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Literature Review: Comparison of Leptospirosis Diagnostic Methods in Southeast Asia

Tinjauan Literatur: Perbandingan Metode Diagnosis Leptospirosis di Asia Tenggara

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Abstract

Introduction: Leptospirosis remains a significant zoonotic challenge in tropical areas, including nations within the Southeast Asian region, accompanied by persistently high rates of morbidity and mortality. Accurate diagnosis is crucial, as clinical manifestations often mimic other tropical febrile illnesses. **Objective:** This study aims to comprehensively review various diagnostic methods utilized across Southeast Asia by applying a One Health framework to synergize data from human, animal, and environmental sectors. **Methods:** A systematic literature review was conducted on 33 selected articles published between 2013 and 2025. **Results:** The results indicate that the Microscopic Agglutination Test (MAT) remains the reference standard (specificity 75-86%), despite its low sensitivity during the acute phase (14-68%). Meanwhile, molecular techniques such as PCR and LAMP demonstrate the highest accuracy (sensitivity 90-100%), with LAMP exhibiting a minimal detection threshold suitable for resource-limited settings. **Conclusions:** This review concludes that an integrated diagnostic strategy combining rapid screening with molecular confirmation, alongside cross-sectoral surveillance, is essential for the effective control of leptospirosis in Southeast Asia.

Keywords: comparison; diagnostic; leptospirosis

Abstrak

Pendahuluan: Leptospirosis tetap menjadi tantangan zoonosis yang signifikan di kawasan tropis, termasuk negara-negara di wilayah Asia Tenggara, disertai tingkat kejadian penyakit serta angka kematian yang tetap signifikan. Diagnosis yang akurat sangat krusial karena manifestasi klinisnya sering menyerupai demam tropis lainnya. **Tujuan:** Penelitian ini bertujuan untuk meninjau secara komprehensif berbagai metode diagnosis yang digunakan di Asia Tenggara dengan menerapkan kerangka kerja Kesehatan Tunggal (*One Health*) guna mensinergikan informasi dari sektor lingkungan, populasi hewan, serta kesehatan manusia. **Metode:** Tinjauan literatur sistematis dilakukan terhadap 33 artikel terpilih yang diterbitkan antara tahun 2013 dan 2025. **Hasil:** Hasil menunjukkan bahwa *Microscopic Agglutination Test* (MAT) tetap menjadi standar referensi (spesifisitas 75-86%), meskipun sensitivitasnya rendah pada fase akut (14-68%). Sementara itu, teknik molekuler seperti PCR dan LAMP menunjukkan akurasi tertinggi (sensitivitas 90-100%), di mana LAMP memiliki ambang batas deteksi minimal (*minimal detection threshold*) yang sangat baik untuk wilayah dengan sumber daya terbatas. **Kesimpulan:** Kajian ini menyimpulkan bahwa strategi diagnostik terpadu yang menggabungkan skrining cepat dengan konfirmasi molekuler serta pengawasan lintas sektor sangat penting untuk pengendalian leptospirosis yang efektif di Asia Tenggara.

Kata kunci: perbandingan; diagnosis; leptospirosis

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1. Introduction

Leptospirosis has become one of the significant zoonotic infection in tropical regions, stemming from Gram-negative bacteria within the *leptospira* genus (Anggraini, 2024); Grillová et al., 2021). It is reported an annual global incidence exceeding 1.03 million cases with mortality reaching 58,900 annual fatalities on a global scale (Nugroho et al., 2023), and about 73% of cases occur in tropical areas such as Southeast Asia (Rajapakse, 2022). In 2019, 920 cases with 122 deaths due to leptospirosis were recorded in Indonesia (WHO, 2020). The annual incidence during 2010-2020 ranged from 8.6-17.2 per 100,000 residents in Malaysia (Philip & Ahmed, 2023). Meanwhile, Thailand reported a total of around 25,006 cases (average ~3.8 per 100,000 population) during 2013–2022 (Sawangpol et al., 2025). Some other countries have also experienced increasing trends. For example, the Philippines recorded a large surge from 182 instance in 2020, rising to 1,661 and 2,794 cases during 2021 and 2022 respectively (Nazir et al., 2024). These case data indicate that leptospirosis represents an important disease burden in Southeast Asia, with variations in incidence influenced by climate, population density, and local surveillance practices.

The disease is transmitted to humans through direct contact with the urine of infected animals (especially rats) or indirectly through water and environments contaminated by infected urine (Rajapakse, 2022). The symptoms range from mild (fever, muscle pain) to severe illness such as Weil's syndrome with icterus and systemic organ dysfunction, so it often resembles other tropical febrile illnesses

(dengue, typhoid, malaria, rickettsioses) (Samrot et al., 2021); (Rajapakse, 2022). This makes clinical diagnosis difficult and unreliable on its own. The difficulty of clinical diagnosis is an important problem in controlling leptospirosis. The ambiguous clinical symptoms of leptospirosis, which often mimic other diseases, make it highly dependent on accurate diagnostic methods and laboratory confirmation (Valente et al., 2024).

In general, leptospirosis diagnosis involves an integration of primary and secondary diagnostic approaches. Direct identification involves bacterial culture and PCR, while indirect diagnostic techniques consist of serological tests such as MAT, ELISA, or lateral flow rapid tests (RDT) (Sykes et al., 2022). However, the implementation of diagnostics in Southeast Asia is hindered by limitations in facilities and skilled personnel. Access to leptospirosis diagnostics in Indonesia is limited because only a few laboratories provide MAT and PCR (Gasem et al., 2020). Similarly in Malaysia, most diagnoses rely on serology because culture and PCR are rarely available and few can perform MAT due to limitations in live culture and experienced staff (Hii et al., 2021). This situation causes many leptospirosis cases to go undetected and be diagnosed late.

Considering the high disease burden and various challenges in the diagnostic process, an in-depth review of leptospirosis diagnostic methods in the Southeast Asia region is very important. To address the complexity of its transmission, this review integrates diagnostic data from human populaton, animal reservoirs, and contaminated habitats, adhering to an integrated One Health framework. This holistic strategy is crucial because precise detection within animal populations and surrounding ecosystems directly informs human risk assessment and early outbreak surveillance in the region (WHO, 2022). This study comprehensively reviews the diagnostic methods used, taking into account the high incidence and limitations of laboratory facilities in tropical areas. The focus of this review is to discuss the advantages and disadvantages of methods such as bacterial culture, PCR, and serological testing in the context of limited resources commonly faced by countries in Southeast Asia. This synthesis seeks to establish a robust scientific foundation for formulating strategic recommendations in selecting and developing rapid, effective diagnostic methods tailored to local conditions, thereby supporting early detection and enhanced control of leptospirosis.

2. Method

A systematic literature review (SLR) methodology was implemented for this study to evaluate and compare diagnostic methods for Leptospirosis in Southeast Asia. The research began with a comprehensive electronic search across three major academic databases: PubMed, ScienceDirect, and Google Scholar, covering the period from 2013 to 2025. To ensure a focused and precise search, specific keywords were utilized in combination with Boolean operators: *(*Leptospirosis or *Leptospira)* and *(*diagnostic methods or laboratory diagnosis OR sensitivity and specificity) and (Southeast Asia: Indonesia, Malaysia, Thailand, Philippines and Vietnam).

To maintain the scientific rigor of the synthesis, strict eligibility criteria were applied. Inclusion criteria required that the articles be original research or primary studies, focusing on the clinical efficacy of diagnostics in humans, zoonotic hosts, or environmental samples within the Southeast Asian region, and available in open-access full-text format. Conversely, certain papers were disqualified if they were case reports, brief notes, or focused solely on clinical symptoms without providing laboratory diagnostic data. Additionally, any research published before 2013 or conducted outside the geographical scope of Southeast Asia was omitted from the analysis.

The process of study selection followed a path guided by the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines. Identification during the primary search resulted in 420 initial records, with 120 PubMed entries, 150 ScienceDirect, and 150 Google Scholar. After the removal of 127 duplicate records, 293 articles underwent title and abstract screening. During this phase, 130 articles were excluded for failing to align with the core thematic focus. The remaining 163 complete manuscripts underwent rigorous assessment for eligibility against the inclusion and exclusion criteria. Ultimately, 33 articles were selected for qualitative synthesis and inclusion in the systematic analysis. Data extraction centered on aspects of diagnostic techniques, values for sensitivity and specificity, and various regional contexts. The detailed steps of how the literature was selected are illustrated in the PRISMA flow diagram in figure 1.

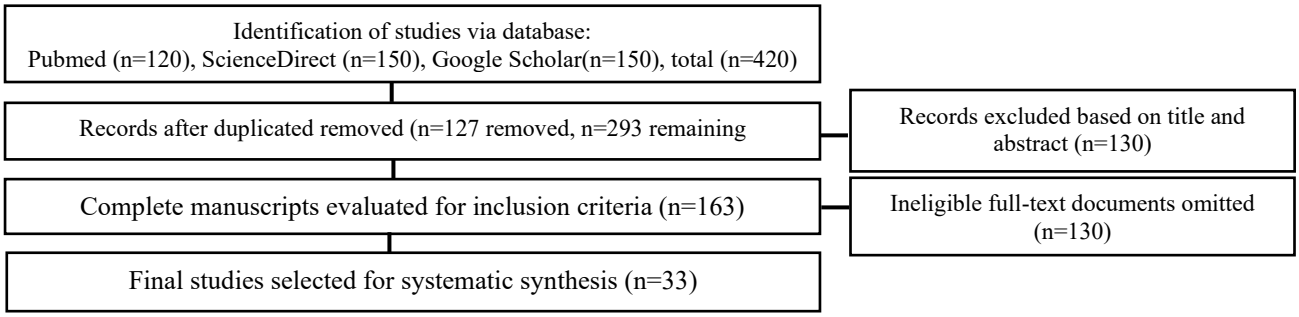

Figure 1. PRISMA Flow diagram outlining to identify and choose research focusing on *Leptospira* detection techniques across Southeast Asia

3. Result and Discussion

The systematic selection process identified 33 articles that satisfied the predefined eligibility requirements. The essential attributes of these selected studies, such as authorship, diagnostic techniques, study locations, and major outcomes, are synthesized in table 1. This table summarizes literature review results from various studies, detailing the diagnostic methods and main findings across the region. The diagnostic accuracy of several screening tools applied for identifying leptospirosis in the region is detailed in Table 2, focusing on their respective sensitivity and specificity rates in tabel 2.

The identification of leptospirosis relies on combining both primary and secondary diagnostic approaches. Primary techniques consist of PCR and bacterial cultivation, whereas secondary options involve serology-based assessments like ELISA, lateral flow rapid tests (RDT), or the microscopic agglutination test (MAT) (Sykes et al., 2022). For serological diagnosis, MAT is widely recognized as the definitive reference standard (Oyamada et al., 2021). Between 2010 and 2015, a study in Thailand by Chadsuthi et al. (2017) examined various *leptospira*l serovars across human and animal populations (including pigs, cattle, and buffalo). A 23-serovar MAT panel was utilized to screen all serum samples for the presence of *Leptospira*-specific antibodies. The findings indicated that the Shermani serovar prevailed in both humans and livestock, with specific seroprevalence rates of 11.3% in pigs, 24.8% in buffalo, 28.1% in cattle, and 23.7% in humans (Chadsuthi et al., 2017). Additionally, research by Mai et al. (2021) employed a 25-serovar MAT and observed elevated antibody levels in animals living near human settlements. They identified a total of 17 different serovars, with prevalence data showing 16% in rats, 12.2% in cats, 32.9% in dogs, 10.2% in pigs, 24.9% in cattle, and 44.2% in water buffalo (Mai et al., 2021). MAT has a sensitivity of 14% in single acute-phase samples (specificity 86%) and increases in paired sera up to 68% (specificity 75%) and remains the validation standard because it can identify many serovars (Valente et al., 2024). However, this test is less effective for early diagnosis, has a complex procedure, requires local serovar cultures, and can only be performed in reference laboratories (Mgode et al., 2015). The infrastructure limitations in Southeast Asia (e.g., limited MAT facilities in Indonesia/Malaysia) reduce widespread use of MAT. The advantage of MAT is its relatively high specificity (~75-86%), so positive results are generally reliable. However, because antibody response takes time, MAT is less helpful for early diagnosis in acute patients.

Meanwhile, the Enzyme-Linked Immunosorbent Assay (ELISA) functions as a serology-based technique that detects specific antigens or antibodies through an enzymatic reaction that produces a color change read by a spectrophotometer (Amini et al., 2023). Introduced in 1971, ELISA has been widely used in medical diagnostics and research due to its high sensitivity, low cost, and ability to process many samples, although it is prone to cross-reactions that can yield false positives (Ipandi et al., 2019). An Indonesian study on an in-house ELISA using the LipL32 antigen showed high effectiveness as an alternative to MAT (Sumarningsih et al., 2024), while in Thailand and Myanmar, the commercial Panbio ELISA showed a sensitivity of 71.4% but low specificity, making it less ideal as a standalone test (Dhawan et al., 2021). Dependence on ELISA is also seen in Malaysia and the Philippines due to limited laboratory resources, although it still carries the risk of false positives (Philip & Ahmed, 2023); (Nazir et al., 2024). In Vietnam and Thailand, ELISA is used for dengue and Japanese Encephalitis detection with sensitivities of 85-95%, although it is difficult to distinguish primary and secondary infections (Samrot et al., 2021). Overall, ELISA in Southeast Asia shows sensitivities of 70-99%, making it effective for diagnosis and surveillance, but it has limitations in specificity and speed of testing.

Furthermore, serological diagnosis with RDTs is widely used to evaluate rapid antibody-detection tests (IgM-based) as a quick screening alternative in primary healthcare settings, since gold-standard tests like MAT and culture are not practical in the field. A study in Indonesia by Handayani et al. (2025) evaluated four types of RDTs on 364 serum samples from acute febrile patients using MAT as the gold standard. It showed that although RDT results can be obtained quickly (±15-20 minutes) and are useful in endemic areas with limited resources, their sensitivity and specificity vary, and interpretation is influenced by fever onset time and reader agreement (Handayani et al., 2025). A similar situation was found in Thailand, where RDTs are considered relevant for early diagnosis but still have limitations because leptospirosis symptoms are not specific (Hwee et al., 2022). Dinhuzen et al. (2021) emphasized that RDT sensitivity ranges from 1.8–75%, with the best performance observed on days 4-6 after fever onset (Dinhuzen et al., 2021). The low sensitivity of 1.8% reported in some instances is consistent with this prospective study, which confirms that RDT performance is heavily influenced by the diagnostic window in the first three days of fever and the choice of reference standards used for evaluation. Therefore, while useful for initial screening, RDTs cannot be used as a standalone test and still require confirmation by MAT, culture, or qPCR (Dinhuzen et al., 2021).

Culture methods are also used in leptospirosis diagnosis and are considered the gold standard for *leptospira* isolation in diagnosis and research. In Indonesia, research by Prasetyo et al. (2024) on culturing rat kidney tissues showed that centrifugation at 3000 RPM for 5 minutes is effective in reducing contamination while maintaining *Leptospira* viability, with EMJH + STAFF media outperforming EMJH + V5FU (Prasetyo et al., 2024). Meanwhile, a study in Thailand successfully isolated 17 *Leptospira* strains from waterfall water using EMJH + 5-FU media, comprising pathogenic strains like *L. kmetyi* and

Table 1. Core attributes of research regarding leptospirosis diagnosis in Southeast Asia

Study title & authors	Diagnostic method used	Country	Main findings
(Mai et al., 2021). *Leptospira* infection among human-close-contact animals in different geographical areas in Vietnam	MAT (panel 25 serovars)	Vietnam	High *leptospira* antibody prevalence in contact animals: water buffalo 44.2%, cattle 24.9%, pigs 10.2%, with rats at 16%, dogs at 32.9%, and cats showing 12.2% (a total of 17 serovars were identified).
(Chadsuthi et al., 2017). Investigation on predominant *Leptospira* serovars and its distribution in humans and livestock in Thailand, 2010–2015	MAT	Thailand	Seroprevalence: humans 23.7%, water buffalo 24.8%, cattle 28.1%, pigs 11.3%; Shermani serovar dominant (humans and animals).
(Dhawan et al., 2021). Evaluation of the Panbio *Leptospira* IgM ELISA among Outpatients Attending Primary Care in Southeast Asia	ELISA IgM (Panbio)	Thailand, Myanmar	Sensitivity 71.4%, specificity 36.4% (compared to PCR). Specificity 42.7% when compared to MAT, indicating low accuracy as a single test.
(Sumarningsih et al., 2024), Development of in-house ELISA using recombinant LipL32 for detection of human leptospirosis in Indonesia	ELISA IgM in-house (LipL32 antigen)	Indonesia (Bogor)	9 of 11 MAT-positive sera were detected by ELISA LipL32; 1 of 3 MAT-negative sera showed a false reaction on ELISA (based on immunoblot).
(Handayani et al., 2025)., Accuracy of four rapid diagnostic tests (RDTs) for human leptospirosis diagnosis in Indonesia	RDT IgM (4 commercial products)	Indonesia	RDT-4 sensitivity (IgM *Leptospira* test) 83.6% (highest); other products 78.2%, 74.3%, 30.9%; combination of two RDTs improved diagnostic performance.
(Hwee et al., 2022) Rapid diagnostic test (Leptocheck-WB) for detection of acute leptospirosis: a meta-analysis of diagnostic accuracy	RDT Leptocheck-WB (meta-analysis)	Multicountry (Asia)	Meta-analysis of 10 studies: pooled sensitivity 75% (95% CI: 64-84%), specificity 87% (95% CI: 77–94%) against MAT as reference.
(Dinhuzen et al., 2021). A prospective study to evaluate the accuracy of rapid diagnostic tests for diagnosis of human leptospirosis (THAI-LEPTO AKI study)	RDT IgM (5 products)	Thailand	Overall RDT sensitivity low (1.8-75%), specificity 52.3-97.7%; Leptocheck-WB reached sensitivity 75% (highest).
(Utami et al., 2013) Examination of *Leptospira* bacteria in human blood samples suspected of leptospirosis using PCR method	PCR (PU1/SU1 primers, rrs)	Indonesia	Identification of DNA bands: 615 bp (pathogenic *Leptospira*) and 316 bp (saprophytic *Leptospira*).
(Krairojananan et al., 2020) Low prevalence of *Leptospira* carriage in rodents in leptospirosis-endemic northeastern Thailand	Real-time PCR (lipL32)	Thailand	*Leptospira* prevalence in rats 3.6% (18/495); species detected: L. borgpetersenii (n=3) and L. interrogans (n=15).
(Suwancharoen et al., 2016). Evaluation of loop-mediated isothermal amplification method (LAMP) for pathogenic *Leptospira* spp. detection with leptospires isolation and real-time PCR	Loop-Mediated Isothermal Amplification (LAMP)	Thailand	LAMP sensitivity 96.8%, specificity 97.0% (accuracy 97.0%), much better than real-time PCR (sens 74.2%, spec 93.0%). Low detection limit (10-100 copies).
(Tubalinal et al., 2018). Evaluation of LAMP for detection and/or screening of *Leptospira* spp. infection among domestic animals in the Philippines	Loop-Mediated Isothermal Amplification (LAMP)	Philippines	LAMP positivity rate: 29.0% (40/140 samples). Very high sensitivity with detection limit down to two *leptospira*l cells per reaction. LAMP results via gel electrophoresis also showed higher sensitivity than nPCR.
(Prasetyo et al., 2024). Effect of different speed and time of centrifugations on decontamination of *Leptospira* spp. cultures from rat's kidney	Culture with EMJH + STAFF & EMJH + V5FU media	Indonesia	Centrifugation 3000 rpm/5 minutes optimal to reduce contamination & maintain viability; EMJH+STAFF better than EMJH+V5FU.

(Chaiwattanarungruengpaisan et al., 2018). Potentially Pathogenic *Leptospira* Species Isolated from a Waterfall in Thailand	Culture EMJH + 5-FU	Thailand	A total of 17 isolates were recovered; these included pathogenic species (L. alstonii, L. kmetyi), intermediate (L. wolffii), as well as non-pathogenic strains; waterfalls may serve as natural reservoirs."
(Azali et al., 2016) Molecular Characterization of *Leptospira* spp. in Environmental Samples from North-Eastern Malaysia Revealed a Pathogenic Strain, *Leptospira* alstonii	Culture EMJH + 5-FU	Malaysia	33 isolates (22.9%) positive: 1 pathogenic (L. alstonii, 3%), 8 intermediate (L. wolffii, L. licerasiae), 22 non-pathogenic (L. meyeri). All MAT negative. Potential public health risk from pathogenic strains in market & recreational environments.

Table 2. Comparative performance of different techniques for detecting leptospirosis based on sensitivity and specificity within Southeast Asian countries

Method	Sensitivity (approx.)	Specificity (approx.)
MAT (Microscopic Agglutination Test)	14% (single acute-phase sample) -68% (paired sera)	75%-86%
ELISA	70%-99% (depending on kit & antigen)	Varies, 60%-95% (potential cross-reaction)
RDT (IgM rapid test)	1.8%-75% (highly dependent on onset day & kit)	52.3%-97.7% (kit-dependent)
Culture (EMJH etc.)	Low–moderate sensitivity (depends on sample quality & timing)	100% (conclusive isolation, highly specific)
PCR / qPCR / MLST	High during bacteremia phase, generally 70%-100% depending on method & target	High, 95%-100%
LAMP	Reported high; studies report 90%-97%	High; 95%-97%

L. alstonii, intermediate types such as *L. wolffii*, alongside non - pathogenic ones (*L. idonii* and *L. meyeri*), suggesting that waterfalls can serve as natural reservoirs for pathogenic *leptospira* (Paisan et al., 2018). In Malaysia, isolation from environmental samples (markets and recreational areas) also showed the presence of pathogenic *L. alstonii* (Azali et al., 2016). All isolates in that study were MAT-negative, highlighting a possible threat to public health due to the existence of virulent strains in the environment.

Besides serology and culture, molecular methods like PCR and LAMP are used in leptospirosis diagnosis. PCR serves as a genetic tool for amplifying particular DNA targets in order to obtain more copies (Gayathri et al., 2022). In Indonesia, Utami et al. (2013) showed that *leptospira* DNA could be isolated from patient blood samples, yielding a 615 bp band for pathogenic *Leptospira* and 316 bp for saprophytic *Leptospira*, using target genes like rrs (16S rRNA), flaB, and B64I/B64II (Utami et al., 2013). Research conducted by Krairojananan et al. (2020) in Thailand reported a low prevalence (3.6%) of *Leptospira* in rodent kidney samples utilizing *lipL32* quantitative PCR (Krairojananan et al., 2020). An investigation in Malaysia led by Amran et al. (2023) found 36 out of 46 samples positive, with a dominance of *L. interrogans*, using PCR-based MLST and rrs2 gene sequencing (Amran et al., 2023). In general, PCR and its variants (real-time PCR, MLST, sequencing) have proven high sensitivity and specificity in detecting *Leptospira*. While in Indonesia research is mostly literature-based, field surveys and molecular characterization in Thailand and Malaysia have shown genetic variation of *Leptospira* in nature.

The DNA amplification technique known as Loop-mediated Isothermal Amplification (LAMP) has been demonstrated to be swift, sensitive, and capable of detecting low copy numbers, making it a promising molecular approach for diagnosing various infectious diseases (Garg et al., 2022). Suwancharoen et al. (2016) in Thailand demonstrated that LAMP achieved a high sensitivity of 96.8% and specificity of 97.0%, significantly exceeding the performance of quantitative PCR for identifying *Leptospira* species. This advantage is supported by its minimal detection threshold of just 10–100 target DNA copies, and the ability to display results visually by a color change, making it suitable for resource-limited settings (Suwancharoen et al., 2016). Comparable results were documented within the Philippines by Tubalinal et al. (2018), demonstrating that LAMP could detect *Leptospira* spp. with a 29.0% positivity rate (40/140 samples), compared to only 9.3% with nested PCR. In that study, LAMP's sensitivity was shown to detect down to two *leptospira* cells per reaction, reinforcing its diagnostic potential in supporting disease surveillance in domestic animals. LAMP results were interpreted by two visual methods: agarose gel electrophoresis and color change with a dye, both yielding higher sensitivity than nested PCR (Tubalinal et al., 2018)

The diagnostic performance data synthesized in Table 2 highlights the stark contrast between different methods across nations within the Southeast Asian region. To illustrate this, the high sensitivity of PCR in detecting early-phase infections in Thailand as well as Malaysia (Amran et al., 2023; Dinhuzen et al., 2021) demonstrates its superiority for acute diagnosis compared to serological methods. Conversely, while ELISA and RDTs show broader variability, their high specificity in certain Indonesian and Vietnamese studies (Sumarningsih et al., 2024; Samrot et al., 2021) confirms their utility for large-scale screening in endemic areas. These comparisons in Table 2 reinforce the necessity of selecting a diagnostic tool based on the duration of fever and available laboratory infrastructure.

Overall, each method has its strengths and weaknesses. MAT excels for epidemiological research (identifying serovars) but is not practical for clinical use. ELISA and RDTs are useful for rapid screening in low-resource areas, but their sensitivity and specificity are not ideal, so they must be confirmed. Culture is very specific but slow and technical, thus more suited for environmental research. PCR/LAMP provide reliable early detection and are suitable for rapid diagnosis, but require molecular facilities. In the context of Southeast Asia, where reference laboratories are limited and case burden is high, an integrated strategy is needed: use ELISA/RDT for initial screening in primary care or regional hospitals, followed by confirmation with PCR or MAT in reference centers. Combining methods (e.g., RDT+PCR or ELISA+MAT) can improve diagnostic accuracy. Strengthening laboratory capacity (MAT training, providing PCR/LAMP) is also essential. The final method choice should consider resources, time, and purpose (acute diagnosis vs. surveillance) so that leptospirosis can be detected early and controlled more effectively in Southeast Asia.

When comparing diagnostic practices across the region, a distinct pattern emerges based on national healthcare infrastructure. In Thailand, the diagnostic approach is more integrated, as evidenced by large-scale prospective studies such as the THAI-LEPTO AKI study, which combine molecular (qPCR) and serological methods to improve early detection accuracy (Dinhuzen et al., 2021). In contrast, Indonesia and the Philippines still rely heavily on serological screening (RDTs) due to their practicality in rural and flood-prone areas, although this necessitates careful timing of the test (Handayani et al., 2025; Hwee et al., 2022). Meanwhile, the emergence of environmental surveillance studies in Malaysia reflects a shift toward a One Health approach. These regional variations, as summarized in Table 2, highlight that while the diagnostic challenges are similar, the choice of method is heavily dictated by the specific socioeconomic and geographical contexts of each country.

4. Conclusion

Leptospirosis diagnosis in Southeast Asia faces major challenges due to high case burden, limited laboratory facilities, and significant variations in diagnostic performance. This review highlights that each method plays a specific role: MAT is superior for epidemiological surveillance and serovar identification; ELISA and RDTs are effective for rapid screening but require careful interpretation regarding the fever onset timing, while PCR and LAMP provide reliable early detection. The regional variation in diagnostic practices from Thailand's integrated molecular-serological approach to the heavy reliance on RDTs in Indonesia and the Philippines reflects the influence of local socioeconomic and geographical contexts. Therefore, an integrated One Health diagnostic strategy is the most effective approach for the region. This involves initial screening at primary care settings followed by molecular or MAT confirmation at referral laboratories. Strengthening laboratory capacity and promoting environmental surveillance are crucial steps to accelerate accurate diagnosis and support more effective leptospirosis control across Southeast Asia.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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Authors' contributions

GMK, PC performed conceptualization, methodology, writing, review and editing. VS, NT performed investigation, supervision, visualization. A and DF performed revision, editing, analyzed and writing.

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