

SYSTEMATIC REVIEW

Molecular prevalence and diagnostic gene targets of *Anaplasma*, *Babesia*, *Theileria*, and *Trypanosoma* in cattle and buffaloes across Southeast Asia (2015–2025): A systematic review and meta-analysis



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ABSTRACT

Background and Aim: Hemoparasitic infections caused by *Anaplasma*, *Babesia*, *Theileria*, and *Trypanosoma* adversely affect cattle and buffalo health, resulting in economic losses and reduced livestock productivity in Southeast Asia. Although numerous molecular epidemiological studies have been conducted in individual countries, a comprehensive regional synthesis of polymerase chain reaction (PCR)-confirmed prevalence and molecular diagnostic targets has been lacking. This study aimed to estimate the pooled molecular prevalence of major hemoparasites in cattle and buffaloes across Southeast Asia, summarize the PCR target genes used for their detection, and investigate the effects of geographical region and host species on prevalence.

Materials and Methods: A systematic review and meta-analysis were conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines. PubMed, ScienceDirect, and Scopus were searched for studies published between 2015 and December 31, 2025. Cross-sectional studies conducted in Southeast Asia that used PCR-based methods and reported extractable prevalence data were included. Pooled prevalence estimates were calculated using random-effects meta-analysis. Heterogeneity was assessed using Cochran's Q test and the I^2 statistic, whereas meta-regression was performed to evaluate the influence of geographical region (mainland versus maritime Southeast Asia) and host species. Publication bias was assessed for *Babesia* spp.

Results: Twenty-two cross-sectional studies involving 5,789 cattle and 901 buffaloes met the inclusion criteria. The pooled prevalence was 12% (95% confidence interval [CI]: 3%-38%) for *Anaplasma* spp., 18% (95% CI: 11%-28%) for *Babesia* spp., 11% (95% CI: 3%-35%) for *Theileria* spp., and 11% (95% CI: 4%-28%) for *Trypanosoma evansi*. Species-specific pooled prevalences were 5% for *Anaplasma marginale*, 10% for *Babesia bigemina*, 9% for *Babesia bovis*, and 10% for *Theileria orientalis*. Significant between-study heterogeneity was observed across all analyses. Meta-regression identified a significantly higher prevalence of *B. bovis* in maritime than mainland Southeast Asia ($p = 0.0048$), whereas no significant regional or host species effects were detected for the remaining hemoparasites. The review also compiled the principal PCR target genes used for molecular diagnosis, including 16S rRNA, *groEL*, *MSP4*, *RAP-1a*, *AMA-1*, *SBP-2*, *SBP-4*, *MSA*, *MPSP*, *RoTat 1.2 VSG*, *ITS1*, *ITS2*, and satellite DNA.

Conclusion: Major bovine hemoparasites remain widely distributed across Southeast Asia, highlighting the need for strengthened molecular surveillance and standardized diagnostic approaches. This study provides the first comprehensive regional synthesis of PCR-confirmed prevalence and molecular diagnostic targets for major hemoparasites in cattle and buffaloes, offering valuable evidence to support veterinary diagnostics, surveillance programs, and disease control strategies based on a One Health approach.

Keywords: *Anaplasma*, *Babesia*, cattle, meta-analysis, molecular epidemiology, polymerase chain reaction, Southeast Asia, *Theileria*.

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INTRODUCTION

The agricultural sector is an important contributor to the economy of Southeast Asia accounting for approximately 11%–22% of the gross domestic product (GDP) of Southeast Asian countries in 2022 [1]. In some countries, such as Thailand and Vietnam, the agriculture, forestry, and fishing sector grew by 3.6% and 3.4%, respectively, in 2025 [2, 3]. In recent years, global population growth and economic development have accelerated the demand for red meat, particularly in Southeast Asian countries, making food security a continuing regional priority [4]. In Thailand, large ruminants (cattle, dairy cattle, and buffaloes) are among the five most important livestock species contributing to national livestock production [5]. Beyond commercial dairy and beef production systems, indigenous cattle are widely raised in rural communities as a source of household income and financial security. Similarly, livestock production is an integral component of rural livelihoods in Vietnam [6] and plays an important role in agricultural sustainability.

Ticks are important arthropod vectors of pathogens affecting both veterinary medicine and public health in Southeast Asian countries, where diverse geographical and ecological conditions support a rich diversity of tick species [7, 8]. Tick-borne pathogens include viruses, bacteria, protozoa, and helminths [7], among which *Anaplasma*, *Babesia*, haemotropic *Mycoplasma*, and *Theileria* are major pathogens affecting livestock health [8, 9]. *Anaplasma marginale* causes anemia in cattle through erythrophagocytosis by splenic macrophages following infection of erythrocytes, resulting in anemia without hemoglobinemia or hemoglobinuria [10]. In contrast, *Babesia* spp. invade and replicate within erythrocytes, causing direct erythrocyte destruction that leads to anemia, jaundice, hemoglobinuria, and fever [11]. *Theileria orientalis* infection is generally considered subclinical or asymptomatic [12]. However, cases of fever, hemolytic anemia, and mortality have been reported in Australia and New Zealand [13].

Another important blood-borne pathogen of cattle and buffaloes is *Trypanosoma evansi*, a salivarian trypanosome that causes surra [14]. This protozoan parasite is mechanically transmitted by tabanid and *Stomoxys* flies, of which 128 and 14 species, respectively, have been reported in Thailand [15]. Because the regional environment favors arthropod vector development and vector species are highly abundant [8, 15], the transmission of economically important hemoparasites remains a persistent challenge in Southeast Asian countries. Therefore, updated information on the molecular prevalence and diagnostic detection of these pathogens is needed to strengthen disease surveillance, improve veterinary diagnostic capacity, and support effective management of large ruminant health. Although a previous meta-analysis evaluated zoonotic tick-borne pathogens in Southeast Asian countries [16], and another systematic review focused on trypanosomes in Indonesia [17], no previous study has synthesized polymerase chain reaction (PCR)-confirmed prevalence data for the four major hemoparasite genera (*Anaplasma*, *Babesia*, *Theileria*, and *Trypanosoma*) in cattle and buffaloes across Southeast Asia during 2015–2025. Furthermore, no previous review has compared molecular prevalence between mainland and maritime Southeast Asian countries.

Despite increasing reports on bovine hemoparasites from individual Southeast Asian countries, comprehensive regional evidence integrating PCR-confirmed prevalence, molecular diagnostic targets, and sources of epidemiological heterogeneity remains unavailable. Consequently, regional differences in hemoparasite distribution and the molecular markers used for diagnosis have not been systematically evaluated, limiting evidence-based surveillance and diagnostic standardization across Southeast Asian countries.

Therefore, this systematic review and meta-analysis aimed to (i) estimate the pooled molecular prevalence of *Anaplasma marginale*, *Babesia bigemina*, *Babesia bovis*, *Theileria orientalis*, and *Trypanosoma evansi* in cattle and buffaloes across Southeast Asian countries; (ii) summarize the molecular diagnostic gene targets used for the detection of major hemoparasites during the past decade; (iii) investigate mainland–maritime and host species heterogeneity using meta-regression analyses; and (iv) provide evidence-based reference data to support veterinary diagnostic laboratories, disease surveillance, and hemoparasite control programs in Southeast Asia.

MATERIALS AND METHODS

Ethical approval

This systematic review and meta-analysis was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [18].

Study period and location

The literature search, study selection, data extraction, data analysis, and interpretation of the findings were

conducted from January 2026 to April 2026 at Akkhraratchakumari Veterinary College, Walailak University, Nakhon Si Thammarat, Thailand.

Protocol registration, database searching, and article screening

The review protocol was registered with the Open Science Framework (OSF; DOI: 10.17605/OSF.IO/BFWQR). Relevant articles reporting the prevalence of hemoparasites in cattle and buffaloes (*Anaplasma*, *Babesia*, *Theileria*, *Trypanosoma*, and *Mycoplasma*) were retrieved from three bibliographic databases: PubMed, ScienceDirect, and Scopus. The search strategy included the following search terms: ("vector-borne diseases" OR "tick-borne diseases") AND ("cattle" OR "buffaloes"); ("*Anaplasma*" OR "*Babesia*" OR "*Theileria*" OR "*Trypanosoma*") AND ("cattle" OR "buffaloes"); and ("prevalence" OR "occurrence"). Articles published between 2015 and December 31, 2025, were eligible for inclusion. Three reviewers (NP, NS, and PP) independently screened titles and abstracts.

Study selection and eligibility criteria

Studies were included in the systematic review if they met the following criteria: (i) cross-sectional study design; (ii) target population comprising cattle, buffaloes, or both; (iii) conducted in Southeast Asia; (iv) sample size ≥ 25 [19]; (v) use of PCR-based detection methods; and (vi) reporting the exact numbers of examined and positive animals or prevalence with a 95% confidence interval (CI). Studies were excluded if they (i) were not relevant to the prevalence of hemoparasites; (ii) investigated animal species other than cattle or buffaloes; (iii) used non-PCR-based detection methods; (iv) were case reports or review articles; (v) provided insufficient data for effect-size calculation; (vi) included fewer than 25 animals; (vii) were conducted outside Southeast Asia; or (viii) contained duplicate data published elsewhere. Full-text assessment and final study selection were independently performed by two reviewers (NP and NS).

Quality assessment and data extraction

Two reviewers (NS and TNT) independently extracted data, and discrepancies were resolved through discussion with a third reviewer (PP). The extracted information included (i) study characteristics (authors and publication year); (ii) methodological characteristics (sampling location, study period, and parasite detection method); (iii) host characteristics (host species, age, sex, total number of animals examined, and number of positive animals); and (iv) parasite characteristics (parasite species). The methodological quality of the included studies was independently assessed by two reviewers (NS and PP), with disagreements resolved through discussion. The quality assessment criteria were adapted from a previously published study [19]. Studies with quality scores of 0–8 were classified as low quality, whereas those with scores of 9–14 were classified as high quality.

Data synthesis and data analysis

Quantitative data, including the total numbers of cattle and buffaloes examined and the numbers infected with *Anaplasma marginale*, *Anaplasma centrale*, *Anaplasma bovis*, *Anaplasma platys*, *Babesia bigemina*, *Babesia bovis*, *Babesia ovata*, *Theileria orientalis*, *Theileria annulata*, *Theileria sinensis*, *Theileria* sp., *Trypanosoma evansi*, *Mycoplasma wenyonii*, and *Candidatus Mycoplasma haemobos*, were extracted and pooled for analysis. Pooled prevalence estimates were calculated for parasite groups represented by at least three eligible studies. Eight pooled prevalence analyses were performed, including overall estimates for *Anaplasma* spp., *Babesia* spp., and *Theileria* spp. infecting cattle and buffaloes. Species-specific pooled prevalence estimates were also calculated for *A. marginale*, *B. bigemina*, *B. bovis*, *T. orientalis*, and *Tr. evansi*. In addition to estimating pooled prevalence, this study systematically summarized the PCR gene targets used by laboratories across Southeast Asian countries for the molecular detection of major hemoparasites during 2015–2025.

A meta-analysis of proportions was performed using a random-effects model in R (version 4.5.3) [20] according to established methodological guidelines [21]. Between-study heterogeneity was assessed using Cochran's Q test and the I^2 statistic. Low heterogeneity was defined as $p \geq 0.10$ or $I^2 < 50\%$, whereas high heterogeneity was defined as $p \leq 0.10$ or $I^2 > 50\%$ [22]. Forest plots were generated to visualize pooled prevalence estimates. Where substantial heterogeneity was detected, meta-regression analyses were performed to evaluate differences in the prevalence of *A. marginale*, *B. bigemina*, *B. bovis*, *T. orientalis*, and *Tr. evansi* according to geographical region (mainland vs. maritime Southeast Asian countries) and host species (cattle vs. buffaloes). Publication bias was assessed using funnel plots and Egger's regression test only for *Babesia* spp., because fewer than ten studies were available for the remaining parasite groups [23].

RESULTS

Characteristics of included studies

Database searching initially identified 4,803 articles (Figure 1). After removing 1,572 duplicate records, 3,157 articles were excluded during title and abstract screening because they did not meet the eligibility criteria. The full texts of 74 articles were subsequently assessed for eligibility, of which 51 were excluded for the following reasons: 12 were not relevant to the study objectives [24–35], seven provided insufficient data for effect-size calculation [36–42], ten used detection methods outside the scope of this review [43–52], one included a sample size of fewer than 25 animals [53], one was not a cross-sectional study [54], two were review articles, systematic reviews, or meta-analyses [55, 56], and 18 were conducted outside Southeast Asia [57–74].

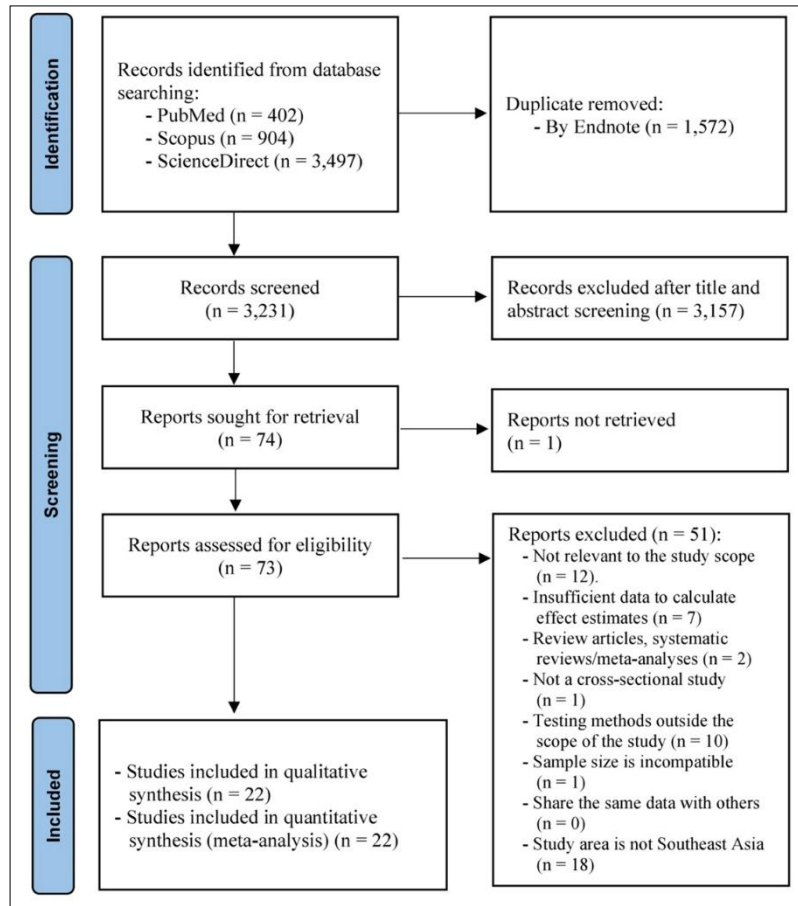


Figure 1: Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flow diagram illustrating the identification, screening, eligibility assessment, and inclusion of studies in the systematic review.

A total of 22 cross-sectional studies were included in the qualitative and quantitative analyses, and their characteristics are summarized in Supplementary File 1. Among these studies, 14 investigated hemoparasites in both beef and dairy cattle [9, 75–87], three investigated buffaloes only [88–90], and five included both cattle and buffaloes [91–95]. *Anaplasma* spp. were reported in nine studies [9, 75, 76, 80–83, 85, 88], *Babesia* spp. in ten studies [77–81, 84, 87, 88, 90, 95], *Theileria* spp. in seven studies [9, 79–81, 88, 90, 92], *Trypanosoma evansi* in five studies [9, 86, 89, 91, 93], and *Trypanosoma theileri* in one study [91]. Two other pathogens, *Candidatus Mycoplasma haemobos* [9] and *Mycoplasma wenyonii* [94], were also reported.

Four *Anaplasma* species were identified, namely *Anaplasma centrale* [82], *Anaplasma bovis* [85], *Anaplasma marginale* [9, 75, 76, 80–83, 85, 88], and *Anaplasma platys* [9, 76, 85]. Three *Babesia* species were reported, including *Babesia bigemina* [77–81, 84, 87, 88, 90], *Babesia bovis* [77–81, 87, 88, 90, 95], and *Babesia ovata* [88]. Likewise, three *Theileria* species were identified, namely *Theileria orientalis* [9, 79–81, 88, 90], *Theileria sinensis* [9], and *Theileria* sp. [92]. These hemoparasites were reported from Indonesia [78, 86], Malaysia [9, 77, 82–84], the Philippines [88, 89, 91, 94], Thailand [75, 79–81, 85, 87, 90, 92, 93], and Vietnam [76, 95]. Quality assessment demonstrated that all 22 included studies were of high methodological quality (Figure 2).

Among the included studies, nine reported hemoparasite co-infections [9, 78–82, 88, 90, 91]. Three studies described co-infections in buffaloes, including dual infections [88, 90, 91] and triple infections [90]. The reported

dual co-infection patterns included *A. marginale* with *B. bigemina*, *A. marginale* with *B. bovis*, *B. bigemina* with *B. bovis*, *B. bigemina* with *T. orientalis*, *B. bovis* with *T. orientalis*, and *Tr. evansi* with *Tr. theileri*. The only reported triple co-infection in buffaloes comprised *B. bigemina*, *B. bovis*, and *T. orientalis*.

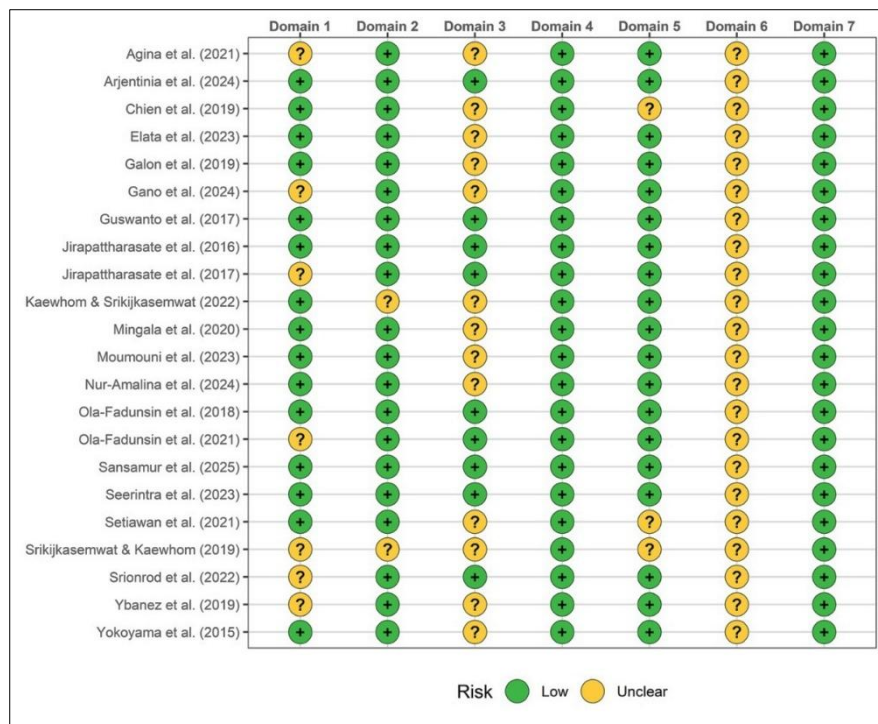


Figure 2: Quality assessment of the included studies. The assessment domains comprised selection bias (Domain 1 = Was the research question clearly described?; Domain 2 = Were the animal characteristics clearly described?), performance bias (Domain 3 = Was the sampling method clearly described?; Domain 4 = Was the polymerase chain reaction-based detection method clearly described?), detection bias (Domain 5 = Were the study subjects categorized into appropriate subgroups?; Domain 6 = Were outcome measurements performed using a blinded method?), and reporting bias (Domain 7 = Was selective reporting bias present?). The maximum quality assessment score was 14 (seven domains). Studies scoring 9–14 were classified as high quality, whereas those scoring 0–8 were classified as low quality. Each domain was scored as 2 (low risk of bias), 1 (unclear risk of bias), or 0 (high risk of bias).

Six studies reported co-infections in cattle, including dual [9, 78–82], triple [9, 79–81], and quadruple infections [81]. The dual co-infection patterns included *A. centrale* with *A. marginale*, *A. marginale* with *B. bigemina*, *A. marginale* with *B. bovis*, *A. marginale* with *T. orientalis*, *A. platys* with *T. sinensis*, *B. bigemina* with *B. bovis*, *B. bigemina* with *T. orientalis*, *B. bovis* with *T. orientalis*, *T. orientalis* with *T. sinensis*, and *T. sinensis* with *Candidatus Mycoplasma haemobos*. Triple co-infections included *A. marginale*, *B. bigemina*, and *B. bovis*; *A. marginale*, *B. bigemina*, and *T. orientalis*; *A. marginale*, *B. bovis*, and *T. orientalis*; *A. marginale*, *T. sinensis*, and *Candidatus Mycoplasma haemobos*; and *B. bigemina*, *B. bovis*, and *T. orientalis*. The only reported quadruple co-infection consisted of *A. marginale*, *B. bigemina*, *B. bovis*, and *T. orientalis*.

Crude prevalence of hemoparasites in cattle and buffaloes in Southeast Asia

The 22 included studies investigated 5,789 cattle and 901 buffaloes. Hemoparasite infections included *Anaplasma* spp. (932 cattle and 29 buffaloes), *Babesia* spp. (781 cattle and 77 buffaloes), *Theileria* spp. (141 cattle and 40 buffaloes), *Tr. evansi* (128 cattle and 99 buffaloes), *Candidatus M. haemobos* (25 cattle), and *M. wenyonii* (32 cattle and 80 buffaloes).

Among the identified parasite species, *A. centrale* was detected in 23 cattle, *A. marginale* in 868 cattle and 29 buffaloes, and *A. platys* in 41 cattle. Among *Babesia* species, *B. bigemina* was detected in 472 cattle and 33 buffaloes, whereas *B. bovis* was detected in 309 cattle and 44 buffaloes. Among *Theileria* species, *T. orientalis* was detected in 73 cattle and 37 buffaloes, whereas *T. sinensis* was detected in 22 cattle.

The highest reported prevalence of each parasite group was 72.63% for *Anaplasma* spp. in Malaysia [83], 71.13% for *Babesia* spp. in Indonesia [78], 32.79% for *Theileria* spp. in Malaysia [9], 35.45% for *Tr. evansi* in Thailand [93], and 12.58% for *Tr. theileri* in the Philippines [91]. *Candidatus M. haemobos* was reported only in Malaysia, with a prevalence of 40.99% [9], whereas *M. wenyonii* was reported only in the Philippines, with a prevalence of 80.00% [94].

Crude prevalence of hemoparasites in beef and dairy cattle

Anaplasma spp. infections in dairy cattle were reported in three studies [75, 81, 82], with the highest prevalence (31.40%) reported in Malaysia. In beef cattle, *Anaplasma* spp. infections were reported in two studies [9, 80], with the highest prevalence (26.23%) also reported in Malaysia.

Babesia spp. infections in dairy cattle were reported in two studies [81, 87], with the highest prevalence (8.39%) reported in Thailand. In beef cattle, *Babesia* spp. infections were reported in five studies [77, 79, 80, 84, 95], with the highest prevalence (37.14%) reported in Malaysia.

Theileria spp. infections in dairy cattle were reported in one study from Thailand [81], with a prevalence of 23.40%. In beef cattle, *Theileria* spp. infections were reported in five studies [9, 79, 80, 91, 92], with the highest prevalence (72.13%) reported in Malaysia.

Tr. evansi infection was reported exclusively in beef cattle in four studies [9, 86, 91, 93], with the highest prevalence (36.65%) reported in Thailand.

Target genes for PCR detection of hemoparasites

The PCR target genes identified for each hemoparasite are summarized as follows. *A. centrale* was detected using the 16S ribosomal ribonucleic acid (16S rRNA) gene [82]. *A. marginale* was detected using the 16S rRNA, *groEL*, and major surface protein 4 (*MSP4*) genes [9, 75, 76, 80–83, 85, 88]. *A. platys* was detected using the 16S rRNA gene [9].

For *Babesia* spp., *B. bigemina* was detected using the rhoptry-associated protein 1a (*RAP-1a*) and apical membrane antigen 1 (*AMA-1*) genes [77–81, 84, 87, 88, 90], whereas *B. bovis* was detected using spherical body protein 2 (*SBP-2*), spherical body protein 4 (*SBP-4*), and merozoite surface antigen (*MSA*) genes [77–81, 87, 88, 90, 95].

Among *Theileria* spp., *T. orientalis* and *T. sinensis* were detected using the major piroplasm surface protein (*MPSP*) gene [9, 79–81, 88, 90].

For *Tr. evansi*, the target genes included *RoTat* 1.2 variant surface glycoprotein (*VSG*) [9, 89], internal transcribed spacer (*ITS*) 1 [91], *ITS2* [86], and satellite DNA [93]. *Tr. theileri* was detected using *ITS1* [91]. *Candidatus M. haemobos* and *M. wenyonii* were detected using the 16S rRNA gene [9, 94].

This systematic review provides the first comprehensive, region-wide inventory of molecular diagnostic targets for major hemoparasites infecting cattle and buffaloes in Southeast Asia. The identified molecular targets included 16S rRNA, *groEL*, *MSP4*, *RAP-1a*, *AMA-1*, *SBP-2*, *SBP-4*, *MSA*, *MPSP*, *RoTat* 1.2 *VSG*, *ITS1*, *ITS2*, and satellite DNA. Collectively, these findings represent the first integrated regional synthesis of molecular diagnostic targets for these pathogens and the first comprehensive summary of molecular diagnostic evidence across multiple hemoparasite genera infecting both cattle and buffaloes.

Pooled prevalence of hemoparasites in cattle and buffaloes in Southeast Asia

The pooled prevalence estimates for the major hemoparasites are presented in Figure 3. *Anaplasma* spp. had a pooled prevalence of 12% (95% CI: 3%-38%; nine studies), with a 95% prediction interval (PI) of 0.37%-89.50% and substantial heterogeneity ($I^2 = 98.6\%$). *Babesia* spp. had a pooled prevalence of 18% (95% CI: 11%-28%; ten studies), with a 95% PI of 3.16%-61.30% and substantial heterogeneity ($I^2 = 97.0\%$). *Theileria* spp. had a pooled prevalence of 11% (95% CI: 3%-35%; seven studies), with a 95% PI of 0.43%-84.85% and substantial heterogeneity ($I^2 = 94.8\%$).

Meta-analysis was not performed for *Candidatus M. haemobos* or *M. wenyonii* because fewer than three eligible studies were available. Species-specific analyses showed that *Tr. evansi* (Figure 3) had a pooled prevalence of 11% (95% CI: 4%-28%; five studies), with a 95% PI of 0.72%-70.64% and substantial heterogeneity ($I^2 = 95.1\%$). *A. marginale* (Figure 4) had a pooled prevalence of 5% (95% CI: 1%-21%; nine studies), with a 95% PI of 0.29%-72.55% and substantial heterogeneity ($I^2 = 94.7\%$). *B. bigemina* (Figure 4) had a pooled prevalence of 10% (95% CI: 5%-18%; nine studies), with a 95% PI of 0.88%-54.90% and substantial heterogeneity ($I^2 = 94.6\%$). *B. bovis* (Figure 4) had a pooled prevalence of 9% (95% CI: 4%-17%; nine studies), with a 95% PI of 0.95%-52.07% and substantial heterogeneity ($I^2 = 97.9\%$). *T. orientalis* (Figure 4) had a pooled prevalence of 10% (95% CI: 3%-31%; six studies), with a 95% PI of 0.77%-73.70% and substantial heterogeneity ($I^2 = 90.2\%$).

Meta-regression and publication bias

The I^2 values for all pooled analyses exceeded 50% (Figures 3 and 4), indicating substantial between-study heterogeneity. Meta-regression analysis explored potential sources of heterogeneity and showed that the

prevalence of *A. marginale*, *B. bigemina*, *T. orientalis*, and *Tr. evansi* was not significantly influenced by geographical region or host species (Table 1). In contrast, the prevalence of *B. bovis* differed significantly between mainland and maritime Southeast Asian countries (meta-regression, $p = 0.0048$), with a higher pooled prevalence observed in maritime countries.

Visual inspection of the funnel plot (Figure 5) suggested slight asymmetry, indicating possible publication bias. However, Egger's regression test did not detect statistically significant publication bias ($p = 0.1293$), suggesting no strong evidence of small-study effects. These findings should be interpreted with caution because of the limited number of included studies. Funnel plot assessment and Egger's regression test were performed only for *Babesia* spp., as fewer than ten studies were available for the remaining hemoparasite groups.

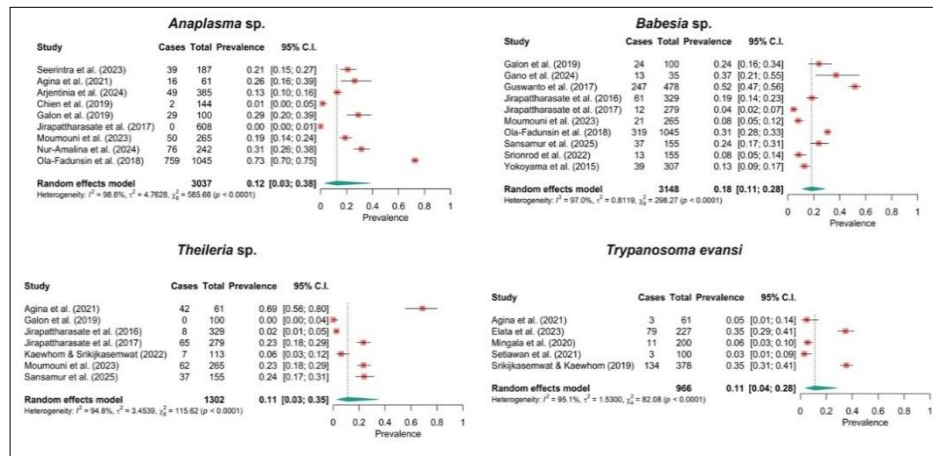


Figure 3: Forest plots showing the pooled prevalence of infections caused by *Anaplasma* spp., *Babesia* spp., *Theileria* spp., and *Trypanosoma evansi* in cattle and buffaloes in Southeast Asia. CI = Confidence interval; PI = Prediction interval.

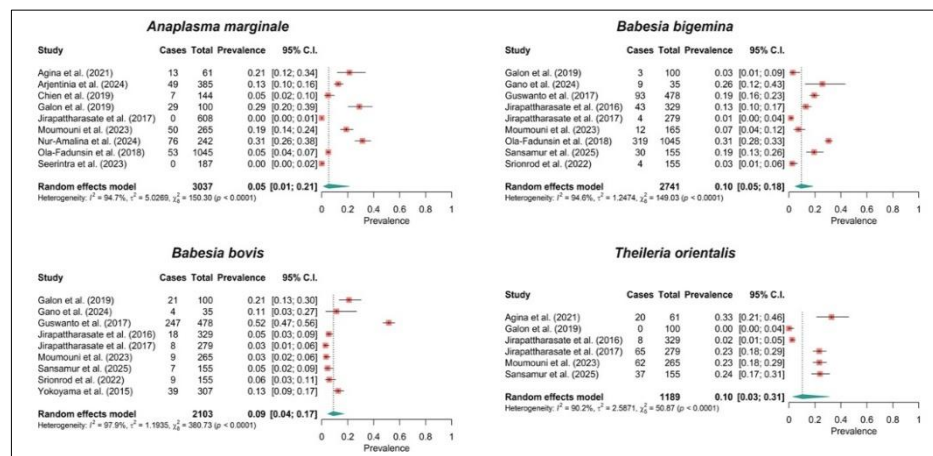


Figure 4: Forest plots showing the pooled prevalence of *Anaplasma marginale*, *Babesia bigemina*, *Babesia bovis*, and *Theileria orientalis* infections in cattle and buffaloes in Southeast Asia. CI = Confidence interval; PI = Prediction interval.

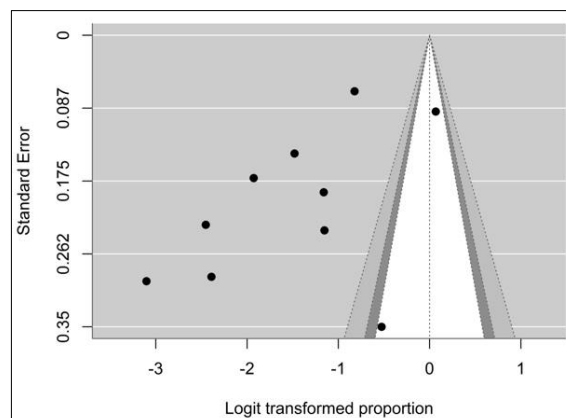


Figure 5: Funnel plot showing publication bias among studies reporting the prevalence of *Babesia* spp. infections in cattle and buffaloes in Southeast Asia.

Table 1: Pooled prevalence, heterogeneity, and meta-regression of major hemoparasites in cattle and buffaloes in Southeast Asia.

Variable	Category	No. of studies	No. examined	No. positive	Pooled prevalence (95% CI)	Q (χ^2)	p-value	I ² (%)	Meta-regression (p-value)
<i>A. marginale</i>	Region								
	Mainland	8	2,937	931	6.49% (1.93–19.70%)	625.95	<0.001	98.90	0.5522
	Maritime	1	100	29	29.00% (20.11–37.89%)	NA	NA	NA	
	Host species								
	Cattle	8	2,937	931	6.49% (1.93–19.70%)	625.95	<0.001	98.90	0.5522
<i>B. bigemina</i>	Buffaloes	1	100	29	29.00% (20.11–37.89%)	NA	NA	NA	
	Region								
	Mainland	7	2,263	409	9.55% (3.87–21.68%)	149.50	<0.001	96.00	0.9222
	Maritime	2	578	96	16.61% (13.58–19.64%)	NA	NA	NA	
	Host species								
<i>B. bovis</i>	Cattle	7	2,586	484	9.57% (3.88–21.72%)	151.81	<0.001	96.00	0.9112
	Buffaloes	2	255	33	12.94% (8.82–17.06%)	NA	NA	NA	
	Region								
	Mainland	7	1,525	94	9.55% (3.87–21.68%)	54.44	<0.001	87.10	0.0048*
	Maritime	2	578	268	46.37% (42.30–50.43%)	NA	NA	NA	
<i>T. orientalis</i>	Host species								
	Cattle	7	1,799	318	9.57% (3.88–21.72%)	358.68	<0.001	98.30	0.4133
	Buffaloes	3	304	44	15.72% (4.65–41.64%)	23.06	<0.001	91.30	
	Region								
	Mainland	5	1,089	192	17.31% (7.14–36.29%)	50.87	<0.001	92.10	0.0463
<i>Tr. evansi</i>	Maritime	1	100	0	0.00% (0.00–0.00%)	NA	NA	NA	
	Host species								
	Cattle	4	934	155	15.79% (4.93–40.40%)	50.42	<0.001	94.00	0.5197
	Buffaloes	2	255	37	14.51% (10.19–18.83%)	NA	NA	NA	
	Region								
<i>Tr. evansi</i>	Mainland	2	439	137	31.21% (26.87–35.54%)	NA	NA	NA	0.6143
	Maritime	4	527	93	13.35% (3.64–38.58%)	61.44	<0.001	95.10	
	Host species								
	Cattle	4	518	131	13.24% (3.52–38.94%)	38.48	<0.001	92.20	0.6194
	Buffaloes	3	448	91	20.61% (5.80–52.25%)	45.26	<0.001	95.60	

CI = Confidence interval; χ^2 = Cochran's Q statistic; I² = Percentage of total variation attributable to between-study heterogeneity; NA = Not available because fewer than three studies were included in the subgroup analysis; *A. marginale* = *Anaplasma marginale*; *B. bigemina* = *Babesia bigemina*; *B. bovis* = *Babesia bovis*; *T. orientalis* = *Theileria orientalis*; *Tr. evansi* = *Trypanosoma evansi*; $p < 0.05$ indicates a statistically significant meta-regression result.

DISCUSSION

Prevalence and environmental factors

This systematic review advances the understanding of hemoparasite epidemiology in Southeast Asia in four important ways. First, it provides the first PCR-based pooled prevalence estimates of multiple hemoparasite genera infecting cattle and buffaloes across Southeast Asia during 2015–2025. Second, it presents the first comprehensive inventory of molecular diagnostic targets used in regional studies. Third, it identifies a significant difference in *B. bovis* prevalence between mainland and maritime Southeast Asian countries. Finally, it translates these findings into practical recommendations to strengthen regional veterinary diagnostic capacity, extending the evidence beyond previous single-country reviews.

The pooled prevalence estimates were 12% for *Anaplasma* spp., 18% for *Babesia* spp., 11% for *Theileria* spp., and 11% for *Tr. evansi*. These hemoparasites adversely affect livestock health and negatively impact the livelihoods of resource-poor farming communities in developing countries [96]. The tropical climate of Southeast Asian countries, characterized by high temperatures and humidity [97], provides favorable conditions for arthropod vector development [7]. In addition, the region harbors high arthropod vector diversity [8, 15], including 19, 61, 11, 48, 62, and 65 tick species reported from Cambodia, Indonesia, Laos, Malaysia, Thailand, and Vietnam, respectively [7]. Thailand alone has reported 128 tabanid species and 14 *Stomoxys* species, which serve as important vectors of hemoparasitic diseases [15]. Collectively, these environmental and ecological factors emphasize the continued risk of vector-borne diseases throughout Southeast Asian countries.

Meta-regression analysis further demonstrated a significant difference in *B. bovis* prevalence between mainland and maritime Southeast Asian countries ($p = 0.0048$). The higher prevalence observed in maritime countries may reflect ecological conditions that favor tick survival and *B. bovis* transmission. Differences in cattle

movement and livestock trade may also contribute to the observed regional variation. However, the interpretation of this finding is limited by the availability of data on vector abundance, environmental conditions, livestock movement, and management practices across the two regions. These knowledge gaps highlight the importance of adopting a One Health approach, together with consideration of socioeconomic factors, to improve the understanding, surveillance, and control of hemoparasitic and other zoonotic diseases in Southeast Asian countries.

Co-infection patterns, economic impacts, and zoonotic potential

The synthesized evidence demonstrated diverse hemoparasite co-infection patterns in both cattle and buffaloes. Cattle exhibited dual, triple, and quadruple co-infections, whereas buffaloes exhibited only dual and triple co-infections. Co-infections involving multiple hemoparasites are likely to result in more severe clinical manifestations than single-species infections because of the combined pathogenic effects of different parasites. For example, *A. marginale* induces anemia through splenic clearance of infected erythrocytes, whereas *Babesia* spp. directly destroy erythrocytes during intracellular replication [10, 11]. Concurrent infection with these pathogens may therefore contribute to more severe disease.

Previous studies have reported substantial production losses associated with *Anaplasma*, *Babesia*, and *Theileria* infections, resulting in considerable economic losses due to reduced productivity and increased mortality [98]. In addition, *A. platys* and *Tr. evansi* have been reported in human infections, suggesting potential zoonotic importance, although their zoonotic roles remain to be fully established [14, 99]. The findings of the present study provide baseline information to support future epidemiological and molecular investigations of transmission pathways, zoonotic potential, vector control, acaricide resistance, and the effects of climate change on hemoparasite distribution. A One Health approach will be essential to strengthen surveillance systems and improve the prevention and control of these diseases in Southeast Asia.

Diagnostic techniques for hemoparasites in cattle and buffaloes

The diagnosis of hemoparasite infections has traditionally relied on clinical signs together with laboratory detection of the causative agents [96]. PCR has been demonstrated to be a sensitive and reliable method for the molecular detection of hemoparasites in animals [100]. This systematic review provides the first comprehensive, region-wide synthesis of molecular diagnostic targets for the major hemoparasites infecting cattle and buffaloes in Southeast Asia by integrating evidence across multiple parasite genera. The compiled molecular targets may serve as a useful reference for veterinary diagnostic laboratories and help improve laboratory diagnostic capacity and technical competency.

However, PCR-based assays may not be readily available in all settings because of financial and technical constraints. In such circumstances, conventional methods, including blood smear and buffy coat smear examinations, remain practical and cost-effective diagnostic alternatives [101], particularly in rural areas where cattle and buffaloes are raised as sources of household income and financial security. The molecular and parasitological information summarized in this review may also help veterinary technicians and laboratory personnel improve their diagnostic competency for hemoparasitic diseases.

Limitations of the systematic review and meta-analysis

This systematic review and meta-analysis has several limitations. First, substantial between-study heterogeneity was observed, with I^2 values of 98.60%, 97.00%, 94.80%, and 95.10% for *Anaplasma*, *Babesia*, *Theileria*, and *Trypanosoma*, respectively. This heterogeneity may reflect differences in geographical location, study populations, host species, sample size, and study methodology. Although meta-regression identified geographical region and host species as significant contributors to heterogeneity for *Babesia* infections, a considerable proportion remained unexplained.

Second, although visual inspection of the funnel plot suggested possible publication bias for *Babesia* studies, Egger's regression test did not provide statistically significant evidence of publication bias. Moreover, these analyses could not be performed for the remaining hemoparasites because fewer than ten studies were available for each group, limiting the ability to assess potential publication bias and the possibility of underestimated pooled prevalence estimates.

Finally, the inclusion criteria restricted the analysis to PCR-based studies published in the English language and excluded studies using serological methods. These selection criteria may have introduced publication and language biases and should be considered when interpreting the findings of this review.

CONCLUSION

This systematic review and meta-analysis provides the first comprehensive synthesis of PCR-confirmed prevalence and molecular diagnostic targets of major hemoparasites infecting cattle and buffaloes across Southeast Asia during 2015–2025. Analysis of 22 cross-sectional studies demonstrated pooled prevalences of 12% for *Anaplasma* spp., 18% for *Babesia* spp., 11% for *Theileria* spp., and 11% for *Tr. evansi*, indicating that these pathogens remain widely distributed throughout the region. The study further identified a significantly higher prevalence of *Babesia bovis* in maritime than mainland Southeast Asian countries and compiled the principal molecular targets currently used for PCR-based diagnosis, including 16S rRNA, *groEL*, *MSP4*, *RAP-1a*, *AMA-1*, *SBP-2*, *SBP-4*, *MSA*, *MPSP*, *RoTat 1.2 VSG*, *ITS1*, *ITS2*, and satellite DNA.

The findings have important practical implications for veterinary diagnostics and disease surveillance. The consolidated molecular diagnostic targets provide a valuable reference for selecting appropriate PCR assays and may help harmonize molecular diagnostic protocols among veterinary laboratories in Southeast Asia. At the same time, conventional diagnostic approaches, such as blood smear and buffy coat smear examinations, remain practical and cost-effective alternatives for routine screening in resource-limited settings where molecular techniques are unavailable.

A major strength of this study is that it represents the first PRISMA-guided, region-wide meta-analysis integrating PCR-confirmed prevalence estimates, molecular diagnostic targets, co-infection patterns, and meta-regression analyses across multiple hemoparasite genera in cattle and buffaloes.

Future research should expand molecular surveillance to underrepresented Southeast Asian countries, standardize molecular diagnostic methodologies, integrate vector ecology and livestock movement data with molecular epidemiology, and investigate the influence of environmental and management factors on hemoparasite transmission using a One Health framework. Longitudinal studies and genomic surveillance will further improve understanding of pathogen diversity, transmission dynamics, and emerging epidemiological trends.

In conclusion, this study provides robust regional evidence on the molecular epidemiology of major bovine hemoparasites in Southeast Asia and establishes a comprehensive reference for molecular diagnosis and disease surveillance. The findings contribute to strengthening veterinary diagnostic capacity, supporting evidence-based surveillance programs, and improving the prevention and control of hemoparasitic diseases in cattle and buffaloes, thereby enhancing livestock productivity, food security, and the sustainability of the livestock sector across the Southeast Asian countries.

DATA AVAILABILITY

The data generated during the study are included in the manuscript.

GENERATIVE AI DECLARATION

The authors declare that no generative AI was used in the preparation of this manuscript.

AUTHORS' CONTRIBUTIONS

NP, NS, and PP: Conceptualization, methodology, data collection, and data curation. NP, NS, TNT, and PP: Investigation and formal analysis. PP: Software and validation. NS: Writing—original draft. NP, TNT, and PP: Writing—review and editing. All authors have read and approved the final manuscript.

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COMPETING INTERESTS

The authors declare that they have no competing interests.

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REFERENCES

1. Trakem V, Fan H. Agricultural trade liberalization, governance quality, and technical efficiency in the agricultural sector of Southeast Asia. *Heliyon*. 2024;10:e39553.
2. General Statistics Office of Vietnam. Socio-economic situation in the fourth quarter and 2025. 2026.
3. Office of the National Economic and Social Development Council. Gross domestic product of Thailand: Q4/2025. 2026.
4. Bush RD. Advances in smallholder large ruminant production and profitability in Southeast Asia over the past decade – lessons from the Mekong region: a review. *Anim Prod Sci*. 2024;64(13):AN23406.
5. Office of Agricultural Economics. Agricultural statistics of Thailand 2025. 2026.
6. Sivakumar T, Lan DT, Long PT, Yoshinari T, Tattiyapong M, Guswanto A, *et al*. PCR detection and genetic diversity of bovine hemoprotozoan parasites in Vietnam. *J Vet Med Sci*. 2013;75(11):1455–1462.
7. Yean S, Prasetyo DB, Marcombe S, Hadi UK, Kazim AR, Tiawsirisup S, *et al*. Challenges for ticks and tick-borne diseases research in Southeast Asia: insight from the first international symposium in Cambodia. *PLoS Negl Trop Dis*. 2024;18(7):e0012269.
8. Yean S, Krib D, Chea R, Sum S, Ren T, San S, *et al*. Tick diversity and tick-borne pathogens in livestock and companion animals in Cambodia: a narrative review and new field data (2022–2023). *Vet Parasitol Reg Stud Rep*. 2026;67:101407.
9. Agina OA, Shaari MR, Nur-Mahiza NMM, Ajat M, Zamri-Saad M, Mazlan M, *et al*. Molecular detection of *Theileria* species, *Anaplasma* species, *Candidatus Mycoplasma haemobos*, *Trypanosoma evansi* and first evidence of *Theileria sinensis*-associated bovine anaemia in crossbred Kedah-Kelantan × Brahman cattle. *BMC Vet Res*. 2021;17(1).
10. Ierardi RA. A review of bovine anaplasmosis (*Anaplasma marginale*) with emphasis on epidemiology and diagnostic testing. *J Vet Diagn Invest*. 2025;37(4):517–538.
11. Hakimi H, Yamagishi J, Kawazu SI, Asada M. Advances in understanding red blood cell modifications by *Babesia*. *PLoS Pathog*. 2022;18(9):e1010770.
12. Gebrekidan H, Perera PK, Ghafar A, Abbas T, Gasser RB, Jabbar A. An appraisal of oriental theileriosis and the *Theileria orientalis* complex, with an emphasis on diagnosis and genetic characterisation. *Parasitol Res*. 2020;119:11–12.
13. Morrison WI. The aetiology, pathogenesis and control of theileriosis in domestic animals. *Rev Sci Tech Off Int Epiz*. 2015;34(2):599–611.
14. Desquesnes M, Holzmüller P, Lai DH, Dargantes A, Lun ZR, Jittaplapong S. *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *BioMed Res Int*. 2013;2013:194176.
15. Phetcharat Y, Wongtawan T, Fungwithaya P, Amendt J, Sontigun N. Species diversity and seasonal abundance of Stomoxyinae (Diptera: Muscidae) and tabanid flies (Diptera: Tabanidae) on a beef cattle and a buffalo farm in Nakhon Si Thammarat province, Southern Thailand. *Insects*. 2024;15:818.
16. Soe BK, Huertas-Lopez A, Sato H, Adisakwattana P. Ticks and tick-borne pathogens with zoonotic importance in animals in Southeast Asia: a decade of evidence from systematic review and meta-analysis. *Acta Trop*. 2026;274:107974.
17. Firdausy LW, Fikri F, Wicaksono AP, Çalışkan H, Purnama MTE. Prevalence of trypanosomiasis in domesticated animals in Indonesia: a systematic review and meta-analysis. *Vet World*. 2025;18(5):1333–1344.
18. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, *et al*. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. 2021;372:n71.
19. Zhou S, Sang Z, Wang L, Zhang T. Seroprevalence of *Toxoplasma gondii* in cats in mainland China 2016–2020: a meta-analysis. *J Vet Sci*. 2022;23(1):e13.
20. R Core Team. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing; 2026.
21. Wang N. Conducting meta-analyses of proportions in R. *J Behav Data Sci*. 2023;3:64–126.
22. Huedo-Medina TB, Sánchez-Meca J, Marín-Martínez F, Botella J. Assessing heterogeneity in meta-analysis: Q statistic or I² index? *Psychol Methods*. 2006;11(2):193–206.
23. Sukati S, Kotepui KU, Masangkay FR, Tseng CP, Mahittikorn A, Anabire NG, *et al*. Elevations in D-dimer levels in patients with *Plasmodium* infections: a systematic review and meta-analysis. *Sci Rep*. 2025;15:858.

24. Abd-Elrahman SM, Kamel FA, Abdel-Hakeem SS, Khedr AA, Mohamed SM, Abdelgaber AA, *et al.* Piroplasm infestations in cattle: exploring tick control using *Chrysanthemum* extract and neem oil emulsion. *Front Vet Sci.* 2025;12:1543162.
25. Bibi S, Abbas S, Zaman MA, Gul R, Batool AI, Khalil I, *et al.* Comparative efficacy of imidocarb dipropionate with additive in naturally infected cattle against bovine babesiosis. *Vet Parasitol.* 2025;337:110495.
26. Elata A, Galon EM, Moumouni PFA, Ybanez RHD, Mossaad E, Salces CB, *et al.* First molecular detection and identification of *Trypanosoma evansi* in goats from Cebu, Philippines using a PCR-based assay. *Vet Parasitol Reg Stud Rep.* 2020;21:100414.
27. Ma Q, Liu J, Li Z, Xiang Q, Wang J, Liu A, *et al.* Clinical and pathological studies on cattle experimentally infected with *Theileria annulata* in China. *Pathogens.* 2020;9(9):727.
28. Park MH, Cho SJ, Kim Y, Cho HC, Park YJ, Ji MJ, *et al.* First molecular evidence of bovine hemoplasmas in houseflies (*Musca domestica*). *BMC Vet Res.* 2024;20(1):497.
29. Sharma A, Singla LD, Ashuma, Batth BK, Kaur P. Clinicopatho-biochemical alterations associated with subclinical babesiosis in dairy animals. *J Arthropod Borne Dis.* 2016;10(2):258–266.
30. Ahlawat S, Choudhary V, Kaur R, Arora R, Sharma R, Chhabra P, *et al.* Unraveling the genetic mechanisms governing the host response to bovine anaplasmosis. *Gene.* 2023;877:147532.
31. Arnuphappasert A, Nugraheni YR, Aung A, Asada M, Kaewthamasorn M. Detection of *Babesia bovis* using loop-mediated isothermal amplification (LAMP) with improved thermostability, sensitivity and alternative visualization methods. *Sci Rep.* 2023;13:1838.
32. El-Alfy ES, Abbas I, Elseadawy R, El-Sayed SAE S, Rizk MA. Genetic diversity of merozoite surface antigens in global *Babesia bovis* populations. *Genes.* 2023;14(10):1936.
33. Muriuki R, Ndichu M, Githigia S, Svitek N. Novel CRISPR-Cas-powered pen-side test for East Coast fever. *Int J Parasitol.* 2024;54(10):507–521.
34. Muriuki R, Ndichu M, Githigia S, Svitek N. CRISPR-Cas-based pen-side diagnostic tests for *Anaplasma marginale* and *Babesia bigemina*. *Microorganisms.* 2024;12(12):2595.
35. Hamid PH, Cahyadi M, Wardhana AH, Sawitri DH, Setya NNR, Insyariati T, *et al.* First autochthonous report on cattle *Babesia naookii* in Central Java, Indonesia, and identification of *Haemaphysalis bispinosa* ticks in the investigated area. *Pathogens.* 2023;12(1):59.
36. Aktas M, Özübek S. Outbreak of anaplasmosis associated with novel genetic variants of *Anaplasma marginale* in a dairy cattle. *Comp Immunol Microbiol Infect Dis.* 2017;54:20–26.
37. Chaisi ME, Baxter JR, Hove P, Choopa CN, Oosthuizen MC, Brayton KA, *et al.* Comparison of three nucleic acid-based tests for detecting *Anaplasma marginale* and *Anaplasma centrale* in cattle. *Onderstepoort J Vet Res.* 2017;84(1):e1–e9.
38. Ganguly A, Maharana BR, Ganguly I. Pentaplex PCR assay for rapid differential detection of *Babesia bigemina*, *Theileria annulata*, *Anaplasma marginale* and *Trypanosoma evansi* in cattle. *Biologicals.* 2020;63:81–88.
39. Gebrekidan H, Nelson L, Smith G, Gasser RB, Jabbar A. An outbreak of oriental theileriosis in dairy cattle imported to Vietnam from Australia. *Parasitology.* 2017;144(6):738–746.
40. Sharma B, Ganta RR, Stone D, Alhassan A, Lanza-Perea M, Belmar VM, *et al.* Development of a multiplex PCR and magnetic DNA capture assay for detecting six species pathogens of the genera *Anaplasma* and *Ehrlichia* in canine, bovine, caprine and ovine blood samples from Grenada, West Indies. *Pathogens.* 2021;10(2):192.
41. Zhang B, Sambono JL, Morgan JAT, Venus B, Rolls P, Lew-Tabor AE. An evaluation of quantitative PCR assays (TaqMan® and SYBR green) for the detection of *Babesia bigemina* and *Babesia bovis*, and a novel fluorescent-ITS1-PCR capillary electrophoresis method for genotyping *B. bovis* isolates. *Vet Sci.* 2016;3(3):23.
42. Lakew BT, Kheravii SK, Wu SB, Eastwood S, Andrew NR, Nicholas AH, *et al.* Detection and distribution of haematophagous flies and lice on cattle farms and potential role in the transmission of *Theileria orientalis*. *Vet Parasitol.* 2021;298:109516.
43. Garcia MC, Walshe E, Steketee PC, Paxton E, Lopez-Vidal J, Pearce MC, *et al.* Comparative sensitivity and specificity of the 7SL sRNA diagnostic test for animal trypanosomiasis. *Front Vet Sci.* 2022;9:868912.
44. Jansaento W, Osiriphan M, Kongta N, Modethed W, Rompo T, Duangmano S. Enhanced detection of *Trypanosoma evansi* in cattle: superior performance of LAMP compared to PCR and CATT/T. *evansi* test. *J Assoc Med Sci.* 2025;58(3):278–287.
45. Jenkins C, Bogema DR. Factors associated with seroconversion to the major piroplasm surface protein of the bovine haemoparasite *Theileria orientalis*. *Parasit Vectors.* 2016;9:106.

46. Shahzad M, Garg R, Yadav S, Devi A, Ram H, Banerjee PS. Comparative evaluation of *Babesia bigemina* truncated C-terminal rhoptry associated protein-1 and 200 kDa merozoite protein in indirect enzyme-linked immunosorbent assay. *Ticks Tick Borne Dis.* 2021;12(5):101783.
47. Wibowo SE, Andityas M, Nuraini DM, Zurbein, Nugraheni YR, Awaludin A, *et al.* Spatial analysis of bovine anaplasmosis in Jambi province, Indonesia: 2018-2022. *Vet Integr Sci.* 2024;22(3):857–869.
48. Bitrus AA, Abba Y, Jesse FFA, Pei JLX, Peter ID, Hambali IU, *et al.* Seroprevalence of anaplasmosis in dairy cattle from Peninsular Malaysia. *Adv Anim Vet Sci.* 2018;6(2):70.
49. Koh FX, Kho KL, Kisomi MG, Wong LP, Bulgiba A, Tan PE, *et al.* *Ehrlichia* and *Anaplasma* infections: serological evidence and tick surveillance in Peninsular Malaysia. *J Med Entomol.* 2018;55(2):269–276.
50. Tong Q, Chen R, Kong Q, Goossens J, Radwanska M, Lou D, *et al.* DNA detection of *Trypanosoma evansi*: diagnostic validity of a new assay based on loop-mediated isothermal amplification (LAMP). *Vet Parasitol.* 2018;250:1–6.
51. Ryskeldina A, Korobeinikov A, Tursunbay N, Berdikulov M, Shevtsov A, Bauer C, *et al.* A novel recombinant *Theileria annulata* surface protein as an antigen in indirect enzyme-linked immunosorbent assay for the serological diagnosis of tropical theileriosis. *Vet World.* 2024;17(8):1936–1942.
52. Paliwal S, Sudan V, Shanker D. Comparative diagnostic performance of recombinant Tams1 protein based dot-ELISA in detecting tropical theileriosis in naturally infected cattle. *Sci Rep.* 2025;15(1):431.
53. Nugraheni YR, Ariyadi B, Rochmadiyanto, Kesumaningrum N, Imran K, Kartiko BP, *et al.* Molecular detection of *Babesia* infection in cattle in Yogyakarta, Indonesia. *Biodiversitas.* 2023;24(7):4192–4198.
54. Bohman W, Chooruang N, Sakuna K, Jarujareet W, Areekit K, Chantip D. Prevalence of hemoparasitic infections and influencing factors among fighting bulls in Southern Thailand: a retrospective analysis. *Open Vet J.* 2024;14(10):2587–2598.
55. Firdausy LW, Fikri F, Wicaksono AP, Çalışkan H, Purnama MTE. Prevalence of trypanosomiasis in domesticated animals in Indonesia: a systematic review and meta-analysis. *Vet World.* 2025;18(5):1333–1344.
56. Flores-García DL, Aguilar-Díaz H, Amaro-Estrada I, Martínez-Ocampo F, Quiroz-Castañeda RE. An update of bovine hemoplasmas based on phylogenetic and genomics analysis. *Microorganisms.* 2022;10(10):1916.
57. Fiorin FE, Casa MS, Griebeler LB, Goedel MF, Do Nascimento LFN, Das Neves GB, *et al.* Prevalence of natural infection by *Trypanosoma evansi* in Crioula LAGEANA cattle. *Microb Pathog.* 2023;180:106143.
58. Getange D, Mukaratirwa S, Bargul JL, Khogali R, Ng'uela J, Kabii J, *et al.* Molecular characterisation of tick-borne pathogens in cattle in Kenya: insights from blood, ticks, and skin swab analyses. *BMC Vet Res.* 2025;21:552.
59. Giglioti R, Filho AEV, Domingos AG, Silva SS, Cunha RC, Ibelli AMG, *et al.* Detection and quantification of *Babesia bovis* and *Babesia bigemina* using different target genes. *Res Vet Sci.* 2024;168:105122.
60. Giglioti R, Okino CH, Azevedo BT, Wedy BCR, Gutmanis G, Veríssimo CJ, *et al.* Semi-quantitative evaluation of *Babesia bovis* and *B. bigemina* infection levels estimated by HRM analysis. *Ticks Tick Borne Dis.* 2021;12(5):101753.
61. Guo QY, Cao WL, Wang BJ, Wang ZB, Wang S, Bayin C. Development and application of real-time PCR assay for detection of *Theileria annulata* infection in cattle in Xinjiang Uygur Autonomous Region, China. *Trop Biomed.* 2016;33(1):27–34.
62. Hussain MF, Qamar M, Malik MI, Hussain M, Saeed Z, Shaikh RS, *et al.* Molecular detection of *Anaplasma* in apparently healthy Cholistan breed of cattle from the Bahawalpur district, Pakistan. *Trop Biomed.* 2017;34(1):37–44.
63. Kamani J, Vieira TSWJ, Vieira RFC, Shekaro A, Nahum-Biala Y, Olubade T, *et al.* Molecular detection of *Theileria annulata*, *Theileria mutans* and *Theileria velifera* but no evidence of *Theileria parva* infected or vaccinated cattle in Nigeria despite extensive transboundary migrations. *Vet Parasitol Reg Stud Rep.* 2023;41:100887.
64. Kaur R, Ahlawat S, Choudhary V, Kumari A, Kumar A, Kaur M, *et al.* Expression profiling of cytokine genes in peripheral blood mononuclear cells from *Anaplasma marginale* infected and healthy cattle. *Anim Biotechnol.* 2023;34:4281–4290.
65. Kumar P, Kumar A, Sarma K, Sharma P, Kumari RR, Kumar M. Development of novel multiplex PCR for diagnosis of co-infected hemo-parasites in cattle. *Indian J Anim Res.* 2021;55(2):1504–1509.
66. Lim MM, Jia LJ, Cao SN, Moumouni PFA, Jirapattarasate C, Wang GB, *et al.* Molecular detection of *Theileria*

- species in cattle from Jilin province, China. *Trop Biomed.* 2017;34(3):598–606.
67. Nazar M, Khan MA, Shah AA, Rahman SU, Khan I, Ullah A, *et al.* Occurrence and transplacental transmission of *Anaplasma marginale* in dairy cattle. *Slovenian Vet Res.* 2018;55(3):183–191.
 68. Paoletta MS, Arias LLS, DLF S, Guillemi EC, Luciani C, Sarmiento NF, *et al.* Epidemiology of *Babesia*, *Anaplasma* and *Trypanosoma* species using a new expanded reverse line blot hybridization assay. *Ticks Tick Borne Dis.* 2018;9(2):155–163.
 69. Paucar-Quishpe V, Berkvens D, Pérez-Otáñez X, Rodríguez-Hidalgo R, Cepeda-Bastidas D, Perez C, *et al.* What is the value of testing for tick-borne diseases in cattle in endemic areas? A case study of bovine anaplasmosis. *PLoS One.* 2025;20(3):e0315202.
 70. Romero-Salas D, Mira A, Mosqueda J, García-Vázquez Z, Hidalgo-Ruiz M, Vela NAO, *et al.* Molecular and serological detection of *Babesia bovis*- and *Babesia bigemina*-infection in bovines and water buffaloes raised jointly in an endemic field. *Vet Parasitol.* 2016;217:101–107.
 71. Saad ASA, Hegab AA, Osman MM. Insights into some tick-borne pathogens in cows. *J Adv Vet Res.* 2025;15(3):320–324.
 72. Salazar A, Ochoa-Corona FM, Talley JL, Noden BH. Recombinase polymerase amplification (RPA) with lateral flow detection for three *Anaplasma* species of importance to livestock health. *Sci Rep.* 2021;11:15962.
 73. Sinha S, Behera BK, Padhi SK, Sahoo S, Sahoo N, Biswal S, *et al.* Comparative evaluation of diagnostic methods for detection of *Theileria* spp. in cows. *Anim Biotechnol.* 2023;34(8):3514–3518.
 74. Sudan V, Shanker D, Jaiswal A, Singh A, Pandey V. Standardization and validation of simple PCR, duplex PCR and RAPD in comparison to blood smear examination for diagnosing bovine tropical theileriosis. *Biologicals.* 2017;46:88–91.
 75. Arjentina IPGY, Keomoungkhoun B, Thamrongyoswittayakul C, Sangmaneedet S, Taweenan W. First report on the molecular detection and genetic diversity of *Anaplasma marginale* in healthy dairy cattle in Khon Kaen province, Thailand. *Vet World.* 2024;17(2):389–397.
 76. Chien NTH, Nguyen TL, Bui KL, Nguyen TV, Le TH. *Anaplasma marginale* and *A. platys* characterized from dairy and indigenous cattle and dogs in northern Vietnam. *Korean J Parasitol.* 2019;57(1):43–48.
 77. Gano AI, Ramanoon SZ, Aziz NA, Mazlan M, Shaari MR, Aliyu A, *et al.* Genetic characterization of the RAP-1A and SBP-4 genes of *Babesia* species infecting cattle from Selangor, Malaysia, and Ribah, Nigeria. *Pathogens.* 2024;13(3).
 78. Guswanto A, Allamanda P, Mariamah ES, Sodirun S, Wibowo PE, Indrayani L, *et al.* Molecular and serological detection of bovine babesiosis in Indonesia. *Parasit Vectors.* 2017;10(1).
 79. Jirapattharasate C, Adjou Moumouni PF, Cao S, Iguchi A, Liu M, Wang G, *et al.* Molecular epidemiology of bovine *Babesia* spp. and *Theileria orientalis* parasites in beef cattle from northern and northeastern Thailand. *Parasitol Int.* 2016;65(1):62–69.
 80. Jirapattharasate C, Adjou Moumouni PF, Cao S, Iguchi A, Liu M, Wang G, *et al.* Molecular detection and genetic diversity of bovine *Babesia* spp., *Theileria orientalis*, and *Anaplasma marginale* in beef cattle in Thailand. *Parasitol Res.* 2017;116(2):751–762.
 81. Moumouni PFA, Galon EM, Tumwebaze MA, Byamukama B, Ngasaman R, Tiwananthagorn S, *et al.* Tick-borne pathogen detection and its association with alterations in packed cell volume of dairy cattle in Thailand. *Animals.* 2023;13(18).
 82. Nur-Amalina N, Nur-Sabrina AM, Muhamad-Ali HAM, Mohammad-Sabri AR, Nor-Azlina AA, Basripuzi NH. Molecular identification and phylogenetic analysis of *Anaplasma marginale* and *Anaplasma centrale* isolated from commercial Mafriwal cattle in Johor, Malaysia. *Trop Biomed.* 2024;41:385–391.
 83. Ola-Fadunsin SD, Gimba FI, Abdullah DA, Sharma RSK, Jesse FFA, Sani RA. Epidemiology and risk factors associated with *Anaplasma marginale* infection of cattle in Peninsular Malaysia. *Parasitol Int.* 2018;67(6):659–665.
 84. Ola-Fadunsin SD, Sharma RSK, Abdullah DA, Gimba FI, Jesse FJF, Sani RA. The molecular prevalence, distribution and risk factors associated with *Babesia bigemina* infection in Peninsular Malaysia. *Ticks Tick Borne Dis.* 2021;12(3).
 85. Seerintra T, Saraphol B, Thanchomnang T, Piratae S. Molecular prevalence of *Anaplasma* spp. in cattle and assessment of associated risk factors in Northeast Thailand. *Vet World.* 2023;16(8):1702–1707.
 86. Setiawan A, Nurcahyo W, Priyowidodo D, Budiati RT, Susanti DSR. Genetic and parasitological identification of *Trypanosoma evansi* infecting cattle in South Sulawesi, Indonesia. *Vet World.* 2021;14(1):113–119.

87. Srionrod N, Nooroong P, Poolsawat N, Minsakorn S, Watthanadirek A, Junsiri W, *et al.* Molecular characterization and genetic diversity of *Babesia bovis* and *Babesia bigemina* of cattle in Thailand. *Front Cell Infect Microbiol.* 2022;12.
88. Galon EMS, Adjou Moumouni PF, Ybañez RHD, Ringo AE, Efstratiou A, Lee SH, *et al.* First molecular detection and characterization of tick-borne pathogens in water buffaloes in Bohol, Philippines. *Ticks Tick Borne Dis.* 2019;10(4):815–821.
89. Mingala CN, Abenoja JA, Rivera CV, Balbin MM, Venturina VM, Villanueva MA. *Trypanosoma evansi* and *Neospora caninum* among water buffaloes (*Bubalus bubalis*) in the Philippines. *Arch Vet Sci.* 2020;25(1):10–19.
90. Sansamur C, Boonchuay K, Ngasaman R, Olana KOA, Punyapornwithaya V. Epidemiology and factors associated with the infection of *Babesia bigemina*, *Babesia bovis*, and *Theileria orientalis* in Thale Noi Wetland buffaloes (*Bubalus bubalis*), Southern Thailand. *BMC Vet Res.* 2025;21(1).
91. Elata A, Galon EM, Moumouni PFA, Ybañez RH, Mossaad E, Salces CB, *et al.* Molecular detection of animal trypanosomes in different animal species in the Visayas Region of the Philippines. *Acta Parasitol.* 2023;68(3):604–611.
92. Kaewhom P, Srikijsakemwat K. A molecular survey of *Theileria* spp. in ruminants in the Thailand-Cambodia border region. *Int J Agric Technol.* 2022;18(1):205–214.
93. Srikijsakemwat K, Kaewhom P. Prevalence and genetic diversity of *Trypanosoma evansi* infection causing abortion among cattles and buffaloes in Eastern border area of Thailand-Cambodia. *Int J Agric Technol.* 2019;15(6):1021–1032.
94. Ybañez AP, Ybañez RHD, Armonia RKM, Chico JKE, Ferraren KJV, Tapdasan EP, *et al.* First molecular detection of *Mycoplasma wenyonii* and the ectoparasite biodiversity in dairy water buffalo and cattle in Bohol, Philippines. *Parasitol Int.* 2019;70:77–81.
95. Yokoyama N, Sivakumar T, Tuvshintulga B, Hayashida K, Igarashi I, Inoue N, *et al.* Genetic variations in merozoite surface antigen genes of *Babesia bovis* detected in Vietnamese cattle and water buffaloes. *Infect Genet Evol.* 2015;30:288–295.
96. Salih DA, Hussein AME, Singla LD. Diagnostic approaches for tick-borne haemoparasitic diseases in livestock. *J Vet Med Anim Health.* 2015;7(2):45–56.
97. Swangneat K, Srikacha N, Soulinthone N, Paudel S, Srisanyong W, Stott CJ, *et al.* Molecular prevalence of avian haemosporidian parasites in Southeast Asia: systematic review and meta-analysis. *Animals.* 2025;15:636.
98. Koonyosying P, Rittipornlertrak A, Chomjit P, Sangkakam K, Muenthaisong A, Nambooppha B, *et al.* Incidence of hemoparasitic infections in cattle from central and northern Thailand. *PeerJ.* 2022;10:e13835.
99. Mnisi ZTH, Makgabo SM, Byaruhanga C. *Anaplasma platys* beyond canines: a systematic review of host range, phylogenetic relatedness, and knowledge gaps in Africa. *Parasit Vectors.* 2026;19:128.
100. Bhagwan J, Singh Y, Jhambh R, Chaudhari M, Kumar P. Molecular and microscopic detection of haemoprotozoan diseases in dogs from Haryana, India. *Parasitol Res.* 2024;123:354.
101. Pornpanom P, Valkiūnas G, Paudel S. Morphological and molecular characterization of avian trypanosomes in domestic chickens (*Gallus gallus domesticus*) in Southeast Asia and review of the parasite morphometry in different avian hosts. *Avian Pathol.* 2025;54(4):489–497.
