

Opinion

'Hang on a Tick' – Are Ticks Really the Vectors for Australian Trypanosomes?

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Trypanosomes are global blood parasites that infect a wide range of vertebrate hosts. Several species of *Trypanosoma* cause disease in humans and domesticated animals, and the majority are transmitted between hosts by haematophagous invertebrate vectors. Ticks have long been speculated as vectors for Australian trypanosomes. Recent studies using advanced molecular techniques have refocused attention on these arthropods, and whilst they have renewed discussions about *Trypanosoma* species and their vectors, these reports have simultaneously led to premature conclusions concerning the role of ticks as vectors. Here the controversy surrounding ticks as trypanosome vectors is discussed. We highlight the unanswered questions concerning the role played by ticks in trypanosome transmission and suggest future approaches to resolving these key knowledge gaps.

Ticks and Their Predestined Fate as Disease Vectors

Found throughout the world, ticks belong to the largest and most diverse phylum, the Arthropoda. Ticks are obligate **haematophagous** (see [Glossary](#)) invertebrates of a diverse array of vertebrate hosts [1,2]. Although ticks feed on blood as a way of obtaining essential nutrients for the purpose of moulting and reproducing, it is this promiscuous blood feeding lifestyle that has predestined the tick to a life of efficient 'taxi service' for a plethora of opportunistic, hitchhiking microorganisms [3]. Similarly, highly complex relationships have evolved between ticks and several of their parasites, such as *Babesia microti* and *Theileria parva*, whose life cycles are purposely adapted to coincide with that of their tick host [4,5]. Whilst several important vectors such as mosquitoes and biting flies have a **holometabolous** life cycle, ticks undergo a **hemimetabolous** development [2,6]. This can be an important factor for the survival of several microorganisms within the tick, because although various internal structures are involved in the cell degeneration and regeneration that form part of the metamorphosis process, the midgut remains unaltered [2,7,8]. This 'spectatorship' carried out by the midgut, and anything within it, affords parasites harboured in ticks an opportunity to endure the precarious phases of the moult [9].

Ixodid 'hard' ticks are by far the most frequently studied ticks in respect to **vector capacity** [10]. Specifically, it is the multi-host ixodid ticks that are some of the most efficient vectors for numerous pathogens because of their interactions with different vertebrate hosts throughout their life cycle [1,3,11]. Ixodids are the predominant tick family associated with Australian wildlife and have several biological features that enhance their **vector competence** (Box 1). There are 71 currently recognized Australian tick species (57 ixodid ticks), 55 of which parasitize wild animal hosts [12–14]. The majority of these tick species have a non-host-specific lifestyle coupled with a cosmopolitan distribution, making them prime suspects in the transmission cycle for several infectious diseases in Australia [15,16].

Highlights

Trypanosomes (genus *Trypanosoma*) are blood-borne protozoan parasites of vertebrates that typically require a haematophagous invertebrate as a vector. Triatomine bugs and several biting flies are recognized as their main vectors.

Ticks, as blood feeders, are predisposed to the ingestion of various parasites during feeding and have therefore long been proposed as vectors for *Trypanosoma* species.

Trypanosomes are reported in Australian wildlife, and early indications suggest that some species may adversely affect the health of native hosts. The vectors responsible for transmitting Australian *Trypanosoma* species between vertebrate hosts are unknown.

Ticks are suggested as vectors for several Australian trypanosomes based on microscopic and molecular detection of trypanosomes in ticks removed from wildlife. However, there are no experimental transmission studies to support this hypothesis to date.

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Box 1. What Makes Ticks Efficient Vectors?

Most of the 900 or more recognized species of ticks (order Ixodida) belong to two major tick families: the Ixodidae, or 'hard ticks', and the Argasidae or 'soft ticks' [62]. Despite their difference in physical appearance, which has rooted their respective 'hard versus soft' tick terms, ixodid and argasid ticks further differ in anatomy and life cycle [2]. As a consequence, though argasids are certainly capable of and indeed attributed to transmitting several disease-causing microorganisms, ixodids are by far the most frequently studied ticks in terms of vector potential [1–3,63].

Table I summarizes the biological characteristics of ixodid ticks that facilitate their efficient vector capacity for a multitude of parasitic microorganisms. Most ixodid species are three-host ticks, acquiring a blood meal at three different times corresponding to their three-stage life cycle (larva, nymph, and adult), consequently feeding from three different animal hosts that are often of different taxa [2,11]. This three-host life cycle is significant for dissemination of disease-causing microorganisms between infected and uninfected animal hosts [3]. Similarly, one-host ticks also take three blood meals throughout their life cycle; however, feeding is restricted to the same individual animal, making parasites ingested by one-host ticks dependent on transovarial transmission for successful circulation between animal hosts [2,10].

Table I. The Main Biological Characteristics That Enhance the Vector Potential of the Three-Host Ixodid Ticks

Biological characteristic	Amount/frequency
Number of blood meals	3
Size of blood meals	Large (ticks can engorge on blood by a factor of 100–200x for adult ticks)
Duration of blood meals	2–12 days
Digestion process	Commences a few hours after feeding is initiated but can last for several weeks or months ^a
Metamorphosis	Two hemimetabolous moults
Host specificity	For several tick species, feeding involves a great variety of different vertebrates
Distribution	Many occupy diverse habitats

^aSince digestion is so prolonged in ticks, microorganisms acquired from the blood meal have an increased likelihood of successful passage from the midgut barrier of the tick to the body tissues and organs.

Trypanosomiasis: A Tick-transmitted Infection?

The majority of known vectors of trypanosomes are haematophagous invertebrates within the class Insecta, and include the orders Diptera (e.g., flies), Hemiptera (e.g., triatomine 'reduviid' bugs), and Siphonaptera (e.g., fleas) [17], and for aquatic and semi-aquatic vertebrates, the leech (order Hirudinea) is the implicated vector [18,19]. For several years other haematophages, including ticks, have been associated, albeit infrequently, with trypanosome transmission [20–23] (Box 2). However, recent findings of trypanosome DNA in ticks using modern molecular techniques [24–28] has once again reignited the debate about their role as vectors for trypanosomes.

Flagellates of different morphologies, believed to be trypanosome life stages, have been observed in tick species removed from various host animals since 1857 [29–39]. However, C.A. Hoare, in his 1972 detailed monograph on trypanosomes, clearly denounced any suggestion of ticks as trypanosome vectors, instead proposing these parasite forms observed within ticks to be the result of the arthropod haemolymph acting as a culture medium [17]. Nevertheless, with a founding [34] and agricultural interest in the bovine parasite *Trypanosoma theileri*, several publications ensued suggesting multiple tick genera as hosts [40–43]. Similarly, investigations concerning ticks that parasitize wildlife hosts [38,44] implicated ticks as vector candidates for other trypanosome species. In 1986, the first biological transmission of *T. theileri*-like flagellates to cattle by the ixodid tick *Hyalomma anatolicum anatolicum* was reported [21] (see below) but never confirmed. It was not until 2007, with advances in molecular technologies,

Glossary

Biological vector: a vector that is essential to the multiplication and maturation of a parasite to a stage that is infective to a subsequent host.

Dixenous: a parasite with a life cycle that is completed between two different hosts.

Ectoparasite: a parasite that resides on the outside (external surface) of the body of a host.

Haematophagous: an organism that feeds on blood. Haematophagy is the act of feeding on blood.

Hemimetabolous: a type of metamorphosis in which an organism passes through partial 'incomplete' life stages, and where the immature stages are similar to the mature adult stage.

Holometabolous: a type of metamorphosis in which an organism passes through distinct 'complete' life stages, and where the immature stages differ significantly from the mature adult stage.

Infection: the invasion and multiplication of an infectious microorganism within a body. In the context of trypanosomes, an infectious state within ticks has not been confirmed in any present studies.

Mechanical vector: a vector that simply carries a parasite to a susceptible host and is not essential to the development of that organism.

Salivarian: in the context of trypanosomes: a parasite that is transmitted to the recipient vertebrate host through inoculation of the blood with the vector's saliva.

Stercorarian: in the context of trypanosomes: a parasite that is transmitted to the recipient vertebrate host through inoculation of a wound or mucus membrane with the vector's infected faeces.

Transovarial transmission: in the context of ticks: the passage or transmission of a parasite from a female tick to its progeny (eggs) via the ovaries.

Trans-stadial transmission: in the context of ticks: the passage or transmission of a parasite from one tick life stage to the next (vertical transmission). In some contexts, it is also used to describe parasite transmission from a tick to its vertebrate host (horizontal transmission).

Vector capacity: a measure of the transmission capability of a vector for a parasite, influenced by factors including vector behaviour, population density,

Box 2. Trypanosomes and Their Transmission

Trypanosomes are obligate protozoan parasites that are responsible for a group of diseases referred to as the trypanosomiasis [64]. The trypanosome life cycle is **dixenous**, involving a vertebrate that acts as a definitive host, called a reservoir, and an invertebrate vector that acts as the intermediate host and is responsible for transferring the parasite between different vertebrates [17]. Species of *Trypanosoma* are divided into two groups: the **salivarian** and the **stercorarian** trypanosomes, a grouping determined by their final infective location within the invertebrate vector, prior to transmission [17]. The extent to which the invertebrate vector serves as a host can vary and is dependent on several factors, including the invertebrate species, its ecology, which encompasses the invertebrate-vertebrate relationship, the number of vertebrate hosts and haematophagous invertebrates present, as well as the species of trypanosome [17,65]. In some cases, the invertebrate serves purely as a 'bridge' for transferring the parasite between successive vertebrate hosts, typically in the course of interrupted feeding. Referred to as a **mechanical vector**, transmission largely relies on the accidental uptake of the infective parasite by a haematophagous arthropod. In most instances, however, the trypanosome parasite remains within a **biological vector** and undertakes various stages of development necessary to multiply and become infective to a subsequent vertebrate host. This biological transmission concludes at the time of the vector's next feed [66,67]. In the tick life cycle (Figure 1), transmission would be successful if: (i) it feeds on an infected vertebrate host, (ii) it acquires the parasite during feeding, (iii) it maintains the parasite throughout its life cycle (trans-stadial 'vertical' transmission) and/or transovarial 'vertical' transmission), and (iv) it can transfer the parasite to another host when feeding (trans-stadial 'horizontal' transmission) [2,4,6,9].

host preferences, feeding habits and frequency, lifespan, and vector competence.

Vector competence: the intrinsic ability of a vector to acquire a parasite from the reservoir host, maintain infection, and later transmit the parasite to a subsequent host.

Xenodiagnosis: a method for diagnosing an infection that uses the vector, acting as a biological culture medium, for the detection of subsequent parasite infection.

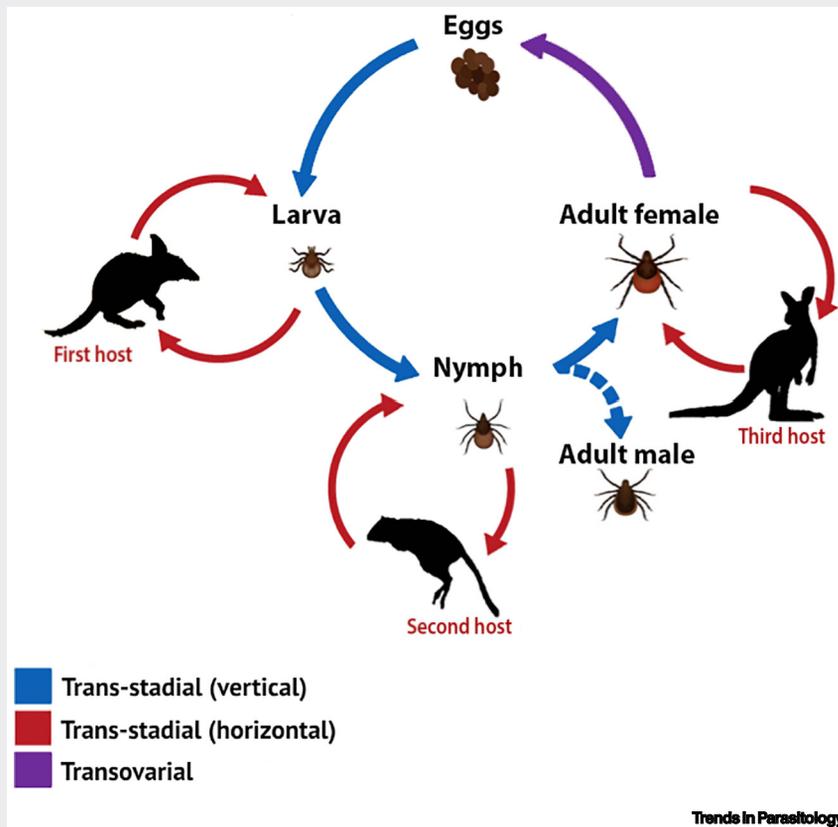


Figure 1. The Differences between Trans-stadial and Transovarial Transmission Routes in the Tick Life Cycle.

that the vectorial potential of ticks re-emerged with the first report of trypanosome DNA isolated from the tick *Haemaphysalis hystricis* [24]. More recently, there have been sporadic accounts of trypanosome **infection** in ticks [25–28], raising the question again of whether ticks play an important role in trypanosome transmission.

Ticks as Vectors of Trypanosomes: The Microscopy Era

The hypothesis that ticks could be vectors of trypanosomes was first made following observations of trypanosome-like flagellates within a tick removed from infected cattle in 1932 [35]. Several reports followed, all presenting varying degrees of morphological evidence depicting the presence of different trypanosome-like forms (trypomastigotes, amastigotes, epimastigotes, promastigotes, sphaeromastigotes) in various ixodid and argasid ticks (Box 1) from different geographical locations [36–43]. However, as appropriately denoted by C.A. Hoare, ticks cannot be identified as a vector because the animal infective forms of the parasite – metacyclic trypanosomes – have not been observed in ticks [17]. In addition to confirming infective trypanosome forms, recognition of the manner in which trypanosomes are transmitted to vertebrate hosts is imperative to confirming vector status.

Evidence of Transmission by Tick Bite and Feeding

The only published report describing the biological transmission of trypanosomes by tick bite was in 1986 [21]. Conducted in Sudan, this study involved cattle and their ticks infected with a trypanosome-like parasite believed to be *T. theileri* based on morphological similarities to flagellates previously described in the tick *Amblyomma americanum* [41]. Several different flagellate forms, not unlike those reported in previous studies [40,41], were documented from engorged ticks. The authors proceeded to inoculate three presumably uninfected calves with either a suspension containing trypanosome-like flagellates from ground-up infected tick-gut contents, or through the application of infected adult ticks to the calves' ears. Only one of the calves showed any trypanosome-like flagellates, which were observed on one blood smear from a biopsy of an enlarged lymph node. This report, which tantalisingly hints at *H. a. anatolicum* as a possible vector for the bovine parasite *T. theileri*, unfortunately falls short of confirming vector status.

An Alternative Transmission Route: Ingesting Infected Ticks

Transmission via either salivary inoculation, faecal contamination of a wound or mucus membrane, or through infected mouthparts during interrupted blood feeding are the primary routes of infection employed by trypanosome parasites through their haematophagous invertebrate vector [17]. Nonetheless, other routes of transmission do occur, and in 2011 the potential for transmission of *Trypanosoma evansi* to laboratory rodents via the oral route was investigated [45]. First, rodents were fed *T. evansi*-infected blood, resulting in 70% of the experimental rodents developing parasitaemia. For the second experiment, uninfected rats were fed engorged *Rhipicephalus sanguineus* adult ticks that had originally fed on the blood of parasitaemic rats. Despite ingestion of up to 75 infective engorged ticks over three consecutive days, no trypanosomes were observed in any of the rats [45]. Several reports have emerged of trypanosome species being spread by way of the host ingesting various infected invertebrates, but none have implicated ticks [17,46,47].

Indications of Trypanosome Passage between Tick Life Stages and Their Hosts

There are several important factors to consider when attempting to understand the transmission dynamics of a particular parasite within a tick vector. For ixodid ticks, feeding only occurs once per developmental life stage prior to undergoing an incomplete metamorphosis. Additionally, an ixodid tick's subsequent feed may only take place several weeks to months later (Box 1) [2]. Successful **trans-stadial transmission** of a parasite from a tick to a vertebrate host depends on whether the microorganism can survive the moulting process as well as an extended duration within the tick vector. The trans-stadial (vertical) passage of trypanosome parasites between life stages in ticks and subsequent trans-stadial (horizontal) transmission to an animal host (Box 2) has been claimed in several early studies. In 1980, D.J. Weilgama reported trans-stadial transmission of the parasite *Trypanosoma thylacis* by the native tick *Ixodes tasmani* to the Australian

short-nosed bandicoot (*Isoodon macrourus*) [44]. Results indicating the presence of an apparent *T. thylacis* infection in the moulted ticks suggested a successful trans-stadial transmission between tick life stages. Moreover, findings suggested that, subsequent to the application of what we can only assume were *T. thylacis* infected ticks, the experimental bandicoots presented with a peripheral blood *T. thylacis* infection several days later. However, as *T. thylacis* in natural conditions presents only as a very low-grade infection in bandicoots (Weilgama, D.J., PhD thesis, University of Queensland, Australia, 1979), there remains a possibility of misdiagnosis or a transient (non-replicative) infection. Yet, without evidence to suggest an alternative route such as venereal transferal (e.g., *Trypanosoma equiperdum* in horses) or maternal transferal (e.g. *Trypanosoma cruzi* in humans) [48,49], transmission of this parasite via tick feeding is plausible, albeit in need of further investigation.

The next record of trans-stadial transmission came from researchers in India who were investigating the role of ticks (in this case *H. a. anatolicum*) as vectors of *T. theileri* [43]. Larvae and nymph ticks were fed on experimentally infected calves, before being removed for moulting under laboratory conditions. Flagellates of various developmental forms were reported in 31% of moulted ticks post infection, indicating that trypanosome-like flagellates could persist within the tick from one life stage to the next. This is an important finding when contemplating the feasibility of ticks as trypanosome vectors; however, their report that 'infective nymphs lost their infections upon feeding on the [subsequent] animals' is confusing [43], as it would be expected that they would have continued to harbour the insect infective flagellate forms, such as epimastigotes.

Early microscopy studies [21,43,44,50] have clearly demonstrated that, for certain trypanosome species, such as *T. theileri*, ticks appear capable of retaining trypanosome forms following moulting from one life stage to another. Similarly, it would seem that maintenance of some wildlife trypanosome species in ticks between life stages is plausible [44]. However, evidence of tick infectiousness under natural conditions remains deficient and in serious need of validation.

Another Potential Route: Ovarian Infection

Transovarial transmission is an alternative route for parasite maintenance in nature [51]. This was proposed for the tick *H. a. anatolicum* based on the observation of flagellates within the ovaries of female ticks [39], and others have also reported the presence of flagellate forms within this reproductive tissue [34,40,41]. It was not until 2008 that a detailed examination of tick eggs for trypanosomes was conducted [52]. This study described flagellates, believed to be *T. theileri*, in the ovaries of the cattle tick *Boophilus microplus* (syn. *Rhipicephalus microplus*). However, following the oviposition of eggs by the same colony of female ticks, no flagellate forms were present in any of the eggs. As tick species have shown potential to harbour flagellates in their reproductive tissues [34,39–41] we cannot fully discount the possibility of transovarial transmission based on what remains a solitary investigation [52].

The Molecular Detection of Trypanosomes in Ticks: A New Era

The first use of molecular tools for the identification of trypanosomes in ticks utilized contemporary sequencing technologies to detect and subsequently deduce the phylogeny of the tick isolate *Trypanosoma* KG1 [24]. The midgut contents from field collected ixodid *Haemaphysalis hystricis* were cultured and subsequently inoculated into several argasid *Ornithodoros moubata* via injection or artificial feeding. The authors further inoculated several animals (mice, rats, rabbits, sheep) with their trypanosome cultures. Injected ticks showed the insect infective epimastigote forms only, whereas artificially fed ticks presented with the 'animal infective' trypomastigote forms in the midgut and salivary glands. However, no flagellates were microscopically observed or genetically detected in their animal experiments.

The next study to incorporate molecular detection methods in the trypanosome–tick context was by Australian researchers in 2011 [25]. They reported developmental forms of *Trypanosoma copemani* and positive DNA detection of the parasite within the haemolymph and midgut of *Ixodes australiensis*, 49 and 117 days post removal from infected quokkas (*Setonix brachyurus*). In addition, the authors reported seeing flagellates in the dried faeces of collected ticks, though not the ‘animal infective’ trypomastigote forms that would have suggested a potential **stercorarian** transmission route [25]. The tick faeces, however, were negative for *T. copemani* DNA. There is no mention of a post-feed moult for any of the ticks, so the capacity for this parasite to survive a trans-stadial transmission, necessary prior to their next blood meal, is unknown. To date, no transmission studies have been conducted to determine whether *I. australiensis* could in fact transmit *T. copemani* to a subsequent animal host.

In 2017 Australian researchers described mixed trypanosome infections within ticks [26] by analysing the trypanosome DNA present in ticks (*Ixodes holocyclus* and *I. tasmani*) and their wild koala hosts (*Phascolarctos cinereus*). Several trypanosomes were detected concurrently in ticks and koalas, including *T. copemani*, which was the dominant trypanosome found in ticks. The authors concluded that these two tick species are vector candidates for these trypanosome species. However, this proposal is premature as it is unknown whether the DNA detected was from viable trypanosomes or if the trypanosomes were remnants from an infective blood meal.

In 2018, novel trypanosomes were described in South American ticks *Amblyomma brasiliense* and *R. microplus* [27,28]. The ticks were directly removed from wild animal hosts and the detected epimastigotes were subsequently cultured. The authors inferred that the ticks harboured a natural infection, but this cannot be concluded as in both cases the trypanosomes were most likely derived from a recent blood meal.

Vector Competence and Prevalence of Trypanosomes in Field Ticks Remains Unknown

Evaluating the vector competence of ticks for trypanosomes is fundamental to establishing whether they can act as a potential source of infection to their vertebrate hosts. Concerning prevalence, the majority of published studies either analyse a small number of field-collected ticks [24] or they refer to ticks collected directly from infected animals; the latter significantly increases the probability of finding trypanosomes within the tick [25,26,38] without necessarily establishing that an infection is present. To date only two studies have conducted a prevalence screening of ticks for trypanosome parasites. The first investigation was carried out over a 6-year period and examined more than 3000 *Ixodes ricinus* ticks that had been collected from forests in Lithuania [53]. Dissected, the gut contents of ticks were smeared and microscopically examined for flagellates. Only three ticks (0.1%) presented with trypanosome-like flagellates. Additionally, only insect stages, including epimastigotes and some amastigote forms, were reported. Unfortunately, a morphological description of these flagellates was not provided so there is no way of knowing the trypanosome species observed. Furthermore, since the level of engorgement was not stated, it is not known how recently the ticks had dropped off their host, or if they were freshly moulted and thus questing for their next blood meal. The second study was in Australia and involved a preliminary investigation of several Australian haematophagous invertebrates. The ixodid tick was identified as a hypothetical vectorial candidate of *T. copemani* as well as another native parasite, *Trypanosoma vegrandis*. Removed directly from animal hosts originating from a trypanosome endemic area, it is not surprising that the reported infection rates in these ticks were high at 14% and 12% for *T. copemani* and *T. vegrandis*, respectively (C. Thompson, PhD thesis, Murdoch University, 2014). Apart from Žygutienė [53], no study has since determined the prevalence of trypanosome infection in questing ticks from any global arena. This is of utmost

importance in Australia where the tick has been repeatedly suggested as a likely vector for trypanosomes implicated in the poor health and population decline of several native Australian mammals [25,26].

The Australian Trypanosome Conundrum: No Confirmed Vectors

Very few published reports have detailed the screening of Australian invertebrates for trypanosome parasites. Studies to date have all relied on opportunistic sampling, and the majority have been from native hosts intended for other research purposes or as part of population surveys. The consequence of this random and 'by chance' screening of invertebrates throughout the years has meant that the identity of vectors for Australian trypanosomes remains unconfirmed. Several studies have examined and subsequently suggested a number of potential invertebrate vectors (e.g., fleas, ticks, leeches) for some of Australia's native trypanosomes [25,26,38,44,54–57]. However, none have conclusively determined the vectorial capability of these invertebrates beyond observing flagellates in their tissue contents and/or detecting trypanosome DNA from invertebrates that have been directly removed from infected animal hosts. Perhaps of most concern though is that anecdotal reports have exacerbated the situation by arguably proposing various vector candidates (e.g., the tick *I. australiensis*) prematurely [58–61]. The danger lies in that these suggestions can give undue bias toward a particular invertebrate candidate, and for Australia's native trypanosomes this has meant that information about their life histories has continued to remain largely neglected.

Why Do We Continue to Assume That Ticks Are Vectors for Australian Trypanosomes?

Ticks have been reported as potential vectors for Australian trypanosomes based on various forms of incomplete scientific evidence as well as anecdotal suggestions (Table 1). As common

Table 1. Studies Suggesting Ticks as Vectors for Australian Trypanosomes

Trypanosome species	Recorded vertebrate host(s)	Tick species	Tick life stage(s)	Vector evidence			Refs
				Anecdotal	Microscopic	Molecular	
<i>Trypanosoma thylacis</i>	Southern brown bandicoot (<i>Isodon obesulus</i>); Northern brown bandicoot (<i>Isodon macrourus</i>)	<i>Ixodes holocyclus</i> ; <i>Ixodes tasmani</i>	L; N; A ^a		✓ ^b		[38,44]
<i>Trypanosoma binneyi</i>	Platypus (<i>Ornithorhynchus anatinus</i>)	<i>Ixodes ornithorhynchi</i>	N/A	✓		✓	[38,61]
<i>Trypanosoma irwini</i>	Koala (<i>Phascolarctos cinereus</i>)	<i>Ixodes holocyclus</i> ; <i>Ixodes tasmani</i>	A	✓		✓	[26,59]
<i>Trypanosoma gilletti</i>	Koala (<i>Phascolarctos cinereus</i>)	<i>Ixodes holocyclus</i> ; <i>Ixodes tasmani</i>	A	✓		✓	[26,59]
<i>Trypanosoma copemani</i>	Wombat (<i>Vombatus ursinus</i>); Quokka (<i>Setonix brachyurus</i>); Gilberts potoroo (<i>Potorous gilbertii</i>); Koala (<i>Phascolarctos cinereus</i>); Quenda (<i>Isodon obesulus fusciventer</i>); Common brush-tailed possum (<i>Trichosurus vulpecular</i>); Woylie (syn. Brush-tailed bettong, <i>Bettongia penicillata</i>)	<i>Amblyomma triguttatum</i> ; <i>Ixodes australiensis</i> ; <i>Ixodes holocyclus</i> ; <i>Ixodes tasmani</i>	A		✓	✓	[25,26] [†]
<i>Trypanosoma vegrandis</i>	Chuditch (syn. Western quoll, <i>Dasyurus geoffroii</i>); Woylie (syn. Brush-tailed bettong, <i>Bettongia penicillata</i>)	<i>Amblyomma triguttatum</i> ; <i>Ixodes holocyclus</i> ; <i>Ixodes tasmani</i>	A			✓	[26] [†]

^aAbbreviations: A, adult; L, larvae; N, nymph; N/A, not available.

^b✓ indicates available evidence.

ectoparasites of Australian mammals, the collection and subsequent analysis of ticks is more achievable than other, less noticeable, haematophagous invertebrates. For instance, *Ixodes ornithorhynchi* is host-specific to the platypus, a monotreme frequently infected with the parasite *Trypanosoma binneyi*. As a consequence of their common association with their host, this tick species has been assumed to be a likely vector for *T. binneyi* [38]. However, platypuses are equally exposed to other haematophages, including freshwater leeches and biting flies [38]. In fact, the molecular screening of unengorged and partially fed platypus ticks was negative for trypanosome DNA whereas a platypus freshwater leech was trypanosome positive [56].

To date, all published studies have investigated ticks directly removed from hosts where the potential contamination from a blood meal cannot be ruled out. Therefore, a key outstanding question remains: do ticks collected from the field (i.e., those that have moulted and are questing for a new blood meal) carry trypanosome infections (Box 3)?

Critical Comments

Presently, no published report provides a comprehensive assessment of the vectorial capability of ticks for trypanosome parasites. The suggestion that the Australian tick *I. australiensis* is a vector for *T. copemani* [25] was based on **xenodiagnosis**, an established method for monitoring *T. cruzi* infection in the reduviid bug. This test, however, is not appropriate for monitoring trypanosome progression in ticks as reduviid bugs belong to a taxonomically distinct group – invertebrates differ in their incubation period, hence their vectorial capacity for trypanosomes

Box 3. Why the Examination of Questing Ticks for Trypanosomes Is So Important

Ticks that are actively searching for their next blood meal are referred to as questing. Questing ticks can either be specialists – focusing on a limited number of host species and typically found in close proximity to animal burrows – or they can be opportunistic generalists – not host-specific, and therefore capable of feeding on a vast variety of vertebrate hosts [2,9,11]. This active host-seeking behaviour occurs between blood meals and after the moulting period. For three-host ixodid ticks, questing typically occurs at three different periods that correspond to their life cycle [2,9] (Figure 1). As such, the examination of questing ticks is important for establishing whether ticks can maintain a trypanosome infection in nature, both between host feeding and throughout a moult. Currently, no study has properly assessed the prevalence of trypanosome infection in questing ticks. Published studies refer to a limited number of ticks opportunistically collected from infected animal hosts [21,25–28,38]. Consequently, the reported infection rate in these ticks is relatively high and cannot exclude a blood meal contamination. As a result, there is a presently unresolved question as to whether trypanosomes are found naturally occurring in questing ticks and, if so, whether they represent an important source of infection.

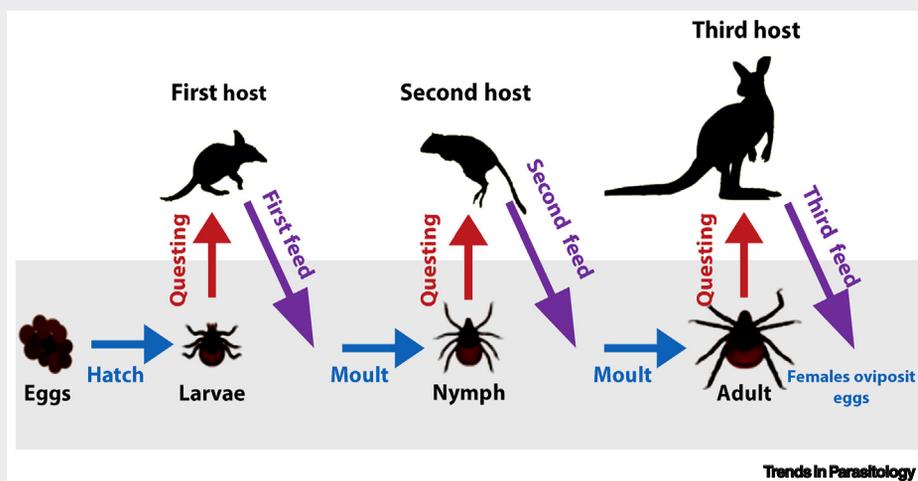


Figure 1. Questing Behaviour in Relation to the Tick Life Cycle.

cannot be correlated. Next, the suggested stercorarian transmission of trypanosomes from tick to vertebrate host [25] is unlikely for several reasons. Firstly, tick faeces dry rapidly and therefore do not provide the moist medium that is required for maintaining trypanosome viability [2,17]. For this reason, O'Farrell [34] considered faeces an impractical source of infection. While contamination through infected faeces is common for fleas, biting flies, and reduviid bugs as a result of their intermittent, interrupted feeding patterns and constant passage of fluid faeces while feeding, the feeding and defaecation habits of ticks are not conducive to successful transmission by faecal contamination [2]. Ultimately, because of the efficient plug of cement substance produced by several ixodid ticks for secure adhesion to their host during feeding, it is highly improbable that trypanosomes could enter by this route as long as the tick is attached.

Concluding Remarks

Currently there is no conclusive evidence that ticks are involved in the transmission of trypanosomes to Australian wildlife. Existing evidence using advanced molecular technologies suggest that trypanosomes found within Australian ticks collected from parasitaemic hosts could be viable several weeks post blood feeding [25], and researchers have likewise established that the trypanosome DNA present within ticks and their hosts correlate [26]. However, blood meal contamination remains the primary obstacle in discerning a blood meal infection from a naturally acquired infection in the fed tick. The practical approach to overcoming this problem is by examining ticks that have already digested a previous blood meal – questing ticks. This remains an unexplored field with no investigation having yet assessed the presence of trypanosomes in Australian questing ticks. Evaluating whether viable trypanosomes are present as an infection within ticks that have moulted, and are seeking their next blood meal, is fundamental to establishing whether they can act as a source of infection to subsequent hosts (see Outstanding Questions).

Remaining questions are centred upon the post-ingestion, internal environment of ticks and whether they support the survival and subsequent establishment, multiplication, and development of the *Trypanosoma* species in question. The need to verify viability and the presence of infective developmental stages – metacyclic trypomastigotes – go hand in hand. Clearly, solving these questions will be dependent on a fusion of existing molecular and microscopy techniques that afford us information concerning species identity as well as morphology and structural integrity. Finally, experimentally confirming transmission of infective trypanosomes from infected tick to uninfected host would be the 'icing on the cake' for validating vector capability. With Australian trypanosomes, however, this is difficult to achieve given the ethical dilemma of dealing with native species, many of which are critically endangered. Nevertheless, perhaps resolution is achievable through extensive sampling of ticks on and off hosts and exploring their vectorial capacity using a combination of molecular and ultrastructural analyses.

In conclusion, when considering the available evidence (Table 1), what is lacking is systematic and correlative experimental data that marry physical evidence with molecular information. We advocate the need for a holistic approach using molecular and ultrastructural analyses to better understand both the vertebrate and invertebrate aspects of the life cycle of Australian trypanosomes, and hence to work toward the holy grail of confidently identifying their invertebrate vector(s) and modes of transmission. Until then, the significance of ticks in the life cycle of Australian trypanosomes and their potential impact upon endangered wildlife populations will remain nothing more than speculation.

Resources

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Outstanding Questions

Do trypanosomes survive the tick moult and subsequent timespan between blood meals?

- (i) Where are trypanosomes located within the tick tissues?
- (ii) What trypanosome developmental form do they represent?
- (iii) At what prevalence are trypanosomes found in questing ticks?

Are trypanosomes transmitted by ticks to vertebrate hosts during a blood meal?

- (i) If so, by what route(s) does transmission take place?
- (ii) Are these forms found in ticks infective for vertebrate hosts?
- (iii) What is their epidemiological significance in trypanosome transmission?

Does the life cycle of trypanosomes and Australian ticks match up?

- (i) Are trypanosomes ingested during a blood meal capable of surviving the tick's subsequent moulting period?
- (ii) Can Australian ticks maintain a trypanosome infection during the several months between successive blood meals?

References

- Parola, P. and Raoult, D. (2001) Ticks and tickborne bacterial diseases in humans: an emerging infectious threat. *Clin. Infect. Dis.* 32, 897–928
- Sonenshine, D.E. and Roe, R.M. eds (2013) *Biology of Ticks*, Oxford University Press
- Jongejan, F. and Uilenberg, G. (2004) The global importance of ticks. *Parasitology* 129, S3–S14
- Chauvin, A. et al. (2009) *Babesia* and its hosts: adaptation to long-lasting interactions as a way to achieve efficient transmission. *Vet. Res.* 40, 37
- Nene, V. et al. (2016) The biology of *Theileria parva* and control of East Coast fever – current status and future trends. *Ticks Tick Borne Dis.* 7, 549–564
- Marquardt, W.C. ed (2005) *Biology of Disease Vectors*, Elsevier Academic Press
- Tarnowski, B.I. and Coons, L.B. (1989) Ultrastructure of the midgut and blood meal digestion in the adult tick *Dermacentor variabilis*. *Exp. Appl. Acarol.* 6, 263–289
- Kahl, O. et al. (1990) Gross morphological changes in the salivary glands of *Ixodes ricinus* (Acari: Ixodidae) between blood meals in relation to active uptake of atmospheric water. *Exp. Appl. Acarol.* 9, 239–258
- Sonenshine, D.E. and Mather, T.N. eds (1994) *Ecological Dynamics of Tick-borne Zoonoses*, Oxford University Press
- Kahl, O. (2018) Hard ticks as vectors – some basic issues. *Wien. Klin. Wochenschr.* 130, 479–483
- Kolonin, G.V. (2007) Mammals as hosts of Ixodid ticks (Acarina, Ixodidae). *Entomol. Rev.* 87, 401–412
- Roberts, F.H.S. (1970) *Australian Ticks*, CSIRO Publishing
- Barker, S.C. et al. (2014) A list of the 70 species of Australian ticks; diagnostic guides to and species accounts of *Ixodes holocyclus* (paralysis tick), *Ixodes cornuatus* (southern paralysis tick) and *Rhipicephalus australis* (Australian cattle tick); and consideration of the place of Australia in the evolution of ticks with comments on four controversial ideas. *Int. J. Parasitol.* 44, 941–953
- Ash, A. et al. (2017) Morphological and molecular description of *Ixodes woyliei* n. sp. (Ixodidae) with consideration for co-extinction with its critically endangered marsupial host. *Parasit. Vectors* 10, 70
- Barker, S.C. and Walker, A.R. (2014) Ticks of Australia. The species that infest domestic animals and humans. *Zootaxa* 3816, 1–144
- Graves, S.R. and Stenos, J. (2017) Tick-borne infectious diseases in Australia. *Med. J. Aust.* 206, 320–324
- Hoare, C.A. (1972) *The Trypanosomes of Mammals: A Zoological Monograph*, Blackwell Scientific Publishing
- Hayes, P.M. et al. (2014) Morphological and molecular characterization of a marine fish trypanosome from South Africa, including its development in a leech vector. *Parasit. Vectors* 7, 1–11
- Lemos, M. et al. (2015) Phylogenetic and morphological characterization of trypanosomes from Brazilian armoured catfishes and leeches reveal high species diversity, mixed infections and a new fish trypanosome species. *Parasit. Vectors* 8, 1–17
- Ayala, S.C. (1971) Trypanosomes in wild California sandflies, and extrinsic stages of *Trypanosoma bufophlebotomi*. *J. Protozool.* 18, 433–436
- Morzaria, S.P. et al. (1986) Transmission of a *Trypanosoma* sp. to cattle by the tick *Hyalomma anatolicum*. *Vet. Parasitol.* 19, 13–21
- Kato, H. et al. (2010) Natural infection of the sand fly *Phlebotomus kazeruni* by *Trypanosoma* species in Pakistan. *Parasit. Vectors* 3, 10
- Svobodová, M. et al. (2017) Biting midges (Ceratopogonidae) as vectors of avian trypanosomes. *Parasit. Vectors* 10, 1–9
- Thekiso, O. et al. (2007) A trypanosome species isolated from naturally infected *Haemaphysalis hystrix* ticks in Kagoshima Prefecture, Japan. *Parasitology* 134, 967–974
- Austen, J.M. et al. (2011) Vector of *Trypanosoma copemani* identified as *Ixodes* sp. *Parasitology* 138, 866–872
- Barbosa, A.D. et al. (2017) Increased genetic diversity and prevalence of co-infection with *Trypanosoma* spp. in koalas (*Phascolarctos cinereus*) and their ticks identified using next-generation sequencing (NGS). *PLoS One* 12, 1–20
- Marotta, C.R. et al. (2018) *Trypanosoma amblyommi* sp. nov. (Protozoa: Kinetoplastida) isolated from *Amblyomma brasiliense* (Acari: Ixodidae) ticks in Rio de Janeiro, Brazil. *Parasitol. Open* 4, 1–7
- Marotta, C.R. et al. (2018) *Trypanosoma rhipicephalis* sp. nov. (Protozoa: Kinetoplastida) isolated from *Rhipicephalus microplus* (Acari: Ixodidae) ticks in Rio de Janeiro, Brazil. *Parasitol. Open* 4, 1–8
- Leydig, F. (1857) *Lehrbuch der Histologie des Menschen und der Thiere*, Verlag von Meidinger Sohn & Comp.
- Schaudinn, F. (1904) Generations- und Wirtswechsel bei *Trypanosoma* und *Spirochaete* (Vorläufige Mitteilung). *Arb. Kaiser Lichen Gesundheitsamt* 20, 387–439
- Novy, F.G. et al. (1907) Trypanosomes of mosquitoes and other insects. *J. Infect. Dis.* 4, 223–276
- Swelengrebel, N.H. and Strickland, C. (1910) The development of *Trypanosoma lewisi* outside the vertebrate host. *Parasitology* 3, 360–389
- Bishop, C.F. (1911) Notes on a trypanosome, found in sheep tick, and its probable connection with the disease known as louping-ill. *Vet. J.* 67, 709–715
- O'Farrell, W.K. (1913) Hereditary infection with special reference to its occurrence in *Hyalomma aegyptium* infected with *Crithidia hyalommae*. *Ann. Trop. Med. Parasitol.* 7, 454–562
- Carpano, M. (1932) Localisations du *Trypanosoma theileri* dans les organes internes des bovins son cycle évolutif. *Ann. Parasitol. Hum. Comp.* 10, 305–313
- Rodhain, J. (1942) Au sujet du développement intracellulaire de *Trypanosoma lewisi* chez *Ornithodoros moubata*. *Acta Biol. Belgica* 2, 413–415
- Rodhain, J. (1942) Au sujet du développement intracellulaire de *Trypanosoma pipistrelli* chez *Ornithodoros mouhata*. *Acta Biol. Belgica* 2, 416–423
- Mackerras, M.J. (1959) The haematzoa of Australian mammals. *Aust. J. Zool.* 7, 105–135
- Arifdzhanov, K.A. and Nikitina, R.C. (1961) Detection of *Crithidia hyalommae* (O'Farrell 1913) in *Hyalomma anatolicum anatolicum* (Koch 1844) ticks. *Zool. Zhurnal.* 40, 20–24
- Burgdorfer, W. et al. (1973) Detection of *Trypanosoma theileri* in Ethiopian ticks. *Acta Trop.* 30, 340–346
- Krinsky, W.L. and Burgdorfer, W. (1976) Trypanosomes in *Amblyomma americanum* from Oklahoma. *J. Parasitol.* 62, 824–825
- Aeschlimann, A. et al. (1979) Aspects nouveaux du rôle de vecteur joué par *Ixodes ricinus* L. en suisse. *Acta Trop.* 36, 181–191
- Shastri, U.V. and Deshpande, P.D. (1981) *Hyalomma anatolicum anatolicum* (Koch, 1844) as a possible vector for transmission of *Trypanosoma theileri*, Laveran, 1902 in cattle. *Vet. Parasitol.* 9, 151–155
- Weilgama, D.J. (1980) Cyclical development of trypanosomes in the tick and their transmission. In *Haemo-Protozoan Diseases of Domestic Animals* (Gautum, O.P., et al., eds), pp. 81–97, Proceedings of a Seminar held by Haryana Agricultural University, Department of Veterinary Medicine, October 27–November 1, 1980, at Hissar, India
- Vergne, T. et al. (2011) Attempted transmission of *Trypanosoma evansi* to rats and mice by direct ingestion of contaminated blood and via engorged ticks. *Acta Protozool.* 50, 133–136
- Argañaraz, E.R. et al. (2001) Blood-sucking lice may disseminate *Trypanosoma cruzi* infection in baboons. *Rev. Inst. Med. Trop. São Paulo* 43, 271–276
- Votýpka et al. (2012) *Trypanosoma culicavium* sp. nov., an avian trypanosome transmitted by *Culex* mosquitoes. *Int. J. Syst. Evol. Microbiol.* 62, 745–754
- Norman, F.F. and López-Vélez, R. (2013) Chagas disease and breast-feeding. *Emerg. Infect. Dis.* 19, 1561–1566
- Gizaw, Y. et al. (2017) Dourine: a neglected disease of equids. *Trop. Anim. Health. Prod.* 49, 887–897
- Latif, A.A. et al. (2004) High infection rates of the tick *Hyalomma anatolicum anatolicum* with *Trypanosoma theileri*. *Onderstepoort. J. Vet. Res.* 71, 251–256

51. Randolph, S.E. (1994) The relative contributions of transovarial and transstadial transmission to the maintenance of tick-borne diseases. In *Lyme Borreliosis* 260 (Axford, J.S. and Rees, D.H. E., eds), pp. 131–134
52. Martins, J.R. *et al.* (2008) Tripanosomatides like *Trypanosoma theileri* in the cattle tick *Boophilus microplus*. *Rev. Bras. Parasitol. Vet.* 17, 113–114
53. Žygutienė, M. (1998) Ticks as vectors of Trypanosomae. *Acta Zool. Lituonica* 8, 117–120
54. Richardson, L. and Hunt, P. (1968) Trypanosomes in the crop of an haemadipsid leech. *Aust. J. Sci.* 30, 374–375
55. Hamilton, P.B. *et al.* (2005) A new lineage of trypanosomes from Australian vertebrates and terrestrial bloodsucking leeches (Haemadipsidae). *Int. J. Parasitol.* 35, 431–443
56. Paparini, A. *et al.* (2014) Novel genotypes of *Trypanosoma binneyi* from wild platypuses (*Ornithorhynchus anatinus*) and identification of a leech as a potential vector. *Exp. Parasitol.* 145, 42–50
57. Thompson, C.K. and Thompson, R.C.A. (2015) Trypanosomes of Australian mammals: knowledge gaps regarding transmission and biosecurity. *Trends Parasitol.* 31, 553–562
58. McInnes, L.M. *et al.* (2009) *Trypanosoma irwini* n. sp (Sarcostigophora: Trypanosomatidae) from the koala (*Phascolarctos cinereus*). *Parasitology* 136, 875–885
59. McInnes, L.M. *et al.* (2011) Novel trypanosome *Trypanosoma gilletti* sp. (Euglenozoa: Trypanosomatidae) and the extension of the host range of *Trypanosoma copemani* to include the koala (*Phascolarctos cinereus*). *Parasitology* 138, 59–70
60. Smith, A. *et al.* (2008) Trypanosomes in a declining species of threatened Australian marsupial, the brush-tailed bettong *Bettongia penicillata* (Marsupialia: Potoroidae). *Parasitology* 135, 1329–1335
61. Jakes, K.A. *et al.* (2001) Phylogenetic relationships of *Trypanosoma chelodina* and *Trypanosoma binneyi* from Australian tortoises and platypuses inferred from small subunit rRNA analyses. *Parasitology* 123, 483–487
62. Guglielmone, A.A. *et al.* (2010) The Argasidae, Ixodidae and Nuttalliellidae (Acari: Ixodida) of the world: a list of valid species names. *Zootaxa* 2528, 1–28
63. Fritz, C.L. (2009) Emerging tick-borne diseases. *Vet. Clin. North Am. Small Anim. Pract.* 39, 265–278
64. Desquesnes, M. *et al.* (2013) *Trypanosoma evansi* and surra: a review and perspectives on origin, history, distribution, taxonomy, morphology, hosts, and pathogenic effects. *Biomed. Res. Int.* 2013, 1–22
65. Lehane, M. (2005) *The Biology of Blood-Sucking in Insects*, Cambridge University Press
66. Vickerman, K. (1985) Developmental cycles and biology of pathogenic trypanosomes. *Br. Med. Bull.* 41, 105–114
67. Dyer, N.A. *et al.* (2013) Flying tryps: survival and maturation of trypanosomes in tsetse flies. *Trends Parasitol.* 29, 188–196