final report

Project code: B.AHE.0212

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Date published: 31 October 2017

PUBLISHED BY
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NORTH SYDNEY NSW 2059

Cattle vaccination studies using novel anti-cattle tick antigens developed during Beef CRC research

Meat & Livestock Australia acknowledges the matching funds provided by the Australian Government to support the research and development detailed in this publication.

Abstract

Cattle ticks (*Rhipicephalus (Boophilus) microplus*) cost Australian cattle industries approximately $175m in economic losses through Ill-thrift of cattle, hide damage, tick fever transmission and the cost of treatments required to control infestations. The previous TickGARD vaccine was discontinued in 2010 due to poor uptake by the beef industry in northern Australia where several boosts of the vaccine were required per year to develop adequate protection from tick infestations. The Beef CRC (2005-2012) developed a research program to identify novel tick vaccine candidates and mixtures of vaccines demonstrated promising efficacies in cattle tick challenge trials. The objective of this project was to screen the components of these mixtures and identify potential lead antigens for development and commercialization for an effective tick vaccine in Australia. Approximately 20 vaccine components were tested in tick challenge cattle trials in various formats, as short protein peptides (majority), some mixtures of short peptides, and as whole large proteins. The highest efficacies observed ranged from 45-66% and mixtures of peptides did not produce synergistic higher efficacies. Future research should determine the best method to produce either a mixture of the top 3 peptides or the whole protein derivatives to obtain efficacies >80% for commercial adoption.

Executive summary

The early introductions of arthropod pests into Australia proved to be enormously costly with the cattle tick the most expensive. The cattle tick, *Rhipicephalus (Boophilus) microplus*, affects *Bos taurus* susceptible breeds of cattle (and their mixes) and was introduced into Australia in 1872 by the importation of 12 Brahman cattle from Jakarta. It first appeared in Queensland in 1891, Western Australia in 1895 and New South Wales in 1906. Cattle ticks were introduced to Victoria in 1914 via horses from Queensland, however, did not become established and Victoria, Tasmania and South Australia remain free of the cattle tick. The distribution of the tick is determined by temperature and humidity, and thus is confined to northern Western Australia, the northern half of the Northern Territory, coastal Queensland, and northern New South Wales. Two blood protozoan parasites (*Babesia* species), and a blood-borne bacterium, *Anaplasma marginale* are transmitted by the tick causing babesiosis and anaplasmosis respectively. The economic costs (estimated at ~$175m p.a. in 2005) of cattle tick include:

- Direct effects of the tick on cattle: loss of condition, anaemia and deaths, susceptibility to drought, damage to hides, slow growth rate;
- Risk of market being compromised by unacceptable chemical residues in meat or milk;
- Cost of controlling ticks, increased stock handling, costs of acaricides, cost of government regulations; and
- Costs of tick-borne diseases: deaths, slow growth, vaccine costs, treatment costs, handling costs

The gross value of Australian cattle and calf production in 2015-2016 was estimated at $14.3b with ~47% of this production originating from Queensland (ABARES 2016). Hence management of cattle ticks in northern affected regions is a high priority. *Bos indicus* breeds of cattle are naturally immune to cattle ticks and several tropically adapted *Bos taurus* breeds for the north have been developed. In 1994, a tick vaccine (TickGARD) based on a tick gut protein called 'Bm86' became commercially available. As the vaccine required 3-4 boosts per year, adoption by extensive beef producers was not successful. By 2004, production had ceased and the Queensland Dairy Organisation bought the remaining doses and eventually stopped distribution by 2010. Bm86 was also commercialised in Cuba (1993) under the name 'GAVAC' and continues to be produced and sold. Research undertaken by Cuban researchers demonstrated that geographic variation to this 'concealed' antigen was contributing to Bm86 vaccine failure in other Latin American countries i.e. Brazil.

The CRC for Beef Genetic Technologies developed a research program to identify potential new tick vaccine candidates that would promise to be more protective than Bm86 and more 'generic' or 'less variable' internationally. The research focussed on screening a newly available library of tick sequence data developed by the US Department of Agriculture published in 2007. Bm86 was considered to be a 'concealed' antigen as natural tick challenge did not 'boost' the vaccine response. The Beef CRC research focussed on identifying antigens that had characteristics, which were:

- predicted to be associated with 'exposure' (not 'concealed') – using informatics tools – thus potentially boosted responses following natural tick challenge,
- recognised by tick resistant cattle (resistant only, or resistant and susceptible),
- predicted to possess specific regions known to stimulate an immune response (i.e. B cell epitopes),
- found in ~3 stages of ticks (larvae, nymphs, adults), and several female organs (gut and/or salivary glands), and,
- able to produce high efficacies (internal gut damage – evidenced by blood feed spreading throughout whole tick; and non-viable egg production) following antibody feeding using a laboratory tick feeding model.

This 'reverse vaccinology' research approach led to the identification of approximately 20 vaccine candidates (short peptides representing the above 'B cell epitopes') with 12 of these candidates tested as mixtures in successful efficacy trials undertaken in Australia or Brazil towards the end of the Beef CRC program. The objective of this MLA project therefore was to screen the Beef CRC identified candidates and to identify potential lead antigens for development and commercialization of an effective tick vaccine in Australia. The project was to complete Australian based efficacy trials with the aim of acquiring relevant efficacy data to secure interest from an animal health company.

Five cattle tick challenge trials were undertaken from April 2014 and completed by July 2017 using the specific tick moated cattle pen facilities at UQ Gatton campus (Queensland Animal Science Precinct), except for trial 4, where the infestation was undertaken at UQ's Pinjarra Hills Farm. Synthetic peptides (short proteins) were produced with the desired sequence (B cell epitope) and conjugated to a carrier protein for vaccination. The vaccination regime for each trial consisted of 3 tick naïve steers (sourced from tick free areas at approximately 250kg in weight) per vaccinated group and the control group (adjuvant only) using the standard 3 boost vaccination method (Day 0, 4 weeks and 7 weeks). Short peptides are not highly immunogenic thus Freund's Complete Adjuvant was used for the first boost, followed by Incomplete Freund's Adjuvant for the next 2 boosters. A week after the final vaccination, cattle were moved into moated pens to acclimatise prior to larval infestation two weeks after the final vaccination. Cattle were infested with 2,500 larvae twice on 2 consecutive days (total 5,000 larvae). After ~20-21 days, adult female ticks were collected from each pen each day and brought back to the laboratory for counting, weighing and for incubation to monitor egg laying (weights) and larval emergence (percent). Eggs were weighed, and incubated further to determine the extent of larval emergence to measure the fecundity of the ticks collected and thus the potential vaccine effects on the tick life cycle. To calculate vaccine efficacy, average tick numbers, average weights of eggs per number of ticks, and average percent of larval emergence for the treated groups were compared to the control group. Throughout the trial, blood samples were collected and the sera were screened to measure the level of host antibody responses (ELISA) to each vaccine.

Three antigens (2 as peptides, one as a large recombinant protein) produced singular efficacies of 63-66%. A mixture of these peptides in one group in Trial 5 did not produce a synergistically higher combined efficacy at only 47%. Peptide mixes led to a single peptide dominating the immune recognition (as determined by ELISA), and the combined efficacy was thus lower than when peptides are tested individually. Efficacy calculations based on newly adopted improved practises led to the re-analysis of historic Beef CRC efficacies. The previous trial undertaken with a mixture of peptides in Brazil at 87% efficacy was re-calculated as 72%. This mixture was repeated in Australia (Trial 1) and achieved an efficacy of 30%. One of the top 3 antigens producing an efficacy of over 60% was a component of this mixture but did not appear to stimulate antibody production when tested in the mix in Australia. A polypeptide (mixture of peptides linked together) vaccine tested during the Beef CRC research produced an overall efficacy of 42%, this efficacy remained relatively un-changed at 41%. Two of the top antigens from this study were components of this 'polypeptide'. Upon re-examination of the ELISA data from this trial, the immune recognition of these peptides was also insignificant.

Two provisional patents were lodged in February 2017 and meetings with several companies followed to identify a partner for future research. This report recommends that subsequent studies focus on these top 3 candidates and compare their efficacies as individual peptides using higher doses, as recombinant yeast expressed full proteins, and as a designed multi-peptide protein to combine the 3 together using relevant technologies to produce an immunogenic vaccine product. Testing for longevity of immunity and thus the most appropriate adjuvant should also be investigated. Cattle from trials 4 and 5 have been retained at UQ's Pinjarra Hills farm under natural grazing conditions including tick challenge, and on-going monitoring will assist to determine if host vaccine responses (antibodies)

and tick numbers have been maintained and lowered, respectively. This will provide some preliminary longevity data for at least one of these top 3 antigens.

Other insights for future consideration include the use of tick challenge 'pouches' for a localised infestation challenge instead of the animal infestation protocol used in Australia where pouches are not used and ticks are left to infest cattle housed within pens. Most tick challenge trials undertaken in other countries tend to use these pouches and final efficacies should also be determined using similar methods to compare. Future investment will likely be through a multi-national company due to the global impact of cattle ticks. It is thus essential results obtained here can be compared internationally. In addition to this, confirmation of the conservation of all tick vaccine antigens needs to be determined to ensure the final vaccine will not be geographically variable. At this stage Elanco Animal Health have provided an EOI to develop these vaccines further through MLA's Donor Company model of funding (under development).

Finally, a new cattle tick vaccine with a longer duration of immunity (at a minimum of a single boost every 6 months, with annually desired) is a priority for the Australian meat industry. This will lead to opportunities to produce organic meat by minimising the use of acaricides, the ability to farm productively desirable cattle breeds (*Bos taurus*) in more regions, and to pre-vaccinate live export cattle.