Chapter

Oriental Theileriosis

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Abstract

Theileria orientalis, the causative agent of oriental theileriosis, is an apicomplexan haemoparasite and is one of several tick-borne *Theileria* spp. infecting cattle. Unlike the highly pathogenic transforming *Theileria* species (*T. annulata* and *T. parva*) which induce uncontrolled lymphocytic proliferation, *T. orientalis* is a non-transforming strain exerting its major pathogenic effects via erythrocyte destruction. Clinical symptoms associated with oriental theileriosis are largely consequences of the underlying anaemia. Because of its non-transforming nature, *T. orientalis* was previously considered a benign parasite, however, in the recent years, clinical outbreaks of *T. orientalis* have been increasingly observed throughout Asia and Australasia. Recent rapid spread of clinical theileriosis has been linked to a pathogenic genotype of the parasite, genotype Ikeda (Type 2). The geographic distribution of clinical outbreaks correlates to the range of the major vector tick, *Haemaphysalis longicornis*, although other vectors and modes of transmission are possible. This review includes discussion of *T. orientalis* epidemiology, transmission, pathogenesis, treatment and control and provides an update on the taxonomy of this organism which is still under debate.

Keywords: *Theileria orientalis*, cattle, taxonomy, epidemiology, transmission, control

1. Introduction

T. orientalis has been reported to cause mortality in up to 5% of infected cattle. Clinical outbreaks commonly occur when naïve cattle are introduced into endemic herds, when animals undergo stress through transportation or are immunosuppressed. Pregnant heifers and calves are particularly susceptible to infection, with late term abortions also commonly reported. The parasite is globally spread but countries impacted by clinical theileriosis include Australia, New Zealand, Japan, Korea, China and Vietnam [1–3]. Oriental theileriosis represents a major economic burden to cattle production. In Australia in 2010 the economic impact of the parasite was estimated at $20 million AUD per annum. However, the costs associated with disease are likely to have increased substantially since that time with the subsequent spread of bovine theileriosis into new areas of the country. In New Zealand, although the total economic impact has not been well established, clinical outbreaks were estimated to cost up to $NZ 1 million on a single large dairy farm [4]. Recently, clinical outbreaks of theileriosis were documented for the first time in dairy cattle undergoing transport stress during importation to Vietnam from Australia [3], highlighting the potential importance of this disease in the live cattle trade. In countries like Japan and China where multiple tick species have been identified as potential disease vectors, economic impacts have been significant [5, 6].

The lack of preventive measures or suitable vaccines complicates the management of *T. orientalis*. Currently, there are limited therapeutic options available for

treatment of oriental theileriosis and no vaccines available for this disease globally. Vaccine and/or therapeutic development has been identified as a research priority for bovine theileriosis; however as in malaria studies, an understanding of the taxonomy and genetic variability within parasite populations is essential to ensure vaccine and therapeutic efficacy.

2. Taxonomy of *T. orientalis*

2.1 Taxonomic history of *T. orientalis*

Historically, the taxonomy of *T. orientalis* (formerly referred to as the *Theileria orientalis/sergenti/buffeli* group) has been a subject of some confusion, due to similarity in strain morphology, variability of host animals and transmission vectors, occurrence of mixed infections, parasite genetic diversity and the difficulty in extracting pure isolates for studies, especially in benign infections where parasitaemia is low [7]. Originally, these parasites were classified based on geographic origin [8, 9]. Further attempts to classify this group of parasites led to suggestions that the group should be classified into one species [1, 8–11]. More recently, variations in the major piroplasm surface protein (MPSP) gene have been used to classify members of the *T. orientalis* group, separating it into 11 genotypes [1].

Members of the *Theileria orientalis* group were first identified in Australian cattle in 1910 and the organism classified as *T. mutans* [12] due to the morphological similarity to the previously described African species [13]. Some years later, Wenyon [14] made the first description of a similar blood parasite from sheep and named it *Babesia sergenti*. The morphological drawings of *B. sergenti* [9] corresponded to *Theileria* spp. morphology and it was later found that the parasite he described was indeed a theilerial parasite of sheep [15, 16]. However, in the intervening years, a new parasite of cattle in Eastern Siberia was described and *T. sergenti* [17]. The sheep parasite thus has precedence with respect to the name *T. sergenti,* rendering this name invalid for the cattle parasite; nonetheless the name *T. sergenti* had been used widely for this organism in the literature. Following the initial description of "*T. sergenti*" in Siberian cattle, a similar cattle haemoparasite was found in the same area and the authors named it *T. orientalis* [18].

Serological and morphological studies [19] later revealed that the *T. mutans* isolate identified in Australia [12] was the same species as "*T. sergenti*" [17] and not the African *T. mutans* described by [8]. Authors [15] suggested that the Australian isolate was either *T. orientalis* [18] or *T. buffeli* [20]. Serological and morphological studies conducted on *Theileria* stocks from Australia, Britain, Iran, Japan, USA and a higher pathogenicity stock from Korea concluded that the nomenclature of Australian *Theileria* should be *T. orientalis* [8]. But, a few authors still propose that the name *T. buffeli* should be designated due to the transmission of parasite from buffalo to cattle and the fact that isolates characterised at that point of time were all infective for buffalo [11, 21, 22]. Studies in Japan suggested *T. orientalis* and *T. buffeli* to be separated from *T. sergenti* and be classified as a different group due the serological and transmissibility differences [9, 23, 24]. Regardless of these findings, it was concluded that designation of the name *T. sergenti* should not be used for any blood parasite of ruminants with the exception of sheep [15, 16, 22].

2.2 Taxonomic classification using molecular techniques

As serological and morphological techniques were not suitably discriminatory for distinguishing isolates from the *Theileria orientalis/sergenti/buffeli* group,

molecular techniques became more prevalent. The use of the MPSP and 18S rRNA genes further clarified the relationships within this taxonomic group. Early PCR analysis of the MPSP gene revealed four major genotypes, Ikeda, Chitose and Buffeli and Thai type [25, 26]. The Buffeli genotype type was also separated into sub-genotypes B1 and B2 due to variability observed between these isolates [25]. Genotyping of the V4 variable region with the 18S rRNA gene which was previously shown to enable classification of *Theileria* spp. [27] revealed seven genotypes (Genotypes A to G) [28]. Subsequent examinations of *Theileria orientalis/sergenti/buffeli* group taxonomy utilised MPSP sequences due to greater observed sequence variation, producing stronger branch support in phylogenetic analyses [29, 30]. By 2010, eight MPSP genotypes (1–8) were classified including the unclassified genotype from Brisbane, Australia (*T. buffeli* Warwick) [29–31]. MPSP genotype 6 found in cattle and yak was reclassified and the taxonomic name *Theileria sinensis* was suggested to reflect divergence from the other members of the *Theileria orientalis/sergenti/buffeli* group [6, 32]. Three new genotypes from sheep, water buffalo and cattle were further identified [33] in Vietnam (N1, N2 and N3 respectively) bringing to current number of classified MPSP genotypes to 11 (Types 1–8 and N1–N3). Retrospective analysis of the genotypes previously identified with the 18S rRNA gene [28] against the current MPSP genotyping scheme shows that genotype A corresponds to Chitose while genotypes B and E correspond to Ikeda. 18S rRNA Genotypes C and D correspond to the Buffeli and Type 6 MPSP genotypes respectively. Further analysis revealed the 18S rRNA genotypes F and G identical to *Theileria cervi* a species found in elk. Buffeli sub-genotypes B1 and B2 identified in [25] correspond to the MPSP buffeli genotype and Type 4 respectively.

Molecular examinations have considerably clarified the taxonomy of *T. orientalis*. Asian isolates previously referred to as *T. sergenti* were found to be a mix of MPSP genotypes that were also commonly found in *T. buffeli* and *T. orientalis* isolates. Both Types 1 (Chitose) and 3 (Buffeli) were commonly found in both Australia and East Asia, with Type 3 spread globally. Hence more recent studies have begun to refer to this group by the common name *T. orientalis* [1, 34, 35].

Great efforts have been made by researchers to genetically characterise *T. orientalis*. However, current genetic characterisation methods utilise relatively few molecular markers. It has been well established that the primary mechanism driving genetic diversity in apicomplexans is through the sexual recombination; in the case of *Theileria* parasites, this occurs within the tick vector. Recombination has been relatively poorly studied in *T. orientalis*, however it has been suggested that recombination between MPSP genotypes is unlikely due to the low sequence identities between types [33] and high sequence identities within each clade [1, 31, 36, 37].

2.3 Current taxonomic state of *T. orientalis*

Genetic diversity within and between the MPSP genotypes should be further investigated as it has the potential to resolve the controversy surrounding the taxonomic classification of *T. orientalis,* elucidate virulence factors driving differential pathogenicity, and has implications for vaccine design. A complete genome of *T. orientalis* (Ikeda) has now been sequenced and annotated and is available for further research [34], and whole genome sequencing of large numbers of isolates is now feasible. A recent study which presented draft genomes of Australian isolates of Ikeda, Chitose and Buffeli genotypes confirmed the MPSP phylogenies indicating that the apathogenic Chitose and Buffeli genotypes are more closely related to each other than to the pathogenic Ikeda genotype [37]. That study further suggested that *T. orientalis* may indeed encompass multiple species and subspecies. The average nucleotide identity (ANI) between the Ikeda genome

and those of the Chitose and Buffeli genotypes (82%) was comparable to that of *T. annulata* and *T. parva* (80%). While sequencing of additional representatives of these genotypes is desirable, the evidence from the ANIs combined with the differential pathogenicity of these genotypes suggests that *T. orientalis* Ikeda is a separate species to *T. orientalis* Chitose and *T. orientalis* Buffeli. Moreover, the ANI between *T. orientalis* Chitose and *T. orientalis* Buffeli (86%) was comparable to that of the murine *Plasmodium* spp. suggesting that there may be further species or subspecies-level diversity within *T. orientalis* genotypes. [37]. Whole genome sequencing of additional *T. orientalis* genotypes is warranted to determine whether a new species designation should be applied to *T. orientalis* Ikeda and whether this may extend to include the phylogenetically related Type 7 which has also been associated with clinical disease [38]. Additional genome-wide studies will also enable researchers to formulate vaccine strategies by characterising possible vaccine targets and allow genetic diversity investigations within parasite populations [1]. Current efforts to understand the recombination mechanisms of other species of *Theileria* that lead to genetic diversity and taxonomic uncertainties [39–41] have been fruitful and it warrants researchers to conduct further investigations to answer the taxonomic questions surrounding *T. orientalis*.

3. Epidemiology

T. orientalis is a cosmopolitan parasite of cattle that also affects buffaloes and yaks [8]. *T. orientalis* infections have been globally reported in Australia [42–45], New Zealand [46], Southeast Asia [3, 33, 47–50] East Asia [6, 29–31, 36], South Asia [38, 51–53], Middle East [54–56], Africa [57–59], Europe [8, 60–65] and the Americas [1, 10, 66]. The distribution of *Theileria* species is dependent on the availability and competence of suitable tick vectors [7]. The principle vector of *T. orientalis*, *H. longicornis,* can be found in most of the countries where disease outbreaks have been reported (**Table 1**). In countries where distribution of *H. longicornis* is sparse or where the species is not known to occur, other *Haemaphysalis* spp. or other genera of ixodid ticks (**Table 1**) have been identified to be capable of transmitting the parasite, although the comparative competency of these species is unclear. The significance of these ticks as vectors of *T. orientalis* warrants further investigation.

3.1 Clinical disease outbreaks: Japan, Australia, New Zealand

In Japan, *T. orientalis* sourced from grazing cattle in Hokkaido was reported to cause 0.1% and approximately 2.5% of mortality and morbidity respectively [83]. In 2009, PCR analysis of the MPSP and p23 gene of *T. orientalis* revealed the presence of at least four genotypes (1, 2, 4 and 5) [29]. Further analysis [68] revealed Type 3 (Chitose) to be present in Japan and with earlier studies the authors suggested a total of seven genotypes (1–3, 4, 5, 7 and 8) to be present [69, 84]. Studies conducted over a number of years implicated *T. orientalis* Ikeda (Type 2) as being linked to clinical disease [42, 83, 85].

In recent years, outbreaks of oriental theileriosis have been increasingly observed in a number of different countries and are usually identified as being associated with MPSP genotype Ikeda (Type 2) [29, 45, 46, 68]. Australia and New Zealand recently experienced major disease incursions linked to *T. orientalis* Ikeda despite other genotypes of the parasite being present in these countries for many years.

T. orientalis was first observed in Australian herds in 1910, and the introduction was linked to the importation of *T. orientalis* infected *H. longicornis* ticks on

Country	*T. orientalis* MPSP genotypes	Host species	Vectors	References
Australia	1, 2, 3, 5	Cattle	*H. longicornis*	[1, 42, 44]
New Zealand	1, 2, 3, 5	Cattle	*H. longicornis*	[46, 67]
Japan	1, 2, 3, 4, 5, 7, 8	Cattle	*H. longicornis*, *H. mageshimaensis*, *H. douglasi*, *I. persulcatus*, *I. ovatus*	[5, 29, 34, 36, 68–70]
Korea	1, 2, 3, 4, 5, 8	Cattle	*H. longicornis*	[30, 71–74]
Taiwan	3	Cattle	*H. longicornis*	[31]
Vietnam	1, 2, 3, 5, 7, N3	Cattle	*Rhipicephalus microplus*	[3, 33, 49]
	5, N1, N2	Water buffalo	Unspecified	
	N1	Sheep	Unspecified	
Indonesia	7	Cattle	Unspecified	[48]
Thailand	1, 3, 5, 6, 7, N3	Cattle	Unspecified	[10, 47, 75]
	1, 3, 4, 5, 7, N2, N3	Water buffalo	Unspecified	
Cambodia	1, 3	Cattle	Unspecified	[49]
Myanmar	1, 3, 4, 5, 7, N3	Cattle	*R. microplus*, *Haemaphysalis* spp.	[50]
Philippines	Unspecified, but possible Type 1 and/or Type 3	Cattle	Unspecified	[76, 77]
India	1, 3, 7	Cattle	*H. bispinosa, R. microplus*	[38, 52]
	N2	Water buffalo	*R. microplus*	[53]
Sri Lanka	1, 3, 5, 7	Cattle	Unspecified	[1, 51]
	N1, N2	Water buffalo	Unspecified	
China	1, 2, 3, 5, 6, 8	Cattle	*H. longicornis, H. qinghaiensis*	[6, 32, 78–80]
	3	Water buffalo	*H. longicornis*	
	6	Yak	*H. qinghaiensis*	
Mongolia	1, 3, 5, 7, N3	Cattle	*Dermacentor nuttalli*	[36]
Russia	1	Cattle	*H. longicornis*	[60]
Egypt	1, 2	Cattle	Unspecified	[56]
	2	Water buffalo	Unspecified	
Kenya	3, 5	Cattle	Unspecified	[58]
United Kingdom	3	Cattle	*H. punctata*	[1, 8]
Italy	1, 3	Cattle	*R. bursa*	[62]
Hungary	Unspecified, PCR of 18S rRNA was done to identify presence of *T. orientalis*	Cattle	*H. punctata*	[65]
Portugal	Unspecified, RLB assay was done to identify presence of *T. orientalis*	Cattle	*H. punctata*	[64, 81]

Country	*T. orientalis* MPSP genotypes	Host species	Vectors	References
Spain	Unspecified, RLB assay was done to identify presence of *T. orientalis*	Cattle	*Haemaphysalis* spp.	[63]
Greece	Unspecified, IFAT—Indirect fluorescent antibody test for *T. orientalis* antigens	Cattle	*H. punctata*	[61]
Brazil	1, 2, 3, 4, 5, 7, N2 ,N3	Cattle	*R. microplus*	[1]
	Unspecified	Water buffalo	*R. microplus*	[66]
USA	6	Cattle	Unspecified	[1, 10]
Ethiopia	1, 2, 3, 5	Cattle	Unspecified, but *T. orientalis* DNA found in *Amblyomma* and *Rhipicephalus* species	[59]
Iran	Unspecified	Cattle	*H. punctata*, *H. longicornis*	[55, 82]
Turkey	1, 3	Cattle	*Hyalomma excavatum*, *R. annulatus*	[54, 62]
Central Africa	Unspecified	Cattle	*A. variegatum*	[57]

Majority of the unspecified vectors were suggested to be Haemaphysalis spp. MPSP genotypes Type 1 = Chitose, Type 2 = Ikeda, Type 3 = Buffeli. The other eight genotypes (4–8 and N1–N3) have yet to be named.

Table 1.
The global distribution of T. orientalis MPSP genotypes reported in four different host species and the possible transmission vectors.

cattle from Japan [86, 87]. Surveys of cattle in New South Wales (NSW, Australia) performed in the mid-20th century revealed the presence of *T. orientalis* in 60% of examined blood smears [42, 86] and later studies found herd and individual animal seroprevalence of 75% and 41% respectively in endemic parts of Queensland [42, 87]. The parasite was considered to be relatively benign as it caused only mild anaemia [42]. Prior to 2006, reports of clinical theileriosis in Australia were rare and experimental transmission studies were unable to establish clinical infection in test animals, suggesting that Australian strains of *T. orientalis* were of the benign Buffeli genotype [8, 22, 42, 88]. Samples from cattle imported into Japan from Australia were shown to be positive for the Chitose genotype by MPSP restriction fragment length polymorphism (RFLP), showing evidence that Chitose was present in Australia prior to 1998 [31]. However, since 2006, there was a large increase in clinical *T. orientalis* outbreaks in coastal and highlands regions of NSW [44, 89] and other parts of Australia such as Queensland [43], Victoria [90, 91], Western Australia [92] and South Australia [93, 94] (**Figure 1A**). Most clinical theileriosis outbreaks were linked to the movement of periparturient cattle from inland areas to the coast and the introduction of naïve cattle into endemic areas and/or introduction of infected cattle to *T. orientalis* non-endemic areas [2, 42, 89]. Large scale surveillance efforts identified the Ikeda genotype as the sole infecting type or as a mixed infection with other genotypes in all herds examined [43–45, 90].

T. orientalis was first reported in New Zealand in 1982 [95] with suggestions that the parasite could have been introduced through the importation of cattle from Britain or Australia where the parasite was prevalent. Prior to 2012, the Ikeda genotype was not associated with clinical theileriosis in New Zealand. Since then outbreaks of *T. orientalis* of the Ikeda genotype have been reported in beef and dairy

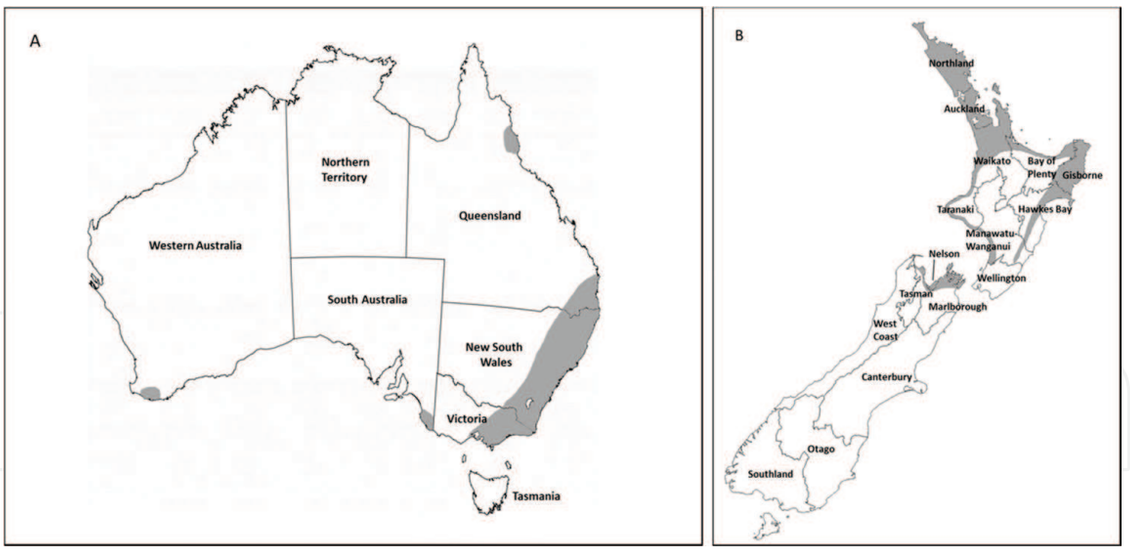

Figure 1.
Map of Australia (A) and New Zealand (B) showing the extent of spread of theileriosis during the recent disease incursions in each respective country. The areas in which T. orientalis Ikeda is enzootic closely mirrors the distribution of the vector tick H. longicornis.

cattle herds in multiple regions of the North Island [96, 97]. In 2012, genotyping tests conducted on affected cattle herds of *T. orientalis* outbreaks further revealed three other genotypes present, Chitose, Buffeli and Type 5 [97]. Of the four genotypes, Ikeda was identified to be more pathogenic than Chitose and Buffeli in New Zealand [67]. Prevalence and spatial distribution studies showed *T. orientalis* Ikeda to predominantly occur in the Northland (33 out of 35 herds; 94%) and Auckland and Waikato regions (63 out of 191 herds; 33%) where the transmission vector, *H. longicornis* is known to occur [96, 98] (**Figure 1B**). Only 2 out of 204 (1%) herds tested positive for *T. orientalis* Ikeda in the South Island of New Zealand where the distribution of *H. longicornis* is sparse and less common [96, 98].

3.2 Global distribution of *T. orientalis*

The geographic distribution of *T. orientalis* MPSP genotypes was previously reviewed by [1]. Since then, new clinical cases have been reported in Ethiopia [59] where *T. orientalis* was not known to occur, Type 5 was identified in cattle in Kenya [58], Type 2 Ikeda was recently identified in Vietnam via cattle imported from Australia [3], and studies in Kerala, India, revealed for the first time that MPSP genotype N2 to cause clinical theileriosis in Asian water buffaloes [53]. The majority of molecular distribution studies are based on the genetic characterisation of the *T. orientalis* MPSP gene. Some studies utilise other molecular markers such as the ITS 1, ITS 2, COX III and 18S rRNA genes to identify or characterise the parasite [46, 66, 99]. Studies based on molecular markers other than MPSP could not accurately classify MPSP genotypes, therefore, the identity of the MPSP genotypes found in some studies remain unclear [1].

As described above, most studies have implicated *T. orientalis* Ikeda (Type 2) in oriental theileriosis outbreaks [29, 43, 46, 68]. However, some studies have suggested MPSP genotypes Chitose (Type 1) [46, 74] and 7 [38] to be associated with clinical disease. The clinical relevance of these genotypes cannot be confirmed as COX III and 18S rRNA genes were used to characterise the samples instead of the MPSP gene in one study [46] or the possibility of mixed infections with Ikeda genotype was not investigated [1, 38, 74]. Nonetheless, Type 7 is phylogenetically related to the Ikeda genotype [1], and may indeed represent a pathogenic genotype

and should be the subject of further study. MPSP genotype N2 seems to be predominant among water buffalo populations although it has also been reported in cattle in Brazil. Type N2 was identified to cause fatal oriental theileriosis in Asian water buffaloes [53] but its virulence against cattle and other animals is unclear. Further distribution studies are required in order to determine host specificity of type N2. Cross-infection profiles between host animals in different countries may vary. For example, in India, Types 1, 3 and 7 are found in cattle and only type N2 is found in water buffaloes. But, in Thailand, Types 1, 3, 5, 7 and N3 can be found in both cattle and water buffaloes [47, 75]. This suggests that the tick vectors of a specific region may display host specificity limiting transmission to the preferred host or the tick vectors may have different preference for different genotypes. Previously, studies on *T. parva* have demonstrated that different tick populations have different preference for particular genotypes [39]. Whether this holds true for *T. orientalis* remains unclear, and warrants further investigation.

3.3 Vectors of *T. orientalis*

Although, the ixodid tick, *Haemaphysalis longicornis*, is considered to be the principal vector of *T. orientalis* [5, 67, 89, 94], the parasite has been detected in other arthropods such as mosquitoes [100] and lice [94, 101, 102]. Several studies have also revealed several possible tick vectors other than *H. longicornis* (**Table 1**). Prior to the recent Australian *T. orientalis* outbreak, *H. bancrofti* and *H. humerosa* [103–106] were found to be more competent and efficient vectors compared to *H. longicornis* under experimental conditions, although it is noted that the *H. humerosa* used in these studies were latterly believed to be *H. bremneri* [106, 107]. These studies employed the 'Warwick stock' of *T. orientalis* which is of the Buffeli genotype. Interestingly, the extent of spread of clinical theileriosis in Australia (**Figure 1A**) caused by *T. orientalis* Ikeda corresponds very well to the known range of *H. longicornis* rather than to that of *H. bremneri*, *H. bancrofti* or indeed, *H. humerosa* Furthermore, studies on a range of tick species collected from the Gippsland region of Victoria, within the theileriosis endemic zone, only detected the presence of *T. orientalis* in *H. longicornis* [94]. Similarly, in New Zealand, disease is only detected within the known range of *H. longicornis* (**Figure 1B**) and indeed, *H. longicornis* is the only *Haemaphysalis* tick present in that country [98]. In parts of Australia, *T. orientalis* Buffeli and Chitose are known to occur outside the areas in which disease in enzootic and outside the known range of *H. longicornis.* Together, these findings suggest that different ticks transmit different genotypes of *T. orientalis* with different efficiencies or that the tick species displays variable selection for the different genotypes. In *T. parva*, particular genotypes have been shown to be favoured when passaged through different tick clones, suggesting that these genotypes are selected for in tick vectors [39]. Also in China, *T. sinensis* is limited to the surrounding regions of the Tibetan plateau [108] as the vector *H. qinghaiensis* is limited to this region [6]. Indeed, recent genome sequencing studies revealed that the Ikeda, Chitose and Buffeli genotypes are sufficiently divergent to be considered different species or subspecies [37] and therefore may be adapted to different tick hosts. Vector competency for the different genotypes aside from *T. orientalis* Buffeli [87, 104, 105] have not yet been investigated in detail.

Currently, information on tick species transmitting disease is somewhat confounded because the vector competency for the different genotypes has not been thoroughly investigated. In Japan, *H. megaspinosa*, *H. douglasi*, *I. persulcatus* and *I. ovatus* have been identified as other potential vectors of *T. orientalis* [5]. Additionally, these four ticks were found to preferentially transmit the pathogenic

T. orientalis Ikeda [42]. In Europe, *H. punctata* seems to be the predominant tick vector to transmit *T. orientalis* [8, 61, 64, 65, 81], but in other geographical locations such as East Asia [5, 6], Australia [94] and New Zealand [98], *H. longicornis* is identified as the predominant tick vector. Although only limited molecular surveys have been undertaken in Europe, *T. orientalis* Ikeda has not been identified in this region. The specific relationship between type Ikeda and *H. longicornis,* in Japan [5], China [6], Australia [94] and New Zealand [98] where the Ikeda genotype is limited to *H. longicornis* distribution, combined with the absence or sparse distribution of *H. longicornis* in Europe [109], suggests that the Ikeda genotype may have a specific relationship with *H. longicornis*.

The epidemiology of *T. orientalis* is important as it enables researchers to understand distribution patterns and set up appropriate biosecurity measures. It is clear that there are gaps in the current knowledge of *T. orientalis* transmission and distribution. Further research is essential to identify potential tick vectors that may preferentially transmit certain MPSP genotypes of *T. orientalis*. Molecular characterisation and investigations of the MPSP genotypes coupled with whole genome studies could provide insights on the pathogenicity and genetic diversity, therefore enabling the implementation of efficient control strategies against this emerging disease agent.

4. Lifecycle and transmission

Evidence suggests that *H. longicornis* is a major vector of *T. orientalis*. This species is a three host tick meaning that each life stage of the tick will feed on a different host before each moult. *H. longicornis* parasitises cattle and other domestic ruminants [98, 110] and it undergoes obligate parthenogenesis to reproduce, as the adult female is able to lay fertile eggs in the absence of a male [111]. The three host lifecycle of *H. longicornis* has four life stages, an egg, larvae, nymphal and adult stage. Eggs hatch 30–90 days after being laid. The hatched larvae begin questing for its blood meal by climbing vertically on blades of grass to seek a host. *H. longicornis* have enhanced survivability as they are not specific in feeding even though they have a preference for cattle. Each engorgement occurs for 3–4 days before the tick falls to the ground and moults to the next stage.

In the tick vector, the *Theileria* lifecycle begins with blood engorgement on a mammalian host during which infected erythrocytes containing piroplasms are ingested by the tick. These piroplasms differentiate into gametocytes in the midgut of the tick and undergo a brief sexual stage to form zygotes that enter the gut epithelial cells. Motile kinetes are developed by meiotic division within the gut epithelial cells. Following meiosis, the parasite escapes into the haemolymph during the tick moulting phase and migrates to the salivary glands where sporogony occurs. *Theileria* kinetes invade salivary cells, develop into sporoblasts, and then into infectious sporozoites which are injected into the mammalian host when the moulted tick feeds again [112]. Sporozoites are inoculated into the mammalian host through the hypostome of the feeding tick. In *T. parva* and *T. annulata*, sporozoites invade the mammalian host leukocytes to develop multinucleate syncytial schizonts. At this point *Theileria* spp. can be separated into two evolutionary groups based on their ability to transform host leukocytes leading to clonal expansion of infected lymphoid cells [113]. Unlike *T. parva* and *T. annulata, T. orientalis* does not transform the invaded leukocytes. The schizonts undergo merogony to develop merozoites and rupture the leukocytes to invade the erythrocytes and form piroplasms [114]. When the tick feeds on the infected mammalian host, the *T. orientalis* lifecycle is completed.

Transmission of *T. orientalis* in the tick is transstadial, as the parasite can be transmitted from one instar to the next. Ticks that ingest erythrocytes infected with piroplasms transmit the parasite when they moult to the next instar [115]. Transovarial transmission, parasite transmission from adult female to the next generation of eggs, has yet to be scientifically demonstrated [103] by any transmission studies although some researchers have speculated that *Theileria* might involve transovarial transmission in ticks [116, 117].

Interestingly, *T. orientalis* infection dynamics varies depending on the genotype transmitted. A study on *T. orientalis* temporal dynamics in 10 animals revealed that Ikeda was detected first when naïve animals are exposed to herds infected with a mix of Ikeda, Chitose and Buffeli genotypes [35]. Thus the Ikeda genotype possesses a shorter pre-patent period than the other two genotypes, which may be due to a faster growth rate, out-competition of the other genotypes, or perhaps more efficient transmission by the tick vector [35]. Similar observations were made in temporal monitoring of mixed Ikeda and Chitose infections in experimentally infected cattle in Japan [25, 118].

Transplacental parasite transfer from pregnant cattle to offspring through the placenta has been confirmed through molecular and serological methods for a range of *Theileria* spp. This mode of transmission has been demonstrated in species such as *T. annulata* [119] *T. equi* [120, 121], and *T. lestoquardi* [122]. Transplacental transmission also occurs in *T. orientalis* infection [71, 123, 124]. Early studies [123] used blood film examination to demonstrate that transplacental transmission occurs in calves but at a low rate of 5% (5/100 calves that are 1–2 days old). The authors also determined the parasitaemia of newborn calves and post-grazing calves to be similar and suggested the low levels of parasitaemia in newborn calves to be ineffective in producing immunity against *T. orientalis* [123]. In contrast, 100% of the calves (n = 5) from experimentally infected dams were demonstrated to be *T. orientalis* positive and infected dams sometimes aborted the calves (two out of five dams) at approximately 6–7 months of gestation [71]. However, the dams in the study had an extremely high tick burden of approximately 200 ticks which had been artificially fed on cows with high parasitaemia [71]. In contrast, another recent study in New Zealand [125] did not detect transplacental transmission despite using sensitive molecular techniques. Recently, an Australian study [124] used molecular methods to confirm transplacental transmission of *T. orientalis* in field-affected cattle, but at low rate of approximately 2% (2/98 calves) similar to the study of [123]. In that study, abortion did not appear to correlate with transplacental transmission of *T. orientalis*, instead the authors posited that, abortion may occur due to hypoxia in the foetal calves due to maternal anaemia, placental insufficiency, or other factors related to maternal pathology [123].

In addition to ticks, *T. orientalis* can also transmit mechanically through the inoculation of infected blood [8, 101] or via other biting arthropods such as the sucking louse (*Linognathus vituli*) [102, 126] and potentially the horse flies (*Tabanus trigeminus*) and stable flies (*Stomoxys calcitrans*). These biting arthropods have been hypothesised to be able to mechanically transmit *T. orientalis* through the proboscis of the biting flies or regurgitation of blood into the animal host [101, 102, 126]. In Australia, *Theileria* DNA was not detected in March flies (*Dasybasis* sp.) collected in outbreak regions in Gippsland, Victoria [94]; however, *T. orientalis* was detected in mosquitoes collected from the same area. In addition, a xenosurveillance study in the United Kingdom has revealed *T. orientalis* in 16 out of 105 (15.2%) blood meals in mosquitoes [100]. The risk of transmission by mechanical vectors is likely to be dependent on the parasitaemia of the infected blood being transferred by these biting arthropods [101].

Mechanical transmission through routine husbandry practices is another potential method of *T. orientalis* transmission. A recent Australian study showed that *T. orientalis* could be mechanically transmitted with volumes as low as 0.1 mL of blood and persist for at least 5 months in the infected bovine after blood inoculation [101]. Thus, injuries sustained during yarding and transport of cattle, or routine husbandry procedures such as vaccination, blood transfusion, castration or ear notching performed where contaminated instruments are re-used can result in iatrogenic transfer of *T. orientalis* infection. Aside from blood transmission, there is potential for mechanical transfer of the parasite via the oral route. Dam to calf transfer of the apicomplexan *Neospora caninum* has been suggested to occur via the colostrum with pathogen entry via the oral mucosa. Recent findings that *T. orientalis* is present in colostrum raise the possibility that a similar mode of transfer may be possible by this species in calves, although this is yet to be confirmed [101].

Although there is now clear evidence from a number of studies that *T. orientalis* can be transmitted mechanically, including by haematophagous arthropods, this mode of transmission would not be expected to maintain the parasite life cycle. Mechanical transfer bypasses the sexual stage of the lifecycle where genetic recombination occurs. The direct transfer of haploid stage piroplasms from one host to another may result in reduced genetic diversity, a feature of apicomplexans which facilitates immune evasion [127–129]. Thus, mechanical transmission of *T. orientalis* may allow the organism to persist in the herd when tick numbers are low, but passage through the tick is likely to be important for the overall survivability of the parasite [101].

Although different forms of *T. orientalis* transmission have been identified, more research is required in order to increase awareness and formulate efficient control and preventive strategies to reduce disease incidence and stress on livestock.

5. Pathogenesis

Unlike *T. parva* and *T. annulata* that transform host leukocytes leading to fatal lymphoproliferation [130–132], the major pathogenic effect caused by *T. orientalis* is through the destruction of host erythrocytes and subsequent anaemia. Schizonts can be detected transiently in the lymph nodes, spleen and liver of infected cattle approximately 10 days post-inoculation with sporozoites [132]. However, schizonts in *T. orientalis* are rarely associated with major pathogenic effects as the schizont-infected cells are not commonly found in the peripheral blood [132]. Piroplasms can be detected in the host erythrocytes approximately 10 days post-inoculation and anaemia develops approximately 10 days later following detection of piroplasms when parasite load and serological response peaks [133]. Host animals sometimes also experience transient pyrexia and reduction in white blood cell count as anaemia develops [132, 134]. Animals that have been immunologically exposed to *T. orientalis* have lower parasitaemias and recover from infections earlier and with less morbidity. However, the haemoparasites can persist in the host, potentially until death, and can cause relapse through the resumption of piroplasm proliferation when animals face stress from pregnancy, lactation or rapid changes of environmental or rearing conditions [3, 132].

The pathogenic effects of anaemia consequent to infection although not well established [135]; have been studied extensively. Splenic capture of erythrocytes is likely the primary cause of anaemia rather direct lysis of erythrocytes by the pathogen [133]. In malaria infection, splenic clearance of both infected and uninfected erythrocytes is known to occur and may be the consequence of activation of splenic

macrophages or altered red pulp resulting in an increase in mechanical erythrocyte retention [136]. Yagi et al. [137] demonstrated that survival of both infected and uninfected erythrocytes decreased in *T. orientalis* infected calves and suggested that denaturation of blood plasma may play a role in this reduced survivability as reported for other protozoan infections [138–140]. Studies of *T. annulata* have demonstrated that anaemia might be an immune-mediated process as indicated by the presence of a haemagglutinin [141]. However, in *T. orientalis* infection the destruction of erythrocytes can occur in the absence of immunoglobulin or the involvement of complement [142]. Oxygen radicals released from the lysed erythrocytes may also play a role in pathogenesis as observed for *Plasmodium* infections [140]. Indeed, [143] demonstrated the development of anaemia in association with elevated levels of methemoglobin, a product of haemoglobin oxidation. Oxidative damage of erythrocytes occurs when superoxide radicals are released simultaneously to the increased levels of methemoglobin which may result in their removal from circulation by the reticuloendothelial (mononuclear phagocyte) system [132, 143].

As described in detail in Section 3, the pathogenicity of *T. orientalis* is genotype-dependent unlike the transforming theilerias *T. parva* and *T. annulata* [1]. However this may reflect the fact that the *T. orientalis* genotypes display species-level divergence [37] and pathogenicity of *T. orientalis* Ikeda may be driven by as-yet unidentified virulence factors.

6. Clinical disease, infection dynamics and the immune response

In the early stages of clinical oriental theileriosis, signs of muscle weakness, ataxia, and abortion are observed in infected animals. A variety of clinical findings such as the lack of appetite, pyrexia, elevated heart rate, abnormal breathing, pale mucous membranes and jaundice have been reported [89]. Aggression in clinically affected animals has occasionally been observed and may be caused by the alteration of mentation as a result of cerebral hypoxia [89]. All of these symptoms are a result of the anaemia in the host animal. Identification of anaemia can be achieved by measuring haematocrit (packed cell volume), which in severely infected cattle can be as low as 8% [144]. In *T. annulata* infections, bovine cerebral theileriosis associated with aggression was identified as a result of lymphocytic proliferation and blood vessel inflammation [145].

In *T. orientalis* both clinical and subclinical infections are known to frequently occur as a combination of genotypes [29, 42, 44, 146]. In Japan and Australia, *T. orientalis* Ikeda occurs with Chitose genotypes at high frequency with or without the presence of benign genotypes [45, 90, 147] and surveys from the Eastern coast of Australia have revealed genotypes Buffeli and Chitose occur in most subclinical infections [43]. The Ikeda genotype has been linked with higher parasite load and is evident in 100% of the samples that are clinically infected with Ikeda only or a mixture of Ikeda and Chitose [146]. In the clinically mixed infections, semiquantitative data revealed Ikeda to be the dominant genotype (58%) [146]. Within the genotype Chitose, there are two subtypes, Chitose A and Chitose B [35, 146]. In clinical samples from Australia, Chitose A was been noted to commonly occur with Ikeda at a high frequency (approximately 95% of cases examined) and is often detected at high parasite loads, while Chitose B occurs with Ikeda at a lower frequency [35]. Whether Chitose A is contributing to pathogenesis remains unclear. Although, the genotype Chitose was suggested to be able to solely establish a clinical infection in New Zealand cattle [46], the cytochrome oxidase III and 18S rRNA genes rather than the MPSP gene were used to characterise the samples, therefore the genotype of the parasite involved in that study remains unconfirmed. Nonetheless, if the

cattle were naïve to Chitose genotype it is possible that this may have led to clinical disease. Regardless, the Ikeda genotype has been associated with recent clinical outbreaks in New Zealand [67, 96, 97]. Another Korean study [74] also suggested Chitose to independently establish clinical infection in cattle, but mixed infections were not accounted for in the study.

Higher susceptibility to clinical theileriosis is observed in association with cattle movements; especially where naïve cattle are newly introduced to an endemic area, and/or infected animals are introduced to a non-endemic area with competent vectors [43]. Naïve cattle become rapidly infected in the presence of infected vector ticks, with time to patency (as determined by qPCR) as early as 11 days post-introduction to an infected herd [35]. Overall parasite load peaks around 40 days post-introduction with the onset of anaemia occurring 8–10 days later, although drops in haematocrit commence at the onset of the patent period. Interestingly, in mixed infection with Ikeda and Chitose genotypes (with or without Buffeli genotype), the Ikeda genotype is detected first and also peaks first. Declines in Ikeda genotype are then followed by an increase in the Chitose suggesting a genotype switching mechanism which may be driven by the host immune response [35, 148].

Additional factors may drive disease susceptibility in cattle such as breed or the age of the animal. In Japan, beef cattle of the Wagyu breed have been reported as being less susceptible to clinical infections [149]. Although potentially a factor in disease susceptibility, the effect of age has not been well-studied. Some cases occurring in regions where adult cattle had previously been exposed *T. orientalis* reported calves at 6 to 14 weeks of age to have high mortality and severe morbidity [150, 151] which coincides with high parasitaemias which are consistently observed in calves from *Theileria*-endemic areas [124]. While MPSP antibodies are sometimes detectable in the colostrum of dams and appear to be transferred to calves [101], any passive immunity appears to be short lived, with antibodies undetectable in calves by 4 weeks of age [124]. Lack of protection from maternal antibodies likely explains the high infection intensities and clinical disease observed in calves.

In adult cattle, seroconversion to the MPSP occurs approximately 14 days after patency and humoral responses to this protein persist for at least 11 weeks post-infection [133]. However, a study of 256 *T. orientalis*-infected animals showed that humoral responses to the MPSP are much more frequently observed in animals experiencing clinical anaemia (89%) versus those with subclinical infections (45%). It is unsurprising therefore that seroconversion to the MPSP is also strongly correlated with both parasite load and the Ikeda genotype [133]. Another study demonstrated that humoral responses to experimental infection with *T. orientalis* (via mechanical transfer) are variable and only established after persistent infection [152]. The role of humoral immunity in protecting against *T. orientalis* infection in adult cattle is unclear. Cell-mediated rather than humoral immunity is generally considered more important in responding to intracellular pathogens; however once established, humoral immunity may assist in preventing the pathogen from gaining cell entry, as for *Babesia bovis* [153]. Further work is required to determine whether animals that have experienced clinical theileriosis are immune to disease recrudescence and whether immunity against one genotype confers protection against another.

Studies of the transforming theilerias, *T. parva* and *T. annulata*, have shown that cattle that recover from infection are able to establish immunity against homologous strains but succumb to heterologous strains suggesting that immune responses are highly specific for particular parasite epitopes [154, 155]. Immunity is mediated via cytotoxic T lymphocytes (CTL) which target parasitized lymphocytes but allow parasitized erythrocytes to persist [129]. Thus the immune pathways important in protection against non-transforming theilerias such as *T. orientalis* may be more akin to those of *Babesia* species [133].

7. Diagnosis

Oriental theileriosis can be diagnosed by various methods such as microscopy, serology, molecular techniques and xenodiagnosis. Bovine erythrocytes are anucleate, therefore those infected with piroplasms can be visualised under a light microscope using DNA stains (such as Giemsa or Diff-Quik) [156]. In carrier-state animals, erythrocyte infections are commonly observed in the low parasitaemia range of 0.02–0.03% [85, 157]. Parasitaemia in clinically affected animals suffering severe anaemia and other related clinical signs may range from >1–30% [46, 89]. Light microscopy is a quick and inexpensive method for the initial differential diagnosis of possible clinical theileriosis [89]. It has been used to describe many of the first species of *Theileria* after Koch's [158] initial description of *T. parva* [159]. However, the technique is limited as a diagnostic tool as it is considerably less sensitive than PCR and does not enable the differentiation of morphologically similar piroplasms [160, 161]. The differentiation between similar piroplasms is important to distinguish the clinically important species such as *T. parva; T. annulata* and *T. orientalis* from other less clinically significant species such as *T. taurotragi* and *T. mutans.* Light microscopy is unable to differentiate between pathogenic and apathogenic genotypes of *T. orientalis*. Furthermore, light microscopy lacks the sensitivity to adequately detect clinically-benign carrier animals [45, 159].

While a number of serological tests exist for the detection of *T. orientalis* [29, 87, 133, 152, 162], these assays are currently not genotype-specific and in some cases also cross-react with other *Theileria* species [152, 162]. Serological tests are of a similar sensitivity to blood smear examination and are most reliable when the animals are clinically affected, but are unsuitable for testing newly infected animals that have not yet seroconverted [133]. Currently, serological methods do not offer any advantage over molecular methods for determining whether animals have been exposed to *T. orientalis* since this organism establishes lifelong infections and can be detected in the blood well beyond the initial infection period.

PCR is currently the gold standard for sensitive detection of *T. orientalis* [133]. PCR can detect infection in cattle up to 2 weeks before the infected erythrocytes can be observed under a light microscope [29]. Conventional PCR methods have high sensitivity and have been validated for diagnostic use [43–45, 69]. However, conventional PCR assays are laborious to perform and do not provide information on parasite load making it impossible to distinguish between clinically infected animals and subclinical carriers. To address these problems, a number of real time semi-quantitative and quantitative PCRs have been developed for the detection of *T. orientalis* [146, 163–166]. The majority of these assays have been designed to specifically detect the pathogenic Ikeda genotype [93, 146, 163, 164, 166] and in some cases several genotype specific assays have been multiplexed [146, 163, 166]. Genotype discrimination has been most successfully achieved using assays targeting the MPSP gene [146, 163] while some other molecular markers have been shown to be insufficiently discriminatory [167].

The high prevalence of subclinical carrier animals infected with clinically-relevant genotypes [43] makes accurate quantification critical to correct diagnosis, particularly in the presence of confounding factors. In order to address this, a TaqMan probe-based assay targeting the *T. orientalis* MPSP was used to establish clinical thresholds for disease to facilitate diagnosis [146]. Using this assay, animals with *T. orientalis* gene copy numbers above 300,000 are highly likely to display clinical signs; while those with gene copy numbers below 15,000 are considered subclinical carriers. Cattle with gene copy numbers between 15,000 and 300,000 are frequently clinically affected but may also be recovering from disease or in-contact with clinically affected cohorts [146].

8. Treatment and control of *T. orientalis*

8.1 Chemotherapy

The increase in oriental theileriosis outbreaks in recent years highlights the need for effective treatment and control measures for this disease. Chemotherapy remains an important strategy in combating protozoan diseases [168]. Chemotherapeutics such as imidocarb, oxytetracyclines and halofuginone have been used to treat oriental theileriosis [2]. In Australia, imidocarb and oxytetracyclines are some of the registered chemicals which in some studies, appeared to have a positive response on cattle with low parasitaemia, but a poor response in severely infected cattle [169]. Menoctone, a hydroxynaphthoquinone compound was discovered to have anti-theilerial properties [170] and two active analogues, parvaquone [171] and buparvaquone [172] were developed shortly thereafter; which treated *Theileria* infections in cattle with high efficacy [173]. Total elimination of *T. orientalis* infection was achieved in splenectomised calves by a chemical mixture of primaquine and buparvaquone, or primaquine with halofuginone [174]. In Japan, buparvaquone was demonstrated to be effective enough to be used as a single chemical treatment [175]. A single intramuscular injection dose of 2.5 mg/kg buparvaquone was sufficient to treat the Buffeli, Chitose and Ikeda genotypes [2, 176]. In contrast, imidocarb was identified to have little effect on *T. orientalis* infection [177]. Prior to 2010, buparvaquone resistance in *T. annulata* has never been documented [173]. However, in *P. falciparum* and *Toxoplasma gondii*, resistance against atovaquone, a hydroxynaphthoquinone compound, was well documented to be caused by the mutation of the mitochondrial cytochrome b gene [178, 179]. The mode of action of buparvaquone in *T. orientalis* is not well established, but a study in coccidian parasites suggests an effect on the generation of energy [180]. While buparvaquone treats *Theileria* infections with great efficacy when used in the early stages of disease, resistance observed in apicomplexan infections are a growing concern and is a problem with chemotherapeutic agents in general.

An Australian study [169] showed that treatment with buparvaquone leads to the retention of residues in cattle tissue. The tissue residues were present up to 147 days post treatment with buparvaquone and as such this chemotherapeutic has long withholding periods and has not been approved for use at all in Australia. Previously in Japan, chemicals such as pamaquine and primaquine phosphate were commonly used treat *T. orientalis* infections but due to declining efficacy, its usage was discontinued [177]. This declining efficacy further revealed the inefficacy of primaquine phosphate to eliminate *T. orientalis* alone [174]. It requires a combination of chemicals as discussed above to successfully eliminate *T. orientalis* infection. As such, chemotherapy options have been limited due to the variations of drug efficacy. The development and identification of chemical compounds suitable for the treatment of *T. orientalis* is important, however, drug discovery is both time consuming and expensive. There are other important preventive measures worthy of investigating such as the control of competent vectors and management of animals that can facilitate the reduction of *T. orientalis* outbreaks.

8.2 Vector control and animal management

Vector control is important to reduce the rapid spread of *T. orientalis* outbreaks. Restriction of grazing cattle movements may assist in reducing exposure to infected *H. longicornis* ticks. Control of this vector can also be achieved by using acaricides such as multi-seasonal pour-on flumethrin [83]. This method has been successfully

demonstrated in Japan to reduce *T. orientalis* infection [83], but it is not permitted for use in Australia due to the possibility of unacceptable residues [181]. Currently, the acceptable methods of tick control in Australia are the application of synthetic pyrethroids in the form of short-acting dips and sprays that can contain amitraz, cypermethrin with chlorfenvinphos and deltamethrin with ethion [2, 181]. The usage of these cheap and common acaricides although economic (A$0.50–A$1.50 per head), might lead to resistance in the tick vectors, which in the long run will incur higher cost due to the requirement for more expensive macrocyclic lactones (approximately A$600 per treatment for a 100-cow herd) for tick control [182]. *H. longicornis* as described above is a three-host tick; therefore it is a challenge for these acaricides to be effective at controlling this species due to a limited host attachment period.

An alternate method to control *T. orientalis* transmission by ticks would be the development of a vaccine that targets exposed antigens of the tick [132]. Currently, there is a commercialised vaccine against *B. microplus* [183] and similar attempts have been made by utilising tick saliva proteins (p29, p34 and p35) against *H. longicornis* to produce a vaccine [184, 185]. The immunised animals when exposed to ticks, display interference that reduce tick growth and increase mortality of the ticks.

Besides controlling the tick vectors, proper management of animals can also reduce *T. orientalis* infection or re-infection. Infected animals are susceptible to relapses when faced with stress factors as discussed above. Supportive therapy such as blood transfusion can be performed to improve the anaemic conditions in affected animals; however, these therapies are time-consuming and expensive and may only be practical to treat valuable stud animals. Animal movement should be kept to a minimum to prevent elevated blood pressure which can cause the animal to collapse [186]. Intravenous fluids and nutritional supplements may also benefit affected animals [2] and intramuscular injection with iron dextran over the course of 3 days can aid recovery of infected animals [187]. The treatment and control of *T. orientalis* is multi-faceted and it requires all of the different elements discussed in order to be effective.

8.3 Vaccine development

Vaccination is viewed as the preferred method of control for oriental theileriosis. Unfortunately no vaccines currently exist for this disease; however, live vaccines for *T. parva* and *T. annulata* have been successfully used to treat East Coast fever and tropical theileriosis for over 40 years. Vaccination with highly passaged macroschizont-infected cell lines is possible for *T. annulata* due to the stimulation of immunity with low doses of attenuated cells which do not induce clinical disease. In contrast, for *T. parva*, the doses required to stimulate an immune response also induce clinical disease, therefore vaccination against *T. parva* involves simultaneous vaccination with sporozoites (homogenised ticks) and treatment with long-acting formulation of oxytetracycline to suppress disease. Because vaccination with a single strain of *T. parva* leaves animals susceptible to heterologous challenge, immunisation involves a mixture of three isolates which provides broad protection against disease [188].

The vaccination strategy employed for *T. annulata* is not directly transferrable to *T. orientalis* due to the non-transforming nature of this species and a lack of cultivation methods for this organism. The "infect and treat" method used for *T. parva* has potential promise for control of *T. orientalis* but is currently somewhat limited by a lack of suitable chemotherapeutic agents for parasite suppression.

Vaccination against tick fever, caused by the closely related piroplasmids, *Babesia bovis* and *B. bigemina,* also employs live attenuated organisms and is administered

to calves between 3 and 9 months of age when they are less susceptible to disease. This vaccination strategy has not been attempted with *T. orientalis* but unlike for tick fever, calves are highly susceptible to oriental theileriosis [150, 151]. Nonetheless, live vaccination is still considered one of the most promising approaches for control of oriental theileriosis. It has been suggested that vaccination with benign genotypes of *T. orientalis* may provide cross protection against the pathogenic genotypes [189]; however recent genome studies suggesting that the differences between genotypes are at the subspecies or species level make this more doubtful [37]. Furthermore, despite a relatively high seroprevalence *T. orientalis* in Australia (due to the presence of benign strains), extensive outbreaks caused by *T. orientalis* Ikeda occurred across the entire range of the vector tick. Combined with data showing that infections with *T. orientalis* are usually of mixed genotype [45, 90, 146, 190], there is little evidence to suggest that vaccination with *T. orientalis* Buffeli, Chitose or other genotypes would provide cross protection against *T. orientalis* Ikeda.

Development of a subunit vaccine is another possible avenue for control of oriental theileriosis. Early studies showed that passive immunisation of calves with an anti-MPSP monoclonal provided protection against development of disease upon challenge [191]. Therefore the MPSP was selected for use in subunit vaccine formulations consisting of recombinant baculovirus-expressed MPSP or synthetic MPSP peptides (containing KEK motifs) mixed with Freund's adjuvant or encapsulated in mannan-coated liposomes. Following immunisation with these vaccine formulations, calves were splenectomised and challenged with Ikeda or Chitose sporozoite stocks. Animals immunised with high dose peptide or recombinant MPSP had reduced parasitaemias relative to control calves and were protected from clinical signs of oriental theileriosis [190]. Despite these promising preliminary results, a subunit vaccine for *T. orientalis* has not been pursued further. Subunit vaccines are generally considered problematic when working with apicomplexans due to genetic diversity among strains. Indeed, in their subunit vaccine trial Onuma et al. observed homologous rather than heterologous protection between *T. orientalis* MPSP genotypes [190]. Furthermore, antigens such as the MPSP which are immunogenic are also under immune pressure, resulting in genetic drift. These issues may be overcome by using multiple antigens in the subunit vaccine formulation or targeting antigens which are not normally immunogenic. A greater understanding of how the bovine immune system responds to *T. orientalis* is required before further work on vaccine development can meaningfully proceed. Despite the hurdles in developing a vaccine for *T. orientalis*, it remains a worthy goal given the ongoing burden that this disease imposes on cattle production throughout Asia and Australasia.

9. Conclusion

T. orientalis is an apicomplexan parasite of economic significance around the world to both beef and dairy industries. This review has highlighted several knowledge gaps surrounding oriental theileriosis from taxonomic uncertainties, vector preferences and treatment and control measures. Development of effective therapeutics or prophylactic measures such as vaccines remains a priority due to recent spread of oriental theileriosis into new areas across the Asia Pacific region. Advancements in whole genome sequencing technologies promise to provide new insights into the *T. orientalis* taxonomy, genetic diversity and the underlying mechanisms of pathogenesis, all of which underpin successful development and implementation of efficient control strategies against this emerging parasite.

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