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MODELLING THE FIELD EFFICACY OF RECOMBINANT rDNA (Bm86) ANTI-TICK VACCINE TICKGARD™ AGAINST *BOOPHILUS MICROPLUS* AND *BOOPHILUS DECOLORATUS* TICKS

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SUMMARY

Markov chain model was applied in modelling the field efficacy of rDNA vaccine based on recombinant Bm86 (TickGard™) a membrane bound protein gut antigen from *Boophilus microplus* against *Boophilus microplus* and *Boophilus decoloratus* tick species. Data were imputed into a Markov Chain modelling system using the EXCEL programming tool in a mathematical equation that has two major components namely, states and transitions. Simulation of Vaccinated versus Control against the Two tick species at 25ºC with relative humidity of 85% showed an initial fluctuation then equilibrium was gained on day 46 for the Control model. Equilibrium was gained on days 30 and 36 for the vaccinated cattle population against *B. microplus* and *Boophilus decoloratus* respectively. There was a shortened decadal period of 10 days earlier which has a direct impact on the spread of *B. microplus* and *B. decoloratus* in subsequent generations.

KEY WORDS: ***Boophilus*** **ticks, Markov Chain, Model, Efficacy, TickGard™**

INTRODUCTION

Ticks and tick borne diseases affect animals and human health worldwide and are the cause of significant economic losses. Approximately 10% of the currently known 867 ticks species act as vectors of a broad range of pathogens of domesticated animals and humans and are also responsible for damage directly due to their feeding behaviour. The impact of the global economy is considered to be high and although some estimates are given, there is a lack of reliable data, (Jongejan and Uilenberg, 2004). Ticks can cause severe toxic conditions such as paralysis and toxicosis, irritation and allergy. The diseases transmitted by ticks to livestock are also a major constraint to animal production predominantly in (sub) tropical areas of the world. Generally, tick borne protozoan diseases (e.g. Babesiosis and Theileriosis) and Rickettsial diseases (e.g. Anaplasmosis and Heartwater or Cowdriosis) are pre eminent health and management problems of cattle and all ruminants as well as buffaloes, affecting the livelihood of farming communities in Africa, Asia and Latin America (Walker *et al*, 2003).

Tick-borne diseases rank high in terms of their impact on the livelihood of resource poor farming communities in developing countries (Perry *et al*., 2000; Minjauw and McLeod, 2003). This is particularly relevant in parts of sub-Sahara Africa, Asia and Latin America where the demand for livestock is increasing rapidly, (Delgodo *et al*., 1999, Tonnesen *et al*., 2004). *Boophilus* species are one-host ticks that take about three weeks to complete their cycles on the host from unfed larva to engorged female, preferably on cattle. Although *Boophilus* ticks have short mouth-parts, damage to hides and skin is considerable as the preferred feeding sites are of good leather potential.

B. microplus is the most important species; others are *B. annulatus, B. decloratus, and B. geigyi* and they continue to be a major threat to cattle in the tropics and sub-tropics acting both as debilitating agents and as vectors of organism that transmits disease such as babesiosis, anaplasmosis and theileriosis. The tick-host relationship is complex with great sensitivity to environmental conditions such as temperature, humidity and evaporation rate in different parts of the tick's geographical range.

Control of tropical ticks and tick-borne diseases, especially in more susceptible and productive exotic or upgraded breeds of livestock, still depend mainly on intensive tick control using acaricides. However, these chemicals are toxic, leave residues in meat and milk and cause environmental pollution. The resistance of ticks to acaricides poses an increasing threat to livestock range. Vaccination using concealed antigen, was proposed by Galun, (1978) and a protective antigen, Bm86 was subsequently identified and synthesized using recombinant DNA technology. The efficacy of available anti *Boophilus microplus* vaccines (TickGard®, Hoechst Animal Health, Australia and Gavac®, Heber Biotec S.A., Havana, Cuba) against *Boophilus annulatus* in two independent trials with two different vaccines was interesting to note (Fregoso *et al*.,1998; Pipano *et al*., 2003). The anti-tick vaccines reduces fecundity of adult female ticks rather than causing high mortalities, as has been the case with chemicals, therefore research into novel, ecologically sound, practical tick control methods should be intensified and implementation of existing methods to vaccinate against ticks and tick- borne diseases (Redondo *et al*., 1999; De Vos ea al., 2001; Rodriguez *et al*., 1995 and De La- Fuente *et al*., 1998).

The objectives of the study was to model the field efficacy of recombinant DNA vaccine based on recombinant Bm86 gut antigen (TickGard®) from *Boophilus microplus* against *Boophilus microplus* and *Boophilus decoloratus* tick species.

MATERIALS AND METHODS

Data collection

Data for the model were retrieved from work done by (Odongo *et. al*., 2007 and Garcia-Garcia *et. al*., 2000) Table 3 and experts' opinion in regions where the recombinant Bm86 vaccine trials have been conducted on cattle.

Programming Markov chain model

A Markov Chain model is a mathematical equation that has two major components: states and transitions. The model represents a system or process that moves between a number of states through transition. In this Markov vaccination model, the transitional matrix has nine states based on *Boophilus* life cycle using the EXCEL 2003 soft-ware package.

Modelling methods

The Markov chain Matrix modelling technique was employed to estimate the effect of vaccination on a naive population dynamics at different booster time of 10 days decadal period at various vaccine efficacies probabilities from literature on *Boophilus decoloratus* and *Boophilus microplus (*Caswell, 2001). The basic model was developed and used as control, compared with the immunized model. The components of Markov chain Model (MCM) were the (1) Equation system (2) Survival Rates on Host and (3) Eggs hatchability. Simulations were made by calculating the number of individuals entering into next age class interval (state) based on the survival rates probability, fecundity, development rates, mating probability of the adult females, and eggs hatchability in the present state. For consistency of the model a time step of 10 days is used in this model as well as vaccination booster intervals (Caswell, 2001). Also in this model three approaches were adapted. Firstly, the population growth pattern was observed at 25^0C and relative humidity >85% control model. Secondly, the model was simulated for *B. decoloratus* and *B. microplus* until an equilibrium stage was seen using the constant parameters and lastly, vaccination parameters were introduced in the model and the behaviour of the population dynamics were observed for both ticks concurrently.

Equation Systems

The equations used for the model are given below which explains the nine (9) states of the developmental or life cycle of *Boophilus* species which is a one host tick.

$$L1_{(i+1)} = s_2Q_{2(i)} + s_3Q_{3(i)}$$

(Success rate of questing larva on host)

$$H_{(i+1)} = \theta \times L_{(i)}$$

(Probability rate of larva moulting to male nymphs)

$$X_{(i+1)} = \mu \times H_{(i)} + k_1 \times X_{(i)}$$

(Rate of nymphs surviving as adult males)

$$Y_{(i+1)} = \mu \times H_{(i)}$$

(Probability rate of female nymphs moulting into adult females)

$$P_{(i+1)} = f \times Y + k_1 x P$$

(Survival rate of engored females ovipositing)

$$E_{(i+1)} = (n \times P_i) \times g + k_2 \times E_{(i)}$$

(Rate of ovipositing eggs hatchibility to larva)

$$Q_{1(i+1)} = h \times E_{(i)}$$

(Survival rate of newly hached larva without cuticle)

$$Q_{2(i+1)} = m_1 \times Q_{1(i)}$$

(Survival rate of questing larva with developed cuticle)

$$Q_{3\,(i+1)} = m_2 \times Q_{2\,(i)} + k_3 \times Q_{3\,(i)}$$

(Survival rate of questing larva finding a host)

RESULTS

3.1 Simulations of Immunized versus Control against the two tick species

Comparisons between simulations of the tick population density at 25ºC for vaccinated cattle and unvaccinated on *Boophilus microplus* is shown in Fig1. Initial fluctuation in the model was observed and equilibrium was achieved on day 46. In the control simulation, equilibrium was gained on day 30 for the immunized cattle population which shows a sharp drop in the slope of the graph as compared with simulation on *Boophilus decoloratus* while equilibrium was gained on day 36 for the immunized under same conditions as with control Fig 2. Similarly, a pooled simulation was done to compare between vaccinated and control groups irrespective of the species in which differences between the tick population density form the slope of the graph where equilibrium was attained much faster Fig 4. The effect of climatic conditions on tick population in the field a temperature dependent simulation at 25ºC at 85% relative humidity was performed Fig 3.

TABLE I: Parameters and constants in the unvaccinated model

Parameter	**B. decoloratus**	**B. microplus**
Larvo_nymph moult **θ**	0.91	0.91
Numphal_ Adult moult (Male) **µ**	0.335	0.335
Numphal_ Adult moult (Female) **µ**	0.335	0.335
Decadal survival prob. Of male **P_1**	0.35	0.35
Survival prob. of Adult females	0.5	0.5

TABLE II: Temperature dependent parameters in the unvaccinated model at different temperatures including 25^0c as optimum for the two tick species

	10^0C		**15^0C**		**20^0C**		**25^0C**		**30^0C**		**35^0C**	
	Bd	***Bm***	***Bb***	***Bm***	***Bb***	***Bm***	***Bb***	***Bm***	***Bb***	***Bm***	***Bb***	***Bm***
Pre_oviposition + Oviposition period	40	40	58	56	34	34	**25**	**18.5**	24	16	23	12
Period in decade	4d	4d	6d	6d	4d	4d	**3d**	**2d**	3d	2d	3d	2d
Cumulative Survival rate	.9	.9	.9	.9	.9	.9	**.9**	**.9**	.85	.8	.8	.7
Decadal Survival rate of females **n**	.7	.7	.78	.78	.7	.7	**.63**	**.47**	.61	.44	.59	.41
Decadal rate of ovipositing females	0	.0	.11	.11	.19	.19	**.27**	**.42**	.23	.35	.28	.28
No. of eggs **g**	0	0	1100	1000	2000	2500	**2500**	**3000**	2000	2500	1500	2000
Incubation period	100	100	100	100	55	40	**26**	**30**	20	18	20	19
Period in decade	10d	10d	10d	10d	6d	5d	**3d**	**3d**	2d	2d	2d	2d
Cumulative Hatchability	0	0	0	0	.7	.7	**.9**	**.9**	.95	.95	.95	.95
Decadal rate of delayed incub.$\mathbf{k_2}$	.69	.69	.69	.69	.65	.6	**.63**	**.63**	.48	.48	.48	.48
Decadal hatchability **h**	0	0	0	0	.04	.09	**.26**	**.26**	.46	.46	.46	.46
Survival rate of Qa $\mathbf{m_1}$	.9	.9	.9	.9	.9	.9	**.9**	**.9**	.2	.2	.1	.1
Surviv. Rate of Qb $\mathbf{m_2}$	.8	.88	.8	.88	.86	.86	**.2**	**.2**	.2	.2	0	0
Host finding rate of Qb $\mathbf{s_2}$	.25	.25	.25	.25	.25	.25	**.25**	**.25**	.25	.25	0	0
Host finding rate of Qc $\mathbf{s_3}$	.05	.05	.05	.05	.05	.05	**.05**	**.05**	.05	.05	0	0
Survival rate of Qc $\mathbf{k_3}$	.8	.87	.8	.87	.78	.84	**.16**	**.19**	.16	.16	0	0

TABLE III: Parameters in the Vaccination Models

Parameter	***Survival Probability***	***Number of eggs***	***Vaccine efficacy***	***Reference***
Female ticks *Control*	*0.65*	*2500*	*0*	*Odongo , 2007*
Female ticks *immunized*	*0.38*	*1525*	*0.61*	
Female ticks *Control*	*0.5*	*4500*	*0*	Garcia - Garcia *et al.* (2000)
Female ticks *immunized*	*0.23*	*720*	*0.84*	

Mortality in eggs of 0.43 is constant for both species

Fig.1: Immunization with Bm86 and control groups of *Boophilus microplus*

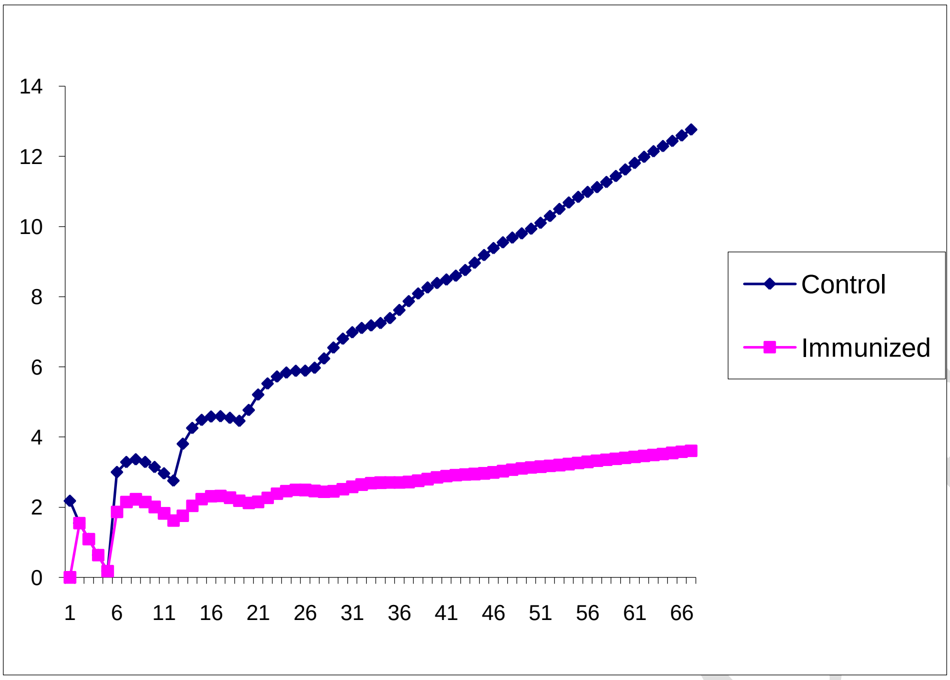

Fig.2: Immunization with Bm86 and control groups of *Boophilus decoloratus*

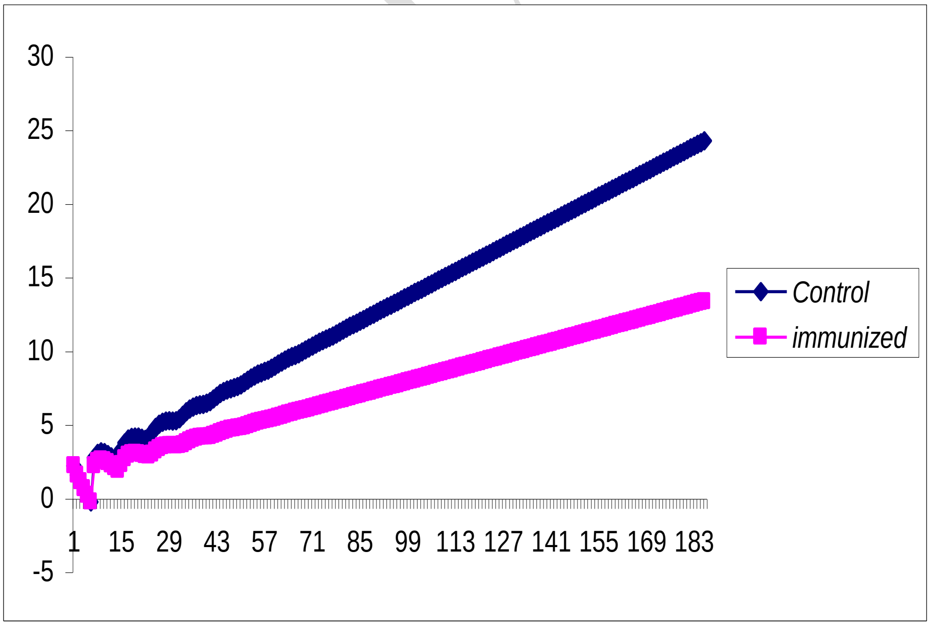

Fig. 3: Simulation at different temperatures showing ticks population dynamics

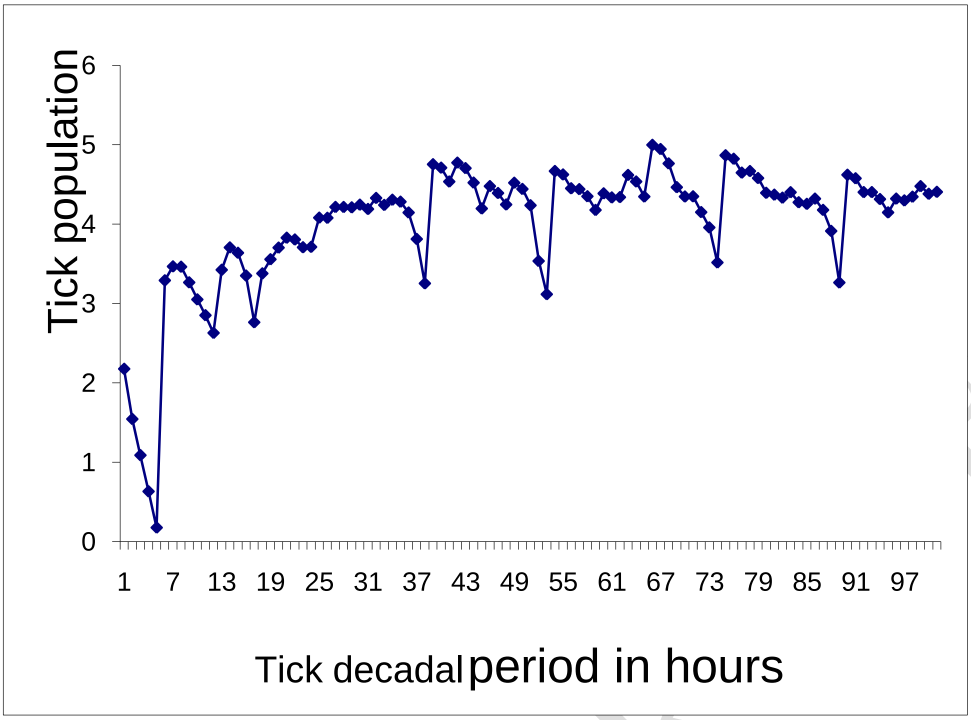

TABLE IV: CONTROL AND VACCINATION TRANSITIONAL STATE MATRIX OF *B. DECOLORATUS*

	L1	H1	X1	Y1	P1	O1	E1	Q11	Q12	Q13
L1	0	0	0	0	0	0	0	0	0.25	0.05
H1	0.91	0	0	0	0	0	0	0	0	0
X1	0	0.335	0.35	0	0	0	0	0	0	0
Y1	0	0.335	0	0	0	0	0	0	0	0
P1	0	0	0	.5	0	0	0	0	0	0
O1	0	0	0	0	0.63	0	0	0	0	0
E1	0	0	0	0	675	0	0.63	0	0	0
Q11	0	0	0	0	0	0	0.26	0	0	0
Q12	0	0	0	0	0	0	0	0.9	0	0
Q13	0	0	0	0	0	0	0	0	0.2	0.16
	L2	H2	X2	Y2	P2	E1	E2	Q21	Q22	Q32
L2	0	0	0	0	0	0	0	0.25	0.05	0.05
H2	0	0	0	0	0	0	0	0	0	0
X2	0.335	0.35	0	0	0	0	0	0	0	0
Y2	0.335	0	0	0	0	0	0	0	0	0
P2	0	0	0.29	0	0	0	0	0	0	0
O2	0	0		0.47	0	0	0	0	0	0
E2	0	0	0	400	0	0.51	0	0	0	0
Q21	0	0	0	0	0	0.14	0	0	0	0
Q22	0	0	0	0	0	0	0.9	0	0	0
Q23	0	0	0	0	0	0	0	0.2	0.006	0.16

TABLE V. CONTROL AND VACCINATION TRANSITIONAL STATE MATRIX OF *B. MICROPLUS*

	L1	**H1**	**X1**	**Y1**	**P1**	**O1**	**E1**	**Q11**	**Q12**	**Q13**
L1	0	0	0	0	0	0	0	0	0.25	0.05
H1	0.91	0	0	0	0	0	0	0	0	0
X1	0	0.335	0.35	0	0	0	0	0	0	0
Y1	0	0.335	0	0	0	0	0	0	0	0
P1	0	0	0	0.5	0.47	0	0	0	0	0
O1	0	0	0	0	1260		0.51	0	0	0
E1	0	0	0	0	0	0	0	0	0	0
Q11	0	0	0	0	0	0	0.14	0	0	0
Q12	0	0	0	0	0	0	0	0.9	0	0
Q13	0	0	0	0	0	0	0	0	0.2	0.19
	L2	**H2**	**X2**	**Y2**	**P2**	**E1**	**E2**	**Q21**	**Q22**	**Q32**
L2	0	0	0	0	0	0	0	0	0.25	0.05
H2	0.91	0	0	0	0	0	0	0	0	0
X2	0	0.335	0.35	0	0	0	0	0	0	0
Y2	0	0.335	0	0	0	0	0	0	0	0
P2	0	0	0	.23	0	0	0	0	0	0
O2	0	0	0	0	0.63	0	0	0	0	0
E2	0	0	0	0	400	0	0.63	0	0	0
Q21	0	0	0	0	0	0	0.26	0	0	0
Q22	0	0	0	0	0	0	0	0.9	0	0
Q23	0	0	0	0	0	0	0	0	0.2	0.16

DISCUSSION

The relative influence of climate is often difficult to discern amongst the noise created by variations in other factors that are not directly climatic. To study the effect of vaccination on tick population, sub-models describing vaccination effect were added to population dynamic models (Lodos *et al* 1995, 1998; Labarta *et al*. 1996). The effect of vaccination with Bm86 has been studied for several geographical locations in Australia and the Americas (de la Fuente *et al*., 1995). Computer modelling has been used to study the ecology of *Boophilus microplus* under different climatic conditions (Dallwitz, 1987; Jorge *et al*., 2000; Patarroyo *et al*., 2002;Lodos *et. al*., 1995, 1998) and Floyd *et al*.(1995) simulated the effect of a vaccine on tick populations, using TICK2 model (Dallwitz, 1987) with incorporated vaccination sub model. Considering the complexity of the tick-host-environment relationship, it is nearly impossible to predict the effect of vaccination on field tick population without realistic models for tick population and the effect of vaccination on population dynamics. A Markov chain model was developed for the very first time to predict the efficacy of TickGard® in a multi tick infested environment. Parameters incorporated in the initial model as shown in (Table 1) served as control natural environment. The basic assumption in the model as it is in any model was that, the host resistance was same for both tick species at 25ºC with the relative humidity of 85%. The efficacy of the anti tick vaccine TickGard ™ against *B. decoloratus* was 61% (Odongo, *et. al*., 2007) and *B. microplus* was 84% (Garcia – Garcia *et. al*., 2000) were used in vaccination models and were compared with the non vaccination model. These results and others given by (Lodos *et. al*., 2000) who developed models that show the effect of vaccination on the tick population dynamics using Bm86 antigen proved that tick populations can be predicted and control strategies designed targeted at effectively eliminating the *Boophilus* species in areas which harbour the parasite. There is also the need to further test these models by practically conducting field efficacy trials in areas or regions where these tick species co-exist.

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