

## Effect of *Theileria Parva* Marikebuni Vaccine on Feeding and Reproductive Success of *Rhipicephalus Appendiculatus*

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### Abstract

Marikebuni vaccine is a live parasite vaccine used to immunize cattle against East Coast Fever (ECF), which is caused by *Theileria parva* parasite, transmitted by a three-host tick *Rhipicephalus appendiculatus*. This study investigated the effect of live parasite vaccine on *Rhipicephalus appendiculatus* feeding and reproductive success. Three groups of ECF naïve calves ( $n=3$  per group) were used: an immunized group receiving 1 ml of Marikebuni vaccine and a long-acting oxytetracycline blocking agent and two control groups receiving either uninfected tick material and the blocking agent or uninfected tick material only. Uninfected adult *Rhipicephalus appendiculatus* were counted and weighed before and after feeding on the calves and incubated at 27-28 °C optimum temperature and 80%-85% humidity. Tick feeding success, oviposition success and larval hatchability were analysed using linear regression, logistic regression and ANOVA ( $p<0.05$ ). Ticks that fed on immunized calves had lower feeding success compared to the control groups though the difference was not statistically significant (number of successfully fed ticks and blood meal weight =  $p> 0.05$ ). However, a significant negative relationship occurred between the unfed tick weight and the blood meal imbibed by ticks fed on immunized calves ( $\beta = 0.4918$ ,  $R^2 = 0.7261$ ,  $F (1, 7) = 22.21$ ,  $p = 0. 002176$ ). These ticks produced significantly lower egg mass weight compared to controls. ( $F = 7.993$ ;  $p$ -value = 0.023). Immunization reduced the absolute number of viable larvae produced, though the difference was not statistically significant ( $P>0.05$ ). The vaccine therefore indirectly reduce tick reproductive output, offering potential added value for vector control.

**Keywords:** ECF, Marikebuni Vaccine, *Rhipicephalus Appendiculatus*.

### Introduction

East Coast Fever (ECF) is a tick-borne protozoal ailment that occurs in cattle and domesticated buffaloes in East, Central, and South Africa and is caused by the *Theileria parva* parasite. The three-host tick vector, *Rhipicephalus appendiculatus*, transmits this parasite transstadially. The cyclical change of this parasite occurs within the larvae, nymphs, and adult stages during the

development of the vector. Exotic cattle are the major source of dairy milk in Kenya, but their production is challenged by their high susceptibility to ECF, which causes high morbidity and mortality. The treatment of ECF is expensive. Available data indicates an annual mortality of approximately one million cattle, equivalent to a financial loss of approximately 300 million USD [1-4].

Acaricide is the major control strategy for the ECF tick vector. Although effective to some extent, this control method has significant drawbacks, such as high costs running into a million dollars per year, the development of relative resistance, and being environmentally unfriendly. The high cost of control is also attributed to tick resistance developed with continued use of the limited range of acaricide. Besides, acaricide residues also find their way into the environment and animal products like meat and milk and pose a high risk to human health. To reduce reliance on acaricide, research on alternative control options, specifically the use of vaccines, has made good progress. Infection and Treatment Method (ITM) has been ongoing in the East African region since the 1970s [5-7].

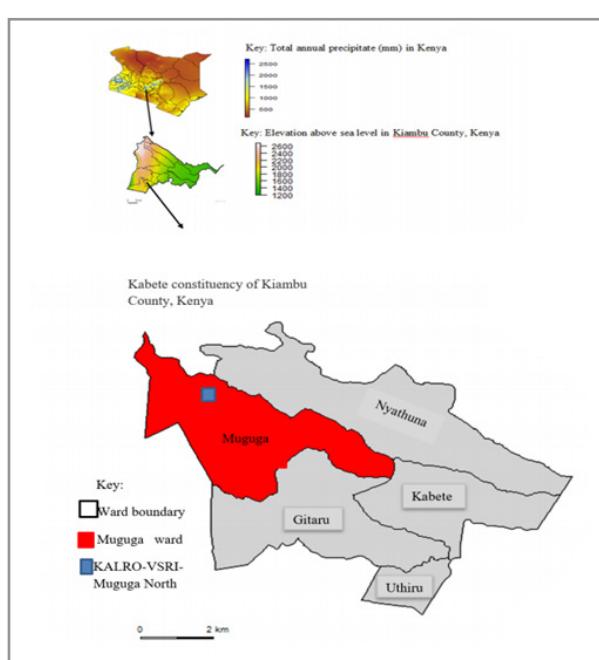
Based on the available clinical data, the vaccine was approved by the governments of Kenya, Malawi, and Tanzania in May 2010. In Kenya, there are two such types of ITM vaccines: the Marikebuni vaccine and the “Muguga Cocktail” vaccine. The Marikebuni vaccine is based on live parasites of *T. parva* and is manufactured and marketed by the Kenya Agricultural and Livestock Research Organization (KALRO Annual Report, 2015-2016). At present, there are no documented findings showing the impact of this vaccine on *Rhipicephalus appendiculatus*. The blood feeding of the tick vector is vital. The blood obtained by adult

male and female ticks is utilized to produce sperm and eggs, respectively, and also carry out other physiological processes. Hindrances to proper blood feeding can have a detrimental effect on the reproductive success of *R. appendiculatus* ticks. The main objective of this work was to evaluate the effect of the *Theileria parva* Marikebuni vaccine on the vector tick after feeding on immunized cattle. This was done by determining the blood feeding success of adult *R. appendiculatus*, oviposition success, and viability of eggs laid between vectors that fed on immunized and non-immunized cattle.

## Methods

### The Study Site

The author of this work conducted the study at Kenya Agricultural and Livestock Research Organization -Veterinary Sciences Research Institute (KALRO-VSRI) laboratories. The laboratories are located in Muguga, Kiambu County, along the Nairobi-Naivasha highway and approximately 30 km north of Nairobi city. The site has an altitude of 1675M above sea level, at a latitude 10 13'S, and a longitude 360 38' E. Average annual rainfall is approximately 1200 mm with a mean humidity of 1716 mm. The climate in this region is quite ideal for the thriving of the ticks [8].



**Figure 1:** Location of the study site - KALRO-VSRI-Muguga North. (Created by Author based on GADM version4).

### The Parasite Stock, Uninfected Tick Material, and Ticks used in the Experiment

*Theileria parva* infectious material (Marikebuni vaccine) was obtained from KALRO-VSRI. Sporozoites stabilate stock of *T. parva* Marikebuni was used to immunize the experimental cattle (treatment group). Isolated this *Theileria parva* parasite stock from Kilifi County, Kenya, which was shown to provide good protection against severe challenge with other *Theileria* strains. A dose estimates of *Theileria parva* sporozoites using a 1:20 immunizing dilution of the original stabilate, combined with treatment using long-acting oxytetracycline was used. The Marikebuni vaccine viability test was conducted following the procedure described by to verify that the *Theileria parva* sporozoites were still alive and fit for use in the study. Uninfected tick

material was prepared using adult *Rhipicephalus appendiculatus* ticks fed on New Zealand white rabbits, as described by Uninfected *Rhipicephalus appendiculatus* adult ticks (Muguga Tick Colony) were obtained from KALRO-VSRI [9-13].

### Experimental Animals

New Zealand white rabbits from KALRO-VSRI were used to prepare uninfected tick material. The rabbits were properly housed in cages and given pellets, mineral supplements, and water throughout the study. The author purposely selected calves from KALRO herds as per the inclusion criteria: naïve to ECF and between 3 and 12 months of age (World Organization for Animal Health). A total of fifteen calves were selected and screened for the presence of *Theileria parva* parasites and any

other tick-borne infections such as Babesiosis and Anaplasmosis using microscopy. Only healthy calves qualified to be enrolled in the study. Any calf exposed to acaricide treatment for 8 weeks before enrollment was excluded. Six Boran calves were used to carry out the Marikebuni vaccine viability test. The remaining nine calves were randomly sampled comprising 3 Boran and 6 *Bos taurus* (3 Ayrshire and 3 Friesian cattle breeds), all females, and assigned them to the immunized and control groups. All the calves were dewormed with Force Oneplus broad-spectrum anthelmintic containing 1.5% Levamisole hydrochloride and 3.0% Oxydiazine with cobalt. To ensure reproducible experimental results, the calves were acclimatized and their health assessed. This allowed them to stabilize psychologically, nutritionally, and physiologically [14, 15].

### Experimental Study Design

The author utilized three groups of ECF naïve calves aged between 3 and 12 months. The calves were randomly sampled and assigned to three groups, as indicated in Table 1. The vaccination of the calves followed the procedure described by Briefly, two vaccine straws were removed from the liquid nitrogen canister. Each straw consisted of 0.5 ml of *Theileria parva* parasite suspension. Rubbing the straws between the palms of warm hands helped thaw them. One end of each straw was cut with scissors, and the contents were emptied into a bottle containing 20 ml of diluent. The bottle was gently shaken to allow the contents to mix. The diluted stabilate was then allowed to stand for 30 minutes to equilibrate in readiness for use. Daily rectal temperatures were recorded for each animal. A weighing band determined the

weight of each animal. The long-acting oxytetracycline dose calculation involved 1 ml per 10 kg of body weight plus 10% more long-acting oxytetracycline than the calculated dose due to the risk of underestimating animal body weight. Oxytetracycline was injected intramuscularly, followed by the injection of vaccine subcutaneously close to the superficial lymph node. The injection of Marikebuni vaccine and uninfected tick material was done on the right prescapular lymph node for all animals in each group for purposes of monitoring the infection. Calves in the immunized group received 1 ml of Marikebuni vaccine and a long-acting oxytetracycline blocking agent at 30 ml/kg body weight.

The control group 1 calves received uninfected tick material and the blocking agent, while the control group 2 calves received the uninfected tick material only (Table 1). Two controls in this study design were needed to isolate the effect of the Marikebuni vaccine itself (specific immunization effect). Control group 1 ensured that any effect observed in the vaccinated group is due to the vaccine antigens and not due to the blocking agent or other injection components. It controlled for the effect of blocking agent (non-specific effect) because the blocking agent can sometimes alter immune responses or physiology of the animal. The control group 2 provided a true negative baseline/normal baseline response without vaccine antigens or blocking agents. This way, one is able to see what the normal tick feeding and reproduction looks like in absence of any experimental manipulation [11].

**Table 1:** Vaccination of calves with Marikebuni vaccine and the number of adult *R. appendiculatus* ticks applied in determination of effect of *Theileria parva* Marikebuni vaccine on feeding and reproductive success of *Rhipicephalus appendiculatus*.

Group name	Immunized group	Control group1	Control group2
Treatment	1 ml of Marikebuni vaccine + 30% long-acting oxytetracycline	1 ml of uninfected tick material + 30% long-acting oxytetracycline	1 ml of uninfected tick material only
Number of calves	3	3	3
sex	F	F	F
Breed	Boran, Ayrshire, and Friesian	Boran, Ayrshire, and Friesian	Boran, Ayrshire, and Friesian
Adult ticks per calf	60 males 60 females	60 males 60 females	60 males 60 females

The calves were closely monitored to ensure a successful immunization. The clinical signs used to monitor the vaccination included an enlarged lymph node, a rise in temperature above 39.5 °C, anorexia, loss of condition, lacrimation, corneal opacity, coughing, terminal dyspnea, diarrhea, nasal discharge, and petechial hemorrhages on the mucus membrane [13]. Calves in the immunized group showed mild *T. parva* reactions with swelling of the lymph nodes on days 7–14 post-immunization. The control group 1 calves showed no apparent clinical reactions to inoculation with non-infected tick material, probably due to co-treatment with oxytetracycline.

The microscopy technique detected the presence of *Theileria parva* parasites. The identification of white blood cells present in the blood smear was also done. Calves in the immunized group were observed to have macro-schizonts in their lymph node smears. The white blood cell count was observed to increase in immunized group calves. One calf in control group 3 showed an increase in white blood cell count on day 14 post-inoculation,

an indication of a reaction to the uninfected tick material inoculated. The indirect fluorescent antibody test (IFAT) assessed the exposure of the calves to the parasite by detecting antibodies against *T. parva* after vaccination. All calves in the immunized group developed *T. parva* schizont antibodies by day 21 post-immunization, while those in the control groups did not show any exposure to *T. parva* parasites [14].

### Feeding and Reproductive Success of *Rhipicephalus Appendiculatus*

The tick rearing unit was surrounded by a water moat to ensure no ticks entered or escaped to the surrounding area following recommended arthropod containment practices. The author applied *Rhipicephalus appendiculatus* adult tick stages to the neck region of the immunized and control calves after counting and weighing, following the procedure described by (Table 1). The calves in each group were washed and shaved on the neck region using an electric shaver. The muslin bags were glued to the neck patch using an adhesive (Conta) and allowed to dry as the

strong odor disappeared. A total of 120 adult ticks were applied to each calf in the immunized and control groups; each received 60 males and 60 females. The male ticks were applied to stimulate the engorgement of female ticks and were not part of the study. The muslin bag was tied on the other end to restrict the ticks on the shaved neck region [16-18].

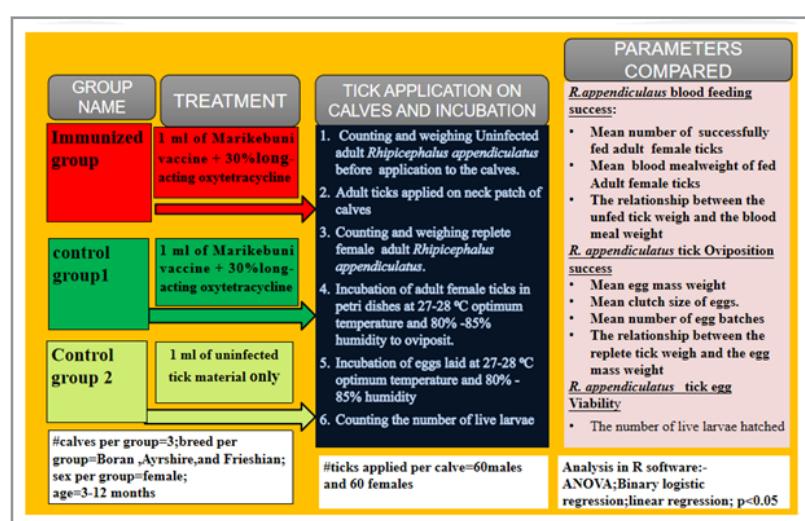
Harvesting, counting and weighing of adult *R. appendiculatus* female ticks that fed on immunized and control group 1 and 2 calves was done after feeding. They were then placed in Petri dishes to lay eggs. The 90-mm-diameter Petri dishes were labeled, sealed with tape, and stored at an optimum temperature of 27–28 °C and a relative humidity of 80–85% [16]. Each tick was put in its own Petri dish. We used an electronic balance to weigh the eggs after incubating the female ticks for 21–28 days. The number of egg batches was counted and recorded. Observation under a dissecting microscope and counting using a hand-held 4-digit counting device determined the clutch sizes of the eggs [19].

To determine the viability of the eggs obtained above, they were incubated in petri dishes sealed with tape at an optimum temperature of 27–28 °C and 80–85% relative humidity until hatching. The number of live larvae that hatched was counted. The viability was determined based on the ability of larvae to walk; those that could walk were considered live, while the immobile ones were considered non-viable. Gentle breathing on the larvae was used to stimulate movement. We used a dissecting microscope to identify the larvae that could not walk. The live

larvae were removed from the Petri dish with a paint brush and immobilized using cotton wool moistened with water. The live larvae trapped in the cotton wool were counted using the hand-held 4-digit counting device. Where all larvae were evidently alive, counting was not performed; instead, the total number of eggs initially recorded in the petri dish was taken as the number of viable larvae. [20].

## Data Analysis

The author entered the data in Excel and performed statistical analysis using R statistical software Version 4.1.3 (2022-03-10), at a  $p<0.05$  significance level. A binomial logistic regression model fitted the data to predict the effect of the vaccine on the feeding success of *R. appendiculatus*. A linear regression analysis determined the relationship between the unfed female tick weight and the blood meal weight. Parameters compared between the immunized and non-immunized cattle to determine the egg-laying success of *R. appendiculatus* included the weight of egg mass, clutch sizes of eggs, and number of egg batches. ANOVA test compared the weight of egg mass. A linear regression analysis determined the relationship between the weight of the egg mass and the female tick's weight after the blood meal. A binomial logistic regression model fitted the data to predict the effect of the vaccine on the number of egg batches and clutch sizes of the eggs. To determine the viability of *R. appendiculatus* eggs, a binomial logistic regression model was fitted to predict the effect of the vaccine on the number of live larvae that hatched successfully [21, 22].



**Figure 2:** Summary of methodology used to determine effect of Theileria parva Marikebuni vaccine on feeding and reproductive success of *Rhipicephalus appendiculatus*.

## Ethical Statement

The Institutional Animal Care and Use Committee of KALRO-VSRI approved this work upon compliance with all resources evaluated and coded: KALRO-VSRI/IACUC 023/04062021

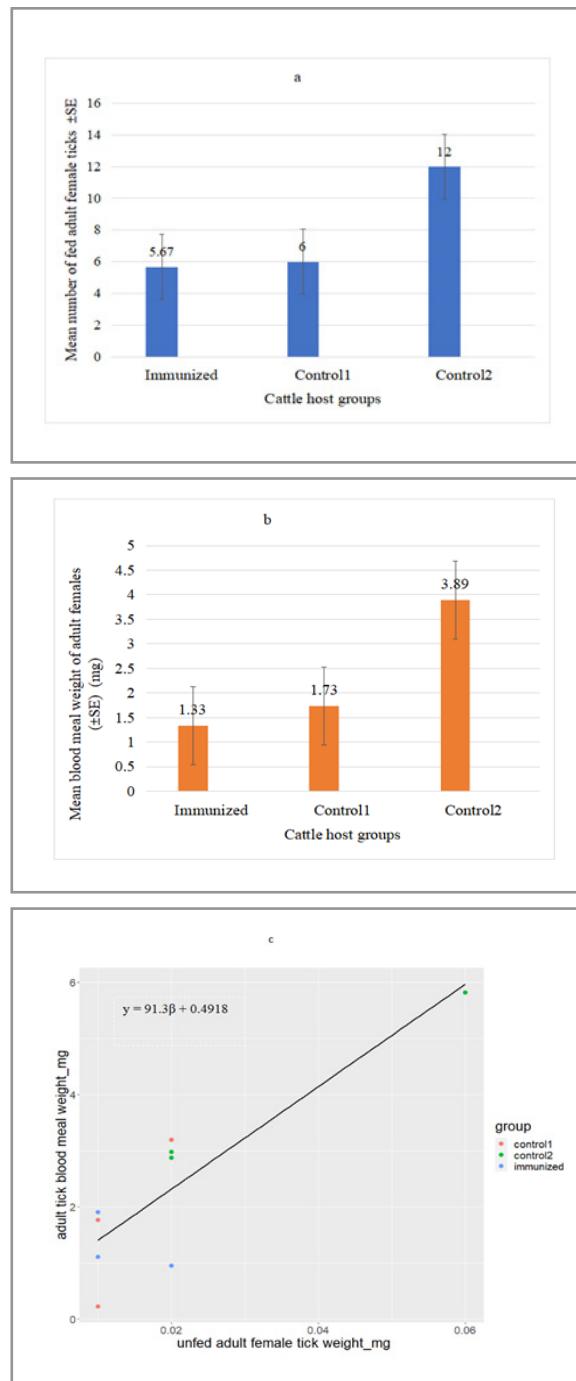
consistent reduction in tick vector fitness. Though some of the parameters measured were not statistically significant, they were biologically significant.

## Assessment of the Blood Feeding Success of Adult *Rhipicephalus appendiculatus* fed on Cattle Immunized with Marikebuni Vaccine

Ticks that fed on immunized calves had lower feeding success compared to the control groups. The mean number of replete adult female ticks was lowest in the immunized group ( $5.67 \pm 3.61$ ) compared to the control group 1 ( $6 \pm 3.61$ ) and control group 2 ( $12 \pm 2.65$ ) (Figure 2a). The blood meal weight also

followed the same pattern and was lowest in the immunized group ( $1.33 \pm 0.51$  mg), compared to the control group 1 ( $1.73 \pm 1.49$  mg), and control group 2 ( $3.89 \pm 1.67$  mg) (Figure 2b). These differences, however, were not statistically significant (mean number of replete adult female ticks: OR = 1.05, p-value = 0.93; blood meal weight: OR = 0.32, p value = 0.48; F = 3.26, p value = 0.11). Regression analysis showed that unfed female tick weight significantly predicted the blood meal weight ( $\beta$

= 0.4918, p = 0.002176)(p < 0.05), explaining 72.6 % of the variance ( $R^2 = 0.7261$ ,  $F(1, 7) = 22.21$ ). Heavier ticks ingested more blood in control calves. In contrast, a negative correlation was observed for the ticks that fed on immunized calves (Figure 2c). This indicates that there is impaired engorgement in larger ticks following host immunization. These observations suggest that Markebuni vaccine may limit blood intake efficiency, a key determinant of tick reproductive output [23].



**Figure 2:** Feeding success of *Rhipicephalus appendiculatus* adult female fed on calves immunized with *Theileria parva* Markebuni vaccine.

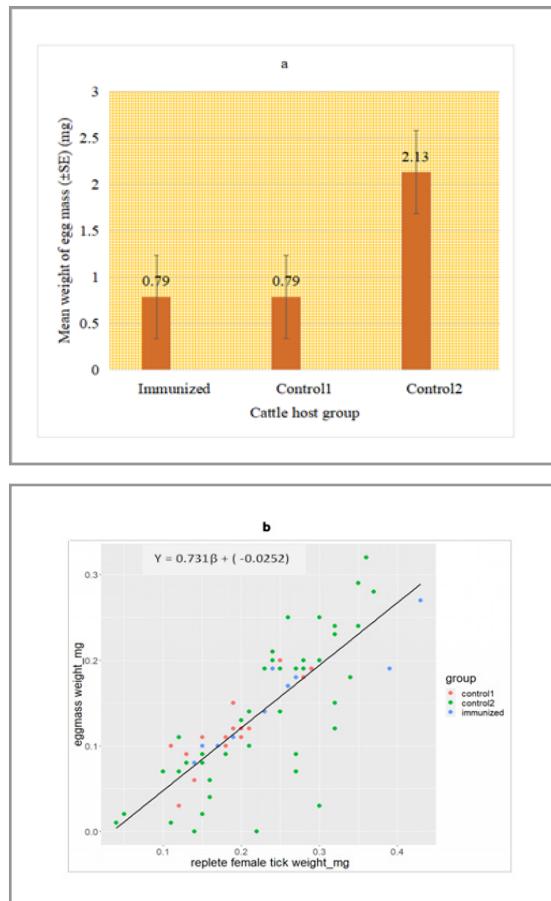
**Key :** a. Mean number of successfully fed adult females in immunized, control 1, and control 2 calves. b. Mean blood meal weight of adult females in immunized, control 1, and control 2 calves. c. Relationship between *Rhipicephalus appendiculatus* weight before feeding and the weight of the blood meal (mg) after feeding.

#### Determination of Oviposition Success of *Rhipicephalus appendiculatus* Fed on Cattle Immunized with Markebuni Vaccine

The egg mass weight was one of the most robust findings in this study which was significantly affected by host immunization. Ticks from immunized calves laid smaller egg masses

$(0.79 \pm 0.02)$  compared to the control group 2  $(2.13 \pm 0.73)$  and control group 1  $(0.79 \pm 0.33)$  (Figure 3a). ANOVA test confirmed significant difference  $(F=7.993, p=0.023)$  (Table 2), with Tukey HSD test on ANOVA results showing significant pairwise differences between immunized and control group 2 ticks (adjusted p-value=0.031). The statistically significant reduction in egg mass weight suggests that this trait is particularly sensitive to

impaired feeding. The regression analysis indicated that the egg mass weight was strongly predicted by female tick engorgement weight (mg)  $(\beta = -0.025, R^2 = 0.643; F (1, 79) = 145.1; p = < 2.2 \times 10^{-16})$  (Figure 3b), underscoring the link between feeding success and fecundity. The blood meal weight appeared to be a key driver of egg mass as heavier engorged females produced heavier egg masses (Figure 3b) [24].



**Figure 3:** Oviposition success of *Rhipicephalus appendiculatus* adult female fed on cattle immunized with *Theileria parva* Marik-buni vaccine.

**Key:** a. Mean weight of egg mass of *R. appendiculatus* fed on immunized, control1 and control2 calves.

b. Regression analysis of *R. appendiculatus* egg mass weight (mg) against replete female tick weight (mg) after feeding on

calves immunized with Marik-buni vaccine and the control calves. Egg mass weight was strongly predicted by female tick engorgement weight (mg)  $(\beta = -0.025, R^2 = 0.643; F (1, 79) = 145.1; p = < 0.05)$

**Table 2:** ANOVA test on oviposition success of *Rhipicephalus appendiculatus* fed on cattle immunized with Marikbuni vaccine (Egg mass weight (mg))

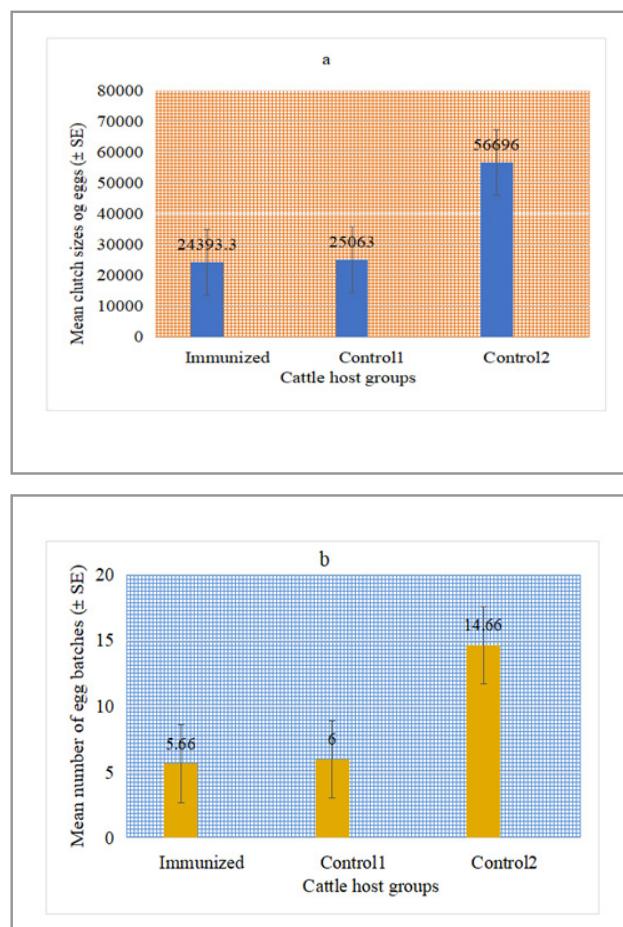
Parameter		Degrees of freedom	Sum of squares	Mean sum of squares	F- value	Pr(>F)
Egg mass weight (mg)	Ind	2	3.609	1.8045	7.993	0.023*
	Residuals	6	1.355	0.2258		

**Key:** Ind=independent variable, Residuals=all variation not explained by independent variable, Sum of squares =variation between the group averages and overall average, df= degrees of freedom, F-value=test statistic from F test, Pr (>F) = p-value of F –statistic. p-value of F-statistics with asterisks \* was significantly different. Tukey HSD test on ANOVA results showed significant pairwise differences between immunized and control group 2 ticks (adjusted p-value=0.031).

The other reproductive parameters such as clutch size also trended downwards in the immunized group ( $24393 \pm 4911.23$  eggs) compared to the control groups 1 ( $(25063 \pm 16109.94$  eggs) and 2 ( $56896.33 \pm 28263.08$  eggs) (Figure 4a). Similarly, egg batches produced by ticks from immunized calves were fewer ( $5.66 \pm 1.53$ ) compared to those from control group 1 ( $6 \pm 3.61$ ) and 2 ( $14.66 \pm 7.23$ ) calves (Figure 4b). While these reductions suggested impaired reproductive potential, neither clutch sizes (OR=1.0; p=0.40) nor number of egg batches

(OR=0.08;p=0.35) differed significantly across the groups(Table 3) The observed trends may reflect reduced feeding efficiency in immunized hosts translating into diminished reproductive out-

put, though not strong enough to achieve statistical significance under current experimental conditions.



**Figure 4:** Oviposition success of *Rhipicephalus appendiculatus* adult female fed on cattle immunized with *Theileria parva* Marikbuni vaccine. a. Mean clutch sizes of eggs of *R. appendiculatus* fed on immunized, control1 and control2 calves; b. Mean number of egg batches of *R. appendiculatus* fed on immunized, control1 and control2 calves.

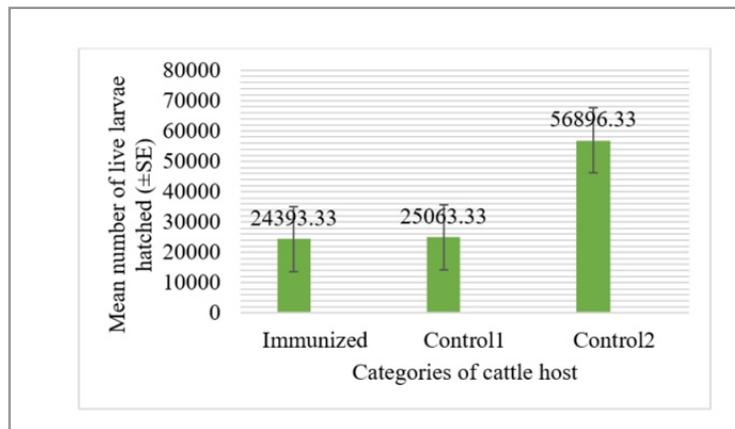
**Table 3:** Logistic regression analysis of oviposition success *Rhipicephalus appendiculatus* fed on cattle immunized with the Marikbuni vaccine

COEF (SE)	97.5%CI for Odds Ratio			z-value	p-value
	Lower 2.5 %	Odds Ratio (OR)	Upper 97.5 %		
Constant (intercept)	1.184(2.14)			0.55	0.58
Egg batch	-2.53(2.73)	0.00006	0.08	3.2	-0.93
Egg clutch sizes	0.0005 (0.0006)	0.99	1.0	1.0	0.85
					0.40

**Key:** COEF-coefficient, SE-standard error, CI-confidence lev-el. Note:  $R^2 = 0.27$  (Hosmer& Lemeshow), 0.29 (Cox&Snell), 0.40 (Negelkerke). Model  $\chi^2 = (2) = 3.04$ ,  $p > 0.05$  ( $p = 0.23$ ) A positive constant (intercept) in the logistic regression model signals that the probability of the ticks that fed on immunized calves being affected by the vaccine is  $> 0.5$ . The number of egg batches had a negative coefficient, indicating that the egg batches of the ticks that fed on immunized calves were less likely to be altered by the vaccine. The clutch sizes of eggs had a positive coefficient, which means that the egg clutch sizes originating from ticks fed on the immunized host were more likely to be altered by the vaccine.

Egg viability was assessed as the number of live larvae that hatched. This was lowest in tick feeding on immunized calves ( $24140 \pm 4916$ ) compared to control 1( $24783.33 \pm 15946.87$ ) and control 2 ( $56376.33 \pm 27162.81$ ) calves (Figure 5). The observed variation was, however, not statistically significant ( $p > 0.05$ ) ( $OR = 0.99$ ;  $p\text{-value} = 0.33$ ) (Table 4). The logistic regression analysis indicated a negative coefficient for number of live larvae that hatched ( $-6.454\text{e-}05$  ( $6.611\text{e-}05$ )), suggesting that increased larval counts slightly reduced odds of viability. However the effect size was negligible and statistically non-significant. This implies that while immunization reduced the absolute number of viable larvae produced, the overall hatching capacity was not significantly compromised.

#### Determination of Viability of Eggs of *Rhipicephalus Appendiculatus* Fed on Cattle Immunized with Marikbuni Vaccine



**Figure 5:** Mean number of live larvae hatched ( $\pm$  standard error) in immunized, control 1, and control 2 calves in an experiment to determine the viability of eggs of *Rhipicephalus appendiculatus* fed on cattle immunized with *Theileria parva* Marikebuni vaccine.

**Table 3:** Logistic regression analysis of the viability of eggs of *Rhipicephalus appendiculatus* fed on cattle immunized with the Marikebuni vaccine

COEF (SE)	97.5%CI for Odds Ratio			z-value	p-value
	Lower 2.5 %	Odds Ratio (OR)	Upper 97.5 %		
Constant (intercept)	1.26 (1.94)			0.65	0.52
#of Live larvae	-6.454e-05 (6.611e-05)	0.999	0.999	1.0	0.98

**Key:** COEF-coefficient, SE-standard error, CI-Confidence level. Note.  $R^2 = 0.14$  (Hosmer & Lemeshow), 0.17 (Cox & Snell), 0.23 (Nagelkerke). Model  $\chi^2 = (1) = 2.53$   $p > 0.1$  ( $p = 0.11$ ).

reduced tick populations and transmission potential of *T. parva* in field setting [19-24].

### Recommendations

*Theileria parva* Marikebuni vaccine impacts the fecundity and fertility of *R. appendiculatus*, which indirectly amounts to some degree of vector control. This calls for an increase in Marikebuni vaccine production and distribution in endemic regions of sub-Saharan Africa. Future studies should employ larger cohorts, extended follow-up, and immunological assays to confirm the dual role of Marikebuni vaccine in controlling both pathogen and its vector [29, 30].

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### Conflict of Interest

The authors declare no conflict of interest.

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