Final report

Evaluation of anti-tick vaccines for tick immunological control in cattle

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Prepared by: Professor Timothy J Mahony
The University of Queensland

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Abstract

Cattle-ticks are the most important parasite affecting the Australian cattle industry, resulting in annual losses exceeding $160M. Sustainable and effective control of cattle-ticks through vaccination is a well-established approach, but there are no vaccines registered for the control of cattle ticks in Australia. To address this issue, this project evaluated the capacity of three Cuban cattle tick vaccine formulations to protect cattle from cattle-tick larval infestations. The first formulation evaluated was Gavac™ which is registered for use in Cuba and parts of South America. Gavac™ is similar in composition to a vaccine that was once registered for use in Australia, with both containing the cattle-tick antigen Bm86. The second formulation consisted of the recently discovered cattle-tick antigen, P0 peptide, linked to an immune carrier. The third formulation contained the P0 peptide linked to Bm86. All three formulations elicited detectable antigen-specific antibody responses in immunised cattle. The capacities of these immune responses to protect cattle from infestation by cattle tick larvae were also evaluated. Vaccine efficacy estimates were determined for each formulation based on the reproductive characteristics of engorged adult female ticks recovered from the infested cattle. The reproductive efficacy estimates for Gavac™, P0-KLH, and P0-Bm86 were 49.3%, 22.3% and 31.4%, respectively. Based on these estimates Gavac™ maybe of use in combating cattle tick infestations in Australia.

Executive summary

Cattle ticks are the most important parasite affecting the Australian cattle industry, resulting in annual losses estimated to be $161M. Currently cattle tick are controlled by relying on the indicine genotype and using chemicals. However, there are increasing reports of cattle-ticks developing resistance to the current range of acaricides. Previously, an effective cattle-tick vaccine was available in Australia but is no longer available. A similar vaccine, Gavac™, is manufactured in Cuba for domestic use and some parts of South America. Both vaccines contain the well-characterised cattle tick antigen Bm86.

The objective of this project was to evaluate the capacity of Gavac™ to protect cattle from induced infestation with cattle tick larvae. The performance of Gavac™was also compared to two other cattle tick prototype vaccines that have been developed in Cuba. One of these prototypes contains a recently identified cattle tick antigen known as P0 peptide (P0-KLH). While the third formulation contains the P0 peptide conjugated to Bm86 (P0-Bm86). All three vaccines elicited antigen specific antibody responses following the completion of the immunisation regimes in groups of cattle (n=6). The cattle were subsequently infested with cattle tick larvae to evaluate the protective capacity of the formulations. While there was a trend for each formulation to reduce the number of engorged adult female ticks dropping off trial cattle, these reductions were not significant. However, the ticks recovered from Gavac™ immunised cattle were significantly lighter and phenotypically damaged compared to ticks recovered from unimmunised control cattle. The reproductive efficacies of each formulation were also estimated based on evaluations of laboratory analyses of the recovered adult ticks. The reproductive efficacy estimates for Gavac™, P0, and P0-Bm86 were 49.3%, 22.3% and 31.4%, respectively. These estimates are well below those reported for these formulations in published studies. Further research would be needed to determine if the efficacy of Gavac™ is high enough to impact on the population dynamics of cattle ticks in the field.

An effective and usable cattle tick vaccine would reduce industry reliance on chemical control methods, and might allow the introduction of the taurine genotype to the Northern herd, in response to consumer demand for better beef eating quality. It has been estimated that optimal control of cattle ticks would return $61M in lost productivity to northern Australian cattle producers. An effective cattle tick vaccine would be a vital component of an integrated pest-management system to deliver this return to industry.

Table of contents

1. Background

The cattle tick (*Rhipicephalus australis*) is the costliest pest of cattle in northern Australia, with an estimated annual impact of $161M (Lane et al., 2015). This impact is made up of blood loss, hide damage, behavioural disturbance and the transmission of parasites associated with tick fever. Cattle tick control over recent years has relied on the more resilient *Bos indicus* genotype and the use of chemicals (either tick growth regulators, which provide up to three months of protection, or more frequent application of short-acting acaricide products). Control relying on chemicals is becoming increasingly difficult in many areas because resistance to all families of acaricide, including the growth regulators, has now been recorded in Australia, whereas the indicine genotype suffers the disadvantage of inferior beef eating quality. To address these issues and ensure that producers can continue to effectively control cattle tick infestations it is necessary to develop complementary strategies for the development of multi-faceted integrated cattle tick control systems. One such strategy is the application of a cattle tick vaccine whereby the immune systems of cattle are harnessed to control infestations. It has been estimated that if all cattle at risk of cattle tick infestation could be reduced to the lowest risk of infestation there would be an annual return of $61 million to industry (Lane et al., 2015).

A cattle tick vaccine, TickGard^{PLUS}, based on the recombinant Bm86 antigen, was previously developed and commercialised in Australia (Willadsen et al., 1995). While effective, TickGard^{PLUS} was difficult to utilise in the northern cattle herd as the initial vaccination protocol recommended three doses with frequent booster vaccinations to ensure continued protection. Despite these limitations the vaccine did achieve good market penetration in Australia, becoming the highest selling cattle tick pharmaceutical in the first four years it was on the market (de la Fuente et al., 2007). The results of one Australian on-farm study of the impact of the vaccine on acaricide usage reported on average 2.4 times fewer treatments and over 25% of properties completely eliminating treatments (Cobon et al. 1995 as reported by de la Fuente et al., 2007). Furthermore a long-term review of TickGard^{PLUS} and similar products in other countries demonstrated that vaccination was an economically viable method of tick control and minimised the use of chemical residue issues (de la Fuente et al., 2007). While specific reasons for the demise of TickGard^{PLUS} in Australia were not documented, two main causes have been proposed. Firstly, the application of the vaccine was difficult due to the technical requirements for multiple initial injections and frequent booster shots to maintain protection being incompatible with the mustering practices used in the Northern Australia extensive cattle industry. Secondly, the sequential veterinary health company mergers and acquisitions and resulting changes in product focus are also thought to have contributed to TickGard^{PLUS} being withdrawn from the market. The vaccine continued to be available to the Queensland dairy industry until 2010 with supply of remaining stocks of the vaccine being facilitated by the industry peak body. The continued successful use by the dairy industry suggests vaccination can be an effective method for sustainable control of cattle ticks.

The purpose of the current project was to evaluate the capacity of three cattle tick vaccines which had been developed and manufactured by the Center for Genetic Engineering and Biotechnology (Havana, Cuba) to protect Australian cattle from experimental infestation with cattle tick larvae.

The first formulation to be tested was, Gavac™, which is very similar in composition to Tickguard^{PLUS}, as it contains the well characterised protective cattle tick antigen, Bm86, as key active component. This vaccine has been used to effectively control cattle ticks in the Cuban cattle industries for many decades. The application of the vaccine is mandated by the Cuban government. Gavac™ is also exported and used in some countries in South America. Similar, to TickGard^{PLUS}, Gavac™ is a multi-dose vaccine, it was included in the current study for two reasons. Firstly, as it contains the well characterised Bm86 antigen and as such it provides an efficacy reference point to compare the two more experimental anti-tick vaccine formulations. These formulations are reported to provide higher efficacy estimates compared Gavac™ (Rodríguez-Mallon et al., 2015, 2020). Secondly, if immunisation with Gavac™ provides strong efficacy estimates (equivalent to or exceeding that of TickGard^{PLUS}) under local conditions it might attract interest from a veterinary health company to undertake its registration in Australia. An important consideration would be the company would only have to invest in registration trials as Gavac™ is manufactured under GMP conditions in Cuba. As the company would not need to invest in the

manufacturing of the vaccine this could considerably reduce costs associated with bringing it to market in Australia.

The second formulation tested contained the P0 peptide (pP0) as the protective antigen covalently linked to the widely used immunological carrier molecule keyhole lymphocyte hemocyanin (KLH). pP0 is a recently identified 20 amino acid peptide from the cattle tick ribosomal protein. Rodriguez-Mallon et al. (2015) reported that vaccines formulated with pP0-KLH were 96% effective in protecting cattle from larval infestation. In this study the cattle were immunised on Day 0, 21, 36 and 60, followed by larval infestations on Day 75, 76 and 77 with 1,000 larvae per day. Formulations which include the pP0 are also of interest as are reported to provide protection from a broader range of ticks compared to Bm86 (Rodríguez-Mallon et al., 2012).

Finally, the third formulation tested, contain the pP0 peptide covalently linked to the protective antigen Bm86 (pP0-Bm86). Rodriguez-Mallon et al. (2020) recently reported that immunisation of cattle with either the pP0-Bm86 formulation or the pP0-KLH formulation afforded 84% and 89% protection from infestation cattle tick larvae, respectively. The cattle were immunised on Day 0, 21 and 36, followed by larval infestation at Day 51, 52, and 53 with 1,000 larvae per day.

This project addressed the MLA RD&A priority - **reducing the economic impact of cattle tick; define production benefits from effective tick treatment strategies**. The availability of an effective cattle tick vaccine would also reduce the risk of chemical contamination of beef products resulting from chemical anti-tick treatments. Currently the withholding periods and export slaughter intervals for these treatments are 42 days, with additional time for calves from treated cows. Perhaps more importantly the availability of an effective cattle tick vaccine that provides sustainable long-term control of infestations could permit producers to reduce the Brahman content of their cattle and this would have flow on effects of improved meat quality and easier compliance with MSA programs thus providing the opportunity to increase production value. In a study of dairy cattle, Jonsson et al. (2000) demonstrated significant differences in liveweight gain of TickGard^{PLUS} vaccinated cattle (52.5 kg) compared to unvaccinated cattle (33.9 kg) during the course of a six-month period of natural cattle tick exposure. Prior to this, Holroyd et al. (1988) reported that Droughtmaster cattle had higher conception rates and weaned heavier calves when cattle ticks were effectively controlled. As Droughtmasters have a high *Bos indicus* genetic content they would be expected have strong natural resilience to cattle ticks, thus demonstrated boosting of production through pest control suggests a vaccine could be equally effective. As a vaccine could provide longer term protection compared to chemical treatments additional benefits might be achieved.

The project outcomes strongly align to the Meat Industry Strategic Plan (MISP 2020) priority to improve the ***welfare of animals in our care*** by addressing the imperative to **minimise the impact of endemic disease**. This target aims to reduce the negative impacts of pests and diseases by $50 million and $250 million by 2020 and 2030 respectively through the use of various strategies including vaccines. Improving control of cattle tick could provide a $61 million returned to the cattle industry (Lane et al., 2015). An effective cattle tick vaccine is one way to sustainably achieve this return.

The project outcomes also contribute to the Beef Industry Strategic Plan (BISP 2020) imperative to **minimise the impact of endemic disease** by accelerating the application of **proven industry practice (R&D) for on-farm disease management to contribute to year-on-year reductions in the cost of endemic disease** control. Vaccines are a well proven approach to controlling endemic diseases and are routinely used by the cattle industry to control a range of endemic diseases. Moreover, as discussed previously a cattle tick vaccine was successfully developed and adopted in Australia despite having a sub-optimal vaccination protocol it was utilised by industry.

The aim of this study was to evaluate the capacity of the three cattle tick vaccine formulations manufactured in Cuba to protect cattle from experimental infestation with cattle tick larvae under Australian conditions.

2. Objectives

The first objective of this project was to evaluate the protective efficacy of the new antigen candidate pP0 compared with Gavac against the Australian Cattle tick. Including the suite of vaccines tested was the pP0 linked to Bm86 as a third formulation. The objective was successfully completed with the three formulations shown to induce antigen specific antibodies in the immunised cattle. Furthermore, the results of the cattle tick larvae infestation enable the estimation of protective efficacy for each formulation.

The second objective was to determine if the efficacy estimates of any of the vaccine formulations exceeded the expected threshold efficacy will be 70% for each single antigen (pP0 and Gavac), and 80% for the combined formulation (pP0-Bm86). These efficacy estimates were based on reducing the number of engorged adult female cattle tick, tick weights, tick survival to oviposition and egg hatchability. The reproductive efficacy estimates for the Gavac, pP0, and pP0-Bm86 were 49.3%, 22.3% and 31.4%, respectively. Thus none of the vaccine formulations reached the expected efficacy threshold.

3. Methodology

3.1 Approvals

3.1.1 Animal ethics approval

The trial protocol was approved by the University of Queensland's Production and Companion Animal Ethics Committee (Approval Number QAFFI/QASP/283/20/DAF).

3.1.2 In vivo permit

The importation and *in use* permit of the Cuban vaccines was also approved by the Department of Agriculture and Water Resources (Permit No 0003642843).

3.2 Cattle immunisation and infestation trial

3.2.1 Description of vaccines

Three vaccine formations were manufactured by the Center for Genetic Engineering and Biotechnology (Havana, Cuba). Brief descriptions of these formations are provided below:

Vaccine 1: Gavac® (Batch M91015), cattle tick vaccine registered for use in Cuba and some countries in South America. It is similar in composition to TickGard^{PLUS} that was once marketed in Australia. The main active is the recombinant Bm86 antigen that was discovered in Australia by CSIRO in the 1980's emulsified in the oil-based adjuvant Montanide 888 (SEPPIC).

Vaccine 2: pP0-KLH. It is a chemical conjugate of a synthetic peptide from the cattle tick P0 ribosomal protein (pP0) and KLH (Hemocyanin from *Megathura crenulata*, Keyhole Limpet) in an oily formulation with Montanide ISA 50 (SEPPIC).

Vaccine 3: pP0-Bm86. It is a chemical conjugate of a synthetic peptide from the cattle tick P0 ribosomal protein (pP0) and Bm86 (GAVAC active pharmaceutic ingredient) in an oily formulation with Montanide ISA 50 (SEPPIC).

The data from the temperature logger included in the shipment are shown in Appendix A. During one period of the shipment, the temperature was outside the desired parameters note, with the temperature exceeding 8°C for 2 Days, 7 hours and 46 minutes (Appendix A). during this period, the temperature went from 7.9°C to 8.4°C, and subsequently peaked at 12.9°C.

3.2.2 Immunisation schedule

The immunisation study was conducted to evaluate the capacity of the Cuban formulations to protect cattle from infestation with cattle tick larvae. Angus steers (n=24) were randomly allocated to one of four treatment groups and immunised as shown in Table 3.1.

Table 3.1 Vaccination schedule used in cattle trial to evaluate Cuban vaccine formulations.

	Vaccine formulation (Group number)			
Time	GAVAC® (1)	pP0-KLH (2)	pP0-Bm86 (3)	Control (4)
Day 0	Nil	Dose 1	Dose 1	Nil
Day 7	Dose 1	Dose 2	Dose 2	Nil
Day 42*	Dose 2	Dose 3	Dose 3	Nil

*Final immunisations were delayed by one week to avoid tick collections occurring across the institutional shutdowns (25 Dec 2020 to 1 Jan 2021).

The complete trial schedule is shown in Appendix B.

3.2.3 Cattle tick infestation

Cattle were infested with 5,000 cattle tick larvae (strain Yeerongpilly NRFS) on Day 56, Day 58, Day 61, Day 63, Day 65, Day 68. From Day 75 to Day 93. Briefly, cattle were housed in individual pens with the larvae being applied along the neck/back of each animal. Pens were cleaned daily with all waste material passing through fine-meshed stainless basket located in the drainage systems of each pen. The pens were constructed with an impervious lining to prevent the escape/loss of ticks dropping of animals. Between Day 75 and Day 93, engorged adult female cattle ticks dropping from the trial cattle were recovered from the baskets, and washed. After transport to the laboratory, the recovered ticks were counted, weighed, and assessed for damage typically associated with ingestion of anti-Bm86 antibodies. Data were recorded on an individual animal basis. A random selection of ticks (up to 50) from each animal were selected for further analyses, including survival to oviposition, mass of eggs laid, and hatchability of eggs laid Ticks were incubated in a incubator at 27°C with 85% relative humidity.

Egg hatchability was assessed seven days after lay by visual inspection. The viability of the eggs laid by the incubated cattle ticks was visually assessed at Day 35 post-lay

The potential impact of the vaccines on cattle ticks and their reproduction capacities were evaluated using the parameters listed in Table 3.2 which efficacy estimates calculated using the Equations 1 to 4. The equations were used to calculate daily efficacy estimates and cumulative efficacy estimates for the duration of the trial. The final efficacy estimates utilised the cumulative data from Day 76 to 93 (Equation 4.3) . If the data suggested a protective effect in the control group (e.g. more ticks recovered from vaccinated animals compared to control animals) compared to the vaccinated group, it was deemed the vaccine formulation had no effect and the efficacy estimate was arbitrarily designated as zero.

Table 3.2 Parameters used to estimate the impact of vaccine formulations on the growth and reproductive capacity of cattle ticks recovered from trial cattle.

Parameter	Abbreviation	
Control Arithmetic Mean Tick Count	cTC	C
Vaccinated Treated Arithmetic Mean Tick Count	vTC	D
Control Arithmetic Mean Weight (g) of Ticks Incubated	cTW	I
Control Arithmetic Mean Egg Weight (g) (post 7 days)	cEW	J
Vaccinated Arithmetic Mean Weight (g) of Ticks Incubated	vTW	K
Vaccinated Arithmetic Mean Egg Weight (g) (post 7 days)	vEW	L
Control Arithmetic Mean % Viability of Eggs at Hatch (35 Days incubation)	cEHV	Q
Control Calculated Weight (g) of Viable Eggs at Hatch	cEHW	R
Vaccinated Arithmetic Mean % Viability of Eggs at Hatch (35 Days incubation)	vEHV	S
Vaccinated Calculated Weight (g) of Viable Eggs at Hatch	vEHW	T
Control Egg Weight per Gram Tick	cEggWperTg	
Vaccinated Egg Weight per Gram Tick	vEggWperTg	

Efficacy based on Tick Counts (E_TC):

Equation 1: $$E\_TC = 100 \times (1 - vTC \div cTC)$$

Tick Egg Efficacy based on Tick counts (E_Egg_TC):

Equation 2: $$E\_Egg\_TC = 100 \times 1 - \Big(\big((vEW \div vTW) \times vTC\big) \div \big((cEW \div cTW) \times cTC\big)\Big)$$

Tick Egg Hatch Efficacy based on Tick Count (E_EggH_TC)

Equation 3:

$$E\_EggH\_TC = 100 \times 1 - \Big(\big((vEW \div vTW) \times vEV \times vTC\big) \div \big((cEW \div cTW) \times cEV \times cTC\big)\Big)$$

Reproductive Estimates:

Reproductive Estimate Vaccinated (vRE):

Equation 4.1: $$vRE = vEggWperTg \times vEHV \times vTC$$

Reproductive Estimate Control (cRE):

Equation 4.2: $$cRE = cEggWperTg \times cEHV \times cTC$$

Reproductive Estimate (RE):

Equation 4.3: $$RE = 100 \times \big(1 - (vRE \div cRE)\big)$$

3.2.4 Quantification of antibody responses

Blood samples (5-10 mL) were collected from the jugular vein of each animal on Day 0, Day 21, Day 42, Day 55 and Day 98. After collection, the blood samples were allowed to clot at room

temperature, the sera harvested and stored at -20 °C until required. The levels of antigen specific antibody in each serum sample were determined using a standard ELISA assay. The ELISA plates were coated with one of the following antigens, recombinant Bm86, peptide P0, or KLH. All serum samples were diluted 1:800 for screening purposes. A control serum from a previous trial was used as a reference serum sample (diluted 1:800) to facilitate comparisons between ELISA plates and the reactivity of samples collected at each sampling time-point.

4. Results

4.1 Antibody responses to cattle tick vaccines

4.1.1 Antibody responses for Group 1: Gavac™

Significantly higher Bm86 specific antibody responses were detected in the cattle that were immunised with GAVAC™ compared to unimmunised control cattle (Fig. 4.1). The antibody responses peaked at Day 55, after the second immunisation was administered on Day 42. The antibody responses were trending downwards at the end of the animal trial, Day 98 (Fig. 4.1).

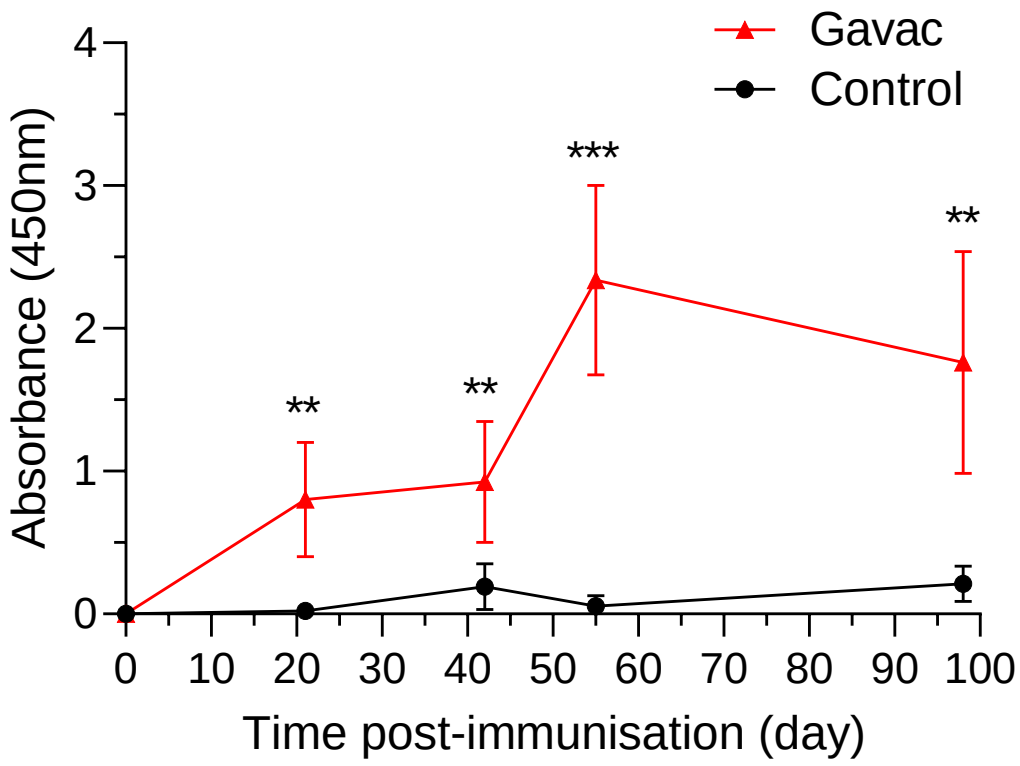

Fig. 4.1 Quantification of the specific antibody responses to the cattle tick antigen Bm86 of cattle (n=6) immunised with GAVAC™ determined using ELISA. Cattle were immunised on Day 7 and Day 42. The reactivity of the Control cattle (Group 4, n=6) to Bm86 is also shown. The mean absorbance for sera (diluted 1:800) for each group at 450nm is shown with one standard deviation. Levels of significance between the mean antibody levels of the groups are indicated by ** p <0.01; *** p <0.001.

4.1.2 Antibody responses for Group 2: P0-KLH

Specific antibody responses to the P0 peptide were detected in cattle immunised with the P0-KLH formulation (Fig. 4.2). The responses peaked at Day 55, following the administration of the third dose on Day 42. The responses of these cattle were highly variable with the absorbance values ranging from 0.134 to 1.689 on Day 55. This high variability is reflected in the large standard deviation for this data point shown on Fig. 4.2. Similar to the responses of the Gavac™ immunised group the P0 antibody responses were decaying by the end of the experiment on Day 98.

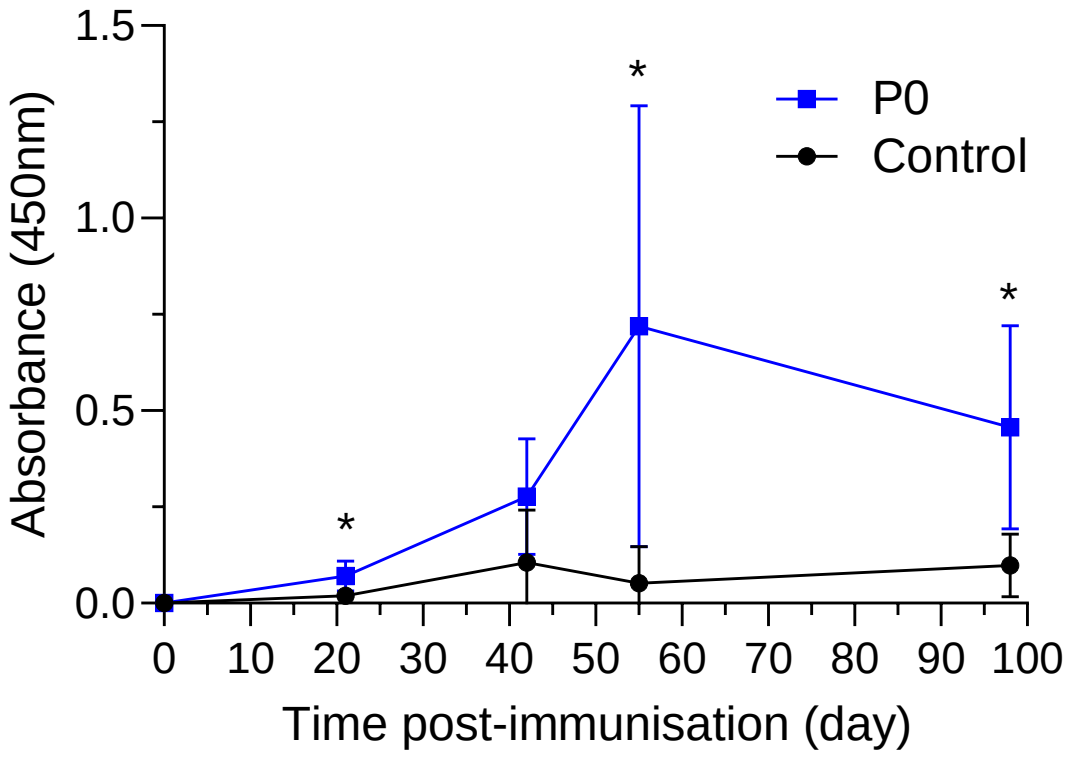

Fig. 4.2 Quantification of the specific antibody responses to the cattle tick peptide antigen P0 conjugated to carrier immunogen keyhole limpet hemocyanin (KLH) of cattle (n=6) immunised with the P0-KLH formulation (Group 2). Cattle were immunised on Day 0, Day 7 and Day 42. The antibody reactivities of the Control cattle (Group 4, n=6) to P0 component of the formulation are also shown. The mean absorbance for sera (diluted 1:800) for each group at 450nm is shown with one standard deviation. Levels of significance between the mean antibody levels of the groups are indicated by * $p < 0.05$.

4.1.3 Antibody responses for Group 3: P0-Bm86

For the cattle immunised with the P0-Bm86 formulation, specific antibody responses were detected to both antigens at Day 55, following the administration of the third dose on Day 42 (Fig. 4.3). The antibody responses to the P0 component of the formulation were highly variable, as observed for the P0 when delivered by itself (compare Fig. 4.3A and Fig. 4.1). Overall, the responses to the Bm86 component of the formulation were stronger and less variable compared to the P0 component (significantly different on Day 55, $p < 0.05$). As observed for the other formulations, after the antibody responses peaked on Day 55, they were decaying by the end of the experiment at Day 98.

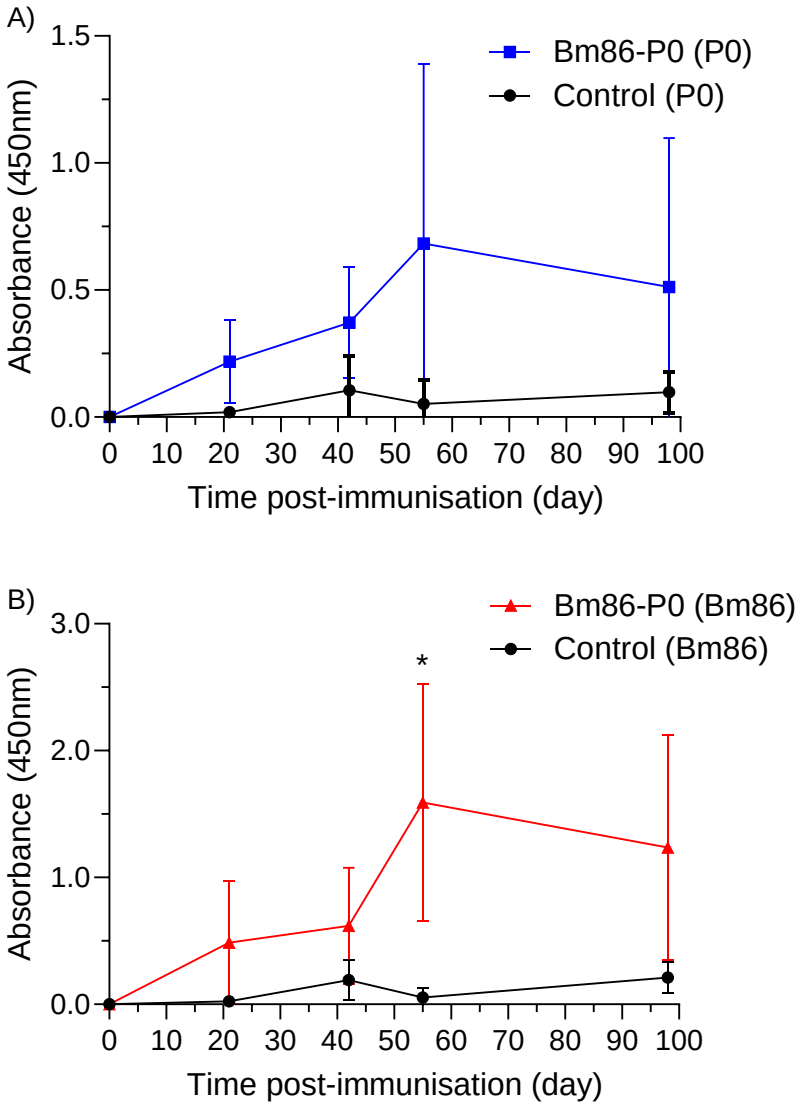

Figure 4.3 Quantification of the specific antibody responses to the cattle tick antigens peptide P0 and Bm86 of cattle (n=6) immunised with the P0-KLH formulation (Group 3). A) P0 specific antibodies; B) Bm86 specific antibodies. Cattle were immunised on Day 0, Day 7 and Day 42. The reactivity of the untreated cattle (n=6) to P0 and Bm86 is also shown in each panel. The mean absorbance for sera (diluted 1:800) for each treatment group at 450nm is shown with one standard deviation. Note different scales on the y-axes of each panel. Statistically significant differences between the mean antibody levels of the groups are indicated by * $p < 0.05$.

4.2 Summary of cattle tick collection data

4.2.1 *Number of adult ticks recovered*

Figure 2.4A illustrates the average number of adult female cattle ticks recovered from the cattle immunised with Gavac™ compared to the unimmunised control cattle. For the first seven days of tick collection there were no clear differences in the number of ticks collected from each group. However, after Day 82, on average fewer ticks were collected from the group immunised with Gavac™. After Day 88, the average number of ticks collected from each group were similar until the end of the collection period (Figure 4A). While these data suggest Gavac™ was effective in reducing the number off ticks dropping of cattle from Day 83 to Day 88, these differences were not statistically significant.

Figure 2.4B illustrates the average number of adult female cattle ticks recovered from the cattle immunised with the P0-KLH formulation compared to the unimmunised control cattle. While in general fewer ticks were recovered from the P0-KLH formulation, the distinction between the groups was not clear. These results suggest the P0-KLH formulation was not effective in reducing the number of ticks dropping off cattle. No statistically significant differences were identified in the number of ticks recovered between these groups.

Figure 2.4C illustrates the average number of adult female cattle ticks recovered from the cattle immunised with the P0-Bm86 formulation compared to the unimmunised control cattle. While in general fewer ticks were recovered from the P0-Bm86 formulation, the distinction between the groups was not clear. These results suggest the P0-Bm86 formulation was not effective in reducing the number of ticks dropping of cattle. The wide standard deviations suggest there may have been a broad range of immune responses in the animals in this group. No statistically significant differences were identified in the number of ticks recovered between these groups.

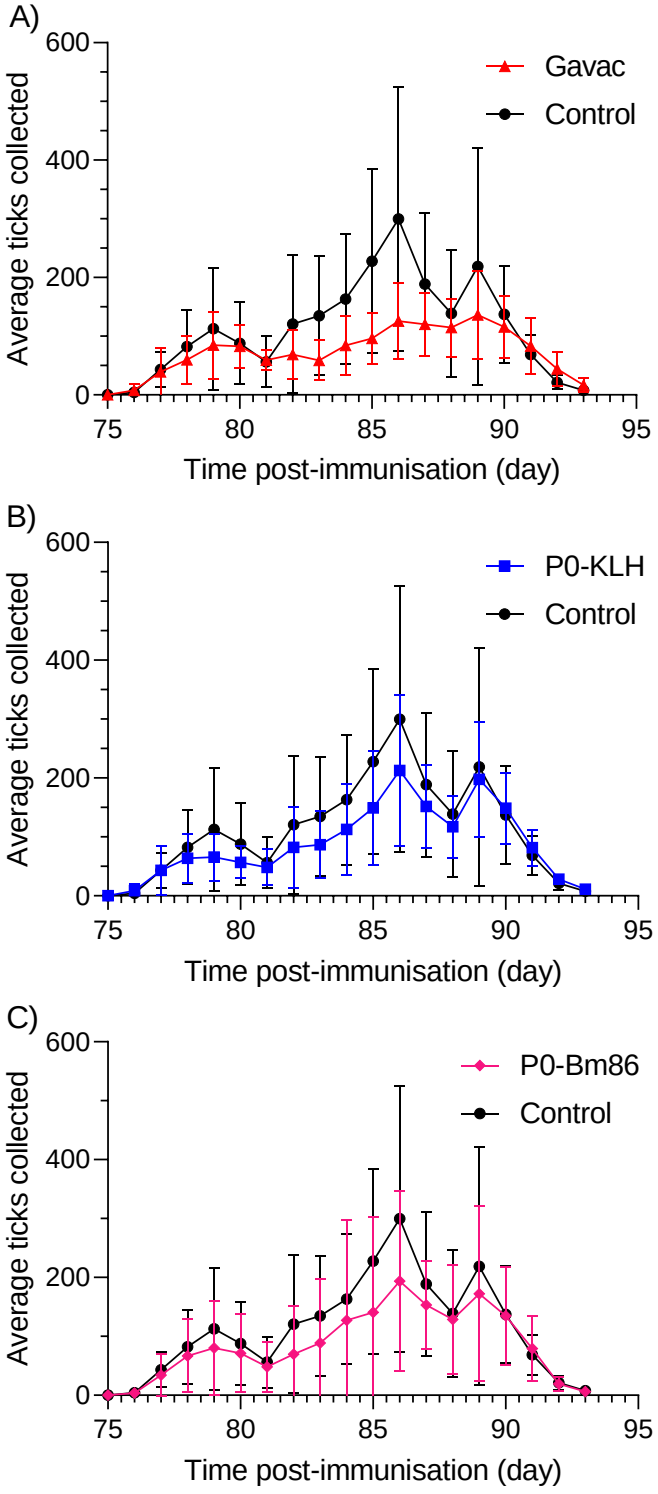

Figure 2.4 Average number of female cattle ticks collected from cattle infested with larvae. A) Cattle (n=6) immunised with the GAVAC™; B) Cattle (n=6) immunised with the P0 formulation; C) Cattle (n=6) immunised with the P0-Bm86 formulation; The mean is shown for each group with one standard deviation. The same data for the control cattle are shown in each panel.

4.2.2 *Weight of adult female ticks recovered*

Figure 2.5A illustrates the average weight of adult female cattle ticks recovered from the cattle immunised with Gavac™ compared to the unimmunised control cattle. The average weights of the ticks collected from the Gavac™ immunised group were significantly different from the average weights of the ticks from the control from Day 77 to Day 90 ($p < 0.05$, Fig. 2.5A). This suggests Gavac™ was effective in reducing the weight of ticks dropping off cattle for most of the experiment.

Figure 2.5B illustrates the average weight of adult female cattle ticks recovered from the cattle immunised with the P0-KLH formulation compared to those recovered from the unimmunised control cattle. No significant differences in the average weights of recovered ticks were identified between these treatment groups (Fig. 2.5B). These results suggest the P0-KLH formulation had no effect in reducing the weight of ticks dropping off cattle.

Figure 2.5C illustrates the average weight of adult female cattle ticks recovered from the cattle immunised with the P0-Bm86 formulation compared to the unimmunised control cattle. While there was a trend for the average weight of the ticks collected from the immunised group to be lighter in comparison to those recovered from the unimmunised control animals, these differences were not statistically significant. These results suggest the P0-Bm86 formulation exhibited some capacity in reducing the weight of adult ticks dropping off immunised cattle, though it was less effective than Gavac™ (Fig. 2.5A and 2.5C).

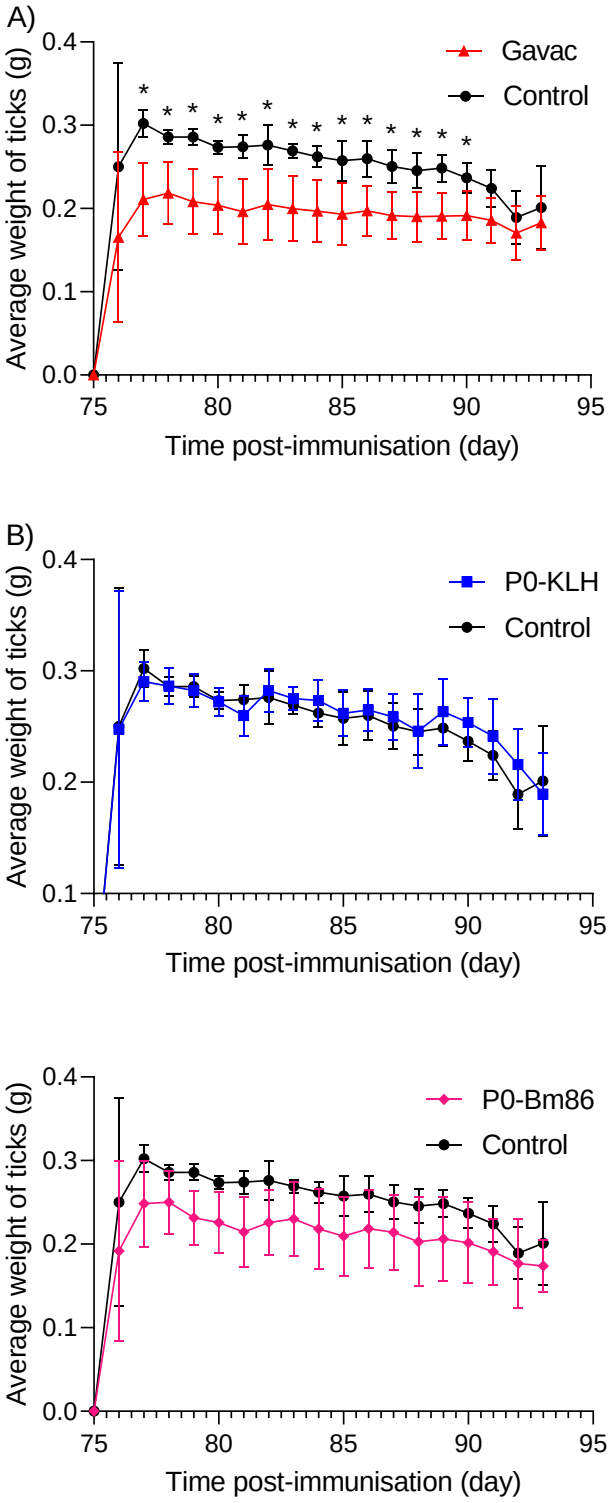

Figure 2.5 Average weight of female cattle ticks collected from cattle infested with larvae. A) Cattle (n=6) immunised with the GAVAC™; B) Cattle (n=6) immunised with the P0 formulation; C) Cattle (n=6) immunised with the P0-Bm86 formulation; The mean is shown for each group with one standard deviation. The same data for the control cattle is also shown in each panel. Statistically significant differences between the mean weight of the recovered ticks are indicated by * $p < 0.05$.

4.2.3 *Percentage damage of recovered ticks*

A representative sample of engorged adult female ticks recovered from each group on Day 97 are shown in Fig. 4.6. The undamaged ticks from the control group were a green-brown colour in appearance with yellowish stripes (urate crystals), wrinkled cuticles and clear/yellow legs (Fig. 4.6A). In contrast the ticks from the group immunised with Gavac™ were red-brown in appearance, few or no yellow stripes, generally smooth cuticles and mostly reddish legs (Fig. 4.6B). Ticks recovered from cattle immunised with the P0-KLH formulation were similar in appearance to those recovered from the control animals (Fig. 4.6C). Ticks recovered from cattle immunised with the P0-Bm86 formulation exhibited similar phenotypic characteristics to those immunised with Gavac™ (Fig. 4.6D). These characteristics included red-brown in appearance, few or no yellow stripes, generally smooth cuticles and mostly reddish legs (Fig. 4.6D).

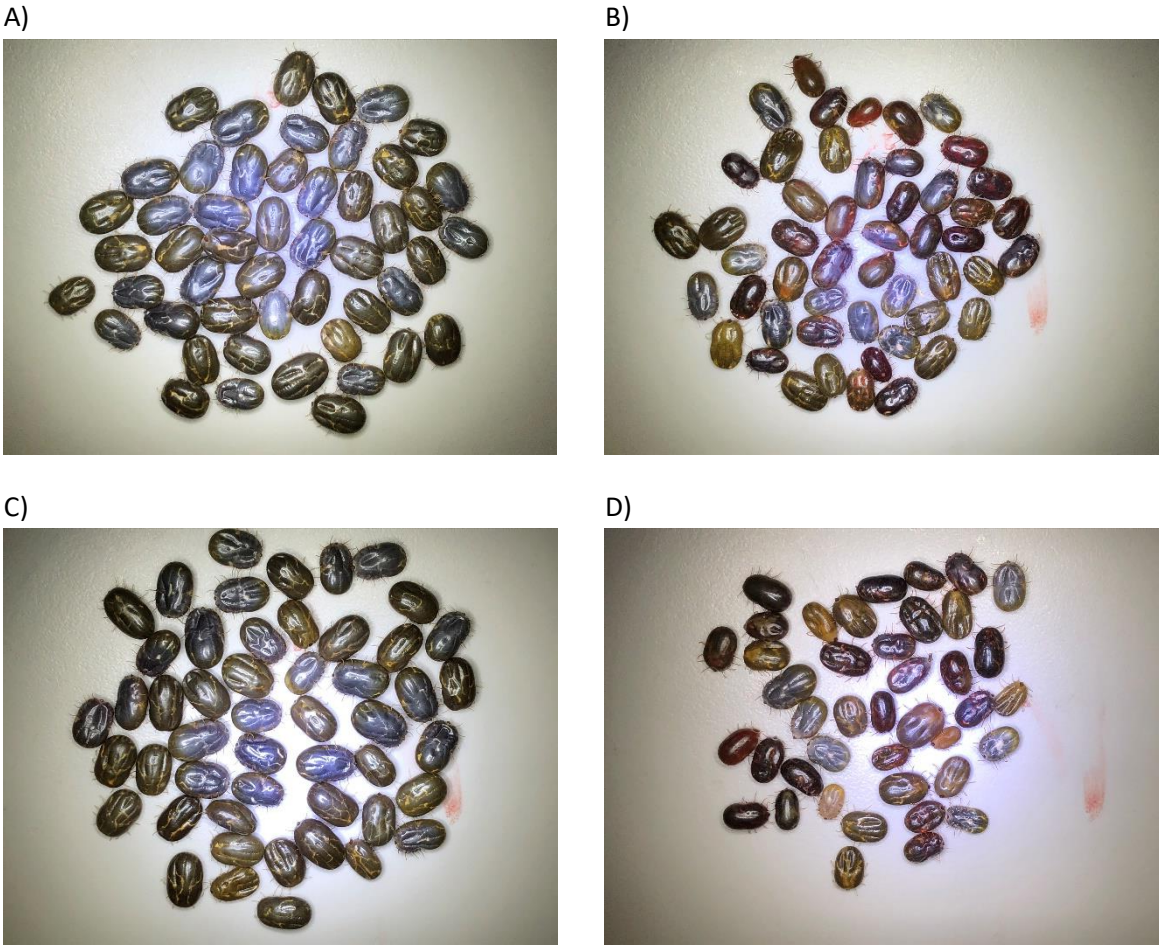

Figure 4.6 Appearance of adult female ticks completing engorgement and dropping off cattle during the infestation trial, collected on Day 97. A) Ticks from an unimmunised animal. B) Ticks from an animal immunised with Gavac™. C) Ticks from an unimmunised animal with P0-KLH. D) Ticks from an animal immunised with P0-Bm86.

The average percentages of affected ticks recovered from each group of cattle are shown in Fig. 4.7. A significantly higher percentage of affected ticks were recovered from the Gavac™ immunised group compared to the control group from Day 77 to Day 93 ($p<0.05$ all days, Fig. 4.7). Few affected ticks were identified in the group immunised with the P0-KLH formulation. The average percentages of damaged ticks collected from this were indistinguishable from the control animals (Fig. 4.7B).

The average percentage of affected ticks collected from the cattle immunised with the P0-Bm86 formulation was less than 25% across the collection period (Fig. 4.7C). While the average percentages of affected ticks tended to be consistent, no significant differences were identified when these data were compared to the control group data.

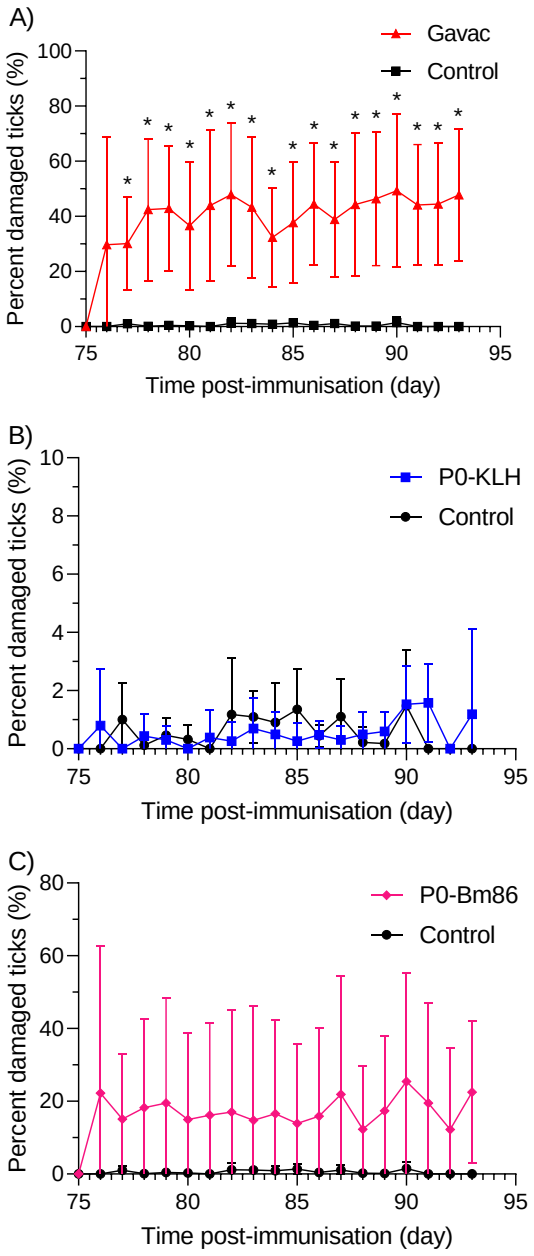

Figure 4.6 Percentage of affected engorged female ticks collected from cattle infested with larvae. A) Cattle (n=6) immunised with the Gavac™; B) Cattle (n=6) immunised with the P0-KLH formulation; C) Cattle (n=6) immunised with the P0-Bm86 formulation. The mean percentage affected ticks is shown for each group with one standard deviation. The same information for the control cattle is shown in each panel. Note differing scales on Y-axis of each panel. Statistically significant differences between the percentage of damaged ticks in the immunised animals compared to the control animals are indicated by * $p < 0.05$.

4.2.4 *Cattle tick survival to oviposition*

To investigate the reproductive capacity of the recovered female adult ticks, random selections of ticks were selected and evaluated for their capacity to survive to oviposition. While there was a trend for a lower percentage of ticks to survive to oviposition for the Gavac™ and P0-Bm86 immunised groups, no significant differences were identified (Fig. 4.7A and 4.7C). The percentage of ticks surviving to oviposition from the P0-KLH immunised group was indistinguishable from that of the control group (Fig. 4.7B).

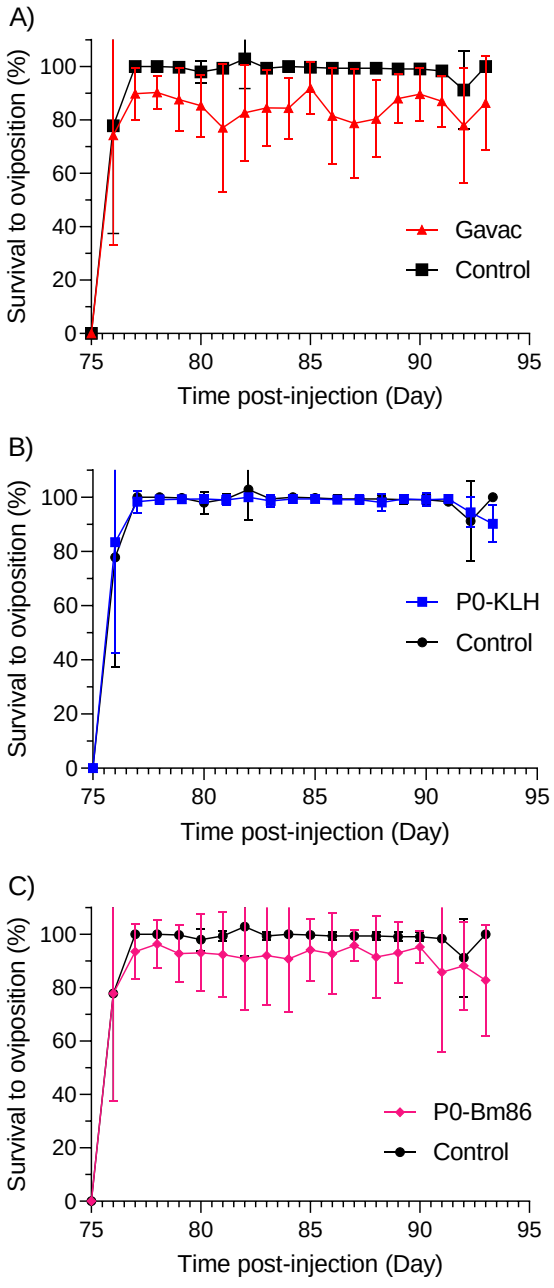

Fig. 4.7: The mean survival percentage of cattle ticks recovered from the trial cattle between Day 75 and Day 92 post-immunisation. A) Cattle (n=6) immunised with the Gavac™; B) Cattle (n=6) immunised with the P0-KLH formulation; C) Cattle (n=6) immunised with the P0-Bm86 formulation. Error bars represent one standard deviation.

4.2.5 *Egg production by recovered ticks*

Across all collection days the cattle ticks from the groups immunised with Gavac™ or P0-Bm86, on average, laid lighter egg masses per gram of incubated cattle ticks compared to the unimmunised control group (Fig. 4.8A and 4.8C). However no statistically significant differences were identified for either group. The average egg masses laid by ticks recovered from the P0-KLH immunised group were indistinguishable from those of the control group (Fig. 4.7B).

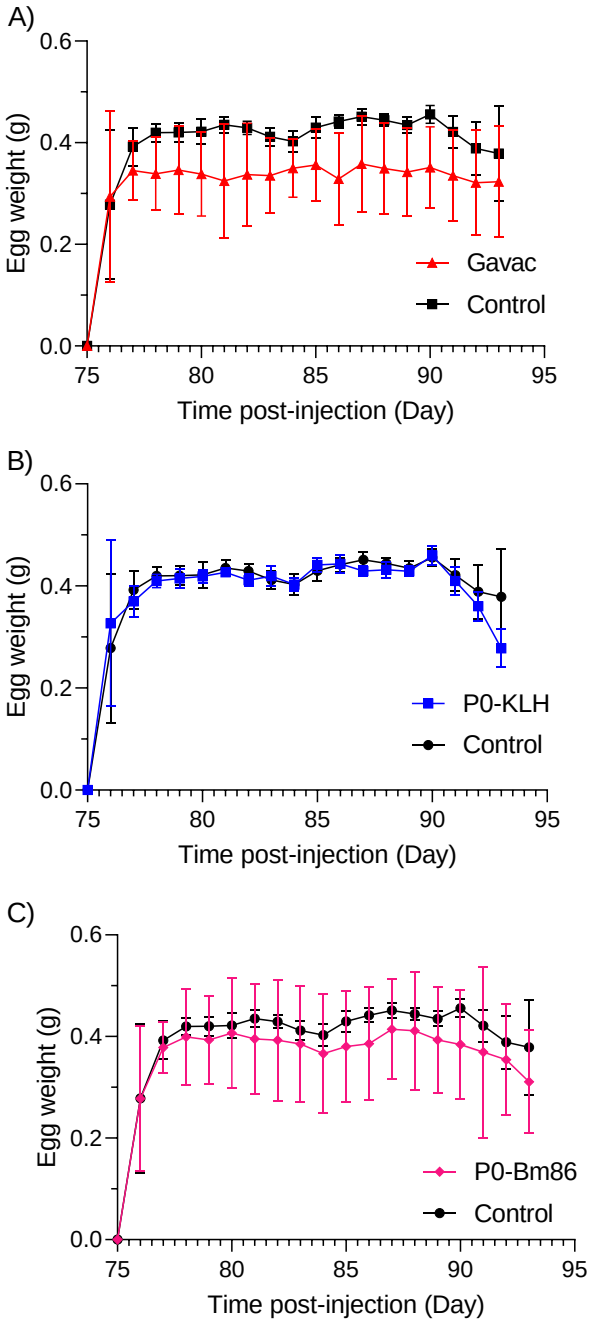

Fig. 4.8 The mean weights of the egg masses laid by ticks recovered from the trial cattle between Day 75 and Day 92 post-immunisation. A) Cattle (n=6) immunised with the Gavac™; B) Cattle (n=6) immunised with the P0-KLH formulation; C) Cattle (n=6) immunised with the P0-Bm86 formulation. Error bars represent one standard deviation. Error bars represent one standard deviation.

4.2.6 *Viability of cattle tick eggs (Day 7)*

Comparison of the average percentages of egg viability (visually assessement), suggested the immunisation groups were indistinguishable from the unimmunised control group (Fig. 4.9).

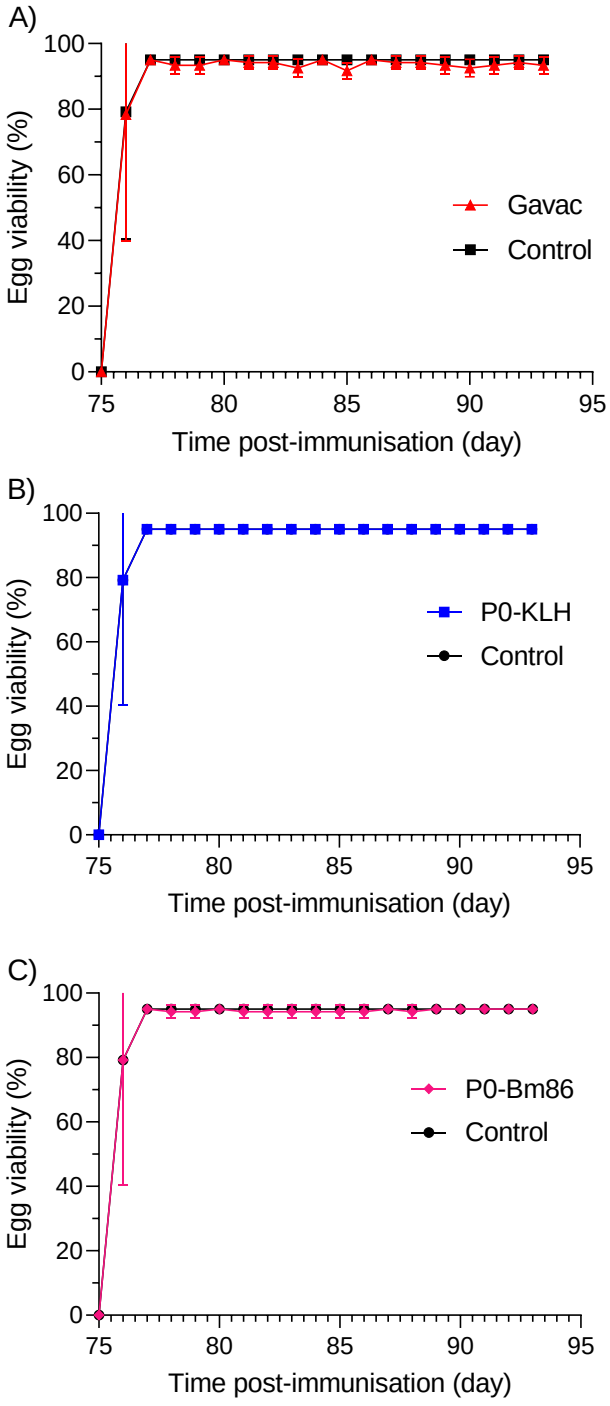

Fig. 4.8: Visual assessment of the viability of cattle tick eggs seven days after laying from the trial cattle between Day 75 and Day 95 post-immunisation. A) Cattle (n=6) immunised with the Gavac™; B) Cattle (n=6) immunised with the P0-KLH formulation; C) Cattle (n=6) immunised with the P0-Bm86 formulation. Error bars represent one standard deviation.

4.2.7 *Viability of cattle tick eggs at hatch (Day 35)*

The average percentages of egg viability at hatch were marginally lower for the Gavac™ and P0-Bm86 immunised groups compared to the unimmunised control group (Fig. 4.9A and 4.9B). However, these differences were not statistically significant. The egg viabilities at hatch for the P0-KLH immunised group were indistinguishable from that of the control group (Fig. 4.9B).

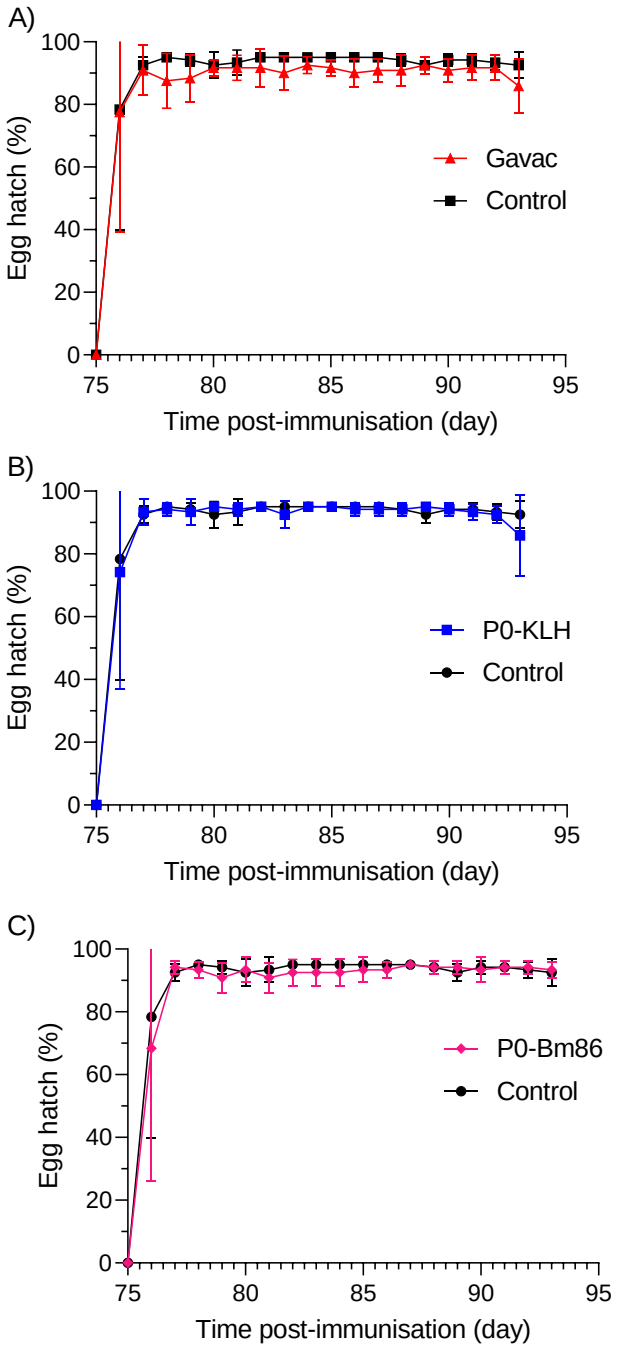

Fig. 4.9: Viability of cattle tick eggs 35 days after laying by cattle ticks collected dropping from cattle between Day 75 and Day 93 post-immunisation. A) Cattle (n=6) immunised with the Gavac™; B) Cattle (n=6) immunised with the P0-KLH formulation; C) Cattle (n=6) immunised with the P0-Bm86 formulation. Error bars represent one standard deviation.

4.3 Estimation of vaccine efficacy – Gavac™

The efficacy estimates for Gavac™ are shown in Fig. 4.10. The cumulative efficacy estimates for Gavac™ all peaked at Day 86 at approximately 50% to 55%. The highest reproductive efficacy estimate was detected on Day 86 was 55.8% (Fig. 4.10D). While this estimate ranged from 35.6% and 49.3% from Day 78 to Day 93 (Fig. 4.10D).

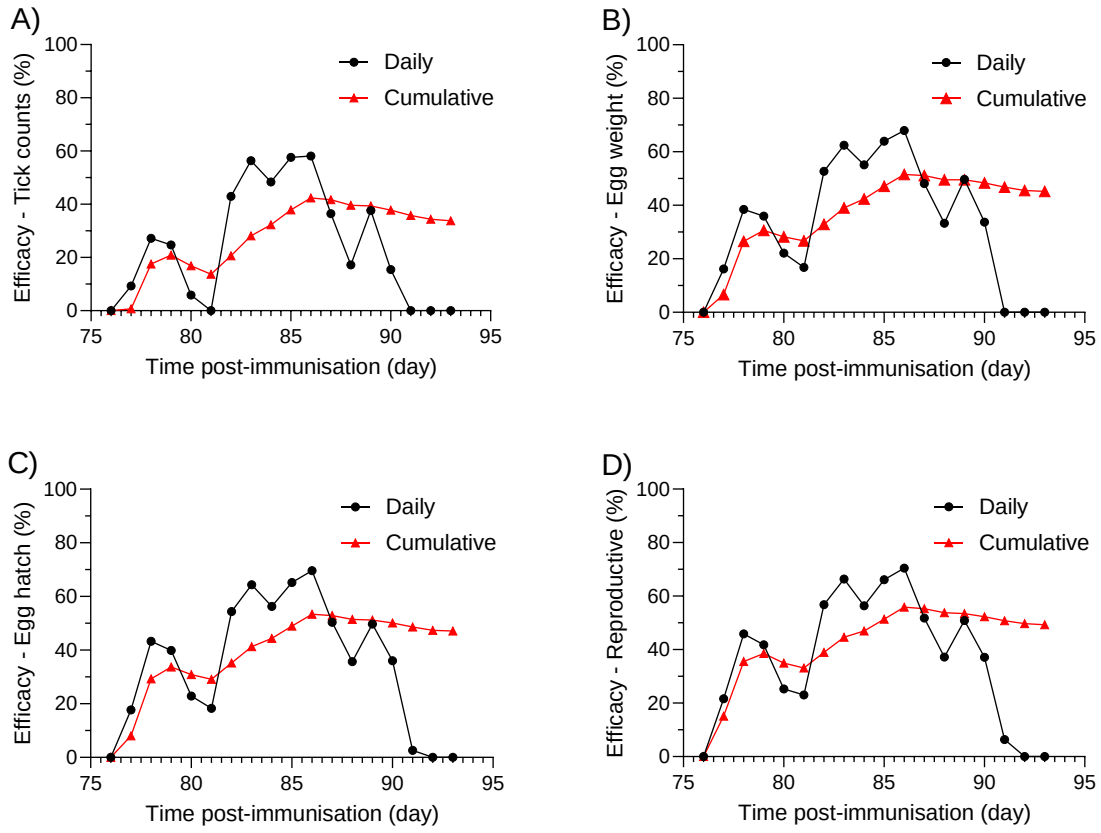

Fig. 4.10. Estimates of vaccine efficacy for Gavac™. The daily efficacy and cumulative efficacy are shown for each estimate. A) Vaccine efficacy based on the number of adult female ticks recovered. B) Vaccine efficacy based on the number of average mass (g) of eggs laid by adult female ticks. C) Vaccine efficacy based on the hatchability (%) of eggs laid by adult female ticks. D) Vaccine efficacy based on tick reproductive capacity.

4.4 Estimation of vaccine efficacy – P0-KLH

The vaccine efficacy estimates for the P0-KLH formulation are shown in Fig. 4.11. The cumulative efficacy estimates for P0-KLH formulation were all consistently around 30% from Day 79 to Day 88. The reproductive efficacy reached 28.4% on Day 79, peaked at 31.2% on Day 85 and decayed to 22.2% by Day 93 (end of the experiment) (Fig. 4.11D).

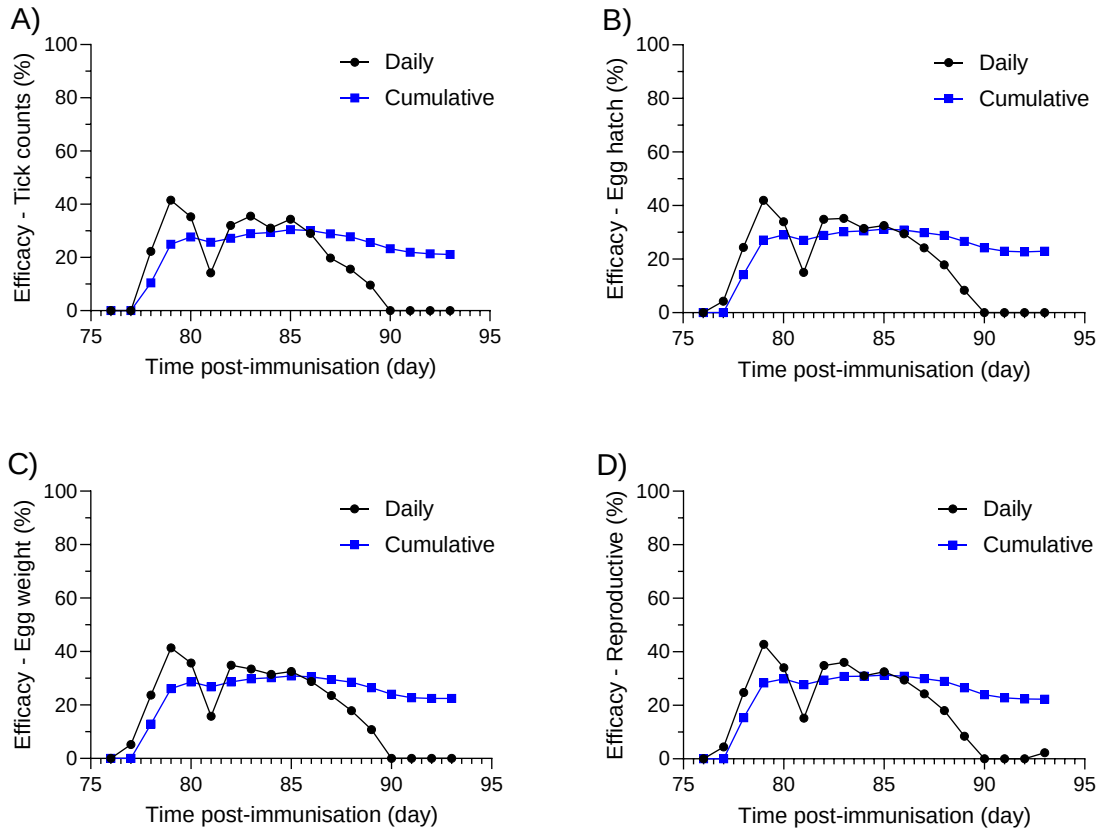

Fig. 4.11. Estimates of vaccine efficacy for the P0-KLH formulation. The daily efficacy and cumulative efficacy are shown for each estimate. A) Vaccine efficacy based on the number of adult female ticks recovered. B) Vaccine efficacy based on the number of average mass (g) of eggs laid by adult female ticks. C) Vaccine efficacy based on the hatchability (%) of eggs laid by adult female ticks. D) Vaccine efficacy based on tick reproductive capacity.

4.5 Estimation of vaccine efficacy – P0-Bm86

The vaccine efficacy estimates for the P0-Bm86 formulation are shown in Fig. 4.12. The cumulative efficacy estimates for P0-Bm86 formulation were consistently above 30% from Day 82 to Day 93. The reproductive efficacy reached 27% on Day 76, peaked at 38.1% on Day 86 and decayed to 31.4% by Day 93 (end of the experiment) (Fig. 4.12D).

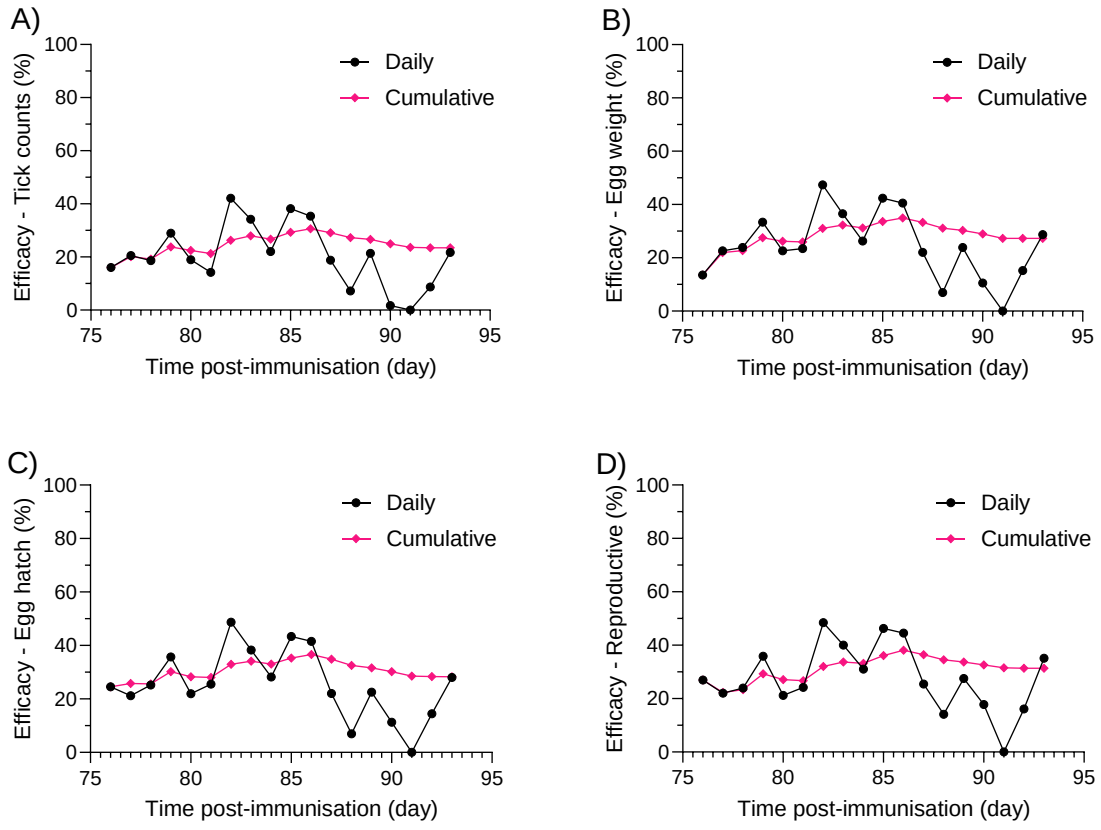

Fig. 4.12 Estimates of vaccine efficacy for the P0-Bm86 formulation. The daily efficacy and cumulative efficacy are shown for each estimate. A) Vaccine efficacy based on the number of adult female ticks recovered. B) Vaccine efficacy based on the number of average mass (g) of eggs laid by adult female ticks. C) Vaccine efficacy based on the hatchability (%) of eggs laid by adult female ticks. D) Vaccine efficacy based on tick reproductive capacity.

5. Conclusion

5.1 Key findings

5.1.1 Gavac™

Antibody responses: Very good Bm86 specific antibody responses were detected in the cattle immunised with Gavac™. These peaked approximately two weeks after the final booster immunisation. Overall, the responses were reasonably consistent between the animals in this group. The peak antibodies levels in some animals were consistent with the antibody responses we have detected in previous experiments. Willadsen et al. (1995) reported a negative correlation between the mass of eggs laid and the antibody titres from TickGuard vaccinated animals. Thus one potential extension of the research report here would be to determine the Bm86 specific-antibody titres of the animals in this study, as this may provide some insights into the low efficacy estimate.

Formulation efficacy estimate: Despite Gavac™ inducing strong antibody responses to Bm86, the reproductive efficacy estimates for this formulation peaked at 55.8% on Day 86, while the cumulative reproductive efficacy estimate was 49.3% on Day 93 (end of experiment).

Previously, the efficacy of Gavac™ has been reported to range from 51 to 91% depending on the strain of cattle tick used (de la Fuente et al., 1999). Although, several studies utilised the Cuban strain Camcord, yielding efficacy estimates ranging from 75 to 91% (See Table 1 in de la Fuente et al., 1999). An efficacy of 75% for Gavac™ against the Australian cattle tick strain Yeerongpilly NRFS has also been reported Penichet et al. (1994). This is the same strain of cattle tick used in the current study.

Conclusion: Gavac™ may be of some value to reducing the impacts of cattle ticks on the Australian red-meat industry. This benefit may only be realised in those production areas where multiple doses are not an impediment to vaccine adoption. It may also require some estimation of what the potential impact of how a 50% reduction in reproductive efficiency would impact on the viability of cattle tick populations in these areas.

5.1.2 P0-KLH formulation

Antibody responses: Significant antibody responses were detected to the P0 peptide were detected after three doses on Day 55 and Day 98. For the P0 peptide, unlike Bm86 being primarily antibody mediated protection, the mechanism through which this antigen affords protection has not been accurately defined. The application of the ribosomal protein P0 as a vaccine candidate was first described in studies focusing on protozoa and bacteria. While antibody was shown to be important in protection from these pathogens, activation of cellular immune responses was also implicated (summarised by Rodríguez-Mallon et al., 2012). The mechanism by which the P0 peptide provides the reported protection from cattle tick infestations has not been determined. The results of the current study suggest antibodies levels maybe a poor indicator of protective efficacy.

Formulation efficacy estimate: Rodríguez-Mallon et al. (2015) reported a remarkably high efficacy of 96% when this formulation was tested in cattle immunisation and infestation studies after four doses administered. In a recent study Rodríguez-Mallon et al (2020) report an efficacy of 89% for this formulation, after three doses administered. In comparison the vaccine efficacy estimate from the current study was 22.3%. It is unclear why the efficacy estimate for the P0-KLH formulation of the current study was well below what has been previously reported for this formulation. Importantly,

strong antibody responses to the peptide were detected which suggest the vaccine formulation retained potency.

Conclusions: In the current study, the P0-KLH formulation failed to provide protection against an induced tick infestation.

5.1.3 P0-Bm86 formulation

Antibody responses: Detectable antibody responses were observed to both components of the P0-Bm86 formulation. However, only the responses to the Bm86 component were significantly different to the control animals on Day 55. The Bm86 specific antibody levels were lower and more variable in this group compared to the antibody levels in the Gavac™ immunised group.

Formulation efficacy estimate: In a recent study Rodríguez-Mallon et al. (2020) reported an efficacy of 84%, after three doses of this formulation. In the current study the reproductive efficacy of the formulation was 31.4%. As this formulation contains both the P0 peptide and Bm86 antigen it was expected this formulation would give the highest level of efficacy or at least equivalent to the Gavac™ estimate of 49.3%.

A potential reason for this reduced estimate is the antigen for this formulation is generated by covalently linking the P0 antigen to the Bm86 antigen. This linking occurs through a chemical reaction between the amine functional groups (the side chains of the amino acid lysine residues and the N-terminus) of Bm86 and P0. This process cannot be controlled and as a result, any, or none of the 54 lysine residues and the N-terminus of a Bm86 molecule could be linked to a peptide. Rodríguez-Mallon et al. (2020) determined that 41 internal residues and the N-terminus of Bm86 were linked to a P0 peptide. The percentage of damaged ticks recovered from cattle immunised with the P0-Bm86 formulation ranged from 12.3% to 25.5% compared to 29.6% to 47.9% for the Gavac™ formulation. The mechanism of protection of the Bm86 antigen is well accepted as being a result of Bm86 specific antibodies binding to the Bm86 protein expressed by epithelial cells lining the tick gut. Subsequent disruption of the gut lining resulting in the uncontrolled ingestion of blood which causes the observable damage to the cattle tick body (e.g. “red legs”). The reduced damage observed to ticks recovered from the P0-Bm86 immunised animals could indicate that the linking of the P0 peptide to the lysine residues in the amino acid backbone of Bm86 has masked some epitopes. Alternatively, the linkages may have interfered with processing of Bm86 by the immune system resulting in the production of less antigen specific antibodies. The Bm86 specific antibody responses detected in the P0-Bm86 immunised animals were, on average, lower and more variable compared to those detected in the Gavac™ immunised cattle with mean absorbance values of 1.56 (±0.93SD) and 2.25 (±0.67SD), respectively. These values were not significantly different ($p=0.1734$), and suggest the cattle responded poorly to this formulation.

Conclusions: In the current study, the P0-Bm86 formulation failed to provide protection against an induced tick infestation.

5.2 Benefits to industry

Although the three vaccines tested in this project failed to provide evidence of efficacy, the benefits to the cattle industry of an effective cattle tick vaccine are as described in the Background section of this report. This is especially true if such a vaccine could provide season-long protection with a single injection.

6. Future research and recommendations

The results of this study clearly suggest that cattle tick vaccines can interfere with the reproductive capacity of this important pest. These results are consistent with the results of other studies in Australia and overseas, albeit that the formulations evaluated in this study were less effective than might have anticipated. Future research and development activities are required on other prototype cattle tick vaccines to determine their value to Australian red-meat producers. As this study has demonstrate a key component of any evaluation is that protection studies for any overseas formulation be conducted in Australia to ensure its effectiveness under Australian conditions.

Considering the possibility of a deterioration in transit of the quality of the imported vaccines, it seems justifiable to perform at least one more efficacy trial in Australia, with locally produced vaccines (recombinant antigen expression and vaccine formulation). Overseas publications describing the broader (than just cattle tick) efficacy spectrum of the P0 antigen, makes this a potentially attractive prospect for the management of other tick infestations, e.g. *Ixodes holocyclus* (Paralysis tick) and *Haemaphysalis* spp. (vectors of *Theileria orientalis*), on more hosts than just cattle. As an example, immunisation with the P0 peptide from *R. sanguineus* yielded an efficacy of 90% in rabbit immunisation/challenge studies (Rodríguez-Mallon et al., 2012). Moreover, the P0 antigen has been shown to elicit high protective efficacy estimates against other pathogens, including several species of babesia, suggesting it may warrant further investigation (as summarised by Rodríguez-Mallon et al., 2012).

Research and development may also be warranted to determine what the target efficacy is for a cattle tick vaccine. While the aspirational target of most vaccine development projects optimistically/arbitrarily set the target efficacy somewhere between 90% and 100% this is generally not achievable, particularly in field studies. Thus, a better understanding of how efficacies at various levels might affect the long-term structure, stability, and resilience of cattle tick populations would allow for vaccine efficacy targets to be set that would be potential achievable and robust across multiple settings (pen trials, field trials, etc).

While Gavac™ was identified as the most effective of the three formulations evaluated in this study, the level of efficacy was approximately 50%, this is lower than other reported estimates for this vaccine, discussed previously (Section 5.1.1). However, this is the first time this vaccine has been used under Australian experimental conditions, combined with Australian cattle and cattle ticks. Previously, Jonsson et al. (2000) reported the reproductive efficacy of TickGaurd^{PLUS} to be 72% in Australian dairy cattle after administration of three doses. Perhaps, if an additional dose of Gavac™ was administered an improved reproductive efficacy for this formulation may have resulted. Although the main target population of a cattle tick vaccine is the northern Australian cattle industry where mustering is only once or twice annually, evaluating the efficacy of Gavac™ after three doses may have over-estimated its value in extensive production systems. Consequently, additional research and development is required to further develop promising single-dose cattle tick vaccines, such as the one recently described by Mahony et al. (2019).

7. References

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8. Appendix

8.1 Appendix A – Data from the temperature logger included in the vaccine shipment from Cuba to Australa

Read-Out document of the Q-tag CLm doc

Q-tag® CLm doc

Identification Number: BHIU01701_01_202010200043

Configuration id number (CID)	228
Start delay	0 min
Alarm status	**Alarm**
Total number of measurements	15374

Alarm	Configuration	Status	Date (dd.MM.yyyy)	Time (GMT-05:00)	Duration
1: Single Event	above 45.0°C for 1h	OK			
2: Accumulated	above 30.0°C for 10h	OK			
3: Accumulated	above 8.0°C for 20h	**ALARM**	14.10.2020	00:17	2d 7h 46min
4: Accumulated	below 2.0°C for 20h	OK			3h 59min
5: Single Event	below -0.5°C for 1h	OK			

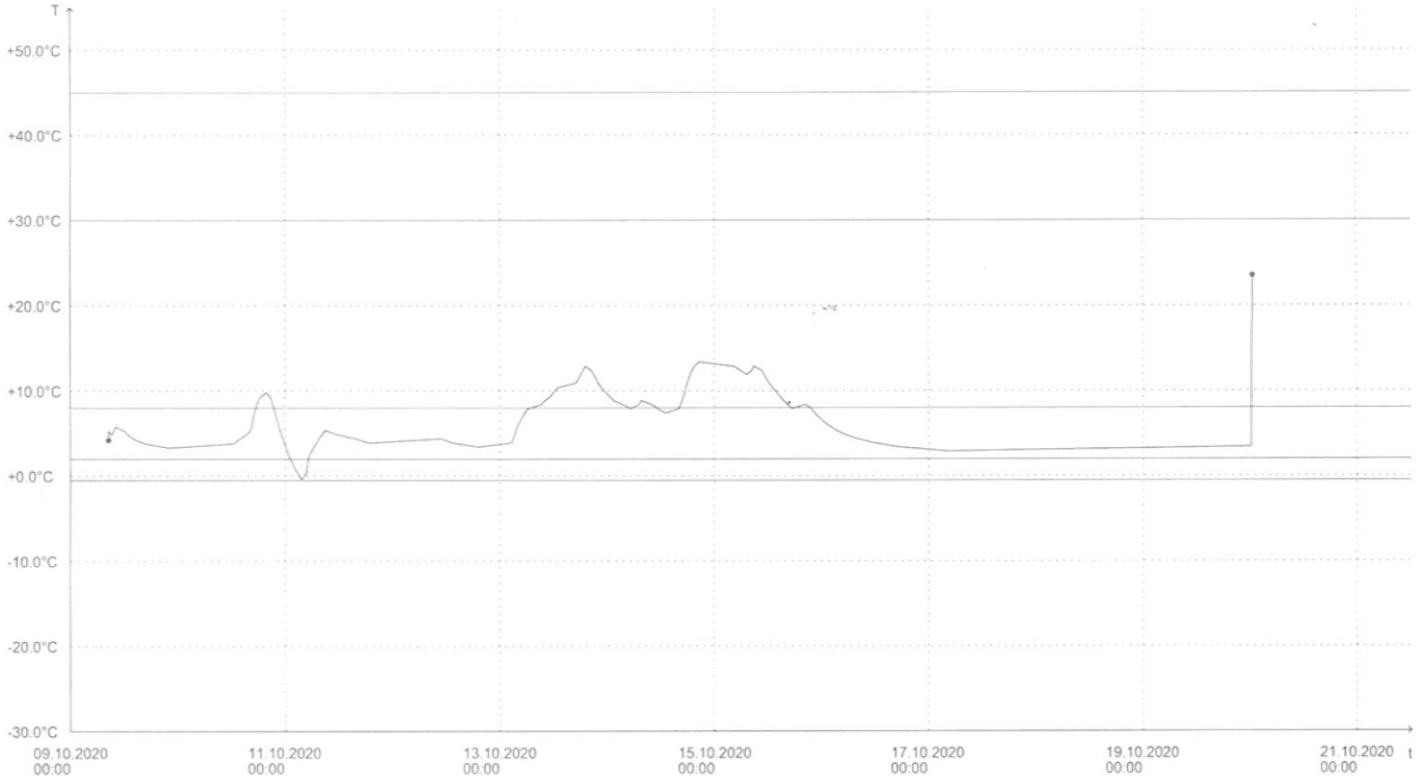
Log Result	Temperature	Date (dd.MM.yyyy)	Time (GMT-05:00)
Start date and time		09.10.2020	08:29
Stop date and time		20.10.2020	00:43
Highest temperature	+23.5°C	20.10.2020	00:42
Lowest temperature	-0.4°C	11.10.2020	03:38
MKT	+6.0°C		
Average temperature	+5.3°C		

Q-tag® CLm doc is manufactured by Q-tag AG Switzerland 1/2

Read-Out document of the Q-tag CLm doc

Q-tag® CLm doc

Identification Number: BHIU01701_01_202010200043



Q-tag® CLm doc is manufactured by Q-tag AG Switzerland 2/2

8.2 Appendix B – Immunisation and infestation trial protocol

Experimental Day	Gavac (n=6)	P0-KLH vaccine (n=6)	P0-Bm86 Group (n=6)	Control Group (n=6)	Comments
Day -5					General Health Assessments
Day -2					General Health Assessments
Day -1					General Health Assessments
Day 0	Nil	Dose 1	Dose 1	Nil	General Health Assessments Bleed all animals
Day 1					General Health Assessments
Day 3					General Health Assessments
Day 5					General Health Assessments
Day 7	Dose 1	Dose 2	Dose 2	Nil	General Health Assessments Bleed all animals
Day 8					General Health Assessments
Day 10					General Health Assessments
Day 12					General Health Assessments
Day 14					General Health Assessments, Bleed all
Day 21					General Health Assessments, Bleed all animals
Day 42	Dose 2	Dose 3	Dose 3	Nil	General Health Assessments Bleed all animals
Day 43					General Health Assessments
Day 45					General Health Assessments
Day 47					General Health Assessments
Day 49					Move into challenge facility
Day 55					Bleed all animals
Day 56	Infestation	Infestation	Infestation	Infestation	Infest all animals with 5000 female cattle tick larvae. Infestation 1 (Day 0)
Day 58	Infestation	Infestation	Infestation	Infestation	Infest all animals with 5000 female cattle tick larvae
Day 61	Infestation	Infestation	Infestation	Infestation	Infest all animals with 5000 female cattle tick larvae

Day 63	Infestation	Infestation	Infestation	Infestation	Infest all animals with 5000 female cattle tick larvae
Day 65	Infestation	Infestation	Infestation	Infestation	Infest all animals with 5000 female cattle tick larvae
Day 68	Infestation	Infestation	Infestation	Infestation	Infest all animals with 5000 female cattle tick larvae
					Infestation 6 (Day 0)
Day 75-76	ITC[1]	ITC[1]	ITC[1]	ITC[1]	
Day 77	ITC[1]	ITC[1]	ITC[1]	ITC[1]	Infestation 1 (Day 21)
Day 78-88	ITC[1]	ITC[1]	ITC[1]	ITC[1]	
Day 89	ITC[1]	ITC[1]	ITC[1]	ITC[1]	Infestation 6 Day 21
Day 90-92	ITC[1]	ITC[1]	ITC[1]	ITC[1]	
Day 93	ITC[1]	ITC[1]	ITC[1]	ITC[1]	Bled all animals.

[1]Individual tick counts