Australian Tick Vaccines

Subjects: Agriculture, Dairy & Animal Science
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Keywords: Australia ; tick vaccines

1. Introduction

Ticks are hematophagous (bloodsucking) arthropods, which have evolved with animal hosts. There are 70 known tick species in Australia, and of these, 14 are soft ticks (Argasidae), and 56 are hard ticks (Ixodidae). Sixteen species are known to feed on humans and domestic animals, while the remaining species feed on wild mammals, birds and reptiles (reviewed by Barker et al., 2014) [1]. The hard tick species, which have the most economic impact on livestock and companion animals in Australia, are *Rhipicephalus australis* (the Australian cattle tick, formerly *Rhipicephalus (Boophilus) microplus*) and *Ixodes holocyclus* (paralysis tick).

The Australian paralysis tick *Ixodes holocyclus* is an indigenous tick species and is distributed along the east coast of Australia . Unlike *R. australis*, *I. holocyclus* has remained the species name since it was first described by Neumann in 1899 [1]. Of the 892 species of ticks worldwide, approximately 27 species are evidenced as causing host paralysis, including two species in Australia, *I. holocyclus* and *Ixodes cornuatus* [4]. *Ixodes holocyclus* has been considered the most toxigenic tick species, producing a family of neurotoxins known as holocyclotoxins, which is currently the only toxin family to date described for a toxin-producing tick species [5]. Unlike the cattle tick *R. microplus*, which is a one host tick species specific to cattle to complete their life cycle, *I. holocyclus* has a three-host life cycle, including hosts, such as Australian wildlife, and humans, livestock and companion animals as primary and incidental hosts, respectively [2]. It has been reported that approximately 10,000–100,000 animals are affected annually, with 5% deaths in affected livestock and companion animal pets [3].

Approaches for the development of anti-tick vaccines have been quite variable, particularly for *R. microplus* and *R. australis* globally. One approach has been to target gut-associated 'concealed antigens', which are usually not recognised by the host during natural infestation; however, when used as a vaccine antigen, the host's antibodies attack the tick gut, disrupting the life cycle [4]. One disadvantage is that these vaccines require several boosts each year as tick challenge does not boost host responses to these types of antigens. Other vaccine approaches have focused on secreted salivary antigens, which conversely are assumed to be recognised by the host during the natural challenge and, thus, may potentially boost vaccine responses [5]. This is particularly relevant for tick species whose tick stages feed on their hosts for shorter periods, i.e., *I. holocyclus* [3][6]; however, these approaches have also been applied in the discovery of *R. microplus* antigens [5].

Australia has led the world in the development of anti-tick vaccines. The first anti-cattle tick recombinant vaccine was developed by researchers from Australia's National Science agency—the Commonwealth Scientific and Industrial Research Organisation (CSIRO) in the late 1980s [4]. Attempts to develop a canine anti-paralysis tick vaccine were also reported in the 1980s in Australia through research, also originally led by CSIRO [6]. This review reports Australian research capacity in the development of anti-tick vaccines throughout the 19th–20th Century with considerable efforts from the 1980s to date.

2. Review Approach

A search using the PubMed database was undertaken with the following terms: ((tick) AND (ticks)) AND (vaccine)) AND (Australia). This search returned 112 articles. Articles that were not related to the topic were deleted (Australian authors, un-related vaccines), resulting in 65 references. Several of these references were reviews, or out of scope for this review, or were published by the same group from which selected articles were highlighted. Following this scrutiny, 20 articles from this search were utilised in this review (See Supplementary Table S1). Other references were added to format the introduction, and together with other article leads (including international tick vaccine research and historic publications), this led to a total of 96 references for this review. These also included Meat & Livestock Australia reports as a relevant source of information available on the website (https://www.mla.com.au/research-and-development/search-rd-reports/ , accessed on 3 June 2021) and available online vaccine patents.

3. Tick Vaccine Research

Tick vaccine research in Australia has been supported since the 1980s for both the cattle tick and the Australian paralysis tick through several groups, see **Table 1**.

Table 1. Summary of tick vaccine research in Australia –the Australian cattle tick *Rhipicephalus australis* and the Australian paralysis tick *Ixodes holocyclus.*

Target Species	Group	Outputs	References
Rhipicephalus australis	CSIRO	Demonstrated protection from challenge using crude gut extracts; Isolation of gut protein Bm86 and demonstrated protection from challenge using native protein; Patent filing in 1985/1986, followed by commercial development and release of TickGARD® (1994) and TickGARD®*PLUS* (1995)	[21–23]
	UQ	Demonstrated protection from challenge using midgut membrane extracts; Demonstration that gut membrane extracts are protective compared to soluble antigens; Showed that IgG1 and complement antibodies correlated with protection	[24,25]
	Beef CRC/MLA	Reverse vaccinology pipeline was developed to identify novel tick vaccine antigens; Full patents filed February 2018; Antigens under Intellectual Property protection, data unpublished	[7,26]
Ixodes holocyclus	CSIRO	Developed paralysis tick toxoid for anti-toxin activity demonstrated in rabbits; Artificial feeding methods for the collection of paralysing toxin; Development of a suckling mouse toxin/anti-toxin assay; Protection shown in dogs	[15,27–30]
	University of Technology Sydney	Isolated three polypeptides bound to synaptosomes; Successfully sequenced and expressed holocyclotoxin-1 (HT-1) in *E. coli*	[31,32]
	UQ	Transcriptome sequence database prepared from feeding adult female tick guts and salivary glands; Based on HT-1 homology, a diverse family of 19 HTs was identified; Proof of concept dog trial with a cocktail of synthetic HTs; Full patent submitted October 2017; Data unpublished	[16]

During the process of this review, it was clear that several researchers in Australia were responsible for seminal studies, which led to the development of the current vaccines against cattle ticks and paralysis ticks. These researchers were mostly employed by CSIRO, and their research legacy paved the way for tick vaccine development in Australia. Each of these three researchers was dedicated to science until they passed with phenomenal legacies.

Dr Ian Clunies Ross completed a bachelor's degree in veterinary science (B.V.Sc.) at the University of Sydney in 1921. Clunies Ross spent 1921 as a temporary lecturer in veterinary anatomy at the university, and in 1922, he was appointed as a Walter and Eliza Hall research fellow. In England, he studied parasites at the Molteno Institute, Cambridge and at the London School of Tropical Medicine. In 1925, he resumed research on parasites and undertook some part-time teaching at the University of Sydney's Veterinary School (https://adb.anu.edu.au/biography/clunies-ross-sir-william-ian-9770, accessed on 1st September 2021) He was employed as a parasitologist at the Council for Scientific and Industrial Research (CSIR—later CSIRO) in the mid1920s. His pioneering research in the paralysis tick laid the foundation for ongoing research into the toxic fractions of the Australian paralysis tick *I. holocyclus,* as described above [59,68,69]. Sir Dr Ian Clunies Ross's publications have been previously summarized [70]. The review by Gordon was included in a special issue dedicated to Sir Dr Ian Clunies Ross in Volume 44, Issue 10 (October 1968) of the *Australian Veterinary Journal*. His research interests included the hydatid parasite *Echinococcus granulosis* and the liver fluke *Fasciola hepatica,* with a strong research focus associated with the development of livestock antihelmintic treatments (see citations in Gordon 1968 [70]). During his research career, he published approximately 70 research publications, including surveys and records, immunity and resistance, pathogenesis, antihelmintics and the application of control measures across many livestock parasite species [70]. In 1954, Clunies Ross was knighted and appointed CMG (Order of St Michael and St George) and became a Foundation Fellow of the Australian Academy of Science. He also held administrative leadership roles as the Chairman of CSIRO from 1946 (formerly CSIR) until his passing in 1959.

In 1954, Bernard Stone joined CSIRO, Division of Entomology, working on cattle tick biocide resistance, and authored his first article about parthenogenesis in the cattle tick in 1963 [71]. He completed his BSc and Master of Science (MPhil) at UQ by 1966 and his PhD with the University of Western Ontario (Canada) by 1968. Dr Stone was awarded a UQ Doctor of Science in 1982. As a researcher, he was quite prolific with 90 research publications (Marian Schneid, personal communication) with a strong impact on the development of *I. holocyclus* toxoid and the standardisation of tick anti-toxin production in Australia for treating paralysis in domestic pets and livestock [17,27,29,67,72]. His publications also focused on *R. australis* drug resistance. In 1994, he was awarded an Order of Australia for his contributions to science, environment, youth and scouting. Following retirement, he led a consultancy business evaluating commercial antitoxins for tick paralysis until his passing in 2005.

After completing his PhD on *Ixodes ricinus* ticks on sheep in Scotland at The University of Edinburgh, Dr Kemp was employed by CSIRO in Australia with the Division of Animal Health. Dr Kemp had an extensive career in cattle tick research with CSIRO, spanning almost 40 years, and he was one of the main protagonists developing the CSIRO TickGARD vaccine [4][7][8]. He also collaborated with Allen, who initially discovered the protective properties of *Dermacentor variabilis* tick gut extracts in 1979 [9][10]. Specifically, together with his technician Joanne Gough, he noted that tick-resistant breeds of cattle carried ticks, which appeared to hemorrhage internally (red legs) (Dr Anne Kemp, personal communication). He was a tick biologist with many interests: improving cattle resistance to ticks, tick histological structure, the taxonomy of Australian ticks (his collection is currently within CSIRO's Australian Insect Collection, Canberra; Australian National Insect Collection-CSIRO) and leading ACTEST—tick acaricide testing facility (Note by author: ACTEST was transferred to QDAF in the 2000s). Approximately 60 of Dr Kemp's publications are recorded in NCBI's PubMed (searched 12/07/2021), mostly associated with *R. australis* (as *B. microplus*), with additional research contributions to I. holocyclus associated with livestock infestations [11][12][13], and other tick/ectoparasite species [14][15]. Research collaboration between Dr Kemp and Dr Stone (as members of different Divisions in CSIRO) was demonstrated with the development of an in vitro feeding system for *I. holocyclus* toxin collection [16].

Dr Kemp also contributed to the commencement of the new Beef CRC/MLA research program (Section 3.1.3) (during his retirement) through his initial suggestions to examine 'frustrated ticks' (ticks in bags sensing the cattle host) in vaccine candidate discovery research. This led to vaccine candidates, which were included in the reverse vaccinology approach by identifying transcripts produced when sensing cattle [17][18]; see **Table 1**.

4. Conclusions

It is clear that, in particular, due to the commercialisation of the cattle tick Bm86 protein, vaccination against ticks is a feasible control option [9]. The research by CSIRO described the impact of Australian research on the development of anti-tick vaccines. For I. holocyclus , a vaccine alternative to companion animal chemical treatments is also a future option for this unique Australian ectoparasite. This author notes that due to the focused impact of this tick species on the east coast

of Australia, international animal health companies deem that commercialisation is not economically feasible. With the shortcomings associated with *R. microplus*/*R. australis* delivery, both with poor global efficacies and the need for multiple boosting, much research has focused on identifying alternative vaccine candidates, as described above. What is disconcerting is the slow commercialisation of promising new cattle tick vaccine candidates, a topic recently reviewed by de la Fuente and Estrada-Pena in 2019 [19]. Indeed, of the anti-cattle tick vaccines described in this review, only the Bm86 antigen vaccine GAVAC ® is currently commercially available. De la Fuente and Estrada-Pena discuss the challenges associated with the fact that the application of generic chemicals is a more attractive alternative to specific vaccines (confirmed by the author's personal experience with *R. australis* vaccine commercialisation as companies strive to develop generic chemicals as alternatives to vaccines); the expense of novel adjuvant delivery systems for livestock; the challenge to produce anti-tick vaccines that can be applied across multiple tick species; and finally, the ability to control tick-vectored pathogens simultaneously [19]. It remains to be seen if new products will become available to the market. Vaccines will always provide a safe alternative to chemicals by reducing environmental residues and costs in the administration of these chemicals.

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