

# **Evaluation of efficacy, effectiveness and safety of the Subolesin anti-tick vaccine in a randomized double-blind Ugandan multi-site field trial**

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**Supplementary Tables 1-5.** Summary of assessment of vaccine safety at different locations throughout the trial.

**Supplementary Table 1.** Assessment of vaccine safety at different locations throughout the trial.

**Supplementary Table 2.** Hematology assessment of blood biomarkers associated with liver function.

**Supplementary Table 3.** Assessment of hematology lipid biomarkers.

**Supplementary Table 4.** Assessment of hematology biomarkers associated with kidney function.

**Supplementary Table 5.** Assessment of hematology biomarkers throughout the trial for all locations.

**Supplementary Table 6.** Target genes, forward (F) and reverse (R) primers and PCR conditions used for the molecular detection of tick-borne pathogens.

**Supplementary Figure 1.** Surveillance of tick infestations in Mbarara and Maruzi for 367 and 251 days, respectively.

**Supplementary Table 1.** Assessment of vaccine safety at different locations throughout the trial.

Treatment	BT (°C)	BC	F	L	R	LR	D	M
<b>Mbarara</b>								
SUB	38.7±0.1	2.7±0.1	10.0±0.1	0.0±0.0	10.0±0.0	10.0±0.1	1.0±0.0	0.0±0.0
Control	38.7±0.1	2.7±0.1	10.1±0.2	0.0±0.0	10.0±0.0	10.0±0.0	1.0±0.0	0.0±0.0
<b>Isimba</b>								
SUB	39.0±0.1	2.8±0.0	10.1±0.4	0.0±0.0	10.1±0.3	10.0±0.1	1.0±0.0	0.0±0.0
Control	39.0±0.1	2.8±0.0	10.0±0.1	0.0±0.0	10.0±0.1	10.0±0.0	1.0±0.0	0.0±0.0
<b>Maruzi</b>								
SUB	38.6±0.1	2.9±0.0	10.0±0.1	0.0±0.0	10.0±0.1	10.0±0.0	1.0±0.0	0.0±0.0
Control	38.7±0.1	2.9±0.0	10.1±0.3	0.0±0.0	10.0±0.1	10.0±0.0	1.0±0.0	0.0±0.0
<b>Kiburara</b>								
SUB	39.0±0.1	2.8±0.0	10.1±0.3	0.0±0.0	10.0±0.1	10.0±0.0	1.0±0.0	0.0±0.0
Control	39.0±0.1	2.8±0.0	10.0±0.1	0.0±0.0	10.0±0.1	10.0±0.0	1.0±0.0	0.0±0.0
<b>Nabuin</b>								
SUB	38.6±0.1	2.9±0.0	10.1±0.4	0.0±0.0	10.1±0.2	10.1±0.1	1.0±0.0	0.0±0.0
Control	38.6±0.2	2.9±0.1	10.2±0.2	0.0±0.0	10.1±0.2	10.0±0.0	1.0±0.0	0.0±0.0

Values and scores (average ± SD) for each treatment and location along the trial were compared by One-way ANOVA with post-hoc HSD test ( $p > 0.05$ ;  $n = 36-37$  animals/group). Abbreviations: BT, body temperature; BC, body condition; F, feeding; L, locomotion; R, respiration/breeding; LR, local reactions/skin coat; D, demeanor; M, mortality. Normal scores: BT, 38.5-39.0 °C; BC, 2-3, thin-moderate; F, 10, normal grazing consistently; L, 0, normal even walking; R, 10, normal breeding; LR, 10, smooth even/regular hair pattern and kempt; D, 1, active when animal generally involves actively in all activities such as feeding; M, 0, no mortality.



0 dpv	2.2±0.5*	2.0±0.0	103.3±80.1	16.2±9.8	13.4±9.2	53.7±32.1	41.6±27.8	25.2±5.8
30 dpv	NA	NA	NA	NA	NA	NA	NA	NA
60 dpv	2.1±0.4*	2.0±0.0	112.2±107.6	13.8±3.2*	17.3±5.8*	51.7±13.8*	32.3±24.1*	22.5±9.9
90 dpv	NA	NA	NA	NA	NA	NA	NA	NA
Control			<sup>&amp;</sup> p = 0.03		<sup>&amp;</sup> p = 0.001		<sup>&amp;</sup> p = 0.03	<sup>&amp;</sup> p = 0.005
0 dpv	3.0±1.0	2.2±0.4	87.6±72.4	17.2±8.0	11.2±4.2	81.6±37.6	37.4±24.2	19.2±6.6
30 dpv	2.5±0.8	2.1±0.3	52.9±36.7	14.0±7.7	13.3±7.9	53.7±30.2	38.4±25.5	17.6±8.7
60 dpv	3.0±0.9	2.4±0.5	224.6±199.8	24.5±10.4	33.5±15.4	97.6±43.6	64.0±8.7	34.3±11.9
90 dpv	NA	NA	NA	NA	NA	NA	NA	NA

Values (average ± SD) for each location were compared between treatments by Student's t-test with unequal variance, \*p<0.05) and for each treatment along the trial by One-way ANOVA with post-hoc HSD test, <sup>&</sup>p<0.05) (n = 3-12 animals/group). Abbreviations: TBIL, total bilirubin; CBIL, conjugated bilirubin; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein level; ALB, albumin; NA, data not available.

**Supplementary Table 3.** Assessment of hematology lipid biomarkers.

<b>Treatment</b>	<b>CHOL</b>	<b>LDLCHOL</b>	<b>HDLCHOL</b>	<b>NONHDL</b>	<b>CHOL/HDL</b>	<b>TRIG</b>
Reference	<5 mmol/l	<3 mmol/l	>1 mmol/l	<3.8 mmol/l	<4.1 mmol/l	0.0-1.7 mmol/l
<b>Mbarara</b>						
<b>SUB</b>						
0 dpv	2.8±0.9	0.8±0.4	1.7±0.6	1.1±0.5	1.6±0.2	0.6±0.3
30 dpv	2.1±0.9	0.6±0.5	1.4±0.5	0.7±0.5	1.5±0.2	0.3±0.2
60 dpv	1.8±0.4	0.6±0.2	1.2±0.3	0.7±0.2	1.6±0.1	0.1±0.0
90 dpv	2.4±0.9	0.9±0.6	1.4±0.5	1.0±0.6	1.8±0.5	0.2±0.1
<b>Control</b>						
0 dpv	NA	NA	NA	NA	NA	NA
30 dpv	2.3±0.3	0.9±0.2	1.4±0.2	0.9±0.2	1.7±0.1	0.1±0.1
60 dpv	NA	NA	NA	NA	NA	NA
90 dpv	2.1±0.8	0.7±0.4	1.3±0.5	0.9±0.4	1.7±0.3	0.3±0.2
<b>Isimba</b>						
<b>SUB</b>						
0 dpv	3.9±1.2*	2.1±1.1	3.2±4.5	2.3±1.0*	2.4±0.6	0.3±0.1
30 dpv	NA	NA	NA	NA	NA	NA
60 dpv	NA	NA	NA	NA	NA	NA
90 dpv	2.8±1.7	1.2±0.8	1.5±0.9	1.4±0.9	1.9±0.2	0.4±0.2
<b>Control</b>						
0 dpv	2.7±1.4	1.3±0.8	1.3±0.6	1.4±0.8	2.0±0.3	0.7±1.2
30 dpv	NA	NA	NA	NA	NA	NA
60 dpv	NA	NA	NA	NA	NA	NA
90 dpv	3.7±1.6	1.6±1.0	1.9±0.7	1.8±1.0	1.9±0.3	0.5±0.2
<b>Maruzi</b>						
<b>SUB</b>						
0 dpv	NA	NA	NA	NA	NA	NA
30 dpv	2.2±1.4	0.7±0.7	1.4±0.8	0.8±0.7	1.6±0.4	0.4±0.2
60 dpv	2.3±0.4	0.4±0.2	1.7±0.3	0.6±0.2	1.3±0.1	0.4±0.1*
90 dpv	NA	NA	NA	NA	NA	NA
<b>Control</b>						
0 dpv	2.9±1.4	0.9±0.7	1.8±0.8	1.1±0.7	1.6±0.2	0.5±0.2
30 dpv	1.7±1.7	0.6±0.6	1.0±1.0	0.7±0.7	1.7±0.4	0.4±0.2
60 dpv	2.6±0.8	0.4±0.4	1.9±0.5	0.7±0.4	1.3±0.1	0.5±0.1
90 dpv	NA	NA	NA	NA	NA	NA
<b>Kiburara</b>						
<b>SUB</b>						
0 dpv	2.9±0.5*	1.0±0.2*	1.8±0.3	1.1±0.2*	1.7±0.1	0.6±0.8
30 dpv	NA	NA	NA	NA	NA	NA
60 dpv	NA	NA	NA	NA	NA	NA
90 dpv	2.9±1.2	1.0±0.6	1.8±0.7	1.2±0.6	1.6±0.3	0.5±0.1
<b>Control</b>						
0 dpv	3.9±1.0	1.5±0.5	2.3±0.6	1.7±0.5	1.7±0.2	0.3±0.1
30 dpv	NA	NA	NA	NA	NA	NA
60 dpv	NA	NA	NA	NA	NA	NA
90 dpv	2.0±1.9	0.6±0.8	1.2±1.1	0.8±0.8	1.6±0.2	0.4±0.1
<b>Nabuin</b>						
<b>SUB</b>						
0 dpv	2.0±1.2	0.6±0.6	1.3±0.9	0.8±0.6	1.7±0.4	0.5±0.5

30 dpv	NA	NA	NA	NA	NA	NA
60 dpv	2.4±0.8*	0.7±0.3	1.6±0.5*	0.8±0.3*	1.5±0.1	0.3±0.2
90 dpv	NA	NA	NA	NA	NA	NA
<b>Control</b>	<sup>&amp;</sup> p = 3e-5	<sup>&amp;</sup> p = 0.002	<sup>&amp;</sup> p = 2e-5	<sup>&amp;</sup> p = 0.002		
0 dpv	5	0.4±0.3	1.0±0.5	0.5±0.3	1.5±0.1	0.2±0.1
30 dpv	1.5±0.8	0.4±0.3	0.9±0.5	0.5±0.4	1.6±0.3	0.3±0.2
60 dpv	1.5±0.9	1.0±0.3	2.8±0.8	1.2±0.3	1.4±0.1	0.4±0.1
90 dpv	4.0±1.0	NA	NA	NA	NA	NA
	NA					

Values (average ± SD) for each location were compared between treatments by Student's t-test with unequal variance, (\*p<0.05) and for each treatment along the trial by One-way ANOVA with post-hoc HSD test, (<sup>&</sup>p<0.05) (n = 3-12 animals/group). Abbreviations: CHOL, cholesterol; LDLCHOL, low-density lipoprotein (LDL) cholesterol; HDLCHOL, high-density lipoprotein (HDL) cholesterol; NONHDL, non-HDL cholesterol; CHOL/HDL, CHOL to HDL ratio; TRIG, triglycerides; NA, data not available.

**Supplementary Table 4.** Assessment of hematology biomarkers associated with kidney function.

<b>Treatment</b>	<b>UREA</b>	<b>CREA</b>
Reference	3.6-10.7 mmol/l	0-50 mmol/l
<b>Mbarara</b>		
<b>SUB</b>		<sup>&amp;</sup> p = 0.01
0 dpv	4.9±1.1	79.6±26.6
30 dpv	3.2±1.8	90.5±24.5*
60 dpv	3.8±0.7	66.0±2.6
90 dpv	4.1±1.8	60.5±14.3
<b>Control</b>		
0 dpv	NA	NA
30 dpv	4.2±0.2	55.5±15.6
60 dpv	NA	NA
90 dpv	4.3±1.5	60.4±16.5
<b>Isimba</b>		
<b>SUB</b>	<sup>&amp;</sup> p = 1e-5	
0 dpv	6.6±1.5	70.9±15.0
30 dpv	NA	NA
60 dpv	NA	NA
90 dpv	2.5±0.8*	69.4±32.9
<b>Control</b>	<sup>&amp;</sup> p = 0.002	
0 dpv	6.1±1.5	60.9±29.9
30 dpv	NA	NA
60 dpv	NA	NA
90 dpv	3.6±1.2	93.9±34.8
<b>Maruzi</b>		
<b>SUB</b>	<sup>&amp;</sup> p = 0.04	
0 dpv	NA	NA
30 dpv	4.4±1.4	80.6±40.5
60 dpv	6.0±0.7*	71.2±5.1
90 dpv	NA	NA
<b>Control</b>	<sup>&amp;</sup> p = 0.0009	
0 dpv	4.7±1.1	76.4±27.3
30 dpv	3.9±1.9	66.0±57.5
60 dpv	6.9±0.9	83.9±15.7
90 dpv	NA	NA
<b>Kiburara</b>		
<b>SUB</b>	<sup>&amp;</sup> p = 0.03	
0 dpv	6.0±1.2	60.3±29.1
30 dpv	NA	NA
60 dpv	NA	NA
90 dpv	4.5±0.7*	82.0±38.3
<b>Control</b>	<sup>&amp;</sup> p = 0.0008	
0 dpv	5.9±0.3	76.2±12.2
30 dpv	NA	NA
60 dpv	NA	NA
90 dpv	3.1±1.3	73.3±63.8
<b>Nabuin</b>		
<b>SUB</b>	<sup>&amp;</sup> p = 2e-5	
	2.5±0.9	84.1±45.7

0 dpv	NA	NA
30 dpv	6.6±1.5	75.5±23.7
60 dpv	NA	NA
90 dpv		
<b>Control</b>	<sup>&amp;</sup> p = 5e-7	
0 dpv	3.5±1.8	85.6±34.2
30 dpv	1.8±0.6	70.2±28.8
60 dpv	8.5±2.4	106.8±40.6
90 dpv	NA	NA

Values (average ± SD) for each location were compared between treatments by Student's t-test with unequal variance, \*p<0.05) and for each treatment along the trial by One-way ANOVA with post-hoc HSD test, <sup>&</sup>p<0.05) (n = 3-12 animals/group). Abbreviations: UREA, urea nitrogen; CREA, creatinine; NA, data not available.



**Supplementary Table 5.** Assessment of hematology biomarkers throughout the trial for all locations.

Biomarkers Reference	Data analysis		Treatment	
			SUB	Control
<b>Liver function</b>				
<b>TBIL</b> 2-26 $\mu\text{mol/l}$	Average $\pm$ SD	0 dpv	2.3 $\pm$ 0.5	2.6 $\pm$ 0.7
		30 dpv	2.7 $\pm$ 0.9	2.6 $\pm$ 0.9
		60 dpv	2.3 $\pm$ 0.5*	3.2 $\pm$ 1.4*
		90 dpv	2.2 $\pm$ 0.4	2.2 $\pm$ 0.5
Student's t-test p<0.05		*p = 0.01, 60 dpv		
One-way ANOVA		p = 0.06	p = 0.004	
post-hoc Tukey HSD p<0.05		---	60 vs. 90 dpv	
<b>CBIL</b> 2-6 $\mu\text{mol/l}$	Average $\pm$ SD	0 dpv	2.0 $\pm$ 0.0	2.0 $\pm$ 0.2
		30 dpv	2.0 $\pm$ 0.2	2.1 $\pm$ 0.3
		60 dpv	2.0 $\pm$ 0.0*	2.3 $\pm$ 0.4*
		90 dpv	2.0 $\pm$ 0.0	2.0 $\pm$ 0.0
Student's t-test p<0.05		*p = 0.02, 60 dpv		
One-way ANOVA		p = 0.28	p = 0.02	
post-hoc Tukey HSD p<0.05		---	0 vs. 60, 60 vs. 90 dpv	
<b>ALP</b> 53-128 $\mu\text{mol/l}$	Average $\pm$ SD	0 dpv	109.1 $\pm$ 55.0	99.8 $\pm$ 57.0
		30 dpv	68.8 $\pm$ 34.6	59.1 $\pm$ 58.1
		60 dpv	114.0 $\pm$ 77.4	177.8 $\pm$ 148.5
		90 dpv	90.6 $\pm$ 62.0	80.9 $\pm$ 51.3
Student's t-test p<0.05		p > 0.05		
One-way ANOVA p-value		p = 0.07	p = 0.0003	
post-hoc Tukey HSD p<0.05		---	0 vs. 60, 30 vs. 60, 60 vs. 90 dpv	
<b>GGT</b> 0-50 $\mu\text{mol/l}$	Average $\pm$ SD	0 dpv	16.7 $\pm$ 7.2	15.1 $\pm$ 6.4
		30 dpv	16.4 $\pm$ 5.9	14.7 $\pm$ 7.6
		60 dpv	15.1 $\pm$ 6.0*	22.9 $\pm$ 9.4*
		90 dpv	16.6 $\pm$ 8.7	16.3 $\pm$ 10.9
Student's t-test p<0.05		*p = 0.01, 60 dpv		
One-way ANOVA p-value		p = 0.92	p = 0.02	
post-hoc Tukey HSD p<0.05		---	0 vs. 60, 30 vs. 60 dpv	
<b>ALT</b> 7-40 $\mu\text{mol/l}$	Average $\pm$ SD	0 dpv	18.0 $\pm$ 8.7	17.3 $\pm$ 7.5
		30 dpv	15.4 $\pm$ 8.0	13.5 $\pm$ 10.4
		60 dpv	17.8 $\pm$ 7.2*	27.6 $\pm$ 12.8*
		90 dpv	16.0 $\pm$ 8.3	17.0 $\pm$ 9.9
Student's t-test p<0.05		*p = 0.01, 60 dpv		
One-way ANOVA p-value		p = 0.65	p = 0.0006	
post-hoc Tukey HSD p<0.05		---	0 vs. 60, 30 vs. 60, 60 vs. 90 dpv	
<b>AST</b>	Average $\pm$ SD	0 dpv	67.3 $\pm$ 22.7	68.7 $\pm$ 29.3
		30 dpv	56.7 $\pm$ 22.0	50.7 $\pm$ 32.4
		60 dpv	59.1 $\pm$ 14.1*	83.1 $\pm$ 35.9*
		90 dpv	63.5 $\pm$ 31.4	64.6 $\pm$ 35.5
Student's t-test p<0.05		*p = 0.01, 60 dpv		

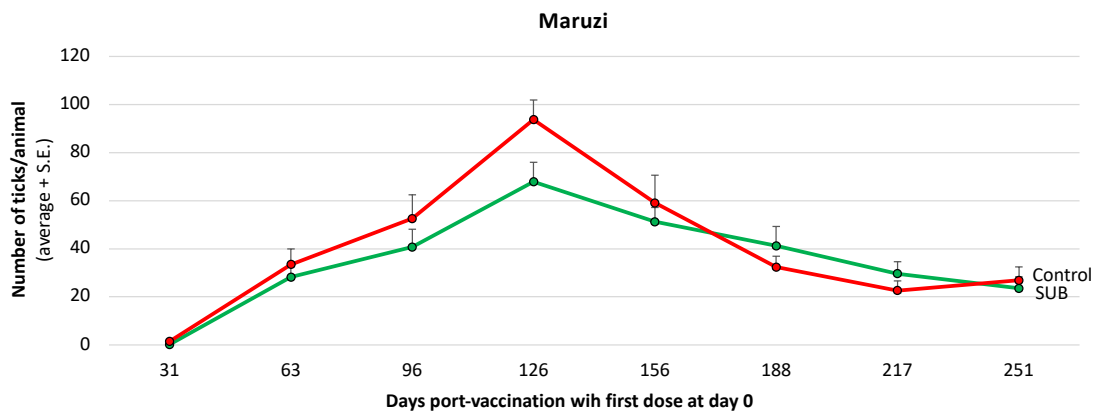
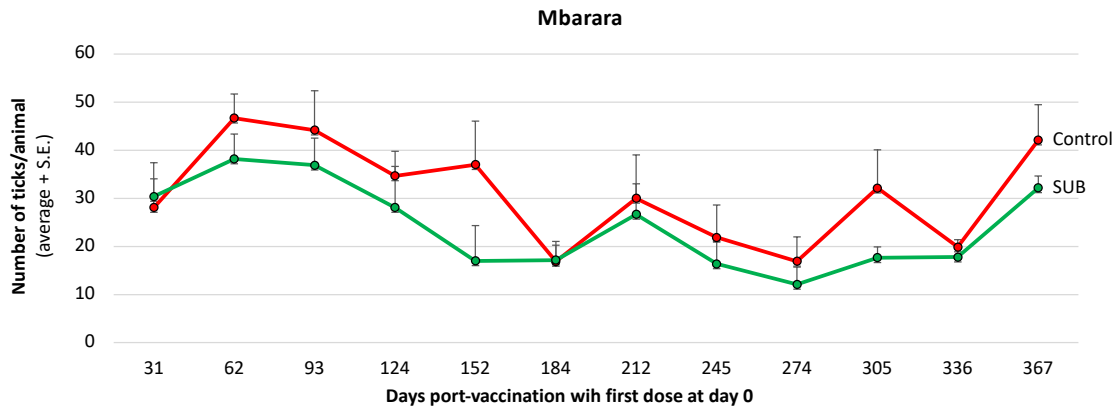
	One-way ANOVA p-value		p = 0.46	p = 0.04
	post-hoc Tukey HSD p<0.05		---	30 vs. 60 dpv
<b>TP</b> 60-80 µmol/l	Average ± SD	0 dpv	44.3±26.5	42.0±19.7
		30 dpv	39.4±23.2	33.7±21.4
		60 dpv	48.2±22.5*	68.7±10.0*
		90 dpv	32.5±24.0	34.0±23.5
	Student's t-test p<0.05		*p = 0.003, 60 dpv	
One-way ANOVA p-value		p = 0.20	p = 0.000002	
post-hoc Tukey HSD p<0.05		---	0 vs. 60, 30 vs. 60, 60 vs. 90 dpv	
<b>ALB</b> 30-40 g/l	Average ± SD	0 dpv	21.4±8.8	20.6±8.3
		30 dpv	22.4±7.1	19.8±11.1
		60 dpv	23.3±7.0*	31.4±8.9*
		90 dpv	22.3±7.3	23.1±7.6
	Student's t-test p<0.05		*p = 0.01, 60 dpv	
One-way ANOVA p-value		p = 0.90	p = 0.0008	
post-hoc Tukey HSD p<0.05		---	0 vs. 60, 30 vs. 60, 60 vs. 90 dpv	
<b>Lipids</b>				
<b>CHOL</b> <5 mmol/l	Average ± SD	0 dpv	2.9±1.2	2.7±1.4
		30 dpv	2.1±1.1	1.7±1.1
		60 dpv	2.2±0.6*	3.3±1.1*
		90 dpv	2.6±1.2	2.6±1.5
	Student's t-test p<0.05		*p = 0.002, 60 dpv	
One-way ANOVA p-value		p = 0.09	p = 0.008	
post-hoc Tukey HSD p<0.05		---	30 vs. 60 dpv	
<b>LDLCHOL</b> <3 mmol/l	Average ± SD	0 dpv	1.2±0.9	1.0±0.7
		30 dpv	0.6±0.6	0.6±0.4
		60 dpv	0.6±0.3	0.7±0.4
		90 dpv	1.0±0.6	1.0±0.8
	Student's t-test p<0.05		p > 0.05	
One-way ANOVA p-value		p = 0.02	p = 0.08	
post-hoc Tukey HSD p<0.05		0 vs. 30 dpv	---	
<b>HDLCHOL</b> >1 mmol/l	Average ± SD	0 dpv	2.0±2.4	1.6±0.8
		30 dpv	1.4±0.6	1.1±0.7
		60 dpv	1.5±0.4*	2.3±0.8*
		90 dpv	1.5±0.7	1.5±0.7
	Student's t-test p<0.05		*p = 0.001, 60 dpv	
One-way ANOVA p-value		p = 0.43	p = 0.00003	
post-hoc Tukey HSD p<0.05		---	0 vs. 60, 30 vs. 60, 60 vs. 90 dpv	
<b>NONHDL</b>	Average ± SD	0 dpv	1.4±0.9	1.2±0.7
		30 dpv	0.7±0.6	0.7±0.5
		60 dpv	0.7±0.3	0.9±0.4
		90 dpv	1.1±0.7	1.2±0.8
	Student's t-test p<0.05		p > 0.05	
One-way ANOVA p-value		p = 0.007	p = 0.052	
post-hoc Tukey HSD p<0.05		0 vs. 30,	---	

			0 vs. 60 dpv	
<b>CHOL/HDL</b> <4.1 mmol/l	Average ± SD	0 dpv	1.9±0.5	1.7±0.3
		30 dpv	1.5±0.3	1.6±0.3
		60 dpv	1.5±0.2	1.4±0.1
		90 dpv	1.8±0.4	1.8±0.3
		Student's t-test p<0.05	p > 0.05	
		One-way ANOVA p-value	p = 0.006	p = 0.0007
		post-hoc Tukey HSD p<0.05	0 vs. 30, 0 vs. 60 dpv	0 vs. 60, 30 vs. 60, 60 vs. 90 dpv
<b>TRIG</b> 0.0-1.7 mmol/l	Average ± SD	0 dpv	0.5±0.4	0.5±0.6
		30 dpv	0.3±0.2	0.3±0.2
		60 dpv	0.3±0.2*	0.5±0.1*
		90 dpv	0.3±0.2	0.4±0.2
		Student's t-test p<0.05	*p = 0.001, 60 dpv	
		One-way ANOVA p-value	p = 0.07	p = 0.40
		post-hoc Tukey HSD p<0.05	---	---
<b>Kidney function</b>				
<b>UREA</b> 3.6-10.7 mmol/l	Average ± SD	0 dpv	4.9±2.0	5.1±1.6
		30 dpv	3.7±1.7	2.9±1.6
		60 dpv	5.8±1.5*	7.7±1.9*
		90 dpv	3.7±1.6	3.9±1.4
		Student's t-test p<0.05	*p = 0.003, 60 dpv	
		One-way ANOVA p-value	p = 0.0008	p = 7.2e-13
		post-hoc Tukey HSD p<0.05	30 vs. 60, 60 vs. 90 dpv	0 vs. 30, 0 vs. 60, 0 vs. 90, 30 vs. 60, 60 vs. 90 dpv
<b>CREA</b> 0-50 mmol/l	Average ± SD	0 dpv	74.8±31.2	72.5±27.3
		30 dpv	83.6±31.6*	65.8±36.9*
		60 dpv	71.9±15.5*	95.3±32.0*
		90 dpv	68.2±27.3	73.7±35.6
		Student's t-test p<0.05	*p = 0.04, 30 dpv *p = 0.01, 60 dpv	
		One-way ANOVA p-value	p = 0.21	p = 0.06
		post-hoc Tukey HSD p<0.05	---	---

Values (average ± SD) for each location were compared between treatments by Student's t-test with unequal variance, (\*p<0.05) and for each treatment along the trial by One-way ANOVA with post-hoc HSD test, p<0.05) (n = 14-32, 22±6 animals/group). Abbreviations: TBIL, total bilirubin; CBIL, conjugated bilirubin; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TP, total protein level; ALB, albumin; CHOL, cholesterol; LDLCHOL, low-density lipoprotein (LDL) cholesterol; HDLCHOL, high-density lipoprotein (HDL) cholesterol; NONHDL, non-HDL cholesterol; CHOL/HDL, CHOL to HDL ratio; TRIG, triglycerides; UREA, urea nitrogen; CREA, creatinine.

**Supplementary Table 6.** Target genes, forward (F) and reverse (R) primers and PCR conditions used for the molecular detection of tick-borne pathogens.

<b>Fragment length</b>	<b>Primer nucleotide sequences (5'-3')</b>	<b>PCR conditions</b>
<b><i>Anaplasma</i> spp. gene <i>RpoB 16S rRNA</i></b>		
577 bp	RpoB 16SF (F): GCTGTTCTAGGCTYTCTTACGCGA	95°C-5 min, 40 cycles (95°C-30 sec, 55°C-45sec, 72°C-45 sec), 72°C-7 min
	RpoB 16SR (R): AATCRAGCCAVGAGCCCCTRTAWGG	
<b><i>Ehrlichia</i> spp. gene <i>16S rRNA</i></b>		
345 bp	EHR16SF (F): GGTACCYACAGAAGAAGTCC	95°C-5 min, 40 cycles (95°C-30 sec, 54°C-30 sec, 72°C-45 sec), 72°C-7 min
	EHR16SR (R): TAGCACTCATCGTTTACAGC	
<b><i>Rickettsia</i> spp. gene <i>16S rRNA</i></b>		
416 bp	FD1 (F): AGAGTTTGATCCTGGCTCAG	95°C-5 min, 35 cycles (95°C-30 sec, 54°C-30 sec, 72°C-30 sec), 72°C-7 min
	Rc16 (R): AACGTCATTATCTTCCTTGC	
<b><i>Piroplasmids</i> gene <i>18S rRNA</i></b>		
408 bp	PIRO A (F): AATACCCAATCCTGACACAGGG	95°C-5 min, 35 cycles (95°C-30 sec, 58°C-30 sec, 72°C-30 sec), 72°C-7 min
	PIRO B (R): TTAAATACGAATGCCCCCAAC	



**Supplementary Figure 1.** Surveillance of tick infestations in Mbarara and Maruzi for 367 and 251 days, respectively. The the prevalence of tick species was recorded in Mbarara (predominant *R. appendiculatus*) and Maruzi (concomitant *R. appendiculatus*, *R. decoloratus* and *A. variegatum*). Results between SUB-vaccinated and control groups were compared Chi2 test ( $p = 1.4E-06$ ,  $n = 9-13$  cattle/day/group, Mbarara;  $p = 0.01$ ,  $n = 9-14$  cattle/day/group, Maruzi).