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Systematic review on molecular diagnosis of cattle tick fever in Brazil: occurrence and future opportunities

Revisão sistemática sobre diagnóstico molecular da tristeza parasitária bovina no Brasil: ocorrência e oportunidades futuras

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Highlights

Molecular occurrence of 98.78% of *Anaplasma marginale* in the state of Rondônia.
Molecular occurrence of 86.25% of *Babesia bigemina* in the state of São Paulo.
Molecular occurrence of 95.47% of *Babesia bovis* in the state of Rondônia.
Study opportunities in the states of Mato Grosso and Pará.
Need for studies quantifying economic losses due to tick fever.

Abstract

Cattle farming in Brazil faces several challenges, among which vector-borne diseases cause significant losses due to their prevalence across the country. Molecular techniques have proven more sensitive in identifying persistently infected animals. Thus, the objective of this study was to compile data on the occurrence of *Anaplasma marginale, Babesia bigemina,* and *Babesia bovis,* which cause cattle tick fever (TF) and can only be detected by molecular techniques. This study also aimed to identify gaps and opportunities for future research in Brazil. To achieve this, a systematic review was conducted, involving the following main inclusion criteria: studies undertaken with samples from Brazil; use of samples from naturally infected bovine animals; accurate data on the number or percentage of positive animals; number of samples per herd ≥ 10; and information on at least the state of origin of the samples. In total, 38 studies were included in the review, employing conventional PCR techniques (multiplex PCR, PCR, nPCR, and snPCR) as well as quantitative or semi-quantitative PCR (qPCR and HRM). The occurrence

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ranges were: 35 to 98.78% for *A. marginale*; 8.19 to 86.25% for *B. bigemina*; and 7.32 to 95.47% for *B. bovis.* The highest occurrences of *A. marginale* and *B. bovis* were recorded in the state of RO, and for *B. bigemina* in the state of SP. Despite the extensive data collected, there remain numerous opportunities to guide future research, particularly concerning the use of molecular techniques and the quantification of losses in herds with higher incidences of TF.
Key words: Absolute quantification. Anaplasmosis. Babesiosis. PCR.

Resumo

A bovinocultura brasileira enfrenta diversos desafios, dentre esses, as doenças transmitidas por vetores que geram prejuízos significativos pela ampla disseminação no país. As técnicas moleculares são mais sensíveis na identificação de animais persistentemente infectados. Dessa forma, o objetivo deste trabalho foi compilar dados referentes à ocorrência de *Anaplasma marginale, Babesia bigemina* e *Babesia bovis,* causadores da tristeza parasitária bovina (TPB), detectados por técnicas moleculares, identificando lacunas e oportunidades para futuras pesquisas no Brasil. Para isso, foi conduzida uma revisão sistemática, onde os principais critérios de inclusão foram: estudos realizados com amostras do Brasil; utilização de amostras de bovinos naturalmente infectados; dados exatos sobre número ou percentual de animais positivos; número de amostras por rebanho ≥ 10; e informações, pelo menos, do estado de origem das amostras. Ao todo, 38 estudos foram incluídos na revisão, utilizando-se de técnicas de PCR convencional (multiplex PCR, PCR, nPCR e snPCR) e PCR quantitativa e semiquantitativa (qPCR e HRM). As faixas de ocorrência para as espécies foram: 35 a 98,78% para *A. marginale*; 8,19 a 86,25% para *B. bigemina*; e de 7,32 a 95,47% para *B. bovis.* Os maiores percentuais de ocorrência de *A. marginale* e *B. bovis* foram no estado de RO, e de *B. bigemina* foi no estado de SP. Apesar da quantidade de dados encontrados, ainda são evidentes inúmeras oportunidades de direcionamento para futuras pesquisas, principalmente no que diz respeito ao uso de técnicas moleculares e quantificação das perdas nos rebanhos de maior ocorrência da TPB.
Palavras-chave: Anaplasmose. Babesiose. PCR. Quantificação absoluta.

Introduction

Brazil hosts the largest commercial cattle herd worldwide, numbering 224.6 million animals, which reflects growing livestock productivity (Instituto Brasileiro de Geografia e Estatística [IBGE], 2021) and provides employment for nearly two million workers (Barros et al., 2022). One of the main economic challenges in both Brazilian and global cattle farming is vector-borne diseases (Grisi et al., 2014; P. A. Oliveira et al., 2017; Almazan et al., 2018; Narladkar, 2018; R. S. Souza et al., 2021). The rickettsia *Anaplasma marginale* and the protozoa *Babesia bovis* and *Babesia bigemina* are intraerythrocytic parasites that commonly infect cattle, causing a disease complex known as tick fever (TF). Clinical manifestations of TF include hemolytic anemia, fever, abortion, stillbirths, weight loss, and reduced milk production (Grisi et al., 2014; P. A. Oliveira et al., 2017; Henker et al., 2020). A survey in Rio Grande do Sul state quantified TF-related losses at approximately BRL 10 million per year (P. A. Oliveira et al., 2017).

The incidence of bovine babesiosis and anaplasmosis in herds correlates with the presence of specific vectors, the *Rhipicephalus microplus* tick and hematophagous flies such as *Haematobia irritans* and *Stomoxys calcitrans* (Hornok et al., 2008). Most of the Brazilian territory is deemed an epidemiological stability zone for ticks, but the southern region displays instability. During winter, the populations of ticks and flies diminish or disappear, leading to increased clinical cases (Santos et al., 2019). A recent review reported a prevalence of 36.6% for *A. marginale* and 62.6% for *Babesia* spp. across Brazil, summarizing data from various diagnostic methods and regional occurrences. However, results vary due to differences in test sensitivity and specificity, as well as parasitemia levels (Ferreira et al., 2022).

The ability of the pathogens to evade the immune system of the host means that treatment does not always completely eliminate the parasites, and animals can become persistently infected (reservoirs). These parasites remain latent in bovine cells (Kieser et al., 1990; Yokoyama et al., 2006), with potential for parasitemia resurgence during stressful situations, such as transport, high population density, and peripartum, leading to acute disease outbreaks (Silva et al., 2014).

Identifying persistently infected animals is essential for strategic decision-making to minimize herd losses. These animals act as infection sources for ticks and susceptible animals that have not previously encountered TF agents and thus lack an immune response (naïve), particularly in areas of enzootic instability (Santos et al., 2019; Alvarez et al., 2019). The most basic and cost-effective TF diagnostic tool is direct microscopic visualization of parasites in total or peripheral blood smears. However, this method has low sensitivity, and skilled evaluation is essential. Furthermore, carrier animals often exhibit low parasitemia levels, making it impossible to visualize infectious agents with this technique (Alvarez et al., 2019; Aubry & Geale, 2011).

Nevertheless, for greater sensitivity and specificity in diagnosing infections of *A. marginale* and *Babesia* spp., molecular techniques are preferred, particularly for identifying persistently infected animals. Various target genes and techniques have been utilized globally over time in the quest for a gold standard test (Alvarez et al., 2019; Echaide et al., 1998; Al-Hosary, 2017; Parodi et al., 2021; Kovalchuk et al., 2020).

In view of advances in direct detection, this review proposes to compile data regarding the presence of *A. marginale, B. bigemina,* and *B. bovis* in cattle herds, detected solely through molecular techniques, as well as to identify research gaps and opportunities in Brazil.

Material and Methods

Search protocol

For this review, the PRISMA 2020 checklist was followed as suggested by Page et al. (2021). Articles eligible for inclusion were those published using data from samples collected from cattle (*Bos taurus* and/or *Bos indicus*) naturally infected in any state within Brazilian territory. The review focused solely on articles employing molecular techniques

for diagnosing *Anaplasma* spp. and *Babesia* spp. Exclusions were made for dissertations, theses, and abstracts published in conference proceedings, as well as articles that focused exclusively on phylogenetics or reported diagnoses based only on blood smears and/or serology. Additionally, articles with small sample sizes (n ≤ 10) or those involving only experimental infections were excluded.

For bibliographic searches in international databases (PubMed and Scopus), we employed the following keywords: ("*Anaplasma marginale*" OR "*Anaplasma centrale*" OR "*Babesia bovis*" OR "*Babesia bigemina*" OR "*Cattle tick fever*" OR "*Bovine tick sadness*") AND (Cattle OR Bovine OR "*Bos taurus*" OR "*Bos indicus*") AND ("*Molecular diagnos**" OR "*PCR" OR "blotting") AND "Brazil". Additionally, these keywords were utilized in both English and Portuguese for searches on Google Scholar. Initially, titles and abstracts were screened, excluding duplicates, articles with only samples from other countries, or those not published in peer-reviewed journals. The materials and methods sections were then evaluated to exclude studies that did not meet our inclusion criteria. To qualify for inclusion, articles needed to specify at least the sampling site (state), the molecular method used, the target gene and/or nucleotide sequence (or provide a reference), and the exact number or frequency of positive animals. All searches were conducted from April to August 2022.

Two reviewers independently selected eligible records. During the data collection process, predefined information in each included article was verified at least three times. Articles that met all the criteria were imported into Zotero (2022) software. When similarities in sampling data were observed (city/region, number of animals, breed), corresponding authors were contacted to verify whether the samples had been used in more than one published article

Data analysis

Data were compiled in an Excel (2019) spreadsheet, which included details such as the study period, municipality/region and state, geographic coordinates, herd size, breed or species, number of animals sampled, age, sex, type of production system, infectious agent investigated, target gene, nucleotide sequence, molecular method used, number of positive animals, other diagnostic methods employed, number of symptomatic animals, type of sample utilized, number of fatalities due to anaplasmosis and/or babesiosis, post-mortem evaluations, treatment protocols at the farm (symptomatic or prophylactic), and co-infections with more than one TF agent (Supplementary File 1).

For the analysis of occurrence data, to avoid using duplicate data when information about the same animal groups was reported in more than one article, priority was given to those with larger sample sizes or more sensitive molecular methods. For longitudinal studies on TF occurrence, either the mean value provided by the studies was used, or it was calculated by dividing the total number of positive samples by the total number of samples analyzed, irrespective of the sampling period. From studies employing more than one molecular method, frequency data were derived from the method with

the highest specificity and/or sensitivity. Only pre-treatment results were considered in studies involving treatments. In studies evaluating both naturally and experimentally induced infections (via ticks or direct inoculations), data from naturally infected animals were exclusively considered. To create the maps, studies were categorized into four groups based on sample size: A – 10 to 50 animals; B – 51 to 100; C – 101 to 200; D – 201 to 400. Occurrence was calculated by dividing the number of positive animals by the total number evaluated and multiplying by 100. Herds were categorized into four groups based on the percentage of positive animals: ≤25%; >25% to ≤50%; >50% to ≤75%; and >75%.

In the occurrence map of *B. bovis,* data from studies that utilized primers for *Babesia* spp. were included. Each Brazilian state was categorized according to the frequencies obtained for each infectious agent, identifying geographic coordinates and sample sizes of the herds using Geographic Information System [QGIS] (2022) software.

The types of studies were categorized based on the objectives described in the articles and their Material and Methods sections into: (i) Influence of breed (TF); (ii) Epidemiology (prevalence); (iii) Genetic characterization; (iv) Genetic characterization (epidemiology); (v) Genetic characterization (longitudinal); (vi) Genetic characterization (outbreak); (vii) Genetic diversity; (viii) Longitudinal study; (ix) Comparison between methods; (x) Outbreak investigation; (xi) Relationship between ticks and TF; and (xii) Evaluation of treatment efficacy. Data were compiled into tables according to the infectious agent.

Results and Discussion

Through database searches, 298 studies were identified, and after initial screening of titles and abstracts, only 76 articles had their Material and Methods sections assessed. Of these, 49 were evaluated for eligibility and 38 were included in the review (Figure 1). None of the studies included diagnosis for *A. centrale* or utilized blotting techniques. The studies were conducted from 2000 to 2019 and were published between 2005 and 2022.

The selected studies encompassed data on 6,282 animals across 72 herds, with ages ranging from birth to nine years. The prevalence of *A. marginale* was the most extensively investigated (n = 4,648), followed by *B. bovis* (n = 2,164), *B. bigemina* (n = 3,647), and *Babesia* spp. (n = 45). The majority of the animals assessed were dairy crossbreeds (n = 2,272), followed by taurine breeds including Aberdeen Angus, Crioula Lageana, Limousin, Charolais, and Nellore (n = 977); crossbreeds of taurine breeds (n = 362); and two herds of Holstein cattle (n = 80). Regarding rearing systems, 1,105 animals were kept in an extensive system, 135 in a semi-intensive system, and 15 in an intensive system.

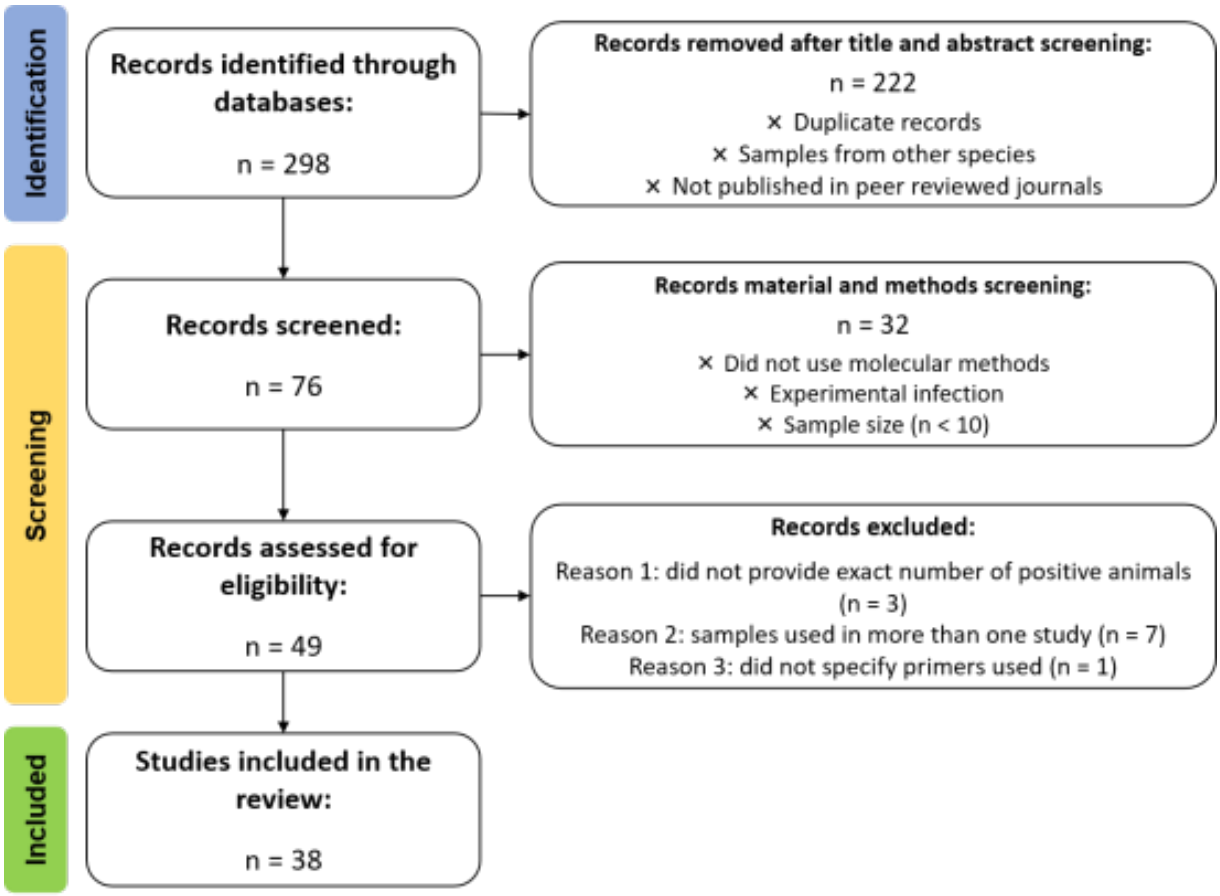

Figure 1. PRISMA 2020 diagram for identification and selection of registries for the systematic review on molecular diagnosis of cattle tick fever in Brazil.

Forty-five herds across 14 Brazilian states were evaluated for infection with *A. marginale*. Nineteen herds used the smallest sample size category (10 to 50 animals). Samples from 51 to 100 cattle were collected in 13 herds, and more than 100 animals were included in the evaluations of twelve herds: five herds ranging from 101 to 200 animals, and eight herds from 201 to 400 animals (Figure 2). The molecular occurrence of *A. marginale* in each state was: 68.68% in Acre, 46.97% in Bahia, 38% in Goiás, 95.78% in Maranhão, 57.74% in Minas Gerais, 68.47% in Mato Grosso do Sul, 60.98% in Paraíba, 76.24% in Piauí, 75.00% in Paraná, 80.59% in Rio de Janeiro, 98.78% in Rondônia, 35.00% in Rio Grande do Sul, 55.35% in Santa Catarina, and 97.90% in São Paulo.

Babesia bigemina was investigated in 22 herds across nine states, with 10 to 50 animals sampled in 10 herds, 51 to 100 in five, 101 to 200 in three, and 201 to 400 in four herds (Figure 2). The molecular occurrence of this agent was: 26.57% in Bahia; 66.67% in Goiás; 8.19% in Maranhão; 19.05% in Minas Gerais; 74.46% in Mato Grosso do Sul; 17.07% in Paraíba; 50.66% in Piauí; 39.98% in Santa Catarina; and 86.25% in São Paulo.

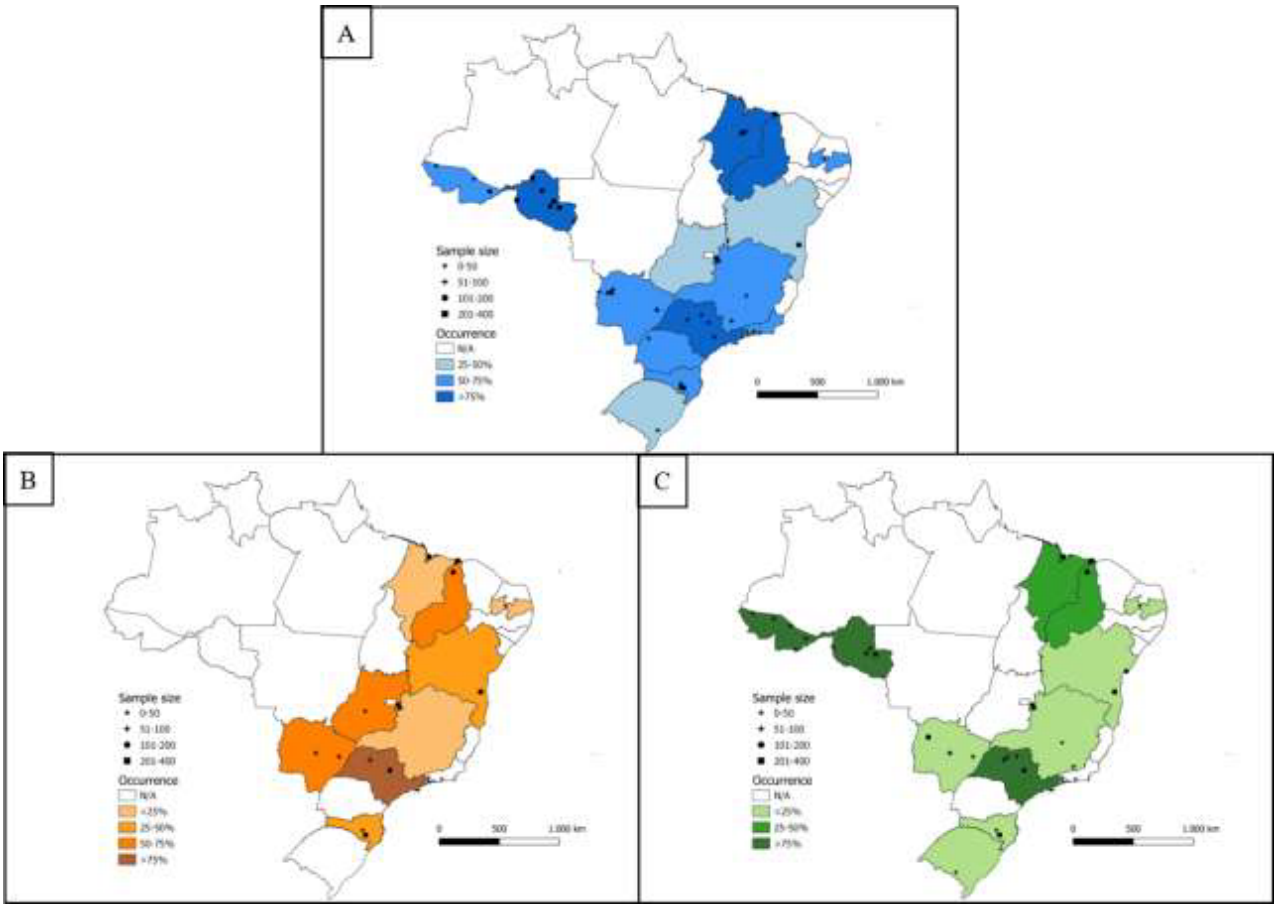

Figure 2. Occurrence (%) of animals infected with *Anaplasma marginale* (A), *Babesia bigemina* (B), and *Babesia* spp. and *Babesia bovis* (C) in cattle herds in Brazil, according to studies that used molecular methods for diagnosis. The maps were created using QGIS (2022) software. Individual state maps of specific cities/regions where sampling took place are available in Supplementary File 2.

In the two herds where primers for *Babesia* spp. were used, fewer than 50 cattle were sampled in the states of Minas Gerais and Acre. For the detection of *B. bovis*, samples were collected from 16 herds of 10 to 50 animals, from eight herds of 51 to 100, from three herds of 101 to 200, and from two herds of 201 to 400 animals. The molecular occurrence of *Babesia* spp. and *B. bovis* was: 87.47% in Acre, 24.17% in Bahia, 32.38% in Maranhão, 19.64% in Minas Gerais, 9.28% in Mato Grosso do Sul, 7.32% in Paraíba, 37.72% in Piauí, 95.47% in Rondônia, 10.00% in Rio Grande do Sul, 23.99% in Santa Catarina, and 91.13% in São Paulo.

Compiling data from 12 articles, which reported cattle infected with more than one TF agent (Vieira et al., 2019; F. A. L. Souza et al., 2013; S. C. L. Costa et al., 2016; Reckziegel et al., 2022; Andreotti et al., 2018;

F. B. Costa et al., 2015; F. A. L. Souza et al., 2018; Giglioti et al., 2021; M. C. S. Oliveira et al., 2005; Giglioti et al., 2016, 2018b), it was observed that 31.9% of the animals (342/1,071) exhibited co-infections. The co-infections comprised 53.5% (n = 183) with *B. bovis* + *B. bigemina*; 30.7% (n = 105) with *Babesia* spp. + *A. marginale*; 6.4% (n = 22) with *A. marginale* + *B. bigemina*; 5.6% (n = 19) with *A. marginale* + *B. bovis*; and 3.8% (n = 13) with *A. marginale* + *B. bigemina* + *B. bovis.*

Animals exhibiting clinical symptoms were reported in 17 herds due to *A. marginale* (n = 218 animals), in two herds due to *B. bovis* and *B. bigemina* (n = 5 animals), and 15 symptomatic cattle within a single herd were diagnosed using primers for *Babesia* spp. Symptomatic treatment was discussed in five articles (Bahia et al., 2020; Silva et al., 2016; Garcia et al., 2022; Reckziegel et al., 2022; Alberton et al., 2015), and chemoprophylaxis to prevent babesiosis was reported only on one farm (F. A. L. Souza et al., 2018) in cattle at 70 days of age. Tick control was reported in three studies (Casa et al., 2020; F. A. L. Souza et al., 2013; Andreotti et al., 2018). Deaths due to *A. marginale* infection were mentioned only on three farms (Silva et al., 2016; Machado et al., 2015; S. C. L. Costa et al., 2016). One study mentioned that the animals in the herd died during an outbreak, but it was not specified whether the samples analyzed were from any of the dead animals (Machado et al., 2015). None of the studies presented data on post-mortem evaluations.

This is the first study with occurrence data obtained solely through molecular diagnostics. By examining the occurrence maps presented here, gaps and opportunities for future research on TF in Brazil were identified. The three Brazilian states with the largest cattle herds Mato Grosso (MT), Goiás (GO), and Pará (PA) represent 36%of the national total (IBGE, 2021). Among the studies reviewed, samples were collected from only one herd in GO to investigate *A. marginale* and one for *B. bigemina*, with no studies using samples from MT or PA. This suggests that, although molecular diagnostics are a valuable tool, they are primarily used for research purposes and not routinely in production systems. The infrequent use of molecular techniques is hindered by high costs, as most equipment and consumables are imported, and the requirement for specialized equipment and personnel.

Regarding the types of studies involving the diagnosis of *A. marginale,* most were aimed at performing genetic characterization of this agent in different contexts (n = 8), such as epidemiological, longitudinal, and outbreak investigation studies (Table 1). Six articles provided information on the epidemiology of *A. marginale* (prevalence), followed by four longitudinal studies, three of which investigated transplacental transmission. Two outbreak investigations were reported, one using multiplex PCR, and three studies focused on evaluating the relationship between tick infestation and the occurrence of TF agents.

Table 1
Type of studies conducted in Brazil to investigate the occurrence of Anaplasma marginale in cattle using molecular methods

Study type	Molecular method	Ref.
Epidemiology (prevalence)	multiplex PCR	Vieira et al., 2019
	PCR	Brito et al., 2010; Casa et al., 2020
	nPCR	Amorim et al., 2014*; F. A. L. Souza et al., 2013
	snPCR	Bahia et al., 2020
Genetic characterization	nPCR	Silvestre et al., 2016
Genetic characterization (epidemiology)	qPCR	Pereira et al., 2021; I. A. S. Ramos et al., 2019
Genetic characterization (longitudinal)	qPCR	Silva et al., 2015, 2016
	Real time PCR	Pohl et al., 2013
Genetic characterization (outbreak)	snPCR	Machado et al., 2015
	qPCR	Machado et al., 20151; Garcia et al., 2022
Longitudinal (transplacental transmission)	PCR	Grau et al., 2013
	snPCR	*Silva et al., 2014; S. C. L. Costa et al., 2016*
Longitudinal study	PCR	V. M. M. Costa et al., 2018
Outbreak investigation	multiplex PCR	Canever et al., 2014
	PCR	Reckziegel et al., 2022
Relation between ticks and TF	PCR	Andreotti et al., 2018; Martins et al., 2020
	qPCR	Martins et al., 2020; Giglioti et al., 2018a
Evaluation of treatment efficacy	PCR	Alberton et al., 2015

*nPCR was only used for negative samples in the first PCR reaction
1The authors mentioned that qPCR was performed to amplify the *msp1α* fragment, but referred to another study (M. C. S. Oliveira et al., 2008) that used primers to amplify the *msp1β* gene.

A single study included in this review applied conventional PCR to evaluate the effectiveness of various treatments for *A. marginale.* It was observed that, over the evaluation period, the number of positive animals within each group decreased, yet no treatment protocol completely eradicated the agent from all animals (Alberton et al., 2015).

In most studies employing conventional PCR, the *msp5* gene was targeted (n = 9), followed by *msp1α, msp4,* and *msp1β,* respectively. For quantitative methods, the primary target was *msp1,* with only three and five studies targeting *msp1α* and *msp1β,* respectively. Calibration curves for DNA quantification were constructed using plasmid techniques or synthetic DNA fragments (gBlocks™), with absolute DNA quantifications ranging from 1.04×10^{1} to 4.36×10^{12} copies μL^{-1}.

Research focusing on the prevalence of *B. bigemina* and its correlation with tick infestation accounted for the majority

of the publications, with five studies in each category (Table 2). None of the studies primarily aimed at the genetic characterization of *B. bigemina;* however, two utilized target genes of this pathogen to compare molecular methods. This agent was also studied in epidemiological surveys and outbreak investigations employing multiplex PCR.

Additionally, qPCR was used in three studies to explore the relationship between the number of *R. microplus* at various life stages and *B. bigemina* DNA copies. Conventional PCR targeting hypothetical partial mRNA protein and 18S ribosomal RNA (*18SrRNA*) was most common (n = 6 and n = 5, respectively), while mitochondrial cytochrome B (*mt-cyB*) was the primary gene investigated in qPCR. Standard curves were constructed using plasmids, gBlocks™, and cultures of experimentally inoculated red blood cells, with *B. bigemina* DNA copy numbers ranging from 6.6×10^{-1} to 4.4×10^{3} μL^{-1}.

Non-specific primers targeting the *18SrRNA* gene were employed for genetic characterization studies of the lines present in in Holstein calves and in investigating an outbreak among Nellore cattle in the state of Acre (Table 2). Overall, 21 articles utilized molecular techniques to detect *B. bovis,* including seven epidemiological surveys; four examining the relationship between tick counts or tick parasite loads and TF; three involving method comparisons; two focusing on genetic diversity and longitudinal studies; one on transplacental transmission; and one investigating a disease outbreak.

For *B. bovis* detection, *rap1α* was the chosen gene in eight studies, *18SrRNA* in four, and *sbp2* and sbp1 in two and one studies, respectively, and *mt-cyB* for only one of the experiments that used conventional PCR. *Mt-cyB* was the most investigated gene in qPCR techniques, whereas *18SrRNA* and *msa2c* were each targeted in one study. Regarding absolute quantification, the number of parasites per µL of blood in cattle in the state of Mato Grosso do Sul ranged from $2.5 \times 10^{2} – 2.5 \times 10^{3}$. In other studies, DNA copy numbers ranged from 3.09 to 5.62×10^{5} μL^{-1}. Standard curves were constructed using plasmids, gBlocks™, and cultures of *B. bovis*-positive red blood cells.

Table 2
Type of studies conducted in Brazil to investigate the occurrence of *Babesia bigemina, Babesia* spp., and *Babesia bovis* in cattle using molecular methods

Babesia bigemina		
Study type	**Molecular method**	**Ref.**
Breed influence (TF)	nPCR*	M. C. S. Oliveira et al., 2008
Epidemiology (prevalence)	multiplex PCR	Vieira et al., 2019
	nPCR*	Amorim et al., 2014; Bahia et al., 2020
	PCR	F. A. L. Souza et al., 2013; F. B. Costa et al., 2015
Longitudinal (transplacental transmission)	snPCR	S. C. L. Costa et al., 2016
Longitudinal study	PCR	V. M. M. Costa et al., 2018; Santana et al., 2008; F. A. L. Souza et al., 2018
Method comparison	HRM**	Giglioti et al., 2021
	nPCR	Kim et al., 2007
	qPCR	Giglioti et al., 2021; Kim et al., 2007
Outbreak investigation	multiplex PCR	Canever et al., 2014
Relation between ticks and TF	PCR	Andreotti et al., 2018
	nPCR*	*M. C. S. Oliveira et al., 2005*
	qPCR	Giglioti et al., 2018a, 2016, 2018b
Babesia* spp. and *Babesia bovis		
Study type	**Molecular method**	**Ref.**
Epidemiology (prevalence)	multiplex PCR	Vieira et al., 2019
	PCR	F. A. L. Souza et al., 2013; F. B. Costa et al., 2015
	nPCR*	Amorim et al., 2014; Bahia et al., 2020; Brito et al., 2013
	qPCR	Bilhassi et al., 2014
Genetic characterization	nPCR	Silvestre et al., 20161
Genetic diversity	nPCR	Mendes et al., 2019; Nagano et al., 2013
Longitudinal (transplacental transmission)	snPCR	S. C. L. Costa et al., 2016
Longitudinal study	PCR	V. M. M. Costa et al., 2018; F. A. L. Souza et al., 2018
Method comparison	HRM**	Giglioti et al., 2021
	PCR	C. A. N. Ramos et al., 2011
	PCR multiplex	Vieira et al., 2019
Outbreak investigation	PCR	Brito et al., 2010; Casa et al., 2020
	nPCR	Amorim et al., 2014*
Relation between ticks and TF	snPCR	Bahia et al., 2020
	nPCR*	Silvestre et al., 2016
	qPCR	Pereira et al., 2021; I. A. S. Ramos et al., 2019

*nPCR was only used for negative samples in the first PCR reaction
**Semi-quantitative technique – HRM: "High-resolution melting"
[1]Primers used for *Babesia* spp.

One gap identified from the analysis of the studies presented here concerns the quantification of production losses due to infections by agents such as *A. marginale* and *Babesia* spp. Researchers commonly note significant losses for producers resulting from disease, the need for treatment, abortions, and animal deaths (Kocan et al., 2010). However, none of the studies reviewed aimed to assess the damage caused by the presence or intensity of infection in the evaluated herds. On this topic, a study conducted in Minas Gerais, Brazil, assessed the rectal temperatures and blood smears of 395 Holstein calves for detecting *A. marginale* and *Babesia* spp. The researchers advocated for the rational use of antibiotics and antiparasitics in these animals, concluding that this approach could save approximately 77.99% on the costs of TF control drugs (R. S. Souza et al., 2021). This finding highlights the value of employing diagnostic measures for the detection of these parasites in routine farm operations to refine control strategies and reduce costs.

No studies have yet determined how low levels of infection impact or threaten cattle at different life stages and for various purposes (weaning, transition period of dairy cows, etc.). Additionally, other critical applications for molecular techniques, particularly quantitative ones, include assessing the efficacy of chemoprophylactic management and evaluating vaccination effectiveness (C. A. N. Ramos et al., 2011) and monitoring variations in infection levels before and after transportation and during reproductive management (TAI).

Molecular biology techniques, which offer higher sensitivity and specificity, help overcome limitations associated with other diagnostic tools, such as false positives due to staining artifacts in blood smears (Silaghi et al., 2017). By using quantitative and semi-quantitative PCR, it is possible to identify not only the parasite involved but also the intensity of the infection (Silva et al., 2014). Nevertheless, due to the more complex procedures and higher costs associated with molecular techniques, several studies in Brazil have explored infections by *Babesia* spp. and *A. marginale* using direct diagnosis through blood smears and/or hematocrit, the latter serving as a complementary method to indirectly assess the impact on animal health (R. S. Souza et al., 2021; Bastos et al., 2021; Lopes et al., 2021; Heller et al., 2022; Teixeira et al., 2022).

Multiplex PCR was employed in only two studies (Vieira et al., 2019; Canever et al., 2014), neither of which reported using this approach in quantitative PCR techniques. This tool can be invaluable, given the likelihood of co-infection in animals and its potential to optimize time in epidemiological surveys by processing a larger number of samples more quickly (Al-Hosary, 2017). Regarding sample types, two epidemiological studies that examined infections by *A. marginale* and *B. bovis* in cattle herds in southwestern Amazonia utilized blood clots for DNA extraction. These clots were provided by the official veterinary service responsible for monitoring bovine diseases in the states of Rondônia and Acre (Brito et al., 2010, 2013). Other studies used whole blood samples collected in tubes with anticoagulants. Collaboration between research centers and official public veterinary services can lead to cost savings in project execution, enabling the extension of research to more farms without additional costs.

Conclusion

Of the 38 articles reviewed, the lowest molecular occurrence of *A. marginale* was 35% in the state of RS, while the highest was 98.78% in RO. For *B. bigemina,* the occurrences ranged from 8.19% in MA to 86.25% in SP. *Babesia bovis* showed a high occurrence in RO, with 95.47% of the evaluated animals testing positive for this parasite, whereas the lowest was in PB at 7.32%.

Despite the extensive data available, significant gaps remain, particularly the lack of data from states with large cattle herds like MT and PA. There are also opportunities for future research in the use of molecular techniques, the evaluation of the efficacy of preventive treatments and protocols, and the quantification of losses in herds with a high occurrence of clinical cases of TF.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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