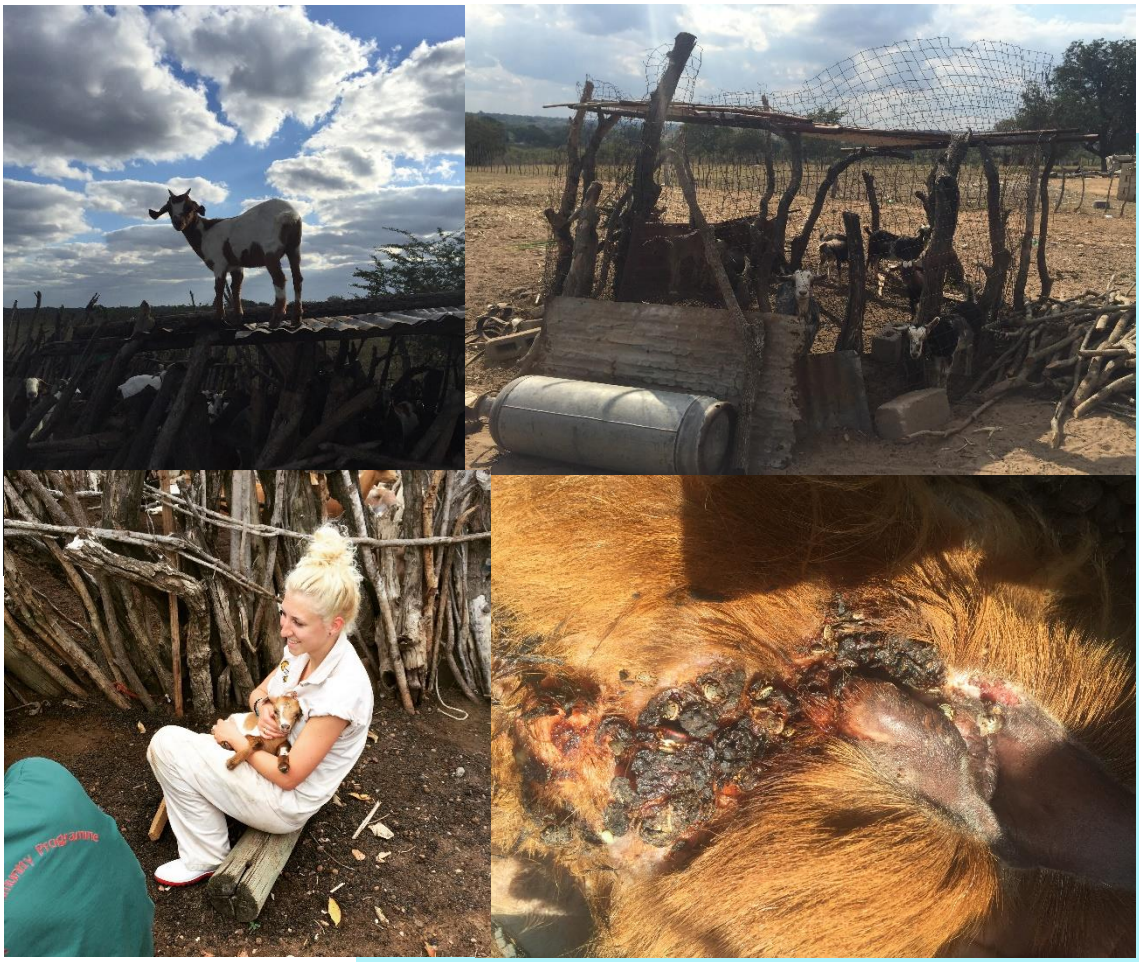
Can DELETE ALL® serve as an adequate method for goats in *Amblyomma* tick control management in the area of Mnisi (Mpumalanga), South Africa?

By Anne van Renssen, May/ June 2015



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Abstract

The present study was undertaken to determine whether treatment with the acaricide DELETE ALL® could serve as an adequate tick control method on indigenous goats in the Mnisi area (Mpumalanga), South Africa. Ticks were collected from 79 goats of fifteen different households, which were divided in two groups, namely the control group and the DELETE ALL group. A total number of 21,367 ticks, of which only 38 were adult *A. hebraeum* ticks, were collected. Ticks were mostly found on the feet of the goats, especially in the inter-digital space. A total number of 10,286 ticks were found on the front legs and 8,282 ticks on the hind legs. Other predilection sites for *A. hebraeum* ticks were the ears (n=1,239), udder/ genitals (n=1,071), armpits (n=450) and tail (n=39).

There was a statistically significant difference in the number of collected ticks from the goats of the control group and from the goats that were treated with DELETE ALL®. The mean number of ticks in the DELETE ALL group was respectively 2,39 and 2,29 in week 2 and 5, respectively. Goats in the control group carried 33,57 and 31,03 ticks in week 2 and 5, respectively. The decrease in the number of ticks in the DELETE ALL group reached almost 96 percent in the first week after treatment.

However, the treatment did not prevent that the number of ticks increased again over the following weeks. A shorter treatment interval than 4 weeks may therefore be more effective.

Although goats play an important role for the farmers in the Mnisi area, it will probably be difficult for farmers to obtain it and it is an expensive product. Therefore it would be better to make the product more accessible for farmers first, to use it for tick control management in the Mnisi area.

1. Introduction

Livestock farming is the main agricultural activity in the area of Mnisi, South Africa. Cattle are by far the most important species, however goats account for a significant part of the total population as well. Since the Mnisi area lies in a semi-arid region livestock farmers struggle with challenges in the form of water and grazing availability. An additional challenge for the farmers in this area are several tick species, since the right conditions for the survival and maintenance of ticks are present due to the sub-tropical climate and high densities of hosts in the Mnisi area. Ticks are important vectors of protozoal, bacterial, viral and rickettsial diseases.[1] Ticks and tick-borne diseases have a negative impact on the development of the livestock industry resulting in loss of food and income for farmers. The main reason why farmers want to control ticks is because of the lameness that is caused by ticks feeding on their hosts. To feed themselves, the ticks must attach using their mouthparts to insert the skin. Once the ticks detach from the host, the tick feeding lesion can get irritated and may cause a secondary infection. These infections can also damage teats and this can make cattle lose one or more udder quarters. Another problem farmers are concerned about is the possibility for ticks to serve as a vector for tick-borne diseases. This study is focused on the bont tick, *Amblyomma hebraeum*. It is a three-host tick, this means it has three life stages: larvae, nymphs and adults. Many mammals and birds can serve as a host for *A. hebraeum*, however the tick is able to transmit the important disease Heartwater in cattle, sheep, goats, antelope and buffalo.[1,9]

Heartwater (also known as Cowdriosis) is an important tick-borne disease caused by the rickettsia *Ehrlichia ruminantium*, which is an obligate intracellular gram-negative organism. This disease of ruminants is common in sub-Saharan Africa, Madagascar and in some Caribbean Islands.[3]

According to the World Organization of Animal Health (OIE), heartwater is considered as one of the most important vector-borne diseases of wildlife and domestic livestock in Sub-Saharan Africa. With mortality rates up to 90% in non-indigenous sheep and goats the mortality for heartwater is three times higher than in cattle. This does not apply for all the breeds, since annual mortality in indigenous goats of an endemic area has been estimated at around 10%. [11, 12]

Since heartwater is such an important tick-borne disease of livestock it is important to reduce exposure of livestock to the target ticks as much as possible. Firstly the question of endemic stability/instability needs to be resolved. It is typically seen that livestock in endemically stable areas, where tick control is not practiced already, have a high level of immunity. When acaricidal programs will be implemented in these areas, the level of herd immunity that results from exposure to ticks, will decrease. Therefore intensive tick control in an endemically stable area may even increase losses due to heartwater. Eradication is only feasible in endemically unstable or climatically marginal areas and in such cases, intensive tick control or immunization are recommended.[9]

Thus far, heartwater has not been diagnosed in the Mnisi area, neither in cattle nor in small ruminants. Although there is circumstantial evidence for the presence of the disease, a definitive diagnosis based on stained and positive

brain-crush smears has not been made. It could be imagined that when ticks are not intensively controlled, a stable endemic situation might result. Endemic stability refers to a state of a tick-host-pathogen interaction, wherein clinical cases are rarely seen despite a high level of transmission.[16]
This concept originates from theoretical models based on the cattle-Boophilus - Babesia system in Australia developed by Mahoney et al. However, the very short duration of age-related resistance to heartwater (one to two weeks in newborn shoats) does not allow all or even a majority of the young animals to become infected before the resistance wanes. A stable endemic situation without apparent disease problems can probably not be attained in populations that are genetically susceptible to the disease. Moreover, since in general infection rates in ticks are low, there will be large numbers of ticks required for a stable situation and this would be unacceptable because of the direct harmful effects of the ticks to the animals. Thus, it is questionably if a concept as endemic stability can be applied to *Amblyomma*-goats-*Ehrlichia* relationships as has been done for bovine babesiosis.[17, 18]

There is already a vaccine available that is actually a virulent strain of *E. ruminantium* in sheep blood. Immunization is accomplished by infecting animals with this vaccine and then treating the infection with anti-rickettsial drugs as tetracyclines for instance.[9]

The current method mostly used for heartwater control is the control of the tick vector through acaricides. Acaricides used to control ticks on livestock or in the environment should be applied in such a manner that the ticks will be killed, the treatments will not harm livestock or applicators, the tissues of treated animals will not contain toxic residues, and the environment will not be adversely affected. Although the toxicity is low for mammals, acaricides are toxic for aquatic organisms such as fish. One way in which ticks are controlled is immerse animals in acaricides in dipping tanks. However, high initial cost of construction of the dipping tanks, their immobility and the cost of the acaricides may make vats impractical for many small ranching operations. However *A. hebraeum* ticks mostly inhabit a limited area of an animals' body, therefore acaricides may also be applied to these areas by hand. There are also alternative control methods that include the use of household disinfectant, used engine oil and paraffin, which are highly questionable.[9, 13]

One of the acaricides that is available as a tick-control intervention method to prevent *Amblyomma* tick infestations in the Mnisi area is DELETE ALL® produced by MSD Animal Health. DELETE ALL® is a ready to use pour-on product which contains Amitraz 2,0% m/v, Deltamethrin 0,50% m/v and Piperonyl Butoxide 2,0% m/v. [14]
DELETE ALL® can be used for tick-control on cattle, goats, sheep and wildlife. One of the components, Amitraz, is a formamidine widely used as an insecticide and acaricide.

So far, seven students of Utrecht University have studied the relative abundance and seasonal dynamics of *A. hebraeum* ticks and the prevalence of *E. ruminantium* in goats.:

1. Francine van der Steen, started her research in July 2013. She found a total of 1276 nymphs and only 23 adult *A. hebraeum* ticks. In a later phase these

nymphs and adult ticks were identified at the Department of Veterinary Tropical Diseases (DVTD). Part of the adult ticks and nymphs went to Utrecht University and were examined at the Utrecht Centre for Tick-borne Diseases (UCTD) to determine the rate of infection with *E. ruminantium*. The results showed that 11.8% of nymphs and 13% of adult ticks tested positive for this infectious agent by using reverse line blot (RLB) and PCR.[2]

2. Suzanne Busser continued the research by attempting to determine the relative abundance of *A. hebraeum* ticks on goats during the summer of 2013, taking the geographical distribution into account. The study aimed to learn more about the epidemiology of heartwater in livestock and the role of ticks in the transmission of the disease. Suzanne found a total of 522 *A. hebraeum* adults and discovered that 25% of those adults were positive on heartwater. However more nymphs were found (n=1430) and even 23.5% of all nymphs tested positive for *E. ruminantium*.[3]
Since it became clear that many goats showed signs of lameness and several goats showed clinical signs suspected of heartwater, the main focus of the study was changed.[3]

3. Iris Deetman continued the research on the seasonal distribution of ticks in February, the rainy season in South Africa. During this study Iris found that 9.8% of adults and 46.7% of nymphs were tested positive for heartwater. Next, she had drawn up a standardized sampling method with the help of Suzanne, therefore the sampled results could be compared.[4]

4. Barry de Sitter was the next student that went to the Mnisi area to sample goats in the months July and August of 2014. Barry collected a total of 4808 ticks of the species *A. hebraeum, Rhipicephalus microplus and Rhipicephalus appendiculatus*. A total of 3,823 of the ticks were nymphs, only 158 of them were adults and a total of 823 were larvae. Since there were goats that died with symptoms of heartwater, Barry made three crushed brain smears. Two of these samples came back negative and the third remained inconclusive.

5. Tiffany Leenders continued in the footsteps of Barry, as regards to the crushed brain smears. With her study Tiffany also wanted to monitor the seasonal abundance of A. hebraeum ticks on the goats. She also examined a few brain smears, however these turned out to be negative.[5]

Overview of sampling times	
1. F. Van der Steen	June and July 2013
2. S. Busser	October and November 2013
3. I. Deetman	March and April 2013
4. B. De Sitter	July and Augustus 2014
5. T. Leenders & Nikky Kok	November and December 2014
6. M. de Vos & M. King	February, March and April 2015
7. Y. Nobel, M. Jochems & A. Van Renssen	May, June and July 2015
8. 3 new students	April, May and June 2016

Just like Tiffany, Nikky Kok went to Mnisi as well in November 2014. With her study Nikky tried to gain better insight in the acaricide resistance level and she collected base line data in order to create a better understanding of the resistance of the concerning ticks to different classes of acaricides.[6]

Finally two additional students, Maria King and Marjan de Vos, continued the research in February 2015. Maria's study was about monitoring the effect of an acaricide, tick grease, on goats. Tick grease contains Cypermethrin and is one of the most predominant acaricides used for tick control. Marjan was monitoring the effect of another acaricide, DELETE ALL®. [7,8]

2. Research questions

2.1 Main research question

Can DELETE ALL® serve as an adequate method for goats in *Amblyomma* tick control management in the area of Mnisi (Mpumalanga), South Africa?

2.2 Sub-questions

- What is the effectiveness of DELETE ALL® in a period of four weeks compared to the non-treated control group?
- What is the total cost of the intervention?
- Are there any side effects?

3. Materials and methods

3.1 Study area

In order to create a valuable and comparable continuation of the previous studies, some of the former study methodologies were applied in this study. Just like the other studies, the study and tick collection was conducted in the Mnisi area, province of Mpumalanga, South Africa. This area is situated in the north-eastern corner of the Bushbuckridge Municipal Area and covers about 29500 hectare. The Mnisi area has a mainly Shangaan-speaking community of more than 40.000 people. These residents are divided over an estimated 8555 households of which 917 different goat farmers own a total of approximately 6000 goats. The Hluvukani Animal Clinic is in the centre of the Mnisi area and the Hans Hoheisen Wildlife Research Station in the Great Limpopo Transfrontier Park at the Orpen Gate into the Kruger National Park. The area falls within the savannah ecosystem and in the Mpumalanga Province the life cycle of *A.hebraeum* continues throughout the year. Over two thirds of the study area's land is on the interface with the adjacent Andover and Manyeleti provincial game reserves, the Kruger National Park and several private game reserves. The only barrier between livestock in this area and wildlife of the game reserves is a feeble yet very important fence. [16]

The area is part of the Mnisi Community Programme, an initiative by the University of Pretoria and the Mnisi Traditional Authority. A total of five villages located in the Mnisi area were visited, those were the following: Welverdiend A (24°34'46.3"S 31°19'26.7"E), Gottenburg (24°38'21.6"S 31°24'38.8"E), Utah A (24°41'36.0"S 31°26'49.5"E), Share (24°40'36.0"S 31°18'46.5"E) and Athol (24°42'34.2"S 31°20'45.8"E).[15] (SEE APPENDIX A)

3.2 Study animals

Five different villages each week were used for this study, where every village was assigned with its own day of the week: Welverdiend A on Monday, Gottenburg on Tuesday, Share on Wednesday, Athol on Thursday and Utah on Friday. Within one village a total of three households were used to examine the goats. And within each household five different goats were used as a DELETE ALL group and five different goats were used as a control group. Unfortunately most households had only ten or less goats and therefore the control and DELETE ALL group contained five or less goats. However, even this was not always possible as sometimes the goats were out grazing. A total of 79 goats

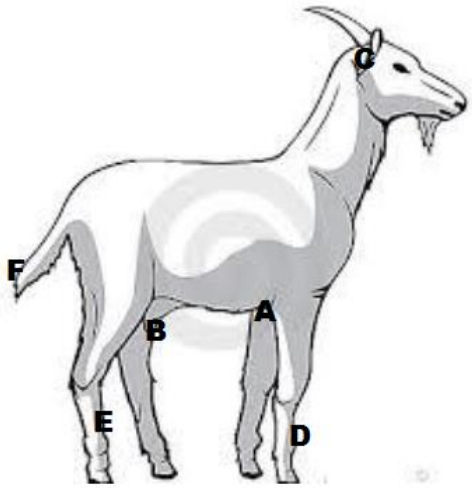
were sampled in this study. The goats were kept in a traditional manner, mostly in a kraal (an enclosure for livestock, located next to an African settlement surrounded by a fence of thorn-bush branches, roughly circular in form). In order to recognize the goats from the two different groups, the goats were marked with a special marker pen for livestock. All the goats in the DELETE ALL group were marked with a big cross on their thighs and the goats in the control group were marked with a big circle on their thighs. This was repeated every week to make sure that the marks would sustain until the next week.

3.3 The acaricide

The goats of the DELETE ALL® group were treated with the acaricide DELETE ALL® which is a ready to use pour-on product that contains Amitraz 2,0% m/v, Deltamethrin 0,50% m/v and Piperonyl Butoxide 2,0% m/v produced by MSD Animal. The DELETE ALL® group contained 41 goats in total and these goats were treated with the acaride in week 1 and 4. The acaricide was applied between and around the hooves of the goats by using a brush.

3.4 Tick collection

From the first week nymphs and adult ticks were collected from goats in the DELETE ALL® groups and the control groups at the five different villages. This was repeated every week, for six weeks long. The tick collection was performed manually by using tweezers and wearing protective clothes and boots. In order to check the goats for ticks properly and to collect each of them, two environmental monitors were present to restrain the goats. The ticks were collected from the predilection sites of the goats, which are the legs, udder, genitals, ears and the armpits. The collected ticks were counted in order to monitor for tick re-infestation in the two different groups.



A	Armpits
B	Udder/Genitals
C	Ears
D	Front legs
E	Hind legs
F	Tail

Figure 1: The predilection sites that were assessed.

Thereafter the ticks were stored in room temperature and preserved in labeled bottles containing 70% ethanol until further analysis. The name of the village, the owners name of the household where the ticks were collected, the research group where the goat was in and the date were written down on every bottle with ticks. The number of ticks found on the individual goats was recorded for every predilection site where the ticks were found. In order to distinguish adult ticks from the nymphs, they were counted separately. Subsequently the data of the tick collection was used for monitoring the tick re-infestation of the two

different groups and to examine if a three to four week treatment interval is reliable.

3.5 Examination of the collected ticks

The amount of adult ticks found on every sampled goat was specified. After collection of the ticks, they were identified with a stereoscopic microscope at Hans Hoheisen Research Station. 'Tick of Domestic Animals in Africa: a Guide to Identification of Species' guide by Walker, A. R. & Bouattour, is one of many sources that may help with this identification.

4 Results

4.1 Tick Collection

Ticks were collected from goats from different villages. Five villages in total were visited, respectively Welverdiend A, Gottenburg, Share, Athol and Utah A. The most numerous tick that was found was *A. hebreaum.* Performing whole-body tick collection was necessary on all the goats in week 1 (n=71), since there was not any goat free of ticks at that moment. Mainly larvae and nymphs (n=21,329) were found and the few adult ticks (n=38) that were found came from only one of the five villages, namely Utah A. Thirty-seven of the adult ticks came from one goat of the owner Hazasah Moyahn. This goat had a great abscessing wound in its inguinal region what may have attracted the adult ticks, since they were only found in and around the wound.

Although this particular goat had a great wound, there were several goats with skin lesions as well. A largest number of small wounds were found, particularly around the hooves. *A. hebraeum* nymphs were mostly found in the inter-digital space and it was clear that these nymphs were responsible for the several purulent abscesses or ulcers that were found in this region. Some of these skin lesions could bleed a little and a couple of goats were showing symptoms of lameness. The predilection sites were mainly around the front legs (n=10,286), followed by the hind legs (n=8,282), ears (n=1239), udder/ genitals (n=1,071), armpits (n=450) and tail (n=39).

The number of ticks collected from the goats is shown for each predilection site separately in table 1.1

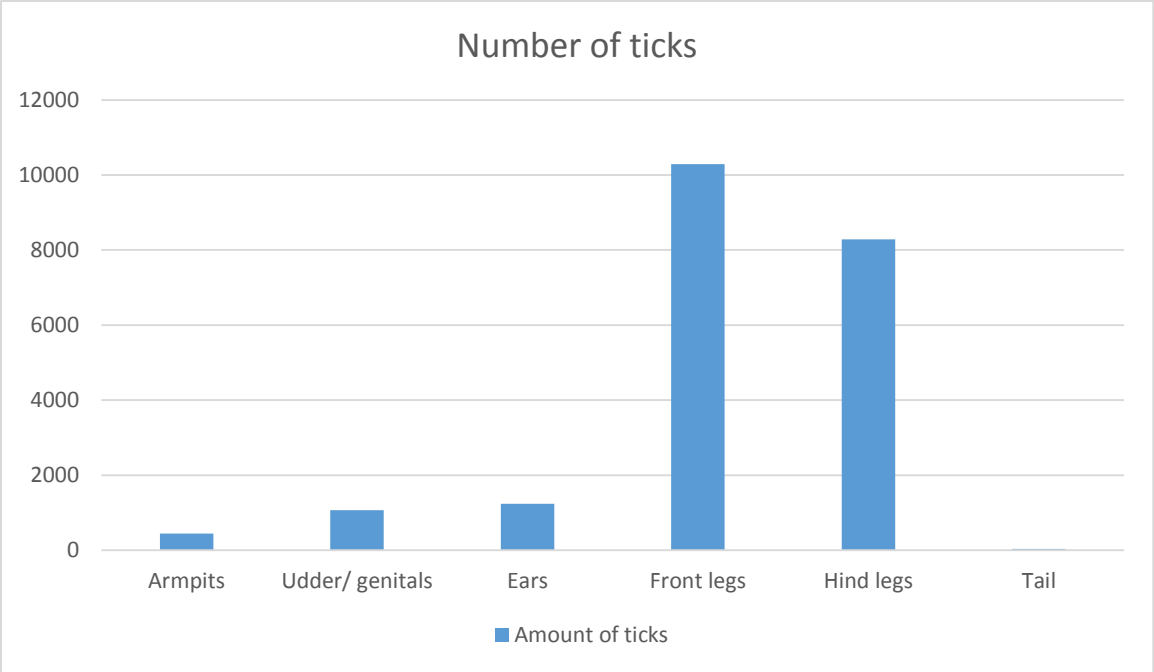


Table 1.1. Tick numbers counted in the different predilection sites in the period of six weeks.

Each week three different households in each village were visited. In the first week all the ticks were removed and counted from the goats of the Mnisi area. After removing the ticks, a couple of goats were treated with the acaricide DELETE ALL® and marked with a cross afterwards. As many goats as possible were used for the study, however most of the households had only ten or less goats and therefore the control and DELETE ALL® group contained five or less goats.

The number of ticks collected from goats in these five villages in a period of six weeks, is summarized in tables 2.1a, 2.1b, 2.2a, 2.2b, 2.3a, 2.3b, 2.4a, 2.4b, 2.5a and 2.5b.

Welverdiend A

Control	Ticks	Goats	Total ticks	Total goats	Ratio	
Renias[1]	39	4	241	7	34,43	
Frank[1]	202	3				
Renias[2]	113	4	148	7	21,14	
Frank[2]	35					
Renias[3]	117	4	169	7	24,14	
Frank[3]	52	3				3
Renias[4]	204	4	285	7	40,71	
Frank[4]	81	3				
Lion[4]*						

Renias[5]	57	4	81	8	10,13
Frank[5]	4	3			
Lion[5]	20	1**			
Renias[6]	90	4	187	8	23,38
Frank[6]	75	3			
Lion[6]	22	1			

Table 2.1a. Tick numbers counted in the control group in a period of six weeks from goats in the Mnisi area. The week numbers are denoted by the superscripts.
**In week 1 the owner Lion wasn't present, this household was included in the study from week 4. Counting of the ticks started in week 5.*
***There was only one goat, one goat was sold and one goat ran away.*

DELETE ALL	Ticks	Goats	Total ticks	Total goats	Ratio
Renias[1]	129	4	620	8	77,5
Frank[1]	491	4			
Renias[2]	7	4	44	8	5,5
Frank[2]	37	4			
Renias[3]	38	4	107	8	13,38
Frank[3]	69	4			
Renias[4]	110	4	202	8	25,25
Frank[4]	92	4			
Lion[4]*					
Renias[5]	8	4	20	11	1,82
Frank[5]	2	4			
Lion[5]	10	3			
Renias[6]	69	4	138	11	12,55
Frank[6]	46	4			
Lion[6]	23	3			

Table 2.1b. Tick numbers counted in the DELETE ALL group in a period of six weeks from goats in the Mnisi area. In week 1 and week 4 goats received acaricide treatment with DELETE ALL.
**In week 1 the owner Lion wasn't present, this household was included in the study from week 4. Counting of the ticks started in week 5.*

Gottenburg

Control	Ticks	Goats	Total ticks	Total goats	Ratio
Gracias[1]	77	4	688	8	86
Esta[1]	611	4			
Gracias[2]	39	4	635	8	79,38
Esta[2]	596	4			
Gracias[3]	68	4	734	8	91,75
Esta[3]	666	4			
Gracias[4]	51	4	752	8	94
Esta[4]	697	4			
Gracias[5]	20	3*	475	7	67,86
Esta[5]	455	4			
Gracias[6]	33	3	375	7	53,57
Esta[6]	342	4			

Table 2.2a. Tick numbers counted in the control group in a period of six weeks from goats in the Mnisi area. The week numbers are denoted by the superscripts.
** One goat switched from the control group to the DELETE ALL group*

DELETE ALL	Ticks	Goats	Total ticks	Total goats	Ratio
Gracias[1]	58	4	646	8	80,75
Esta[1]	588	4			
Gracias[2]	4	3*	16	7	2,29
Esta[2]	12	4			
Gracias[3]	20	3	35	7	5
Esta[3]	15	4			
Gracias[4]	45	2**	138	6	23
Esta[4]	93	4			
Gracias[5]	3	3***	17	7	2,43
Esta[5]	14	4			
Gracias[6]	6	3	40	7	5,71
Esta[6]	34	4			

Table 2.2b. Tick numbers counted in the DELETE ALL group in a period of six weeks from goats in the Mnisi area. In week 1 and week 4 goats received acaricide treatment with DELETE ALL.
** There were only three goats, one of the goats ran away.*
*** There were only two goats, one of the goats ran away.*
**** One goat switched from the control group to the DELETE ALL group*

Share

Control	Ticks	Goats	Total ticks	Total goats	Ratio
Merita[1]	63	1	255	5	51
Melias[1]	20	2			
Welcome[1]	172	2			
Merita[2]	30	1	57	3	19
Melias[2]	27	2			
Welcome[2]*					
Merita[3]	55	1	165	5	33
Melias[3]	11	2			
Welcome[3]	99	2			
Merita[4]	68	1	138	3	46
Melias[4]*					
Welcome[4]	70	2			
Merita[5]	21	1	112	3	37,33
Welcome[5]	91	2			
Merita[6]	15	1	125	3	41,67
Welcome[6]	110	2			

Table 2.3a. Tick numbers counted in the control group in a period of six weeks from goats in the Mnisi area. The week numbers are denoted by the superscripts.
** In week 2 the owner let the goats out already and the goats weren't back the following day.*
***In week 4 the owner let the goats out already and the goats weren't back the following day. The owner was excluded from the study from week 4.*

DELETE ALL	Ticks	Goats	Total ticks	Total goats	Ratio
Merita[1]	23	1	225	5	45
Melias[1]	42	2			

Welcome[1]	160	2			
Merita[2]	2	1	3	3	1
Melias[2]	1	2			
Welcome[2]*					
Merita[3]	9	1	74	5	14,8
Melias[3]	3	2			
Welcome[3]	62	2			
Merita[4]	17	1	84	3	28
Melias[4]*					
Welcome[4]	67	2			
Merita[5]	1	1	11	3	3,67
Welcome[5]	10	2			
Merita[6]	11	1	52	3	17,33
Welcome[6]	41	2			

Table 2.3b. Tick numbers counted in the DELETE ALL group in a period of six weeks from goats in the Mnisi area. In week 1 and week 4 goats received acaricide treatment with DELETE ALL. The week numbers are denoted by the superscripts.
** In week 2 the owner let the goats out already and the goats weren't back the following day.*
***In week 4 the owner let the goats out already and the goats weren't back the following day. The owner was excluded from the study from week 4.*

Athol

Control	Ticks	Goats	Total ticks	Total goats	Ratio
Obe[1]	60	5	187	10	18,7
Nonhlanhla[1]	99	3			
Steffelina[1]	28	2			
Obe[2]	133	5	170	10	17
Nonhlanhla[2]	33	3			
Steffelina[2]	4	2			
Obe[3]	131	5	209	10	20,9
Nonhlanhla[3]	62	3			

Steffelina[3]	16	2			
Obe[4]	243	5	304	8	38
Nonhlanhla[4]	61	3			
Steffelina[4]*					
Obe[5]	36	4	69	7	9,86
Nonhlanhla[5]	33	3			
Obe[6]	80	4	109	7	15,57
Nonhlanhla[6]	29	3			

Table 2.4a. Tick numbers counted in the control group in a period of six weeks from goats in the Mnisi area. The week numbers are denoted by the superscripts.
** In week 4 the goats broke out and the goats didn't receive second treatment, therefore this household was excluded from the study from week 4.*

DELETE ALL	Ticks	Goats	Total ticks	Total goats	Ratio
Obe[1]	47	5	155	10	15,5
Nonhlanhla[1]	73	3			
Steffelina[1]	35	2			
Obe[2]	10	5	13	10	1,3
Nonhlanhla[2]	1	3			
Steffelina[2]	2	2			
Obe[3]	63	5	72	10	7,2
Nonhlanhla[3]	5	3			
Steffelina[3]	4	2			
Obe[4]	91	5	111	8	13,88
Nonhlanhla[4]	20	3			
Steffelina[4]*					
Obe[5]	17	5	17	7	2,43
Nonhlanhla[5]**	0	2			
Obe[6]	31	5	33	7	4,71
Nonhlanhla[6]	2	2			

Table 2.4b. Tick numbers counted in the DELETE ALL group in a period of six weeks from goats in the Mnisi area. In week 1 and week 4 goats received acaricide treatment with DELETE ALL. The week numbers are denoted by the superscripts.
** In week 4 the goats broke out and the goats didn't receive second treatment, therefore this household was excluded from the study from week 4.*
*** There were only two goats, one of the goats ran away.*

Utah A

Control	Ticks	Goats	Total ticks	Total goats	Ratio
Hazasah[1]	130	3	419	5	83,8
Sarah[1]	289	2			
Hazasah[2]	27	3	98	5	19,6
Sarah[2]	71	2			
Hazasah[3]	151	3	442	5	88,4
Sarah[3]	291	2			
Hazasah[4]	67	3	257	5	51,4
Sarah[4]	190	2			
Leonard[4]*					
Hazasah[5]	32	3	105	7	15
Sarah[5]	27	2			
Leonard[5]	46	2			
Hazasah[6]	46	3	169	6	28,17
Sarah[6]	51	1**			
Leonard[6]	72	2			

Table 2.5a. Tick numbers counted in the control group in a period of six weeks from goats in the Mnisi area. The week numbers are denoted by the superscripts.
**In week 1 the owner Leonard wasn't present, the owner was included in the study from week 4. Counting of the ticks started in week 5.*
***There was only one goat, one goat ran away.*

DELETE ALL	Ticks	Goats	Total ticks	Total goats	Ratio
Hazasah[1]	300	3	433	5	86,6
Sarah[1]	133	2			
Hazasah[2]	3	3	15	5	3
Sarah[2]	12	2			
Hazasah[3]	23	3	95	5	19
Sarah[3]	72	2			
Hazasah[4]	28	3	149	5	29,8
Sarah[4]	121	2			

Leonard[4]*						
Hazasah[5]	7	3		10	7	1,43
Sarah[5]	3	2				
Leonard[5]	0	2				
Hazasah[6]	4		2**	36	6	6
Sarah[6]	17	2				
Leonard[6]	15	2				

Table 2.5b. Tick numbers counted in the DELETE ALL group in a period of six weeks from goats in the Mnisi area. In week 1 and week 4 goats received acaricide treatment with DELETE ALL. The week numbers are denoted by the superscripts.
**In week 1 the owner Leonard wasn't present, the owner was included in the study from week 4. Counting of the ticks started in week 5.*
***There were only two goats, one goat ran away.*

To give a more schematic perspective on the differences between the control group and the DELETE ALL group, a couple of graphs have been added below (see figures 2, 3, 4, 5 and 6).

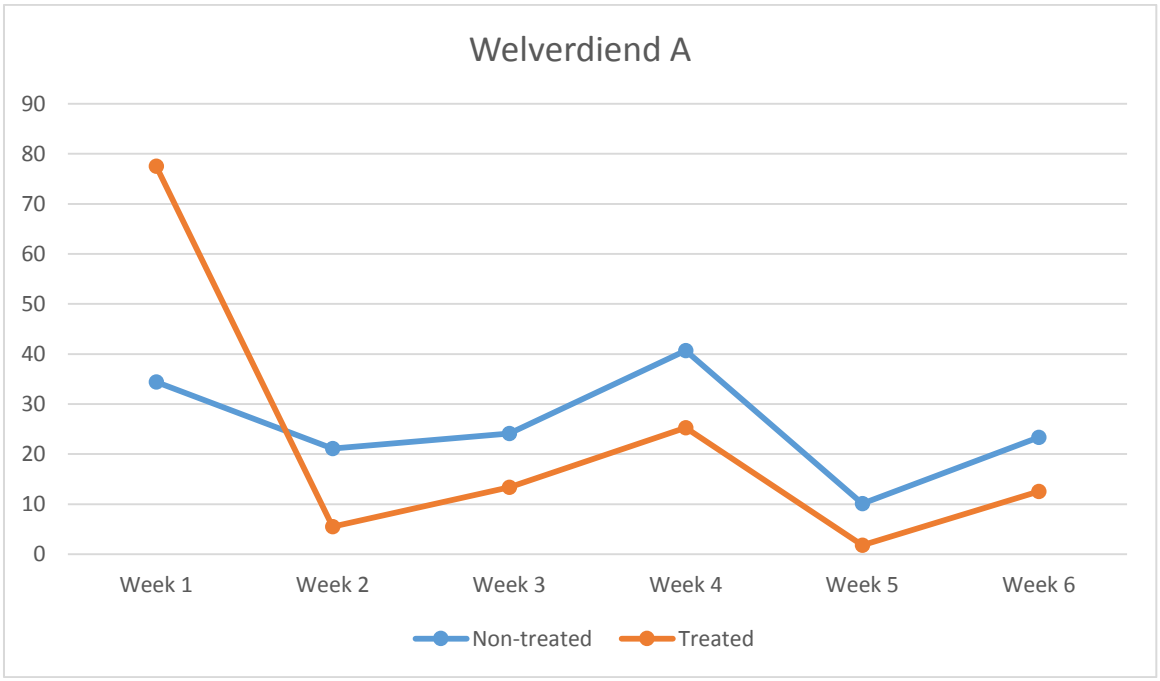


Figure 2. The tick/goat ratios of the control group and the DELETE ALL group of Welverdiend A.

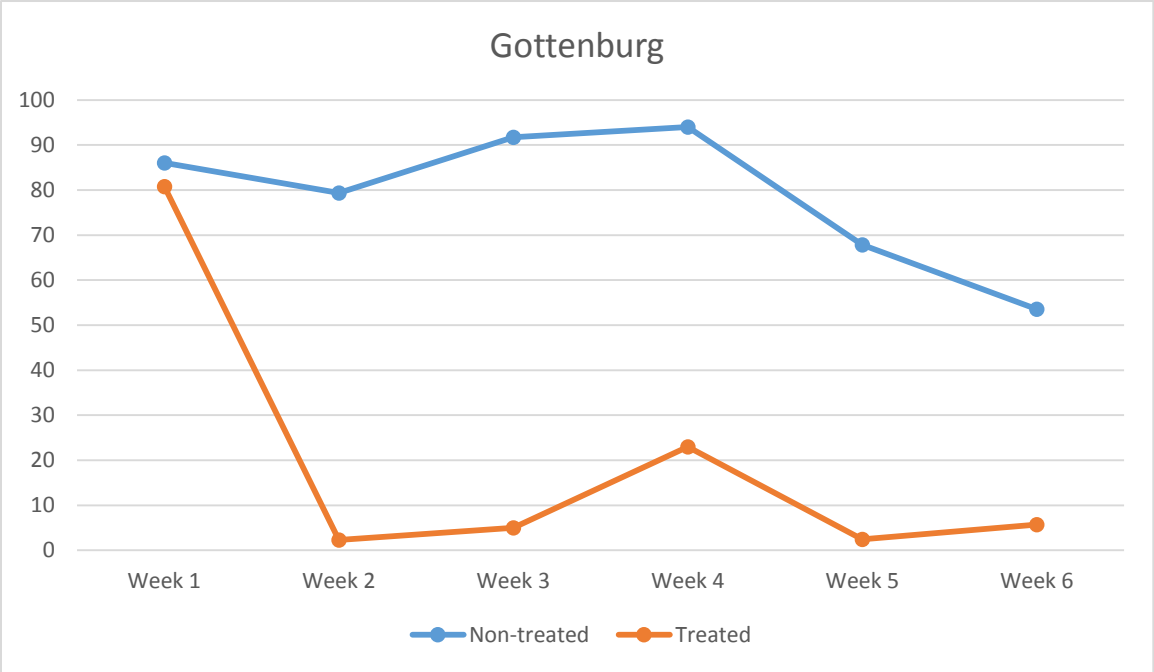


Figure 3. The tick/goat ratios of the control group and the DELETE ALL group of Gottenburg.

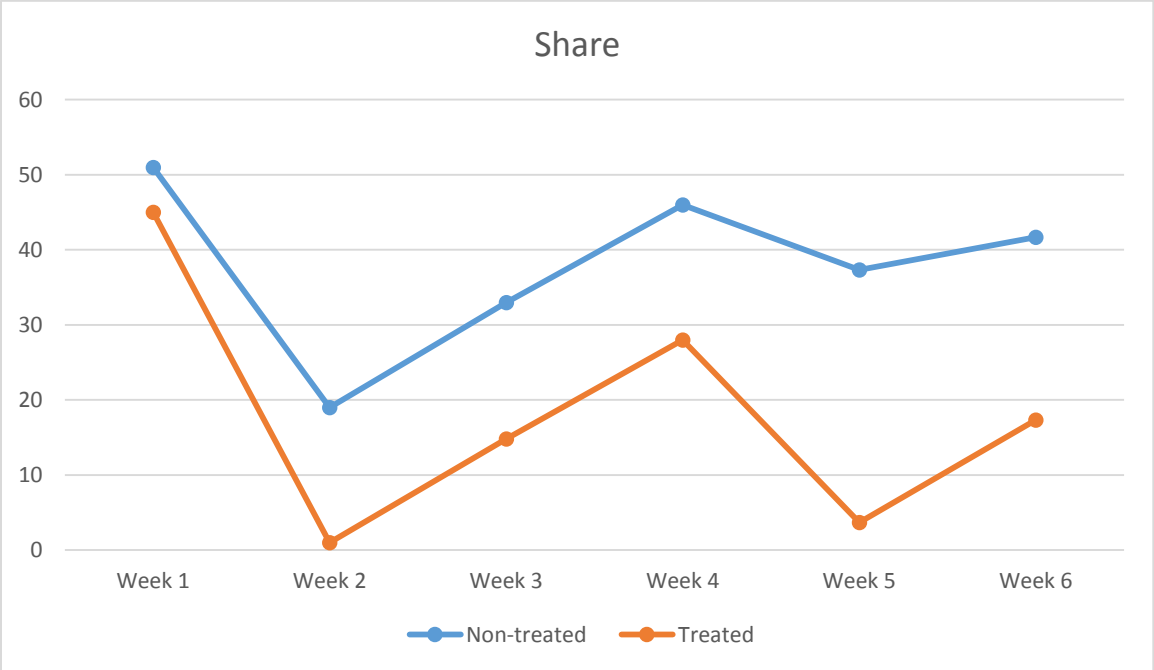


Figure 4. The tick/goat ratios of the control group and the DELETE ALL group of Share.

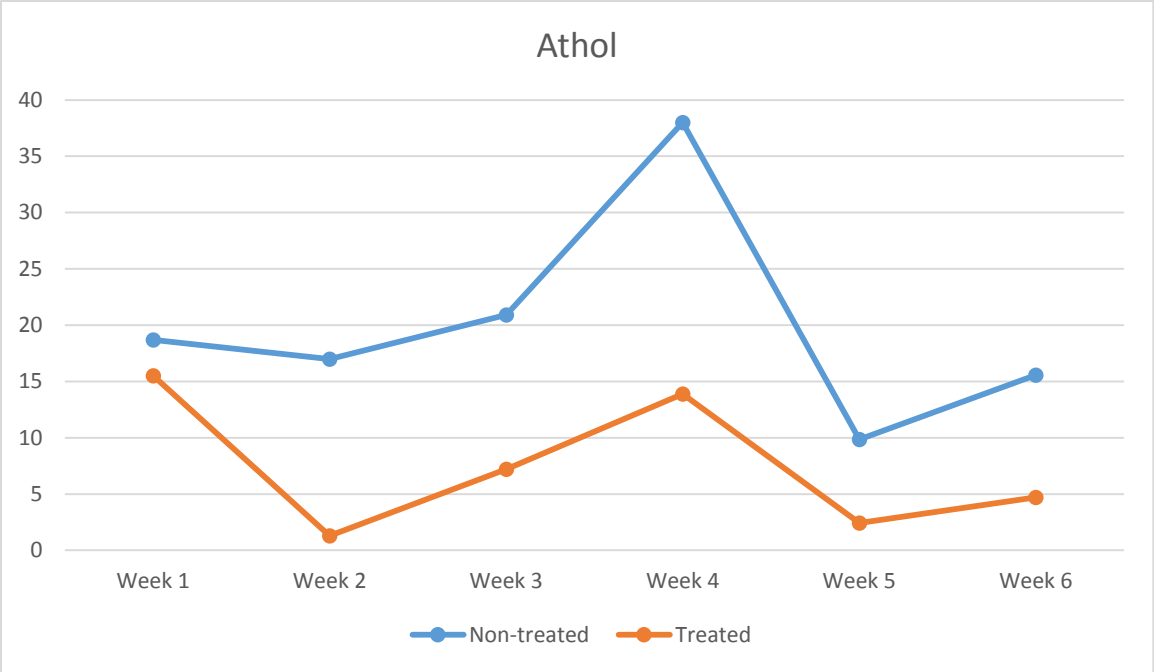


Figure 5. The tick/goat ratios of the control group and the DELETE ALL group of Athol.

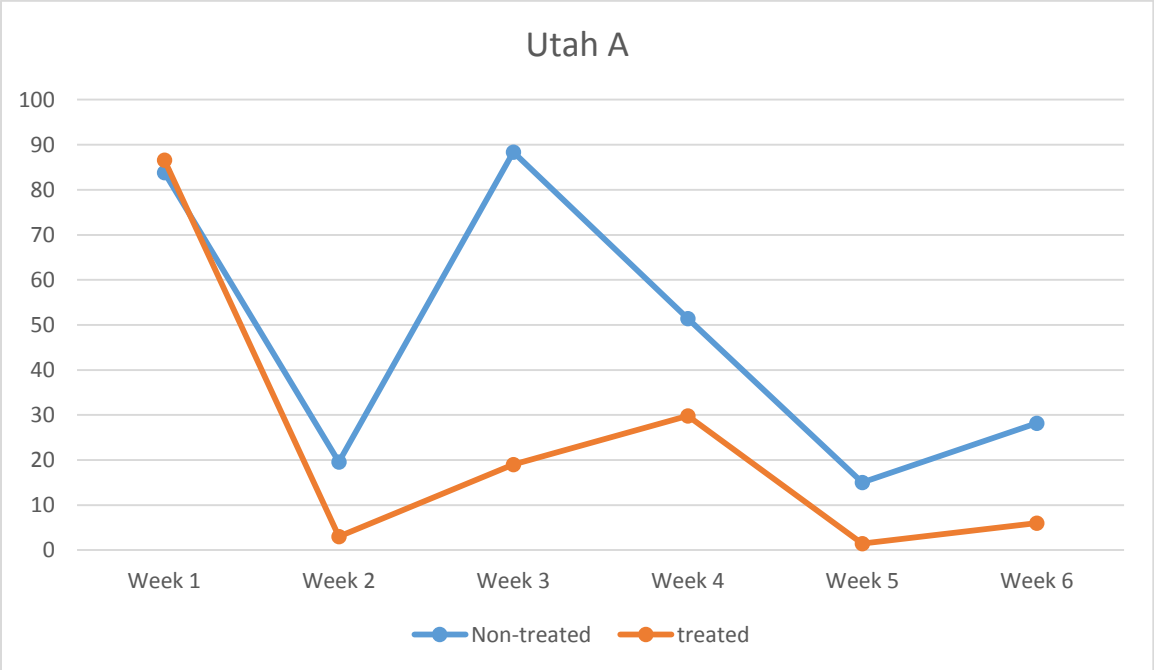


Figure 6. The tick/goat ratios of the control group and the DELETE ALL group of Utah.

4.2 effectiveness of DELETE ALL®

To determine if DELETE ALL® really works, the two group means are compared with each other. Table 2.1 shows that the mean difference of the two groups is 31,18 ticks in week 2, that is one week after treatment with the acaricide DELETE ALL®. These results show that a goat in the DELETE ALL group generally had 31,18 less ticks on its body in week 2 than a goat in the control group. Therefore it seems that the acaricide DELETE ALL® really works to reduce the number of ticks one week after treatment with a factor 14,03.

In order to tell if the difference between the control group and the DELETE ALL group isn't just a chance occurrence a test for statistical significance needs to be performed. An independent T-test is performed to conclude if the difference between the control group and the DELETE ALL group is significant. At first the Levene's Test for Equality of Variances is performed to determine if the two groups have about the same or different amounts of variability between scores. Since the Sig. value is less than 0,05 the top row can be used (equal variances assumed). The Sig. (2-Tailed) value of 0,008 is less than 0,05 and therefore one can conclude that there is a statistically significant difference between the control group and the DELETE ALL group in week 2.

Table 2.2 shows the results for week 3, where it seems that DELETE ALL® reduced the number of ticks with a factor 4,67 compared to the control group. A test for statistical significance is performed for this week as well, with a sig. value of 0,001. Since the sig. value is less than 0,05 the conclusion can be made that there is a statistically significant difference between the control group and the DELETE ALL group in week 3.

The results for week 4 are shown in table 2.3. Compared to the control group it seems that DELETE ALL® reduced the number of ticks with a factor 2,45 in week 4. The sig. value is less than 0,05, namely 0,013. Therefore one can conclude that there is a statistically significant difference between the two groups in week 4.
Table 2.4 shows the results of week 5, which is the week after the second treatment with DELETE ALL®. It seems that this acaricide reduced the number of ticks with a factor 11,51 compared to the control group. The sig. value of the test for statistical significance is 0,001 so the conclusion that there is a statistically significant difference between the control group and the DELETE ALL group in week 5 can be made.

The results for week 6 are shown in table 2.5. Compared to the control group it seems that DELETE ALL® reduced the number of ticks with a factor 3,65 in week 6. The sig. value of 0,001 is less than 0,05. Therefore one can conclude that there is a statistically significant difference between both groups in week 5.

Week 2

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Total	Control	33	33,5758	65,67678	11,43286
	DELETE ALL	33	2,3939	2,39713	,41729

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Total	Equal variances assumed	8,033	,006	2,726	64	,008	31,18182	11,44047	8,32685	54,03679
	Equal variances not assumed			2,726	32,085	,010	31,18182	11,44047	7,88077	54,48287

Table 3.1. Output IBM SPSS Statistics Viewer, independent samples T-test.

Week 3

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Total	Control	35	49,7714	64,06021	10,82815
	DELETE ALL	35	10,6571	12,63269	2,13531

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Total	Equal variances assumed	15,694	,000	3,544	68	,001	39,11429	11,03669	17,09092	61,13765
	Equal variances not assumed			3,544	36,640	,001	39,11429	11,03669	16,74442	61,48415

Table 3.2. Output IBM SPSS Statistics Viewer, independent samples T-test.

Week 4

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Total	Control	31	55,9355	67,96123	12,20620
	DELETE ALL	30	22,8000	18,83760	3,43926

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Total	Equal variances assumed	5,403	,024	2,576	59	,013	33,13548	12,86399	7,39470	58,87627
	Equal variances not assumed			2,613	34,726	,013	33,13548	12,68147	7,38346	58,88750

Table 3.3. Output IBM SPSS Statistics Viewer, independent samples T-test.

Week 5

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Total	Control	32	26,3125	40,91726	7,23322
	DELETE ALL	35	2,2857	2,53877	,42913

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Total	Equal variances assumed	20,033	,000	3,469	65	,001	24,02679	6,92586	10,19488	37,85869
	Equal variances not assumed			3,316	31,218	,002	24,02679	7,24594	9,25279	38,80078

Table 3.4. Output IBM SPSS Statistics Viewer, independent samples T-test.

Week 6

Group Statistics

	Group	N	Mean	Std. Deviation	Std. Error Mean
Total	Control	31	31,0323	36,96709	6,63948
	DELETE ALL	34	8,5000	6,56552	1,12598

Independent Samples Test

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Total	Equal variances assumed	12,057	,001	3,497	63	,001	22,53226	6,44390	9,65515	35,40936
	Equal variances not assumed			3,346	31,727	,002	22,53226	6,73428	8,81033	36,25418

Table 3.5. Output IBM SPSS Statistics Viewer, independent samples T-test.

4.3 Side effects of DELETE ALL®

DELETE ALL® is a relatively new ready to use pour-on product which contains several components. The deltamethrin, which is a pyrethroid substance, kills ticks that get in contact with it. However DELETE ALL® does not only kill the ticks since another substance, namely amitraz, detaches the ticks from the host to make them drop off.
The product details of MSD describe that one need to treat animals strictly according to body weight. In case of an overdosage, clinical symptoms such as salivation and lethargy can be shown by the animals.[14] In fact, several cases of Amitraz poisoning in humans and animals are reported. Different mechanisms of Amitraz toxicity have been described, such as oxidative stress and neurotoxicity. Amitraz toxicity and the lethal dose (LD50) have been evaluated in acute toxicity studies. The LD50 for rats for instance is 600 mg/ kg/ day that can be taken orally. For a rat with an average weight of 500 gram the LD50 is 300 mg a day. The dosage that is advised for sheep and goats is only 5 ml/ kg. When you have a goat with a body mass of 50 kg for instance, this goat will only receive 100 mg of Amitraz so this is not a lethal dosage for a rat.[24]

4.4 Costs of the treatment

Farmers of the Mnisi area can buy a bottle of DELETE ALL® at the 'Obaro' store in Hoedspruit without any prescription of a veterinarian. DELETE ALL® comes in amounts of 200 ml, 1 litre, 5 litre or 20 litre.[14] Farmers can only buy the one litre variant at the 'Obaro' store for 443 ZAR (South African Rand) which is the equivalent of 29,76 euro's. With one litre of this acaricide 100 goats can be treated once (10 ml per goat). Therefore a farmer with 5 goats for instance could treat his animals 20 times. Since the recommended treatment interval is 3-4 weeks, a farmer with 5 goats can use the one litre bottle of DELETE ALL® for 60-80 weeks in total.[25]

Another acaricide named tick grease is also sold by the 'Obaro' store in Hoedspruit. Tick grease contains cypermethrin which is another pyrethroid substance that kills the ticks. This is less expensive than the acaricide DELETE ALL®, since the cheapest option for a pot of 500 gr of tick grease is 58 ZAR (4,08 euro's). However, former studies have shown that the effect of tick grease is not as great as the effect of DELETE ALL®. Although one would expect a decreased number of ticks on goats that were treated with tick grease, Maria King has shown that there even was an increase in tick numbers on the goats of the tick grease group.[7]

Although DELETE ALL® is an effective tick-control method, it is not a matter of course that every farmer has this acaricide on the shelf. A questionnaire survey that was performed by the student M. King, showed that none of the farmers that were visited used any kind of tick control.[7] It is difficult for farmer to even get to the 'Obaro' store, since most of the farmers in the Mnisi area travel by foot. It is approximately an hour's drive from the veterinary clinic in Hluvukani to Hoedspruit, what makes the store beyond the reach of the farmers.

5. Discussion

A recent performed questionnaire survey on diseases and problems affecting goats and sheep in communal farming regions of the Eastern cape Province showed the importance of agriculture to the standard of living. There were four different groups of participants involved in this survey, namely livestock farmers, their livestock production advisors, animal health technicians and veterinarians. The farmers scaled their goats as of most importance in this survey. Both internal and external parasites emerged as important disease categories with heartwater as one of the most important diseases.[17] Despite this information the occurrence of heartwater in the Mnisi area is thus far under-reported. Heartwater has not been diagnosed yet in the Mnisi area, neither in cattle nor in small ruminants. Since the definitive diagnosis of heartwater is based on the observation of rickettsial inclusion bodies within endothelial cells in stained brain crush smears it is not easy to subscribe clinical symptoms to heartwater. Mostly farmers are unable or unwilling to pay for definitive diagnoses, what makes the economic impact of heartwater disease difficult to quantify.[20]

Another reason for heartwater to be underscored could be that farmers did not notice the clinical symptoms of the disease or that they simply do not have enough knowledge about it. In the questionnaire survey performed by Gareth F. Bath, farmers were asked to award high scores for the main challenges that were identified for controlling diseases. Lack of information for farmers received the highest scores with a 9,7 out of 10. The veterinarians and technicians rated lack of farmer training the highest and the advisors awarded the highest score to lack of expert assistance. These answers taken together indicate that lack of knowledge and skills and especially the inability to access them are bottlenecks of most importance for controlling diseases in small ruminants.[17]

It is clear that ticks form a major burden for the farmers in the Mnisi area, since there was not even a single goat that was free of ticks in the first week of this study. The questionnaire surveys that were conducted by M. King in a prior study showed that seventy percent of the farmers that were visited have seen lameness or infected feet in some or all of their goats.[7] The presence of *A. hebraeum* in the inter-digital space can be associated with purulent ulcers and abscesses accompanied by lameness in goats. This tick species has a tendency to form clusters and can cause serious damage to feet, udders and scrota because of its long mouthparts. [18] Several cases of goats with non-purulent or purulent ulcerations on other parts of the body are described, especially in the peri-anal region. Adult ticks can cause serious wounds that become secondarily infected by bacteria and the screwworm *Chrysomyia bezziana.* This explains why the goat of the owner Hazasah Moyahn with the great abcessing wound in its peri-anal region had a large amount of adult *A. hebraeum* ticks in this area.[19, 21]

However, only a few adult ticks were found compared to the total number of collected ticks. This might be due to the season in which this study has taken place. The *A. hebraeum* adults are mainly found during the summer period and less during autumn or winter.[18] The fact that mostly larvae and nymphs were found in the period from May to July is in agreement with previous observations by K.D.F. Maclvor.[22] As most ticks were attached on the legs, especially in the inter-digital space of the hooves, acaricide applied in a footbath or DELETE ALL®

applied on the feet would probably provide adequate control and prevent damage.

Results of this study show that there is a statistically significant difference in the number of collected ticks from the goats of the control group and from the goats that were treated with DELETE ALL®. Figures 2 to 6 show that there is a decrease in the number of ticks in the week after treatment with DELETE ALL®. Although there is a marked decrease, the goats of the DELETE ALL group are still not entirely free of ticks the week after treatment. Tables 3.1 and 3.4 show a mean in number of ticks for the goats of the DELETE ALL group of respectively 2,39 and 2,29. Roughly taken a goat treated with DELETE ALL® has 2 ticks on its body though a goat in the control group had 33,57 and 31,03 ticks on its body in week 2 and 5 respectively. The decrease in number of ticks within the DELETE ALL group is most noticeable, with a mean in this group of 57,75 ticks per goat in the first week. This means that there is a decrease in ticks of almost 96 percent for the goats in the DELETE ALL group just one week after treatment.

Notwithstanding the results that show a statistically significant difference in the number of ticks between both groups two or even three weeks after treatment, the 3-4 week interval that is advised for treatment with DELETE ALL® can still be discussed.[14] Figures 2 to 6 show that the number of ticks increases from the second week after treatment. Because of the treatment with DELETE ALL® the treated goats in week 3 and 6 severally had 4,67 and 3,65 times less ticks than the goats of the control group. However the number of ticks in the control group and the DELETE ALL group only differ with a factor 2,45 three weeks after treatment. Therefore a shorter treatment interval seems to be more efficient in the use of this product. In this study, it was obvious that the effects of DELETE ALL® did reduce the number of ticks infesting the goats in the Mnisi area, especially the week after treatment with the acaricide.

It is remarkable that there is not only a decrease in the number of ticks shown in the DELETE ALL group, since figures 2 to 6 show a decrease in the control group as well. In a study on the effect of tick-control for free-living populations of *A. hebraeum* kudu, scrub hares and helmeted guinea fowls, which were on the farm with domestic stock were compared with the burdens of similar animals in an adjacent nature reserve. The cattle, sheep and goats from this farm were treated with acaricide every 4 weeks for several years. Not only the animals from the different areas were assessed on the amount of ticks, since the dragging method was performed as well to collect the free-living ticks. Results of this study show a clear depression of the population size of all life stages of *A. hebraeum* on the farm due to acaricidal treatment of livestock.[22] Therefore it is likely that the treatment with DELETE ALL® caused a decrease in the number of ticks found in the control group as well.

Although DELETE ALL® has proven itself to be effective, there are several cons for this product. Since the 'Obaro' store is too far from Hluvukani to travel by foot, it is hard for farmers to obtain the acaricide. The present study has shown that a treatment interval of 1 week would be most efficient, which means that a farmer with 5 goats for instance can only treat the goats for 20 weeks. This would make the product relatively even more expensive. This might encourage farmers to use alternatives for tick controlling such as used engine oil. Components in used engine oil can not only harm the environment, they are toxic for animals and

humans as well.[23] This does not mean that one should not be cautious in the use of DELETE ALL®, at least this is a product with clear directions for use that people should take into account.

There were a couple of things that did not go according to plan during this study. Firstly, the number of goats at the households. According to the study design, 10 goats per household should be used (5 for the DELETE ALL group and 5 for the control group). However there was only one owner with enough goats to meet these requirements. There were several households with only 2 goats or households with unequal groups, which make the outcome of this study less reliable (see tables 2.1 to 2.5). Another problem was the absence of the owners Lion of the village Welverdiend A and Leonard of Utah A (see tables 2.1 and 2.5) the first day of the study. Several visits to these households were paid during the first week without any success. Since we were not able to treat these goats in week one, these households needed to be excluded from the study for the first period of 3 weeks.

The problem that there were not enough goats for the study even got more serious over time, because of goats that were sold by the owner and goats that ran away. In week 4 for example, there were 3 goats of the owner Lion in the control group and there was only 1 goat left in week 5 (see table 2.1a). For this reason, adjustments had to be made in the composition of both groups. These adjustments were necessary for the goats of the owner Gracias of the village Gottenburg (see table 2.2).

Many owners let their goats out of the pen during the day. Although the environmental monitors tried to emphasize the importance of keeping the goats in the pens until the goats were examined, it occurred several times that the goats were out already. Therefore it was necessary to visit several households a day or even several days later than planned. Because of this there were eight or more days between examinations of a household, which can influence the number of collected ticks by the next examination. Whether the goats of the owner Steffelina of Athol were let out or broke out was not sure, however these goats did not receive treatment in week 4 and were excluded from the rest of the study (see table 2.4).

The goats within the two different groups were marked with a special marker aerosol for livestock. Unfortunately the tin was empty in week 2 already, so a livestock marker pen was used from that moment as an alternative. However it became more difficult to distinguish goats from both group, since the marks made with this pen were not able to sustain that well for a week. A few times when the marks were not visible anymore, goats were ascribed to a group based on memory. For this reason it might be possible that some goats were allocated to the wrong group.

Lastly, it may be that DELETE ALL® was not applied correctly to the predilection sites what might be the reason that the goats of the DELETE ALL group were not entirely free of ticks the week after treatment. A brush was used to apply the DELETE ALL® which is a liquid substance. Despite the cautious manner used to apply the acaricide, a certain amount of this substance dripped on the ground. Because of this it could be that not enough of the substance was applied per

goat. When applying DELETE ALL® one should make sure to pay extra attention to the inter-digital space of the hooves.

Conclusion

Goats play an important role for the standard of living of the farmers in the Mnisi area. Although heartwater is still not found in goats of the Mnisi area, one cannot conclude that these goats do not get infected by *A. hebraeum* ticks. The disease seems to be underscored due to lack of knowledge of farmers and the difficulty to make a definitive diagnoses. This makes it even more important to always include goats in a tick control program.

Results of this study have shown that DELETE ALL® can serve as an adequate method for goats in *Amblyomma* tick control management in the area of Mnisi. Every week of the study, there was a significant difference in the number of ticks collected from the goats in the control group and from the goats in the DELETE ALL group. Especially one week after treatment with the acaricide the number of ticks collected from treated goats almost went down to zero. In the following weeks the effect of DELETE ALL® got less, therefore a shorter treatment interval should be used for a more effective treatment with this acaricide. This study also shows a remarkable effect of DELETE ALL® on goats that were not even treated. The number of ticks collected from the goats of the control group was reduced as well, what might be due to acaricidal treatment of the other goats in the same pen.

Although DELETE ALL® can serve as an method for goats in *Amblyomma* tick control management, it will probably not be a matter of course that every farmer will use it. It is an expensive product, especially when farmers need to treat their goats more often than recommended by the animal health company MSD. The problem that the 'Obaro' store is too far away, makes it difficult for farmers to even obtain the acaricide. Therefore it would be better to make the product more accessible for farmers first, to use it for tick control management in the Mnisi area.

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APPENDIX A: Study area

Hluvukani (24°38'42.9"S 31°20'47.5"E), Welverdiend A (24°34'46.3"S 31°19'26.7"E), Gottenburg (24°38'21.6"S 31°24'38.8"E), Share (24°40'36.0"S 31°18'46.5"E), Athol (24°42'34.2"S 31°20'45.8"E) and Utah A (24°41'36.0"S 31°26'49.5"E).