2016), Sri Lanka (Yathramullage & Meegaskumbura, 2016), Cambodia (Ivanova *et al.*, 2012) and Kajang, Malaysia (Azhari *et al.*, 2018).

Based on the age category, adults were significantly more likely to be infected with *Leptospira* than sub-adult and juvenile hosts. Studies by Krojgaard *et al.* (2009) also found that the prevalence of *Leptospira* increased with age. The finding is in agreements with other research studies which showed that adults have higher infection rate of *Leptospira* compared to immature individuals (Ivanova *et al.*, 2012; Benacer *et al.*, 2013; Costa *et al.*, 2015). This is further exemplified in the work undertaken by Himsworth *et al.* (2013) that proved mature rats were infected more frequently with *L. interrogans* compared to immature rats in an inner-city neighbourhood of Canada. Similar results have been reported by Loan *et al.* (2015) in Vietnam in which higher prevalence of infection among older rats were detected through RT-PCR and MAT tests. Furthermore, Minter *et al.* (2019) found that rat characteristics (age and wounds) are associated with *Leptospira* infection among Norway rats in two different cities of Brazil. He found that older rats with more wounds were more likely became carrier of *L. interrogans*. Besides, specific behaviour characters such as fighting and biting among adult rats accelerate the transmission events of *Leptospira* among them (Mohammed-Hassan *et al.*, 2012). Longer period of movement and wider coverage areas by adult host also increased the risk of exposure to the *Leptospira* infection (Benacer *et al.*, 2016). Meanwhile, the movement pattern among juvenile hosts mostly limited around areas of nesting burrows, thus, the infection rate was low (Benacer *et al.*, 2013).

Based on our results, highest infection of *Leptospira* among host were significantly correlated with sampling phases especially during dry period (March and June), in concordance with the dry season in Malaysia which occurred between March until September (Benacer *et al.*, 2016). These findings were contrasting with other previous studies in Cambodia (Ivanova *et al.*, 2012), Vietnam (Loan *et al.*, 2015) and Malaysia (Benacer *et al.*, 2016) which found that the incidence of leptospirosis among rats were significantly higher during wet season compared to dry season. Weather trends in certain areas affects the incidence of leptospirosis as there has been an increasing number of positive *Leptospira* host from dry season (68%) to wet season (78%) in the urban environment (Biscornet *et al.*, 2017). According to Garba *et al.* (2018), majority of leptospirosis outbreak cases occurred after heavy rainfall and flooding which has caused the rise in the number of reported cases in this country. An overflow of water from flooding events help washed out the animal's burrow and facilitate the environmental transmission of *Leptospira* among rats and humans (Himsworth *et al.*, 2013). Temperature was proven to be one of the environmental factors that influence the transmission of leptospirosis disease (Lau *et al.*, 2010). However, higher temperatures and

warm weather are able to maintain and prolong the survival of leptospires for longer periods of time (Levett, 2001; Lau *et al*., 2010). A surveillance studies conducted from 1999 to 2005 in Argentina revealed that 76% of reported leptospirosis cases occurred during warmer and wetter periods (Vanasco *et al*., 2008). Furthermore, higher temperatures tend to reduce surface water availability by evaporation process and thus encourage water-based activities for humans and animals such as bathing, swimming and drinking, thereby increased the chances of contact between humans, livestock, pets and wildlife through more intense sharing of shrinking surface water sources (Dufour *et al*., 2008; Lau *et al*., 2010). Rats that have a constant contact with contaminated water have higher prevalence of *Leptospira* (Krojgaard *et al*., 2009). Therefore, our study suggest that dry and warm weather are crucial for *Leptospira* transmission.

CONCLUSION

This case study highlights the important insights on population structure of small mammals towards *Leptospira* prevalence. March to June which conincide with periods of low rainfall and low relative humidity were proven to harbour higher incidence of *Leptospira* prevalence among small mammal, which could potentially be transmitted to human. Therefore, specific control measure needs to be taken in these periods to control for the hosts, as well as their habitat. Massive eradication of these small mammal *Leptospira* reservoirs was particularly effective especially in the urban areas during these periods, to control for the disease transmission. Thus, these could be a key point to consider in future risk management and prevention efforts in urban environments.

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