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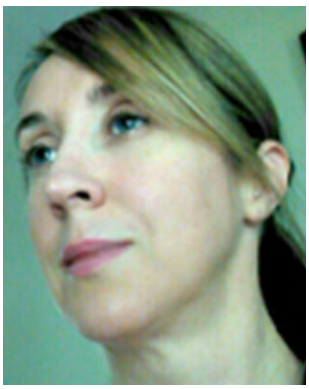
## Biography

**Professor Beaman** graduated from the University of Western Australia and trained in Clinical Microbiology and Infectious Diseases at Sir Charles Gairdner Hospital. He completed a Post Doctoral Fellowship at Stanford University under Professor Remington and then established the first Infectious Diseases Department in Western Australia at Fremantle Hospital. He joined Western Diagnostic Pathology in 2002, where he was Medical Director and Deputy CEO until recently. He is currently an Infectious Diseases specialist at Joondalup Health Campus in Perth.

# Bovine theileriosis in Australia: a decade of disease



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**Theileriosis refers to the clinical disease caused by organisms from the genus *Theileria*, tick-borne haemoprotozoans infecting a diverse range of mammalian hosts. In Australia, *Theileria* spp. have been identified in both domestic and wildlife species but the bovine parasite, *Theileria orientalis*, has received the most attention due to the emergence and spread of clinical disease over the past 12 years, particularly in cattle herds on the east coast. At an estimated $20 million per annum, the burden to cattle production is significant but despite over a decade of disease, there are still no effective chemotherapeutic treatments or vaccines available in Australia. Recent insights from genome sequencing studies reveal species level diversity within *T. orientalis*, which may help direct efforts at disease control.**

## Clinical presentation

*Theileria orientalis* is an apicomplexan parasite that requires both a bovine and a tick host in order to complete its lifecycle (Figure 1).

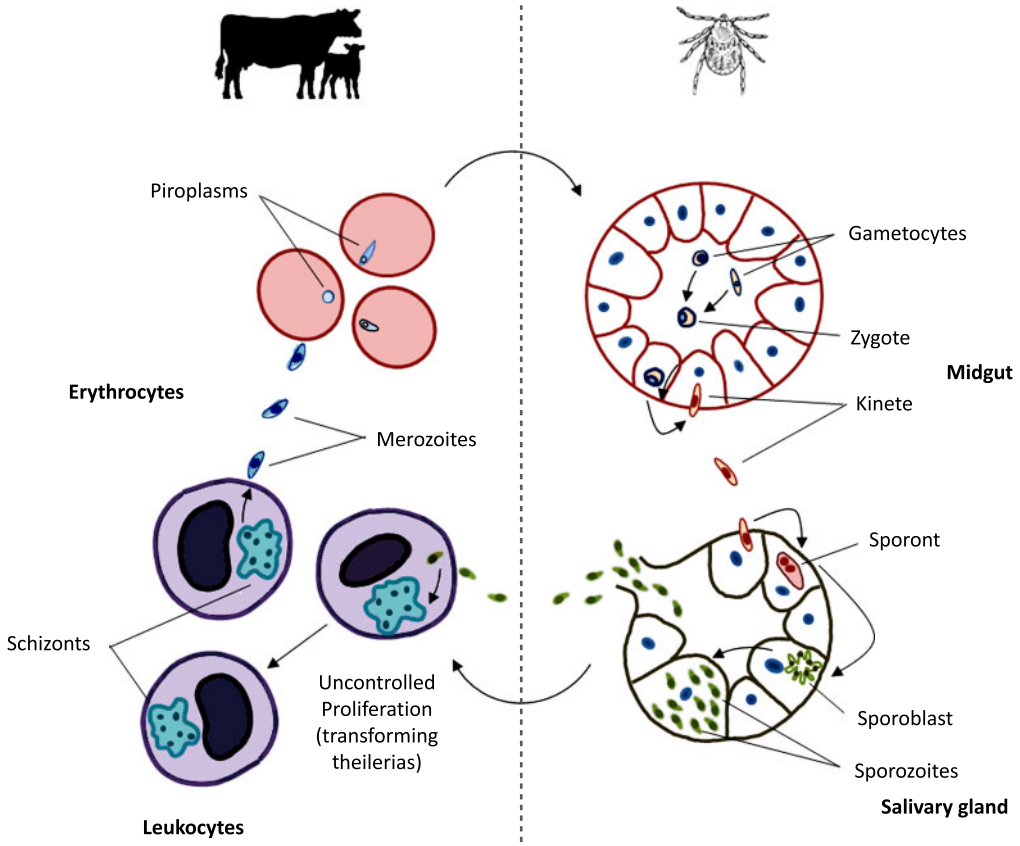


Figure 1. The theilerial intraerythrocytic (piroplasm) phase in the mammalian host is ingested by the tick as it feeds, with gametogenesis occurring in the tick midgut. *Theileria* gametocytes combine to form zygotes in a brief diploid stage within the tick gut lumen. Zygotes which have entered the gut epithelium undergo meiotic division to form motile kinetes which then migrate to the tick salivary gland acini where they differentiate into sporozoites. Sporozoites are the infective stage for the mammalian host and inoculation is achieved as the tick feeds. Sporozoites quickly invade the mammalian host's lymphocytes and develop into multinucleated schizonts. In some species of *Theileria*, known as the transforming theilerias, schizonts induce uncontrolled proliferation of the infected lymphocytes resulting in a lethal cancer-like state. In all *Theileria* species, whether transforming or non-transforming, schizonts go on to produce merozoites that invade erythrocytes to form the piroplasm phase, thus completing the lifecycle.

In Australia, bovine theileriosis is sometimes referred to as bovine anaemia caused by *Theileria orientalis* group (BATOG) to distinguish it from the more severe, exotic bovine theilerial diseases East Coast fever and tropical theileriosis (caused by *Theileria parva* and *Theileria annulata* respectively). Both *T. parva* and *T. annulata* are known as transforming theilerias in that they have their major proliferative stage within bovine leukocytes, inducing a lethal cancer-like state. While *T. orientalis* causes less severe disease than the transforming theilerias, this organism is nonetheless capable of causing up to 5% mortality in affected cattle herds. The major pathogenic effects of *Theileria orientalis* are elicited through the destruction of infected erythrocytes and subsequent anaemia. Therefore, the red blood cell phase (piroplasm), rather than the leukocyte phase (schizont) drives pathogenesis in this species. An enlarged spleen is frequently observed upon post-mortem, along with a large ochre-coloured liver and generalised jaundice[1] brought about by excessive bilirubin from broken down erythrocytes. Animals frequently present with symptoms related to underlying anaemia including lethargy, ataxia and an increased heart and respiratory rate.

## The epidemiology of theileriosis in Australia

Prior to 2006, infections with *T. orientalis* in Australian cattle were considered benign. The organism had been observed for over 100 years in blood smears but was considered an incidental finding with very few reports of clinical disease. Early serological surveys suggested the parasite was widespread in NSW and QLD but researchers were unable to induce clinical disease experimentally[1,2]. *T. orientalis* is currently classified into genotypes based on the sequence of the major piroplasm surface protein (MPSP). Up until 2006, MPSP genotypes identified in Australia were Buffeli and Chitose.

Between 2006 and 2008 theileriosis cases were reported in NSW cattle herds with a history of pregnancy or introduction to new herds. Animals presented with abortion, lethargy, jaundice and anaemia. Attention turned to *Theileria* as a cause due to the unusually high numbers of parasites observed in blood smears and after alternative causes of anaemia were ruled out. Follow up molecular testing revealed the presence of a new genotype, *T. orientalis* Ikeda, which was linked to disease in Japan[3]. We

undertook surveillance of a large number of cattle in Australia revealing that this genotype was associated with disease either as the sole agent or in mixed infections with Buffeli and Chitose genotypes[4]. Reports of BATOG increased substantially, and in the intervening years the disease spread throughout coastal NSW and Victoria, south east Qld and into isolated parts of SA, WA and far north Qld (Figure 2). New disease cases have consistently been associated with *T. orientalis* Ikeda.

## Immunity

Since 2015, incursions into new areas of the country ceased, although movement of naïve animals into areas where the disease is enzootic remains a major risk factor for disease. Subclinical infections with *T. orientalis* Ikeda are common. In areas where the disease is enzootic it is not unusual to find 100% prevalence in the absence of disease implying a level of immunity, although the immune mechanisms are poorly understood. Animals affected by clinical theileriosis usually seroconvert to the MPSP while subclinically infected animals often lack a detectable humoral response[5]. Calves acquire little protection from the dam via antibodies in colostrum and are highly susceptible to infection[6, 7]. While calves can sometimes be infected transplacentally, this does not appear to be a major route of transmission. Infection dynamics in calves are consistent with tick transmission and animals routinely become highly parasitaemic between 4–9 weeks of age[7]. Given the intracellular nature of the parasite, immunity is likely to be cell-mediated although the potential mechanisms behind this are yet to be explored.

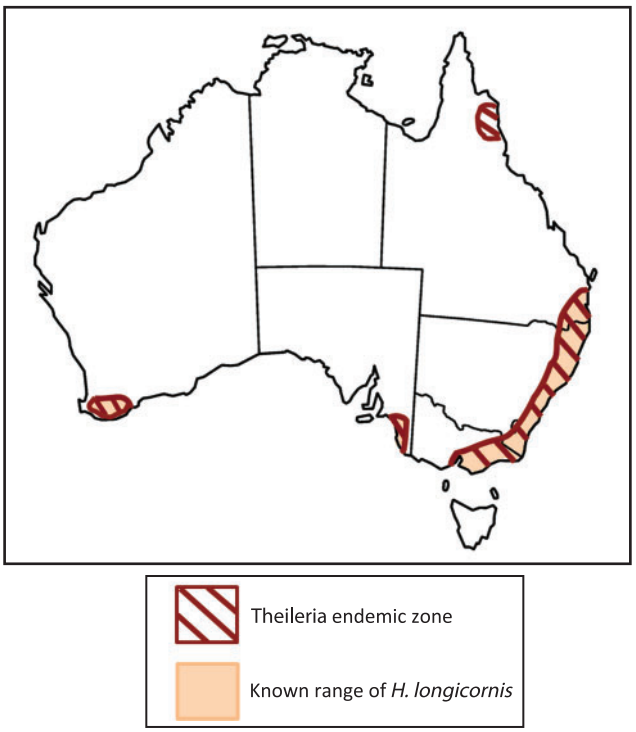


Figure 2. The *T. orientalis* endemic zone and the known range of *H. longicornis* in Australia.

## Transmission

A range of tick species have been implicated in transmission of theileriosis overseas, but members of the genus *Haemaphysalis* are considered the main vectors. Studies conducted in Japan demonstrated transmission of *T. orientalis* Ikeda with *Haemaphysalis longicornis*, which was introduced to Australia in the 19th or 20th century. Conversely, transmission work conducted in Australia in the 1980s demonstrated that *H. bancrofti* and *H. humerosa* (latterly believed to be *H. bremneri*) were competent transmitters of *T. orientalis*, while *H. longicornis* was not[2]. The likely explanation for this discrepancy lies in the fact that Japanese studies were conducted with *T. orientalis* Ikeda stock, while studies in Australia were conducted with *T. orientalis* Buffeli. To investigate this further we undertook sampling of ticks from cattle and other domestic and wildlife species within the endemic area, identified the tick species with DNA barcoding, and screened the mouthparts by PCR for *T. orientalis*. A total of 135 ticks were collected representing eight different species; however, only *H. longicornis* ticks tested positive for *T. orientalis*, lending further weight to *H. longicornis* as the likely vector for theileriosis in Australia[8]. Indeed the extent of disease spread in Australia is almost perfectly defined by the known range of *H. longicornis*, which prefers the wetter areas of east coast and is rarely found west of the Great Dividing Range (Figure 2). Small pockets of *H. longicornis* occur in the moist areas of southwest WA and southeast SA where *T. orientalis* Ikeda outbreaks have also been reported. Thus, while never directly demonstrated, the evidence overwhelmingly points to *H. longicornis* as the major vector for bovine theileriosis.

In addition to tick transmission, mechanical transmission can be achieved experimentally with as little as 0.1 mL of blood when parasite levels are high and can induce clinically relevant levels of *Theileria* in the recipient animal[6]. While merozoites can undergo several rounds of proliferation within erythrocytes, mechanical transfer (via non-tick arthropods or iatrogenic means) is unlikely to support parasite persistence in the long term as it bypasses the sexual stage of the lifecycle that is required to maintain genetic diversity within the population.

## Control

There are currently no effective chemotherapeutics or vaccines for the control of bovine theileriosis in Australia. Treatment of

(a)

| *T. orientalis* Ikeda (Australia) | *T. orientalis* Chitose (Australia) | *T. orientalis* Buffeli (Australia) | *T. parva* | *T. annulata* | |
|---|---|---|---|---|---|
| 99.2% | 82.6% | 82.6% | 66.1% | 66.0% | *T. orientalis* Shintoku (Japan) |
| | 82.8% | 83.1% | 66.1% | 66.1% | *T. orientalis* Ikeda (Australia) |
| | | 86.5% | 66.3% | 66.2% | *T. orientalis* Chitose (Australia) |
| | | | 67.0% | 66.9% | *T. orientalis* Buffeli (Australia) |
| | | | | 79.5% | *T. parva* |
| | | | | | *T. annulata* |

(b)

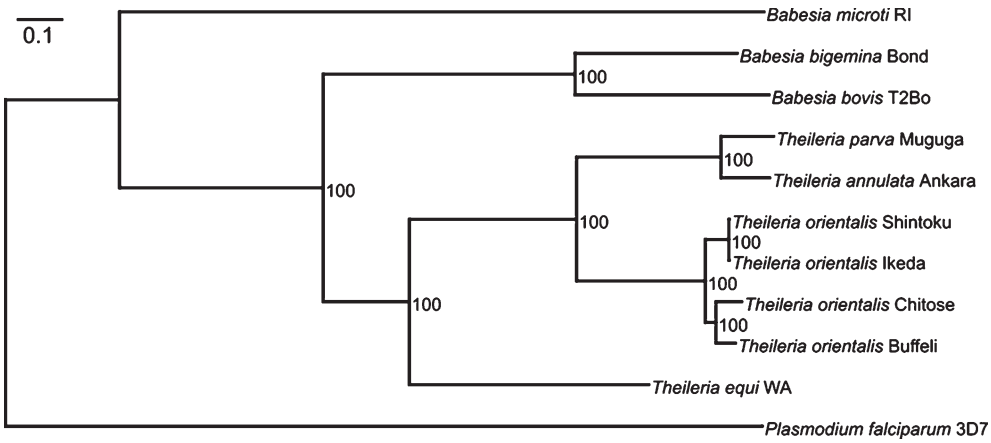


Figure 3. (*a*) Average nucleotide identity of key *Theileria* strains as determined from genome sequences. (*b*) Phylogenomic analysis of *T. orientalis* Ikeda, Chitose and Buffeli relative to reference strains, including *T. orientalis* Ikeda (Shintoku strain) from Japan, based on 654 protein coding genes. Figure adapted from Bogema *et al*.[13].

vector ticks via acaricides and minimising the movement of cattle from non-endemic to endemic areas are the main methods of disease management. Imidocarb, erythromycin or oxytetracycline are sometimes administered to affected animals, but to little effect[9]. In New Zealand, blood transfusion is regularly undertaken on animals that are moderately to severely anaemic, but this practice is costly and time consuming. Buparvaquone, a known anti-protozoal is used to treat BATOG in New Zealand and is also used to treat East Coast fever in Africa. When administered in a timely fashion, buparvaquone is effective against *T. orientalis*, yet this drug is not approved for use in Australia due to its tendency to leave residues in meat and milk[10] and the need to observe lengthy withholding periods. Vaccination would be the preferred option for disease control but there has been little progress towards a vaccine for this disease worldwide. Despite assertions that a live vaccine based on the benign Buffeli genotype would be a potential way forward[11], there is little hard evidence that this genotype provides protection in naturally infected animals. In other *Theileria* species, immunisation with one variant does not result in heterologous immunity against other variants. Furthermore, high seroprevalence of animals to *T. orientalis* Buffeli and/or Chitose in NSW prior to 2006[1] failed to prevent widespread outbreaks of disease caused by *T. orientalis* Ikeda. Development of subunit vaccines is generally regarded as problematic for apicomplexan parasites due to genetic diversity within parasite populations. Nonetheless some early work in Japan demonstrated partial protection against theileriosis using a subunit vaccine formulation of the Ikeda MPSP antigen with Freund's adjuvant or liposomes[12]. Despite these

initially promising results, no further vaccine development has been undertaken with this or with other antigens.

## Lessons from genome sequencing

Recent draft genomes of Ikeda, Chitose and Buffeli genotypes of *T. orientalis* may assist in providing insights into the differential pathogenesis of these subtypes[13]. Surprisingly, these genomes revealed potential species level diversity within *T. orientalis* with average nucleotide identities almost as low as observed between *T. annulata* and *T. parva* (Figure 3). Phylogenetic analysis of 654 protein-coding genes also showed that *T. orientalis* Ikeda forms its own lineage relative to *T. orientalis* Buffeli and Chitose, while the Japanese and Australian strains of *T. orientalis* Ikeda are remarkably similar[13]. The origin of *T. orientalis* Ikeda in Australia has never been elucidated although the importation of a small number of Wagyu breed cattle into Australia from Japan in the late 1990s has been proposed as one potential route of introduction. Genome sequencing of further international isolates of *T. orientalis* may lend weight to this theory. The origin of introduction is potentially highly relevant to the issue of vaccine development. If *T. orientalis* Ikeda in Australia arose from only a limited parasite population, then genetic diversity would be expected to be relatively low, making the prospect of developing a long-lasting vaccine for this disease more likely. Further genome-based studies are currently being undertaken to establish the genetic diversity within the Ikeda genotype in Australia.

## Conflicts of interest

The author declares no conflicts of interest.

## Acknowledgements

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## Biography

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