Review

Ticks as Vectors of Trypanosomatidae with Medical or Veterinary Interest: Insights and Implications from a Comprehensive Systematic Review and Meta-Analysis

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Systematic Review and Meta-analysis

Ticks as Vectors of Trypanosomatidae with Medical or Veterinary Interest: Insights and Implications from a Comprehensive Systematic Review and Meta-Analysis

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Abstract: Since the 20th century, numerous studies have detected or isolated parasites from the Trypanosomatidae family in various tick species. However, the status of ticks as vectors of medically or veterinary important *Trypanosoma* and *Leishmania* remains unclear. We conducted a systematic review and meta-analysis to provide new insights into the potential vector status of *Leishmania* and *Trypanosoma*, which have medical and veterinary significance. We searched three databases (PubMed, Google Scholar, and Web of Science) from 1912 to June 30, 2023, resulting in 94 and 86 papers included in the qualitative and quantitative analyses, respectively. All identified field studies were conducted in endemic areas and investigated the presence of *Trypanosoma* and *Leishmania* parasite, DNA, or antigen in ticks. We recorded a pooled prevalence of Trypanosomatidae detection in ticks at 15.48% [7.99-24.61%], with significant variations depending on the year, detection method, and geographical area. Most of positive tick species belonged to the genera *Amblyomma*, *Hyalomma*, *Ixodes*, and *Rhipicephalus*. Experimental laboratory work on transmission routes proved potential vector competence in both the Argasidae and Ixodidae tick families. Although our systematic review and meta-analysis provide unambiguous evidence of the natural infection of ticks by Trypanosomatidae parasites with some evidence of non-traditional transmission routes, it does not provide conclusive evidence on the role of ticks as biological or mechanical vectors for veterinary and medically interest protozoan Trypanosomatidae species. All these unambiguously demonstrates the need of additional investigations to address this point.

Keywords: Trypanosomatidae; *Trypanosoma*; *Leishmania*; Ticks; Ixodidae; Argasidae; vector borne disease

1. Introduction

Ticks (Parasitiformes: Ixodida) are obligate hematophagous ectoparasites of all vertebrate classes and are vectors or reservoirs of human and animal pathogenic virus, bacteria, protozoa and fungi fungi [1]. Two families of ticks of numerous genuses possess considerable significance in public health and veterinary matters [2]. Currently, there are 996 known tick species worldwide, which are divided into three families: ~774 hard tick species (Ixodidae), ~221 soft tick species (Argasidae), and the monotypic family of Nuttalliellidae, mixing features of both families [3,4]. The identification of tick fossils in amber has led to the description of two novel families, the Deinocrotonidae and Khimairidae, while many unique genera are described, including *Compluriscututla*, *Cornupalpatum*, *Deinocroton*, and *Khimaira* [5]. Protozoan pathogens carried by ticks and affecting mammals of medical or veterinary interest are classified under the order Piroplasmida (e.g., genera *Babesia*,

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Theileria, and *Cytauxzoon*) or haemogregarines of the *Hepatozoon* genus (Adeleorina: Hepatozoidae) [6].

The Trypanosomatidae family consists of unicellular eukaryotes, including pathogens of humans and animals from the genera *Trypanosoma* and *Leishmania,* as well as *Endotrypanum* and *Porcisia*. The life cycle of these organisms is characterized by the involvement of arthropod vectors from the Hemiptera and Diptera orders, mainly, and for some Trypanosomes from the Siphonoptera orders and for fish Trypanosome of the Arhynchobdellida (Hirudinidae, leech) order. Two *Trypanosoma* subspecies of *T. brucei* (i.e., *Trypanosoma brucei gambiense*, *T. brucei rhodesiense*), and atypical human trypanosomiasis *T. cruzi*, and *T. rangeli,* along with at least 23 species of *Leishmania,* including the recently described *Leishmania* (*Mundinia*) *chancei* species, are pathogenic for humans. They cause human African trypanosomiasis (HAT or sleeping sickness), Chagas disease (CD), and cutaneous (CL), mucocutaneous (MCL), or visceral (VL) Leishmaniases [7–13]. These diseases also affect domestic, feral, or wild animals. Canine visceral Leishmaniases (CVL) are primarily caused by *L. infantum* infection and occasionally by *L. donovani* or *L. tropica* [14]. *Trypanosoma congolense*, *T. evansi*, *T. b. brucei*, *T. vivax*, *T. simiae*, *T. suis, T. theleiri,* and more rarely, *T. godfreyi* infect livestock. *Trypanosoma equiperdum* infects equids [15,16]. In addition, *Trypanosoma theileri* which is considered as non-pathogenic, is found in cattle, buffalo, and antelope worldwide [17]. It has been unfrequently linked to disease resembling nagana in specific cases: a calf [18], cattle [19], or a cow [20]. *Trypanosoma theileri* can cause illness in cattle under severe stress due to concurrent diseases or poor nutrition [21]. *Trypanosoma caninum* was described in 2014 as a new *Trypanosoma* species infecting dogs, in asymptomatic cases with low humoral immune response [22]. Additionaly, *T. lewisi*, that infect *Rattus* sp. Is also an opportistic bllod parasites of human and share common vertebrate hosts with *T. Cruzi* [23]. Altogether, more than 30 million people are infected, and over 48 million cattle are at risk of contracting animal trypanosomiasis in Africa, causing about 3 million deaths in cattle every year [24].

Since the early 20th century, the idea that ticks can transmit protozoan parasites from the genera *Leishmania* and *Trypanosoma* has been investigated, and more recently, molecular biology techniques have been used to revisit this hypothesis (e.g., Next-Generation Sequencing) [25,26]. There is still an ongoing debate within the scientific community regarding ticks' ability to transmit Trypanosomatidae parasites of medical or veterinary interest. To provide an updated perspective on this current debate, we undertook a systematic review and meta-analysis with the following objectives: (i) to gather published field and experimental data on the detection in ticks of Trypanosomatidae with medical and veterinary interests, including *T. theleiri* and *T. caninum*, and to assess their capacity to act as vectors for these pathogens; (ii) to explore factors associated with the transmission of these pathogens by ticks.

he introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance. The current state of the research field should be carefully reviewed and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the principal conclusions. As far as possible, please keep the introduction comprehensible to scientists outside your particular field of research. References should be numbered in order of appearance and indicated by a numeral or numerals in square brackets—e.g., [1] or [2,3], or [4–6]. See the end of the document for further details on references.

2. Materials and Methods

2.1. Protocol and Registration

The current study was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines [166]**.** The protocol was neither registered nor published. The checklist for meta-analysis is provided as Supplementary Material (Check List S1).

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2.2. Information Source

The systematic screening of existing literature was performed using PubMed, Google Scholar, and Web of Science (WOS) databases. Publish or Perish (Harzing.com), a software that retrieves and analyses academic citations was used to retrieve relevant articles from Google Scholar.

2.3. Search

A set of keywords was used: "Trypanosomatidae" and all species of "*Trypanosoma*" or "*Leishmania*", known to be pathogenic for humans or animals and having medical or veterinary interest, including *T. theleiri* and *T. caninum*, in combination with "ticks" "Ixodidae", "Argasidae", and selected tick genus (e.g., "*Rhipicephalus*", "*Hyalomma*", "*Ornithodoros*"). Database search was done between January 1900 and June 30, 2023, without language restrictions. Articles reporting "Trypanosomatidae" in ticks by direct examination (e.g., culture, microscopy, immunohistochemistry "IHC") or molecular method (e.g., conventional Polymerase chain reaction "PCR", real-time PCR "RT-PCR", capillary or next generation sequencing "NGS") were included in the study. We exclude studies that doesn't fall in the scope of our study and reporting, i.e., those whose scope and objective are "Vaccine", "Virus", "Drug", "Treat", "Pest", "Acaricide", "Immunology", "Serology", "Serum", "ELISA", "Antigen".

2.4. Eligibility Criteria and Study Selection

We undertook the review with current recommendations reported in 2015 and reported our findings as per the PRISMA guidelines, taking into account the remarks for "biological" meta-analyses [167]. Two authors, TK and DS, independently conducted a preliminary review of the articles by examining their titles and abstracts. Articles selected by at least one reviewer were retrieved, and duplicated papers were excluded. Two dependent reviewers (TK and DS) performed a second selection based on full-text analysis, and disagreements were resolved through discussion with a third reviewer (BM). Studies were considered eligible based on established inclusion or exclusion criteria.

The selection of eligible articles was based on previously established criteria: (1) articles dealing with the detection or transmission of Trypanosomatidae parasites of medical or veterinarian interest, including *T. theleiri* and *T. caninum*, in ticks; (2) field or experimental studies; (3) with no restrictions on the host of origin; and (4) without language restriction. The exclusion criteria included (1) literature categories (letters, books, and reviews); (2) studies not focusing on ticks as vectors for Trypanosomatidae (e.g., cellular immune responses of ticks, host immunogenetic influences on tick resistance); (3) studies not focusing on Trypanosomatidae of medical or veterinary interest; (4) studies lacking information on tick collection place and origin; and (5) studies not stating the sample size, the number of positive cases, and the identity of tick and Trypanosomatidae species.

2.5. Data Collection

We employed a pre-existing template to obtain data from imported articles, and two of the authors (TK and DS) compiled them in a Microsoft Excel® spreadsheet. The information collected included the author's name, publication year, study's subregion/country, tick family and species, identification methods, number of tested and positive tick samples, and detection methods.

2.6. Quality Assessment

The quality of the selected publications was assessed following the Grading of Recommendations Assessment, Development, and Evaluation (GRADE) [168]. The total score of each article was calculated using the following seven items: (1) the tick family and species identified; (2) the Trypanosomatidae species identified; (3) the detection method stated; (4) the number of tested ticks; (5) the number of positive ticks; (6) the prevalence of Trypanosomatidae parasites in ticks; and (7) the identity of the host where positive tick(s) originate stated. Items 1-5 are quoted for 2 points,

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while items 6 and 7 for 1. Based on this score, each publication was classified as of high quality (score = 8-12), medium quality (score = 5-7), or poor quality (score = 0-4).

2.7. Statistical Analysis

For data related to field studies, we performed a meta-analysis of proportions [169] using the 'meta' and 'metafor' packages in R software version 4.3.1. We performed a Freeman-Tukey transformation with double arc sine (PFT) to convert the proportions before meta-analysis. This transformation is beneficial for standardizing and stabilizing the distribution variance [170] (dat<-escalc(measure="PFT", xi=xi, ni=ni, data=dat). Because of the high heterogeneity expected in the meta-analysis, a random-effects model was performed to combine the total size effect and subgroup analysis. Cochrane statistics I^2 and Q (expressed as X^2 and P, respectively) were used to assess and quantify heterogeneity. $I^2 < 50\%$ corresponds to low heterogeneity, whereas $I^2 > 50\%$ indicates high heterogeneity. Meta-analysis statistics were visualized using a Forest plots representation. The Egger test and Funnel plots were performed to assess publication bias, and the results were further submitted to a stability analysis. This later evaluates the impact of deleting data extracted from an article on the results of the remaining papers.

We carried out subgroup analyses of potential risk factors, namely: sampling year (before 2000, 2001-2009, 2010-2019, 2020 and after), continent (Europe, Asia, Africa, South America, and Iceland), host family (Canidae, Bovidae, etc.), tick genus, tick species, detection methods, parasite type, and the location of the tick's organs. We also performed a meta-regression with the parameters studied used as co-variables to address possible heterogeneity sources.

Concerning the experimental studies, because in a large number of papers, quantitative data are lacking (number of ticks used for the study), we performed a meta-analysis on semi-quantitative data, i.e., detection or not of pathogens in ticks (0: absence, 1: presence). Using this methodology, we also analyzed the detection of pathogens after a blood meal, mechanical transmission, transmission following the injection of homogenate ticks, and vertical transmission. Statistical tests mentioned above were calculated, and subgroup analyses of potential risk factors were performed on the tick family, tick species, donor host, receiving host, family of the parasite tested, and parasite species.

3. Results

3.1. Selection Process Overview: Curating Datasets for Systematic Review

The systematic search on multiple databases, including PubMed, Google Scholar, MEDLINE®, SciELO, and WOS, a total of 29,592 articles were retrieved (**Figure 1**). After the removal of duplicates (n= 21,007) and ineligible papers (n = 8,423), 166 published studies were selected (last updated 30 June 2023). After analysis of their titles, abstracts, and detailed contents, out of those 166 articles, 94 satisfied the eligibility criteria to be included in the systematic review, 86 articles were eligible for the meta-analysis, of which 49 dealt with field studies and 37 with experimental studies. In addition, 46 are focused on the transmission of *Leishmania*, while 40 are focused on *Trypanosoma*. Full-text Portable Document Format (PDF) files not freely accessible online were obtained through the French Development Research Institute (IRD) library. Publications in French, German, Spanish, Portuguese, Russian, and Turkish languages were handled by both authors (TK and DS) and/or native language colleagues.

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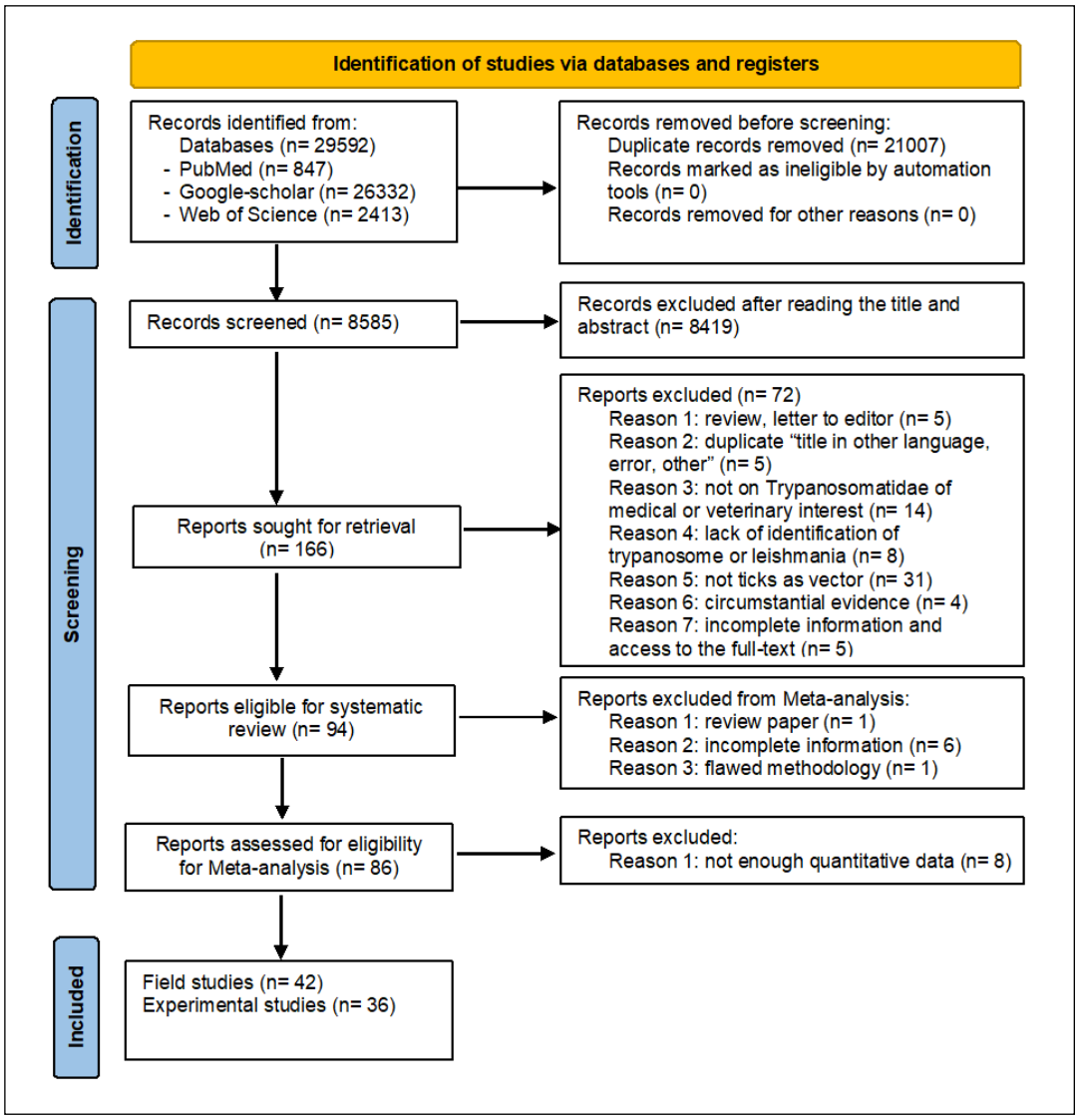

Figure 1. PRISMA Flowchart of the systematic review and meta-analysis.

3.2. Exploring the Historical Presence in Tick of Trypanosomatidae Infecting Human and Animals of Veterinary Interests

The Arthropoda phylum hosts monoxenous and dixenous Trypanosomatidae [27]. Sand flies (Diptera; Phlebotominae) are biological vectors of *Leishmania*, with some exceptions [28]. Diptera (e.g., Tsetse flies, Tabanids Stomoxes), Hemiptera (e.g., triatomine 'reduviid' bugs), Siphonaptera (e.g., fleas), and Arhynchobdellida (Hirudinidae, leech) are biological vectors of *Trypanosoma* [29,30]. Although most Trypanosomatidae colonize members of the Insecta class, *Blastocrithidia* sp. has been reported in ticks (Arachnida class) [31]. Since the late 19th and early 20th centuries, field trials and experiments have been conducted to detect these parasites in ticks (Figure 2). The presence of developmental forms of *Trypanosoma* spp. in ticks by O'Farrell (1913) and the demonstration that ticks can transmit parasites of the *Leishmania* genus are documented long ago [32]. Over the past several years, improved molecular detection of Trypanosomatidae DNA in ticks has generated renewed interest in the role of ticks in the transmission of *Trypanosoma* and *Leishmania*. Specifically, since 2010, the number of studies investigating this topic has increased significantly.

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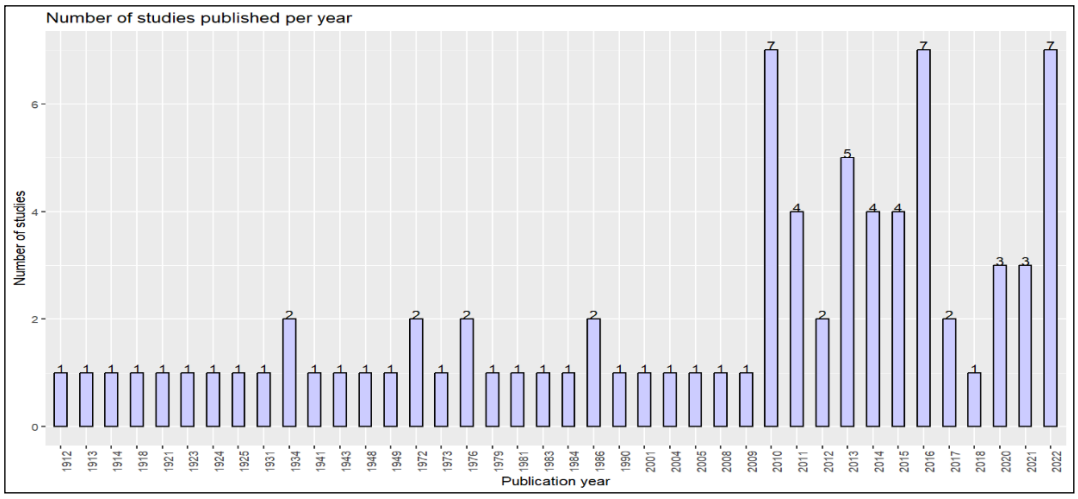

Figure 2. Distribution of the collected and selected scientific papers.

3.2.1. Ticks as Vectors of Trypanosoma: Historical Aspects

The hypothesis that ticks can act as reservoirs and/or vectors of human and animal Trypanosomes has been investigated as early as the beginning of the 20th century [33–47]. In 1972, Hoare reviewed existing data on ticks acting as vectors of trypanosomes and inferred that ticks could not transmit them because of the absence of infective forms, the trypomastigotes [29]. Nevertheless, Burgdorfer et al. (1973) and Shastri and Deshpande (1981) described the presence of various trypanosomes developmental stages in ticks (i.e., amastigotes, sphaeromastigotes, epimastigotes, and trypomastigotes) [48,49].

3.2.2. Leishmania: a Parasite Transmitted by Ticks?

The idea that ticks contribute to the transmission of *Leishmania* emerged in the 20th century. Early experiments by Blanc and Caminopetros suggested that ticks could be vectors for *Leishmania* causing human and canine kala-azar (*L. donovani*/*L. infantum* complex) around the Mediterranean. However, this idea was questioned by Malamos in 1938, that *Leishmania* could survive in the tick gut for more or less extended periods, which called into question the concept of direct transmission [50]. This hypothesis was questioned in 1972 by Rioux et al. who provided evidence contradicting the involvement of ticks in the cycle of *L. infantum* [51]. In the mid-1980s, McKenzie's contradicted this idea by proving the transmission of *Leishmania* during ticks' blood meal [52]. Subsequent field and laboratory studies identified *Leishmania* RNA or DNA within tick organs (including the salivary glands and ovaries) at different development stages (from larvae to nymphs and adults), further supporting the role of ticks in *Leishmania* transmission [53–77].

3.3. Observations of Trypanosomatidae of Medical and Veterinary Interest in Field-Collected Tick Specimens

As detailed in the following sections, many field studies were conducted to investigate the prevalence of pathogenic Trypanosomatidae in ticks by detecting the parasite by direct examination (e.g., culture, microscopy), molecular tools like Polymerase Chain Reaction (PCR), quantitative Polymerase chain reaction (qPCR), capillary sequencing, or Next-generation Sequencing (NGS), or immunological tools (Immuno histochromatography).

3.3.1. Trypanosoma Carriage and Prevalence

Traces of the presence of Trypanosomes of medical/veterinary interest were reported in ticks; these include *Trypanosoma cruzi*, *T. vivax*, *T. evansi*, *T. theileri*, *T. theileri-like*, *T. congolense*, *T. caninum* [36,78,87–91,79–86]. All these studies were performed on hard tick species (Ixodidae). Most tick species positive belonged to four genera, *Amblyomma*, *Hyalomma*, *Ixodes*, and *Rhipicephalus*, including

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A. cajennense [83,90], *A. longirostrum* [36], *A. variegatum* [78], *H. detritum* [88], *H. marginatum* [80], *I. ricinus* [86,88], *Rhipicephalus (Boophilus) microplus* [81,87,90], *R.* (*Boophilus*) spp. [85], *R. sanguineus* [82,88], *R. sanguineus* s.L.[89,91], *Rhipicephalus* sp. [84]. Conversely, other species were negative for *Trypanosoma* sp. (**Table 1**) [84,85,88–92]. Ticks collected on dogs and cattle were most frequently infected by *Trypanosoma* compared to those collected on other domestic animals (e.g., sheep, goats, camels, etc.) or wild animals (e.g., foxes, boars, etc.).

Table 1. Evidences of presence of *Trypanosoma* of medical and veterinary interest in field-collected ticks.

Species	Sampling year	Trypansoma species	Tick statut (P/N)	Localization in the tick	Tick Detection method	Host (Species)	Country	Continent	Author(s)
Amblyomma cajennense	2013	*T. cruzi*	N	H ; Gr	Microscopy, PCR	Dog (*Canis lupus*)	Brazil	South America	[92]
	NS	*T. vivax*	N	H	Microscopy	Bovine (*Bos taurus*)	Cuba	South America	[90]
	NS	*T. vivax*	P	G	Microscopy	Bovine (*Bos taurus*)	Cuba	South America	[90]
	NS	*T. vivax*	P	Gr	PCR, Sequencing	buffalos	Brazil	South America	[83]
Amblyomma longirostrum	NS	*T. cruzi*	P	NS	NS	Cercolabidae (*Arboreal porcupine*)	Venezuela	South America	[36]
Amblyomma spp.	2013-2019	*T. cruzi*	N	Gr	Metagenomics	* Wild ungulates Carnivores	Kenya	Africa	[84]
Amblyomma tigrinum	2013	*T. cruzi*	N	Gr	PCR, Sequencing	Canidae (*Canis lupus familiaris*)	Chile	South America	[89]
Amblyomma variegatum	NS	*T. congolense*	P	Gr	Parasitological analysis, PCR	Cattle	Nigeria	Africa	[78]
Haemaphysalis spp.	2017-2018	*T. evansi*	N	Gr	PCR, Sequencing	Cattle	India	Asia	[85]
Hyalomma detritum	1982	*T. theileri*	P	H	Microscopy	Bovine (*Bos taurus*) Dog (*Canis lupus*)	Algeria	Africa	[88]
Hyalomma dromedarii	2015-2016	*T. evansi*	N	Gr	PCR	**Wild ruminants species	Tunisia	Africa	[91]
Hyalomma excavatum	2015-2016	*T. evansi*	N	Gr	PCR	**Wild ruminants species	Tunisia	Africa	[91]
	1982	*T. theileri, T. evansi*	N	H	Microscopy	Bovine (*Bos taurus*) Dog (*Canis lupus*)	Algeria	Africa	[88]
Hyalomma lusitanicum	1982	*T. theileri, T. evansi*	N	H	Microscopy	Bovine (*Bos taurus*); Dog (*Canis lupus*)	Algeria	Africa	[88]
Hyalomma marginatum	2015-2016	*T. evansi*	N	Gr	PCR	**Wild ruminants species	Tunisia	Africa	[91]

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	NS	*T. theileri*	P	H	Microscopy	Bovine (*Bos taurus*)	Portugal	Europe	[80]
	1982	*T. theileri, T. evansi*	N	H	Microscopy	Bovine (*Bos taurus*) Dog (*Canis lupus*)	Algeria	Africa	[88]
Hyalomma spp.	2017-2018	*T. evansi*	N	Gr	PCR, Sequencing	Cattle	India	Asia	[85]
Ixodes ricinus	2013	*T. caninum*	P	Gr	Culture, PCR, Sequencing	Vegetation	Slovakia	Europe	[86]
	1979-1982	*T. theileri*	P	H	Microscopy	Bovine (*Bos taurus*) Dog (*Canis lupus*)	Switzerland	Europe	[88]
	2015-2016	*T. evansi*	N	Gr	PCR	**Wild ruminants species	Tunisia	Africa	[91]
Rhipicephalus (Boophilus) microplus	NS	*T. theileri-like*	P	H	ND	Bovine (*Bos taurus*)	Brazil	South America	[87]
	NS	*T. vivax*	N	H	Microscopy	Bovine (*Bos taurus*)	Cuba	South America	[90]
	NS	*T. vivax*	P	G	Microscopy	Bovine (*Bos taurus*)	Cuba	South America	[90]
	NS	*T. vivax*	P	Gr	PCR	Bovine (*Bos taurus*)	Venezuela	South America	[81]
	NS	*T. vivax*	P	Gr	PCR, Sequencing	buffalos	Brazil	South America	[83]
Rhipicephalus (Boophilus) spp.	2017-2018	*T. evansi*	P	Gr	PCR, Sequencing	Cattle	India	Asia	[85]
Rhipicephalus bursa	2015-2016	*T. evansi*	N	Gr	PCR	**Wild ruminants species	Tunisia	Africa	[91]
Rhipicephalus sanguineus	2013	*T. cruzi*	N	H ; Gr	Microscopy, PCR	Dog (*Canis lupus*)	Brazil	South America	[92]
	1982	*T. evansi*	P	H	Microscopy	Bovine (*Bos taurus*) Dog (*Canis lupus*)	Algeria	Africa	[88]
	NS	*T. evansi*	P	Gr	PCR	Dog (*Canis lupus*)	Brazil	South America	[82]
	NS	*T. vivax*	P	Gr	PCR	Dog (*Canis lupus*)	Brazil	South America	[82]
Rhipicephalus sanguineus sensu lato	2013	*T. cruzi*	P	Gr	PCR, Sequencing	Canidae (*Canis lupus familiaris*)	Chile	South America	[89]
	2015-2016	*T. evansi*	P	Gr	PCR	Wild ruminants species	Tunisia	Africa	[91]
Rhipicephalus spp.	2013-2019	*T. cruzi*	P	Gr	Metagenomics	* * * Wild ungulates Carnivores Regular domestic	Kenya	Africa	[84]

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Rhipicephalus turanicus	1982	*T. theileri, T. evansi*	N	H	Microscopy	Bovine (*Bos taurus*) Dog (*Canis lupus*)	Algeria	Africa	[88]

NS : unspecified ; **P :** positive ; **N :** negative ; **F:** faeces**; G:** gut**; Gr:** whole body**; H:** haemolymph**; O:** ovaries**; SG:** salivary glands ; **PCR :** conventional Polymerase chain reaction, **Sequencing :** Capillary sanger sequencing.* **Wild ungulates [**Black rhinoceros (*Diceros bicornis*), white rhinoceros (*Ceratotherium simum*), buffalo (*Syncerus caffer*), elephant (*Loxodonta africana*), giraffe (*Giraffa camelopardalis*), Grévy's zebra (Equus grevyi), plains zebra (*Equus quagga*), hartebeest (*Alcelaphus buselaphus*), impala (*Aepyceros melampus*)**]**; **Carnivores [**leopard (*Panthera pardus*), lion (*Panthera leo*), spotted hyena (*Crocuta crocuta*), wild dog (*Lycaon pictus*)**]**; **Regular domestic [**farmed Boran (*Bos indicus*) and cattle (*Bos taurus*)**]. **Wild ruminants species :** Scimitar-horned oryx; Addax antelope; Barbary red deer; Dorcas gazelle*** **Wild ungulates [**plains zebra (*Equus quagga*)**]**; **Carnivores [**lion (*Panthera leo*), spotted hyena (*Crocuta crocuta*)**].**

- *Trypanosoma cruzi*

Since the 1940s, the prevalence of *T. cruzi* in field-collected ticks has remained uncertain. Most of the published studies focus on ticks taken from infected animals. A study by Pifano (1941) on ectoparasites of Cercolabidae in the State of Yaracuy, Venezuela, documented *A. longirostrum*, a parasite of the porcupine, naturally infected with *T. cruzi* [36]. In 2013-2015, ticks were collected from 148 dogs in the urban area of Campo Grande, state of Mato Grosso do Sul, Brazil. This study revealed that *R. sanguineus* and *A. cajennense* were not infected by *T. cruzi* [92]. Recently, Opazo et al. (2022) investigated the presence of *T. cruzi* in dogs and their ectoparasites in a rural area of Central Chile. 57% of blood samples were infected by *T. cruzi,* and 5.4% of the ticks were positive by PCR, particularly *R. sanguineus s.l.* (5/82), whereas all *A. tigrinum* specimens (0/11) were negative [89]. The same year, a study focused on ticks' utility as xenosurveillance sentinels to survey circulating pathogens in Kenyan drylands. Ticks collected from wild ungulates, carnivores, domestic animals, and Boran cattle were screened for pathogens using metagenomics. *Trypanosoma cruzi* DNA was detected in 3/46 (6.5%) pools of *Rhipicephalus* spp. but not in *Amblyomma* spp. [84]. Nevertheless, careful examination of the sequences provides evidence that they belong to the *Schizotrypanum* genus but cannot definitively identify them as *T. cruzi*.

- *Trypanosoma vivax*

Trypanosoma vivax infects wild domestic ungulates and is transmitted mechanically via tabanids and other blood-sucking insects in the Americas [83]. The search for *T. vivax*'s presence has only recently been engaged. Postoyan et al. (2001) analyzed two tick species, *A. cajennense (*Fabricius*) sensu stricto (s.s.)* and *R. (Boophilus) microplus* (Canestrini) (Acari: Ixodidae), collected from cattle (*Bos taurus*) on two farms in Cuba. The gut contents and hemolymph of these ticks were smear-examined for flagellates. In both species, living *T. vivax* forms were observed in the gut for 96 hours after repletion but not in the hemolymph [90]. In 2013, Bolivar et al. conducted a study in which 285 *R. (Boophilus) microplus* ticks were collected from cattle in the livestock areas of the state of Merida, Venezuela. It was found that 7.7% of the samples tested were positive using PCR [81]. In the same year, Cominetti et al. examined 63 specimens of *R. sanguineus* collected from dogs in the city of Campo Grande, Brazil, for the presence of *Trypanosoma* spp. using PCR. 15 (23.8%) were positive for *T. vivax* [82]. Dyonisio et al. (2021) looked for *T. vivax* in both *A. cajennense sensu stricto* and *R. (Boophilus) microplus* collected on cattle. Of the 48 and 45 specimens of each species, 6.25% (3/48) of *A. cajennense s.s.* and 4.5% (2/45) of *R. (Boophilus) microplus* were positive for *T. vivax*. The sequences obtained were 99% identical to those of bovine *T. vivax* from north-eastern Brazil [83].

- *Trypanosoma evansi*

Trypanosoma evansi is primarily transmitted by tabanids and *Stomoxys* spp. It affects a wide range of hosts, including livestock, camelids, equids, carnivores, rodents, and humans, and produces variable clinical symptoms depending on the host and the geographic region [91]. In a study conducted by Monod et al. (1986) on the seasonal dynamics of *R. sanguineus* in dogs in the urban areas of western Algeria, several hemolymph smears were prepared to search for potential microorganisms. Five of the 250 *R. sanguineus* examined were infected with trypanosomes (four

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females and one male), representing 2% of the total. It is worth noting that two infected females displayed a pathological reaction (globular body and milky hemolymph) that may be related to the infection. Based on the observed form and small size of the protozoan, Monod et al. hypothesized that it could be *T. berberum (evansi)*, a parasite of the Camelidae that can also cause deadly trypanosomiasis in dogs [88]. This hypothesis is corroborated earlier by Cominetti et al. (2013), who found 15 out of 63 dogs in the city of Campo Grande, MS, Brazil, positive for *T. vivax* and 7 for *T. evansi* through PCR [82]. In 2021, Said et al. found that 0.2% of the 352 ticks collected from the environment and wild Tunisian ruminants were positive for *T. evansi* by PCR [91]. Krishnamoorthy et al. (2021) reported a prevalence of *T. evansi* in *R. sanguineus s.l* of 0.2%. PCR and sequencing of 240 pooled tick DNA samples display that the prevalence of *T. evansi* was null in Karnataka and 8.3% in Kerala, India [85].

- *Trypanosoma theileri and T. theileri-like*

Multiple studies document the presence of trypanosomes in tick hemolymph [31,48,79,88,93]. Most of these studies identified *T. theileri,* which is typically transmitted by tabanids [94]. *Trypanosoma theileri* was first reported in field-collected ticks in a study published in 1979 in Switzerland. In this study, 0.19% (5 out of 2501) of *Ixodes ricinus* were found to carry trypanosomes morphologically similar to *T. theileri.* [79]. Between 1979 and 1982, the presence of *T. theileri* in the hemolymph of ticks from Switzerland and Algeria was confirmed: 0.24% (37/1570) of *H. detritum* and *I. ricinus* ticks collected from bovines tested positive [88]. In 2008, Martins reported the presence of Trypanosomatidae epimastigote forms in the hemolymph of cattle tick *R. (Boophilus) microplus* from Rio Grande do Sul, Southern Brazil. Again, the microscopic examination suggested that these forms were similar to those of *T. theileri* [87].

- *Trypanosoma congolense*

Animal trypanosomosis is a complex disease caused by one or more species of pathogenic trypanosomes, including *T. congolense*. Clinical symptoms are characterised by intermittent fever, parasitemia, anemia, lymphadenopathy, jaundice, progressive emaciation, weakness, and reduced productivity [95]. Trypanosomes transmitted by tsetse flies (*Glossina spp*.) are responsible for animal African trypanosomoses in livestock in sub-Saharan Africa [96]. In 2016, trypanosomes were detected in engorged adult *A. variegatum* collected from cattle in the Unguwan Rimi and Kaduna state areas in northwestern Nigeria. Of the 33 samples examined under a microscope, 14 were positive for *T. congolense*, representing a prevalence rate of 42.4%. On the first day of parasitemia follow-up, 10 (30.3%) samples were microscopically positive for *T. congolense*, while 23 (69.7%) were negative [78].

- *Trypanosoma caninum*

Trypanosoma caninum, the most recent species within the Trypanosomatidae family, was reported to infect dogs in Brazil [22]. Despite its recent identification, 67 cases of natural infection in dogs have been documented in areas where canine visceral Leishmaniosis is endemic [22,97]. Luu et al. (2020) isolated and partially characterised a novel trypanosome from *I. ricinus* collected in Slovakia, and the resulting sequences suggest that this trypanosome, referred to as *Trypanosoma* sp. Bratislava1 could be a new species closely related to several trypanosomes isolated from, or detected in, ticks in South America and Asia, including *T. caninum* isolated in Brazil [86].

3.3.2. Leishmania Prevalence in Field Collected Ticks

The presence of *Leishmania* is well documented in ticks, including *Leishmania infantum*/*L. donovani* , *L. major*, *L. chagasi* (syn *L. infantum)*, *L. braziliensis*, *L. guyanensis*, and *L. martiniquensis* [55–60,62–64,66–69,74,76,77,92,98–106]. Most of the field studies focus on ticks that parasitise domestic animals in urban areas, like *R. sanguineus* [55,57,58,62,65,67–69,74,76,77,82,98–101,103,104,106]. Ticks collected from more wild ecosystems highlight the diversity of tick species that are positive for the presence of *Leishmania*, including *A. sabanerae* [105], *Amblyomma* spp. [63], *A. tigrinum* [58], *A. variegatum* [56], *Hyalomma aegyptium*, *H. dromedarii* [60], *Ixode ricinus* [62,64,67,102,104], *Ixodes* spp**.** [104], *I. ventalloi* [62,104], *R.* (*Boophilus*) *microplus* [56,63,105], *R. pusillus* [62,104], *R. sanguineus s.l.* and *R. turanicus* [60] (**Table 2**).

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Table 2. Evidences of presence of *Leishmania* of medical and veterinary interest in field-collected ticks.

Species	Sampling year	*Leishmania* species	Tick statut (P/N)	Localisation in the tick	Tick Detection method	Host (Species)	Host Detection method	Country	Continent	Author(s)
Amblyomma cajennense	2013	*L. chagasi* (Syn *L. infantum*)	N	H ; Gr	Microscopy, PCR	Dog (*Canis lupus*)	ND	Brazil	South America	[92]
Amblyomma ovale	2008	*L. infantum*	N	Gr	qPCR	Dog (*Canis lupus*)	IFAT	Brazil	South America	[73]
Amblyomma sabanerae	NS	*Leishmania* sp.	P	Gr	qPCR	Collared peccary (*Pecari tajacu*) Tortoise (*Chelonoidis denticulata*)	ND	Peru	South America	[107]
Amblyomma spp.	2012	*L. guyanensis*	P	Gr	PCR ; HRM-PCR	Tapiridae (*Tapirus terrestris*) Tayassuidae (Pecari tajacu)	ND	Peru	South America	[63]
Amblyomma tigrinum	2010-2013	*Leishmania* sp.	P	Gr	PCR	Wild foxes (Pseudalopex griseus)	PCR, qPCR	Argentina	South America	[58]
Amblyomma variegatum	2014-2015	*L. martiniquensis*	P	Gr	HT-qPCR	Cattle	ND	Guadeloupe	Island	[56]
Dermacentor marginatus	2007-2008	*L. infantum*	N	Gr	PCR	Wild boars	ND	Italy	Europe	[108]
	2007	*L. infantum*	N	Gr	qPCR	Dog (*Canis lupus*)	ELISA, PCR	Italy	Europe	[66]
	2014	*Leishmania* sp.	N	Gr	PCR	Human	ND	Turkey	Asia	[109]
Haemaphysalis longicornis	2012	*L. infantum*	N	Gr	PCR	sheep, cattle and dogs	ND	China	Asia	[110]
Haemaphysalis parva	2014	*Leishmania* sp.	N	Gr	PCR	Human	ND	Turkey	Asia	[109]
Haemaphysalis punctata	2014	*Leishmania* sp.	N	Gr	PCR	Human	ND	Turkey	Asia	[109]
Haemaphysalis sulcata	2007-2008	*L. infantum*	N	Gr	PCR	Sheep; Goats	ND	Italy	Europe	[108]

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	2014	*Leishmania* sp.	N	Gr	PCR	Human	ND	Turkey	Asia	[109]
Hyalomma aegyptium	2015-2018	*L. infantum*	P	Gr	PCR ; Sequencing	Tortoise (*Testudo graeca*) Arabian camels (*Camelus dromedarius*)	ND	Israel	Asia	[59]
	2014	*Leishmania* sp.	N	Gr	PCR	Human	ND	Turkey	Asia	[109]
Hyalomma dromedarii	2015-2018	*L. infantum*	P	Gr	PCR, Sequencing	Tortoise (*Testudo graeca*) Arabian camels (*Camelus dromedarius*)	ND	Israel	Asia	[59]
Hyalomma excavatum	2014	*Leishmania* sp.	N	Gr	PCR	Human	ND	Turkey	Asia	[109]
Hyalomma marginatum	2007-2008	*L. infantum*	N	Gr	PCR	Cattle	ND	Italy	Europe	[108]
	2014	*Leishmania* sp.	N	Gr	PCR	Human	ND	Turkey	Asia	[109]
Hyalomma spp.	2014	*Leishmania* sp.	N	Gr	PCR	Human	ND	Turkey	Asia	[109]
Ixode ricinus	2007	*L. infantum*	P	Gr	qPCR	Dog (*Canis lupus*)	ELISA, PCR	Italy	Europe	[66]
	2007-2008; 2010	*L. infantum*	P	Gr	PCR	Dogs, horses, cat, bovine, humans	ND	Italy	Europe	[64]
	2011 and 2013	*L. infantum*	P	Gr	PCR	Cat (*Felis catus*)	ND	Italy	Europe	[62]
Ixode ricinus (Cont.)	2012-2013	*L. infantum*	P	Gr	qPCR	Cat (*Felis catus*)	PCR, qPCR	Italy	Europe	[104]
	2010	*L. infantum*	P	Gr	PCR ; Sequencing	Flagging	ND	Italy	Europe	[102]
	2014	*Leishmania* sp.	P	Gr	PCR	Human	ND	Turkey	Asia	[109]
Ixodes spp.	2011 and 2013	*L. infantum*	N	Gr	PCR	Cat (*Felis catus*)	ND	Italy	Europe	[62]
	2012-2013	*L. infantum*	P	Gr	qPCR	Cat (*Felis catus*)	PCR, qPCR	Italy	Europe	[104]

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Ixodes ventalloi	2011 and 2013	*L. infantum*	P	Gr	PCR	Cat (*Felis catus*)	ND	Italy	Europe	[62]
	2012-2013	*L. infantum*	P	Gr	qPCR	Cat (*Felis catus*)	PCR, qPCR	Italy	Europe	[104]
Rhipicephalus (Boophilus) microplus	2012	*L. guyanensis*	P	Gr	PCR ; HRM-PCR	Tapiridae (*Tapirus terrestris*) Tayassuidae (*Pecari tajacu*)	ND	Peru	South America	[63]
	2012	*L. infantum*	N	Gr	PCR	Sheep, cattle and dogs	ND	China	Asia	[110]
		Leishmania sp.	P	Gr	qPCR	Collared peccary (*Pecari tajacu*); Tortoise (*Chelonoidis denticulata*)	ND	Peru	South America	[105]
	2014-2015	*L. martiniquensis*	P	Gr	HT-qPCR	Cattle	ND	Guadeloupe	Island	[56]
	2014-2015	*L. martiniquensis*	P	Gr	HT-qPCR	Cattle	ND	Martinique	Island	[56]
Rhipicephalus bursa	2014	*Leishmania* sp.	N	Gr	PCR	Human	ND	Turkey	Asia	[109]
	2007-2008	*L. infantum*	N	Gr	PCR	Sheep, goats, cattle, horses, deer	ND	Italy	Europe	[108]
Rhipicephalus pusillus	2011 and 2013	*L. infantum*	P	Gr	PCR	Cat (*Felis catus*)	ND	Italy	Europe	[62]
	2007-2008	*L. infantum*	N	Gr	PCR	Hedgehogs	ND	Italy	Europe	[108]
	2012-2013	*L. infantum*	P	Gr	qPCR	Cat (*Felis catus*)	PCR, qPCR	Italy	Europe	[104]
Rhipicephalus sanguineus	2012-2013	*L. infantum*	P	Gr	qPCR	Cat (*Felis catus*)	PCR, qPCR	Italy	Europe	[104]
	2016-2017	*Leishmania major*	P	Gr	PCR, Sequencing	Rodents (*Rhombomys opimus; Nesokia indica*)	ND	Iran	Asia	[98]

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	2013	*L. chagasi* (syn *L. infantum)*	P	H ; Gr	Microscopy, PCR	Dog (*Canis lupus*)	ND	Brazil	South America	[92]
	NS	*L. braziliensis*	P	Gr	PCR, qPCR	Dog (*Canis lupus*)	ND	Brazil	South America	[76]
	2007	*L. infantum*	P	Gr	PCR ; qPCR	Dog (*Canis lupus*)	IFAT, PCR, qPCR	Brazil	South America	[100]
	2007	*L. infantum*	P	Gr, SG	PCR ; qPCR ; sequencing	Dog (*Canis lupus*)	ND	Italy	Europe	[74]
	2008	*L. infantum*	P	Gr, SG	PCR; qPCR; sequencing	Dog (*Canis lupus*)	ND	Brazil	South America	[74]
	2007-2008	*L. infantum*	P	Gr	PCR	Dog (*Canis lupus*)	ELISA	Brazil	South America	[103]
	2007-2008	*L. infantum*	N	Gr	PCR	Dog (*Canis lupus*)	ND	Italy	Europe	[108]
	2008-2009	*L. infantum*	P	Gr	PCR, RT-PCR, Sequencing	Dog (*Canis lupus*)	ELISA, PCR	Brazil	South America	[65]
Rhipicephalus sanguineus	2007	*L. infantum*	P	Gr	qPCR	Dog (*Canis lupus*)	ELISA, PCR	Italy	Europe	[66]
	2006-2007	*L. infantum*	P	Gr	qPCR	Dog (*Canis lupus*)	ELISA, PCR	Italy	Europe	[67]
	2011-2012	*L. infantum*	N	NS	Infestation	Dog (*Canis lupus*)	IFAT, ELISA	Brazil	South America	[111]
	NS	*L. infantum*	P	Gr	PCR, qPCR, seuqencing	Dog (*Canis lupus*)	PCR, qPCR	Brazil	South America	[77]
	2012	*L. infantum*	P	Gr	PCR	Dog (*Canis lupus*)	IFAT, ELISA	Brazil	South America	[54]
	2013	*L. infantum*	P	Gr	PCR, Sequencing	Dog (*Canis lupus*)	PCR, Sequencing	Brazil	South America	[55]
	2011	*L. infantum*	P	G	PCR, RFLP, seuqencing, Parasit culture	Dog (*Canis lupus*)	DPP, IFAT, ELISA, PCR	Brazil	South America	[57]
	2011	*L. infantum*	P	SG	PCR, RFLP, Sequencing,	Dog (*Canis lupus*)	DPP, IFAT, ELISA, PCR	Brazil	South America	[57]

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					Parasit culture					
	2011 and 2013	*L. infantum*	P	Gr	PCR	Cat (*Felis catus*)	ND	Italy	Europe	[62]
	2002	*Leishmania* sp.	P	G	Microscopy	Dog (*Canis lupus*)	IFAT	Brazil	South America	[106]
	2009	*Leishmania* sp.	N	NS	present of tick or no	Dog (*Canis lupus*)	IFAT	Brazil	South America	[61]
	2012-2014	*Leishmania* sp.	N	Gr	qPCR	Dog (*Canis lupus*)	qPCR	China	Asia	[112]
	NS	*Leishmania* sp.	P	G ; O ;SG	IHC, qPCR, IHC	Dog (*Canis lupus*)	ND	Brazil	South America	[69]
	NS	*Leishmania* sp.	P	G ; O ; SG	IHC, qPCR, IHC	Dog (*Canis lupus*)	ND	Brazil	South America	[68]
	NS	L. kala azar£	P	NS	NS	Human	NS	France	Europe	[101]
	2008	*L. infantum*	N	Gr	qPCR	Dog (*Canis lupus*)	IFAT	Brazil	South America	[73]
Rhipicephalus sanguineus s.L.	NS	*L. infantum*	P	Gr	PCR, Sequencing	$Flagging & *Animals	ND	Israel	Asia	[59]
Rhipicephalus turanicus	2007-2008	*L. infantum*	N	Gr	PCR	Sheep; Goats	ND	Italy	Europe	[108]
	2014	*Leishmania* sp.	N	Gr	PCR	Human	ND	Turkey	Asia	[109]
	NS	*L. infantum*	P	Gr	PCR, Sequencing	$Flagging & *Animals	ND	Israel	Asia	[59]

NS : unspecified ; **P :** positive ; **N :** negative ; **F:** faeces**; G:** gut**; Gr:** crushing**; H:** haemolymph**; O:** ovaries**; SG:** salivary glands ; **PCR :** conventional Polymerase chain reaction, **RFLP :** restriction fragment length polymorphism ; **qPCR :** real time PCR; **RT-PCR** : reverse transcriptase PCR; **HRM-PCR** : High Resolution Melting PCR ; **HT-qPCR** : High-throughput microfluidic PCR; **Sequencing :** Capillary sanger sequencing, **IFAT :** Indirect Fluorescent Antibody Test ; **ELISA :** Enzyme Linked Immunosorbent Assay ; **DPP :** rapid immunochromatographic test ; **IHC** : immunohistochemistry * **Animals :** dogs (*Canis familiaris*); Tortoise (*Testudo graeca*); hedgehogs (*Erinaceus concolor*); badger. * **L. kala azar** refers to members of the *L. donovani* complex (*L. infantum* and *L. donovani*) without any other information on parasite typing at the time of the study. We therefore use the term proposed by the author. $**Flagging**: refers to the use of a method to collect questing ticks.

- *Leishmania infantum and L. chagasi (Syn L. infantum)*

The hypothesis that ticks can be vectors of *L. infantum* was first proposed in the early 20th century [113]. Domestic dogs play a central role as reservoirs of *L. infantum* in the peridomestic zoonotic transmission cycle. The brown dog tick, *R. sanguineus* (Latreille 1806), has been the subject of extensive research due to its prevalence among urban dogs [114]. The overall *L. infantum* prevalence given by molecular techniques in *R. sanguineus* collected on dogs [54,57,65–67,74,77,100,115] or cats

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[62,104] ranged from 2.5% to 70.3% in Brazil and Italy. Significant *L. infantum* presence is recorded in species of the *Rhipicephalus* genus, including 9.2% of *R. sanguineus s.l.* ticks collected with the flagging technique that collects questing ticks and 23.5% of *R. turanicus* collected from dogs, hedgehogs, and tortoises in Israel [60]. Additionally, 10.9% and 17.6% of infections were reported in *R. pusillus* from cats in Italy. Studies reported the detection of *L. infantum* in *I. ricinus* (1.5% to 50%) [62,64,67,102,104]**,** *I. ventalloi* (19% and 62%) [62,104]**,** *Ixodes sp*. (10.9%) [98], *H. aegyptium* (38.7%), and *H. dromedarii* (55.6%) [60]**,** collected from dogs, horses, cats, bovines, tortoises, camels, and humans in Italy and Israel. In the majority of these studies, *L. infantum* DNA was detected in whole ticks and, in some cases, in the gut or salivary glands [57,74]. *Leishmania chagasi* (Syn *L. infantum*), which causes american visceral leishmaniasis, is widely distributed in Latin America [116]. It is thought that *L. chagasi* (syn *L. infantum*) was introduced in South America along with conquistadors' dogs [117]. In Campo Grande, state of Mato Grosso do Sul, Brazil, de Almeida et al. (2013) identified two tick species, *R. sanguineus* and *A. cajennense,* on dogs in urban areas. Tick samples from 36 dogs were positive for *L. chagasi*, and all were *R. sanguineus* [92].

- *Leishmania major*

Zoonotic Cutaneous Leishmaniasis (ZCL), caused by *Leishmania major*, is a public health problem in several countries, affecting a large number of people. Azarmi et al. (2022) suspected various arthropods, including ticks, to act as secondary vectors of *Leishmania.* They found one nymph of *R. sanguineus* positive for *L. major* in the rodent in Segzi plain in Esfahan Province, Iran [98].

- *Leishmania braziliensis*

American cutaneous leishmaniasis (ACL) is caused mainly by *Leishmania braziliensis*. De Morais et al. (2013) proposed that difficulties in controlling ACL might be related to the complex epidemiology of this disease, which involves the participation of various vector species, including ticks. Two rural areas in Pernambuco, northeastern Brazil, where ACL is endemic, were investigated for canine ectoparasites. Genomic DNA was extracted from 75 *R. sanguineus* ticks; 32 of them (42.67%) were positive for *L. braziliensis* using conventional PCR and real-time PCR (RT-qPCR) [76].

- *Leishmania guyanensis*

Leishmania guyanensis is a causal agent of American Tegumentary Leishmaniasis (ATL). In 2017, 81 *R.* (*Boophilus*) *microplus* and *Amblyomma* collected from three *Tapirus terrestris* and three *Pecari tajacu* in Madre de Dios, Perú, disclosed the presence of DNA belonging to the *Leishmania* (*Viannia*) subgenus after kDNA (kinetoplast DeoxyriboNucleic Acid) amplification **[55]**. *Leishmania* (*Viannia*) kDNA was detected in three *R. microplus* collected from a collard peccary (*P. tajacu*) hunted in the forests of Madre de Dios. The HRM-PCR (High Resolution Melting PCR) indicates that one positive sample displays a kDNA melting curve compatible with *L.* (*V*) *guyanensis* [63].

- *Leishmania martiniquensis*

Leishmania martiniquensis was first isolated in 1995; its taxonomical position was established in 2002, and it was named in 2014 [11,118–120]. The vectors and reservoirs have not yet been described, although biting midges are suspected to be involved in transmission of Leishmania belonging to the *Mundinia* subgenus [118,121]. In 2020, a high-throughput microfluidic real-time PCR system suitable for screening parasites at the genus-level was applied to 132 adult specimens of *A. variegatum* and 446 of *R. microplus* collected in Guadeloupe and Martinique. It was found that 0.7% of the *R. microplus* ticks from Martinique were positive for *Leishmania* spp., and the sequences identified *L. martiniquensis* [56].

- *Other/unidentified Leishmania parasites*

In 2010, the presence of *Leishmania* promastigotes forms in ticks parasitizing domestic dogs was reported in the Municipality of São Vicente Férrer, located in the Northern Agreste of Pernambuco, Brazil [106]. In 2015 and 2016, the presence of *Leishmania* was reported in the intestines, ovaries, and salivary glands by immunohistochemistry (IHC) but also by real-time PCR in *R. sanguineus* from dogs in Brazil. IHC detected *Leishmania* in 98% of the intestines, 14% of the ovaries, and 8% of the salivary glands. Real-time PCR indicates that the intestines, ovaries, and salivary glands were mostly positive

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[68,69]. *Leishmania* DNA (most likely belonging to the *L. donovani* complex) was detected in 11 out of 17 pools (64.7%) of *A. tigrinum* were collected from eight foxes (six grey foxes, *Pseudalopex griseus* and two culpeo foxes *P. culpaeus*) captured in Argentinean Patagonia [58]. The fact that sandflies were trapped only 2000 km and 750 km north of the study area suggests that their distribution may be wider than currently believed or that *Leishmania* is able to persist in another vector in this area of transmission. In 2022, 95.7% of *R. (Boophilus) microplus* and 90% of *A. sabanerae* collected from *Pecari tajacu* and *Chelonoidis denticulata* of the Leishmaniasis endemic zones in the Peruvian Amazon, with a load of 34.1 and 5428.6 parasites per arthropod [122].

3.4. Experimental infection and Transmission of Trypanosomatidae by Ticks

In the early 20th century, laboratory infections were conducted to probe the vector competence of ticks for trypanosomes. Several reports have demonstrated the presence of different forms of *Trypanosoma* or *Leishmania*, including trypomastigotes, amastigotes, epimastigotes, promastigotes, and sphaeromastigotes, in ixodides and argasides. The transmission routes for infestation were investigated from the bite of the tick and its blood meal to other alternative routes such as ingestion of infected ticks, passage between ticks at different life stages and their hosts (transstadial passage), or ovarian infection (transovarial passage).

3.4.1. Transmission of Trypanosoma following tick bites and feeding

The role of ticks as vectors of trypanosomes has been increasingly strengthened by recent studies that have provided additional evidence that ticks can transmit distinct clades of trypanosomes to various mammalian species [26]. Reports on the detection of parasites of the genus *Trypanosoma* after blood feeding on an infected animal in an experimental setting are available [40,43,45,46,123,124]. Trypanosomes of medical or veterinary interest were detected in hard and soft ticks from three tick genera (*Rhipicephalus*, *Hyalomma*, and *Ornithodoros*) and four species: *R. sanguineus*, *H. anatolicum anatolicum*, *O. crossi*, and *O. lahorensis*. Experimental infection of ticks following a bite or a meal was reported for *T. cruzi*, *T. evansi*, and *T. theileri* [125].

- *Trypanosoma cruzi*

The first available reports on dog infection by *T. cruzi*-infected *R. sanguineus* ticks date back to 1913 [40]. Mayer and Roca-Lima later attempted to infect rats with infected soft ticks of the genus *Ornithodoros*, but the results were negative [41]. Several other studies have failed to show the transmission of *T. cruzi* by soft ticks in laboratories (i.e., *O. moubata*, *O. talaje*, and *O. turicata*) [35,39,42,47].

- *Trypanosoma evansi*

Investigations into the biological transmission of *T. evansi* by ticks were initiated at the dawn of the 20th century. Studies have given clues on the capacity of a soft tick, *O. crossi*, infected by *T. evansi* to transmit the parasite after a blood meal to non-infected animals [43,44,46]. But, in 1924, Yorke and Macfie did not confirm the transmission of this protozoan by *O. crossi* [45]. In 1976, Taylor-Lewis also reported the non-transmission of *T. evansi* by *H. dromedarii* to rodents after a blood feeding [126]. Recently, Mahmoud et al. (2020) did not detect *T. evansi* DNA after blood-feeding infected *O. savignyi* on uninfected rodents [127].

- *Trypanosoma theileri and Trypanosoma theileri-like*

Shastri and Deshpande (1981) conducted experimental work on cattle and attempted unsuccessfully to infect calves with *H. a. anatolicum* carrying *T. theileri* [49]. A few years later, Morzaria succeeded in infecting calves and rats with *H. a. anatolicum* ticks infected with *T. theileri-like* [123]. In the early 2000s, Latif et al. reported the ability of *Hyalomma* ticks to transmit *T. theileri* but could not confirm its transmission to calves [124].

3.4.2. Transmission of Leishmania Following Tick Bites and Feeding

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To date, sandflies (*Phlebotominae*) are recognized biological vectors of *Leishmania* [128], but the role of ticks, fleas, and leaches as potential secondary vectors has been evoked [71,129]. Five laboratory studies have attempted to infect ticks by allowing them to feed on vertebrates experimentally infected with *Leishmania* [32,51,52,70,130]. All experiments were performed on hard ticks (Ixodidae), and only ticks of the *Rhipicephalus* genus were involved because of their close relationship with dogs. Ticks were fed on dogs or rodents, and two species were used: *R. turanicus and R. sanguineus*. *R. sanguineus* was the only one to have been infected by *Leishmania*, referred to as *L. infantum, and Leishmania* sp. [52,70].

- *Leishmania infantum*

The transmission of *L. infantum* by ticks was first reported in 1930 by Blanc and Caminopétros, who infected squirrels after exposition to infected *R. sanguineus* ticks infected with what they named "*Leishmania kala azar"* at the time, which today most probably corresponds to parasites of the *L. donovani* complex [32]. Nevertheless, this was not confirmed by Joyeux and Sautet in 1938 [131] nor by Rioux et al. (1972) who failed to establish experimentally the transmission of *Leishmania* to rats by *R. sanguineus* [51]. More recently, de Almeida et al. (2016) were able to infect 14 hamsters with *R. sanguineus* infected with *L. infantum* [70]. In contrast, Rakhshanpour et al. (2017) could not confirm the transmission of *L. infantum* by *R. sanguineus* ticks collected from experimentally infected dogs [130].

3.4.3. Host Infection Following Ingestion of Infected Ticks or Injection with Trypanosoma-Infected Ticks

Documented transmission of trypanosomes following the ingestion of an infected invertebrate suggests that they may also be transmitted through the ingestion of infected ticks [29,132,133]. To date, nine studies on the Argasidae family and five on the Ixodidae family have investigated the transmission of trypanosomes in the laboratory through ingestion of infected ticks or infection through injection of an infected material [35,37–39,41,42,45,47–49,93,123,124,126]**.** Of these 14 studies, nine confirmed transmission by the injection of infected tick materials and only one by the ingestion of infected ticks. However, it is difficult to draw clear conclusions from these results that have not been obtained on the same tick genera or species, parasites, and hosts on which ticks have been fed or exposed to infected ticks. Seven tick species were tested, *O. moubata, O. crossi, O. venezuelensis, O. turicata, O. furcosus, R. pulchellus*, and *H. a. anatolicum excavatum*. Five *Trypanosoma* species were tested.

- *Trypanosoma cruzi*

Experimental *T. cruzi* transmission through ingestion or injection of tick infected material began in the 20th century. Mayer and Roca-Lima (1914) reported that injection of *O. moubata* infected with *T. cruzi* caused rodent infection [41]. The same author confirmed their findings in 1918 and mentioned that laboratory rats also became infected after injection of *O. moubata* infected material [42]. Later reports confirmed the infection by injection of *T. cruzi* infected *O. venezuelensis, O. turicata, O. furcosus,* and *O. moubata* ticks into rodents [35,37,39,47].

- *Trypanosoma theileri and T. theileri-like*

In 1973, Burgdorfer and colleagues unsuccessfully attempted to transmit *T. theileri* by injecting infected hard tick material collected from cattle into rodents [48]. On the other hand, Krinsky and Burgdorfer (1976) showed that *T. theileri* can be transmitted by injecting *O. moubata* infected material [93], as Shastri and Deshpande (1981) showed that injection of *T. theleiri H. a. anatolicum* infected material causes infection in cattle [49]. In 1986, Morzaria et al. reproduced the work of Shastri and Deshpande, but could not demonstrate the transmission of *T. theileri*-like by this route. Latif and colleagues recently succeeded in infecting cattle by injecting *H. a. anatolicum* infected material [117, 118].

- *Other Trypanosoma*

As early as 1924, Yorke and Macfie attempted to transmit *T. rhodesiense* to rodents by injecting them with infected *O. crossi* material [45]. In 1948, Packchanian did not succeed in infecting animals

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with *T. brucei* using the same route [38]. Finally, in 1976, Taylor-Lewis did not succeed in infecting rodents by injecting *H. a. excavatum-T. lewisi* infected material [126].

3.4.4. Infection after Ingestion or Injection with Leishmania- Infected Ticks Matertial

Several studies reported the presence of *L. infantum* in *R. sanguineus* and *I. ricinus* [57,65,67,74,99] and the transmission of other protozoa (*Hepatozoon canis*) in dogs after ticks ingestion [134]. Six studies experimentally investigated the transmission of *Leishmania* via the oral route and the infective capacity of injecting material from infected ticks [50–52,71,73–75]. Of these, five investigated infection by injecting, and one examined oral transmission. There were five confirmed infections in these seven laboratory reports (four after injection and one after ingestion). All these studies focused exclusively on ticks of the Ixodidae family, specifically *R. sanguineus*, which were infected with various *Leishmania* species and fed on rodents or dogs.

- *Leishmania infantum and Leishmania chagasi (Syn: L. infantum)*

In 2010, Dantas-Torres et al. reported an experimental infection of *L. infantum* by injecting infected *R. sanguineus* material [74], which was also supported by the work of Coutinho and colleagues on dog ticks infected with *L. chagasi (syn: L. infantum)* in rodents [71].

- *Leishmania*

In 1984, McKenzie studied the infection of two hunting dogs, crossbred between a German Shepherd and a Labrador Retriever, by injecting them with material from crushed, infected *R. sanguineus* ticks. *Leishmania* sp. was detected by several diagnostic methods in the recipient dogs, indicating the possibility of infection through the injection of tick infected material [52].

3.5 Transstadial Transmission in Ticks

The ability of a microorganism to survive the shedding process and persist for an extended period within the tick is crucial for successful transstadial transmission. Weilgama reported the transstadial transmission of the parasite *Trypanosoma thylacis* in the native tick *I. tasmani* and then to the Australian short-nosed bandicoot (*Isoodon macrourus*) [135].

3.5.1. Transstadial transmission of *Trypanosoma*

Shastri and Deshpande (1981) observed the transstadial passage of *T. theileri* in *H. a. anatolicum* ticks. Larvae and nymphs feeding on infected calves were found to be positive after passage to the adult stage. However, their vectorial competence was not confirmed [49]. The study by Latif et al. (2004) also reported the transstadial passage of *T. theireri* in ticks of the genus *Hyalomma* [124].

3.5.2. Transstadial Transmission of *Leishmania*

Transstadial transmission of *L. infantum* into *R. sanguineus* has been documented since the 1930s [32]. Recent work again provides evidence of the persistence of *L. infantum* kDNA in *R. sanguineus* nymphs and adults that had fed on infected dogs before molting [61]. However, the presence of the parasites could not be confirmed by microscopic examination or culture of infected tick material [61].

3.6. Transovarial Passage

3.6.1. Transovarial Passage of Trypanosoma

The detection of flagellates and Trypanosomes in tick ovary was reported as early as 1961 [48,93,136]. Therefore, a transovarial transmission has been proposed as an alternative route for maintaining the parasite in the wild. The first experimental reports describing the transovarial transmission of *Trypanosoma* in ticks are the work of Mayer and Rico-Lima in Germany, who worked with *T. cruzi* in the early 1900s [41]. In 1972, Lapierre attempted to reproduce Mayer's work but could not detect transovarian transmission in *O. moubata* [39]. One year later, Burgdorfer et al. (1973) also failed to observe transovarian transmission in *Rhipiciphalus* hard ticks [48]. To demonstrate the

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likelihood of *T. theileri* transovarian transmission, Shastri and Deshpande (1981) conducted an experiment on *H. a. anatolicum* but were unable to yield a conclusive outcome [41]. It wasn't until 2004 that a laboratory study provided evidence of pathogenic trypanosomes being transmitted through the ovaries of ticks [124]. Latif et al. discussed the transovarial transmission of *T. theileri* in *H. a. anatolicum.* Their findings implied that the transmission of *T. theileri* in ticks was only sporadic. Subsequently, Vergne et al. tried to establish ovarian transmission in *R. sanguineus* without conclusive evidence collected [137].

3.6.2. Transovarial passage of Leishmania

Recent reports have confirmed the transovarial passage of *Leishmania* in *R. sanguineus*, but McKenzie first pointed out this mode in the early 1980s [52]. Experimental studies by Paz et al. (2010) and Dantas-Torres and colleagues (2010, 2011) demonstrated the presence of *L. chagasi* and *L. infantum* kDNA, respectively, in *R. sanguineus* larvae four months after experimental infection of females [61,75,113]. Coutinho et al. (2005) did not confirm the transovarial passage of *L. chagasi (syn L. infantum)* [71] unlike Dabaghmanesh et al. (2016), who reported the occurrence of such mechanism [72]. Finally, Bilgiç et al. failed to demonstrate *L. major* transovarial passage [138].

3.7. *Other Circumstantial Evidence*

Significative associations between tick infestation prevalence and the presence of anti-*Leishmania* antibodies in dogs in endemic areas for canine Leishmaniosis are reported [73,111,139–141]. According to Paz et al. (2010), the prevalence of *R. sanguineus* infestation was significantly higher (p = 0.04) among seropositive dogs (38.5%) compared with their seronegative counterparts (29.0%). The probability of seropositivity for *Leishmania* is 1.5 times higher in tick-infested dogs than in non-infested animals [61]. Such findings were corroborated by Bernardino et al. (2020) and Nakkoud et al. (2022) [140,141]. On the contrary, the odds infection ratio does not significantly differ between non-infested and *R. sanguineus*-infested dogs in the study of Paz et al. (2013) [111].

Finally, the almost perfect correlation recorded between *Leishmania* detection in dogs by fine-needle aspiration biopsy of the lymph node and the detection of *Leishmania* in the tick's intestine by immunohistochemistry is additional circumstantial evidence [142].

4. Meta-analyses Results

4.1. Field Detection

4.1.1. Overview of the Meta-Analysis

For the field studies, we identified 42 publications for meta-analysis and meta-regression (**Figure 3**). The use of a random-effects model is justified by the P and I^2 statistics, which showed significantly high heterogeneity (X^2=3551.8296 and I^2=98.99%, p=0.0001; **Figure 3**). We identified publication bias in the selected studies based on visual inspection of the graph's asymmetry (**Figure 4**), which is confirmed by Egger's test (t= 4.15, p= 0.0002) (**Supplementary File**, **Table S1**). The elimination, one by one or simultaneously, of the four publications that show bias (**Figure 4)** did not affect the cumulative prevalence recorded.

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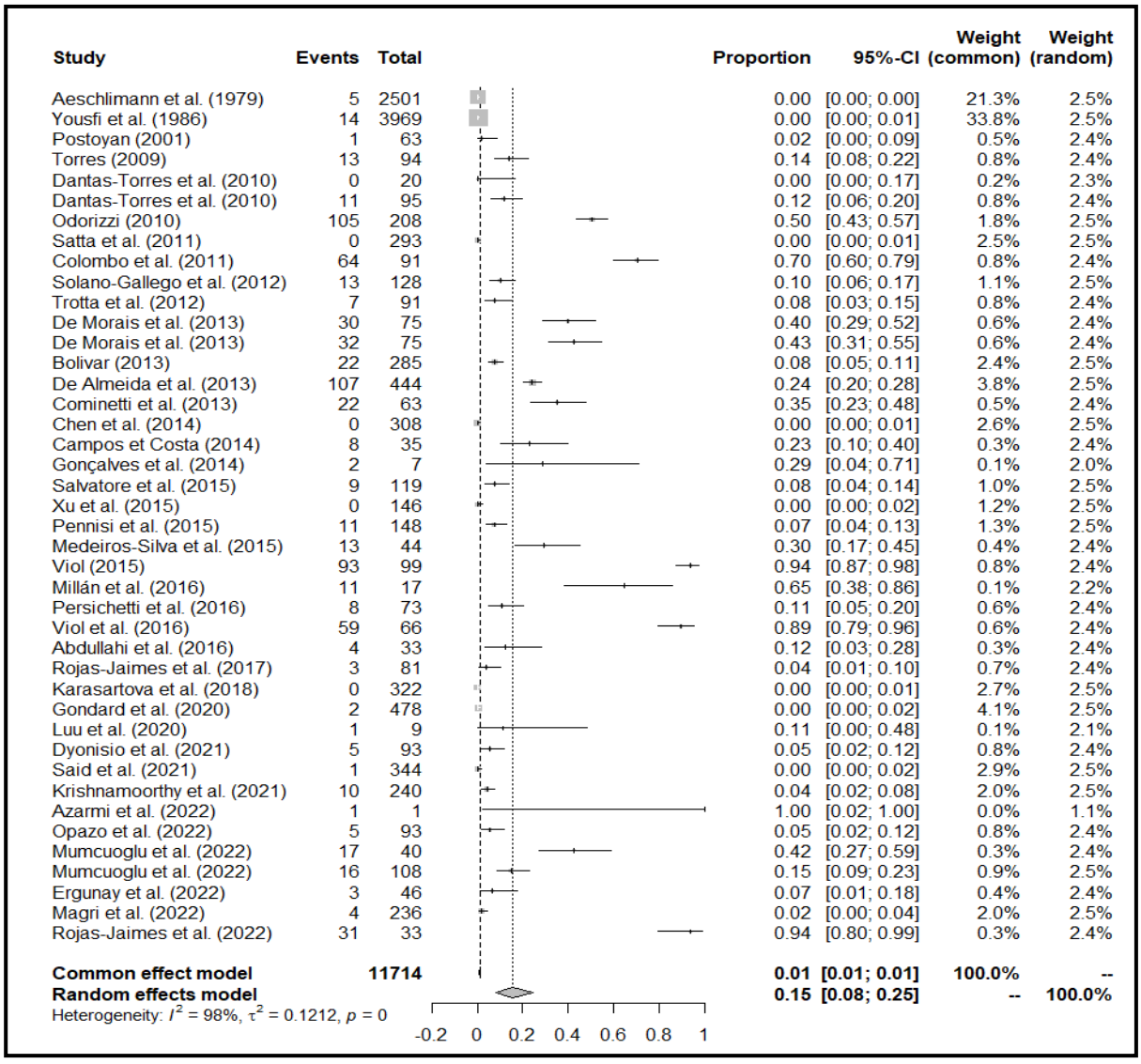

Figure 3. Forest plot of prevalence of Trypanosomatidae detection in field ticks. The length of the horizontal line represents the 95% CI; the diamond represents the summarized effect.

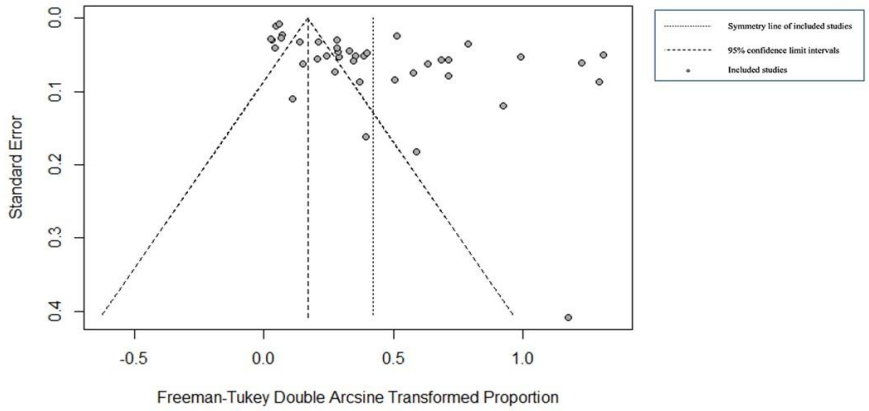

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Figure 4. Funnel plot with 95% confidence limit intervals for the examination of publication bias.

The 42 studies selected included approximately 12,000 ticks collected worldwide. However, in some publications, it was not possible to determine the exact number of ticks used for the experiment. Therefore, the data we used for our analysis was the number of pools, which may overestimate the prevalence. Our results depicted an overall cumulative prevalence for Trypanosomatidae detection of 15.48% (95% CI: 7.99-24.61%) (**Figure 3**).

We then examined factors associated with the detection of *Leishmania* and *Trypanosoma* having medical/veterinary interest in ticks. Factors examined included the sampling decade, study area (continent), animal host (family), tick genus or species, the detection method, parasite species, and location in the tick's organs (**Table 3**). We recorded high heterogeneity in all subgroups; therefore, the pooled seroprevalence estimate for each subgroup was calculated using the random-effects model.

Table 3. Pooled prevalence of parasites detections in ticks by sampling year, continent, host, tick genus/species, method, pariste genus and species, localization.

Category	Variable	No. of studies	No. of tested	No. of positive	% [95% CI]	Heterogeneity			Univariate meta regression		
						χ2	*P*-value	I^2 (%)	*P*-value	R^2 (%)	I^2-res (%)
Sampling year									0.000***	0.00	98.74
	2000 or before	2	6470	19	0.22 [0.16-0.45]	1.14	<0.0001				
	2001-2010	5	480	130	12.19 [0.87-32.08]	126.37	<0.0001	12.0			
	2011-2019	23	3043	518	21.13 [7.7-23.77]	818.31	<0.0001	96.8			
	2020 or after	12	1721	96	11.65 [0.3-30.84]	289.5	<0.0001	97.3			
								96.2			
Continent									0.0056**	23.77	97.78
	Africa	4	4392	22	2.44 [0.00-9.54]	23.24	< 0.0001				
	Asia	7	1165	44	3.11 [0.00-20.83]	131.61	< 0.0001	85.8			
	Europe	10	3693	69	4.36 [1.49-9.06]	158.52	< 0.0001	95.4			
	Island	1	478	2	0.42 [0.01-1.26]	0.000	< 0.0001	94.3			
	South America	20	1986	626	33.35 [14.27-37.83]	828.68	< 0.0001	--			
								96.1			
Host (Family)									0.0007**	0.00	98.34
	Several#	6	4894	43	2.83 [0.0-8.60]	80.46	< 0.0001				
	Bovidae	6	1192	44	3.92 [1.20-7.85]	24.26	< 0.0001	93.8			
	Canidae	20	2184	595	27.92 [14.51-43.57]	1071.56	< 0.0001	88.2			
	Felidae	2	221	19	8.47 [5.07-12.60]	0.81	< 0.0001	98.2			
	Other	7	722	57	21.23 [0.0-62.24]0	252.480	< 0.0001	0.0			
								97.6			
Tick Genus									0.0001***	2.20	97.09
	Amblyomma	10	328	27	8.78 [0-29.41]	100.12	< 0.0001				
	Dermacentor	3	23	0	0.00 [0-5.25]	0.41	< 0.0001	91.0			
	Haemaphysalis	4	360	00	0.00	1.58	< 0.0001	0.0			
	Hyalomma	6	639	17	1.20 [0-14.55]	70.55	< 0.0001	0.0			
	Ixodes	9	6906	42	1.91 [0-10.06]	71.41	< 0.0001	92.9			
	Rhipicephalus	33	3271	668	19.06 [9.64-30.39]	1412.59	< 0.0001	88.8			
								97.7			
Tick species									0.0001***	3.29	97.50
	A. cajennense	1	131	0	0.00 [0-7.04]	3.86	< 0.0001				
	A. ovale	1	10	0	0.00 [0-16.52]	0.00	< 0.0001	--			
	A. sabanerae	1	10	9	90.00 [61.91-100]	0.00	< 0.0001	0.0			
	A. tigrinum	2	28	0	24.86 [0-95.91]	16.89	< 0.0001	--			
	A. variegatum	2	165	11	3.09 [0-24.26]	0.43	< 0.0001	94.1			
	Amblyomma spp.	2	43	4	0.00 [0-0.64]	11.54	< 0.0001	91.3			
								0.0			
	D. marginatus	3	21	0	0.00 [0-5.25]	0.59	< 0.0001				
								0.0			
	H. aegyptium	2	32	12	32.69 [11.66-56.75]	0.46	< 0.0001				
	H. detritum	1	1	1	100 [0-100]	0.00	< 0.0001	0.0			
	H. dromedarii	2	45	5	16.56 [0-88.80]	17.10	< 0.0001	--			
	H. excavatum	3	291	0	0.00	1.45	< 0.0001	94.2			
	H. lusitanicum	1	3	0	0 [0-50]	0.00	< 0.0001	0.0			
	H. marginatum	4	171	0	0.00	2.04	< 0.0001	--			
	Hyalomma sp.	2	81	0	0.00 [0-2.36]	0.01	< 0.0001	0.0			
								0.0			
	Ha. longicornis	1	308	0	0.00 [0-0.58]	0.00	< 0.0001				

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	Ha. parva	1	41	0	0.00 [0-4.15]	0.00	< 0.0001	--		
	Ha. punctata	1	6	0	0.00 [0-26.80]	0.00	< 0.0001	--		
	Ha. sulcata	2	4	0	0.00 [0-78.74]	0.07	< 0.0001	--		
	Haemaphysalis. sp.	1	13	0	0.00 [0-12.82]	0.00	< 0.0001	0.0		
								--		
	I. ricinus	8	6580	32	1.36 [0-10.39]	64.66	< 0.0001			
	I. ventalloi	1	62	3	6.45 [1.42-14.19]	0.00	< 0.0001	87.6		
	Ixodes sp.	1	5	0	0.00 [0-31.73]	0.00	< 0.0001	--		
								--		
	R. (Boophilus)	6	1025	62	0.00 [0-3.92]	0.51	< 0.0001			
	R. bursa	3	23	0	13.54 [0-47.12]	185.13	< 0.0001	0.0		
	R. pusillus	2	18	3	17.65 [2.57-39.97]	0.02	< 0.0001	22.2		
	R. sanguineus	21	2222	583	25.15 [11.67-41.19]	1092.57	< 0.0001	0.0		
	R. turanicus	4	158	10	2.48 [0-1467]	23.70	< 0.0001	97.9		
	Rhipicephalus sp.	1	43	3	6.98 [0.9-16.93]	0.00	< 0.0001	87.3		
								--		
Detection method									0.000***	5.37	98.84
	Microscopy	3	6533	20	0.1 [0.0-0.23]	3.47	< 0.0001			
	Molecular	39	5181	743	17.55 [9.31-27.44]	1937.17	< 0.0001	42.4		
								98.1		
Parasite genus									0.0046**	7.10	98.88
	Leishmania	30	4569	719	18.87 [9.12-30.75]	1642.76	< 0.0001			
	Trypanosoma	12	7739	93	4.62 [1.14-9.79]	203.03	< 0.0001	98.2		
								94.6		
Parasite species									0.000***	9.25	98.97
	L. braziliensis	1	75	32	42.67 [31.64-64.66]	0.00	< 0.0001			
	L. chagas (syn L. infantum)	1	444	107	24.10 [20.23-28.19]	0.00	< 0.0001	--		
	L. guyanensis	1	81	3	12.12 [2.79-25.84]	0.00	< 0.0001	--		
	L. infantum	18	2124	323	15.44 [7.18-25.89]	679.35	< 0.0001	--		
	L. major	1	1	1	100 [0-100]	0.08	< 0.0001	97.5		
	L. martiniquensis	1	578	2	0.35 [0-1.04]	0.00	< 0.0001	--		
	Leishmania sp.	6	683	194	51.82 [8.42-93.57]	0.00	< 0.0001	--		
								94.4		
	T. caninum	1	9	1	11.11 [0-41.77]	0.00	< 0.0001			
	T. congolense	1	33	4	3.70 [0.47-9.18]	0.00	< 0.0001	--		
	T. cruzi	3	583	8	2.39 [0-10.25]	895.52	< 0.0001	--		
	T. evansi	4	892	38	6.44 [0-23.33]	13.00	< 0.0001	91.2		
	T. theileri	2	6220	14	0.20 [0.12-0.36]	73.71	< 0.0001	95.9		
	T. vivax	3	441	42	10.88 [2.91-22.73]	22.82	< 0.0001	0.0		
								84.6		
Localization in ticks									0.000***	0.00	98.89
	H	2	6470	19	0.29 [0.16-0.45]	1.14	< 0.0001			
	G	3	209	164	74.32 [28.48-99.99]	312.09	< 0.0001	12.0		
	GR	35	4532	478	13.45 [6.62-21.87]	1234.08	< 0.0001	97.3		
	GR-SG	1	95	11	11.58 [5.82-18.88]	0.00	< 0.0001	97.2		
	H-GR	1	444	107	24.10 [20.23-28.19]	0.00	< 0.0001	--		
	SG	2	110	35	31.79 [23.31-40.91]	0.00	< 0.0001	--		
	O	2	165	74	44.48 [37.27-52.53]	0.67	< 0.0001	0.0		
								0.0		

G: gut**; Gr:** whole body**; H:** haemolymph**; O:** ovaries**; SG:** salivary glands *0.05; **0.01; ***0.001, # In these studies, various tick species were allowed to feed on diverse infected host (Swiss mice, Meadow voles, Rats, Mice, Rabbits, Camel, Dog, Guinea pig or White rat). In these papers, it was not possible to retrieve the information on the host origin of the ticks that were tested. .

4.1.2. Detection Method

Molecular biology yielded the highest detection rates, reaching 17.55% (with a confidence interval of 9.31–27.44%), surpassing the rates detected by microscopic methods, which stand at a mere 0.1% (confidence interval: 0–0.23%). Despite the specificity of microscopy in identifying Trypanosomatidae [16,143] they demand skilled personnel for accurate parasite detection. The efficacy of these methods varies with the type of sample and generally falls short of the sensitivity and specificity offered by PCR and cell culture methods [144]. Due to their superior sensitivity and specificity, molecular techniques are regarded as more effective [16,145].

4.1.3. Temporal Analysis

There were statistically significant differences across the collection decade (**Table 3**). The highest annual prevalence recorded was 14.92% (95% CI: 7.7-23.77%, 518/3043) between 2011 and 2019, and the lowest at 0.29% (95% CI: 0.16-0.45%, 19/6470) was reported before the 2000s. This increasing

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prevalence trend since 2000 is likely due to advances in the performance of molecular detection methods [74,125] and/or the impacts of climate change on vector populations and behaviors [146,147].

4.1.4. Ticks Geographical Origin

We also assessed differences according to geographical origin (continent) (**Table 3**). The highest prevalence, 25.17% (95% CI: 14.27-37.83%, 626/1986) was recorded in South America, while the lowest was in Africa (p = 0.0056). The cumulative prevalence statistics by host animal on which ticks were collected, displayed a significant difference (p=0.0012) between animal hosts, with the highest cumulative prevalence being on Canidae (27.92% [14.51-43.57%], 595/2184).

4.1.5. Tick's Genus

The prevalence of tick infection significantly varies according to the tick's taxonomic status (p=0.0001), from 19.06% (CI: 9.64-30.39%, 668/3271) in *Rhipicephalu*s to 8.78% (95% CI: 0-29.41%, 27/328) in *Amblyomma* or 1.91% and 1.20% in *Ixodes* and *Hyalomma*, respectively, to 0.0% in *Dermacentor* and *Haemaphysalis* (**Table 3**).

4.1.6. Detection Method, Genus and Organ Location

We found statistical differences according to the detection method (p>0.05). The prevalence was higher at 17.55% (95% CI: 9.31, 27.44) with molecular methods and lowest at 0.1% (95% CI: 0-0.23) using microscopy. Moreover, the infection rate was significantly higher (p= 0.0046) at 18.87% [9.12-30.75] for *Leishmania* than for the *Trypanosoma* genus at 4.62% [1.14-9.79]. Infection rate also varied significantly (p=0.000) by organ in ticks: the highest rate is 74.32% (95% CI: 28.24-99.99, 164/209) in the digestive tract, followed by 44.48% [37.27-52.53] in the ovaries, 31.79% [23.31-40.91%] in the salivary glands, and the lowest rate of infection is 0.29% [95% CI: 0.16-0.45%] in the hemolymph **(Table 3**).

4.2. Experimental Studies

For the experimental studies, 37 publications were selected. Data were extracted from these research papers. Quantitative data such, as the number of ticks used for experimental infections, were not considered in the meta-analysis. They were transformed into semi-quantitative ones (presence or absence of parasites following experimental infection or transmission). Since no heterogeneity was detected in the data set, the common-effect model was chosen for the meta-analysis.

4.2.1. Ingestion after Blood Feeding on an Infected Host

The meta-analysis was performed on 36 studies. The analysis showed that the presence of parasites in ticks after their blood meal was reported in 91% [72-100%] of the studies (**Supplementary File, Figure S1**). The factors associated with the detection of *Leishmania* and *Trypanosoma* in the blood meal were analysed taking into account each actor involved in the transmission cycle, tick, animal host and parasite detected (**Table 4**). No heterogeneity was detected in the subgroups. Rate estimates pooled for each subgroup were calculated using a common-effects model.

- *Ticks Family and Genus*

For tick families, positivity rates were 100% (95% CI: 81.65-100) for Argasidae and 78.01 (95% CI: 48.9-98.84%) for Ixodidae, with no statistical difference according to tick family (p > 0.05). Slight variations were recorded for the genus, with no statistically significant difference. For the genera *Ornithodoros* and *Amblyomma*, all publications reported positive detection after the blood meal; for the genera *Rhipicephalus* and *Hyalomma*, detection rates were 74.57% and 82.18%, respectively. A large variation in prevalence is recorded at the species level, mainly related to the low number of studies that were published (**Table 4**).

- *Parasite genus*

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For parasite genera, the highest detection rate is 99.11% (95% CI: 80.73-100) for *Trypanosoma*, whereas *Leishmania* is detected in 69.10% (95% CI: 62.64-97.17) of the studies. However, this difference is not statistically significant (P > 0.05) (**Table 4**).

- *Host family*

  No statistical differences were recorded (**Table 4**).

Table 4. Pooled rate of parasite detections in ticks in experimental transmission experiments.

Acquisition of pathogens via tick blood feeding										
Category	**Variable**	**No. of studies**	**No. of positive**	**% [95% CI]**	**Heterogeneity**			**Univariate meta regression**		
					χ2	***P*-value**	***I*² (%)**	***P*-value**	***R*² (%)**	***I*²-res (%)**
Ticks Family								0.0976	0.00	0.00
	Argasidae	16	16	100 [81.65-100]	0.00	0.9966	0.0			
	Ixodidae	21	15	78.01 [48.91-98.34]	15.86	0.9966	0.0			
Ticks Genus								0.3905	0.00	0.00
	Amblyoma	1	1	100 [0-100]	0.00	0.9938	--			
	Hyalomma	4	3	82.18 [16.67-100]	2.78	0.9938	0.0			
	Ornithodoros	15	15	100 [80.68-100]	0.00	0.9938	0.0			
	Rhipicephalus	16	11	74.57 [40.89-98.60]	12.72	0.9938	0.0			
Ticks species								0.6118	0.00	0.00
	A. americanum	1	1	100 [0-100]	0.00	0.9989	--			
	B. decoloratus	1	1	100 [0-100]	0.00	0.9989	--			
	D. andersoni	2	2	100 [21.62-100]	0.00	0.9989	0.0			
	H. A. anatolicum	3	2	72.14 [2.45-100]	2.47	0.9989	18.9			
	H. A. excavatum	1	1	100 [0-100]	0.00	0.9989	--			
	H. dromaderii	1	1	100 [0-100]	0.00	0.9989	--			
	H. impressum	1	1	100 [0-100]	0.00	0.9989	--			
	O. amblus	1	1	100 [0-100]	0.00	0.9989	--			
	O. crossi	4	4	100 [48.72-100]	0.00	0.9989	0.0			
	O. furcosis	1	1	100 [0-100]	0.00	0.9989	--			
	O. hermsi	2	0	0 [0-78.74]	0.00	0.9989	0.0			
	O. lahorensis	2	2	100 [21.26-100]	0.00	0.9989	0.0			
	O. moubata	7	7	100 [65.46-100]	0.00	0.9989	0.0			
	O. parkeri	1	1	100 [0-100]	0.00	0.9989	--			
	O. savignyi	1	1	100 [0-100]	0.00	0.9989	--			
	O. talaje	1	0	0 [0-100]	0.00	0.9989	--			
	O. turanicus	2	2	100 [21.62-100]	0.00	0.9989	0.0			

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	O. venzualensis	1	1	100 [0-100]	0.00	0.9989	--			
	R. sanguneus	13	11	91.99 [58.62-100]	6.26	0.9989	0.0			
	R. sanguneus s.I.	2	0	0 [0-78.74]	0.00	0.9989	0.0			
	R. pulchellus	1	1	100 [0-100]	0.00	0.9989	--			
Parasite genus								0.0794	0.00	0.00
	Leishmania	14	9	69.10 [62.64-97.17]	11.90	0.9974	0.0			
	Trypanosoma	22	21	99.11 [80.73-100]	3.53	0.9974	0.0			
Parasite Species								0.7192	0.00	0.00
	L. donovani	1	1	100 [0-100]	0.00	0.9928	--			
	L. chagasi (syn L.infantum)	2	2	100 [21.26-100]	0.00	0.9928	0.0			
	L. infantum	5	4	87.58 [29.26-100]	2.96	0.9928	0.0			
	L. kala azar[£]	4	1	17.82 [0-83.24]	2.78	0.9928	0.0			
	L. major	1	1	100 [0-100]	0.00	0.9928	--			
	Leishmania sp.	1	1	100 [0-100]	0.00	0.9928	--			
	T. bruci	1	1	100 [0-100]	0.00	0.9928	--			
	T. cruzi	8	8	100 [68.71-100]	0.00	0.9928	0.0			
	T. evansi	7	6	92.94 [46.20-100]	3.17	0.9928	0.0			
	T. gambiense	1	1	100 [0-100]	0.00	0.9928	--			
	T. lewisi	2	2	100 [21.26-100]	0.00	0.9928	0.0			
	T. rhodesciense	2	2	100 [21.26-100]	0.00	0.9928	0.0			
	T. theileri	4	3	82.18 [16.67-100]	2.78	0.9928	0.0			
	T. theileri like	1	1	100 [0-100]	0.00	0.9928	--			
Donor host family								0.9455	0.00	0.00
	Bovidae	4	3	82.18 [16.67-100]	2.78	0.9471	0.0			
	Canidae	13	9	75.34 [37.81-99.71]	10.25	0.9471	0.0			
	Camelidae	2	2	100 [21.26-100]	0.00	0.9471	0.0			
	Rodentia	15	14	93.73 [63.97-100]	42.76	0.9471	0.0			
	ND	2	2	100 [21.26-100]	0.00	0.9471	0.0			
	Other	1	1	100 [0-100]	0.00	0.9471	--			
	Ticks	1	1	100 [0-100]	0.00	0.9471	--			
Infection via the injection of ticks infected material										
Ticks Family								0.2884	0.00	0.00
	Argasidae	9	7	85.25 [41.62-100]	5.76	0.6062	0.0			
	Ixodidae	13	7	55.23 [18.49-89.66]	11.96	0.6062	0.0			
Ticks Genus								0.5954	0.00	0.00
	Hyalomma	4	2	50.0 [0-100]	3.70	0.5378	18.9			
	Ornithodoros	9	8	85.25 [41.62-100]	5.76	0.5378	0.0			

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	Rhipicephalus	7	4	59.68 [11.06-99.18]	6.34	0.5378	5.4			
Ticks species								0.2229	0.00	0.00
	B. decoloratus	1	0	0 [0-100]	0.00	0.8803	--			
	D. andersoni	2	0	0 [0-78.74]	0.00	0.8803	0.0			
	H. a. anatolicum	3	2	72.14 [2.45-100	2.47	0.8803	18.9			
	H. a. excavatum	1	0	0 [0-100]	0.00	0.8803	--			
	H. dromaderii	1	0	0 [0-100]	0.00	0.8803	--			
	H. impressum	1	0	0 [0-100]	0.00	0.8803	--			
	O. amblus	1	1	100 [0-100]	0.00	0.8803	--			
	O. crossi	1	0	0 [0-100]	0.00	0.8803	--			
	O. furcosus	1	1	100 [0-100]	0.00	0.8803	--			
	O. hermsi	2	0	0 [0-78.74]	0.00	0.8803	0.0			
	O. moubata	4	4	100 [48.72-100]	0.00	0.8803	0.0			
	O. perkeri	1	1	100 [0-100]	0.00	0.8803	--			
	O. talaje	1	0	0 [0-100]	0.00	0.8803	--			
	O. turanicus	1	0	0 [0-100]	0.00	0.8803	--			
	O. turicata	2	1	50.0 [0-100]	1.85	0.8803	46.0			
	O. venzualensis	1	1	100 [0-100]	0.00	0.8803	--			
	R. pulchellus	1	0	0 [0-100]	0.00	0.8803	--			
	R. sanguineus	6	5	90.82 [38.83-100]	3.08	0.8803	0.0			
	R. sanguineus s.l.	1	0	0 [0-100]	0.00	0.8803	--			
Parasite genus								0.7666	0.00	0.00
	Leishmania	7	5	78.10 [27.34-100]	5.29	0.5774	0.0			
	Trypanosoma	14	9	69.10 [32.64-97.17]	11.9	0.5774	0.0			
Donor host Family								0.7557	0.00	0.00
	Bovidae	4	2	50.0 [0-100]	3.70	0.5180	18.9			
	Camelidae	1	1	100 [0-100]	0.00	0.5180	--			
	Canidae	5	4	87.58 [29.26-100]	2.96	0.5180	0.0			
	Rodentia	11	8	68.26 [27.33-98.66]	9.42	0.5180	0.0			
Receiver host Family								0.5494	0.00	0.00
	Bovidae	3	2	72.14 [2.45-100]	2.47	0.5387	18.9			
	Canidae	1	1	100 [0-100]	0.00	0.5387	--			
	Other	1	0	0 [0-100]	0.00	0.5387	--			
	Rodentia	15	11	63.49 [28.31-93.43]	13.32	0.5387	0.0			
	Ticks	2	2	100 [21.26-100]	0.00	0.5387	0.0			

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Parasite species	*L. chagasi (Syn L. infantum)*	1	1	100 [0-100]	0.00	0.8397	--	0.2614	0.00	0.00
	L. infantum	1	1	100 [0-100]	0.00	0.8397	--			
	L. kala azar[£]	4	2	50.0 [0-100]	3.70	0.8397	18.9			
	Leishmania sp.	1	1	100 [0-100]	0.00	0.8397	--			
	T. brucei	1	0	0 [0-100]	0.00	0.8397	--			
	T. cruzi	6	6	100 [61.37-100]	0.00	0.8397	0.0			
	T. evansi	1	0	0 [0-100]	0.00	0.8397	--			
	T. lewisi	1	0	0 [0-100]	0.00	0.8397	--			
	T. rhodeseinse	1	0	0 [0-100]	0.00	0.8397	--			
	T. theileri	4	3	82.18 [16.76-100]	2.78	0.8397	0.0			
	T. theileri like	1	0	0 [0-100]	0.00	0.8397	--			
Transmission through blood feeding										
Ticks Family	Argasidae	10	3	23.71 [0-66.41]	7.77	0.5314	0.9	0.4962	0.00	0.00
	Ixodidae	11	5	34.33 [0.0-94]	10.09	0.5314	0.0			
Ticks Genus	*Hyalomma*	4	2	50.0 [0-100]	3.70	0.4677	18.9	0.7745	0.00	0.00
	Ornithodoros	10	3	23.71 [0-66.41]	7.77	0.4677	0.0			
	Rhipicephalus	7	3	40.32 [0.48-88.94]	6.32	0.4677	5.4			
Parasite genus	*Leishmania*	5	2	36.51 [0-92.81]	4.44	0.9952	9.9	0.5011	0.00	0.00
	Trypanosoma	16	8	33.22 [5.25-67.40]	13.88	0.9952	0.0			
Donnor host family	Bovidae	4	2	50.0 [0-100]	3.70	0.9017	18.9	**0.0405***	0.00	0.00
	Camelidae	2	2	100 [21.26-100]	0.00	0.9017	0.0			
	Canidae	7	4	59.68 [11.06-99.18]	6.34	0.9017	5.4			
	Rodentia	8	0	0 [0-31.29]	0.00	0.9017	0.0			
Receiver host family	Bovidae	3	2	72.14 [2.45-100]	2.47	0.8461	18.9	0.0678	0.00	0.00
	Canidae	3	2	72.14 [2.45-100]	2.47	0.8461	18.9			
	Other	2	2	100 [21.26-100]	0.00	0.8461	0.0			
	Rodentia	13	4	8.01 [0-41.38]	6.26	0.8461	0.0			
Ticks species	*B. decoloratus*	1	0	0 [0-100]	0.00	0.6280	--	0.6077	0.00	0.00
	D. andersoni	1	0	0 [0-100]	0.00	0.6280	--			
	H. a. anatolicum	3	2	72.14 [2.45-100]	2.47	0.6280	18.9			

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	H. a. excavatum	1	0	0 [0-100]	0.00	0.6280	--			
	H. dromaderii	1	0	0 [0-100]	0.00	0.6280	--			
	H. impressum	1	0	0 [0-100]	0.00	0.6280	--			
	O. crossi	4	3	82.18 [16.67-100]	2.78	0.6280	0.0			
	O. hermsi	1	0	0 [0-100]	0.00	0.6280	--			
	O. lahorensis	2	2	100 [21.26-100]	0.00	0.6280	0.0			
	O. moubata	3	0	0 [0-61.92]	0.00	0.6280	0.0			
	O. savigny	1	0	0 [0-100]	0.00	0.6280	--			
	O. talaje	1	0	0 [0-100]	0.00	0.6280	--			
	O. turanicus	1	0	0 [0-100]	0.00	0.6280	--			
	O. turicata	1	0	0 [0-100]	0.00	0.6280	--			
	O. venzualensis	1	0	0 [0-100]	0.00	0.6280	--			
	R. pulchellus	1	0	0 [0-100]	0.00	0.6280	--			
	R. sanguineus	6	3	50.0 [2.78-97.22]	5.55	0.6280	9.9			
Parasite species								0.5529	0.00	0.00
	L. infantum	2	1	50.0 [0-100]	1.85	0.5302	46.0			
	L. kala azar[£]	2	0	0 [0-78.74]	0.00	0.5302	0.0			
	Leishmania sp.	1	1	100 [0-100]	0.00	0.5302	--			
	T. cruzi	6	1	9.18 [0-61.17]	3.08	0.5302	0.0			
	T. evansi	6	3	50.0 [2.78-97.22]	5.55	0.5302	9.9			
	T. lewisi	1	0	0 [0-100]	0.00	0.5302	--			
	T. theileri	3	1	27.86 [0-97.55]	2.47	0.5302	18.9			
	T. theileri like	1	1	100 [0-100]	0.00	0.5302	--			

Vertical transmission

Ticks Family								0.2809	0.00	0.00
	Argasidae	1	0	0 [0-100]	0.00	0.4606	--			
	Ixodidae	12	7	61.27 [22.41-94.58]	10;79	0.4606	0.0			
Ticks Genus								0.5422	0.00	0.00
	Hyalomma	2	1	50.0 [0-100]	1.85	0.3787	46.0			
	Ornithodoros	1	0	0 [0-100]	0.00	0.3787	--			
	Rhipicephalus	10	6	63.49 [21.05-97.58]	8.88	0.3787	0.0			
Ticks species								0.4394	0.00	0.00
	H. a. anatolicum	2	1	50.0 [0-100]	1.85	0.4143	46.0			
	O. moubata	1	0	0 [0-100]	0.00	0.4143	--			

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	R. pulchellus	1	0	0 [0-100]	0.00	0.4143	--			
	R. sanguineus	9	7	72.14 [27.07-100]	7.40	0.4143	0.0			
Parasite genus								0.0634	0.00	0.00
	Leishmania	8	7	82.18 [35.16-100]	5.55	0.6668	0.0			
	Trypanosoma	5	1	12.42 [0-70.74]	2.96	0.6668	0.0			
Donor host family								**0.0425***	0.00	0.00
	Bovidae	3	1	27.86 [0-97.55]	2.47	0.8446	18.9			
	Canidae	7	6	92.94 [46.20-100]	3.17	0.8446	0.0			
	Rodentia	3	0	0 [0-61.92]	0.00	0.8446	0.0			
Receiver host family								0.3582	0.00	0.00
	Bovidae	2	1	50.0 [0-100]	1.85	0.4748	46.0			
	Canidae	1	1	100 [0-100]	0.00	0.4748	--			
	ND	4	3	82.18 [16.67-100]	2.78	0.4748	0.0			
	Rodentia	5	1	12.42 [0-70.74]	2.69	0.4748	0.0			
	Ticks	1	1	100 [0-100]	0.00	0.4748	--			
Parasite species								0.3655	0.00	0.00
	L. chagasi (Syn L. infantum)	2	1	50.0 [0-100]	1.85	0.5046	46.0			
	L. infantum	3	3	100 [38.08-100]	0.00	0.5046	0.0			
	L. major	1	0	0 [0-100]	0.00	0.5046	--			
	L. kala azar[£]	1	1	100 [0-100]	0.00	0.5046	--			
	Leishmania sp.	1	1	100 [0-100]	0.00	0.5046	--			
	T. cruzi	1	0	0 [0-100]	0.00	0.5046	--			
	T. evansi	1	0	0 [0-100]	0.00	0.5046	--			
	T.theileri	3	1	27.86 [0-97.55]	2.47	0.5046	18.9			

*0.05; **0.01; ***0.001 [£] L. kala azar refers to members of the *L. donovani* complex (*L. infantum* and *L. donovani*) without any other information on parasite typing at the time of the study. We therefore use the term proposed by the author. # ticks (receiver host) were infected via the inoculation of *A. americanum*-infected hemolymph (donor host).

4.2.2. Transmission by injection of tick-infected material

The meta-analysis includes 22 scientific papers. The common-effects model was used to analyze associated factors. About 72% (95% CI: 42-95%) of articles show positive transmission results. In 1984, McKenzie studied the infection of two hunting dogs, crossbred between a German shepherd and a Labrador retriever, by injecting them with material from crushed, infected *R. sanguineus* ticks by injection of ticks infected material (**Supplementary File Figure S2**).

- Tick's family, genus and species

The infection rate elicited via the injection of Argasidae-infected material is statistically higher than that of Ixodidae-infected ones (rate of 85.25% vs. 55.23%). The highest rate, 85.25%, was recorded for *Ornithodoros*, followed by *Rhipicephalus* and *Hyalomma*. However, if all these observations are not statistically significant, they interestingly point to some specificity according to the tick genus (**Table 4**).

- Parasite's genus and species

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No significant difference was recorded within parasite genera and species subgroups ($p > 0.05$). Although we observed a generally higher infection rate with *T. cruzi*, *T. theileri*, *Leishmania*. sp., *L. chagasi* (Syn *L. infantum*), and *L. infantum* infected tick material.

- Host family

These analyses disclosed that tick material collected from infected Camelidae was more likely to initiate infection when injected into a noninfected recipient and that Canidae appears to be more susceptible to infection when injected with infected tick material.

4.2.3. Transmission Through Tick's Blood Feeding on a Non-Infected Host

Tick-borne pathogen transmission can occur via mechanical or biological means. In mechanical transmission, ticks act as carriers, transferring pathogens between hosts without mandatory pathogen development within the tick. In contrast, biological transmission involves the pathogen undergoing necessary biological changes or replication within the tick, completing part of its life cycle before infecting the next host. We performed a meta-analysis on data extracted from 21 publications dealing with experimental transmission by ticks via blood feeding (**Table 4**). This analysis showed that 34% (95% CI: 8-64%) of the studies confirmed transmission by ticks of medical and veterinary interest Trypanosomatidae, for which data can be gathered during our study (**Supplementary Figure S3**). The common-effects model was used to analyze factors associated with experimental transmission.

- Ticks family

The Ixodidae appeared to be more able to transmit Trypanosomatidae than Argasidae, with rates of 34.33% (95% CI: 0-94%) and 23.7% (95% CI: 0-66.4%), respectively (**Table 4**). Regarding the genus, minor variations were recorded without significant differences ($p > 0.05$). Regarding tick species, *R. sanguineus*, *O. lahorensis*, *O. crossi*, and *H. a. anatolicum* seemed more able to transmit Trypanosomatidae parasites of medical and veterinary interest. However, the sample size is too small to get insight into the statistical significance of these observations.

- Parasite genus

Although the success rate of transmission attempts is higher with *Leishmania* (82.18% CI:35.16-100%) than with *Trypanosoma* (12.42% CI: 0-70.74%), the meta-analysis does not record a statistically significant difference between *Leishmania* and *Trypanosoma* ($p>0.05$) (**Table 4**).

- Donor and receiver host family

Surprisingly, the donor host appears to be a factor influencing the subsequent transmission of the pathogen during tick blood feeding ($p<0.05$) **Table 4**. The infection rate for ticks varied from 100% to 95% (CI: 21.26-100%) in Camelidae, to 59.68% for Canidae and 50.0% for Bovidae. The eight studies that focused on ticks collected on infected rodents reported no transmission. The host recipient also showed heterogeneity, with Canidae and Bovidae being more frequently infected at a rate of 72.14% [2.45-100%] than rodents (8.01%, [0-41.38%], when infected and allowed to be fed.

4.2.4. Vertical Transmission

Our analysis includes only 13 studies focusing on the vertical transmission of Trypanosomatidae by ticks. The findings revealed that 55% presented evidence supporting the vertical transmission of parasites within tick populations, as detailed in the supplementary file **(Supplementary Figure S4)**. The factors associated with this transmission were evaluated using the common-effect model. Despite the small sample size, vertical transmission is recorded at 61.27% [22.41-94.58] for ticks of the Ixodidae family and 0% for the Argasidae, although only one study deals with this issue. No significant difference exists according to genus and species (**Table 4**). Concerning genus and parasite species, it seems that parasites belonging to the *Leishmania* genus are better adapted to vertical transmission (82.18 [35.16-100]) than those of the *Trypanosoma* genus (12.42 [0-70.74]). Parasites from the *L. donovani* complex (*L. donovani* or *L. infantum*) appear to be adapted to vertical transmission. Also, the host family from which ticks feed plays a significant role in variability ($p=0.0425$). Ticks

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feeding on dogs (Canidae) have the highest vertical transmission rate at 92.94% [46.20-100%], followed by those feeding on Bovida at 27.86% [0-97.55], and lastly, rodents with no vertical transmission of Trypanosomatidae in ticks following blood meal on rodent infected host.

5. Discussion

Biologists have been particularly interested in insects, vectors of Trypanosomatidae, because of the significant infectious impact of these parasite protists on animal species, including humans [147]. While almost all Trypanosomatidae are transmitted by insects, a single publication reported the case of an avian trypanosome vectorized by a non-traditional vector, an arachnid belonging to the subclass of acarians [148]. This anomaly prompted further investigations into the presence of Trypanosomatids within ticks, as evidenced by the early work of Novy et al. (1907), who identified Trypanosomes in ticks [149]. This has led to an increased focus on understanding the potential role of ticks in harboring and transmitting these pathogenic protozoans.

To determine the prevalence of Trypanosomatidae of medical and veterinary interest in wild-caught ticks from endemic areas, both molecular (PCR, qPCR, PCR-HRM) and parasitological/immunological (microscopic examination, IHC) methods were used. Molecular methods that amplify genomic DNA, which can last after parasite death, are more sensitive than parasitological/immunological methods which target living parasites or their proteic determinants that are rapidly degrade upon parasite death. Statistical differences in detection rates were observed according to the method employed. Specifically, the highest prevalence (17.55%) was recorded with molecular methods compared to microscopic detection of parasites (0.1%). The discovery of DNA or parasites in the digestive system of blood-fed ticks collected from hosts has limited predictive value in inferring a vectorial role. The vast majority of publications included in our systematic review and meta-analysis dealt with ticks collected directly from hosts, focusing on detecting pathogens from host blood deposited in tick bodies. DNA detection alone is insufficient, especially in the case of ticks, due to their digestive capacity and other metabolic peculiarities. To further analyze the prevalence of Trypanosomatidae in field-collected tick samples, it will be necessary to find the parasites themselves in these ticks and/or detect parasite-specific mRNA, which is a better indicator of parasite survival in the digestive tract and other tick organs.

Data on tick infection disclose that the detection of Trypanosomatidae of medical or veterinary interest in ticks varies between 0.01-1.26% to 14.27-37.83% depending on countries or continents (**Table 3**). In many studies, pools are used to ascertain the prevalence, which greatly overestimates the results. These provide the first clues on the high contact frequency between infected hosts and ticks, a crucial element to engage transmission by arthropod vectors. Overall, in many places where infections with Trypanosomatidae are common, there's no available information on the infection rate by these pathogenic agents in their proven vectors and/or in ticks. For example, the Mediterranean area has a high rate of visceral Leishmaniasis [150], but no information on the carriage of *Leishmania* by ticks in countries like Egypt, Libya, and Morocco is available. Latin America and the Caribbean are affected by Chagas disease, caused by *T. cruzi* [151], but no data on its presence in ticks is available, which makes it difficult to draw a global analysis of the seasonal activity patterns of ticks in relation to the incidence of Trypanosomatidae pathogen infections in hosts but also on the geographical overlap between tick populations and human or animal infection by Trypanosomatidae pathogens.

Our systematic review and meta-analysis disclose that if 996 species (774 hard ticks and 221 soft ticks) are described worldwide [3,4], with 25 species acting as vectors of major diseases, the presence of Trypanosomatidae pathogens having medical or veterinary interests was investigated on twenty tick species. In South America, 137 species of hard ticks, from 5 genera and 87 species of soft ticks are reported [152,153]**.** All studies we collected focused on the Ixodidae family, with no field data available on the soft ticks of the Argasidae family. The detection rate in members of the *Rhipicephalus* genus is the highest at 17.49%, followed by *Amblyomma*, *Hyalomma*, and *Ixodes* with infection rates of 11.47%, 2.68%, and 1.87%, respectively. Ticks belonging to the *Dermacentor* and *Haemaphysali* genera were negative. Variability in field studies concerning the detection of Trypanosomatidae in ticks can

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often be attributed to a host effect. Specifically, ticks harvested from Canidae, which are alost exclusively non questing ones and belong to the *Rhipicephalus* genus, exhibit a higher pathogens detection rate compared to ticks collected from other mammals (P=0.0007). This discrepancy may be due in part to the close relationship between dogs and humans and their heightened susceptibility to *Trypanosoma cruzi*, which may increase the risk of tick infection [154]. Additionally, dogs serve as a significant reservoir for several *Leishmania* species, such as *L. infantum*, *L. peruviana*, and *L. donovani* [155]. The extent of the geographical distribution of the ticks belonging to the genus *Rhipicephalus*, the broad range of their animal hosts, their known vector competence, and their diverse morphology provide arguments for this meta-analysis on their potential role in the transmission of Trypanosomatidae [156]. *R. sanguineus* is a typical representative of this genus in so far as the close association of this tick species with domestic dogs, which constitute a known reservoir for *Leishmania*, signs its likely involvement in the persistence and transmission of these parasites in natural habitats [157].

The concept of vector competence is defined as the innate ability of an arthropod to harbor and transmit microbial agents [158,159]. Laboratory studies were conducted to establish whether ticks can acquire and maintain the infection after feeding on infected hosts. Therefore, establishing the vector capacity of a tick consists of confirming its ability to become infected during a blood meal on a host, facilitating the multiplication of the pathogen before its subsequent transmission through saliva, and maintaining the pathogen throughout the tick development stages for potential vertical transmission [160]. Regarding parasite acquisition, the meta-analysis reveals that in experimental settings, tick infection after blood feeding is efficient, with 31 successes out of 37 attempts (Table 4). Although no significant factors were associated with parasite acquisition from an infected host (donor host), the analysis does indicate some tendencies that warrant discussion. Firstly, Argasidae ticks are likely to become infected compared to Ixodidae ticks when feeding on an infected host (P=0.0976). The host's nature does not appear to affect tick infection rates. However, the type of parasite does influence tick infection rates, with a higher rate of infection observed when ticks feed on a *Trypanosoma*-infected host compared to a *Leishmania*-infected host; tick infection is more efficient when they take a blood meal from a host infected with *Trypanosoma* (P=0.0794) **Table 4**. *Trypanosoma cruzi* and *T. evansi* are particularly likely to infect ticks following blood feeding. These observations may be related to the intrinsic ability of Trypanosomes to multiply in the blood of the infected host, making them more available to ticks during the blood-feeding process. Additionally, such disparity in infection rates might be attributed to the feeding behaviors differing for the two tick families: Ixodidae ticks attach to the hosts' skin and suck blood slowly over several days, whereas Argasidae ticks rapidly ingest large volumes of blood in a short time (20–70 minutes), facilitating a more efficient uptake of infectious agents, especially under experimental conditions [161]. The rapid feeding behavior of Argasidae ticks makes them more efficient at acquiring blood-circulating parasites like *T. brucei* or *T. evansi*, compared to the long-feeding Ixodidae ticks, which may be less effective at acquiring tissue-located parasites. Finally, the prolonged feeding process of Ixodidae ticks, which lasts up to two weeks, subjects engorged pathogens to the full spectrum of host defense mechanisms, including specific acquired immunity that may impact parasites of the Trypanosomatidae family's survival [162]. Information gathered on the efficiency of infection through the injection of contaminated tick material provides insight into the presence of infectious parasitic stages in infected ticks. Our meta-analysis did not identify any significant factors related to the donor or receiver host, ticks, or parasite identity that play a role in the transmission of Trypanosomatidae by ticks. While Argasidae ticks appear to be more efficient in such transmission than Ixodidae ticks (7 successes out of 9 attempts compared to 7 successes out of 13 attempts), these differences are not statistically significant (P=0.2884). Because ticks feed only once at each life stage, vertical transmission of pathogens is an important factor to consider when addressing the vectorial status of ticks for Trypanosomatidae parasites. Our literature survey indicates that vertical transmission is detected in experimental settings and only in Ixodidae ticks. Although not statistically significant, *Leishmania* appears to be more likely to be vertically transmitted in *R. sanguineus* than *Trypanosoma* species (P=0.0634). Interestingly, protozoan of the *Babesia* genus can undergo transovarial transmission in

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ticks, which suggest potential similar behavior for protozoan of the trypanosomatidae family [163]. However, the limited number of studies on vertical transmission prevents further discussion on the influence of tick identity or parasite species on vertical transmission.

Trypanosoma evansi and *T. vivax* are mechanically transmitted pathogens, meaning they are transmitted from host to host without biological replication within their vector. This transmission mode does not align well with the feeding habits of ticks, except in cases of interrupted feeding [164]. There is no available data on *T. vivax*. However, a relatively good success rate (3 successes out of 6 attempts) was recorded for transmitting *T. evansi* via tick blood feeding. Therefore, the transmission of *T. evansi* or *T. vivax* after interrupted blood feeding should be more thoroughly investigated.

Salivarian Trypanosomes, such as *T. brucei*, *T. cruzi*, *T. evansi*, *T. congolense*, *T. vivax*, and *Leishmania* parasites, are transmitted through blood-feeding by the injection or regurgitation of saliva during the feeding process. Stercocarian trypanosomes such as *T. cruzi* and *T. rangeli* infect their host are deposited on the host's skin during blood-feeding and subsequently infect the host through the scratching of the infected blood onto the host's mucosa. Infection by ingestion is also documented for *T. cruzi* [165]. Considering this aspect and examining the results of the meta-analysis, we noticed that attempts to transmit Trypanosomatidae pathogens by blood-feeding of infected ticks are more successful with Salivarian Trypanosomatidae pathogens, such as *T. evansi*, T. lewisi, and *T. theileiri*, than with Stercorarian Trypanosomatidae pathogens. Specifically, there were five attempts for two successes for *Leishmania*, 11 attempts for five successes for Salivarian Trypanosomes, and 6 attempts for one success for the Stercocarian *T. cruzi* Trypanosome. Data collected indicate a general trend of better infection rates for Salivarian Trypanosomatidae during tick blood meals. However, these differences are not statistically significant, so we cannot draw a definitive conclusion about the impact of the transmission route.

Overall, this systematic review and meta-analysis provide an updated overview of the vector status of ticks for Trypanosomatidae. This updated perspective reveals that partial information is available regarding the presence of Trypanosomatidae infecting humans and animals of veterinary importance in field-collected specimens or experimental studies aimed at probing the vector competence of ticks. Specifically, data are available for 20 species out of 774 recognized hard ticks and 221 soft ticks. Of the 23 *Leishmania* and 11 *Trypanosoma* species of medical or veterinary interest, we gathered information on 6 species belonging to the *Leishmania* and 9 to the *Trypanosoma* genus. Surprisingly, we did collect data on the presence of Trypanosomes involved in human African Trypanosomiasis (*T. b. gambiense* and *T. b. rhodesiense*) in field-collected ticks. Altogether, we could not provide conclusive quantitative evidence regarding the vectorial role of ticks for *Leishmania* and *Trypanosoma* parasites in medical or veterinary contexts. However, for *Rhipcephalus sanguineus* the documentation of the presence of Trypanaosomatidae infecting humans and animals is the larger one (21 studies), the capacity to engorge parasites during blood feeding on an infected host has been successfully attempted (11 successes over 15 experiments), and the presence of the infective parasite stage has been probed through the injection of tick infected material (7 attempts, and 5 successes), as well as re-transmission through infected tick blood feeding (6 attempts and 3 successes). Interestingly and importantly, data on vertical transmission have also been collected (9 attempts 7 successes). The majority of these collected data are for *L. infantum*, but altogether, these data argue for the vectorial competence of *R. sanguineus*, which needs now to be more thoroughly demonstrated using advanced molecular methods in field-collected specimens and with additional experimental information. In addition, our analysis suggests that the presence in ticks of parasites from the Trypanosomatidae family having medical or veterinary interests is not infrequent and that ticks of the Argasidae family might play a role in the transmission of Trypanosomatidae. The exact role of ticks in sustaining both the parasitic developmental and epidemiological cycles of Trypanosomatidae requires further investigation and continuous scrutiny.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org.

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