Exploring the use of an anti-tick vaccine as a tool for the integrated eradication of the cattle fever tick, *Rhipicephalus (Boophilus) annulatus*

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**A B S T R A C T**

Bovine babesiosis, also known as cattle fever, is a tick-borne protozoal disease foreign to the United States. It was eradicated by eliminating the vector species, *Rhipicephalus (Boophilus) annulatus* and *Rhipicephalus (Boophilus) microplus*, through the efforts of the Cattle Fever Tick Eradication Program (CFTEP), with the exception of a permanent quarantine zone (PQZ) in south Texas along the border with Mexico. Keeping the U.S. free of cattle fever ticks in a sustainable manner is a critical national agricultural biosecurity issue. The efficacy of a Bm86-based anti-tick vaccine commercialized outside of the U.S. was evaluated against a strain of *R. annulatus* originated from an outbreak in Texas. Vaccination controlled 99.9 and 91.4% of the ticks 8 weeks and 5.5 months after the initial vaccination, respectively. Computer modeling of habitat suitability within the PQZ typically at risk of re-infestation with *R. annulatus* from Mexico predicted that at a level of control greater than 40%, eradication would be maintained indefinitely. Efficacy and computer modeling data indicate that the integration of vaccination using a Bm86-based anti-tick vaccine with standard eradication practices within the northwestern half of the PQZ could incentivize producers to maintain cattle on pasture thereby avoiding the need to vacate infested premises. Implementing this epidemiologically proactive strategy offers the opportunity to prevent *R. annulatus* outbreaks in the U.S., which would represent a significant shift in the way the CFTEP operates.

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**1. Introduction**

Until the end of the nineteenth century, the cattle fever tick, *Rhipicephalus (Boophilus) annulatus* (Say), was the most economically important tick infesting livestock, particularly cattle, in the United States. In addition to a direct impact on animal health associated with its obligate feeding behavior, *R. annulatus* also transmits *Babesia bigemina* and *Babesia bovis*, which cause bovine babesiosis, also known as cattle fever, and *Anaplasma marginale*, the causative agent of anaemia. Estimates made in 1906 for the direct and indirect economic loss inflicted by *R. annulatus* on the U.S. cattle industry annually were $130 million which in today’s currency would amount to about $3 billion [1].

Since 1943, the U.S. has been free of the cattle fever ticks (CFT), *R. annulatus* and *Rhipicephalus microplus*, after a successful eradication campaign established in 1906. The southern cattle fever tick, *R. microplus*, became a target for eradication after its presence in the U.S. was reported in 1912 within the southern state of Florida [1]. A permanent quarantine zone (PQZ) remains in place, and is managed by the Cattle Fever Tick Eradication Program (CFTEP) in South Texas to buffer incursions from Mexico where CFT are endemic. The maintenance of this buffer zone is a critical national agricultural biosecurity issue. Estimates indicate that the initial economic impact of *R. annulatus* and *R. microplus* extending to their historical range in the U.S. would be at least $100 million [2].

In collaboration with the Texas Animal Health Commission, personnel with the Veterinary Services division of the U.S. Department of Agriculture’s Animal and Plant Health Inspection Service inspect cattle within the PQZ for CFT. Infested and adjacent premises are quarantined when at least one tick is detected. According to current statutes, producers can choose from two options to have the
quarantine lifted. Producers can have their cattle treated with
the organophosphate acaricide coumaphos every 7–14 days for
6–9 months depending on the season. Alternatively, cattle can
be treated with coumaphos at 7–14 day intervals until they are found
clean of CFT for two consecutive inspections. At this time, produc-
ers may choose to move their animals to a new location provided
the vacated pasture is not restocked for 6–9 months, depending on
the time of the year cattle were removed.

The immunological protection of cattle against infestation offers
an opportunity to sustainably prevent CFT re-infestation in the U.S.
if all the cattle in the permanent quarantine zone are treated using
existing technologies and vaccinated with a highly efficacious anti-
tick vaccine [3]. The anti-tick vaccine technology commercially
available outside the U.S. is based on the recombinant form of the
gut protein Bm86 from R. microplus [4]. Bm86-based vaccines rep-
resent the first generation of recombinant anti-tick vaccines to be
commercialized and their use by producers in integrated programs
in some parts of Mexico has been shown to control CFT infesta-
tions [5]. However, there is no Bm86-based vaccine commercially
available in the U.S.

Exploring the potential utility of a Bm86-based vaccine under
controlled conditions is required to assess the impact of imple-
menting the practice of anti-tick vaccination as part of an integrated
strategy by the CFTEP. Here, we report the results of stall experi-
ments where the efficacy of a Bm86-based vaccine, commercialized
outside the U.S., was tested against a strain of R. annulatus
established from an outbreak within the U.S. Additionally, a com-
puterized model, designed to evaluate tick habitat suitability
within the PQZ prone to R. annulatus infestation, was used to predict
the effect of anti-tick vaccination by the CFTEP.

2. Materials and methods

2.1. Ticks

R. annulatus originally collected in 2010 from Dimmit County
Texas (Klien Grass Field strain) was used in this study. It has been
reared in the laboratory using standard procedures [6]. The F1 and
F2 generations were used for this research.

2.2. Cattle immunizations and tick infestations

Research described in this study was approved by the Institu-
tional Animal Care and Use Committee of the USDA-ARS KRUSLIRL,
and it was conducted in accordance with accepted practices
described in the “Guide for the Care and Use of Agricultural Animals
in Agricultural Research and Teaching, 3rd Ed.”, as promulgated by
the Federation of Animal Science Societies. All personnel working
with cattle and ticks were blinded to the treatment groups. Ten
Bos taurus animals (Black Angus) approx. 136–227 kg (300–500 lbs)
were used in this study. All animals were randomly assigned to
treatment groups. Half of the animals were vaccinated with the
Bm86-based anti-tick vaccine Gavac® (Lot M92011-3, 2 ml intra-
muscular injection, neck or rump, 1.5” needle) imported from
Mexico on weeks 0, 4, and 6. The remaining half was injected with
only an adjuvant carrier (1 ml intra-muscular injection, Montanide
50, Seppic, France). On week 8, each animal was infested with 250 mg (≈4500) larvae.

The Gavac® label recommended a 6 months booster. In order to
determine the efficacy of the vaccine before the boosting, all cattle
were returned to the barn at 5.5 months after the initial vaccination
and each infested with 250 mg of tick larvae using the identical
treatment groups as the initial experiment.

Beginning approximately 21 days (d) after each infestation and
continuing daily for 11 consecutive days, engorged females
detached from each calf were collected and counted. From each tick
collection on each animal, a random sample of up to 10 engorged
females (whenever possible) was saved to obtain ovipositional data.
The sampled females were collected, weighed collectively, and
placed together in a coded 9 cm diameter petri dish in an incubator
at 27 ± 2 °C, 92% RH, under a 12:12 h photoperiod (L:D)
Females were allowed to oviposit for 20 days after which time the
females were discarded. The eggs from each petri dish were mixed,
weighed, and drawn into a 10 mm × 10 mm H × W rectangle. Five,
20 mg aliquots of eggs were taken at equidistant intervals through-
out the length of the rectangle (≥10% of the entire combined egg
mass), placed into a coded 15 mm × 40 mm (1-dram) shell vial, and
weighed. The sub-sampled eggs were held in the incubator for 20
days and then placed into a freezer (−80 °C) for 24 h to kill larvae.
The percent hatch was determined by counting the number of un-
challenged eggs from each sub-sample and comparing that number
to the number of eggs originally in the subsample as determined
by weight (54.6 µg per egg for R. annulatus [6,7]). Vaccine efficacy
(E) was calculated after all data (tick counts, female weight, egg
weights, and percent hatch) were collected over the entire 11 days
evaluation period. The efficacy of vaccine was evaluated employing
the following formulae [8,9].

Effect on the number of adult female ticks (DT) = 100I – (NTV/NTC), where NTV is the number of adult females ticks in the vaccinated group and NTC is the number of adult females ticks in the control group.

Effect on tick weight (DW) = 100I – (WTV/WTC), where WTV is the average adult female tick weight in the vaccinated group and WTC is the average adult female tick weight in the control group.

Effect on oviposition (DO) = 100I – [PATV/PATC], where PATV is the average weight of the eggs per survived tick in the vaccinated group and PATC is the average weight of the eggs per survived tick in the control group.

Effect on egg fertility (DF) = 100I – (PPOV/PPOL), where PPOV is the average weight of the larvae per gram of eggs in the vaccinated group and PPOL is the average weight of the larvae per gram of eggs in the control group.

Vaccine efficacy (E) was calculated as

\[ E = \frac{100I - (CRT \times CR0 \times CRF)}{CRT \times NTV / NTC} \]

where CRT = NTV / NTC, CR0 = PATV / PATC and CRF = PPOV / PPOL that represent the reduction in the number of adult female ticks, oviposition, and egg fertility as compared to the control group, respectively.

The dependent measurements of the vaccinated and control
cattle groups over time were analyzed for statistical differences
(P < 0.05) using the GLIMMIX Procedure in SAS (version 9.2). The
daily (repeated) measures were modeled with the unstructured
covariance structure to account for the correlated measures of the
individual calves.

2.3. Determination of serum antibody levels by enzyme-linked immunosorbent assay (ELISA)

Before each immunization, and monthly between boosting
immunizations, blood samples were collected (10 ml) from each
calf into 12.5 ml sterile serum separator tubes (Corvac, Mansfield,
MA). Serum was collected into 14 ml tubes (Falcon, Franklin Lakes,
NJ) after centrifugation (3400 × g for 1 h at 25 °C) and stored at
−4 °C for 24 h and then at −80 °C thereafter. Serum antibody titers
were determined using an antigen-specific indirect enzyme-linked
immunosorbent assay (ELISA). Purified Bm86 antigens were used to
cat ELISA plates overnight at 4 °C. Sera was serially diluted to
1:800, 1:1600, 1:3200, and 1:6400 in Blue Diluent (Assurequality).
The plates were incubated with the diluted sera for 1 h at room
temperature and then incubated with 1:7500 goat anti-bovine
immunoglobulins (IgG) – horseradish peroxidase conjugates (KPL,
Gaithersburg, MD) for 1 h at room temperature. The color reaction
was developed with 3,3',5,5'-tetramethylbenzidine (KPL, Gaithersburg, MD), and the absorbance at 450 nm (A450 nm) determined. Results were expressed as an antibody titer unit that had been calculated using a standard curve of a predetermined titer using the Magellan program (Version 6.6). The standard’s titer was determined as the reciprocal of the dilution where it equals three times the A450 nm of the negative preimmune serum. Final sample titer unit is the geometric mean of the four readings incorporating the dilution factors. The lognormal value of the serum antibody unit measure was analyzed for differences between vaccinated and control cattle over time using the GLIMMIX procedure (P < 0.05) in SAS (Version 9.2). The repeated measurements were modeled with the unstructured covariance structure.

2.4. Computer modeling

Computer modeling was accomplished using a modified model developed for predicting habitat suitability of CFT within the region of Texas that encompasses the permanent quarantine area of the CFTEP [10]. The model was updated to take into account only the areas under the northwestern half of the permanent quarantine zone with the actual number of cattle contained within the quarantine zone. Equations simulating the life cycle of the tick were updated with new data [11]. Vaccine efficacy was simulated by removing a percentage of cattle from the simulated environment, allowing ticks to feed on a reduced number of available hosts. This reduced the chances of feeding and thence the oviposition and next generation production. Simulations were completed for 10 years, 100 times each. Therefore, results for each single year were the result of the average of 100 different simulations. The simulations allowed a population of 100 engorged females to enter into the area of the permanent quarantine zone, with the simulated densities of cattle. Daily climate data were obtained from the New LocClim software (FAO, Rome, Italy). The software contains the complete database of FAO climate recording stations and is able to interpolate daily values of temperature (maximum, minimum, mean), rainfall and water vapor deficit.

3. Results

3.1. Efficacy of Bm86-based vaccine and effects of immunization on biological parameters of Texas outbreak strain of R. annulatus infesting cattle

The overall vaccine efficacy against R. annulatus was 99.9 and 91.4% for the 55 and 163 d infestations, respectively (Table 1). The drop from 99.9 to 91.4% control was due to a single calf (103) in the vaccinated group that did not react to the vaccination like the others. No engorged adult females were obtained from this calf when infested 55 d post initial injection (100% control). However, there were no differences in the numbers of engorged adult females collected from this calf after the 163 d infestation when compared to the control calves (0% control). The main treatment effect was a reduction in engorged female ticks obtained from cattle vaccinated with Bm86 and infested at 55 and 163 d post initial injection (P = 0.0001, Table 1). Engorgement weight, oviposition, and percent hatch were also reduced by vaccination at the 55 d infestation, but not for the 163 d infestation.

Table 1

<table>
<thead>
<tr>
<th>Experimental group†</th>
<th>N</th>
<th>Percent reduction (vaccinated/control)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DT</td>
<td>DW</td>
</tr>
<tr>
<td>55 d infestation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>5</td>
<td>99.5% (3.0 ± 0.0)*</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>(6473 ± 134.0)</td>
</tr>
<tr>
<td>163 d infestation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccinated</td>
<td>5</td>
<td>82.0% (119.8 ± 114.9)*</td>
</tr>
<tr>
<td>Control</td>
<td>4</td>
<td>(6645 ± 46.8)</td>
</tr>
</tbody>
</table>

† Cattle were randomly assigned to experimental groups, vaccinated, and challenged with R. annulatus larvae.

‡ The percent reduction was calculated with respect to the control group: DT, % reduction in tick infestation; DW, % reduction in tick weight; DO, % reduction in oviposition; DF, % reduction in egg fertility. In parenthesis are the average ± SE for adult female tick number, tick weight (g), oviposition (egg weight (g)/tick) and egg percent hatch and were compared by Student’s t-test with unequal variance between vaccinated and control groups (P < 0.05).

³ Vaccine efficacy (E) was calculated as 100[1 – (CRT × CRO × CRF)], where CRT, CRO, and CRF are the reduction in the number of adult female ticks, oviposition, and egg fertility as compared to the control group, respectively.

Cattle immunized with the Bm86-based vaccine developed a specific humoral immune response. Antibody titers in vaccinated cattle increased after successive immunizations until approximately 8 weeks post initial injection and were significantly different from the control (adjuvant only) group (P = 0.0001, Fig. 1).

Fig. 1. Antibody response in vaccinated animals. Serum antibody titers were determined using an antigen-specific indirect enzyme-linked immunosorbent assay (ELISA). Results were expressed as an antibody titer unit that had been calculated using a standard curve of a predetermined titer using the Magellan program (Version 6.6). Final sample titer unit is the geometric mean of four readings incorporating the dilution factors. The lognormal value of the serum antibody unit measure was analyzed for differences between vaccinated and control cattle over time using the GLIMMIX procedure (P < 0.05) in SAS (Version 9.2). The time of vaccination and tick infestation are indicated (arrows).
In agreement with the tick efficacy data described above, calf 103 was observed to have had a low antibody titer throughout the entire experiment (Fig. 1). By 163 d post initial injection, this calf had an antibody titer greater than 21 fold below the average antibody titer of the other 4 vaccinated animals (781.7 versus 16967.9, respectively). The remaining cattle in the vaccinated group retained at least an antibody titer of 2714 and the vaccine efficacy remained at 99.9% for these 4 vaccinated animals when compared to the control (adjuvant only) group.

3.3. Overt safety of cattle immunized with Bm86-based vaccine

Cattle appeared generally healthy following immunization with the Bm86-based vaccine. No overt adverse reactions were observed at the injection site 24–48 h post injection, and during necropsy at 6 months after the initial inoculation. Rectal temperature of the vaccinated animals remained within normal range throughout the study.

3.4. Computer modeling predicts benefit of vaccination against R. annulatus in PQZ

Computer modeling predicted a significant reduction in tick infested cattle due to vaccination at 20 and 40% efficacy. For control values of 40% and lower, the simulation predicted population establishment and growth throughout the first year followed by population stabilization after 2–3 years. After year 3, the simulated populations were stable and seasonally repetitive for the remaining 10 year simulation (Fig. 2). When the control by vaccination was above 40% in the simulation, no R. annulatus populations established within the PQZ. Abiotic factors alone with control by vaccination had a strong influence on tick survival.

4. Discussion

This is the first study to document the short and long-term efficacy of an anti-tick vaccine against an outbreak strain of CFT in the U.S. Although the two technologies differ in their mode of action, the level of efficacy (99.9% reduction in tick infestation) against R. annulatus achieved by day 55 after initial immunization with the Bm86-based vaccine tested here was equivalent to the level of control achieved with chemical acaricides. A significant reduction in the level of tick infestation, engorgement weight, and fecundity was observed. However, the main effect of the vaccine was heavily based on the reduction of the number of engorged females recovered. Modeling the effect of immunization with an anti-tick vaccine on tick populations predicted that a Bm86-based vaccine would allow the CFTEP to sustainably maintain the eradication of R. annulatus from the PQZ indefinitely.

Commercial vaccines based on the recombinant version of the gut molecule from R. microplus termed Bm86 were initially registered in Cuba under the trade name Gavac® in 1993 and in Australia as TickGuard™ in 1994 [5]. Documentation of the benefits of using Bm86-based vaccines in integrated control programs has focused on R. microplus as this is the more invasive of the two CFT species in tropical and subtropical parts of the world where bovine babesiosis and CFT are endemic. Here, we provide evidence for the first time of the potential to use an anti-tick vaccine for CFT eradication purposes within the U.S. CFTEP. Gavac® was used on 588,573 head of dairy cattle infested with R. microplus in Cuba and reduced the need for acaricide treatments by 87%. Additionally, the incidence of babesia infection was reduced from 54 to 1.9 clinical cases per 1000 cattle following the use of the product [12]. On a ranch in Northern Mexico, 2800 head of cattle were vaccinated with Gavac® for over 10 years, the R. microplus population was reduced by 80% and the use of acaricides decreased by 67% [5].

For reasons that remain to be fully understood, Bm86-based vaccines are more efficacious against R. annulatus than on R. microplus. Studies have shown greater than 99.9% control of R. annulatus on artificially infested cattle in Mexico [13]. More recently, experiments completed at the University of Tamaulipas, Ciudad Victoria, Mexico achieved 99.6% and 100.0% control of R. annulatus infested cattle in different trials [8,9,14]. Unlike previous reports from stall tests, we also assessed residual efficacy prior to the first 6-month booster following the first 3 inoculations according to the product label. Efficacy dropped to 91.4% at the 163 d infestation due to one animal in the vaccinated group. This apparently non-reactive animal had a relatively low anti-Bm86 antibody titer throughout the study (781.7 units at 163 d). In contrast, all other calves in the vaccinated group retained an antibody titer of at least 2714 units and the vaccine efficacy remained at 99.9% for these animals. Variation in the antibody response between cattle immunized with a commercial Bm86-based vaccine has been observed before. This variation in antibody levels between vaccinated animals has been ascribed to factors such as age and reproductive status [15]. Formulation may also play a role in the immunogenicity of vaccine products. The residual effectiveness of Bm86 formulated as Tickgard Plus™ has been demonstrated to be long. After just two priming injections, cattle were 100% protected against CFT for over a year (390 days) [16]. The results presented here using an outbreak strain from the U.S. confirm previous findings indicating that Bm86-based vaccines can elicit an efficacious and lasting immune response in cattle herds affording protection against R. annulatus at low antibody titers.

It is hypothesized that incorporating the practice of immunization, using a Bm86-based vaccine, to the existing protocol requiring the use of chemical acaricides would allow the elimination and prevention of outbreaks in the northwestern half (350 km) of the PQZ where R. annulatus is the predominant species. Currently, the CFTEP is based upon removal of cattle from infested pastures or biweekly acaricide dips for a total of 6–9 months. The program has zero-tolerance, with the detection of one tick initiating quarantine and required program treatment. The time frame of treatment is not subject to be altered for it is designed to eliminate CFT from premises (residual free living larval ticks) by treating or removing the host animals. Vaccination with a Bm86-based vaccine in
combination with either a long acting chemical acaricide, e.g., a macrocyclic lactone injectable product, or the standard coumaphos analogue R. annulatus populations while allowing for cattle to remain on pasture without further treatment. As indicated by the model data, the number and intensity of R. annulatus in the PQZ would progressively diminish. Conceptually, R. annulatus eradication would be sustained through anti-tick vaccination. In practice the integration of anti-tick vaccination, where resistance to R. annulatus is induced in cattle, would enable the CFTEP to take a proactive epidemiological approach in the way it operates, and producers would be motivated to maintain their cattle in premises within the PQZ that have been persistently infested.

A current threat to the CFTEP is the involvement of white-tailed deer (WTD), Odocoleus virginianus, in the epidemiology of CFT outbreaks within and beyond the PQZ. Exploding populations of WTD in south Texas have been shown to be an alternative host of CFT and could serve as a dispersing mechanism in the ecosystem [17]. Current technologies to eradicate CFT infesting WTD involve the use of bait stations that incorporate passive methods of treatment with chemical acaricides acting topically or systemically. The potential to develop an anti-tick vaccine to immunize WTD exists as it has been done with other vaccines [18,19]. Research and development of anti-tick vaccines to treat wildlife is an area requiring scientific attention especially with regards to effective delivery systems. In addition to adapting to infest WTD, the more invasive R. microplus has adapted to parasitize other native cervid species in Brazil and New Caledonia. This situation may complicate local CFT control programs [17].

Eliminating R. annulatus from the northwestern half of the PQZ through the continued immunization of cattle using a highly effective anti-tick vaccine including an antigen like Bm86 could arguably create an ecological niche that the R. microplus would occupy. Such a scenario might occur by disrupting the R. microplus/R. annulatus boundary in the PQZ that has been theorized to be maintained through hybrid instability between the two species [20]. However, it is possible that this scenario may have occurred since both species have been highly controlled in the area for over 65 years through the use of coumaphos, essentially eliminating the potential for interspecific mating. It is more likely that the current distribution of both species is due to the environmental conditions of the area rather than reproductive isolation between the species [10]. If this is the case, climate change resulting in warmer and more humid winters in the northwestern half of the PQZ might promote the invasion of those areas by R. microplus through time regardless of the level of artificial control.

This study documents the utility of integrating anti-tick vaccine technology as part of the toolbox available to keep the U.S. free of CFT in a sustainable manner. Field studies are warranted to confirm vaccine performance under operational conditions. Additionally, access to a commercial anti-tick vaccine approved for use in the U.S. is required for its long-term use. Finally, an anti-tick vaccine that is as efficacious against R. microplus as Bm86-based vaccines are against R. annulatus is needed. Realizing these opportunities will enable the evolution of statutes governing CFTEP operations to advance cattle raising in the PQZ and secure the status of the U.S. as bovine babesiosis-free.

Ethical approval

Research described in this study was approved by the Institutional Animal Care and Use Committee of the USDA-ARS KBUSRL and it was conducted in accordance with accepted practices described in the “Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, 3rd Ed.”, as promulgated by the Federation of Animal Science Societies.

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