Prevalence of *Leptospira* spp. in Urine of Rats (*Rattus* spp.) in an Urban Village in the Philippines using LAMP and PCR Assays

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Abstract— *Rats are recognized as key reservoirs and potential transmission sources of leptospirosis. Despite this, limited data exist on the prevalence of Leptospira spp. among rats in urban villages of the Philippines. This study aimed to assess the presence of Leptospira spp. in rats from an urban village in Benguet Province. Urine samples from 50 rats were analyzed using Loop-Mediated Isothermal Amplification (LAMP) and Polymerase Chain Reaction (PCR) assays. Results showed a Leptospira spp. detection rate of 30% using LAMP and 18% using PCR. DNA sequencing confirmed the presence of Leptospira interrogans serovar Icterohemorrhagiae, suggesting that rats may play a significant role in leptospirosis transmission in the study area. Diagnostic performance analysis revealed that LAMP and PCR had substantial agreement, with a Kappa coefficient of 0.677. Compared to PCR, LAMP demonstrated a diagnostic sensitivity of 100% and a specificity of 85.37%. These findings underscore the importance of continuous monitoring of rodent populations in urban settings and highlight the potential utility of LAMP as a rapid screening tool for leptospiral infections*.

Keywords— Prevalence, Leptospira spp., Rats, LAMP, PCR.

I. INTRODUCTION

Leptospirosis is a widespread bacterial zoonosis and a significant public health concern in Southeast Asia, including the Philippines. It is recognized both as a neglected zoonotic disease (NZD) and a neglected tropical disease (NTD), with increasing reports of outbreaks and severe cases globally. The disease primarily affects impoverished urban and rural populations, particularly in environments that support rodent-borne transmission.

Globally, leptospirosis accounts for over one million severe human cases annually, with the Philippines ranked among the countries with high incidence rates. Leptospirosis is endemic throughout the country and poses a serious health threat, especially in densely populated and flood-prone urban areas. Although most reported cases are concentrated in Regions VI, III, and the National Capital Region, the Cordillera Administrative Region (CAR) reports the highest case fatality rate (CFR) at 15%, despite a lower number of cases. This highlights the need for targeted surveillance and control strategies in this region.

Rats are the primary reservoir hosts for pathogenic *Leptospira* spp., playing a critical role in the transmission cycle. Previous studies have detected multiple *Leptospira* serovars in both humans and rats in the Philippines, suggesting zoonotic transmission. However, data on the status of *Leptospira* infection in rats, particularly in urban villages of La Trinidad, Benguet, remain scarce.

This study aims to determine the prevalence of *Leptospira* spp. in rats in an urban village in Benguet Province using molecular detection techniques—Loop-Mediated Isothermal Amplification (LAMP) and Polymerase Chain Reaction (PCR). The study also evaluates the diagnostic agreement and performance of LAMP relative to PCR. By identifying the circulating *Leptospira* strains and estimating infection rates in rats, the study contributes valuable baseline data that may inform local public health interventions and surveillance strategies.

II. MATERIALS AND METHODS

A total of 50 rats (*Rattus* spp.) were captured using Tomahawk live traps strategically deployed in Betag, La Trinidad, Benguet. Twenty-five rats were trapped within a five-meter radius of selected residential dwellings, while the remaining twenty-five were captured from garden areas within the same locality.

Captured rats were humanely sedated, and urine samples were collected via cystocentesis using a 3 mL syringe fitted with a 23-gauge needle. The urine samples were immediately transferred to 2 mL microcentrifuge tubes and stored at 4°C. DNA extraction was performed shortly thereafter, and the extracted DNA was stored under refrigeration for no longer than five days before molecular analysis.

Detection of *Leptospira* spp. was conducted using Loop-Mediated Isothermal Amplification (LAMP), following the protocol described by Koizumi *et al*. (2012), alongside conventional Polymerase Chain Reaction (PCR) assays. Urine samples that exhibited the darkest bands during agarose gel electrophoresis were selected for DNA sequencing to confirm and identify the *Leptospira* species present.

III. RESULTS AND DISCUSSIONS

3.1 Prevalence of *Leptospira* spp. in Rats:

The prevalence of *Leptospira* spp. in the 50 captured rats was determined using two molecular assays: Loop-Mediated Isothermal Amplification (LAMP) and Polymerase Chain Reaction (PCR). As shown in Table 1, LAMP detected *Leptospira* DNA in 15 of 50 samples, yielding a prevalence of 30% (95% CI: 17.3–42.7), while PCR detected 9 positive samples, corresponding to a prevalence of 18% (95% CI: 7.35–28.65).

TABLE 1
PREVALENCE OF *LEPTOSPIRA SPP.* IN RAT URINE SAMPLES (n = 50)

Test	Positive	Negative	Positivity Rate (%) (95% CI)
LAMP	15	35	30% (17.3–42.7)
PCR	9	41	18% (7.35–28.65)

The detection of *Leptospira* spp. in a considerable proportion of rats confirms their role as reservoirs and highlights ongoing environmental circulation of the pathogen. This finding is consistent with previous reports that have emphasized rats as important sources of *Leptospira* transmission in both humans and animals (Koizumi *et al*., 2012; Bonilla-Santiago & Nally, 2011).

Environmental and ecological factors may contribute to the relatively high prevalence observed. Betag is the only barangay in La Trinidad with predominantly flat terrain, potentially allowing the accumulation of contaminated water during rainy months. Floodwaters, which may contain *Leptospira*, are known risk factors for both rodent infections and human exposure (Yanagihara *et al*., 2007). Notably, this study was conducted during the rainy season (August to September), a period associated with peak leptospirosis incidence (Guerra, 2009; Per *et al*., 2011). Previous research has also shown that low-slope areas and flooding increase *Leptospira* prevalence in rodent populations (Ivanova *et al*., 2012).

The presence of infected rats near agricultural zones, such as the La Trinidad Strawberry Fields—currently being developed as an eco-tourism destination—raises concern for zoonotic transmission. Residents and tourists engaging in fieldwork or recreational activities may be exposed to contaminated soil or water, increasing the risk of infection. Additionally, with 35% of the population engaged in farming or livestock production, and an estimated dog population of 800, potential transmission to animals and humans is a significant concern. Leptospirosis in animals can result in reproductive losses and reduced productivity, leading to economic burdens for local farmers (Acha *et al*., 2003).

3.2 Diagnostic Comparison of LAMP and PCR:

The comparative diagnostic performance of LAMP and PCR is shown in Table 2.

TABLE 2
DIAGNOSTIC COMPARISON OF LAMP AND PCR RESULTS (n = 50)

	PCR Positive	PCR Negative	Total
LAMP +	9	6	15
LAMP –	0	35	35
Total	9	41	50

When compared to PCR, LAMP demonstrated a diagnostic sensitivity of 100% and specificity of 85.37% (95% CI: 74.55–96.19). The calculated Kappa coefficient was 0.677 (95% CI: 0.41–0.94), indicating substantial agreement between the two assays and an overall concordance of 88%.

The higher sensitivity of LAMP may be attributed to its ability to detect low concentrations of DNA. Table 3 shows the DNA concentrations in *Leptospira*-positive urine samples. The six samples that were LAMP-positive but PCR-negative had the lowest DNA concentrations, supporting the premise that LAMP performs better in low-DNA scenarios.

TABLE 3
DNA CONCENTRATIONS OF LAMP-POSITIVE URINE SAMPLES

Sample No.	DNA Concentration (ng/µl)	LAMP	PCR
6	46.5	+	+
8	120.0	+	+
11	232.0	+	+
13	88.0	+	+
18	17.9	+	+
20	76.0	+	+
21	83.0	+	+
22	76.0	+	+
41*	2.5	+	–
43*	28.0	+	–
45*	12.0	+	–
46*	9.5	+	–
48	51.0	+	+
49*	2.1	+	–
50*	24.1	+	–

Samples undetected by PCR

These findings align with previous studies reporting the superior sensitivity of LAMP over PCR in detecting *Leptospira* spp., especially in field conditions or low-resource settings (Koizumi *et al*., 2012; Dhama *et al*., 2014). LAMP's ability to operate at a constant temperature and its tolerance to common PCR inhibitors (e.g., hemoglobin, urea, salts) makes it a practical choice for rapid diagnostics in the field (Kaneko *et al*., 2007; Francois *et al*., 2011). Unlike PCR, which requires sophisticated equipment, LAMP is cost-effective, user-friendly, and suitable for decentralized testing in developing countries.

3.3 Identification of *Leptospira* Strain

Sequencing of the PCR-amplified product confirmed the identity of the pathogen as *Leptospira interrogans* serovar Icterohaemorrhagiae. This serovar is a well-known cause of severe leptospirosis in humans and dogs and is commonly associated with rats, particularly *Rattus norvegicus*, as primary reservoir hosts (Adler & de la Peña, 2009; Thiermann, 1981).

Studies have shown that rats infected with this serovar may become chronic carriers, shedding leptospires in urine for over seven months, thereby perpetuating environmental contamination (Thiermann, 1981). Transmission to humans and animals can occur through direct or indirect contact with infected urine or contaminated water or soil, especially during agricultural, recreational, or occupational activities (Wasin´ski & Dutkiewicz, 2013; Koizumi *et al*., 2009).

IV. CONCLUSION

This study is the first to molecularly detect the presence of *Leptospira* spp. in rats within an urban barangay in La Trinidad, Benguet, Philippines, providing novel insights into the epidemiology of leptospirosis in this area. The use of LAMP and PCR assays revealed that 30% and 18% of the 50 captured rats tested positive for *Leptospira* DNA, respectively. LAMP demonstrated higher sensitivity, effectively identifying positive samples with low DNA concentrations that PCR failed to detect. Diagnostic evaluation of LAMP against PCR indicated a sensitivity of 100% and specificity of 85.37%, with substantial agreement between the two assays ($\kappa = 0.677$), highlighting LAMP's potential as a practical and reliable diagnostic tool, particularly in low-resource settings and field conditions.

One of the most significant findings of this study was the identification of *Leptospira interrogans* serovar Icterohaemorrhagiae through DNA sequencing. This highly pathogenic strain is known to cause severe leptospirosis in both humans and animals. The identification of this serovar in rats suggests that it may be circulating within the local rodent population, particularly *Rattus norvegicus*, which are likely the primary reservoirs of the pathogen. Although sequencing was conducted on only one strongly positive sample, the result is consistent with previous reports of *Icterohaemorrhagiae* as a common cause of zoonotic transmission through rats.

The relatively high prevalence of *Leptospira* spp. detected in this study may be influenced by environmental and geographical factors in Betag, a low-lying area with relatively flat terrain, which may promote water stagnation and the persistence of leptospires, especially during the rainy season when the study was conducted. These conditions, combined with agricultural activities and backyard livestock farming, increase the risk of human and animal exposure to contaminated water and soil, underscoring the need for improved environmental management.

The findings of this study offer significant contributions to our understanding of the role of rats as reservoirs of *Leptospira* spp. in urbanizing agricultural settings and suggest a potentially underestimated public health threat in La Trinidad. However, there are limitations in this study, such as the small sample size and the lack of analysis of rat species, age, and sex, which have been shown to influence the carriage of *Leptospira* (Ivanova *et al*., 2012; Levett, 2001). Future studies with larger sample sizes, incorporating these variables, and including human and other animal populations, would provide a more comprehensive understanding of the transmission dynamics of leptospirosis in the region.

Based on the results of this study, several potential applications emerge. First, LAMP's high sensitivity and practical applicability in low-resource settings suggest it could be widely used for early diagnosis and surveillance of leptospirosis in rural and urban settings. This diagnostic approach could prove invaluable for rapidly detecting and controlling outbreaks, especially in areas where PCR testing is not available. Additionally, the identification of *Leptospira interrogans* serovar Icterohaemorrhagiae as circulating in rats underscores the need for ongoing molecular surveillance of the pathogen in rodent populations, which could help monitor the spread of this zoonotic disease. The use of LAMP in regular monitoring programs, alongside molecular surveillance of different Leptospira serovars, would help public health authorities assess and manage the risk of leptospirosis more effectively, ultimately contributing to better prevention and control strategies in endemic regions.

In conclusion, the results of this study highlight the need for continued vigilance and action to mitigate the risk of leptospirosis in La Trinidad, Benguet, and similar urban agricultural settings. The use of LAMP as a diagnostic tool, along with improved rodent control, environmental management, and public health education, will be crucial in reducing the burden of leptospirosis in both human and animal populations. Future research with larger sample sizes and broader surveillance will be essential for further elucidating the transmission dynamics of *Leptospira* spp. and for developing effective control strategies.

RECOMMENDATIONS

Based on the findings of this study, several recommendations are made to mitigate the risk of leptospirosis transmission in La Trinidad, Benguet, and similar areas. First, effective rodent control measures should be implemented in both urban and agricultural areas, including targeted rodent trapping, improved environmental sanitation, and better waste management, to reduce the rat population, which serves as a significant reservoir for *Leptospira*. Additionally, improving flood control infrastructure and drainage systems is crucial, as the low-lying terrain and flooding in the study area may contribute to the persistence of leptospires in the environment. This can help minimize standing water, a key habitat for the survival of *Leptospira*, and reduce the risk of environmental contamination.

Community awareness and education are also critical. Local public health campaigns should focus on educating people, particularly those working in agriculture and areas with high rat populations, about the risks of leptospirosis and preventive measures, such as using protective gear, properly disposing of waste, and avoiding contact with potentially contaminated water sources. Regular surveillance of rodent populations is also recommended to monitor the spread of leptospirosis and identify areas of higher risk. Rapid diagnostic methods such as LAMP can be used for ongoing monitoring to guide public health responses more effectively.

Given the potential for leptospirosis transmission to livestock and domestic animals, veterinary monitoring and health programs should be strengthened. Livestock owners should be educated about the risks of leptospirosis and encouraged to adopt preventive measures to protect both animals and humans. Furthermore, continued molecular surveillance is essential to explore the full range of *Leptospira* serovars circulating in the area, providing valuable information for targeted diagnostic and control strategies. Sequencing additional positive samples will deepen our understanding of the strains in circulation and help inform future public health interventions.

In addition, future research should consider including variables such as the species, age, and sex of rats, as these factors have been shown to influence the likelihood of *Leptospira* carriage (Ivanova *et al*., 2012; Levett, 2001). Incorporating these variables would provide more detailed insights into the epidemiology of leptospirosis and improve understanding of how the disease is transmitted in urban and agricultural environments. Furthermore, studies with larger sample sizes, including both humans and other animals, would be beneficial to determine the full transmission cycle of leptospirosis and assess the status of this zoonosis.

Lastly, a One Health approach that integrates human, animal, and environmental health sectors should be adopted. This approach would facilitate collaboration between local health authorities, agricultural departments, environmental agencies, and the community, ensuring a coordinated response to leptospirosis and enhancing overall public health management. By addressing these recommendations, local authorities can better manage and reduce the risk of leptospirosis in both human and animal populations, ultimately improving public health in La Trinidad and surrounding areas.

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