Journal of Associated Medical Sciences 2025; 58(3): 278-287

JAMS
Journal of Associated Medical Sciences

Scopus Indexed & Thai-Journal Citation Index Centre (TCI)

Journal of Associated Medical Sciences

Journal homepage: https://www.tci-thaijo.org/index.php/bulletinAMS/index

Enhanced detection of *Trypanosoma evansi* in Cattle: Superior performance of LAMP compared to PCR and CATT/*T. evansi* test

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ARTICLE INFO

Article history:
Received 17 June 2025
Accepted as revised 24 July 2025
Available online 29 July 2025

Keywords:
Trypanosomiasis, Trypanosoma evansi, loop mediated isothermal amplification, RoTat1.2

ABSTRACT

Background: *Trypanosoma evansi*, the causative agent of surra, poses a major veterinary concern in tropical regions, particularly affecting cattle and buffalo. The disease leads to reproductive failures, including abortion, and significant economic losses. Early and accurate diagnosis is crucial for effective control, especially in endemic, resource-limited areas.

Objectives: This study aimed to develop a loop-mediated isothermal amplification (LAMP) assay for rapid detection of *T. evansi* and evaluate its diagnostic performance in comparison with conventional polymerase chain reaction (PCR) and the CATT/*T. evansi* card agglutination test.

Materials and methods: Four LAMP primers were designed to target the RoTat 1.2 variant surface glycoprotein (VSG) gene of *T. evansi*. Optimal reaction parameters, including temperature and incubation time, were established. The LAMP assay, conventional PCR, and the CATT/*T. evansi* card agglutination test were performed on 79 blood samples collected from cattle with suspected trypanosomiasis in northern Thailand (Lamphun and Chiang Mai). Diagnostic sensitivity, specificity, and agreement between tests were statistically analyzed.

Results: The LAMP assay detected *T. evansi* in 32 (40.5%, 95% CI: 29.8-51.9%) samples, slightly outperforming PCR, which detected 30 (37.9%, 95% CI: 27.6-49.0%). However, this difference was not statistically significant (McNemar's test, p=0.724). The CATT/*T. evansi* test yielded 45 (56.9%) positives but lacked the ability to differentiate active infection from prior exposure.

Conclusion: The LAMP assay demonstrated high sensitivity, specificity, and rapid detection capabilities under simplified conditions, making it highly suitable for field applications. When paired with colorimetric or lateral flow readouts, LAMP offers a promising point-of-care diagnostic tool for improving trypanosomiasis control in endemic regions.

Introduction

Trypanosoma evansi, the causative agent of trypanosomiasis (commonly known as surra), represents a significant threat to mammalian livestock health and is classified as a notifiable pathogen under the Animal Epidemic Act B.E. 2499 in Thailand. This extracellular hemoprotozoan parasite proliferates within the circulatory and lymphatic systems of vertebrate hosts, frequently resulting in reproductive complications including abortion and breeding failure. The economic impact of *T. evansi*

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doi: 10.12982/JAMS.2025.098
E-ISSN: 2539-6056

infection on the veterinary industry is substantial, primarily due to its rapid transmission dynamics within cattle populations and the subsequent livestock mortality and productivity losses.

The epidemiological significance of *T. evansi* has expanded beyond traditional endemic regions, with documented emergence in previously unaffected geographical areas and infection of novel host species.[1] Recent comprehensive reviews have highlighted the continued global spread of surra, with new cases reported across tropical and subtropical regions, emphasizing the ongoing threat to livestock industries worldwide.[2] While some infected cattle exhibit clinical manifestations, others remain asymptomatic carriers, serving as reservoirs for transmission to diverse animal species including equines, elephants, large felids, canines, and swine.[3,4] Although human infection with animal trypanosomes is typically prevented by trypanolytic factors present in human serum, certain *T. evansi* isolates have demonstrated resistance to human plasma under specific conditions, raising concerns about potential zoonotic transmission.[5] Molecular characterization has identified two principal serotypes of *T. evansi* based on the presence or absence of the *RoTat 1.2 VSG* gene: serotype A, which expresses this gene, and serotype B, which lacks this genetic element. Notably, the majority of *T. evansi* isolates characterized in Thailand possess the *RoTat 1.2 VSG* gene, making this target an important diagnostic marker for serotype A identification in the region.[6] The specificity and prevalence of this genetic marker provide an excellent foundation for the development of molecular diagnostic assays tailored to the Thai epidemiological context.

The diagnosis of *T. evansi* infection encompasses various methodological approaches, including direct microscopic examination, serological testing, animal inoculation, and DNA-based detection techniques.[7–9] In Thailand, the standard diagnostic protocol relies primarily on microscopic examination as the first-line detection method. The hematocrit centrifuge technique (HCT), commonly referred to as the Woo test, represents a simple and cost-effective diagnostic approach for animal trypanosomiasis; however, this method is constrained by inherently low sensitivity due to its dependence on the direct visualization of motile parasites in EDTA-anticoagulated blood specimens. Multiple factors significantly influence the diagnostic performance and reliability of microscopic detection methods. Low parasitemia levels, particularly during early infection stages or chronic disease phases, frequently result in false-negative outcomes due to insufficient parasite density for microscopic detection. Pre-analytical variables also critically impact diagnostic accuracy: elevated storage and transportation temperatures adversely affect parasite viability by accelerating glucose consumption, leading to energy depletion and subsequent loss of motility—a key criterion for microscopic identification. Transportation delays exceeding 24 hours further compromise parasite viability and motility, thereby reducing detection sensitivity.[10] Additionally, examination of Giemsa-stained blood films using conventional microscopy frequently fails to detect patent infections and chronic manifestations of the disease, where parasitemia levels may be below the threshold of microscopic detection.[11] Serological diagnostic approaches, particularly the card agglutination test for *T. evansi* (CATT/*T. evansi*® kit), represent the current reference standard for antibody-based detection as recommended by the World Organisation for Animal Health (OIE). This assay utilizes a freeze-dried suspension of purified, fixed, and stained bloodstream-form trypanosomes expressing the variable surface glycoprotein RoTat 1.2 as the detection antigen. Despite its recognized diagnostic value and international endorsement, the widespread implementation of CATT/*T. evansi* faces significant practical limitations, including restricted importation and commercialization due to varying national regulatory frameworks, usage constraints, and prohibitive costs that limit accessibility in resource-constrained settings.

DNA-based detection methods, particularly polymerase chain reaction (PCR) techniques, demonstrate superior sensitivity and specificity for diagnosing active *T. evansi* infections. The stability of parasitic DNA persists for 24–48 hours post-mortem, allowing reliable detection even after parasite death, with conventional PCR turnaround times of 3–4 hours. However, PCR methodology remains constrained by its requirement for expensive thermal cycling equipment, limiting its implementation in budget-restricted laboratories and remote geographical areas, particularly in developing countries where trypanosomiasis is endemic.[12] Recent advances in LAMP technology have demonstrated successful point-of-care applications in veterinary medicine, including rapid antimicrobial susceptibility testing for bacterial pathogens in dogs[13] and multiplex detection systems for swine pathogens using microfluidic platforms.[14] Contemporary developments in LAMP methodology have focused on improving field deployability through smartphone-based detection systems and colorimetric readouts.[15] These innovations have shown particular promise for parasitic disease detection, with recent studies demonstrating successful point-of-care applications for various parasitic organisms.[16]

Loop-mediated isothermal amplification (LAMP) offers a promising alternative that reduces dependence on sophisticated instrumentation by utilizing a single amplification temperature of 60–65°C throughout the reaction. This technique achieves remarkable amplification efficiency, generating 10^9–10^{10} fold increases in target DNA within one hour without requiring expensive automation or thermal cycling equipment.[17,18] LAMP has demonstrated versatility across veterinary diagnostic applications, including pathogen detection in aquaculture species such as shrimp[19] and livestock including swine.[20] Furthermore, LAMP methodology has proven successful for diagnosing both human and animal trypanosomiasis in previous studies.[21,22] The implementation of LAMP-based diagnostics for *T. evansi* detection could significantly mitigate economic losses in the veterinary sector through early pathogen identification, enabling rapid veterinary

intervention before widespread epidemic development. However, comprehensive comparative studies evaluating LAMP performance against established PCR and ELISA methodologies for *T. evansi* diagnosis remain limited in the literature. Recent advances in loop-mediated isothermal amplification (LAMP) technology have included smartphone-based fluorescence detection, lyophilized reagent formulations for field deployment, and multiplexed assays for simultaneous pathogen detection.[23,24] However, limited comparative data exists for LAMP versus conventional methods specifically for *T. evansi* detection in Southeast Asian cattle populations, representing a significant knowledge gap for regional disease control strategies.

Therefore, the objective of this study was to compare the diagnostic efficiencies of conventional PCR and CATT/*T. evansi* test with LAMP technique to determine the most suitable method for rapid, field-deployable detection of *Trypanosoma evansi* based on amplification of the *RoTat 1.2 VSG* gene target.

Materials and methods

Sample collection and preparation

Archived whole blood specimens stored at -20 °C from dairy cattle were utilized for this comparative diagnostic study. All blood samples represented residual specimens from routine annual health surveillance programs conducted by veterinarians from The Fifth Regional Livestock Office, Chiang Mai, Thailand, during 2020. Due to budget limitations, 79 cattle from Lamphun and Chiang Mai provinces were selected using convenience sampling based on clinical signs suggestive of trypanosomiasis and herd owners' approval for participation. A confirmed positive control sample from cattle with microscopically confirmed *T. evansi* infection was included in the study design to validate assay performance. For detecting a 15% difference in sensitivity between methods (80% vs 65%) with 80% power and α=0.05, approximately 74 samples per group were required. Our sample of 79 meets this minimum requirement for this preliminary evaluation.

Extraction of genomic DNA and PCR amplification using TR3/4 primers

DNA was extracted from blood samples by using Nucleospin blood extraction kit (Macherey-Nagel GmbH & Co. KG, Düren, Germany). Briefly, 200 µL EDTA blood was mixed with 25 µL of proteinase K and 200 µL buffer. Then, the mixture was vortexed vigorously for cell lysis. After that, the mixture was incubated at 70°C for 10-15 minutes. A total of 210 µL absolute ethanol was added to the mixture and vortexed again. Then, the mixture was transferred into a Nucleospin Blood Column and centrifuged at 12,000 rpm for 2 minutes. After that, the silica membrane was washed two times. The dried silica membrane was centrifuged in the column at 12,000 rpm for 2 minutes. Then, 100 µL elution buffer was added, incubated for 1 minute and centrifuged in the column at 12,000 rpm for 2 minutes to elute pure DNA. For PCR amplification, TR3/4 primers were derived from a trypanosome-specific repetitive nucleotide sequence fragment that amplified 257 bp amplicon, Forward primer (TR3: 5'-GCGCGGATTCTTTGCAGACGA-3'), Reverse primer (TR4: 5'-TGCAGACACTGGAATGT-3'). Conventional PCR was performed by using 1x Quick Taq HS Dye mix reagent (Toyobo Life Science, Tokyo, Japan). The PCR conditions were as follows: 30 cycles of denaturation at 95 °C for 30 seconds, annealing at 55 °C for 30 second and extension at 72 °C for 30 seconds. The PCR products were separated by electrophoresis through 2% agarose gels containing Redsafe nucleic staining dye, and the PCR products were visualized under UV illumination.

LAMP assay

LAMP primers recognizing six sections of the *T. evansi RoTat 1.2 VSG* gene (Accession no. AF317914) were designed using Primer Explorer version 3 software (http:// primerexplorer.jp/lampv3e/index.html), F3 primer (5'-AATTCTGCCCGCAGTTGC-3'), B3 primer (5'-CCCTCTAG GTAGCTGTCTCC-3'), FIP primer GGCTTTGCCCACACAAAA CTTGCAGGGGCGATTTCATCG-3'), BIP primer GCACAAATGC CGACGGTAACGCCGAGGTGTCATAGTTGG-3').

LAMP was carried out in a total 25 µL reaction mixture containing 1x isothermal amplification buffer, 0.8 µM each FIP and BIP, 0.1 µM each F3 and B3, 1.4 mM each dNTP, 8 mM $MgSO_4$, 320 U/ml Bst 2.0 WarmStart DNA polymerase (New England Biolabs, MA, USA) and 100 ng of DNA template. Thereafter, incubation at 65 °C for 30 minutes in Mini Heating Dry Bath incubator and heating at 80 °C for 5 minutes to terminate the reaction were performed. Aliquots of 2 µL of LAMP products were observed in a ladder-like pattern in 2% agarose gels containing Redsafe nucleic staining dye and the LAMP products were visualized under UV illumination. LAMP products were also detected with the naked eye by observing white turbidity in the reaction mixture.

Sensitivity of LAMP assay

The detection limit of the LAMP assay was determined using a 10-fold serial dilution of 100 ng of purified genomic DNA from infected cattle and using a 10-fold serial dilution of lysed parasite by double-distilled water to determine the sensitivity of the analytical assay. The sensitivity of the LAMP assay was compared with that of conventional PCR by using TR3/4 primers.

Analysis of cattle samples

A total of 79 genomic DNA from cattle with suspected trypanomiasis were used. The DNA was prepared and stored at -20°C before use. A total of 100 ng of the DNA template was added to compose 25 µL of LAMP reaction mixture, and amplification was performed as in section 2.4.

Serological analysis

Serodiagnosis of animal trypanosomosis due to *T. evansi* was performed by using CATT/*T. evansi* test kit (Free University of Brussels and the Institute of Tropical Medicine, Belgium). The assay was performed following the manufacturer's instruction. Briefly, 25 µL of plasma

or serum were mixed with approximately 45 µL of homogenized CATT antigen (variable surface antigen VSA common to all *T. evansi* stocks) in the supplied plastic card. A stirring rod was used to mixed and spread out the reaction mixture to about 1 mm form the edge of test area. The plastic card was rotated on a flatbed orbital rotator for 5 minutes at 70 rpm. After rotation the agglutination results were compared with the positive control.

Statistical analysis

McNemar's test was used to compare paired proportions between diagnostic methods. Cohen's kappa coefficient was calculated to assess agreement between methods. Statistical significance was set at $p<0.05$. Sensitivity, specificity, and 95% confidence intervals were calculated using PCR as the reference standard. Analyses were performed using SPSS version 20 (IBM Corp.).

Results

Detecting T. evansi by conventional PCR and LAMP assay

Conventional PCR amplification yielded the expected 257 bp amplicon from whole-blood genomic DNA, confirming the presence of *T. evansi* infection as shown in Figure 1. Among the 79 blood specimens analyzed, 30 samples (37.9% , 95% CI: 27.6-49.0%) tested positive for *T. evansi* DNA using conventional PCR methodology.

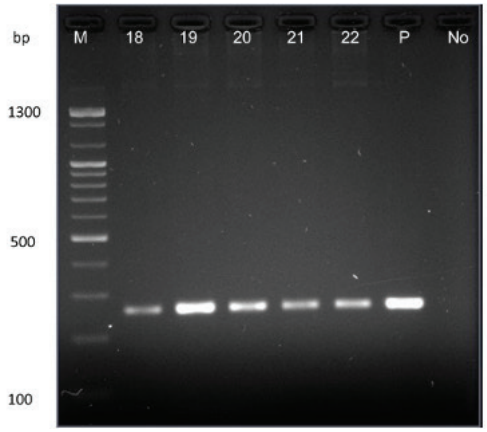

Figure 1. *Detection of T. evansi by conventional PCR using TR3/4 primers. Agarose gel electrophoresis showing 257 bp amplicons. Lanes 18-22: cattle DNA samples with varying band intensities indicating different parasite loads, Lane P: positive control, Lane M: 100 bp DNA marker, Lane No: negative control.*

Optimal LAMP reaction conditions were established using 0.1 µM of each outer primer (F3 and B3), 0.8 µM of each inner primer (FIP1 and BIP1), and 8 mM $MgSO_4$, combined with 1× isothermal buffer, 1.4 mM dNTPs, 320 U/ml Bst 2.0 WarmStart DNA polymerase, and 100 ng of template DNA. The reaction was maintained at 65°C for 30 minutes, with positive and negative reactions clearly distinguishable by visual inspection (Figure 2). Agarose gel electrophoresis analysis revealed distinct patterns characteristic of LAMP amplification products. Positive samples exhibited a DNA ladder-like pattern with multiple bands and smear formations, while negative samples showed no such ladder-like banding pattern. These amplification characteristics were consistent with the positive control (PC), whereas the negative control (NC) and non-infected blood samples displayed single band patterns (Figure 3). Using the optimized LAMP assay, 32 blood samples (40.5% , 95% CI: 29.8-51.9%) tested positive for *T. evansi* DNA, while 47 samples (59.5%) were negative (Table 1). Although LAMP detected 2 additional positive samples compared to PCR (40.5% vs 37.9%), this difference was not statistically significant ($p=0.724$). The clinical relevance of this minimal difference requires evaluation in larger studies.

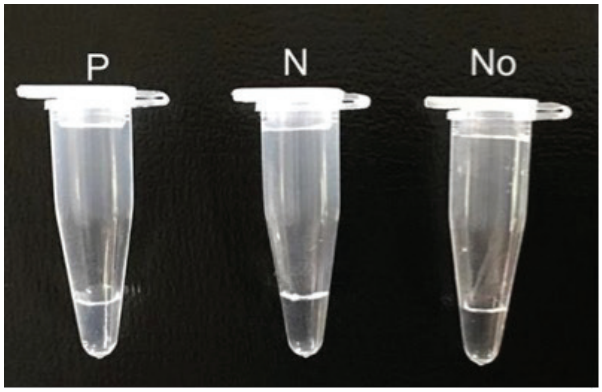

Figure 2. *Detection of T. evansi by LAMP assay. LAMP products detected by naked eye. (P) Positive LAMP products; (N) Negative LAMP products; (No) No template control.*

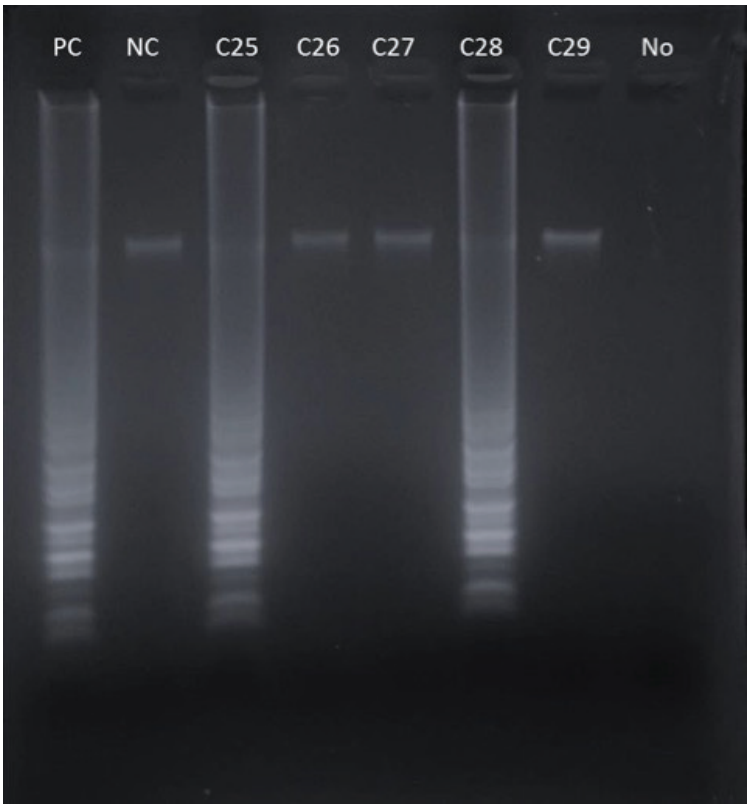

Figure 3. *LAMP assay results showing characteristic ladder-like pattern in positive samples. Multiple bands and smearing indicate successful isothermal amplification. Lane PC: positive control, Lane NC: negative control, Lanes C25-C29: cattle samples.*

Table 1. Detection of *Trypanosoma evansi* infections in cattle using PCR, LAMP assay, and CATT/*T. evansi* kit.

Experiment	Positive N (%)	Negative N (%)	Statistical comparison
PCR	30 (37.9%)	49 (62.1%)	Reference method
LAMP	32 (40.5%)	47 (59.5%)	vs PCR: p=0.724†
CATT/*T. evansi*	45 (56.9%)	34 (43.1%)	vs molecular methods: p<0.001†

Note*: †McNemar's test for paired comparisons agreement between LAMP and PCR, κ=0.89 (95% CI: 0.78–0.95).*

Statistical comparison between diagnostic methods revealed excellent agreement between LAMP and PCR. McNemar's test revealed no statistically significant difference between LAMP and PCR detection rates (χ^2=0.125, p=0.724). Agreement analysis showed excellent concordance between methods (κ = 0.89, 95% CI: 0.78-0.95). Using PCR as reference standard, LAMP demonstrated sensitivity of 96.7% (95% CI: 82.8-99.9%) and specificity of 93.9% (95% CI: 83.1-98.7%).

Analytical sensitivity determination

To evaluate the analytical performance of the LAMP assay, the detection limits of both conventional PCR and LAMP methodologies were determined using 10-fold serial dilutions of purified genomic DNA extracted from *T. evansi*-infected cattle. Comparative analysis by 2% agarose gel electrophoresis demonstrated equivalent analytical sensitivity between conventional PCR (Figure 4A) and the LAMP assay (Figure 4B), indicating comparable detection thresholds for both molecular diagnostic approaches. Both methods showed similar detection limits down to the 10^{-3} dilution (approximately 0.1 ng/µL genomic DNA). However, precise limit of detection in terms of parasite equivalents per µL cannot be determined without standardized parasite quantification. Visual assessment suggests comparable analytical sensitivity, but quantitative confirmation using qPCR standards is recommended for definitive LOD determination.

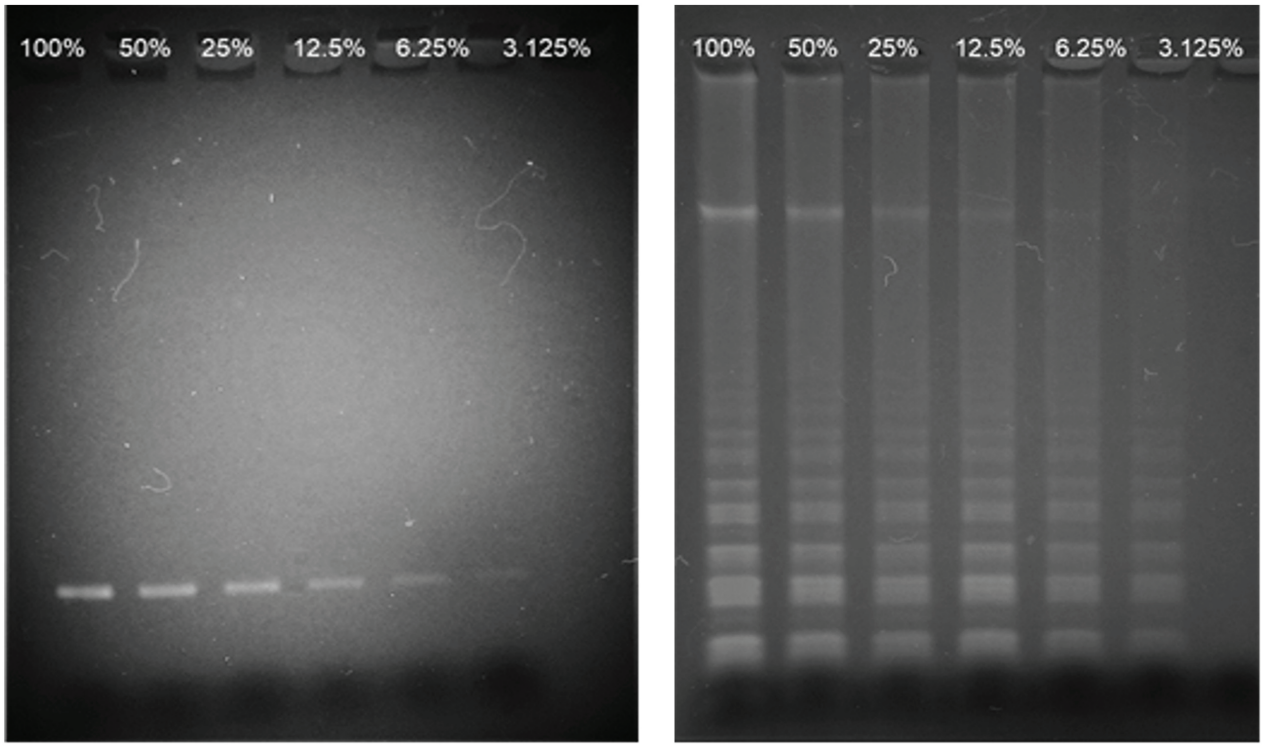

Figure 4. *Comparative sensitivity analysis of PCR and LAMP assays for Trypanosoma evansi detection using serial dilutions of T. evansi DNA template from cattle. A: conventional PCR products, B: LAMP amplification products.*

Serological analysis

Serological testing was performed using the CATT/*T. evansi* kit, which employs a direct agglutination principle for detecting anti-*T. evansi* antibodies in bovine blood specimens. The assay involves mixing plasma or serum samples with variable surface glycoprotein (VSG) RoTat 1.2 antigen reagent, with antigen-antibody complexes observed as aggregates by naked eye examination within 5 minutes. Positive results are characterized by dark-colored clumping (agglutination) around the edges of sample drops, as compared to the positive control (Figure 5). Among the 79 specimens tested, 45 blood samples (57.0%) were positive by CATT/*T. evansi* serological testing. Notably, seven samples that tested positive by CATT showed negative results in both PCR and LAMP molecular assays. This discordance may be attributed to several factors: (1) previous *T. evansi* infection with subsequent parasite clearance, resulting in persistent antibodies but undetectable parasitic DNA; (2) parasitemia levels below the analytical detection limits of the molecular assays employed; or (3) sampling limitations, as the 200 µL sample volume utilized in this study may have contributed to false-negative results in specimens with low parasite density.

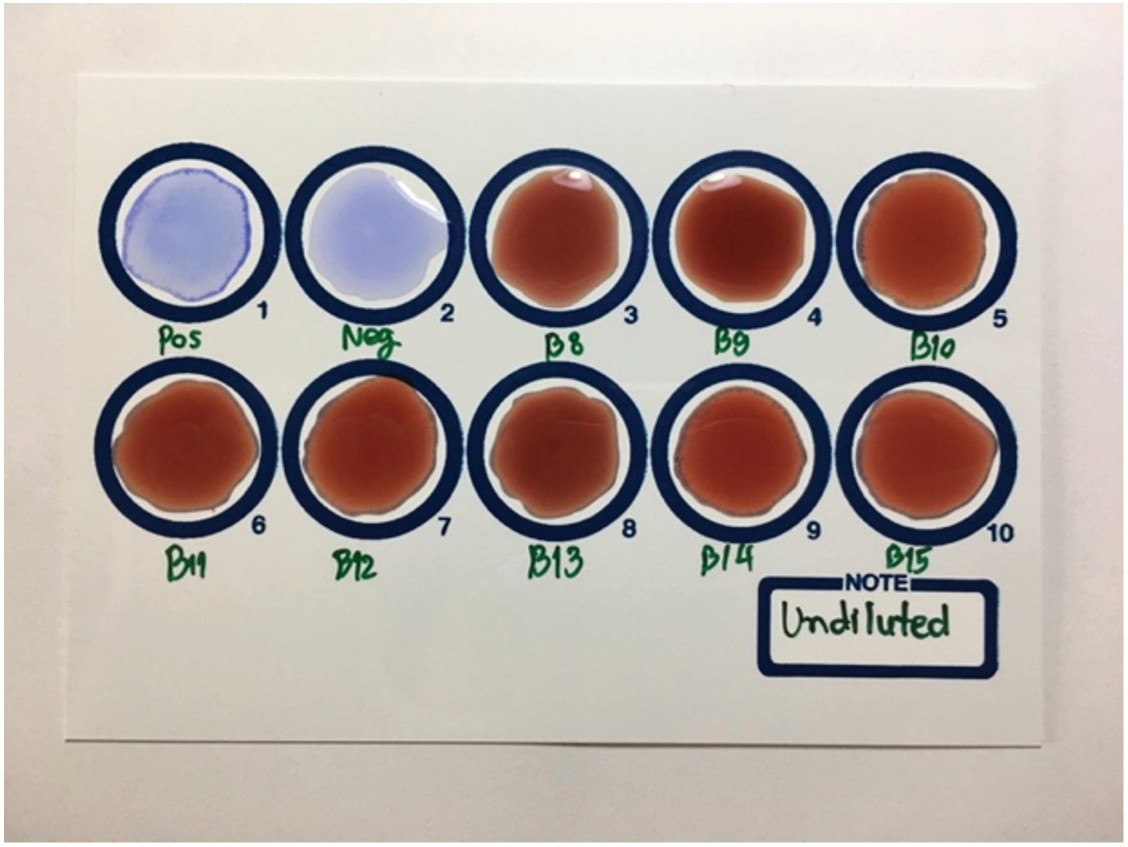

Figure 5. *Serological analysis using CATT/T. evansi kit showing agglutination reactions (characterized by dark-colored clumping around the edges of sample drops). Pos: Positive control, Neg: Negative control, B8-B15: cattle plasma samples.*

Discussion

Trypanosoma evansi represents the most geographically widespread pathogenic trypanosome species globally, necessitating accurate diagnostic capabilities and effective therapeutic interventions for successful disease control, particularly in regions characterized by low-intensity infections. As the prevalence of trypanosomiasis declines through control measures, enhanced diagnostic sensitivity and specificity become increasingly critical to prevent disease re-emergence from undetected outbreaks within animal reservoir populations. Addressing the epidemiological significance of *T. evansi* in Thailand, we developed a rapid and simplified diagnostic assay capable of detecting *T. evansi* genomic DNA using loop-mediated isothermal amplification (LAMP) methodology. Comparative analysis of 79 bovine blood specimens revealed that 32 samples (40.5% , 95% CI: 29.8-51.9%) tested positive by LAMP, compared to 30 samples (37.9%, 95% CI: 27.6-9.0%) positive by conventional PCR, demonstrating superior sensitivity of the LAMP assay over traditional PCR methodology. These findings align with previous studies reporting enhanced LAMP sensitivity for trypanosome detection.[21,25,26] However, the relatively small sample size (n=79) may limit the statistical power for definitive comparisons between diagnostic methods. Future studies with larger sample sizes would strengthen the statistical validity of sensitivity comparisons.

Recent field applications of LAMP technology have demonstrated its practical utility in veterinary diagnostics. Pirolo *et al.* validated a colorimetric LAMP test for bacterial pathogen identification in dogs, achieving near 100% sensitivity and specificity under laboratory conditions, though field performance showed some reduction in accuracy.[13] Ji *et al.* developed a microfluidic-LAMP chip capable of simultaneously detecting multiple swine pathogens with 100% specificity and excellent stability, demonstrating the potential for multiplexed detection systems.[14] These developments highlight both the promise and practical challenges of implementing LAMP technology in real-world veterinary settings. The superiority of LAMP over conventional PCR was further evidenced by more distinct visualization of amplification products on 2% agarose gel electrophoresis, facilitating clearer interpretation of results. Our study demonstrated a detection limit of 3.125% for the LAMP assay, although higher sensitivities have been reported in other investigations.[25,26,27] The inherent advantages of LAMP methodology—including rapid amplification kinetics, single-temperature incubation requirements, and equipment simplicity—position it as a superior alternative to conventional PCR and other amplification strategies such as nested PCR and real-time quantitative PCR for field-based pathogen detection without sophisticated instrumentation requirements.[28]

The practical advantages of LAMP extend beyond sensitivity improvements, with results obtainable within 30 minutes using standard dry bath incubation. To address limitations associated with UV-based result interpretation,

recently developed colorimetric-fluorometric indicators (CFI) enable real-time fluorescence monitoring with visible color changes observable by the naked eye. The progressive consumption of magnesium ions during LAMP amplification causes hydroxynaphthol blue (HNB) within the CFI to undergo a characteristic color transition from purple to sky blue, eliminating the need for gel electrophoresis.[29]

The versatility of LAMP methodology has been demonstrated across diverse parasitological applications in veterinary diagnostics. Kumagai *et al.* demonstrated LAMP's capability to detect schistosomal DNA from primary infections as early as one day post-infection in snails across epidemic areas, establishing it as a robust screening method.[30] Similarly, Plutzer and Karanis successfully applied LAMP for Giardia duodenalis detection in fecal specimens.[31] Kong *et al.* reported excellent specificity of LAMP for Toxoplasma gondii detection in murine blood samples, with no cross-reactivity observed against other parasites and positive detection within one day of experimental infection. [32] Park *et al.* demonstrated that reverse transcription-LAMP (RT-LAMP) provided superior performance compared to conventional LAMP for detecting European and North American porcine reproductive and respiratory syndrome viruses, with reduced turnaround times.[20]

These studies collectively demonstrate the expanding application of LAMP methodology for infectious disease detection in veterinary medicine over the past decade. The enhanced specificity achieved through dual primer pairs binding to six distinct regions on target DNA, combined with single-temperature amplification using strand-displacing Bst DNA polymerase, contributes to the method's robustness, high sensitivity, and specificity while maintaining rapid turnaround times. Furthermore, the reduced requirement for expensive reagents and sophisticated instrumentation significantly decreases the cost of *T. evansi* detection, making it particularly suitable for resource-limited settings.

Examination of 47 cattle revealed that all animals tested negative by LAMP assay. However, serological analysis detected antibodies against *T. evansi* in 13 animals (27.7%), indicating previous exposure to the parasite. Serological testing was performed using the CATT/*T. evansi* kit, which employs the direct agglutination method for detection of anti-*T. evansi* antibodies in bovine blood samples. Test results are interpreted visually by direct observation of agglutination patterns on reaction cards, with accuracy dependent upon the expertise of trained technicians.

The CATT/*T. evansi* kit presents inherent limitations that must be considered in diagnostic applications. Notably, this serological method cannot differentiate between active and resolved infections, rendering it unsuitable as a test-of-cure. This limitation stems from the persistence of anti-Trypanosoma spp. antibodies, which typically appear 4-8 days post-infection and may remain detectable for extended periods following parasite clearance or therapeutic intervention.[33,34]

In contrast, loop-mediated isothermal amplification (LAMP) represents a sensitive and specific molecular diagnostic tool particularly suited for early detection of *T. evansi* infection during the acute phase, when antibody titers may be low or absent.[35] The LAMP assay offers several technical advantages over conventional PCR and ELISA methodologies, including isothermal amplification at a constant temperature of 65 °C, eliminating the requirement for expensive thermocycling equipment. Furthermore, LAMP demonstrates detection limits equivalent to or superior to conventional PCR while providing reduced processing time.[36] While LAMP offers several advantages including rapid results and isothermal amplification, certain limitations must be acknowledged. The high amplification efficiency of LAMP can increase the risk of cross-contamination if strict laboratory protocols are not followed. Additionally, LAMP may be prone to nonspecific amplification if primers are not carefully optimized, potentially leading to false-positive results. Future validation studies should include rigorous specificity testing and standardized protocols to minimize these risks. Despite these considerations, LAMP remains a promising tool for field-based diagnosis when appropriate quality control measures are implemented.

Limitation

Based on agarose gel visualization, both methods showed similar detection limits in this preliminary comparison. However, without quantitative measurement of amplification products or standardized parasite concentrations, we cannot definitively conclude equivalent analytical sensitivity. Additionally, all blood samples were archived specimens stored at -20°C, and prolonged storage may have resulted in DNA degradation, potentially affecting the sensitivity of molecular detection methods and leading to underestimation of true diagnostic performance. Future studies should employ quantitative methods (qPCR, fluorescence measurement) for precise sensitivity comparison.

Conclusion

Based on our findings, the LAMP assay represents a viable alternative to conventional PCR-based diagnostic methods for *T. evansi* detection. The technique's inherent advantages—including rapid processing, high sensitivity, and isothermal amplification requirements—make it particularly suitable for field applications. These characteristics position LAMP as a promising point-of-care testing (POCT) platform for *T. evansi* diagnosis in clinical settings and livestock screening programs, supporting broader trypanosomiasis control and elimination initiatives. The complementary use of both molecular (LAMP) and serological (CATT) methods provides comprehensive diagnostic coverage, with LAMP offering superior performance for acute infection detection and CATT providing epidemiological insights into exposure history within populations.

Ethical approval

Ethical approval was not required as this study utilized archived samples from routine veterinary health surveillance conducted by the Fifth Regional Livestock Office. All samples were anonymized, and no additional animal procedures were performed specifically for this research. The use of archived samples for diagnostic method evaluation falls under institutional guidelines for secondary analysis of surveillance data.

Funding

This research was supported by Agricultural Research Development Agency (ARDA), grant number CRP6105021850. The authors would like to thank the Faculty of Associated Medical Sciences for the research facility support.

Conflicts of Interest

The authors declare no conflict of interest.

CRedit authorship contribution statement

Wuttichote Jansaento: conceptualization, methodology, formal analysis; **Mallika Osiriphan**: formal analysis, data curation, writing – original draft preparation; **Natthariinee kongta**: formal analysis, data curation, writing – original draft preparation; **Wittawat Modethed**: resources, writing – review & editing**; Thanakorn Rompo:** resources, writing – review and editing; **Suwit Duangmano**: conceptualization, methodology, writing – review & editing, project administration, funding acquisition.

Data availability

The corresponding author may provide the data supporting the results of this study upon reasonable request.

Acknowledgements

The authors would like to thank the Faculty of Associated Medical Sciences for the research facility support.

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