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Cattle tick vaccines: Many candidate antigens, but will a commercially viable product emerge?

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ABSTRACT

The cattle tick, *Rhipicephalus microplus*, is arguably the world's most economically important external parasite of cattle. Sustainable cattle tick control strategies are required to maximise the productivity of cattle in both large production operations and small family farms. Commercially available synthetic acaricides are commonly used in control and eradication programs, but indiscriminate practices in their application have resulted in the rapid evolution of resistance among populations in tropical and subtropical regions where the invasive *R. microplus* thrives. The need for novel technologies that could be used alone or in combination with commercially available synthetic acaricides is driving a resurgence of cattle tick vaccine discovery research efforts by various groups globally. The aim is to deliver a next-generation vaccine that has an improved efficacy profile over the existing Bm86-based cattle tick vaccine product. We present a short review of these projects and offer our opinion on what constitutes a good target antigen and vaccine, and what might influence the market success of candidate vaccines. The previous experience with Bm86-based vaccines offers perspective on marketing and producer acceptance aspects that a next-generation cattle tick vaccine product must meet for successful commercialisation.

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1. Introduction

The positive impact that vaccines have had upon human and animal health is without question one of the greatest developments in the history of science and medicine, globally preventing over 6 million deaths and producing direct savings of tens of billions US dollars annually (Ehreth, 2003). Although commercial vaccines generally target diseases with viral or bacterial causes, vaccines against arthropods have been developed in the past. An effective vaccine against the cattle grub, *Hypoderma lineatum*, was developed and patented in the late 1980s (Pruett et al., 1987; Pruett, J.H., Files, J.G., Kuhn, I., Temeyer, K.B., 1989. Vaccines for the protection of animals against hypodermosis. European Patent 0326419, Publication Date August 2, 1989). Despite its efficacy, market factors prevented successful commercialisation of the cattle grub vaccine. This is an example of how business considerations within life sciences and animal health companies can ultimately determine the fate of research and development projects that otherwise meet all non-business technical criteria in a product profile. A vaccine with efficacy against the cattle tick, *Rhipicephalus microplus*, was developed, patented and marketed in Australia in 1994 under the name TickGARD (Willadsen et al., 1995) using the midgut glycoprotein antigen Bm86. More than 12 years of development and trials involving approximately 18,000 cattle led up to the product launch. A similar vaccine using basically the same antigen was developed in Cuba, also in the 1990s, under the trade name Gavac® (Canales et al., 1997). A number of industrial consolidations produced unfavorable outcomes for TickGARD and it is no longer commercially available. However, Gavac® continues to be marketed today, primarily in North and South America, although efficacy results are quite variable and acceptance of this product is not widespread. Tick control is an ongoing problem for both large operation cattle producers and small family farmers. Acaricide resistance is an escalating problem in many countries and tick control strategies that do not rely solely upon chemical acaricides are sorely needed.

2. Why a cattle tick vaccine?

The cattle tick, *R. microplus*, is responsible for huge economic losses to cattle producers, dairy operations and small farmers throughout the world due to direct losses from parasitism of the tick upon the host animal and from diseases vectored by the cattle tick. *Rhipicephalus microplus* transmits bovine babesiosis and anaplasmosis, and the host-pathogen-disease complex of the bovine-cattle tick- *Babesia bovis* (the major causative agent of bovine babesiosis) is likely the most important tick-disease complex in the world (de Castro, 1997). Brazil’s and Australia’s economies suffer annual...
losses of USD $2 billion and AUD $170 million, respectively, due to R. microplus parasitism of cattle (Grisi et al., 2002; Playford, 2005). The United States eradicated cattle ticks in the 20th century and annual savings to their agricultural economy was estimated to be USD $3 billion in today's currency (Graham and Hourrigan, 1977).

In the face of these significant economic costs to global agriculture, control of cattle ticks has become problematic, as these ticks have developed resistance to every class of commercially available acaricide (Rodríguez-Vivas et al., 2007; Perez-Cogollo et al., 2010; Andreotti et al., 2011). Certainly, not all cattle tick populations are multiply-resistant and, in most cases, at least one class of acaricide can be identified to treat resistant field populations. However, there are anecdotal reports of R. microplus populations in Brazil that can no longer be controlled by label-directed application rates of any acaricide. Concerns with worker, environmental and food safety, and the increasing costs of acaricide discovery, development and marketing also factor into what has developed as a real need for novel environmentally-sound control technologies. In Australia, an industry-supported study identified a single dose cattle tick vaccine with 90% efficacy as a priority for their national cattle industry (Playford, 2005). Vaccine research programs are underway in many countries and the remainder of this article will focus on progress of this research and factors that will be critical to bringing a vaccine to the marketplace.

Relative to chemical acaricides, vaccines are non-toxic, non-polluting and less costly to develop and produce. However, they tend to be very species-specific. This is a good characteristic with respect to non-target species that may be threatened or endangered, but a poor characteristic considering marketability of and the desirability for an anti-tick vaccine that is efficacious against multiple tick species. Vaccines have been reported to work well within integrated tick management systems, serving to reduce the number of acaricidal applications per year (de la Fuente et al., 2007). This in turn delays the development of resistance to generally more expensive chemical acaricides and allows the chemical producer to market an effective product for a longer period. While the development of resistance to vaccines cannot be prudently ignored, modifications to vaccines can be produced through sequencing and cloning procedures to isolate novel antigens or to alter the existing antigens such that efficacy is restored. Also, multiple unrelated antigens could be included in a single vaccine product to reduce the rate of resistance development to any single antigen or antigenic determinant. For example, Willadsen et al. (1996) studied the bovine immune responses to vaccination with Bm86 or with the dual antigens Bm86 and Bm91. They found no correlation between the immune response of each antigen within individual bovines. In fact, they theorized that vaccination with a dual antigen product presented an advantage to herd immunity, noting the possibility that individuals that responded poorly to Bm86 would respond well to Bm91, and vice versa. Since the least protected animals in a herd would have the greatest impact on the herd's tick population, the dual antigen Bm86 + Bm91 vaccine would likely have a greater impact on reducing the herd's overall tick count than the single antigen Bm86-containing product.

3. Review of reported vaccine antigen evaluations

Scientific and technological advances in vaccinology during the post-genomic era offer the promise to revolutionise the empirical approach to vaccine development (Oberg et al., 2011). In recent years, the identification of suitable antigens for a cattle tick vaccine and its development has become the subject of research laboratories around the world. As an example of the increased interest in development of this vaccine, we can compare oral presentations at two recent conferences that occurred 6 years apart. In 2005, the Fifth International Conference on Ticks and Tick-Borne Pathogens in Neuchatel, Switzerland met for 4 days and only one oral presentation focused primarily on cattle tick vaccine research. At the recent Seventh International Conference on Ticks and Tick-Borne Pathogens in Zaragoza, Spain, half of the first day was devoted to tick vaccines. Nine of the 14 talks from the Vaccine session, including the conference's keynote presentation, were focused on some aspect of developing a vaccine against the cattle tick. As another specific example of cattle tick vaccine interest, this time solely within Brazil, the XVI Congresso Brasileiro de Parasitologia Veterinaria in Campo Grande, Brazil in 2010 had an entire session devoted to cattle tick vaccines with participation from leaders of three different Brazilian laboratories reporting the results from antigen evaluation trials. Some of the studies reported at these two recent venues evaluated novel candidate tick vaccine antigens such as glutathione S-transferase, vitellin-degrading cysteine endopeptidase, ferritin-like protein, cathepsin, trypsin inhibitor, and regional alleles of Bm86, the primary protective antigen in the vaccines TickGARD and Gavac®. Other, presumably promising, antigens were discussed in general terms, giving care to protection of intellectual property rights. Some of these studies are in their early phases and peer-reviewed publications are not yet available to report the results, while others presumably are reports of antigen evaluations that did not produce results warranting further study. However, there are several antigens that have been described in the scientific literature and these are discussed below.

The performance of the antigen Bm86 has been reviewed several times and many publications report cattle trials with TickGARD and Gavac® or regional full-length versions of Bm86 and will not be the focus of this report (de la Fuente et al., 2007; Willadsen, 2008; Vargas et al., 2010). Instead, the focus herein is on studies of novel antigens that have been evaluated in cattle vaccination trials by various groups around the world (Table 1).

A series of studies by several Brazilian laboratories identified Yolk pro-Cathepsin, an aspartic proteinase expressed in eggs of R. microplus, as a promising antigen for a cattle tick vaccine. However, when expressed as a recombinant protein in Escherichia coli and used as a vaccine antigen in a cattle trial, the efficacy was merely 25% (Leal et al., 2006). A recent review of several Brazilian research efforts aimed at developing a R. microplus vaccine for cattle reported a number of antigens in various evaluation stages. Those antigens that were evaluated in cattle trials showed predominantly disappointing results, generally in the lower efficacy range or below that provided by Bm86-based vaccine antigens (Parizi et al., 2009). Notably, the research approach taken by Patarroyo et al. (2002) yielded promising results whereby cattle were vaccinated with peptides derived from the predicted antigenic determinants of Bm86. Efficacy of over 81% against R. microplus and a robust antibody response was reported in cattle stall trials for peptide vaccine Sbm7462. Further studies have been done by that group on the expression of various peptides and combinations of peptides; some

<table>
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<tr>
<th>Table 1</th>
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<td>Novel antigen cattle vaccination trial results against Rhipicephalus microplus.</td>
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<table>
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<tr>
<th>Antigen</th>
<th>Efficacy (%)</th>
<th>Country</th>
<th>Report</th>
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<tbody>
<tr>
<td>Yolk pro-Cathepsin</td>
<td>25</td>
<td>Brazil</td>
<td>Leal et al. (2006)</td>
</tr>
<tr>
<td>Sbm7462</td>
<td>81</td>
<td>Brazil</td>
<td>Patarroyo et al. (2002)</td>
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<tr>
<td>Subolesin</td>
<td>51</td>
<td>Mexico</td>
<td>Almazan et al. (2010)</td>
</tr>
<tr>
<td>81</td>
<td>Mexico</td>
<td>Almazan et al. (2012)</td>
<td></td>
</tr>
<tr>
<td>ARS Antigen 1</td>
<td>76</td>
<td>Brazil</td>
<td>Guerrero (unpublished data)</td>
</tr>
<tr>
<td>73</td>
<td>Brazil</td>
<td>Guerrero (unpublished data)</td>
<td></td>
</tr>
<tr>
<td>ARS Antigen 2</td>
<td>63</td>
<td>Brazil</td>
<td>Guerrero (unpublished data)</td>
</tr>
<tr>
<td>71</td>
<td>Brazil</td>
<td>Guerrero (unpublished data)</td>
<td></td>
</tr>
<tr>
<td>ARS Antigen 1 + 2</td>
<td>71</td>
<td>Brazil</td>
<td>Guerrero (unpublished data)</td>
</tr>
<tr>
<td>Ferritin 2</td>
<td>64</td>
<td>Mexico</td>
<td>Hajdusek et al. (2010)</td>
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* Bm86 control gave 39% efficacy.  
* Bm86 control + immune enhancer gave 49% efficacy.
of these have been published (Sales-Junior et al., 2005; Peconick et al., 2008) whereas others were presented at the XVI Congresso Brasileiro de Parasitologia Veterinaria in Campo Grande, Brazil in 2010, including a report of the expression of the peptide vaccine candidate SBm7462 in a transgenic plant. Peconick et al. (2008) reported that cDNA isolated from R. microplus from Brazil, Argentina, Colombia, and Uruguay coded for the identical amino acid sequences that corresponded to the peptide sequence of SBm7462, thus providing confidence that the vaccine would have a uniform efficacy across South America, unlike the variable efficacies of vaccines based upon the entire Bm86 coding region. Another very interesting result from Brazilian researchers was the report that recombinant glutathione S-transferase from Haemaphysalis longicornis provided cross-protection to cattle against infestation by R. microplus (Parizi et al., 2011). Although the cattle trial involved six injections per animal, an impractical number for cattle producers, the 57% efficacy against R. microplus showed that this antigen is worthy of further study. Perhaps this antigen might also provide cross protection to cattle from infestations of a wide range of tick species. A number of R. microplus expressed sequence tags (ESTs) with over 80% nucleotide sequence identity to the H. longicornis glutathione S-transferase are present in GenBank and it would be interesting to evaluate the R. microplus orthologue for efficacy as a cattle tick vaccine antigen.

Almazan et al. (2010) reported an efficacy of 51% in a vaccination trial in Mexico that evaluated recombinant subolesin expressed in E. coli. By comparison, the Bm86 antigen tested concurrently yielded an efficacy of 60%. In the same study, the efficacy of subolesin and Bm86 against a second cattle tick species, Rhipicephalus annulatus, was reported as 60% and 100%, respectively. The authors reported suboptimal antibody responses from the vaccinated cattle and speculated that the animals were not in the best health condition for a vaccine evaluation trial, but nevertheless their subolesin results would be considered low, as a 60% efficacious vaccine would only be a marginal improvement over the Bm86-based antigen’s performance. However, recently the same group reported improvements in the antigen expression and purification conditions that boosted the efficacy to 81% against R. microplus (Almazan et al., 2012). This level of efficacy is impressive and presents subolesin as a viable R. microplus vaccine antigen with potential for global impact if commercialised. Although the exact function of subolesin is not precisely defined, it appears to have a role in maintaining the tick’s innate immunity against specific pathogens (Zivkovic et al., 2010). It will be interesting to follow as subolesin is evaluated against cattle ticks from different regions of the world to determine whether the promising results obtained with Mexican cattle and cattle ticks can be reproduced elsewhere.

Another antigen that has been evaluated in published cattle trials is ferritin 2, a predominantly gut-expressed secreted iron storage protein (Hajdusek et al., 2009). RNA interference silencing of ferritin 2 showed significant impacts on tick feeding, oviposition and larval hatch, characteristics that identified ferritin 2 as a candidate tick vaccine antigen. Hajdusek et al. (2010) reported 64% efficacy against R. microplus and 72% efficacy against R. annulatus in cattle vaccination trials in Mexico. In the same trials, Bm86 had 60% and 100% efficacy against R. microplus and R. annulatus, respectively. Perhaps in a fashion similar to the subolesin studies discussed above, adjustments to the expression or purification steps of ferritin 2 would result in significant gains in efficacy. Ferritin 2 features properties that make it an attractive candidate for further evaluations on cattle and ticks globally.

At the recent Seventh International Conference on Ticks and Tick-Borne Pathogens in Zaragoza, Spain, groups from South Africa, Australia and the United States reported the utilisation of a reverse vaccinology approach to identify novel anti-cattle tick antigens that were being evaluated in cattle vaccination trials. This strategy can be summarised as using the tick sample of interest to obtain a transcriptome through mass sequencing. Individual members of the transcriptome are evaluated by various methods to obtain a list of candidate antigens for evaluation in cattle vaccination trials (Fig. 1). While intellectual property concerns precluded the identification of specific antigens by the presenters, the data from cattle vaccination trials demonstrated the impact of applying a rational approach such as reverse vaccinology to the identification and selection of tick molecules for testing as vaccine antigens to elicit solid protection against cattle tick infestation. For example, our group reported results from three cattle vaccination trials that were conducted using two antigens selected by a systems biology approach from transcriptome datasets (Table 1). The trials were conducted with Holstein cattle in stall tests at Campo Grande, Mato Grosso do Sul, Brazil and current trials are underway to evaluate these antigens on cattle from Texas, USA. The University of Pretoria, South Africa, research laboratory of Dr. Christine Maritz-Olivier also reported promising results from their vaccine antigen evaluations and, although some antigen identities were not revealed due to aforementioned intellectual property protection considerations, a

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**Fig. 1.** Reverse vaccinology approach to identify novel anti-tick vaccine antigens. RNAi, RNA interference.
recent publication reported early findings from the group’s reverse vaccinology approach to finding cattle tick vaccine antigens (Barnard et al., 2011). CattleTickBase serves as an updatable public repository and set of bioinformatics tools for the international community’s efforts to sequence the genome of *R. microplus* (Bellgard et al., 2012). CattleTickBase is a valuable resource that can be mined to accelerate the identification of candidate antigens by members of the international research community working on cattle tick vaccines.

### 4. Desirable attributes of antigens in a second generation cattle tick vaccine

Several groups with active cattle tick vaccine discovery research programs have multiple antigens in the pipeline. There is functional and structural diversity in the spectrum of tick molecules tested and their characteristics likely define each group’s definition of what makes a desirable antigen to be a vaccine development candidate. Our group examined the characteristics of Bm86, an antigen that, despite its limitations in some geographic areas, was successfully commercialised by different business concerns in both Australia and Cuba. Bm86 is a concealed antigen (Nuttall et al., 2006), that is, these antigens are normally hidden from the host’s immune system. Conventionally, concealed antigens had been thought of to be primarily gut wall antigens, however, as long as immunoglobulins from the bovine host are able to interact with a protein, the protein could be a candidate concealed antigen for use in a cattle tick vaccine. Of course, the antigen must have a critical function in the tick such that the disruption of function would lead to either lethality or reduced fecundity at levels so as to impact the tick population. Bm86 has shown spectacular efficacies in some cattle vaccination trials and field tests (Canales et al., 2009), which proved the concept of using a concealed antigen as a viable approach. By comparison, the concept of using an exposed antigen for a vaccine has some allure, as these antigens elicit an immune response during tick infestations and a vaccine based on such antigens would prime a vaccinated animal’s immune response against tick infestation. However, in our view, co-evolution of the tick-bovine host interaction has likely led to the tick’s development of means to circumvent the host immune system’s response to exposed antigens (Brake and Pérez de León, 2012). Many of the exposed antigens either proposed or evaluated as cattle tick vaccine components are members of gene families, for example, histamine-binding proteins, serine proteases, and trypsin inhibitors. In our view, a vaccine derived against one member of a gene family may fail because redundancy within the family might allow the function of the targeted family member to be taken up by other family members.

Lackluster results of cattle tick vaccine evaluations using exposed antigens has been noted by Nuttall et al. (2006) and it is our belief that concealed antigens are better antigens to target in vaccine antigen discovery efforts and are more likely to produce a viable cattle tick vaccine, particularly when the antigen is encoded by a single copy or low copy number gene. Trimnell et al. (2002) brought out the concept of dual-action vaccines that utilise an antigen with good efficacy results. Within our own research program, Antigen 2 (Table 1) was isolated as a *B. bovis*-infection associated upregulated protein from an adult female tick gut membrane proteome analysis (Rachinsky et al., 2008). Bioinformatic analysis showed that Antigen 2 had significant sequence similarity to salivary secreted protein from *Ixodes scapularis* and to ESTs from *Rhipicephalus sanguineus* and *I. ricinus* salivary glands. Thus, ferritin 2 and Antigen 2 may each be dual-action antigens and Antigen 2 gave good efficacy in our cattle trials against *R. microplus*.

### 5. From discovery research to commercialisation: moving candidate antigens along the critical path

Acaricide resistance is an ever increasing problem in many regions of the world, having especially severe impacts in Brazil, Australia, Mexico and Argentina. These countries are among the top 10 cattle producing countries in the world. Thus, the market is present for technology that can control the cattle tick and overcome challenges presented by acaricide-resistant tick populations. Anecdotal reports indicate that demand for an effective and safe cattle tick vaccine in South America is such that a crude protein extract of pulverised ticks was registered and marketed as a cattle tick vaccine. The product exhibited unsatisfactory performance in southern Brazil and the registration is under re-review. The overmarketing and failure of products like this engender misperceptions about vaccines as suitable control technology among farmers, especially when coupled with the inconsistent results of the Bm86-based vaccines against *R. microplus* in the Americas.

Of course, product demand is a requisite, but other factors in the animal health enterprise will be crucial to ensure commercial success of the next generation of cattle tick vaccines. Simply having an antigen with good performance in vaccination trials will not suffice. Second generation cattle tick vaccines must meet safety requirements and overcome efficacy challenges under field conditions and the perceived niche-like character of target markets. Scientific proof of concept for candidate antigens is part of the equation to bring a new product to market, as the industrial development of a cattle tick vaccine requires investment decisions by animal health companies that are based on economic principles.
(Heldens et al., 2008). Navigating the funding gap between the development and commercialisation of potentially beneficial innovative technologies by the biotechnology industry is a challenge that applies to the translation of research into the next generation of cattle tick vaccines (National Center for Research Resources, United States National Institute of Health, 2010. CTSA Industry Forum: Promoting efficient and effective collaborations among academia, government, and industry, Final Workshop Report, May 18, 2010.). Regard for the need by commercial partners to preserve intellectual property rights will be crucial for a successful partnership between non-industrial groups, which are currently doing most of the discovery research, and the private sector to develop and market an efficacious vaccine product. Such consideration must be part of the research strategy, and it should be reflected in the project plan as early as possible. Otherwise, inadvertent communications and premature disclosure can destroy the ability to obtain intellectual property protection through patents. Once a superior antigen has been identified, non-industrial discovery research groups will likely have to find a commercial partner to take on the challenges of product development, global evaluations in different cattle and tick populations, and marketing that are necessary for successful commercialisation.

A relatively unexplored area for cattle tick vaccine researchers involves methods to enhance the immunogenicity of a vaccine’s antigen. The intrinsic immunogenicity of an antigen is usually potentiated through the use of adjuvants in vaccine formulations. For example, following the launch of the original Bm86-based vaccine formulations TickGARD and Gavac®, TickGARD Plus and Gavac Plus were developed largely through the application of adjuvant technology to enhance duration of immunity. The efficacy of novel cattle tick vaccines could be enhanced by expanding the mode of immunity through the activation of cytotoxic T cell-, or T cell-mediated mechanisms. Adjuvants are available and are being developed that could be used to activate cell-mediated immunity in vaccines containing tick antigens as is being investigated with other types of vaccines (McKee et al., 2010; Yue et al., 2012). The adjuvanticity of nanoparticles impregnated with cattle tick vaccine antigens could be exploited to augment the antibody response to vaccination with those antigens (Sloat et al., 2010). Novel adjuvants with enhanced capacity to stimulate the bovine immune system, targeted delivery methods such as needleless injection methods to allow intradermal vaccination and the concomitant enhancement of the cellular immune response, and novel antigen presentation in the form of peptides (Patarroyo et al., 2002; Prudencio et al., 2010) are examples of areas where investigations are at an early stage. Pursuing these research avenues could result in enhanced vaccine efficacy. Patarroyo et al. (2009) explored the kinetics of the humoral immune response and changes in lymph nodes of bovines vaccinated with the synthetic peptide vaccine SBm7462®. As SBm7462® showed high efficacy in small-scale cattle trials (>80%, Patarroyo et al., 2002), similar studies in bovines to characterise details of the cell-mediated response accompanying vaccination with SBm7462® or other highly efficacious vaccine antigens would be valuable. It would be very informative to compare the bovine humoral and cellular immune responses to vaccination with highly efficacious antigens against the response to less effective antigens, with the goal of identifying immune system responses that would be predictive of high efficacy and duration of protection. This information might lead to the development of in vitro capabilities to predict efficacy and optimise vaccination schedules and protocols for newly discovered antigen candidates and help prioritise antigens for cattle trials.

The potential to immunise cattle with a commercial vaccine containing DNA coding for protective R. microplus antigens remains to be fully explored. Our cattle vaccination trials with cattle tick DNA vaccines resulted in efficacy that was ~75% of the protection obtained in cattle immunised with the recombinant protein form of the corresponding gene product (F. Guerrero, unpublished results). Priming-boosting through stimulation with CpG oligodeoxynucleotides might augment the immune response to tick antigens or DNA-based cattle tick vaccines, as this has been observed in cattle and sheep studies (Nichani et al., 2004).

Remarkably, the Gavac® product exhibits >90% efficacy against R. annulatus, surpassing control levels achieved by chemical acaricides. This level of efficacy has been shown in cattle vaccination trials using R. annulatus originally collected in Texas (Canales et al., 2009; Almazan et al., 2010), Mexico and Iran (Fragoso et al., 1998). The reasons underlying the dissimilar results of Gavac® (and presumably other Bm86-based vaccines) against R. microplus and R. annulatus are not known, yet should be investigated. In our opinion, understanding the differences in the molecular and immunological interactions between Bm86 and R. microplus and R. annulatus will lead to better cattle tick vaccine antigen selection and design. It is likely that the efforts underway in one or several of the laboratories around the world will find (or may already have found) a novel antigen that can approach this level of efficacy, particularly in combination with other novel developments noted above. However, success should not simply be defined by this efficacy level. In the majority of the cattle tick vaccine markets, the goal is control around an economic threshold, not necessarily total eradication, so that endemic stability with bovine babesiosis can be efficiently maintained. In these markets, success would be defined as developing a vaccine that maintained economic levels of tick control and endemic stability, while achieving consistent performance against R. microplus populations around the globe.

Consumer acceptance will always be crucial for the successful commercialisation of innovative biotechnologies (Aldrich and Bilsard, 1998). Dryden and Payne (2004) emphasised that education of owners about reasonable expectations for tick control products can prevent frustration. As with other anti-parasitic vaccines, it is likely that consumers will expect cattle tick vaccines to perform as veterinary pharmaceuticals which generally exhibit close to 100% efficacy when first introduced. The variation in immunoprotection among vaccinated animals must be minimised to maximise acceptability of a new vaccine product. Major histocompatibility complex (MHC) polymorphisms are known to affect the host immune response to vaccination (Fellay et al., 2011). Sitte et al. (2002) showed that a single amino acid deletion in the antigen recognition site of an MHC class II molecule affected the response to TickGARD. Understanding the genetic basis for the heterogeneous protection against tick infestation experienced with TickGARD and Gavac® will enhance the development and acceptance of a new product among producers immunising their cattle herds with a newly commercialised cattle tick vaccine. To maximise market penetration during launch, cattle tick vaccines could be marketed as part of an integrated control program that includes the use of acaricides. Ecologically-based modelling is a strong approach to address the use of a cattle tick vaccine in control or eradication programs (Lodos et al., 1999). The models can be used to evaluate vaccine efficacy as it varies with immunogenicity of the antigen, number of doses administered, and timing of vaccination(s), among other factors. A thoughtful strategy for field use that includes a campaign to describe the unique mode of action and enhanced safety of a cattle tick vaccine compared with acaricides, and how these technologies can benefit livestock production by complementing each other, will likely maximise acceptance among producers.

6. Conclusions

More than 27 years after the launch of TickGARD, the potential to use anti-tick vaccines as an effective and sustainable approach in the toolbox of technologies available for tick control remains to
be fully realised. The fact that a second-generation vaccine has not been developed and used worldwide for the control of *R. microplus* and *B. annulatus* represents a failure by producers, industry and the scientific community. There is room to achieve efficiencies in the transfer of technology from non-industrial discovery research groups to business concerns to bring the next generation of efficacious and safe cattle tick vaccines to the hands of the producer. While the ideal vaccine would produce >99% efficacy for 1 year after only a single treatment, the current control levels of Bm86-based vaccines, despite their documented variability in field efficacy, have been shown to be useful in integrated management programs by reducing the basal tick burden on cattle and concomitantly decreasing chemical acaricide use and *Babesia* transmission. Somehow, this information has failed to influence producers, industry and scientists to advance this technology to the field on a large scale. We seem content to work within the outmoded paradigm of chemical control which only serves to rapidly render those valuable control tools useless though their indiscriminate use that accelerates the development of resistance. Gone are the days when products containing macrocyclic lactones and insect growth regulators (IGRs) were thought to work forever, no matter the frequency of use, because their active ingredients disrupted critical physiological processes within the target pest. The previously used neurotoxic acaricides, for which resistance drove the discovery of IGR-based pesticides, targeted critical physiological processes within arthropods. The eventual development of resistance to the macrocyclic lactones and IGRs should have been expected and prepared for. In addition to resistance issues, entire classes of neurotoxic chemicals that contained members with acaricidal activity have been, or are in the process of being, banned due to safety and environmental impacts. Natural systems are biologically resilient, however the old paradigm continues unabated in many places, decreasing our already limited pool of chemical agents registered for tick control. There is concern with the registration and use of chemical mixtures as part of the latest marketing strategy in Brazil and Mexico where acaricide resistance to multiple compounds exists. What will producers that rely solely on chemical acaricides for tick control do once resistance to mixtures evolves? Will products with new modes of action that are efficacious against multiply resistant cattle tick populations and safe for livestock, humans and the environment arrive in time? How expensive will they be for the producer who must pass the costs down to the consumer? Discovery research, development and commercialisation of acaricides with new modes of action is a lengthy and expensive process. We must be mindful that bringing a new acaricide to the market that meets all regulatory requirements for claims in the label against cattle ticks can cost over US$100 million (Graf et al., 2004). The use of cattle tick vaccines as part of an integrated pest management system offers the opportunity to extend the useful life of newly developed chemical acaricides. Current advances in genomics and proteomics have created the ability to identify antigens and molecular techniques allow us to manipulate discovered proteins and test new vaccines much more rapidly and cost-effectively than in the past. However, it is critical that the necessary attention be paid to taking antigen discovery into vaccine development and commercialisation. Otherwise, the discovery will remain solely an academic achievement rather than delivery of a much-needed product to the cattle industry. It is anticipated that more efficacious cattle tick vaccines will be produced and will challenge all involved to modernise to new paradigms of the post-genomic era. The visionaries that discovered and developed the Bm86-based products paved the way to enhance the chances of success of a second-generation cattle tick vaccine. A suite of antigens have emerged as viable candidates for vaccine development and are now available. The right moment is now to seize the opportunity to provide the cattle industry with this safe and effective control technology.

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